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ORIGINAL ARTICLE

Chronic Exposure to Polyethylene Terephthalate Microplastics Induces Endocrine and Inflammatory Dysregulation in Female Albino Rats: Implications for Reproductive and Public Health

GODAE FIDELIS BEEGA[1,*], KEMZI ELECHI-AMADI[2], EDNA OGECHI NWACHUKU[2]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Africa, Toru-Orua, Bayelsa State, Nigeria
[2]Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria

Abstract

***Background*:** The widespread use of plastics has led to an increased environmental presence of microplastics, with potential adverse effects. This study was conducted to assess the effects of polyethylene terephthalate (PET) microplastics on reproductive hormones and Interleukin-1β in chronically-exposed female albino rats.
***Methods*:** Forty female albino rats were selected for the study. Polyethylene terephthalate pellets were obtained, crushed, dissolved in water, and passed through a 5µm sieve to obtain particles with a size of ≤ 5µm which were used for the study. Twelve rats were used for a pilot study, while the remaining twenty-eight rats were randomly assigned into four experimental groups (n = 7). The PET treatment was administered orally using gavage tubes for 90-days. 40mg/kg, 80mg/kg, and 120mg/kg PET-microplastics were given to rats in groups 2, 3, and 4, respectively, while group 1 received only food and water (negative control). After the duration of treatments, blood samples were collected for analysis of estradiol, progesterone, prolactin, and IL-1β levels using ELISA methods. Statistical analysis was computed using GraphPad Prism Software. Differences were considered significant at $P \leq 0.05$.
***Results*:** The mean levels of estradiol, progesterone, and IL-1β in the PET-treated rats were significantly higher than the levels in the control group ($p < 0.0001$). However, the mean levels of prolactin in PET-treated groups were not significantly different from those in the control rats ($p = 0.2157$).
***Conclusion*:** These findings suggest that chronic PET-microplastic exposure may induce inflammatory responses and endocrine disruption in the animal model, as evidenced by alterations in hormone levels and IL-1β concentration, highlighting potential health risks that may be associated with prolonged PET microplastic exposure.

Keywords: Estradiol, Progesterone, Interleukin 1β, Plastic Particles, Toxicity

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INTRODUCTION

There is a tremendous increase in the use of plastics worldwide, which has led to an alarming increase in environmental pollution, particularly through the formation of microplastics-plastic particles less than 5 mm in diameter [1]. These particles, which are formed through the break down, splitting, or crushing of larger plastic materials, are now commonly recognized as new environmental pollutants because of their toxicity and persistence [2].

A wide range of environmental matrices, including soil, food items, drinking water, and marine ecosystems, have been reported to contain microplastics (MPs) [3]. Human exposure to microplastics is mostly through inhalation, skin contact, and ingestion, which raises concerns about their accumulation in the body and potential health implications [4]. Among the various plastic polymers, polyethylene terephthalate (PET) is commonly used in packaging, especially in Nigeria due to its strength, transparency, and food safety qualities [5]. Over time, PET materials can breakdown into microplastics that contaminate the environment and may leach toxic chemicals into food and beverages, especially when used in food packaging and bottled products [6].

Notably, studies have revealed that take-out containers and food packages made from polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) release microplastics, with individuals

*Correspondence to: Dr. Godae Fidelis Beega. Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Africa, Toru-Orua, Bayelsa State, Nigeria. Email: godae.beega@uat.edu.ng,
Tel: +2347065968364

who regularly eat takeout food consuming as many as 12–203 microplastic particles weekly [7]. Due to the adverse effects of microplastics, various international organizations have banned the use of certain harmful plastic additives like phthalates in various household products, such as toys and childcare articles, in concentrations above 0.1% [8, 9].

Microplastics often contain endocrine-disrupting chemicals (EDCs), which can affect the functioning of organs that respond to hormonal signals through mimicking natural hormones, antagonizing their action, altering their pattern of synthesis and metabolism, or modifying the expressions of specific receptors, potentially leading to reproductive toxicity and hormone-related disorders [6]. These EDCs can alter hormonal synthesis, metabolism, and receptor expression, ultimately impairing reproductive health and fertility [6].

While there is growing concern and recognition of the toxicological effects of PET microplastics, their long-term effects on female reproductive hormones and inflammatory markers are still greatly under-investigated, especially in Nigeria. Although some studies have suggested that exposure to polystyrene microplastics can induce ovarian inflammation, reduce oocyte quality, and impair pregnancy outcomes via immune dysfunction [10, 11], there is a dearth of literature on the specific effects of PET microplastics on reproductive hormones and inflammation. A limited number of studies have suggested that following environmental exposure, PET microplastics are capable of entering biological systems and accumulating in metabolically active tissues [12, 13]. Once internalized, they can function as physical stressors, triggering the production of reactive oxygen species and innate immunological responses, resulting in the release of pro-inflammatory mediators such as interleukin-1β, creating a chronic low-grade inflammatory state [14]. Inflammatory signaling and oxidative stress may further create a prolonged cellular environment that can disrupt endocrine regulation. In reproductive tissues, inflammatory cytokines may alter the activity of granulosa cells, disrupt the functions of steroidogenic enzymes, and modify the synthesis and feedback control of key hormones such as estradiol and progesterone [15].

Hormones such as progesterone, estrogen and prolactin are crucial in regulating the menstrual cycle, fertility, and breast development [16, 17]. Alterations in these hormones can contribute to infertility and other reproductive health issues. Inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are also known to influence reproductive processes and have been associated with toxicity induced by microplastics [18]. However, the interaction between chronic PET-microplastic exposure, inflammation, and regulation of reproductive hormones in female physiology has not been extensively studied, especially in Nigeria, hence the need for this study.

This study was therefore conducted to evaluate the effects of polyethylene terephthalate microplastics on reproductive hormones and inflammatory marker interleukin-1 beta (IL-1β) in chronically exposed female albino rats. Understanding the effects of these particles on human health, particularly in relation to inflammatory response and reproductive hormones, is necessary for elucidating potential mechanisms of endocrine disruption, identifying environmental risk factors for infertility, guiding public health interventions, and informing regulatory policies aimed at mitigating exposure to microplastics and their associated health impacts.

METHODS

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. Polyethylene terephthalate (PET, PETE), $(C_{10}H_8O_4)_n$, (CAS Number 25038-59-9), pellets used in this study were obtained from Port Harcourt, Rivers State, Nigeria. Rat estradiol, progesterone, and prolactin ELISA Kits were obtained from Calbiotech, Inc., USA (Cat. Nos: ES380S, PG362S and PR234F respectively). Rat-specific IL 1β (Interleukin 1β) ELISA Kit (Catalog No: E-EL-R0012) was obtained from Elabscience Bioinovation Inc. Other equipment used include: MindRay MR-96A Microplate Reader, NewLife Bucket centrifuge (Model: 800D), and digital weighing balance. All chemical and reagents used for all analyses were of good quality and analytical grade.

Experimental Animals

A total of forty female albino rats weighing approximately 150g – 170g were used for the entire study. Twelve rats were used for the pilot study, while the remaining twenty-eight (28) rats were randomly assigned into 4 experimental groups with 7 rats in each study group (n = 7) using a simple randomization method to minimize selection bias. 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 ($28 \pm 2\ ^{\circ}C$) and humidity-regulated (47 ± 2%) location, with a typical 12:12 light-dark photocycle. As there is no institutional Animal Research Ethics Committee at the study site (Rivers State University, Port Harcourt), all experimental procedures were conducted in accordance with internationally recognized guidelines. The animal experiments and handling were in consonance with the National Research Council's Guide for the Care and Use of Laboratory Animals Health [19], and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [20].

Preparation of Polyethylene terephthalate (PET)

Polyethylene terephthalate pellets were crushed thoroughly using a mechanical grinder to obtain tiny particles (microplastics). The crushed PET particles were

weighed using a digital weighing balance (Model: TS500), mixed with water and allowed to stay for 24 hours after which it was passed through a 5 µm sieve to obtain the desired microplastic particle size range/distribution of ≤ 5 µm prior to use.

Dose Determination and Pilot Study

A pilot study was carried out to determine the LD_{50} of polyethylene terephthalate (PET) microplastic administered orally after allowing 14 days of acclimatization using Lorke's method of pilot toxicity testing as described by Lorke [21]. Twelve (12) rats were used for this pilot study. The Highest Dose that caused no death (D_0) was 115mg/kg, the Minimum Dose that caused death (D_{100}) was 130mg/kg while the mean lethal dose (LD_{50}) was calculated to be 122.27mg/kg.

Experimental Design

The study was a chronic study, and after allowing fourteen (14) days for the rats to acclimatise to the new environment, twenty-eight (28) rats were randomly assigned into five (5) groups labelled group 1 to 4 with seven (7) rats in each group. Group 1 was termed the negative control and received food and water only, while group 2, 3 and 4 received 40 mg/kg, 80 mg/kg and 120 mg/kg PET microplastics, respectively, alongside food and water. The PET microplastic treatment was done daily, administered orally using a gavage tube for 90 days.

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, 5ml 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 4000rpm for 5minutes. The serum was collected into another plain bottle and properly labelled for analysis of estradiol, progesterone, prolactin and IL 1β levels.

Estimation of Serum Estradiol, Progesterone and Prolactin

Rat estradiol, progesterone, and prolactin ELISA Kits from Calbiotech, Inc. USA (Cat. Nos. ES380S, PG362S and PR234F respectively) were used to measure the concentration of estradiol, progesterone, and prolactin present in the samples using the ELISA method according to the manufacturer's instructions/procedure as described by Calbiotech Inc. USA [22-24]. Appropriate commercially prepared quality control materials (Randox Acusera Control Samples, Level 1, 2, and 3 Cat Nos: LAL4213, LAN4214 and LAE4215) were included in each analytical run to verify assay precision, and accuracy in line with established laboratory quality-assurance, and bioanalytical validation guidelines [25].

Estimation of Rat Interleukin 1β

Rat-specific IL 1β (Interleukin 1β) ELISA Kit (Catalog No: E-EL-R0012) from Elabscience Bioinovation Inc. was used to measure the concentration of Interleukin 1β in the samples according to the manufacturer's instruction as described by Elabscience Bioinovation Inc. [26]. Quality control materials were equally included in each analytical run to verify assay precision and accuracy.

Statistical Analysis

Data obtained from the evaluation of parameters in this study were presented as mean ± SD. The statistical analysis was computed using GraphPad Prism Software, Version 9.0.0 (121), San Diego, California. Statistical comparison between groups was done using one-way ANOVA, while Tukey's multiple comparison (post hoc) was used to obtain specific differences among the various study groups. Differences were considered significant at $P \leq 0.05$.

RESULTS

Mean ± SD of Levels of Reproductive Hormones in the Rats according to Groups

The mean levels of estradiol (pg/ml) and progesterone (ng/ml) in the rats exposed to polyethylene terephthalate (PET) were significantly higher than the levels in the control group ($p < 0.0001$). However, the mean levels of prolactin in PET-treated groups were not significantly different from the control rats ($p = 0.2157$). A progressive dose-response pattern was observed following PET microplastic exposure. Estradiol and progesterone levels increased in a stepwise manner across the exposure groups, with the highest concentrations recorded in the high-dose group. In contrast, prolactin levels did not demonstrate a consistent dose-dependent change (table 1).

Table 1. Mean ± SD of Levels of Reproductive Hormones in the Rats according to Groups

Groups (n = 7)	Estradiol (pg/ml)	Progesterone (ng/ml)	Prolactin (ng/ml)
1	28.15 ± 4.94^{a}	3.48 ± 1.07^{a}	1.29 ± 0.53
2	48.53 ± 6.77^{b}	7.96 ± 1.17^{b}	1.71 ± 0.55
3	77.61 ± 6.02^{c}	9.12 ± 0.86^{b}	1.76 ± 0.33
4	79.29 ± 3.16^{c}	16.83 ± 1.75^{c}	1.50 ± 0.74
P- value	< 0.0001	< 0.0001	0.2157
F -value	133.0	97.75	1.541
Inference	S	S	NS

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 colum
KEY: S – Significant, NS- Not Significantn do not differ significantly from each other.

Mean ± SD of Levels of IL 1β in the Rats According to Groups

The results of IL 1β, revealed that the mean levels of IL-1β in the rats exposed to PET were significantly higher than the levels in the control group ($p < 0.0001$). Also, there was a graded dose-dependent increase in IL-1β levels with increasing PET exposure (table 2).

Table 2. Mean ± SD of Levels of IL 1β in the Rats According to Groups

Groups (n = 7)	IL 1β (pg/ml)
1	23.34 ± 5.64^{a}
2	34.50 ± 4.81^{b}
3	35.41 ± 3.89^{b}
4	53.40 ± 2.39^{c}
p- value	<0.0001
F -value	78.20
Inference	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: S – Significant; IL 1β – Interleukin 1 β

DISCUSSION

This study investigated the effects of polyethylene terephthalate microplastics on reproductive hormones and the inflammatory cytokine Interleukin 1 β in chronically exposed female albino rats. From this study, as presented in Table 1, there was a significant increase in estradiol ($p < 0.0001$) and progesterone levels in PET-exposed rats compared to controls, with no significant difference in prolactin levels.

As estradiol (a primary estrogen hormone) plays a crucial role in regulating the menstrual cycle, ovulation, and reproductive tissue development, produced mainly by the ovaries and is essential for the proliferation of the endometrial lining in preparation for implantation [27], the increase in estradiol observed in this study suggests that PET microplastics which have been reported to contain or adsorb endocrine-disrupting compounds (EDCs) [28], may interfere with normal hormonal regulation. Importantly, PET as a polymer is generally considered less intrinsically estrogenic than plastics such as polycarbonate [29]; therefore, the observed endocrine effects may arise not only from the PET particles themselves but also from residual monomers, surface-adsorbed environmental contaminants, or leached additives introduced during manufacturing or environmental aging, especially as the present study did not chemically characterize released additives or leachates.

Also, microplastics are known to contain Bisphenol Phenol A (BPA) and phthalates, which are endocrine disruptors that can mimic estrogenic activity and disrupt endocrine function, leading to altered estradiol levels [28]. Hence, the elevated estradiol levels observed in this study could result from microplastic-matrix induced hormonal dysregulation. This finding aligns with a study by Inam [30] who reported that exposure to microplastics, including PET microplastics and their associated chemicals can lead to hormonal imbalances, reproductive toxicity, and increased risk of estrogen-dependent disorders which can result in infertility.

As progesterone is a steroid hormone produced primarily by the corpus luteum in the ovaries and is essential for maintaining pregnancy, regulating the menstrual cycle, and counteracting the proliferative effects of estrogen on the endometrium [31], the increase in progesterone levels observed in this study suggests a potential disruption of normal ovarian function, possibly as a compensatory mechanism in response to elevated estradiol. Research has shown that environmental pollutants, including endocrine-disrupting chemicals, can interfere with ovarian steroidogenic pathways [6]. However, since this study exposed animals to mechanically generated PET microplastic particle suspensions without isolating soluble fractions, the results indicate a biological response to the exposure matrix as a whole rather than to PET polymer toxicity alone, thus, this microplastics matrix, acting as endocrine disruptors, may interfere with this process (ovarian steroidogenesis), causing excessive progesterone secretion or impaired metabolic clearance. Additionally, an imbalance between estradiol and progesterone is associated with menstrual irregularities and hormone-related disorders [32]. The observed increase in progesterone levels may indicate a dysregulated hypothalamic-pituitary-ovarian axis following chronic microplastic exposure. While no direct study has evaluated the effect of PET microplastics on progesterone, results from similar studies indicate that exposure to compounds produced from plastic, such as BPA and phthalates, can alter progesterone secretion and receptor activity, potentially leading to reproductive dysfunction [33].

This study's findings are in alignment with those of Zhang et al. [34], who reported that PET microplastics can trigger reproductive toxicity by activating the p38-MAPK signaling pathway in mice, and with Li et al. [35], who demonstrated that PET microplastic exposure induced reproductive toxicity through oxidative stress and p38 signaling pathway activation in mice. Also consistent with this study, Alahmadi et al. [36] reported that PET nanoplastic exposure impaired ovarian follicle growth and altered expression of genes involved in steroidogenesis and cellular stress responses, demonstrating direct interference with hormonal regulatory pathways.

Unlike estradiol and progesterone, this study found no significant difference in prolactin levels among the experimental groups following PET microplastic exposure. Prolactin is a hormone primarily produced by the anterior pituitary gland and is essential for mammary gland development and lactation [37]. While estradiol typically stimulates prolactin secretion, in this study, despite increased estradiol levels in PET-exposed rats, prolactin levels remained unchanged. This selective endocrine response may suggest that PET microplastic exposure preferentially affects ovarian steroidogenesis rather than pituitary regulation, although confirmation would require

targeted neuroendocrine assessment. This finding contrasts with studies on soluble xenoestrogens such as BPA, which reported increased prolactin levels [38, 39], further supporting the possibility that particulate PET exposure does not replicate the full endocrine profile of freely bioavailable plasticizers.

There was a significant increase in the levels of IL-1β following PET microplastic exposure, suggesting a sustained inflammatory response (table 2). Interleukin 1β is a potent pro-inflammatory cytokine involved in both pathological and physiological reproductive processes [40]. Particle toxicology literature increasingly recognizes that microplastics can act as physical stressors capable of activating innate immune pathways independent of chemical leachates, particularly through macrophage activation and inflammasome signaling [14]. Their long-term increase has been linked to endometrial dysfunction, altered folliculogenesis, decreased ovarian function, and disruptions in the hypothalamic-pituitary-gonadal (HPG) axis [40].

Hence, the increased IL-1β concentrations in PET-exposed rats point to a mechanistic connection between microplastic exposure and reproductive inflammatory signaling. The inflammatory response observed in this study may reflect the combined effects of particle uptake, associated chemicals, and secondary oxidative stress. Specifically, it has been shown that IL-1β disrupts ovarian steroidogenesis by affecting granulosa cell activity, which alters progesterone and estrogen synthesis [41]. This supports the finding that PET microplastics may disrupt hormones through inflammatory mechanisms [42], as evidenced by the significantly increased levels of both progesterone and estradiol that were observed in this study. Increased levels of inflammatory cytokines have been known to trigger apoptosis in ovarian follicles, affecting gonadotropin secretion and the signaling pathways that follow [40, 43]. They have also been connected to anovulation, luteal phase abnormalities, and recurrent implantation failure [44].

The findings of this study are consistent with those of Zhang et al. [34], who demonstrated that exposure to PET microplastics provokes a measurable inflammatory response in mice. Similarly, Ishihara et al. [45] reported that aged PET microplastics induced pulmonary inflammation characterized by neutrophil infiltration and elevated expression of pro-inflammatory cytokines, including IL-6 and IL-33, further supporting the capacity of PET particles to activate immune pathways in vivo. Moreover, Bishop et al. [46] showed that PET microplastics directly stimulated the release of key pro-inflammatory mediators such as IL-1β and TNF-α, alongside increased cellular injury in human immune cells.

Due to resource constraints the inclusion of additional molecular assays such as gene expression profiling and oxidative stress markers, which could have strengthened mechanistic interpretations were limited. It is therefore recommended that future studies involving these markers should be investigated to provide a more comprehensive understanding of PET microplastic toxicity and its implications for human reproductive and public health.

CONCLUSION

The findings from this study revealed that chronic exposure to polyethylene terephthalate (PET) microplastics significantly increases the levels of IL-1β, as well as reproductive hormones such as estradiol and progesterone in female albino rats. The simultaneous elevation of inflammatory cytokine and reproductive hormones suggests that PET microplastics may disrupt reproductive physiology in this animal model through interacting pathways of inflammation and endocrine modulation. These results provide experimental evidence that PET microplastics are biologically active and capable of influencing hormonal regulation under controlled exposure conditions, highlighting potential health risks that may be associated with prolonged exposure and the urgent need for stricter environmental regulations, further investigations, including translational and population-based studies, to further elucidate their potential relevance to reproductive health, as well as public health risk assessment and mitigation strategies.

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