Acute toxicity evaluation of Polyethylene terephthalate (PET) Microplastics in mice, *Mus musculus*, and effects after natural depuration

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled**, "Acute toxicity evaluation of Polyethylene terephthalate (PET) Microplastics in mice, *Mus musculus*, and effects after natural depuration"** is based on the results of investigations carried out by me in the **Zoology Discipline** at the **School Of Biological Sciences And Biotechnology (SBSB)**, Goa University under the Supervision **Dr. Avelyno D'Costa** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will be not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Acute toxicity evaluation of Polyethylene terephthalate (PET) Microplastics in mice, *Mus musculus*, and effects after natural depuration"** is a bonafide work carried out by **Ms Mayuri Manoj Verlekar** under my supervision in partial fulfilment of the requirements for the award of the degree of Master Of Science in **the Zoology Discipline** at the **School Of Biological Sciences And Biotechnology (SBSB)**, Goa University.

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PREFACE

This thesis contains the results of research work carried out by the author for fulfilment of requirement for the degree of Master of Science In Zoology under guidance of Dr. Avelyno D'Costa, Assistant Professor of Zoology, School of Biological Science and Biotechnology (SBSB), Goa University.

This thesis is divided into four main chapters. The first chapter introduces the topic and gives the background of the chosen study. First chapter also deals with the applications of Polyethylene Terephthalate (PET) Microplastics and its sources. It also focuses on the aims and objectives of the study. The future scope of the research is also discussed in this chapter.

The second chapter deals with review of literature. It gives a brief idea of previous studies on Polyethylene Terephthalate (PET) Microplastics in mice and other organisms. It also includes literature survey of microplastics and its toxicology on organisms.

The third chapter contains the details of the materials and methods used for the study. It describes methodology used in the study and the chemical preparations for the test. It also contains the details of experimental set up and exposure. It also describes the maintenance of experimental animal.

The fourth chapter deals with the analysis and conclusion of the research work describes. This chapter contains the results and analysis of the study. It also discusses about the reasons for the results obtained. It also concludes the research findings of the study.

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ABBREVIATIONS

ENTITY	ABBREVIATION
Alkaline Amino Transferase	ALT
Bovine serum albumin	BSA
Catalase	CAT
Dimethyl sulfoxide	DMSO
Ethylenediaminetetraacetic acid	EDTA
Glutathione Peroxidase	GSH-Px
High-Resolution Magic Angle Spinning (HRMAS) NMR Spectra	HRMAS NMR spectra
Hydrochloric Acid	HCl
Institute of Cancer Research	ICR
Macrophage	MΦs
Malondialdehyde	MDA
Micro Fibers	MF
Microplastics	MP
Nano Plastics	NP
Nuclear factor erythroid 2-related factor 2	Nrf2
Parts Per Million	PPM

Phosphate Buffer Saline	PBS
Polyamide	PA
Polyethylene	PE
Polyethylene terephthalate	PET
Polystyrene	PS
Reactive Oxygen Species	ROS
Reduced glutathione	GSH
Revolutions per minute	RPM
Scanning Electron Microscope	SEM
Sodium Chloride	NaCl
Sodium Hydroxide	NaOH
Sodium Dodecyl Sulfate	SDS
Superoxide Dismutase	SOD
Trichloroacetic acid	TCA
Thiobarbituric acid reactive substances	TBARS
X-ray diffraction	XRD

ABSTRACT

There are No previous studies on depuration of PET MP in mice are available. There is no research available on sub-acute PET MP analysis in mice. There are very few studies available on mice model for testing of polyester toxicity hence it is assessed. Hence this study aims to investigate the effects on mice liver caused to acute exposure to Polyethylene terephthalate (PET) microplastics. The study suggests that Polyethylene Terephthalate (PET) Microplastics can have serious toxicological effects on Liver of Swiss Albino Mice, *Mus musculus*. The study focuses on effects of Polyethylene Terephthalate (PET) Microplastics on liver and studies the genotoxic effect. It is evident from the current study that PET MPs can effect the Carbohydrates, Proteins, Albumins, Globulins and Free Sugar content in the mice. Effects on enzymes like Alkaline Amino Transferase (ALT), Catalase and Superoxide Dismutase (SOD) were also observed. Reduced Glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) concentrations were also effected. The study also focuses on the natural depuration of the Polyethylene Terephthalate (PET) Microplastics in Mus musculus. The study revealed that all the other parameters were shifting back to normal after the depuration except for Reduced Glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS).

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CHAPTER 1: INTRODUCTION

1.1. Microplastics

Plastics are long chain of molecules made of synthetic polymers. They are produced by chemically bonding monomers together by a process called polymerization. Plastics can be molded into various shapes and are also known for being lightweight and durable. The other properties of plastics such as low density, low electrical conductivity, transparency, and toughness provide them a wide range of applications. According to United Nations Environmental Programme (UNEP) 19-23 million tonnes of plastic waste leaks into aquatic ecosystems, polluting the water bodies every year. Plastic can effect and alter natural habitats and processes thereby reducing the ecosystems' ability to adapt to climate change. This has raised a concern to plastic pollution becoming a global problem. Most conventional plastics that are in extensive use are not biodegradable and cannot be broken down by natural organisms which leads to their persistent in the environment for a very long time. Although plastics undergo some degradation by processes which include weathering due to sunlight, wind, or waves. This causes more harm as smaller fragments are formed and are the primary cause of microplastics. Microplastics can have more dangerous harm that the normal plastic (Leigh Shemitz & Paul Anastas, 2020).

Microplastics (MPS) are particles derived from any kind of plastics with less than 5 mm of particle (Alimba & Faggio, 2019). There are as many as 51 trillion microplastic particles in the seas according to the United Nations (UN) report. Microplastics can be formed from various sources such as breakdown of larger plastic waste into smaller pieces due to light and oxygen exposure, resin pellets used in plastic manufacturing, run-off from plumbing and leakage from production facilities, microbeads found in

health and beauty products, landfills, households, construction projects, and agricultural activities . These microplastics found in the sea can be easily ingested by marine animals due to its small size. It then gets accumulates in the body and can end up in humans through the food chain.

Microplastics can interact with a variety of environmental pollutants due to the high specific surface area through adsorption and desorption in the environment thereby changing the toxicity, transportation, accumulation and degradation of the pollutants (Yin et al., 2021). These MPs can breakdown further and produce Nanoplastics (NP). Nanoplastics are plastic having a diameter of less than 100 nm. Studies have found that MPs and NPs exhibit unique toxicity mechanisms in different tissues and organs, which may be due to variations in other physical properties. NPs can pass through certain barriers thereby fully contacting the tissues while MPs cannot cross such barriers due to their particle size (Yin et al., 2021).

Since MPs have relatively low density they often float in the ocean waters therefore leading to their pervasive occurrences in the marine environment. Since smaller MP particles have lower rise velocities. The distribution of MP in water is different from the large ones with the same polymer compositions, which often are present on the surface of ocean water. Since MPs have larger specific surface area, it can promote their interactions with organic debris, clays, phytoplankton, and other particles, causing an increase of their densities (Wu et al., 2019).

1.2. Polyethylene terephthalate (PET) and its Sources

Polyethylene terephthalate (PET) is a linear thermoplastic polymer made up of the repeating units of terephthalic acid and ethylene glycol monomers (Yoshida et al., 2016). Polyethylene terephthalate (PET) is known by the trade names Mylar, Decron, terylene,

Recron. It has high crystalline melting temperature of 260°C). The stiff polymer chains that are present imparts high mechanical strength, toughness and fatigue resistance up to 150-175°C. It also provides good chemical, hydrolytic and solvent resistance (Venkatachalam et al., 2012).

Polyethylene terephthalate (PET) is a common plastic in many products that include viscose rayon used for clothing and also in packaging material in the food and beverage industries (Dhaka et al., 2022). More than 50% of synthetic fibres produced across the globe consist of PET. The total usage of PET has been estimated to exceed $17 billion annually (V. Sinha et al., 2010).

PET belongs to a high-molecular-weight thermoplastic, semicrystalline polymer and is made up of terephthalic acid and ethylene glycol (Webb et al., 2012) .The polymer to become a stiff molecule due to the short chain of aliphatic molecules that are coupled with an aromatic ring. Therefore, it finds its applications in food, pharmaceuticals and textile industries. It is used as a food packaging material to store different beverages in food industries whereas it is used as fabric in textile (Venkatachalam et al., 2012). It is also employed to make photographic films, X-ray and electrical insulations (Nanda & Berruti, 2021).

Polyethylene terephthalate (PET) has beneficial properties due to its light weight, high tensile strength, transparency and gas barrier. It's residue can be found in groundwater, drinking water, soils and sediments. physical, chemical and biological methods can degrade Polyethylene terephthalate (PET) (Dhaka et al., 2022). PET MPs can promoted cardiomyocyte apoptosis by inducing oxidative stress and can activate mitochondria-mediated apoptotic processes, ultimately leading to myocardial fibrosis (Lu et al., 2024).

With the increasing global consumption and the natural resistance of these plastics to degradation, their accumulation in the environment is of increasing concern (Webb et al., 2012). There is an increasing concern about plastic pollution and toxicity. Polyethylene terephthalate (PET) is one of the six polymers of plastic with the largest global production volume (Dhaka et al., 2022). Polyethylene terephthalate microplastics (PET MPs) are widespread in environment. They can easily enter organisms and get accumulated in the body yet its toxicity has not been well studied (Lu et al., 2024). PET is highly resistant to environmental biodegradation and can cause many and varied environmental concerns due to its accumulation and absorption associated with concentration of organic pollutants, hazardous effects on marine wildlife and dissemination of potentially invasive species to new environments (Webb et al., 2012).

Previous studies suggest that polystyrene, polyvinyl chloride, Polypropylene and other plastic derivatives can have serious toxicology effects in organisms (Danso et al., 2022) however with respect to Polyester (PET) has rarely been addressed in previous studies in mice model. Ecological risk of PA is yet to be assessed as other MPs such as Polystyrene(PS) (Kik et al., 2020), Polyethylene(PE) (Malafaia et al., 2020) and Polypropylene(PP) (Hwang et al., 2019). No previous studies on depuration of PET MP in mice are available. There is no research available on sub-acute PET MP analysis in mice. There are very few studies available on mice model for testing of polyester toxicity hence it is assessed. Hence this study aims to investigate the effects on mice liver caused to acute exposure to Polyethylene terephthalate (PET) microplastics.

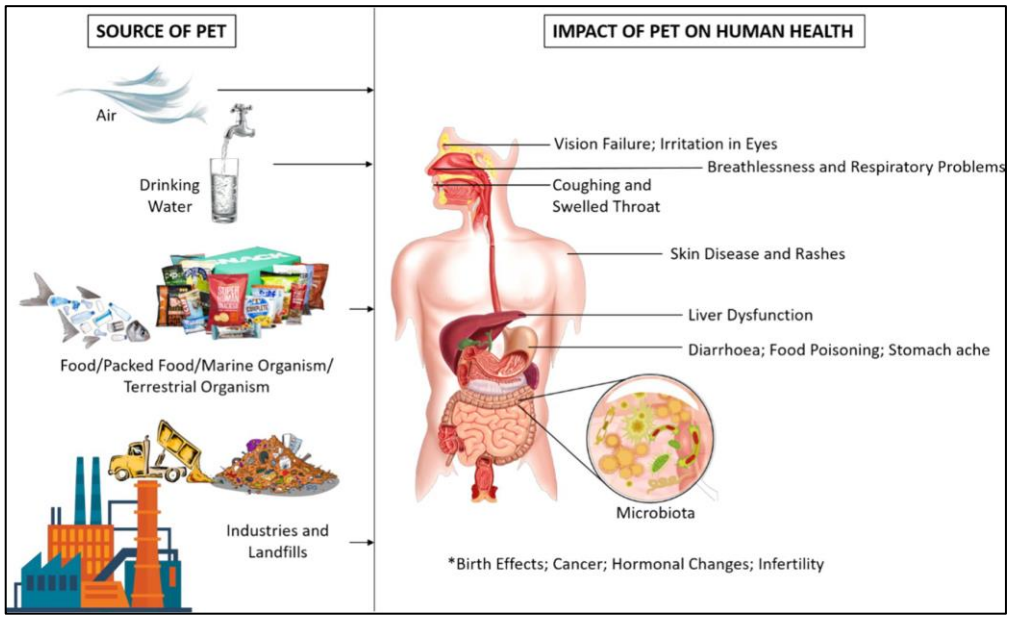


Figure 1: Potential sources of PET and its negative health hazards human organ

1.3. Mice as study model

Mice and rats are preferred species for toxicological and research studies as they have physiological, anatomical and genetic similarities with humans. Mice, rats and humans have 95% common shared genes of approximately 30,000 genes in all three species. Their small size, ease of maintenance, short life cycle and abundant genetic resources are advantageous for using them as model organisms for research studies (Bryda, 2013).

Classification:- Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Rodentia

Family: Muridae

Genus: *Mus*

Subgenus: *Mus*

Species: *M. musculus*

In the current study Swiss Albino Mice, Mus musculus, are used to understand the toxicological effects of Polyethylene Terephthalate (PET) Microplastics because of its similarities with humans.

1.4. Aim And Objectives

a. To analyze effects of PET MP on oxidative stress, hepatotoxicity and genotoxicity.

b. To understand effects caused on biomolecules in the body due to acute exposure to PET MP.

c. To study possible depuration after sub-acute exposure.

1.5. Hypotheses

This study hypothesis that Damage to organ and difference in biomolecules concentration in the body could be observed in mice exposed to Polyester microplastics (PET MP) than those who are not exposed and also that after depuration period the amount of damage caused due to PET MP might be reduced as compared to during exposure period.

1.6. Scope

a. It provides valuable insights into the potential risks posed to human health. Findings from mouse studies can help in risk assessments and regulatory decisions regarding human exposure to PET microplastics since mice share physiological similarities with humans.

b. Since PET microplastics are prevalent pollutants in the environment studying its effects on liver health and genotoxicity in mice contributes to our better understanding

of the broader ecological impacts of microplastic pollution that include effects on wildlife populations and ecosystem health.

c. It can help in overall risk assessment of PET microplastics due to potential hazards associated with exposure. This information is crucial for setting regulatory guidelines and implementing risk management strategies to mitigate the adverse effects of microplastic pollution.

d. The research findings on the toxicological effects of PET microplastics in mice may contribute to public awareness of the potential health risks associated with microplastic pollution. The study findings can support efforts to reduce plastic waste and promote environmentally sustainable practices.

CHAPTER 2: LITERATURE REVIEW

2.1. Microplastics Toxicity in Organisms

Khosrovyan et al. (2020) found in a study to 8uodenum the effects of virgin polyamide microplastics on *Chironomus riparius* adult larvae and adult zebrafish *Danio rerio*. After the depuration period, the number of PA-MP retained assumingly in the gut of fish was remarkably lower than the number of particles found at the end of exposure period, suggesting that the particles did not accumulate in the gut.

Nature and cancer risk of microplastic that originate from e-waste was reported in a study conducted by Madhu D Sharma in 2020. The enrichment of PAHs onto microplastics is determined by physical properties such as particle size, surface morphology and shape. Greater adsorption of PAHs was revealed when the microplastic particles were smaller than 5 mm and prolonged stay of microplastics in water. It was found that the reduction in particle size of microplastic to 10%, the PHAs uptake was increased to 30% (Sharma et al., 2020).

The accumulation of plastic debris has emerged as a global environmental problem, mostly because of plastic's high molecular weight and stable crystal structure, which make it difficult for it to break down. The demand for plastic is growing despite the fact that it pollutes the environment greatly. This plastic over time breaks down into smaller particles such as micro and nanoplastics. In a comparative review of microplastics and nanoplastics it was found that nanoplastics has stronger neurotoxic effect as compared to microplastics. The study also suggested that Nanoplastics are capable of penetrating the barriers in the body easily compared to microplastics. Microplastics and nanoplastics can lead to intense oxidative stress in cells without affecting the cell

viability as confirmed in in-vitro studies. The review also suggested that in intestine, NPs cause higher inflammation and oxidative stress while MPs cause more serious intestinal flora disorders (Yin et al., 2021).

Habio Jin studied the Reproductive toxicity in mice induced by Polystyrene microplastics (PS-MPs). It was found that spermatogenic disorder, decreased testosterone levels, testicular inflammation and destruction of blood testis barrier can be caused. The study revealed that particle size plays an important factor in accumulation of microplastics in different.(Jin et al., 2021).

Non biting midge, *Chironomis riparius*, were exposed to UV-weathered virgin polymide microplastic. Investigation showed that microplastic particles may lead to the accumulation in the internal organs such as liver, kidney and digestive tract due to the ability to cross the biological boundaries. It can also cause inflammation in the liver and intestine, Gill damage, alteration in lipid metabolism and induction of enzymatic responses. Increased acetalcholinesterase enzyme activity was also observed. UV-weathering of MP led to the decrease in the buoyancy of the particles, changed their colour and increased the share of particles of smaller size range (Khosrovyan & Kahru, 2021).

In an experiment conducted to understand the effects of PS MPs on reproductive health of mice, they were feed with water suspended with PS MPs in three different concentrations. The daily water intake was noted. It was found that there was significant reduction in number of viable epididymis sperm and an increased rate of sperm deformity after PS-MPS exposure. Atrophy, shedding, and apoptosis of sperm cells at all levels of the testis were seen on HE and TUNEL staining. The results of Western blot and Qpcr analysis for detection of Nrf2/HO-1 and NF-Kb showed that, the

expression of the pro-inflammatory molecule NF-Kb and that of the inflammatory factors interleukin (IL)-1β and IL-6 increased significantly, whereas that of the anti-inflammatory molecule Nrf2/HO-1 decreased after PS-MPS exposure (Hou et al., 2021).

Intestinal permeability and gene expression after polyester and polyamide microplastic injection in Wister rats was studied by Benuarda Toto in 2022. The aim of the study was to investigate the effect of short term, relatively high oral feeding with polyamide and polyester microplastics on gut permeability, expression and concentration of tight junction proteins and Pro inflammatory proteins. It was found that rats fed with microplastic particles had higher duodenum permeability (Toto et al., 2022).

Qianyu Yang conducted a study on Oral feeding of nanoplastics affects brain function of mice by inducing macrophage IL-1 signal in the intestine. In the study it was found that although nanoplastics were effectively engulfed by MΦs, they were system to degradation within their lysosomes. As a result cascade of events is triggered by lysosomal damage, culminating in the reprogramming of IL-1β-producing MΦs. In NP-fed mice, our unbiased single-cell sequencing identified newly clustered IL-1β+ MΦs, which release a large amount of IL-1β from the gut to the whole body. It was identified IL-1 signaling as a mediator in the cross-talk between the gut and brain, causing neuronal death, microglial activation, and Th17 differentiation. After long-term feeding with NPs, an increased levels of IL-1β in both the intestine and brain of the mice was observed (Yang et al., 2023).

2.2. PET MPs Toxicity

In a study to understand the toxicological impacts of polyethylene terephthalate nano plastics (PET-NP) on zebrafish embryos based on size- and surface coating in terms of hatching rate, heart rate, and ROS generation, it was found that the effects caused varies

based on surface coating. PET NPs having diameter sizes of 20, 60–80, and 800 nm and are capped by two dispersing agents, BSA and SDS. It was found that PET_{BSA} NP treated groups exhibited higher-level abnormalities in heart rate and more severe oxidative damage. The study also suggests novel mechanical preparation protocol for PET NPs from PET bottles (Ji et al., 2020).

A study was conducted to understand the effects caused by Polyester microplastic fibers induce mitochondrial damage, apoptosis and oxidative stress in Daphnia carinata, The reproductive rates were reduced in both the high and low concentration groups. It was observed under fluorescence microscope that PET-MFs were present in the intestine. Obvious signs of cell apoptosis were observed. The ROS content in the low concentration group was significantly higher than that in the control group and the high concentration group (Jiang et al., 2023).

At lower concentration (≥ 50 ppm) of PET NPs no inflict acute toxicity in terms of hatching rate or survival of the embryos was seen whereas higher concentration exposure to PET NPs (100 ppm) influenced the hatching success as well as embryo survival. Also in embryos treated with PET NPs significantly higher levels of ROS were observed specifically in the intestine, liver, and kidney regions. Significant decreases in the levels of acetate, glucose, alanine, leucine, isoleucine, valine, glutamate, glutamine, cysteine, glycine and glutathione was seen in quantitative analysis of the metabolites in HRMAS NMR spectra. On the other hand significant increase was observed in the levels of lactate, choline, glycerophosphorylcholine and ethanolamine, tryptophan, phenylalanine and tyrosine. Increase in exposure groups was also seen for free fatty acids and cholesterol (Bashirova et al., 2023).

2.3. PET MPs Toxicity in Mice

Lu et al. (2024) conducted a study on potential effects of Polyethylene Terephthalate (PET) Microplastics on the heart of mouse. Doses of three different concentrations were given via drinking water and daily dose intake was calculated by the water intake of mice per day. It was found that the group with higher dose showed a significant increase in collagen fibers and malondialdehyde (MDA) levels in the myocardial tissue, whereas the activities of Superoxide Dismutase (SOD), glutathione peroxidase (GSH-Px) and Catalase (CAT) were significantly lower than those in control group. It also suggested that PET MPs can induced changes in the redox status of ICR mice and H9C2 cells. Myocardial apoptosis was also observed due to the excessive accumulation of Reactive Oxygen Species (ROS) caused by PET MPs.

CHAPTER 3: METHODOLOGY

3.1. PET Microplastics

PET microplastics were procured from Metal Fabrik Powder coating factory in Betim-Goa. To confirm the nature of the powder X-ray diffraction (XRD), Raman Spectrometry and Scanning Electron Microscope (SEM) were employed.

3.2. Chemicals

Analytical grade chemicals from Sigma Aldrich, Hemedia, Thermo-Fisher, etc were used.

3.3. Glassware And Instruments

The required glass wares like beakers, conical flasks, test tubes, pipettes, various measuring cylinders etc., were procured from M/s., Borosil Glass Works Ltd. Mumbai, India and other lab wares like plastic reagent bottles, micro vials, centrifuge tubes, pipette stands, micro tips and Eppendorf tubes etc., were obtained from M/s., Tarsons Products Pvt. Ltd., Kolkata, India. All glass wares were soaked in chromic acid and then rinsed thoroughly in distilled water and dried before use. All the glassware were sterilized by keeping in hot air oven for 2 hrs.

3.4. Maintenance Of Experimental Animals

The 24 experimental animals, Swiss Albino Mice *Mus musculus* (Male), were were procured from National Institute of Biosciences, Pune – India (Ref no.GUZ/IAEC/23-24/N3,dated 12/09/2024) and kept in the animal house of the Discipline of Zoology, School of Biological Sciences And Biotechnology (SBSB), Goa University. The animal house (Reg. No. 2104/GO/Re/S/20/CPCSEA dt. 10/08/2020) is been registered under

the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India. The mice were initially acclimatized for a period of two weeks, after which they were they were grouped in a group of 6 mice in each cage of uniform body weight (30-35g). The mice were housed in polypropylene cage (42cm x 25cm x 15cm) with stainless steel lid, having provision for food and water. The mice were maintained in pathogen-free room under controlled conditions (temperature, 22 ± 2 ◦C) and a 12/12 h light/dark cycle (Hou et al., 2021).Paddy Husk was used as bedding and corn meal was given as feed. The paddy husk bedding was changed regularly twice a week to maintain hygiene.

3.5. Experimental Setup

After acclimatisation, the mice were randomly separated in four groups (3 experimental and 1 Control) comprising 6 mice in each cage. PET Microplastics of different concentrations, namely, low, medium and high suspended in distilled water were feed to the mice in experimental groups and only distilled water for mice in control group (Lu et al., 2024). The three concentrations of doses were set as 1mg/ml, 1.5mg/ml and 2mg/ml according to previous studies and LD50 values of PET Microplastics (Hou et al., 2021). The daily water consumptions of mice per cage was recorded to be 5-7ml. The water bottles were changed every day at 11.30am and the amount of water consumed was recorded. The bottles were shook regularly to ensure the mice ingested sufficient amount of microplastics regularly. The mice were exposed for a period of 14 days after which 50% of the mice from each group were sacrificed by Cervical Dislocation. The remaining 50% of mice from each experimental group were given normal water for 7 days post which they were also sacrificed by Cervical Dislocation.

3.6. Collection of Blood and Tissue

After euthanization the mice were dissected and blood was collected by cardiac puncture. The syringe was flushed with EDTA to avoid coagulation. The blood was transferred to Eppendorf tubes that were washed with EDTA. The blood sample was centrifuged at 3000 RPM for 30 minutes until serum and plasma were separated. The serum was stored at cold temperature for further analysis. After dissecting the mice, liver tissue was washed with Phosphate Buffer Saline (0.01M, pH7). The right lobe of the liver was weighed and preserved in 10% Formalin for Histological studied. The remainder was stored in aluminium foil at -20°C for biochemical estimation and other toxicological tests. The bone marrow from the hind limb of the mice was flushed with PBS (0.01M, pH7) which was used for genotoxicity tests. The entire Gut from stomach to large intestine was collected, washed and stored in PBS (0.01M, pH7).

3.7. General Examination

During the entire experimental period of 21 days the mice were routinely examined for any evident behavioural changes and the weight gain or loss.

3.7.1. Behavioural

Mice were observed daily for any behavioural changes such as rolling of tail, isolation, feeding behaviour, water consumption, drowsiness, hyperactivity and other evident behavioural changes.

3.7.2. Weight Change

The mice were weighed before separating them into groups. They were later weighed before sacrificing. The difference between the weight were recorded.

3.8. Biomolecules Estimation

3.8.1. **Total carbohydrates** (Carroll et al., 1956)

Chemicals- Anthrone reagent- 0.2 gm of anthrone was dissolved in 100m1 of concentrated sulphuric acid.

Procedure- 10% liver tissue homogenate in ice-cold water was prepared. The homogenate was deproteinized by adding equal amounts of 0.3 N barium hydroxide and 5% zinc sulphate. And centrifuged at 800 x g for 15 minutes, the supernatant was used for estimation of total carbohydrates and free sugars.

0.1 ml of of supernatant was diluted to 1 ml with distilled water. 4ml of anthrone reagent was added to this and incubated for 10 minutes in boiling water bath. The intensity of the color developed was measured at 620nm against a suitable blank containing 1 ml of distilled water and 4ml of Anthrone. Quantification of the total carbohydrate content was done with the help of a standard curve of total carbohydrate (100µg of glucose)

3.8.2. **Free Sugars** (Smogyi, 1952)

Chemicals- Alkaline copper reagent- A] a)- 12.0g anhydrous sodium carbonate and 6.0g sodium potassium tartarate were dissolved in 125 ml of distilled water.

b)- 2.0 g of copper sulphate was dissolved in 25m1 distilled water. Both the solutions a and b were mixed, 8.0g of sodium bicarbonate was added to it by stirring to prepare solution A.

B] 90.0g of anhydrous sodium sulphate was dissolved in 250 ml of distilled water. Boiled to expel air and then cooled to room temperature to prepare solution B.

Now both the solutions A and B were mixed and the volume was made up to 500ml with distilled water.

Arsenomolybdate colour reagent- 25.0 g of ammonium molybdate was dissolved in 450 ml of distilled water. 21m1 of concentrated sulphuric acid was added slowly while mixing. To this, 3.0 g disodium hydrogen arsenate (already dissolved in 25ml of water) was added. Mixed well, stored in amber coloured bottle at 37°C for 48 hours.

Procedure- 1.0 ml alkaline copper reagent was added to 1 ml of deproteinized supernatant and incubated in the boiling water bath for 20 minutes. l ml of arseno-molybdate colour reagent was added after cooling at room temperature. The mixture was diluted with 7 ml distilled water. The intensity of the colour was read at 540 nm against a suitable blank Quantification of tissue free sugar concentration was calculated with the help of glucose standard curve, prepared by using 200 µg/ml-glucose as a standard solution.

3.8.3. Proteins (Lowry et al., 1951)

Chemicals- Lowry's reagent-To 98.0m1 of 4% sodium carbonate, lml of each 2% copper sulphate and 4% sodium —potassium tartarate were added to make the volume up to 100 ml.

Procedure- 10% tissue homogenate was prepared using equal amounts of ice cold distilled water and 10% PCA. The homogenate was centrifuged at 800 x g for 15 minutes. The residue was re-extracted with chloroform: methanol (2:1) and then with diethyl ether. After collecting the supernatants through centrifugations at 800 x g for 15 min, the supernatants were pooled together. The pooled supernatant was treated with salt wash by adding sufficient 5% sodium chloride solution and keeping the mixture overnight at refrigerated temperature for complete phase separation. The residue was

again treated with 10% perchloric acid at 90°C for 30 minutes and centrifuged. The supernatant was discarded and the residue was dissolved in 1 (N) sodium hydroxide solution and stored for protein estimation.

0.1 ml of supernatant was diluted up to 0.5 ml with distilled water. To this, 5 ml of Lowry's reagent was added and incubated for 15 minutes at room temperature. 0.5 m1 of Folin Cio-Calteau (1:2 dilution) reagent was then added and the mixture was further incubated for a period of 30 minutes. The intensity of the blue coloured complex was measured against a suitable blank at 690nm. Quantification of the protein content of the sample was done with the help of a standard ,curve of bovine serum albumin (250µg/ml BSA in 1N NaOH).

3.8.4. Albumin And Globulins (Doumas et al., 1971)

Chemicals- Albumin reagent- 8.85 g succinic acid, 0.108 g of bromocresol green, 0.1 g of sodium azide and 4.0 ml of Brij-35 were dissolved in 900 m1 of distilled water. pH of this solution was adjusted to 4.1 by using 1N sodium hydroxide. Final volume was made up to l Litre by using distilled water. This reagent can be stored at room temperature (25°C ± 5°C) for one year.

Procedure- To 50 µl of tissue protein extract or serum, 5 ml of albumin reagent was added. The mixture was kept at room temperature for exactly 10 minutes. Intensity of the colour was measured at 640nm against a suitable blank. Albumin present in the samples was quantified with the help of albumin standard curve (4.0g/dl prepared in 0.1g/dl sodium azide).

Globulins present in the samples were calculated by subtracting the albumin values from total proteins.

3.9. Genotoxicity Tests

3.9.1. Comet Essay

Chemicals- Lysis Buffer- 43.806 g of NaCl (2.5M), 11.22 g of Na2EDTA (100mM), 0.36 g of Tris Buffer (10mM) in 210 ml of Distilled water and add 2.4 g of NaOH. Adjust pH to 10. Make volume to 267 ml with distilled water---Solution A. Working Solution (To be freshly prepared)- To Solution A add 30 ml of 10% DMSO and 3 ml of 1% Triton-X.

Electrophoresis Buffer- 20 g NaOH in 50 ml of distilled water---Solution A. 1.48 g EDTA in 20 ml distilled water and maintain pH 10---Solution B. Working Solution- To 27 ml of Solution A, 4.5 ml of Solution B and 1.8 ml of DMSO was added. Make the volume to 900ml with distilled water.

Neutalization Buffer- To 230 ml of distilled water, 14.52 g of Tris Base was added. The pH was adjusted to 7.5 and volume was made to 300 ml. (Chill before use)

Ethidium Bromide- 10mg of Ethidium Bromide in 50 ml Distilled Water (10X Stock Solution). Working Solution- 1ml of 10X Stock solution in 9ml distilled water. (Store in Amber coloured bottle)

Procedure- The alkaline comet assay was performed as per Singh et al., with moderate modifications. All steps were performed in dark to avoid possible DNA damage. Fully frosted microscope slides were immersed in Chromic Acid overnight and washed with distilled water. The slides were then covered coated with a thin layer of 1% high melting-point agarose (500 µL) and allowed it to solidify. Once solidified, 100 µL of bone marrow suspension in PBS (0.01M, pH 7) was mixed with 100 µL of 0.5% low

melting agarose was added onto the pre-coated slides and covered with a cover slip. The agarose layer on the slide was allowed to solidify and after its solidification, the cover slip was removed. A final layer of 0.5% low melting agarose (200 µL) was placed over the second layer and allowed to solidify. The slides were then submersed in a cold, alkaline (4°C, pH 10) lysis solution overnight in cold temperature. The slides were dried and placed in an alkaline electrophoresis buffer for 10 min to induce unwinding of DNA strands. The slides were then transferred to fresh alkaline electrophoresis buffer in an electrophoresis tank and electrophoresis was performed at field strength of 20V/125 mA for 20 min. The slides were then incubated in Neutralization Buffer to neutralize the DNA in the agarose at room temperature (26ºC) for 15 min. DNA was stained by adding 20 µL/mL of ethidium bromide on the agarose and covered with a cover slip and incubated for 5 min in dark. The DNA damage was quantified by observing the cells under 20X objective of a fluorescence microscope (Olympus BX 53, Japan) equipped with an excitation filter of 510-560 nm and an emission filter of 590 nm.. The comets were analysed by the image analysis software CASP.

3.10. Oxidative Stress

3.10.1. **Reduced glutathione (GSH)** (Moron et al., 1979)

2% homogenate was prepared with 5% TCA and centrifuged at 500 x g for 5 minutes to remove the precipitate. To 1.0 ml of tissue extract or serum, 2 ml of 5, 5'- dithiobis, 2-nitrobenzoic acid (DTNB) reagent was added to make the final volume 3.0 ml. Absorbance was read at 412nm against a suitable blank. Reduced glutathione content of the samples was quantified with the help of a standard curve of reduced glutathione (0.2mnole/m1 in 5% TCA).

- 5, 5'- dithiobis, 2-nitrobenzoic acid (DTNB) reagent— 19.8mg of 5, 5'- dithiobis, 2-nitrobenzoic acid was dissolved in 100m1 of 0.1%sodium nitrate to prepare this reagent.

3.10.2. **Thiobarbituric acid reactive substances** (Niehaus & Samuelsson, 1968)

The liver tissue was homogenized in Tris HCl buffer pH 7.0 to prepare a 2% homogenate. 0.1 ml of tissue homogenate or 10 µl serum was treated with 2ml of TBA-TCA-HCl reagent and placed in boiling water bath for 15 minutes, cooled and centrifuged at room temperature for 10 minutes at 500 x g. The absorbance of clear supernatant was measured against a suitable blank at 535 nm. Thiobarbituric acid reactive substance concentration was estimated with the help of standard curve of malondialdehyde (109mole/m1).

- **TBA-TCA-HC1 Reagent-** 0.37% Thiobarbituric acid, 15% Trichloroacetic acid and 0.25(N) Hydrochloric acid were mixed in 1:1:1 ratio to prepare this reagent.

3.11. Enzyme Analysis

3.11.1. **Alkaline Amino Transferase (ALT)** (Reitman & Frankel, 1957)

Chemicals- ALT substrate- To 0.1 ml of 1N NaOH prepared in phosphate buffer (0.01M, pH 7.5), 0.532 g of alanine and 6 mg of a-ketoglutaric acid were added. Final quantity was adjusted to 20 ml with phosphate buffer (pH 7.5).

DNPH reagent-10 mg of dinitrophenyl hydrazine was added to 4.25 ml conc. HC1 and the final quantity was adjusted to 20 ml with distilled water.

Procedure- 0.5 ml of ALT substrate was incubated at 37°C for 5 minutes. 0.1 ml of serum or 5% liver tissue homogenate (in 0.01M phosphate buffer, pH 7.0) was added to it. A enzyme blank was prepared by taking 0.5 ml of substrate and 0.1 ml of distilled water. The homogenate and blank were incubated for 15 minutes. 0.5 ml dinitrophenyl hydrazine reagent was added to stop the reaction. They were mixed thoroughly and incubated at room temperature for 20 minutes. 5 ml of 0.4 N NaOH was added to develop the colour, intensity of which was read against the enzyme blank at 540 nm. The product formed (pyruvate) during this reaction time was quantified with the help of a standard curve of pyruvate (220 µg/ml). The enzyme activity was expressed as µg of pyruvate formed/min reaction/ mg of enzyme protein. Protein content in the enzyme was estimated as described earlier.

3.11.2. Superoxide Dismutase (SOD) (Beauchamp & Fridovich, 1971)

Chemicals- SOD substrate- To 25m1 of 0.2M potassium phosphate buffer(pH 7.0), 149mg of methionine, 4.93m1 of nitroblue tetrazolium chloride (1mg/m1 in 50mM potassium phosphate buffer, pH 7.0) and 0.63m1 of riboflavin (1mg/m1 in 0.05M potassium phosphate buffer) were added and the volume was made up to 100m1 with double distilled water.

Procedure- To 0.1 ml of 5% liver homogenate (prepared in ice cold 10mM of potassium phosphate buffer, pH 7.0, and it was brought down to 2.5% with 50 mM potassium phosphate buffer, pH 7.0 and then centrifuged. The supernatant was used for the assay) 2.9 ml of freshly prepared SOD Substrate was added. Enzyme blank was prepared by adding 0.1 ml 50 mM potassium phosphate buffer, pH 7.0 instead of sample. Illumination was carried out in the aluminium foil lined box fitted with 15 V fluorescent lamp for exactly 10 minutes. . Intensity of the colour was read at 560 nm. Enzyme

activity was quantified with the help of a standard curve of SOD (400µg/ml). Protein content in the enzyme was estimated as described earlier. SOD activity was expressed as lU/mg protein. One unit of the enzyme can be defined as the amount of SOD required to inhibit 50% of NBT reduction at standard condition.

3.11.3. **Catalase** (A. K. Sinha, 1972)

Chemicals- Dichromate acetic acid reagent- 5% potassium dichromate and glacial acetic acid were taken in 1: 3 ratio to prepare this reagent.

Procedure- 1.5 ml of phosphate buffer (0.01 M, pH 7.0) and 0.4 ml of substrate (0.2 M H202) were incubated at 37°C for 5 minutes. To this 0.1 ml serum or 10% liver tissue homogenate (prepared in phosphate buffer) was added and incubated at 37°C for 15 minutes. An enzyme blank was prepared by addition of 1.6 ml phosphate buffer (0.01 M, pH 7.0) and 0.4 ml of substrate (0.2 M H_20_2). 2 ml of dichromate acetic acid reagent was added to stop the reaction. This was then kept in a boiling water bath for 10 minutes. Mixed well and the intensity of the colour was measured against reference blank at 620 nm. Protein content in the enzyme was estimated as described earlier. The enzyme activity was quantified with the help of a reference curve of hydrogen peroxide (2µmole/ ml) and expressed as moles of H_20_2 consumed / min/mg protein.

3.12. Statistical Analysis

GraphPad Prism 8.0.1 was employed for all the statistical calculations. Arithmetic mean of six samples was used to recorded and analyse the observation. Two way ANOVA and Sidak's Test was used for analysis and interpretation of data.

3.12.1. Two-way ANOVA

A two-way ANOVA ("analysis of variance") was used to determine whether or not there is a statistically significant difference between the means of the Control and the three experimental Groups. The F value was used to determine the Significance.

3.12.2. Sidak's Test

Šidák test was used to counteract the multiple comparisons. It is a pairwise multiple comparison test based on a t statistic. It was employed to performs simultaneous joint pairwise comparisons for all possible combinations of means.

3.13. COMET Analysis

CASP Software was used for calculating the Tail percentage DNA for analysis of COMET Nucleus.

CHAPTER 4: ANALYSIS AND CONCLUSION

4.1. RESULTS

4.1.1. PET Characterization

The PET MPs were subjected to X-Ray Diffraction, Scanning Electron Microscope and Raman Spectroscopy to understand and confirm the nature of the substance. The results of X-Ray Diffraction showed that the PET MPs were of crystalline nature. This was confirmed by the appearance of stiff peaks in the graph in Figure 2. The Raman shift as depicted in Figure 3 showed characteristic peaks at 1603.5, 1340.6, 979.2 and 453.5 cm^{-1} confirming that the chemical composition of the MPs used in the experiments was PET (Gedler et al., 2013; Nava et al., 2021). The SEM images revealed irregular shaped grainy structures of size 2-5 um as depicted in Plate 1 and Plate 2.

4.1.2. Behavioural Changes

No drowsiness and hyperactivity was observed during the quarantine period. In few mice in Experimental 3, minute drowsiness was seen post 12^{th} day. Experimental 1 and Experimental 2 did not show as signs of drowsiness. Hyperactivity was in Experimental 3 Group post 7^{th} day while in Experimental 1 and Experimental 2 was seen after 10^{th} day. No other much behavioural changes were seen in experimental groups as compared to control group. Few mice did show signs of isolation at multiple times in Experimental 2 and Experimental 3 Groups. No signs of isolation were observed in Experimental 1 and control Groups. Negligible difference in weight was seen pre and post exposure period. The water intake was seen to decrease over the exposure period and more intake of feed was seen. No much behavioural changes were seen during the depuration period from that of exposure period. During the depuration period the water intake was similar to the start of experiment.

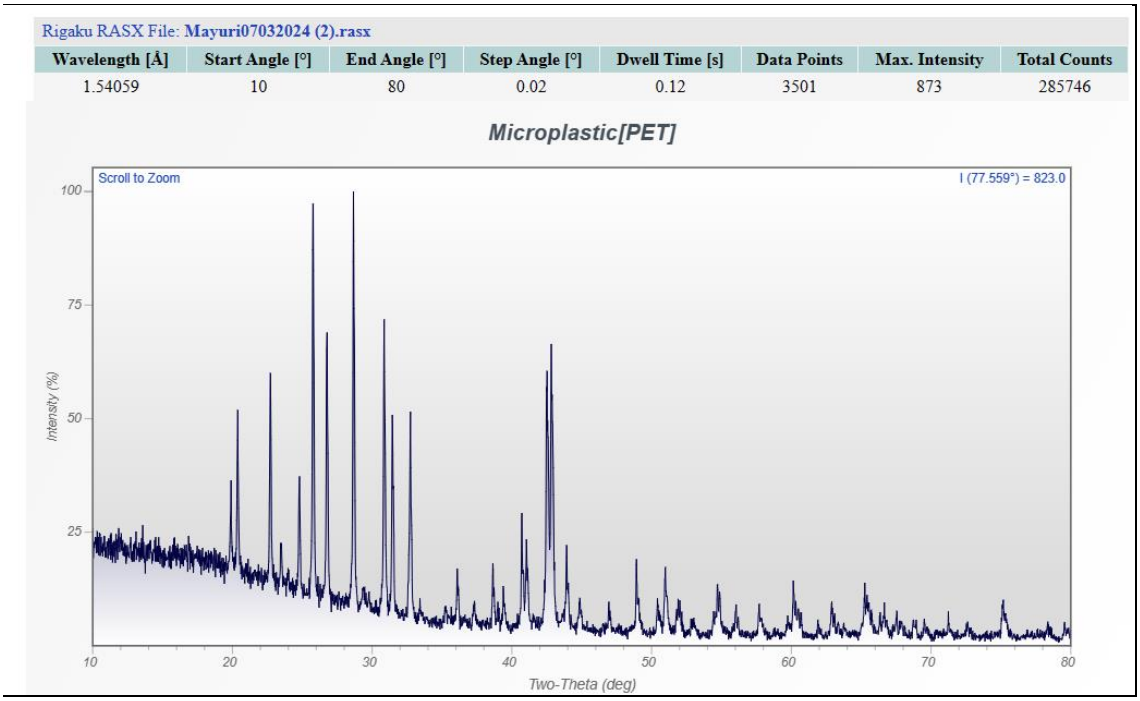


Figure 2: X-Ray Diffractor Graph

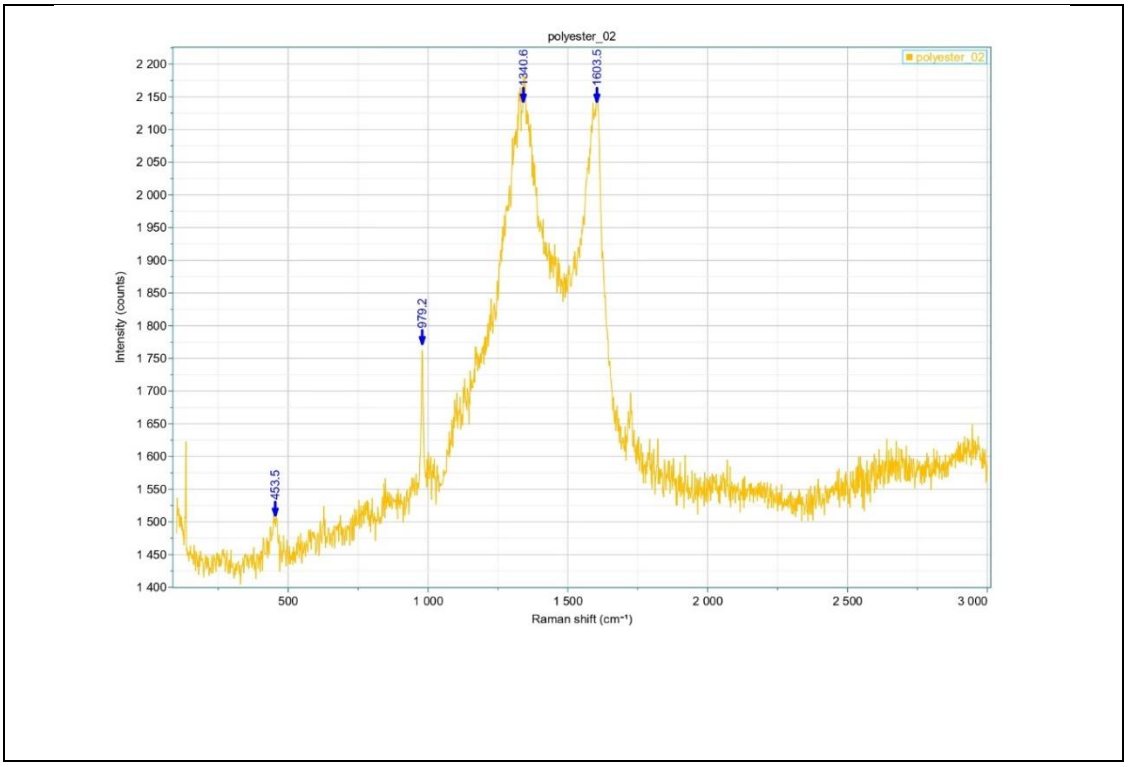
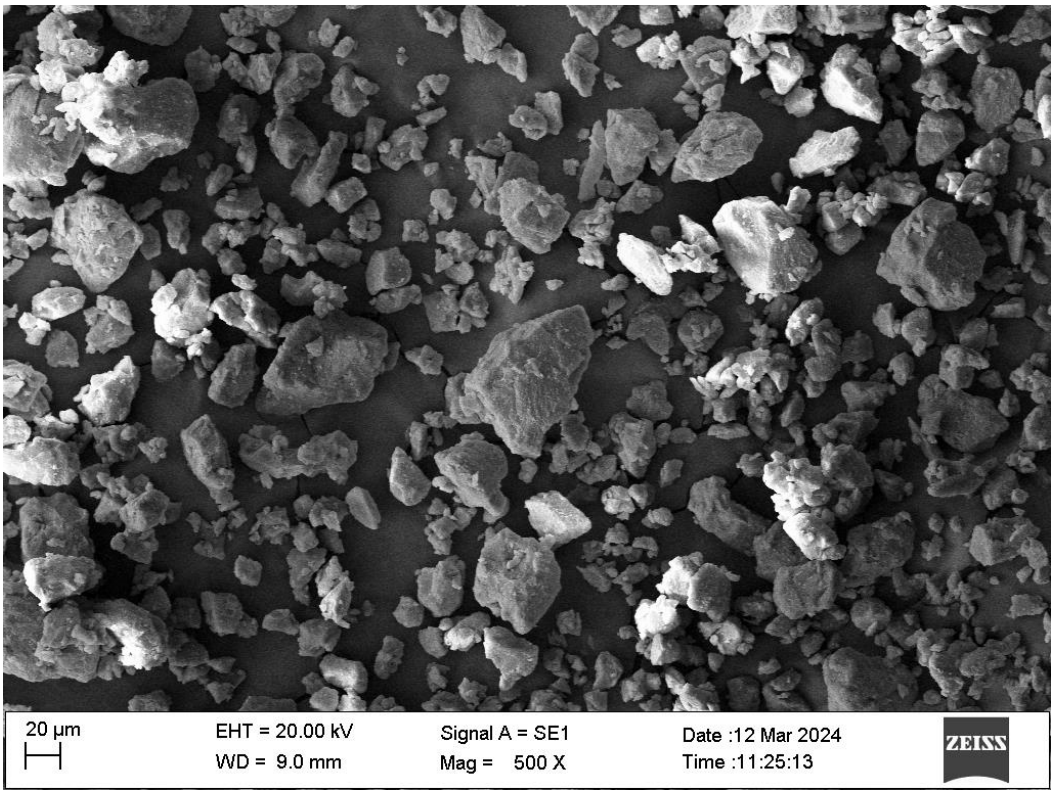


Figure 3: Raman Spectroscopy of PET MP



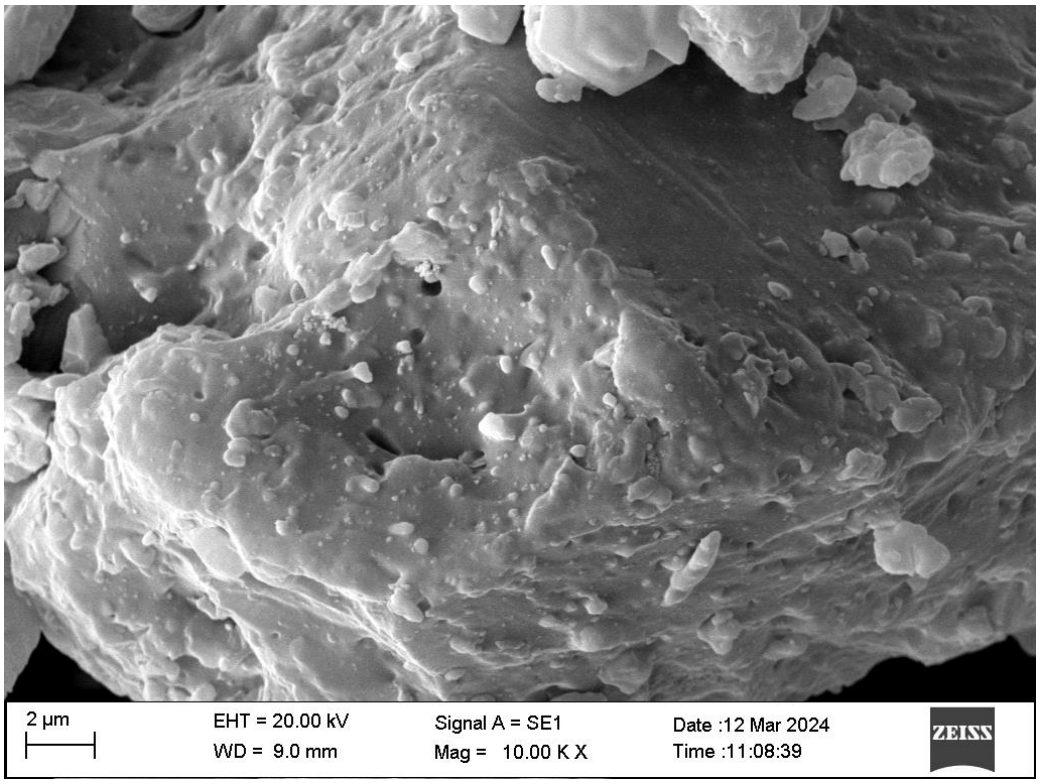


Plate 1: SEM image

4.1.3. Biomolecules Estimation

4.1.3.1. Carbohydrates

The results for carbohydrate estimation as depicted in Figure 4 showed an increase in carbohydrate concentration with increasing concentration of dose. A significant difference in carbohydrate concentration was seen post depuration period of 7 days as depicted in Figure 5.

The two- way ANOVA results showed P value to be P <0.0001 **** for Carbohydrate concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 4.

The two- way ANOVA results showed P value to be P <0.0001 **** for Carbohydrate concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 5.

4.1.3.2. Free Sugars

The results for Free Sugars estimation as depicted in Figure 6 showed an increase in Free Sugars concentration with increasing concentration of dose. A significant difference in in Free Sugars concentration was seen post depuration period of 7 days as depicted in Figure 7.

The two- way ANOVA results showed P value to be P <0.0001 **** for Free Sugars concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 6.

The two- way ANOVA results showed P value to be P ≤ 0.03 * for Free Sugars concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 7.

4.1.3.3. Proteins

As depicted in Figure 8 the results for Protein estimation showed an increase in Protein concentration with increasing concentration of dose. A significant difference in Protein concentration was seen post depuration period of 7 days as depicted in Figure 9.

The two- way ANOVA results showed P value to be P <0.0001 **** for Protein concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 8.

The two- way ANOVA results showed P value to be P ≤0.02 * for Protein concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 9.

4.1.3.4. Albumins

As depicted in Figure 10 the results for Albumin estimation showed an increase in Albumin concentration with increasing concentration of dose. A significant difference in Albumin concentration was seen post depuration period of 7 days as depicted in Figure 11.

The two- way ANOVA results showed P value to be P <0.0001 **** for Albumin concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 10.

The two- way ANOVA results showed P value to be P ≤0.0009 *** for Albumin concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 11.

4.1.3.5. Globulins

The results for Globulins estimation as depicted in Figure 12 showed an increase in Globulin concentration with increasing concentration of dose. A significant difference in Globulin concentration was seen post depuration period of 7 days as depicted in Figure 13.

The two- way ANOVA results showed P value to be P <0.0001 **** for Globulins concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 12.

The two- way ANOVA results showed P value to be P≤0.02 * for Globulins concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 13.

4.1.4. Genotoxicity

4.1.4.1. COMET Assay

As depicted in Figure 14 and 15 the results for COMET assay showed non significant increase or decrease in Tail DNA %.

The two- way ANOVA results showed P value to be P =0.5 Non Significant Tail DNA % when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 14.

The two- way ANOVA results showed P value to be P =0.5 Non Significant Tail DNA % when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 19.

4.1.5. Oxidative Stress

4.1.5.1. Reduced glutathione (GSH)

The results for Reduced Glutathione (GSH) as depicted in Figure 16 showed an increase in Reduced Glutathione (GSH) concentration with increasing concentration of dose. No significant difference in Reduced Glutathione (GSH) concentration was seen post depuration period of 7 days as depicted in Figure 17.

The two- way ANOVA results showed P value to be P <0.0001 **** for Reduced Glutathione (GSH) concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 16.

The two- way ANOVA results showed P value to be P= 0.5 Non Significant for Reduced Glutathione (GSH) concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 17.

4.1.5.2. Thiobarbituric acid reactive substances (TBARS)

As depicted in Figure 18 the results for Thiobarbituric acid reactive substances showed an increase in Thiobarbituric acid reactive substances concentration with increasing concentration of dose. No significant difference in Thiobarbituric acid reactive substances concentration was seen post depuration period of 7 days as depicted in Figure 19.

The two- way ANOVA results showed P value to be P <0.0001 **** for Thiobarbituric acid reactive substances concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 18.

The two- way ANOVA results showed P value to be P =0.5 Non Significant for Thiobarbituric acid reactive substances concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 19.

4.1.6. Enzyme Analysis

4.1.6.1. Alkaline Amino Transferase (ALT)

As depicted in Figure 20 the results for Alkaline Amino Transferase showed an increase in Alkaline Amino Transferase concentration with increasing concentration of dose. A significant difference in Alkaline Amino Transferase concentration was seen post depuration period of 7 days as depicted in Figure 21.

The two- way ANOVA results showed P value to be P <0.0001 **** for Alkaline Amino Transferase concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 20.

The two- way ANOVA results showed P value to be P ≤0.009 *** for Alkaline Amino Transferase concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 21.

4.1.6.2. Superoxide Dismutase (SOD)

The results for Superoxide Dismutase (SOD) as depicted in Figure 22 showed an increase in Superoxide Dismutase (SOD) concentration with increasing concentration of dose. A significant difference in Superoxide Dismutase (SOD) concentration was seen post depuration period of 7 days as depicted in Figure 23.

The two- way ANOVA results showed P value to be P <0.0001 **** for Superoxide Dismutase (SOD) concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 22.

The two- way ANOVA results showed P value to be P <0.0001 **** for Superoxide Dismutase (SOD) concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 23.

4.1.6.3. Catalase

As depicted in Figure 24 the results for Catalase showed an increase Catalase concentration with increasing concentration of dose. A significant difference in Catalase concentration was seen post depuration period of 7 days as depicted in Figure 25.

The two- way ANOVA results showed P value to be P <0.0001 **** for Catalase concentration when compared to control and each experimental group and the Sidak's multiple comparisons test showed similar results as shown in Figure 24.

The two- way ANOVA results showed P value to be P <0.0001 **** for Catalase concentration when compared post exposure and depuration period and the Sidak's multiple comparisons test showed similar results as shown in Figure 25.

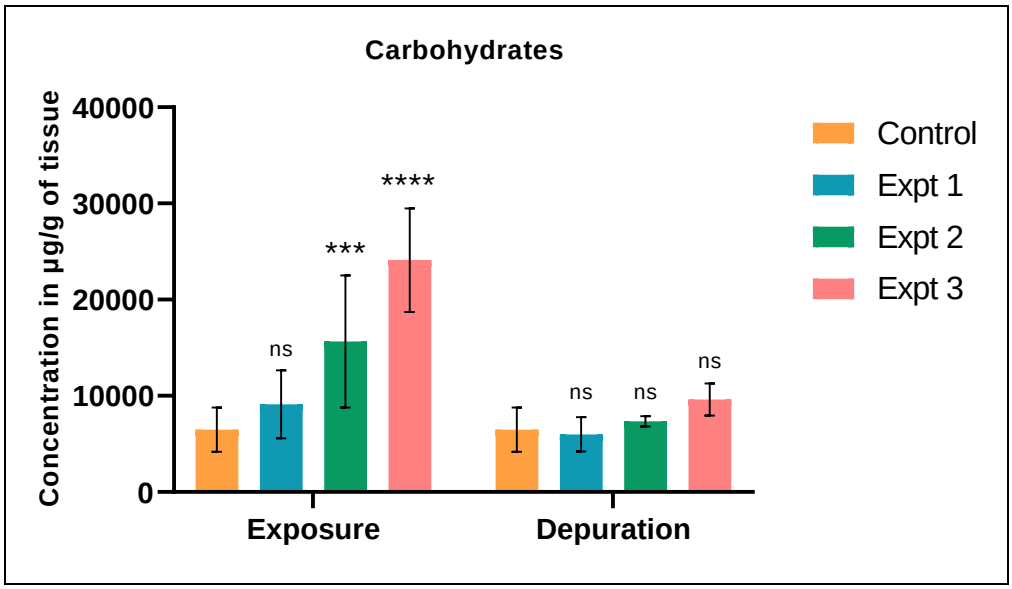


Figure 4: Effect of Acute exposure to PET MP on the Carbohydrates content on the liver of mice. (ns- Non significant; ***-P< 0.005 Moderately significant; ****-P< 0.0001 Highly significant)

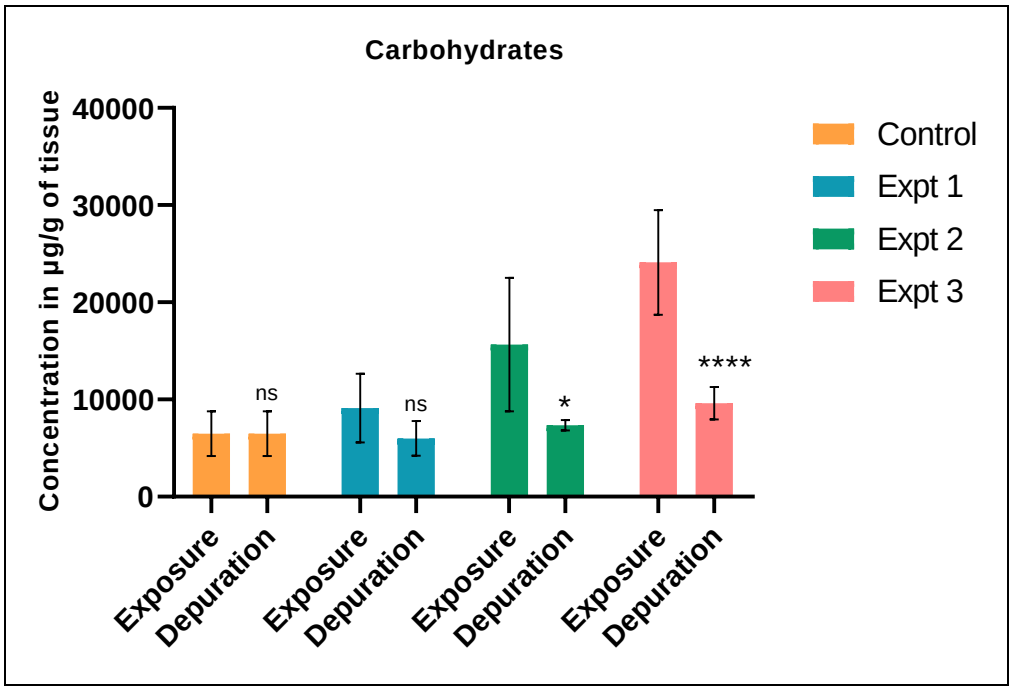


Figure 5: Effect of Acute exposure to PET MP on the Carbohydrates content on the liver of mice post exposure and depuration. (ns- Non significant; *-P< 0.0 significant; ****-P< 0.0001 Highly significant)

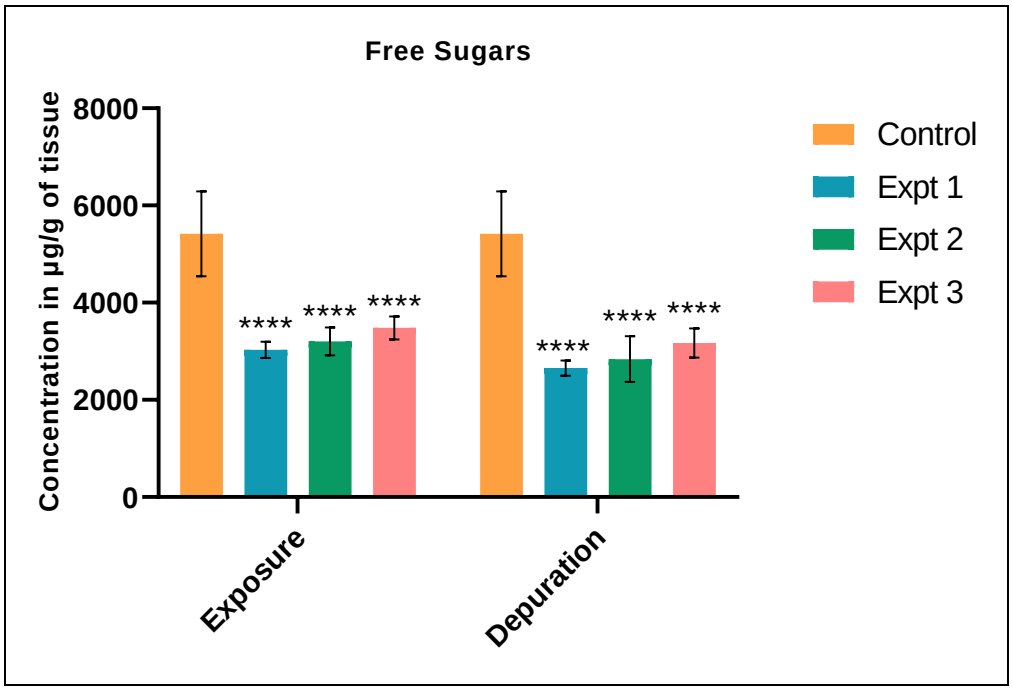


Figure 6: Effect of Acute exposure to PET MP on the Free Sugars content on the liver of mice. (****-P< 0.0001 Highly significant)

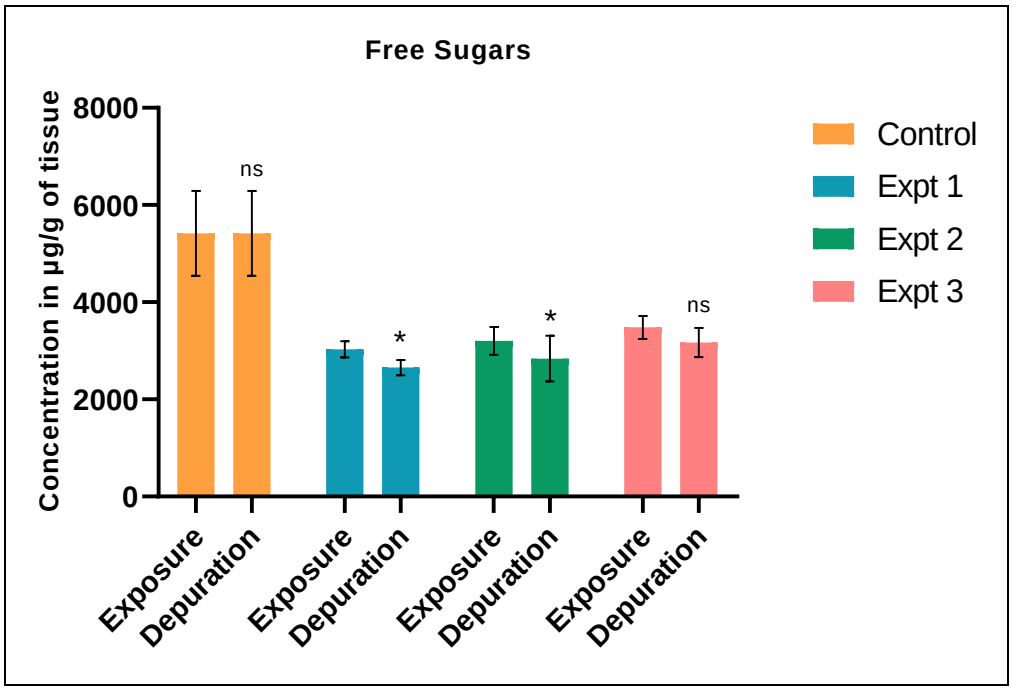


Figure 7: Effect of Acute exposure to PET MP on the Free Sugars content on the liver of mice post exposure and depuration. (*-P< 0.04 Significant)

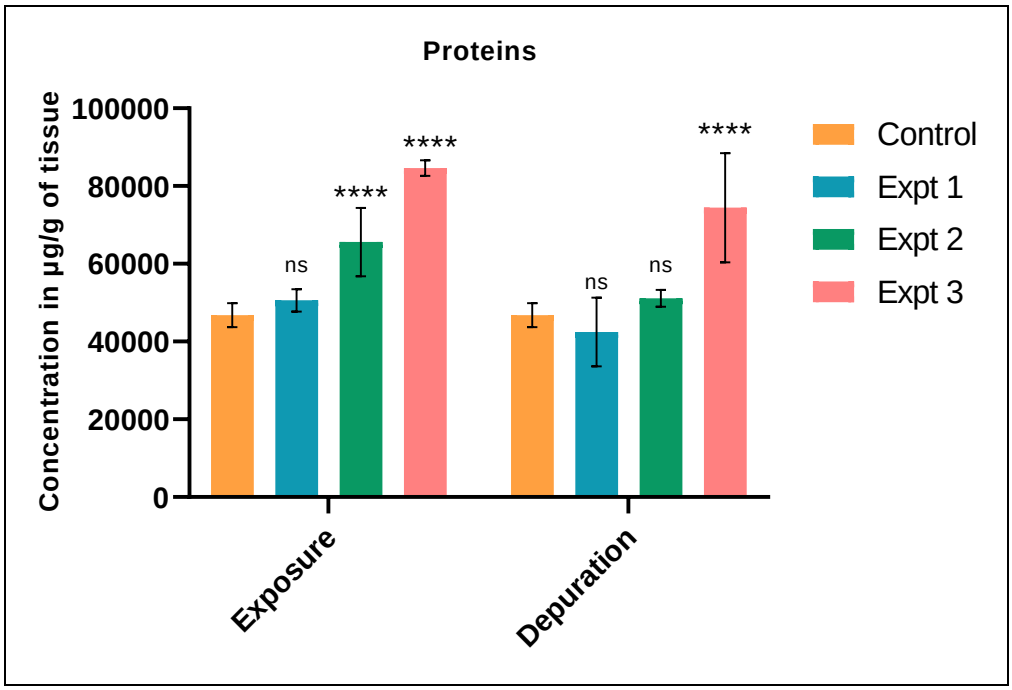


Figure 8: Effect of Acute exposure to PET MP on the Proteins content on the liver of mice. (ns- Non significant; ****-P< 0.0001 Highly significant)

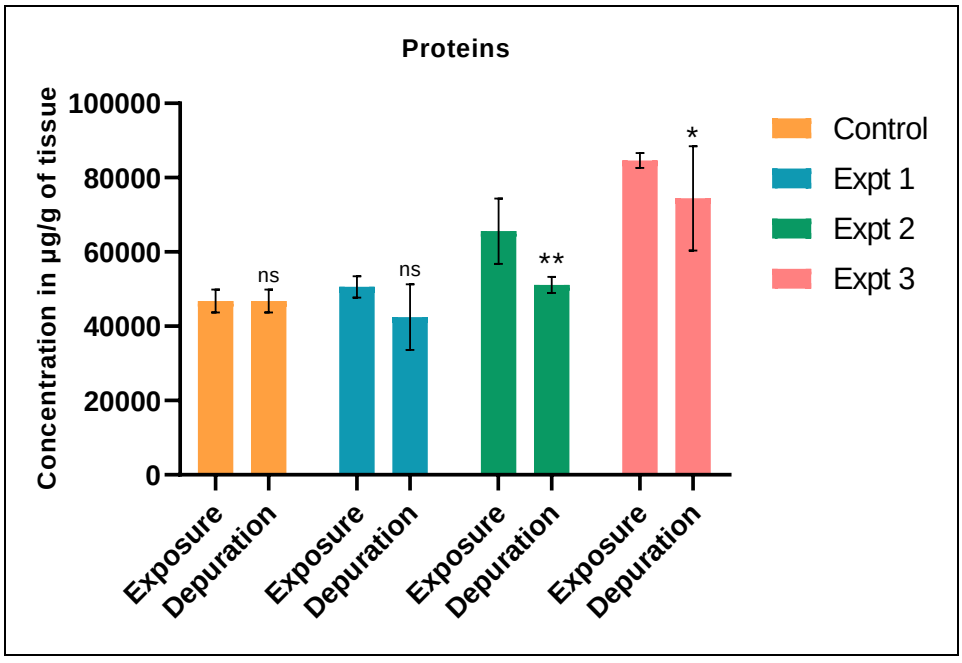


Figure 9: Effect of Acute exposure to PET MP on the Proteins content on the liver of mice post exposure and depuration. (ns- Non significant; -*-P< 0.01 Significant **-P< 0.005 Moderately significant)

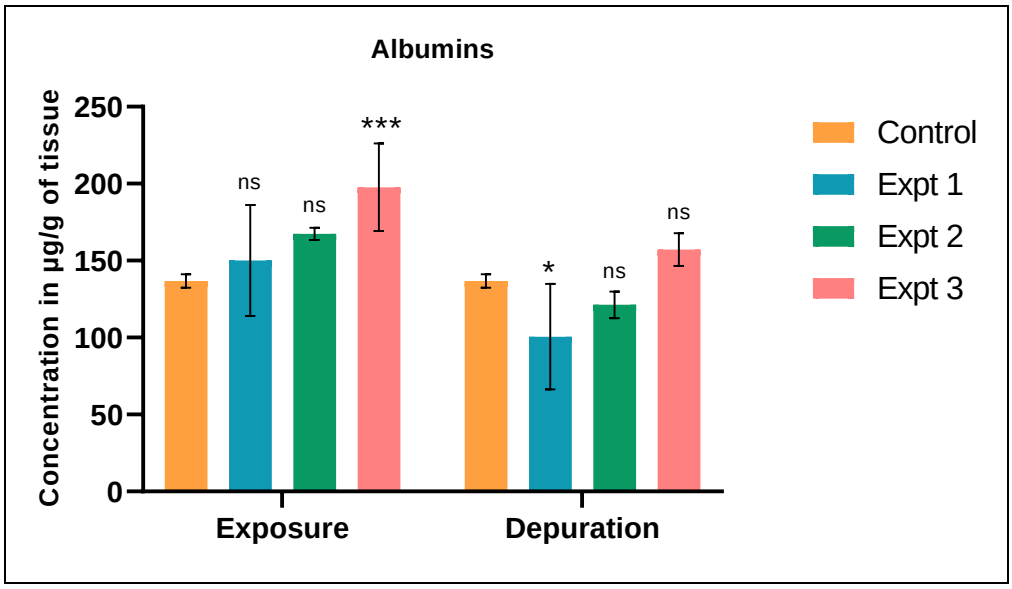


Figure 10: Effect of Acute exposure to PET MP on the Albumins content on the liver of mice. (ns- Non significant; *-P< 0.01 Significant; ***-P< 0.005 Moderately significant)

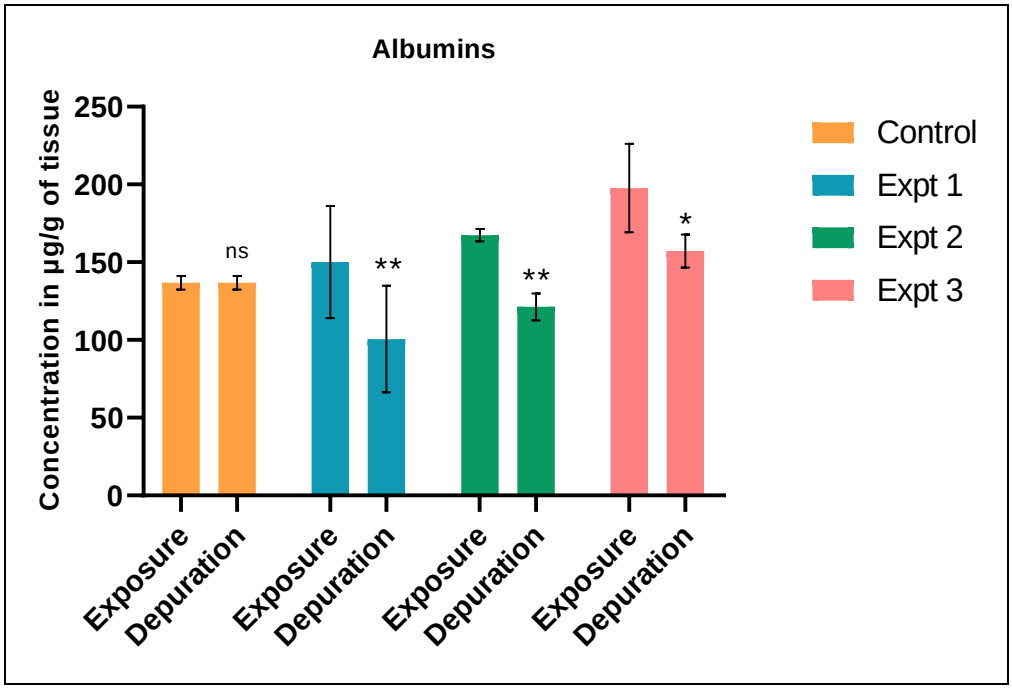


Figure 11: Effect of Acute exposure to PET MP on the Albumins content on the liver of mice post exposure and depuration. (ns- Non significant; *-P< 0.01 Significant; **-P< 0.005 Moderately significant)

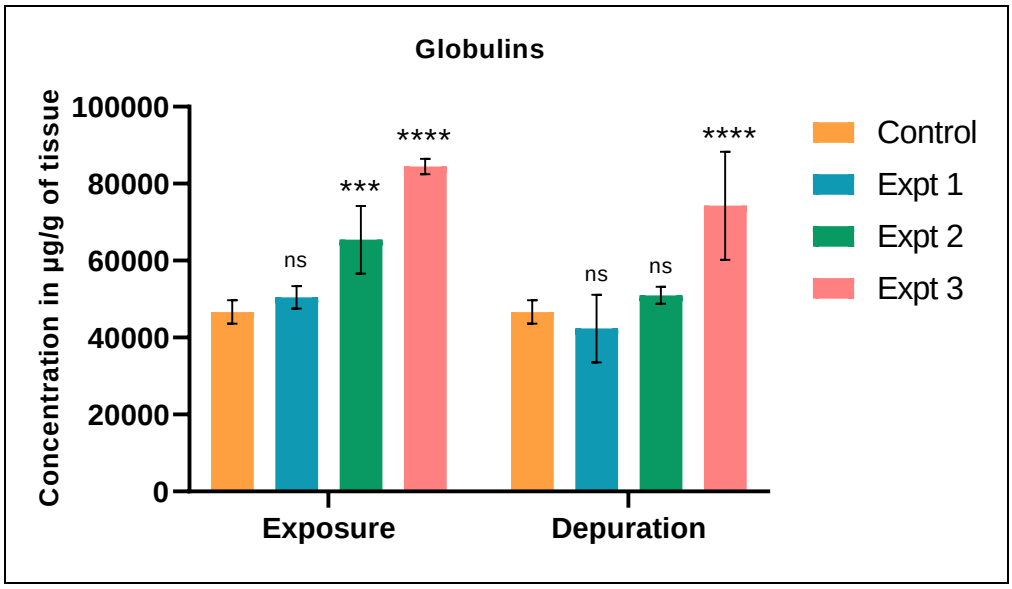


Figure 12: Effect of Acute exposure to PET MP on the Proteins content on the liver of mice. (ns- Non significant; ***-P< 0.005 Moderately significant; ****-P< 0.0001 Highly significant)

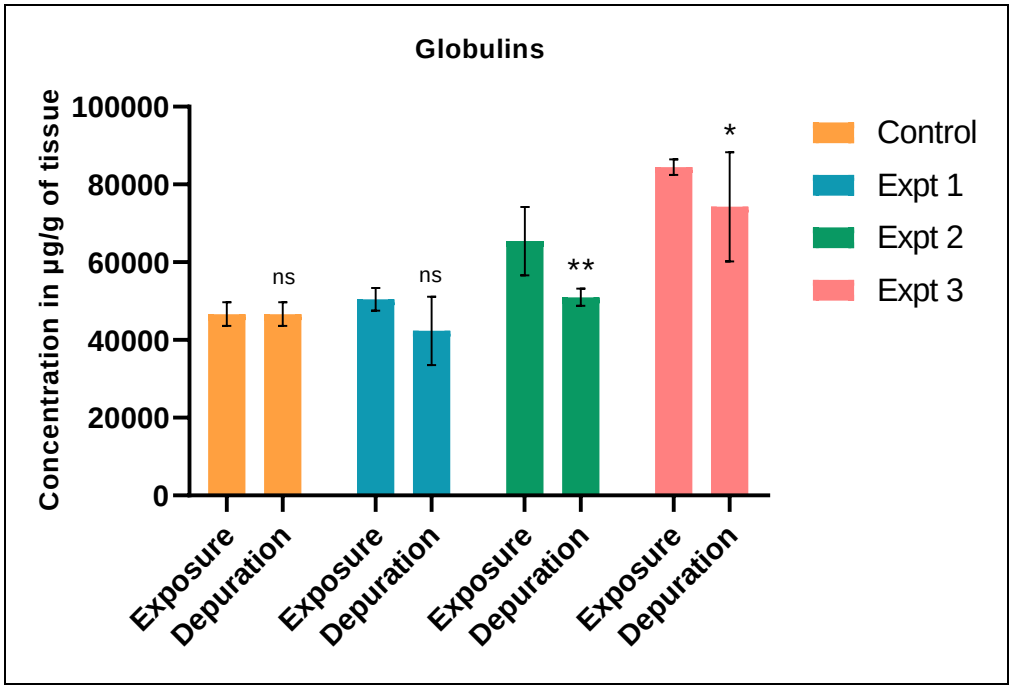


Figure 13: Effect of Acute exposure to PET MP on the Proteins content on the liver of mice post exposure and depuration. (ns- Non significant; -*-P< 0.01 Significant **-P< 0.005 Moderately significant)

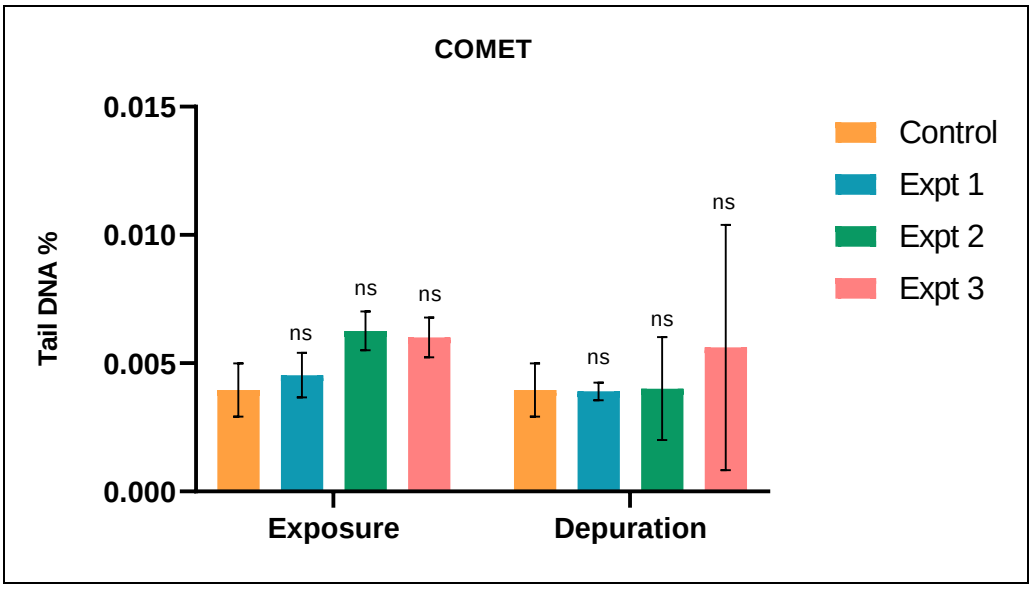


Figure 15: Effect of Acute exposure to PET MP on the Tail DNA % on the Bone marrow of mice. (ns- Non significant)

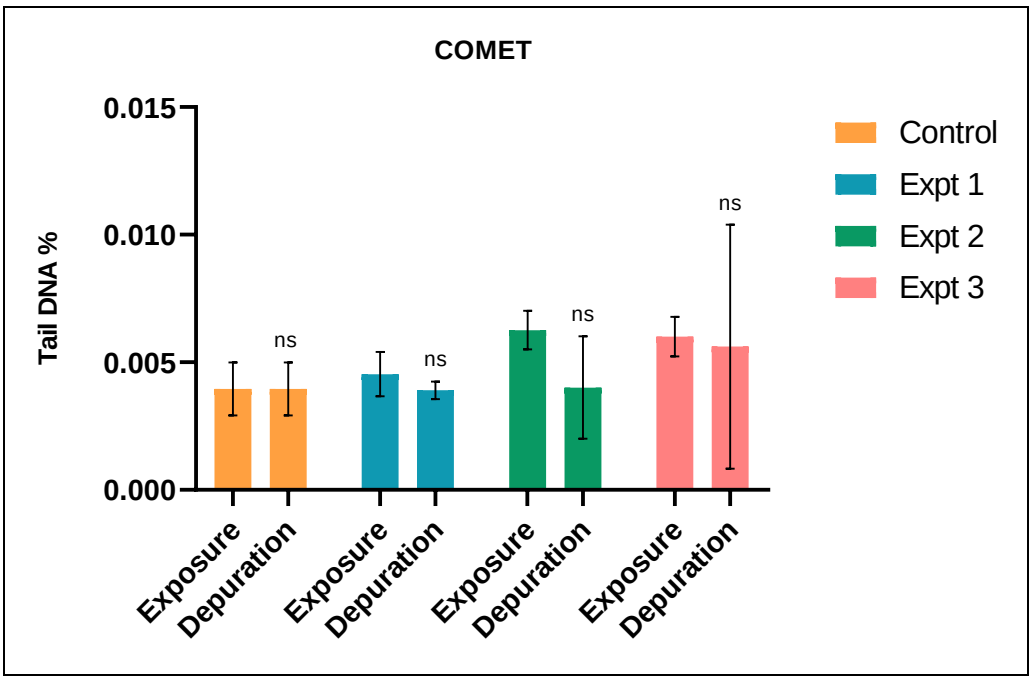


Figure 15: Effect of Acute exposure to PET MP on the Tail DNA % on the Bone marrow of mice post exposure and depuration. (ns- Non significant)

Figure 16: Effect of Acute exposure to PET MP on the Reduced Glutathione (GSH) content on the liver of mice. (ns- Non significant; **-P< 0.008 Significant; ***-P<0.001 Moderately Significant; ****-P< 0.0001 Highly significant)

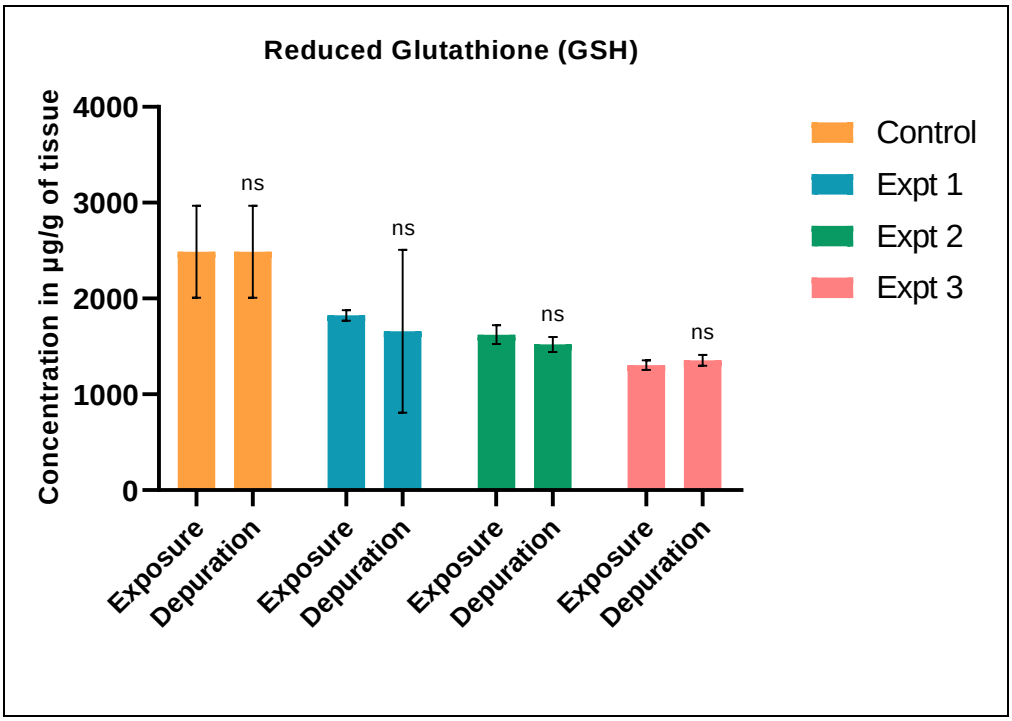


Figure 17: Effect of Acute exposure to PET MP on the Reduced Glutathione (GSH) content on the liver of mice. (ns- Non significant)

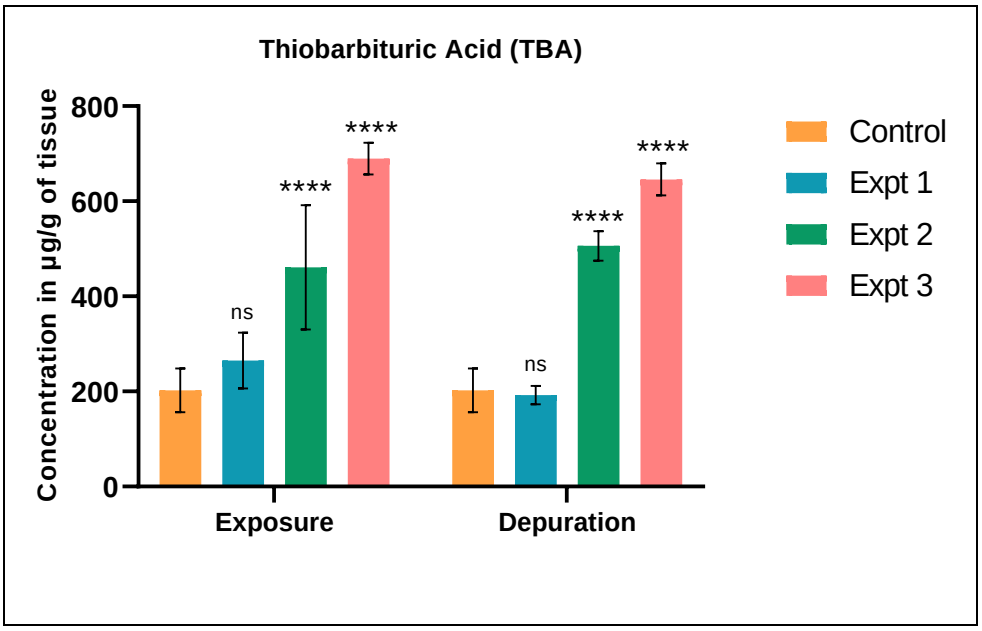


Figure 18: Effect of Acute exposure to PET MP on the Thiobarbituric Acid (TBA) content on the liver of mice. (ns- Non significant; ****-P< 0.0001 Highly significant)

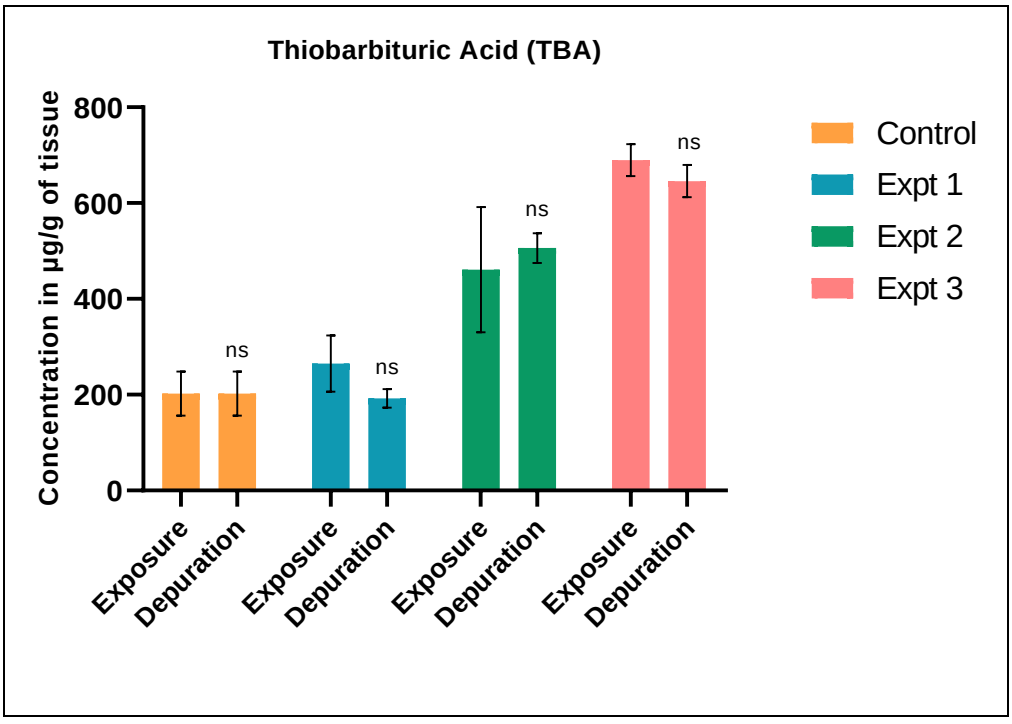


Figure 19: Effect of Acute exposure to PET MP on the Thiobarbituric Acid (TBA) content on the liver of mice. (ns- Non significant)

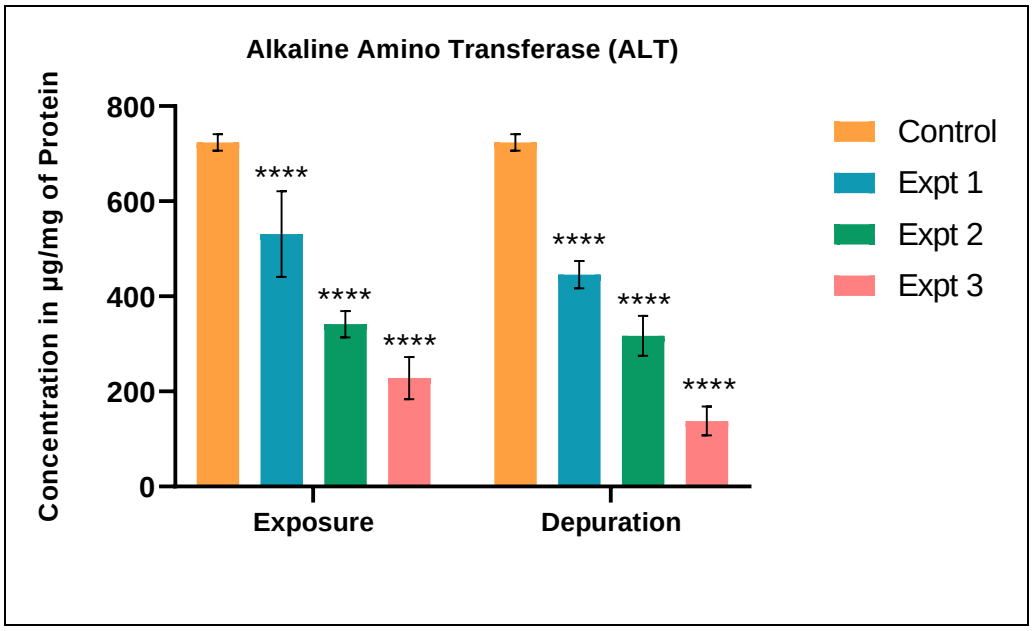


Figure 20: Effect of Acute exposure to PET MP on the Alkaline Amino Transferase content on the liver of mice. (ns- Non significant; **-P< 0.003 Moderately significant; ****-P< 0.0001 Highly significant)

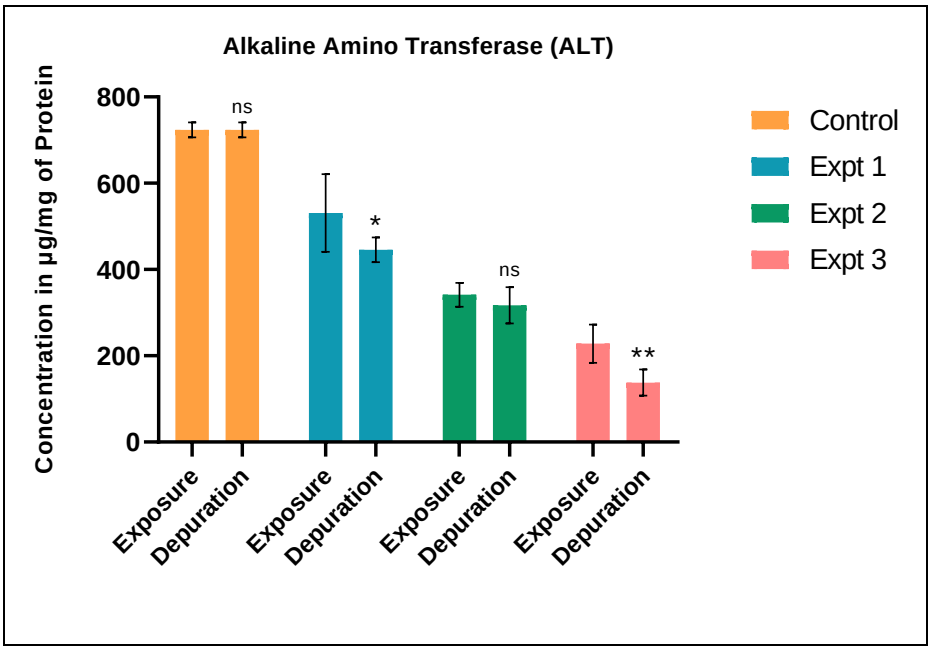


Figure 21: Effect of Acute exposure to PET MP on the Alkaline Amino Transferase content on the liver of mice post exposure and depuration period. (ns- Non significant; -*-P< 0.01 Significant **-P< 0.005 Moderately significant)

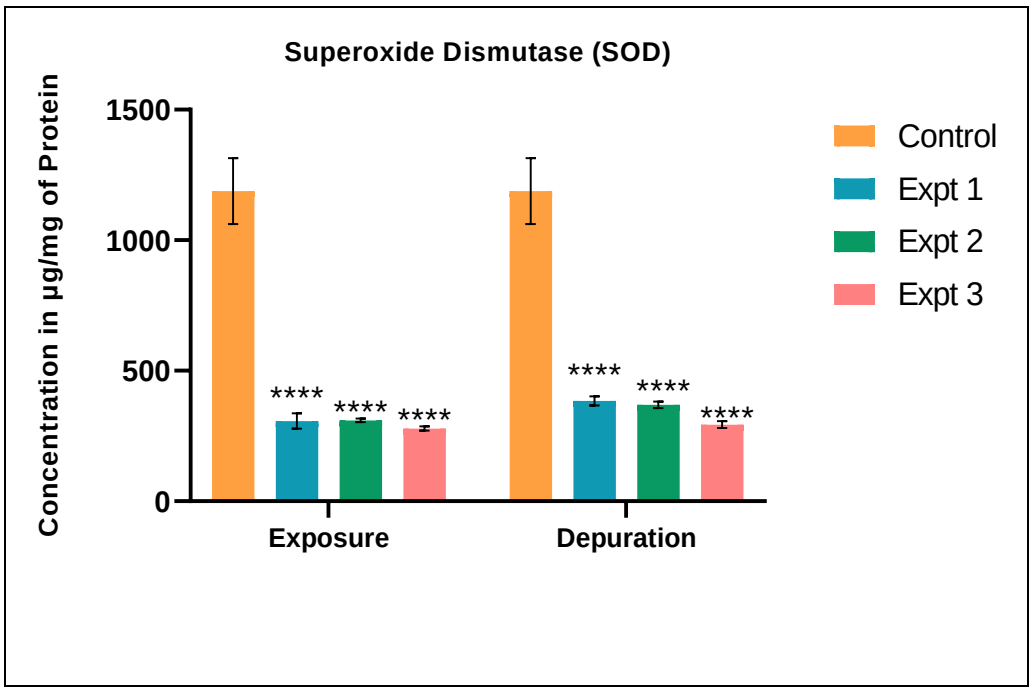


Figure 22: Effect of Acute exposure to PET MP on the Superoxide Dismutase (SOD) content on the liver of mice. (****-P< 0.0001 Highly significant)

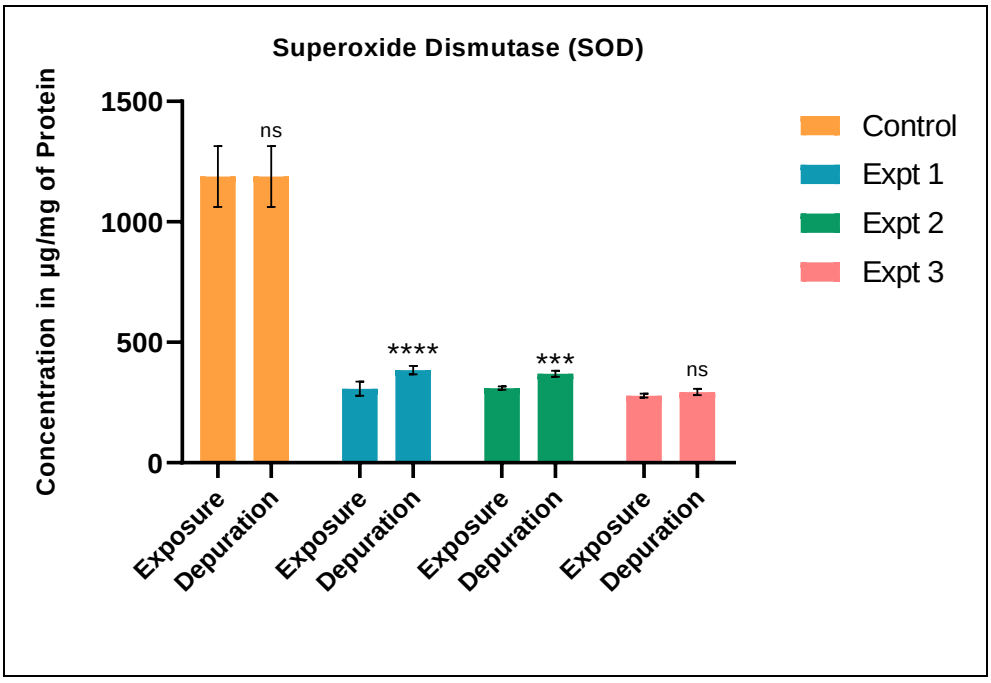


Figure 23: Effect of Acute exposure to PET MP on the Superoxide Dismutase (SOD) content on the liver of mice. (ns- Non significant; **-P< 0.005 Significant; ****-P< 0.0001 Highly significant)

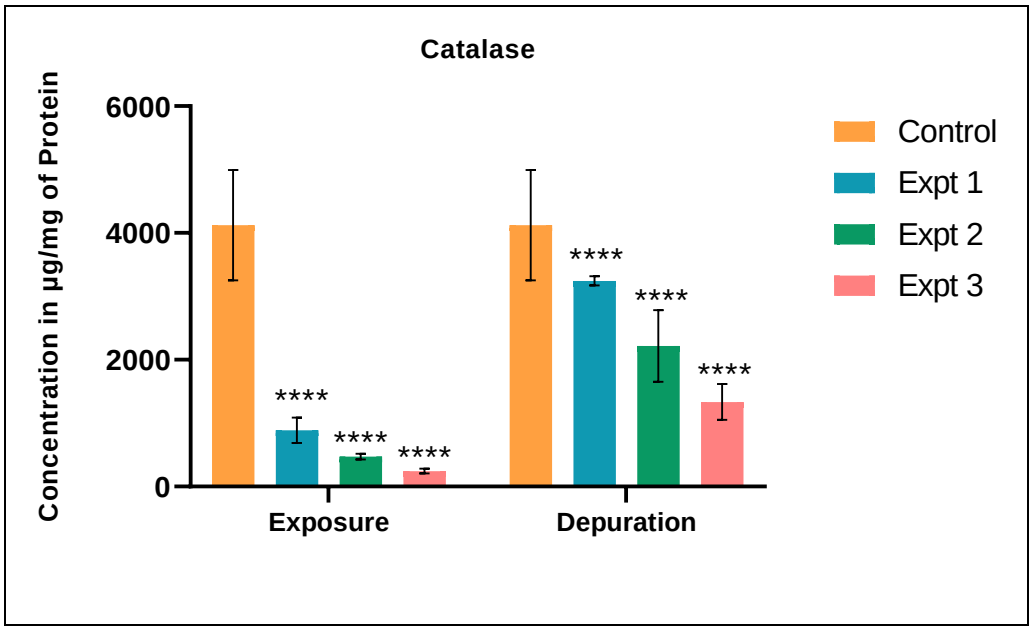


Figure 24: Effect of Acute exposure to PET MP on the Catalase content on the liver of mice post exposure and depuration period. (****-P< 0.0001 Highly significant)

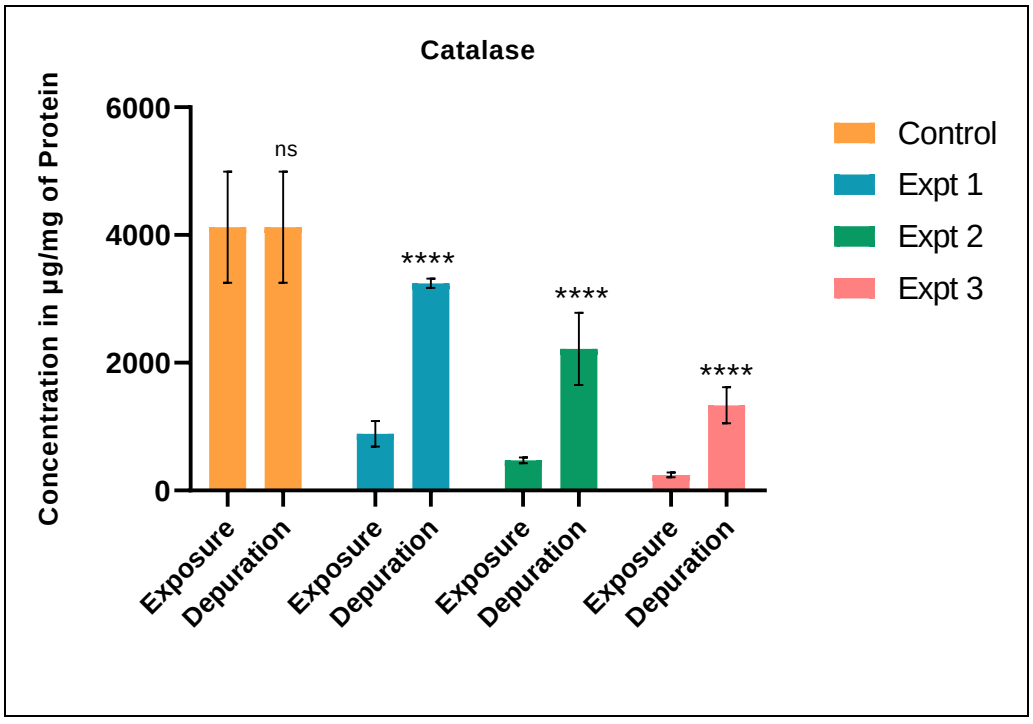


Figure 25: Effect of Acute exposure to PET MP on the Catalase content on the liver of mice post exposure and depuration period. (ns- Non significant ; ****-P< 0.0001 Highly significant)

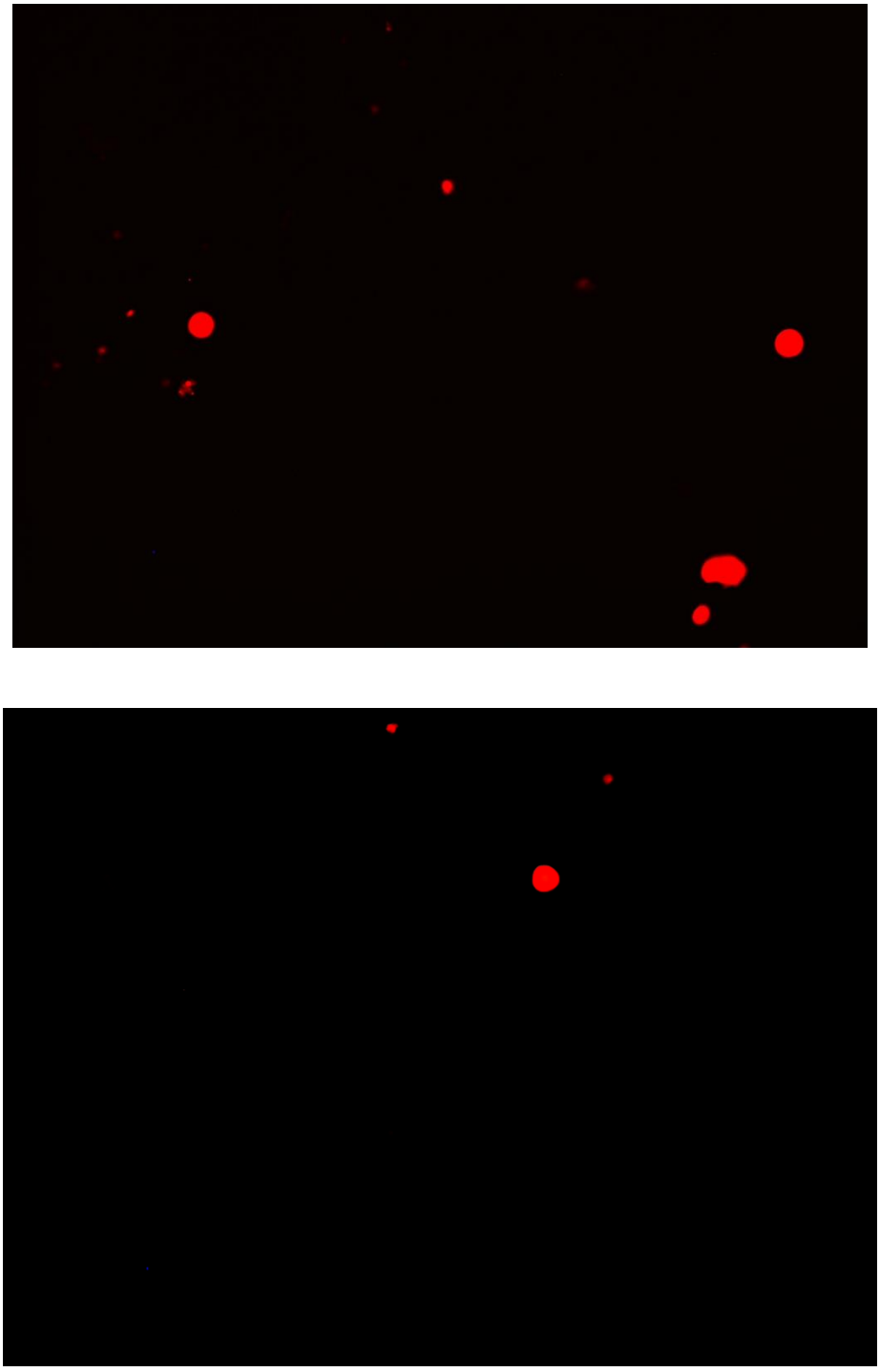


PLATE 2: COMET Assay Cells

4.2. DISCUSSION

Accumulation of plastic in the world's oceans is of increasing environmental concern. Polyethylene terephthalate (PET) which is frequently used in many applications, including textiles and food packaging is one of the major components of plastic waste (Webb et al., 2012). The PET Microplastic used for current study were procured from Metal Fabrik India, Panjim, with finds its applications in powder coating of metals. Raman Spectrometry was employed for identification of the microplastics to be Polyethylene terephthalate (PET) and the same was confirmed by the peaks obtained at 1603.5, 1340.6, 979.2 and 453.5 cm−1 (Gedler et al., 2013; Nava et al., 2021). The X-Ray Diffraction data confirmed the crystalline nature of the substance and the Scanning Electron Microscope confirmed the irregular grainy structure of the PET microplastics.

The behaviour of the mice was also monitored. No drowsiness and hyperactivity was observed during the quarantine period. Experimental 1 and Experimental 2 did not show as signs of drowsiness. In few mice in Experimental 3 minute drowsiness was seen post 12^{th} day. No other much behavioural changes were seen in experimental groups as compared to control group. Few mice did show signs of isolation at multiple times in Experimental 2 and Experimental 3 Groups. No difference in weight was observed pre and post exposure period. A reduced water intake and increased intake of feed was seen over the exposure period. During the depuration period the water intake was similar to the start of experiment.

The present study on Acute exposure to PET microplastics aimed to understand the effects on mice, *Mus musculus*, and the effects after depuration studies. Tests to analyse the biomolecules like Carbohydrate, Free Sugars, Proteins, Albumins and Globulins were carried out using liver tissue of the mice. The Carbohydrates Test showed an

increase in Carbohydrate concentration with increasing concentration of dose. A decrease in carbohydrate concentration was seen during the post depuration period. Similar results were obtained for Free Sugars, Proteins, Albumin and Globulin Concentrations. Thus suggesting that the concentrations of these biomolecules shifts back to normal as the exposure to PET Microplastics is discontinued.

COMET assay was performed to analyse any gene damage caused due to PET Microplastics. COMET analysis showed very minute Tail DNA Percentage thus suggesting PET Microplastics to be not genotoxic at concentrations of 1mg/ml, 1.5mg/ml and 2mg/ml (Hou et al., 2021).

Reduced Glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) were analysed to see the effects of PET Microplastics on Oxidative Stress in mice. The Reduced Glutathione result showed a significant increase in the GSH concentration with increasing dose. There was no significant difference seen post and pre depuration period in Reduced Glutathione (GSH) concentrations. Similar results as Reduced Glutathione (GSH) were recorded for Thiobarbituric acid reactive substances (TBARS).

Alkaline Amino Transferase (ALT) showed an increase in ALT concentration with increasing concentration of dose. A decrease in ALT concentration was seen after the post depuration period. Similar results were obtained for Superoxide Dismutase (SOD) and Catalase tests (Lu et al., 2024). Thus suggesting that the concentrations of Alkaline Amino Transferase (ALT), Superoxide Dismutase (SOD) and Catalase shifts back to normal as the exposure to PET Microplastics is discontinued.

This study is plays a crucial role in understanding the potential health risk associated with Polyethylene Terephthalate (PET) Microplastics. Liver plays an important role in detoxification and metabolism any adverse effects on the organ can have serious health

implications. Hence understanding how PET microplastics effects the mice liver functioning can provide insights into potential health risks. It helps to provide valuable insights into the toxicity, bioaccumulation and consequences of PET Microplastics exposure.

4.3. CONCLUSION

The present study showed that acute exposure to Polyethylene terephthalate (PET) microplastics can have serious toxicological effects on *Mus musculus*. It is also evident from the current study that PET MPs can effect the Carbohydrates, Proteins, Albumins, Globulins and Free Sugar content in the mice. Effects on enzymes like Alkaline Amino Transferase (ALT), Catalase and Superoxide Dismutase (SOD) were also observed. Reduced Glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) concentrations were also effected . No genotoxic effect was observed at the doses in the experiment hence concluding PET MPs to be not effecting at the genetic level at the mentioned concentrations. It was seen that the concentrations of the above mentioned variables were shifting back to normal except for Reduced Glutathione (GSH) and Thiobarbituric acid reactive substances (TBARS) after the depuration period of 7 days. It can be concluded that there PET microplastics can naturally be detoxified from the body as most of the parameters were shifting back to normal after discontinuation of exposure.

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