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Polystyrene Toxic Effects On The Brain Of *Channa Punctatus.*

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ABSTRACT

The pervasive presence of polystyrene microplastics in aquatic environments poses a significant threat to the health of aquatic organisms and ecosystems.This study explores the toxicological impacts of polystyrene microplastics on aquatic organisms, specifically on *Channa punctatus*. Chemically, heavy metals and persistent organic pollutants, which have the ability to bioaccumulate and biomagnify within the food web, are transported by microplastics. The biological effects of microplastic exposure include oxidative stress, inflammatory reactions, and endocrine disruption, which impair aquatic creature's ability to grow, reproduce, and survive.The experiment performed aims to investigate the mechanisms by which microplastic exert harmful effects, including significant differences in the parameters like SOD & Catalase, Necrosis & Vacuolization in the brain's tissue and neurotoxic & physical behavioural responses are seen.

Keywords : Microplastics , Polystyrene , Antioxidant enzyme , *Channa punctatus* , Necrosis , Vacuolization.

INTRODUCTION

Aquatic systems are essential to the complex web of ecosystems that make up our world, supporting life in all its forms. However, a secret hazard that threatens up to disrupt this delicate equilibrium is there beneath the glistening surface of aquatic forms. Plastic pollution is one of the most important environmental issues of our day, which has serious effects and is a major global concern. The amount of plastics in our modern society has increased alarmingly, with disastrous effects on aquatic ecosystems around the globe. Plastics have contaminated every body of water on our world, from vast oceanic

stretches to little freshwater streams and affecting its life forms. Global plastic output has increased over the past 70 years from 1.5 million tonnes to roughly 359.0 million tonnes (Bui et al., 2020), with projections indicating that it will go upto 500.0 million tonnes by 2025 (Huang et al., 2021a).

Plastics' cost-effectiveness, endurance, and flexibility have made them a crucial element of modern life, of particular concern are Microplastics, minute particles measuring less than 5 millimeters in size, which have emerged as a persistent and ubiquitous pollutant in oceans, rivers and other water bodies. The term "microplastics" was initially used 19 years ago by researchers studying plastic pollution in the UK's oceans, Thompson et al. (2004). Since then, governments, non-governmental organizations, the scientific community, and others have become interested in microplastics. Microplastic gradual buildup in aquatic habitats poses significant hazards to human health as well as freshwater and marine ecosystems. One such smicroplastic is Polystyrene (Latex beads – carboxylate modified polystyrene). One of the most prevalent polymer varieties for microplastics found in sediments is Polystyrene which may endanger benthic life. A synthetic polymer made from styrene monomers is called polystyrene. Polystyrene is produced through the polymerization process, which involves connecting these styrene monomers to form long chains. This versatile plastic's desired qualities, such as being stiff, lightweight, and having excellent insulation capabilities, make it commonly used in a variety of industries.

Previous research has demonstrated that Polystyrene microplastics can have detrimental effects on aquatic life. For example, they can cause oxidative stress in river clams (Corbicula fluminea) (Li et al., 2020a) and medaka (Oryzias melastigma) (Kang et al., 2021), inhibit the growth and destroy cell morphology in microalgae (Platymonas helgolandica) (Wang et al., 2020), damage to the intestinal and liver tissue in goldfish (Carassius auratus) (Yang et al., 2020), reduce immunity in Macrobrachium nipponense (Li et al., 2021b), cause inflammation and cardiomyocyte apoptosis in carp (Wu et al., 2022). Consequently, it is crucial to look into the mechanism of action of Polystyrene microplastics that may harm aquatic life.

The focus of this study is to check the toxicity of Polystyrene (Latex beads – carboxylate modified polystyrene) on the brain of *Channa Punctatus*, the spotted snakehead which is a species of freshwater fishes that belongs to the family Channidae. It is found in the Indian Subcontinent and nearby areas, ranging across Afghanistan, Pakistan, India, Bangladesh, Myanmar and Tibet. Its niches are swamps, ponds and brackish water systems. Rich in premium proteins from animals, fish is a cheap and common food source (Maulu et al., 2021a). They are a great source of calcium and fluorine, which are necessary for children's developing bones and teeth (Paital, 2018a). The minerals phosphorus, magnesium, iodine, iron, zinc, and selenium are also highly bioavailable in fish (Thilsted et al., 2016; Maulu et al., 2021a; Maulu et al., 2021b; Ahmed et al., 2022). According to Balami et al. (2019) and Kwasek et al. (2020), all of these nutrients maintain a person healthy and free from cardiovascular, neurodegenerative, and low blood pressure diseases. Hence it is important to study about the aquatic organisms which are getting affected by the microplastics pollution.

MATERIAL AND METHODS

Chemical

L4655-1ML Latex beads, carboxylate-modified polystyrene fluorescent yellow green aqueous suspension, 1.0μm mean particle size of Sigma Aldrich, Co, 3050 Spruce Street (St. Louis, USA) is used for this study which was purchased from local dealer Gyan Scientifics Laboratory Lucknow, India.

Test animal collection and its acclimatization

Because of the ability of bioaccumulating pollutants, having long lifespans, inhabiting diverse environments, exhibiting physiological and behavioural responses to contamination, having economical and ecological importance, and are commonly used as bioindicators in environmental monitoring and regulatory assessments, fishes are an excellent model organism for studying the effects of water and sediment-borne contamination. One such variety of fish named *Channa Punctatus*, the spotted snakehead was brought in the quantity of 108 of both sexes with varying weight (30-35 gm) & of approx. 15-18 cm in length were collected from local lentic habitat of Lucknow city. To give the fish time to adjust to their new environment, they were kept in quarantine in the laboratory for two weeks before the experiment. Prior to the experiment being set up, fish were cleaned by immersing them in a 0.01% $KMnO_4$ solution. They were given commercial fish food and remove waste products every day and aeration were provided with aerator. In addition, throughout the study water's parameters were also observed at regular intervals.

Experimental Setup

In this study, the fishes were divided into four groups out of which group 1 was served as control and the other three was Group 2, Group 3 and Group 4 were treated with the Polystyrene (Latex beads – carboxylate modified polystyrene) aqueous suspension, 1.0μm mean particle size with variant concentration of 5mg/L, 10mg/L, 20 mg/L respectively. The parameters which are analysed in this study are Behaviour of the test fish, Histology of Brain, antioxidant enzyme. The experiment's parameters were collected and evaluated on the scheduled days i.e. 7th, 14th, 21st and 28th day respectively. Throughout the course of the study, the experiment was conducted with restricted feeding schedules and limited water changes to ensure controlled conditions and reliable results. In order to retain the stability in the tanks and reduce fluctuations in the water's chemistry, minor water changes were performed. This created a more controlled environment for assessing the toxicant's effects. With the least amount of possible unintended consequences, this experimental strategy allowed for a comprehensive investigation of the toxicant's effects at different concentration levels. Through the addition of restricted feedings and water alterations, the study intended to highlight the distinctive impacts of the toxicant on the organism under investigation, providing important insights on its toxicological pattern and potential ecological ramifications.

Antioxidant Enzymes

Antioxidant enzymes are proteins produced by living organisms that plays an essential role in scavenging harmful chemicals known as free radicals. These enzymes function by eliminating dangerous substances that may destroy biological components .The antioxidant enzymes which are analysed in this experiment are Superoxide dismutase (SOD) and Catalase. The catalase is a primary antioxidant enzyme present in almost all aerobic organisms. The enzyme's activity is tissue-specific, with increased activity in organs with high oxidative potential.(B. Halliwell, J.M.C. Gutteridge et al. 1989) This was further validated in the current goldfish investigation. Activity of catalase declined in the subsequent order: Red muscle > brain > white muscle > liver > kidneys > heart . During the experiment fishes were subjected to the toxicants under carefully monitored circumstances. Following exposure, tissue samples were taken from the fish, usually from an organ with high concentrations of antioxidant enzymes. The collected tissue samples are homogenized to dissemble the cellular structures and liberate the enzymes into a solution for analysis. The enzyme activity data obtained from the experiments are analyzed statistically. To find out if there are any statistically significant differences between the experimental groups (fish exposed to toxicants) and the control group (unexposed fish). Overall the experiment, these studies offer insightful information about how pollutants impact fish's antioxidant defence mechanism, which is critical to fish survival in contaminated areas.

Histology

Histology is the study of the anatomy at the microscopic level of tissues and cells. It involves the examination of thin sections of tissues under a microscope to observe their structure, organization, and cellular composition. Under this study, brain histology is focused for examination specifically Necrosis and Vacuolization parameters are taken under consideration. Brain histology of fishes exposed to toxicants are analysed to evaluate any morphological changes or deformities caused by the toxicant exposure. Fish were exposed to the toxicants under carefully monitored conditions. The fish's brains were delicately removed after exposure. After that, the brains were preserved using the Bouin’s fixatives in order to maintain their structural integrity for histological examination. Following fixation in Bouin's solution, alcohol was used to hydrate the tissues. Tissue blocks were made with paraffin wax. Haematoxylin and eosin stains were used after the paraffin blocks' sagittal slices, which were 5 m thick, were cut for analysis. Using light microscopy and a Nikon or Eclipse camera equipped with a Spot Insight DS-Fi2 digital camera, photomicrographs of stained sections were obtained. ImageJ software, which includes 64-bit Java 1.8.0_172 image software, was used to analyze the impairment to brain tissue. After histological examination, by analysing the histological findings within the context of toxicant exposure and contrasting them with fish from the control group that did not receive any toxicant exposure, the toxicological effects on brain histology were assessed. Overall, brain histology analysis provides valuable insights into the neurotoxic effects of toxicants on fish and helps in understanding the mechanisms underlying their toxicity.

Analysis of Behaviour of a test fish

Analysing the behaviour of test fish after exposure to toxicants is an important aspect of toxicology research as changes in behaviour is one of the earliest indicators of toxicant exposure. Behavioural analysis enhances other toxicological study objectives and advances our knowledge of how toxicants affect fish health, sustainability, and ecosystem functioning.

Video-based behavioural inspection is a common method used to collect quantified behavioural data for aquatic risk assessment. The commonly used method of video-based behavioural monitoring was employed to observe test fish, which involves filming and recording the fish's activity. The camera used in the experiment was produced in China, Anhui Province, BHR5003 IN. The fish were recorded on video, which was carefully examined to ascertain the number of leaps, chases, bites, and violent, swaying, and quivering displays they made against the intruder.

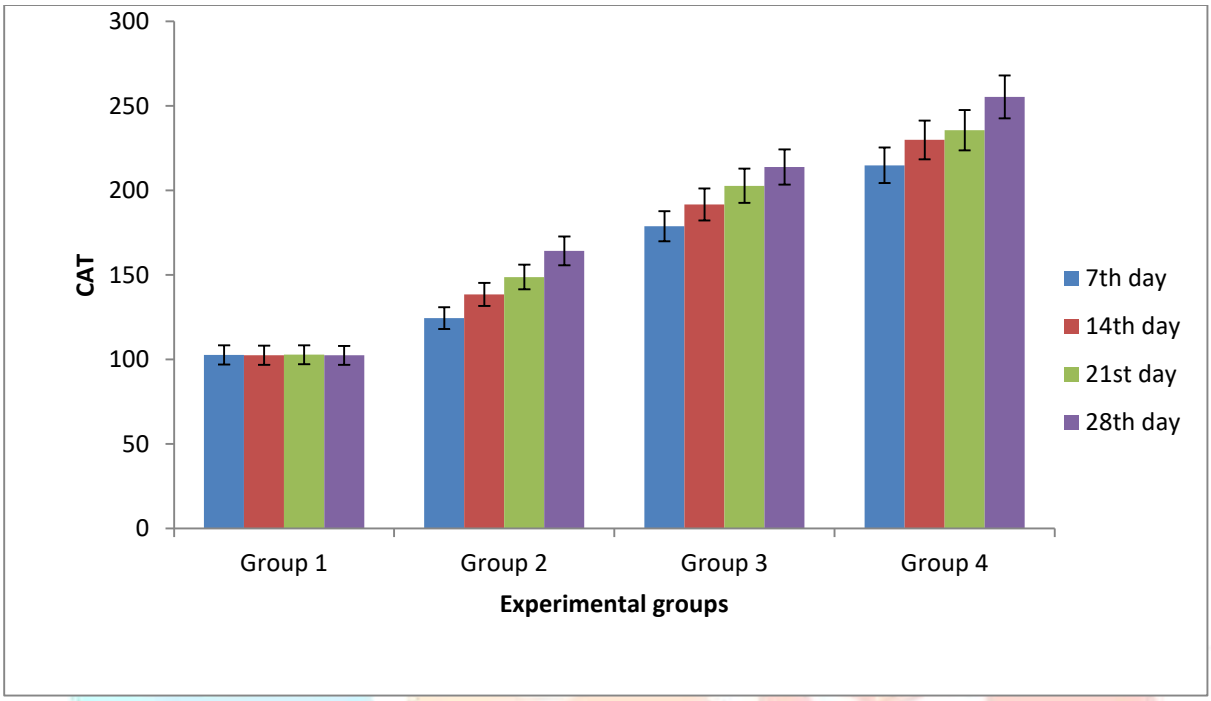
Result and Discussion

After the exposure of fishes to the toxicant Polystyrene (Latex beads – carboxylate modified polystyrene) aqueous suspension,1.0µm mean particle size with variant concentration of 5mg, 10mg, 20 mg respectively. The experiment's parameters were collected and evaluated on the scheduled days i.e. 7th, 14th, 21st and 28th day respectively. There were various statistically significant differences in the parameters like SOD & Catalase, Necrosis & Vacuolization in the brain's tissue and neurotoxic and physical behavioural responses are seen.

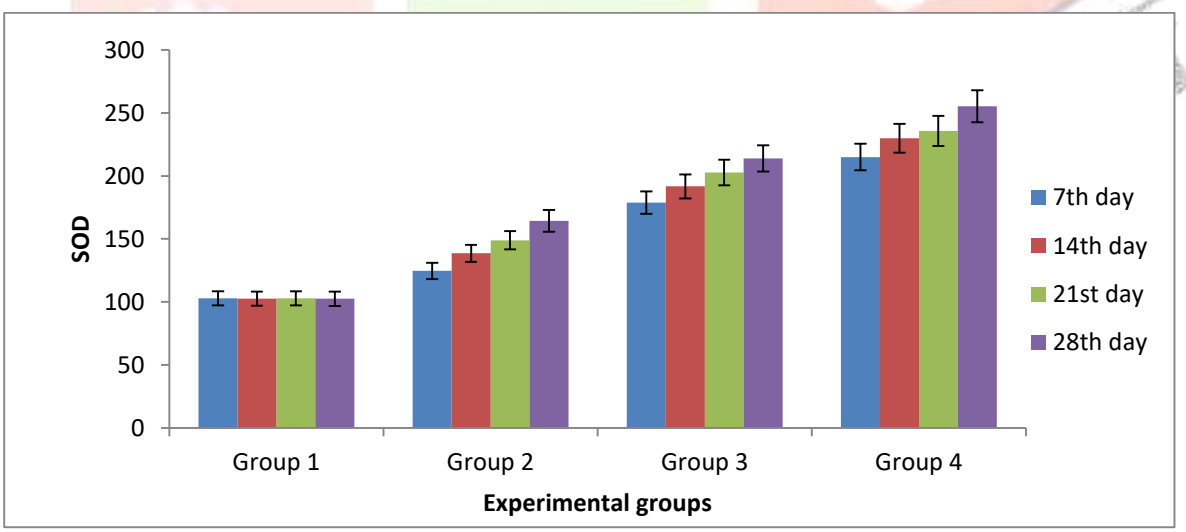
Table 1: Antioxidant Enzymes : SOD & Catalase.

Groups	Conc.(mg/L)	Exposure Period	SOD	CAT
Group 1	0 mg/L of Polystyrene	7 Day 14 Day 21 Day 28 Day	103.65±5.15 103.63±5.13 103.57±5.14 103.54±5.16	102.77±5.64 102.52±5.61 102.82±5.67 102.44±5.64
Group 2	5 mg/L of Polystyrene	7 Day 14 Day 21 Day 28 Day	114.67±5.24 123.42±6.25 136.53±6.83 145.67±7.26	124.56±6.45 138.54±6.78 148.81±7.25 164.33±8.55
Group 3	10 mg/L of Polystyrene	7 Day 14 Day 21 Day 28 Day	156.71±7.65 163.55±8.24 172.73±8.69 183.61±9.33	178.83±8.94 191.72±9.51 202.73±10.12 213.87±10.35

Group 4	20 mg/L of Polystyrene	7 Day	212.61±10.54	214.88±10.54
		14 Day	222.72±11.15	229.9±11.52
		21 Day	232.82±11.75	235.68±11.89
		28 Day	244.81±12.26	255.32±12.65



Graph.1, showing catalase enzyme level by different groups at 7th,14th,21st & 28th day.



Graph.2, showing SOD enzyme level by different groups at 7th,14th,21st & 28th day.

Antioxidant Enzyme Estimation

To examine the biochemical parameters, fish of each control and treatments group were dissected. Organs of dissected fish were placed in ice chilled saline solution. Each tissue was prepared for estimation of superoxide dismutase (SOD) and catalase (CAT). Data was analyzed by ANOVA using (SPSS Inc., Illinois, USA) program. Mean ± SE values for biochemical and antioxidant enzymes among control and treated groups were compared.

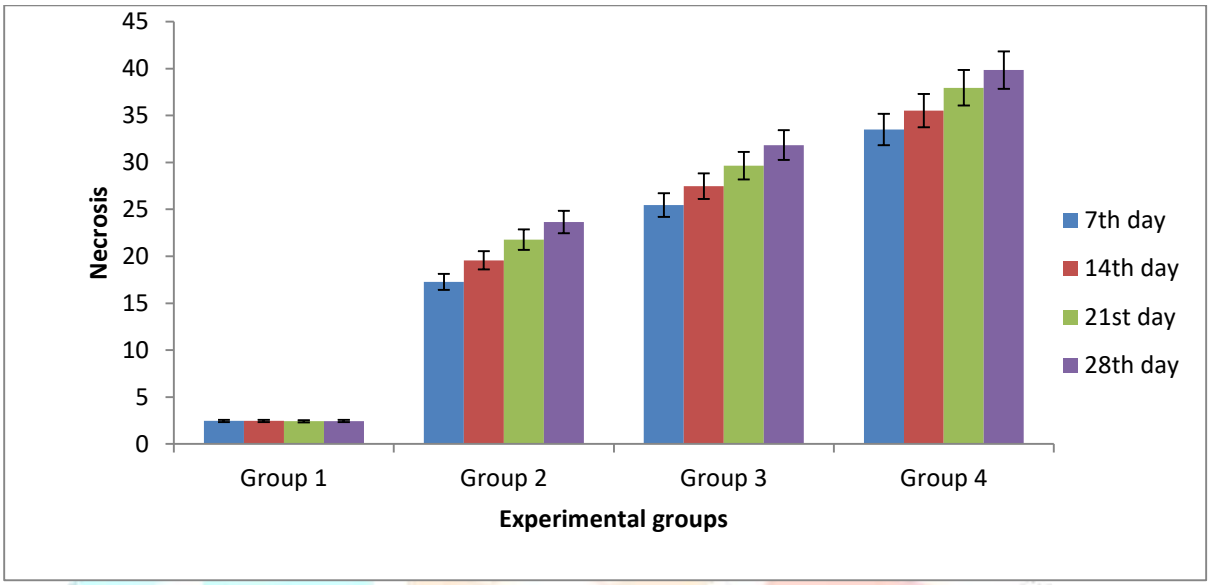
In the above given table of superoxide dismutase (SOD), toxicant ministered groups were contemplated from control group (103.65, 103.63, 103.57, 103.54) mg/L . Group 2nd when evaluated on 7th, 14th, 21st & 28th day to control group, the SOD enzyme was up surged by 10.68% , 19.05% , 31.73% , and 40.66 % respectively where as when group 3rd analyzed on 7th, 14th, 21st & 28th day to control group, the SOD enzyme was escalated by 51.21 % , 57.76 % , 66.71 % , and 77.09 % respectively. Group 4th when compared on 7th, 14th, 21st & 28th day to control group, the SOD enzyme was increased by 101.56%, 114.83%, 124.66% , and 136.25 % respectively.

In the above given table of catalase (CAT), Group 2nd when compared on 7th, 14th, 21st & 28th day to control group (102.77,102.52,102.82,102.44) mg/L , the catalase enzyme was increased by 1.21,1.35,1.45,1.60 folds respectively where as when group 3rd evaluated on 7th , 14th , 21st , & 28th day to control group , the CAT enzyme was up surged by 1.74 , 1.87 , 1.97 , 2.08 folds respectively. Group 4th when analyzed on 7th, 14th, 21st & 28th day to control group, the CAT enzyme was escalated by 2.08, 2.24, 2.29, 2.49 folds respectively.

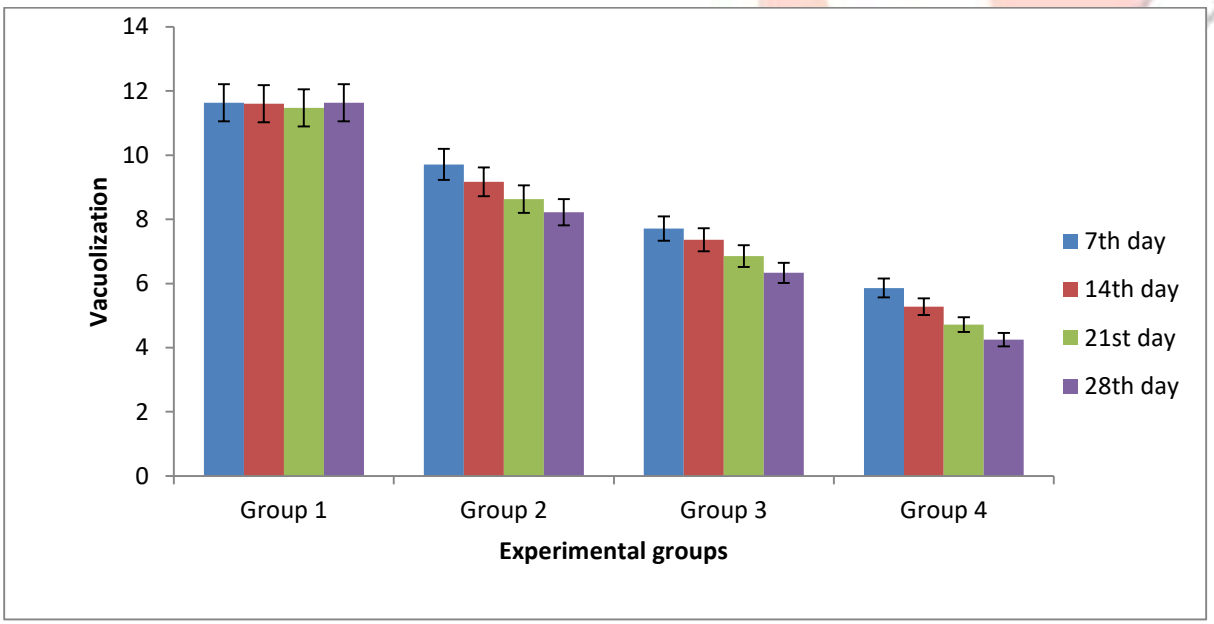
Table 2: Histological Parameters : Necrosis and Vacuolization

Groups	Conc.(mg/L)	Exposure Period	Necrosis	Vacuolization
Group 1	0 mg/L of Polystyrene	7 Day	2.45±0.12	3.44±0.17
		14 Day	2.46±0.12	3.47±0.16
		21 Day	2.42±0.12	3.74±0.17
		28 Day	2.44±0.12	3.67±0.16
Group 2	5 mg/L of Polystyrene	7 Day	17.27±0.86	12.82±0.64
		14 Day	19.56±0.97	15.64±0.78
		21 Day	21.77±1.08	18.43±0.92
		28 Day	23.65±1.18	21.65±10.8
Group 3	10 mg/L of Polystyrene	7 Day	25.45±1.27	23.54±1.27
		14 Day	27.46±1.37	26.56±1.32
		21 Day	29.65±1.48	29.75±1.48
		28 Day	31.84±1.59	32.48±1.62

Group 4	20 mg/L of Polystyrene	7 Day	33.51±1.67	35.48±1.77
		14 Day	35.52±1.77	38.65±1.93
		21 Day	37.95±1.48	41.65±2.08
		28 Day	39.84±1.59	44.65±2.23



Graph.3, showing Necrosis level by different groups at 7th,14th,21st & 28th day.



Graph.4, showing Vacuolization level by different groups at 7th,14th,21st & 28th day.

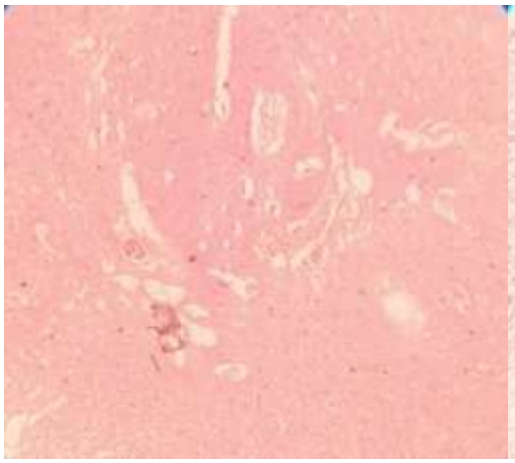


Fig.1. Male brain of *Channa punctatus* at 7th day.

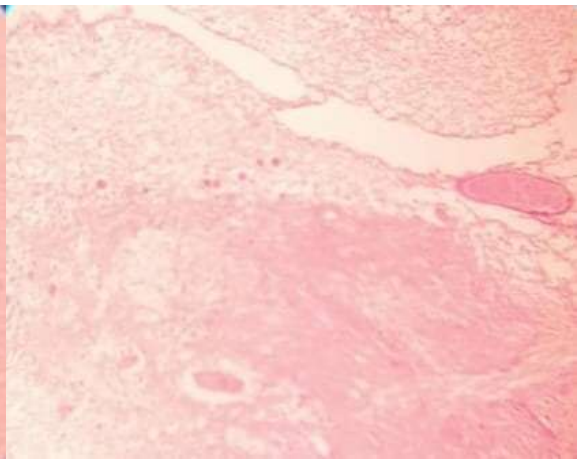


Fig.2. Female brain of *Channa punctatus* at 7th day.

Fig.3. Female brain of *Channa punctatus* at 14th day.

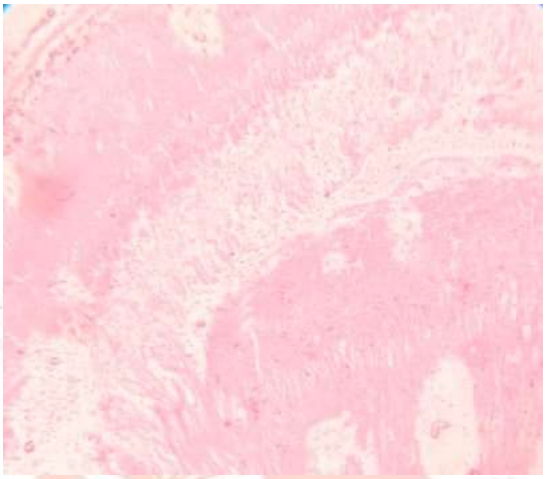


Fig.4. Male brain of *Channa punctatus* at 14th day.

Histological Analysis

The study of histology involves employing specialized imaging methods to examine the microscopic structure of cells and tissues. For information on the alterations caused by chemicals at the tissue and cellular level, toxicological histopathology is helpful .

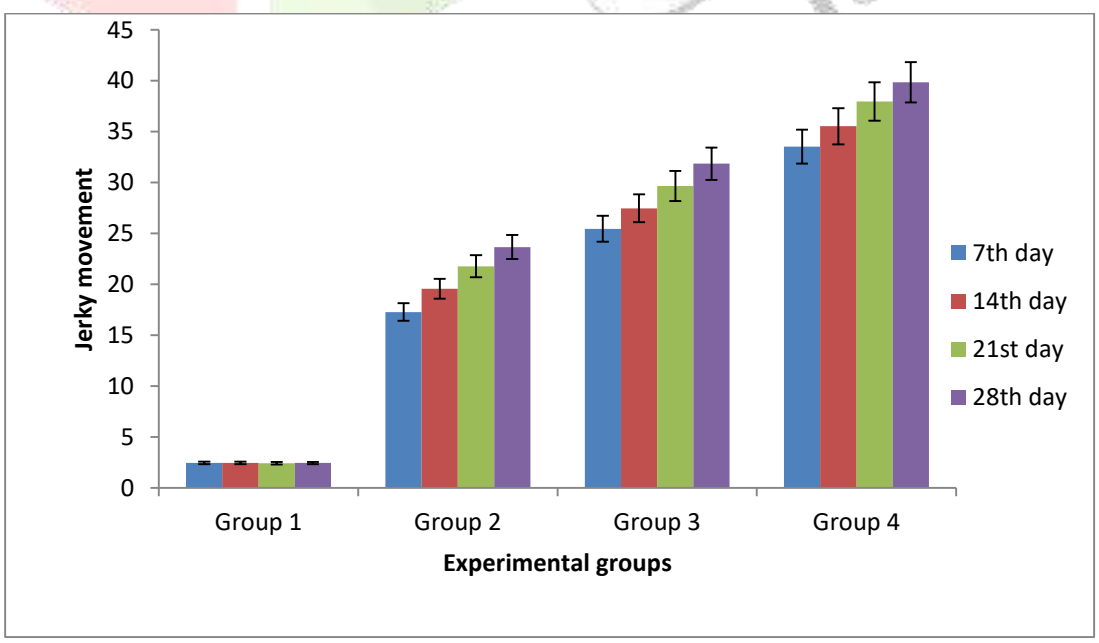
In the above given table of Necrosis factor, toxicant ministered groups were contemplated from control group. Group 1st when evaluated on 7th, 14th, 21st & 28th day to control group (2.45,2.46,2.42,2.44) mg/L , the degree of Necrosis was up surged by 7.02 , 7.87 , 8.95 , and 9.65 folds respectively where as when group 3rd analyzed on 7th, 14th, 21st & 28th day to control group, the degree of Necrosis was escalated by 10.34 , 11.08 , 12.20, and 12.99 folds respectively. Group 4th when compared on 7th, 14th, 21st & 28th day to control group, the degree of Necrosis factor was increased by 13.65, 14.33, 15.61 , and 16.26 folds respectively.

In the above given table of degree of Vacuolization , Group 2nd when compared on 7th, 14th, 21st & 28th day to control group (3.44,3.47,3.74,3.67) mg/L the degree of vacuolization factor was increased by 3.72,4.49,4.92,5.88 folds respectively where as when group 3rd evaluated on 7th , 14th , 21st , & 28th day to control group , the vacuolization was up surged by 6.82 , 7.63 , 7.93 , 8.82 folds respectively. Group 4th

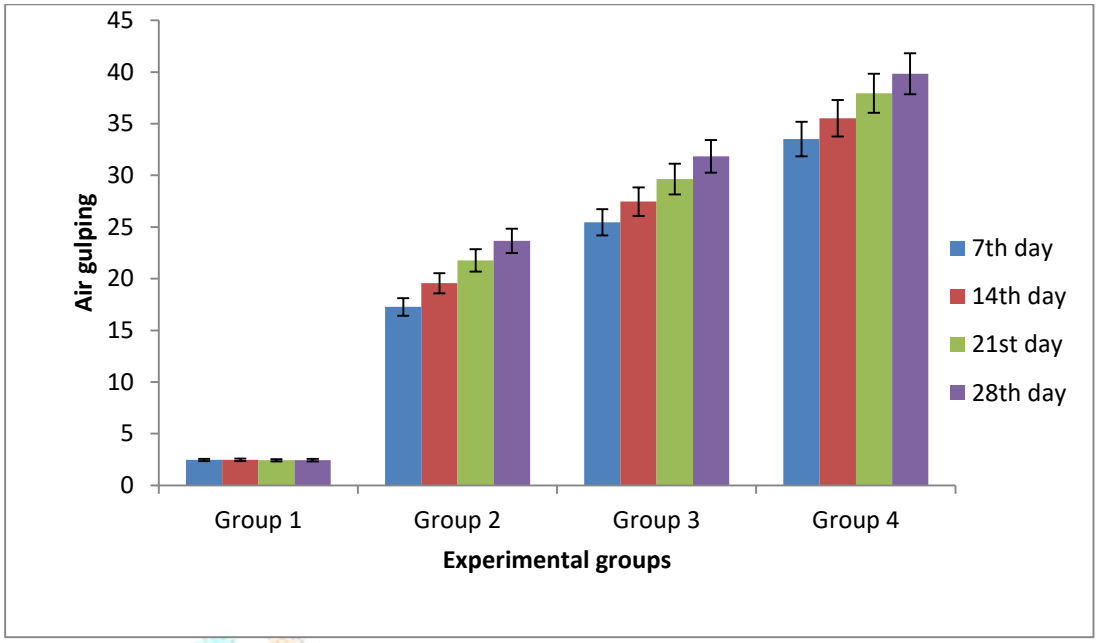
when analyzed on 7th, 14th, 21st & 28th day to control group, the degree of vacuolization was escalated by 2.08, 2.24, 2.29, 2.49 folds respectively

Table 3: Behavioural responses

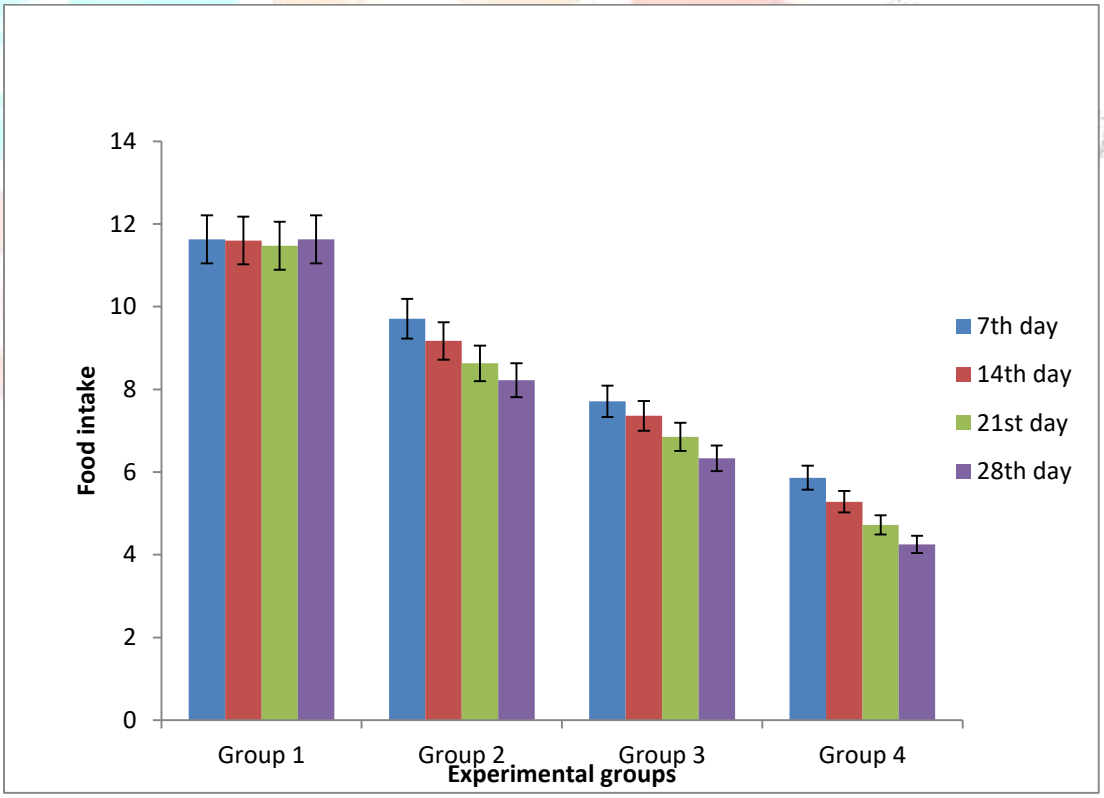
Groups	Conc.(mg/L)	Exposure Period	Jerky Movement	Air Gulping	Food intake
Group 1	0 mg/L of Polystyrene	7 Day	1.53±0.07	3.56±0.19	11.63±0.58
		14 Day	1.59±0.07	3.43±0.24	11.6±0.58
		21 Day	1.57±0.07	3.63±0.26	11.47±0.58
		28 Day	1.55±0.07	3.67±0.29	11.63±0.58
Group 2	5 mg/L of Polystyrene	7 Day	3.46±0.17	4.16±0.19	9.71±0.48
		14 Day	4.65±0.23	4.92±0.24	9.17±0.45
		21 Day	5.47±0.26	5.32±0.26	8.63±0.43
		28 Day	6.84±0.34	5.83±0.29	8.22±0.41
Group 3	10 mg/L of Polystyrene	7 Day	8.51±0.42	6.27±0.31	7.71±0.38
		14 Day	9.42±0.47	6.91±0.34	7.36±0.36
		21 Day	10.83±0.54	7.45±0.37	6.85±0.34
		28 Day	11.92±0.59	7.92±0.39	6.33±0.31
Group 4	20 mg/L of Polystyrene	7 Day	13.82±0.69	8.18±0.41	5.86±0.29
		14 Day	14.51±0.72	8.77±0.43	5.28±0.26
		21 Day	15.48±0.77	9.24±0.46	4.72±0.23
		28 Day	16.58±0.82	9.82±0.46	4.25±0.21



Graph.5, showing Jerky movement level by different groups at 7th,14th,21st & 28th day.



Graph.6, showing air gulping level by different groups at 7th,14th,21st & 28th day.



Graph.7, showing food intake level by different groups at 7th,14th,21st & 28th day.

Behaviour analysis

In the above given table of Jerky movement, toxicant ministered groups were contemplated from control group. Group 1st when evaluated on 7th, 14th, 21st & 28th day to control group (1.53,1.59,1.57,1.55) mg/L , the degree of Jerky movement was up surged by 2.25 , 2.94 , 3.45 , and 4.47 folds respectively where as

when group 3rd analyzed on 7th, 14th, 21st & 28th day to control group, the degree of Jerky movement was escalated by 5.54 , 5.96 , 6.81 , and 7.80 folds respectively. Group 4th when compared on 7th, 14th, 21st & 28th day to control group, the degree of jerky movement was increased by 8.98 , 9.18 , 9.72 , and 10.83 folds respectively.

In the above given table of degree of Air gulping , Group 2nd when compared on 7th, 14th, 21st & 28th day to control group (3.56,3.43,3.63,3.67) mg/L , the degree of air gulping was increased by 1.15,1.44,1.46,1.59 folds respectively where as when group 3rd evaluated on 7th , 14th , 21st , & 28th day to control group , the degree of air gulping was up surged by 1.76 , 2.02 , 2.04 , 2.16 folds respectively. Group 4th when analyzed on 7th, 14th, 21st & 28th day to control group, the degree of air gulping was escalated by 2.29, 2.54, 2.53, 2.66 folds respectively.

In the above given table of Food intake , Group 2nd when compared on 7th, 14th, 21st & 28th day to control group (11.63, 11.6, 11.47, 11.63) mg/L , the degree of food intake was decreased by 1.19,1.25,1.32,1.41 folds respectively where as when group 3rd evaluated on 7th, 14th , 21st , & 28th day to control group , the degree of food intake was decline by 1.51, 1.25 , 1.67 , 1.83 folds respectively. Group 4th when analyzed on 7th, 14th, 21st & 28th day to control group, the degree of food intake was diminished by 1.98, 2.18, 2.41, 2.73 folds respectively.

Conclusion

Until now, few relevant researches on the toxicity of polystyrene toxicity has been conducted. The present study concluded that polystyrene accumulation in *Channa punctatus* led to histological changes in brain, changes in level of antioxidant enzymes and behavioural responses. In the biochemical parameter, when the toxicant is administered then the values of antioxidant enzymes SOD and catalase level keeps increasing which denotes the organism body keeps produsing enzymes to fight with the toxic radicals and protect themselves. whereas when the histological parameters were analysed , the brain tissues were affected by necrosis & vacuolization and it keeps upsurging with time. Apart from this the difference in physical and neurotoxic behavior were seen when the toxicant is administered . The degree of jerky movement & air gulping rises with passing days but the food intake capacity of organism decreases. This shows the organisms was adversely affected by the polystyrene microplastics. In conclusion, the toxicological impacts of microplastics on aquatic organisms are significant and diverse, requiring immediate and broad action. Future research should focus on elucidating the long-term ecological consequences of microplastic exposure, exploring the efficiency of mitigation strategies, and advancing our understanding of the interactions between microplastics and other environmental stressors. By addressing the issue of microplastic pollution through scientific research, policy intervention, and public engagement, we can work towards safeguarding the health and resilience of aquatic ecosystems.

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Author's Contributions

Dr.Vivek Kumar: Experimental designing, Statistical analysis of the data and preparation of graphs, drafting of the manuscript and supervision of the experiment.

Arya Shukla: Collection of test animal and Execution of the experiment.

Sunil P. Trivedi: Final editing of the manuscript.

Compliance with Ethical Standards

As the requirements of the committee for the purpose of control and supervision of experiments on animals (CPCSSEA), Government of India, and Institutional Animal Ethics Committee (IAEC) vide registration no. 1861/GO/Re/S/16/CPCSEA, University of Lucknow.

The authors carried out the experiment in accordance with the CPCSEA's specified procedures.

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