RESEARCH ARTICLE

Dose-dependent alteration in hepatic and cerebral glucose metabolism following exposure to polystyrene microplastic in wistar rats

Sudipta Pal*, Susmita Chakraborty, Sumana Mondal

ABSTRACT

Background: Recently, microplastics (MPs) with dimensions less than 5 mm have gained more attention due to their adverse impact on the environment and living creatures. Polystyrene (PS) particle is a key element of primary microplastics, which are causing numerous health issues such as interruption of energy metabolism, oxidative stress, neurotoxicity, immunotoxicity, digestive gland disorders, reproductive disruption, and genotoxicity in marine living organisms. *Method:* Alteration in carbohydrate metabolism was evaluated in male wistar rats (six weeks of age) after four weeks of oral exposure to polystyrene microplastic (PS-MP) at three different doses (0.5, 5 and 50 mg/L *via* drinking water). *Results:* Polystyrene exposure caused a significant decrease in blood glucose, liver glycogen, and pyruvic acid content in liver and cerebral tissue. Free amino nitrogen content significantly altered in the liver and cerebrum in a dose-specific manner. The LDH activity was found to be decreased in the liver, whereas it increased in the cerebral cortex of rats in a dose-responsive fashion. Enzymes like glucose 6-phosphatase, GOT, GPT, and succinate dehydrogenase activities demonstrated differential effects on the liver and cerebrum of rats in terms of energy metabolism. *Conclusion:* It is suggested that sub-acute polystyrene exposure significantly perturbs glucose metabolism by inducing hypoglycemia associated with decreased glycolysis and increased TCA cycle enzyme function in rat liver in a dose-dependent manner. Gluconeogenesis is also affected differentially by metabolic adjustment in the studied organs.

Keywords: Polystyrene microplastics, Energy metabolism, Glycolysis, Gluconeogenesis, TCA cycle, Transaminase function.

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Introduction

During the last few decades, humans assisted the growing rate of plastic production worldwide. As a result, plastics have spread into the environment, so it could be easily said that we live in a world of plastics. Plastic particles measured as <5 mm in diameter are defined as microplastics (MPs).[1] MPs are spotted in different sizes, shapes, polymers, and concentrations in freshwater, drinking water, food, atmosphere, marine water, biota, and agroecosystem in the environment (Figure 1).[2] MPs are derived from plastic debris through biological and mechanical degradation.[3] In marine surroundings, microplastics were identified for the first time and studied well. There are two main types of MPs, namely primary and secondary microplastics. MPs of microscopic sizes and factory-made are defined as 'primary microplastics,' and those derivatives from large plastic debris are described as 'secondary microplastics.' The diameter of primary MPs is between 1 to 5 µm; these are sphere-shaped and are made of polystyrene (PS), polyethylene (PE), or polypropylene (PP) polymers.[4] MPs are widely spread worldwide due to their low density, strong durability, and small size characteristics.[5] Polystyrene (PS) particle is a key element of primary microplastics. It is a stiff, lightweight, formless thermoplastic polymer written as $[CH_2CH(C_6H_5)]n$.[6] PS is dissolved in organic components and impervious to salts, alcohols, acids, etc.[4] PS can cause a threat to marine living organisms via oxidative stress, neurotoxicity, immunotoxicity, digestive gland disorders, reproductive disruption, and genotoxicity.[7,8] In humans, PS can cause endothelial dysfunction, vascular malformation, autophagy, and other health effects.[9]

Polystyrene microplastics (PS-MPs) cause numerous health issues, including interruption in energy metabolism (Figure 2).[10-12] A significant decrease in lactate dehydrogenase and succinate dehydrogenase functions was reported in mice testicular tissue, indicating deficient energy metabolism in sperm cells after PS-MP exposure.[11] Moreover, altered energy metabolism was reported in terms of increased expression of isocitrate dehydrogenase and lactate dehydrogenase in the muscular tissue of the European seabass. It decreased intestinal lactate production in the zebrafish.[10,13] Additionally, hepatic transcriptome analyses revealed that PS-MP inhibited the expression of genes of Glut2, hexokinase, glucokinase, pyruvate kinase, and PEPCKC involved in glycolysis/gluconeogenesis and decreased fatty acid oxidation in

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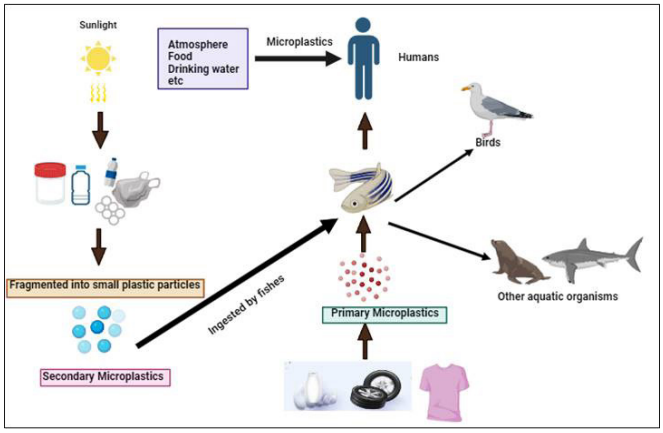


Figure 1: Distribution of microplastics in different organisms

mice and zebrafish.[12,14] PS-MPs induced hepatotoxicity and interrupted lipid metabolism are also evident in the human pluripotent stem cells derived liver organoids.[15] Moreover, LC-MS metabolomics, histopathological, and RNA sequence transcriptomic studies indicated a distinct metabolic interference of PS-MPs in rare minnow fish, suggesting that PS-MP exposure may encourage immune response and oxidative threat and may also disrupt energy and glycolipid metabolisms in that fish model.[16]

Other than the liver, the renal and muscular tissue metabolisms are also compromised by microplastic contamination. Further *in-vitro* studies in human kidney 2 cells as well as Hep G2 cells revealed that PS-MPs could inhibit gene expression of the glyceraldehyde 3-phosphate dehydrogenase (glycolytic enzyme), and antioxidant enzymes like catalase and superoxide dismutase 2, resulting in energy deficit as well as reactive oxygen species (ROS) generation.[17] Skeletal muscle regeneration and lipid deposition in the skeletal muscle were motivated by PS-MP exposure. Moreover, *in vitro* mechanistic research on C2C12 myoblast (skeletal muscle) revealed that PS-MPs caused inhibitory effect on myogenic differentiation by reducing p38 MAPK phosphorylation, promoting adipogenic differentiation by stimulating NF-κB expression and motivating ROS production.[18] The harmful effects of microplastics are mostly reported to disturb lipid and energy metabolisms in different tissue and animal systems.[19-22] However, there is a knowledge gap concerning comprehensive liver and cerebral cortex metabolic reactions triggered by polystyrene exposure. Moreover, the metabolic relationship of the mentioned energy-generating organs in connection with metabolic homeostasis remains unexplored.

Materials And Methods

Chemicals and Reagents

Polystyrene microplastics (1µm diameter) were purchased from Sigma-Aldrich Co., USA, Source BCCJ0518 (Cat no.89904-5ML-F). Other chemicals like diethyl ether, hydrochloric acid, H_2SO_4, acetic acid, ethanol, sodium carbonate, sodium potassium tartrate, sodium citrate, copper sulfate, KOH, ethanol, phenol, magnesium chloride ($MgCl_2$), sodium carbonate, and glucose 6-phosphate were purchased from Merck (India); bovine serum albumin (BSA) and dichlorophenolindophenol (DCPIP) were of analytical grade and procured from Sigma–Aldrich (India); sucrose and trichloroacetic acid (TCA) were purchased from SRL (India). Biochemical kits such as glucose estimation kits were purchased from Transasia Bio-Medicals Ltd, Mumbai, India. Ultrapure water from Millipore was used to prepare all reagents to avoid metal contamination.

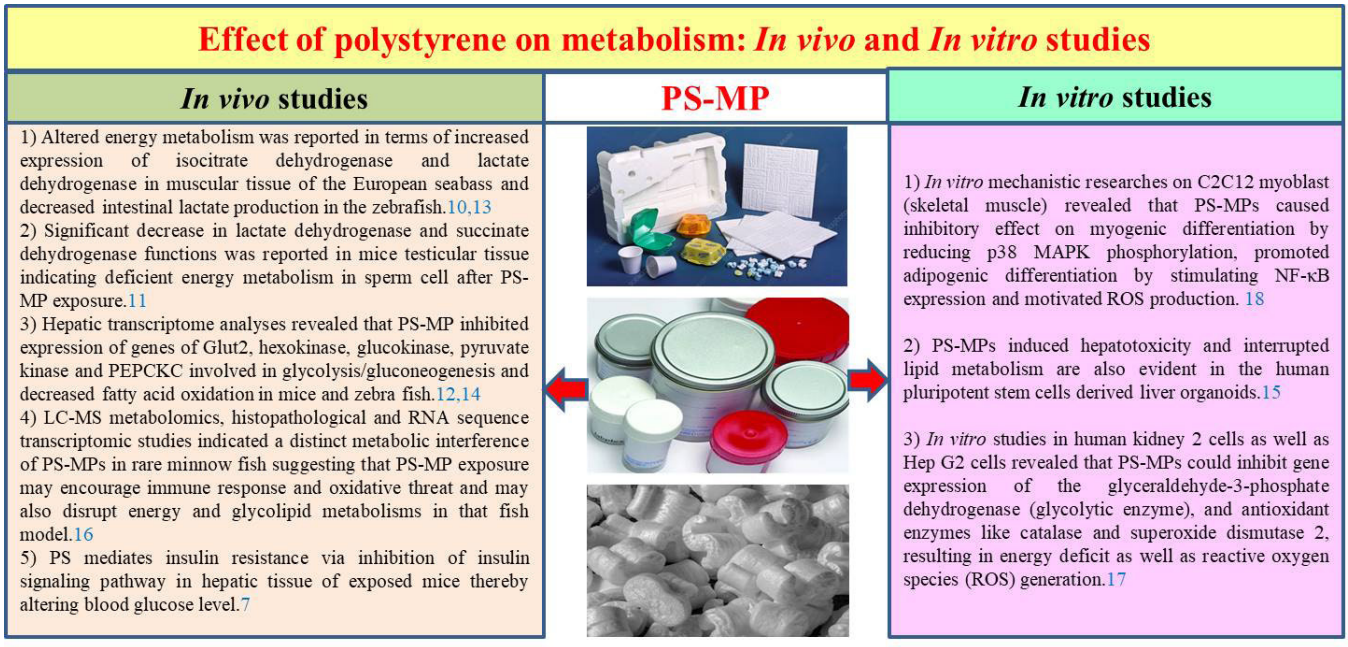


Figure 2: PS-mediated metabolic disintegration: *In-vivo* and *in-vitro* studies

Animal Experiments

A dose-dependent *in-vivo* study was carried out in male growing Wistar rats (six weeks of age) to evaluate the effect of sub-acute polystyrene microplastic (PS-MP) exposure on specific aspects of carbohydrate metabolism. Animals were procured, and experiments were conducted with clearance from the Institutional Animal Ethics Committee (IAEC) registered with the Committee for Control and Supervision of Experiments on Animals (CCSEA) approval no (Ref. No. TU/IAEC/2023/I/2-2 dated 11/12/2023). Twenty-four animals were procured from Chakraborty Enterprise, Kolkata (India), and divided into one control group and three PS-MP exposed groups, PM1, PM2, and PM3, with six animals in each group. The control group received drinking water only, while the exposed groups received polystyrene microplastic (1µm diameter) at graded doses of 0.5mg/L, 5mg/L, and 50mg/L via drinking water for four weeks. After sacrifice, blood was collected from a hepatic vein, and blood glucose was immediately measured. The liver and brain were collected, blotted dry, and stored at -20°C for biochemical analyses. Parameters of carbohydrate metabolism like blood glucose, liver glycogen, glycolytic intermediates, free amino nitrogen, glucose 6-phosphatase, lactate dehydrogenase, succinate dehydrogenase, and transaminase enzyme activities were evaluated by standardized biochemical methods.

Blood Glucose

Blood glucose was estimated by the Erba glucose Kit (Transasia BioMedicals Ltd, Mumbai, India) following glucose oxidase–peroxidase method.[23] In brief, 1 mL of the working reagent was mixed with 10 µL of the sample and incubated for 10 min at 37°C. Similarly, blank and standard solutions were prepared using 10 µL of distilled water and the same volume of standard solution containing 5.55 mmol/L of glucose, respectively. After incubation, the absorbance was taken at 500 nm in a UV-visible spectrophotometer. The blood glucose was expressed as mg/dL.

Liver Glycogen

Liver glycogen content was measured by the method of Montgomery.[24] Approximately 300 mg of hepatic tissue was mixed with 1 mL of 30% KOH solution, heated for 20 min in a boiling water bath, cooled at room temperature, and added with 1 mL of ethanol. The process was repeated and centrifuged at 3000 rpm for 10 minutes. The pellet was collected and washed twice with 1 mL of ethanol, and the resultant precipitate was used to measure glycogen content by mixing with 2.5 mL of concentrated H_2SO_4 and 0.5 mL of 80% phenol. Optical density was read in a UV-visible spectrophotometer at 420 nm. Tissue glycogen content was expressed as mg/100g of tissue.

Tissue Pyruvate Level

Pyruvic acid content was measured in hepatic and cerebral tissue by the method of Segal *et al*.[25] 0.5 mL of 5% tissue homogenate (0.1 M phosphate buffer, pH 7.4) was added with 5% TCA and centrifuged at 3000 r.p.m. for 10 min. The resultant supernatant was mixed with a definite volume of distilled water and 0.1% 2,4 dinitrophenylhydrazine (DNPH) and vigorously shaken for 3 min. Then 2.5 mL of toluene solution was added, mixed by handshaking for a few minutes, and kept standing for a while. The lower layer was collected and added with 10% Na_2CO_3 and 1.5 M NaOH to make the final reaction mixture. Optical density was recorded at 420 nm in a UV-visible spectrophotometer. The observed result was expressed as g/100g of tissue.

Tissue-free Amino Nitrogen

Tissue-free amino nitrogen was estimated by the method of Rosen.[26] The 5% tissue homogenate (in 0.1 M phosphate buffer, pH 7.4) was added with 10% Na-tungstate and 0.67 (N) H_2SO_4 to precipitate proteins, centrifuged to get the protein-free filtrate. The filtrate was treated with 3% ninhydrin solution and cyanide acetate buffer. After that, the solution was heated at 100°C for 5 minutes in a water bath and added with isopropanol immediately after cooling. A violet color was developed, optical density of which was measured in a spectrophotometer at 570 nm. The amino nitrogen content was expressed in terms of g of leucine per 100g of tissue.

Glutamate-Pyruvate Transaminase (GPT) and Glutamate-Oxaloacetate Transaminase (GOT) Activities

The transaminase enzyme activities were measured by the method of Reitman and Frankel using an assay kit (Coral clinical systems, Goa, India).[27] Values were expressed in terms of units/mg of protein.

Glucose 6 Phosphatase (G6PASE) Activity

To measure the G6PASE enzyme function, a tissue homogenate containing 52 mg of the tissue was mixed with 1.8 mL of substrate buffer (pH 6.5) containing 0.1-mmol/L tris-HCl, 0.1-mol/L EDTA, 0.05 mol/L glucose 6-phosphate. The mixture was incubated at 37°C for 10 minutes, followed by the addition of 1 mL ice-cold 10% TCA. It was centrifuged at 3,000 rpm for 10 minutes. The supernatant was taken to estimate the phosphate content according to the method of Plummer.[28] The data were recorded with a spectrophotometer at 880 nm. The unit of glucose 6-phosphatase was expressed as µg of phosphate liberated/min/g tissue protein.

Succinate Dehydrogenase (SDH) Activity

The hepatic SDH activity was determined by the method of Hollywood *et al*.[29] The assay mixture consisted of 1-mL phosphate buffer (pH 7.2) containing sodium succinate (0.15 M), azide (0.2 M) and the liver mitochondrial isolate. The activity of this enzyme was measured by adding DCPIP (6 mg/mL) as a coloring reagent, and finally, the optical density was recorded in a spectrophotometer at 600 nm. The result was expressed as µm of DCPIP reduced/min/mg of tissue protein.

Lactate Dehydrogenase Activity

To measure the LDH activity in the liver and cerebral cortex, the protocol of Bergmeyer *et al*.[30] was followed. 100 µL of tissue homogenate was mixed with 50 µL of reduced diphosphopyridinenucleotide (8×10^3 M β-DPNH) to measure the rate of consumption of pyruvate and DPNH in a total volume of 3 mL, made with phosphate–pyruvate solution (3.1×10^{-4} M pyruvate in 0.05 M phosphate buffer, pH 7.5). The change in optical density due to the oxidation of DNPH was taken for 5 min at 340 nm in a UV-visible spectrophotometer. The enzyme activity was expressed as µmoles/min/mg of protein.

Tissue Protein

Protein was measured using the method of Lowry *et al*. using BSA as a standard.[31]

Statistical Analysis

One-way ANOVA was performed using Microsoft Excel version 10 to compare the changes in biochemical responses between the control and the polystyrene-exposed groups of animals. 'Multiple comparison t-test' was used to differentiate between the mean values of two specific groups. $p < 0.05$ was considered as statistically significant.

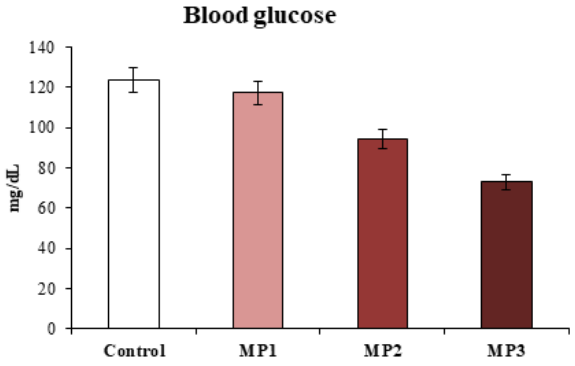
Result

Polystyrene exposure decreased the blood glucose level in Wistar rats in a dose-dependent fashion (Figure 3). The decrease was noted to be 5.1% ($p > 0.05$), 23.93% ($p < 0.01$), 40.95% ($p < 0.001$) in treated 1, 2 and 3, respectively.

Liver glycogen content was significantly decreased in PS-MP-exposed animals (Table 1). The percentage change was calculated as 21.1% ($p < 0.01$), 57.74% ($p < 0.001$), and 31.47% ($p < 0.001$) in the exposed PM1, PM2 and PM3 groups, respectively. Additionally, the hepatic pyruvic acid level was found to be significantly reduced by 48% ($p < 0.001$), 60% ($p < 0.001$), and 92% ($p < 0.001$) in PS-MP-exposed groups PM1, PM2 and PM3 correspondingly in comparison to the control group (Table 1). The result depicted in Table 1 further indicates 11.11% ($p < 0.01$), 27.8% ($p < 0.001$), and 16.7% ($p < 0.01$) decrease in the hepatic free amino nitrogen concentration in response to graded doses of PS-MP.

Additionally, data represented in Table 1 further reveal that PS-MP caused the most significant reduction of G6PASE function in hepatic tissue of rats exposed to 5mg/L of PS-MP (25.9% decrease, $p < 0.001$). The decrease in treated 1 and 3 groups was noted to be 7.73% ($p > 0.05$) and 8.65% ($p < 0.05$) in comparison to the control group. The GOT activity in PS-MP exposed liver was decreased in a dose-dependent fashion (Table 1). The percentage change was observed as 9.4% ($p < 0.05$), 18.1 ($p < 0.01$), and 22.2% ($p < 0.001$) in exposed PM1, PM2 and PM3 groups, respectively. Moreover, GPT activity declined remarkably in PS-MP exposed animals; the most significant change was noted in PM3 animals (15.6%, $p < 0.001$). Furthermore, the SDH function was stimulated in response to PS-MP exposure in a dose-dependent fashion. The increase was calculated as 1.4 fold, 3.3 folds, and five fold in PM1, PM2 and PM3 groups, respectively (Table 1).

In addition to change in hepatic tissue metabolism, polystyrene also caused marked alteration in cerebral tissue energy metabolism. Table 2 represents that PS-MP exposure



[Values are Means ± SEM. $p < 0.05$ was considered statistically significant]

Figure 3: Effect of graded PS-MP doses on rats' blood glucose level

Table 1: Effect of PS-MP on hepatic glycogen, pyruvic acid, free amino nitrogen, G-6-pase, GOT, GPT, and SDH activities in Wistar rats

A	*Groups of animals*	*Liver glycogen (mg/100 g tissue)*	*Pyruvic acid (g/100 g tissue)*	*Free amino nitrogen (g/100 g tissue)*	
	Control	10.01 ± 1.12	0.50 ± 0.04	0.18 ± 0.01	
	PM1	7.90 ± 0.67**	0.26 ± 0.02***	0.16 ± 0.01**	
	PM2	4.23 ± 0.54***	0.20 ± 0.02***	0.13 ± 0.01***	
	PM3	6.86 ± 0.84***	0.04 ± 0.01***	0.15 ± 0.01***	
B	*Groups of animals*	*G-6 pase activity (ng of Pi liberated /min/mg protein)*	*GOT activity (µg/ min/100 mg tissue)*	*GPT activity (µg/ min/100 mg tissue)*	*SDH activity (µmoles/ min/mg protein)*
	Control	144.83 ± 4.62	70.45 ± 1.51	40.50 ± 0.70	8.41 ± 1.17
	PM1	133.63 ± 3.18	63.81 ± 2.20*	36.50 ± 0.42***	12.17 ± 2.5
	PM2	107.35 ± 2.97***	57.90 ± 1.54**	35.33 ± 0.28***	27.93 ± 2.42**
	PM3	132.30 ± 11.39*	54.80 ± 2.00***	34.16 ± 0.54***	42.04 ± 6.02***

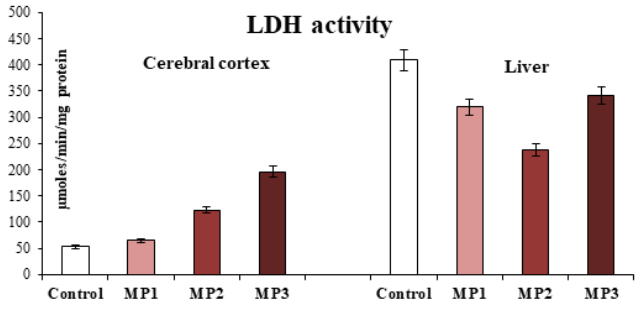
Values are means ± SEM.

Table 2: Effect of PS-MP on cerebral tissue pyruvic acid level, free amino nitrogen content, G-6-pase, GOT, and GPT activities

Groups of animals	*Pyruvic acid (mg/100g tissue)*	*Free amino nitrogen (mg/100g tissue)*	*G-6-pase activity (ng/min/mg protein)*	*GOT activity (µg/ min/100 mg tissue)*	*GPT activity (µg/ min/100 mg tissue)*
Control	448.54±24.41	79.40±3.52	9.43±0.42	15.9±0.7	23.85±0.79
PM1	230.48±17.89***	64.40±3.79**	10.14±0.71	17.08±0.58	16.93±1.11*
PM2	215.82±56.76***	81.92±2.65	12.98±1.2**	18.87±0.51*	26.95±0.93*
PM3	187.48±20.65***	95.65±2.09**	10.33±0.27*	18.61±0.75*	34.87±2.16*

dose-dependently decreased cerebral pyruvic acid level in the exposed rats. The percentage changes were noted as 48.6% ($p < 0.001$), 51.9% ($p < 0.001$) and 58.2% ($p < 0.001$) in treated 1, 2 and 3 groups individually. Additionally, free amino nitrogen content was declined in treated 1 group whereas mild increase was observed in treated 2 and 3 groups of rats (Table 2). The percentage change was calculated as 18.9%, 21% and 3.2% respectively. Glucose 6-phosphatase function in cerebral tissue was increased in PS-MP exposed animals, the most remarkable effect was observed in treated 2 group. The percentage changes were observed as 7.3% ($p > 0.05$), 37.7% ($p < 0.01$) and 8.6% ($p < 0.05$) in group 1, 2 and 3 respectively (Table 2). Additionally, the GOT function remained almost unchanged following exposure to a low dose of PS-MP, though a mild increase was noted in group 2 (18.7% increase) and group 3(17% increase) animals. On the other hand, the cerebral tissue GPT activity declined in low-dose exposed animals, whereas it increased in medium and high doses of PS-MP exposure (Table 2).

Moreover, the LDH function in the liver was found to be significantly declined in PS-MP exposed rats; the most significant effect was noted in the PM2 group (Figure 4). There was 21.9% ($p < 0.001$), 42.2% ($p < 0.001$) and 16.65% ($p < 0.001$) decrease in the mentioned parameter in PM1, PM2 and PM3 groups of animals. On the other hand, in the cerebral cortex of the PS-MP-treated experimental animals, the LDH function was stimulated in a dose-dependent manner. There was 1.2 fold ($p > 0.05$), 2.3 fold ($p < 0.05$), and 3.7 fold ($p < 0.05$) increase in cerebral LDH function in response to the mentioned graded doses of polystyrene as compared with the control group.



Each column represents values as Mean±SEM.

Figure 4: Effect of PS-MP on hepatic and cerebral lactate dehydrogenase function in Wistar rats

Discussion

Polystyrene Disrupted Glucose Homeostasis

Four-week oral exposure to polystyrene microplastic induces hypoglycemia in experimental animals in a dose-dependent fashion. The reason may be the change in food intake patterns in the exposed animals after PS exposure. Polystyrene may induce stress in animals by increasing cortisol release, as reported in larval zebrafish.[32] Due to stress, daily food intake and absorption by the exposed animals may be reduced, causing less gain in body weight and resulting in hypoglycemia. Additionally, it is evidenced that PS causes an increase in insulin levels in the blood, thus reducing the blood glucose level.[33] Another plausible mechanism may be polystyrene-mediated renal injury that may cause renal glycosuria and subsequent hypoglycemia.

Hepatic Energy Metabolism was Altered in Response to Varied Doses of Polystyrene Microplastic

Polystyrene exposure for 28 days significantly decreased hepatic glycogen content and pyruvic acid level in a dose-dependent manner. This may result from hypoglycemia-induced enhanced glycogenolysis and decreased glycolysis in that tissue. Energy scarcity in hepatic tissue thus may promote metabolic stress and subsequent alteration in TCA cycle enzyme function. Decreased glycolysis by polystyrene, as observed in the present study, is supported by the hepatic transcriptome analyses, which revealed that PS-MP could inhibit the expression of genes of Glut2, hexokinase, glucokinase, pyruvate kinase, and PEPCKC involved in glycolysis/gluconeogenesis in mice.[12,14] Altered energy metabolism in terms of reduced ATP production was reported earlier.[34] Additionally, the free amino acid nitrogen was decreased in the liver, suggesting the mobilization of free amino nitrogen from the liver to extrahepatic tissue in response to stress. As mentioned earlier, polystyrene may induce stress in animals by increasing cortisol release,[32] thus promoting the mobilization of free amino acids from the liver to circulation.

Both lactate dehydrogenase and glucose 6-phosphatase activities decreased in the liver after the treatment, indicative of retardation of glycolysis and gluconeogenesis after PS-MP exposure. The present finding conforms with the earlier observation of decreased LDH activity in mice testicular tissue, indicating deficient energy metabolism in sperm cells after PS-MP exposure.[11] Moreover, diminished activity

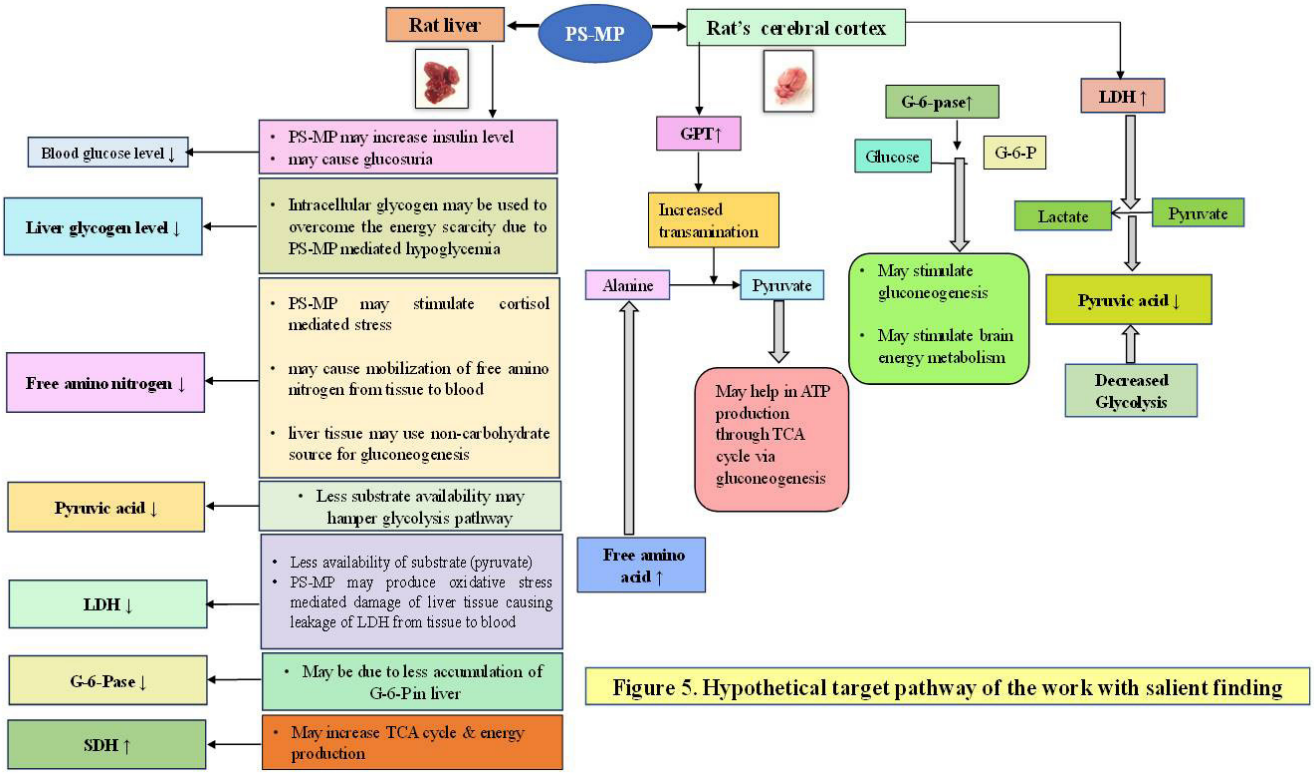


Figure 5. Hypothetical target pathway of the work with salient finding

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of G6PASE may be associated with less availability of glucose 6-phosphate in the hepatic tissue of PS-MP exposed animals. Suppressed G6PASE function is another causative factor for PS-MP-mediated hypoglycemia (Figure 5).

Decreased transaminase function in hepatic tissue may result from leakage of the enzymes from the damaged liver to blood due to PS-MP-mediated metabolic stress. PS-MP exposure at higher doses stimulates succinate dehydrogenase activity in the hepatic tissue of rats, suggesting enhancement of the TCA cycle function, trying to maintain energy level in the hypoglycaemic situation as a compensatory mechanism for the survival of the hepatic tissue. Altered energy metabolism was reported regarding increased expression of isocitrate dehydrogenase and lactate dehydrogenase in the liver and other tissue (muscle) of the European seabass and decreased intestinal lactate production in the zebrafish.[10,13]

Cerebral Tissue Energy Metabolism was Compromised by Sub-Acute Polystyrene Exposure

A dose-dependent decrease in pyruvate content in the cerebral cortex of Wistar rats was associated with increased LDH activity in PS-MP-exposed animals. As hypoglycemia is induced by PS-MP exposure in the present study, the resulting decrease in glycolysis was noted in the cerebral tissue of rats. Increased LDH function in cerebral tissue indicates anaerobic metabolism and energy scarcity. This conforms to earlier studies of Deng *et al*., who reported that PS-MP stimulates liver LDH function in mice.[34] TCA cycle function was compromised, resulting in less energy production. Defective energy production (decrease in ATP content and inhibition of ATP-associated gene expression) by polystyrene nanoplastics (50 nm diameter) exposure for 28 days at repeated doses was evidenced to induce Parkinson's disease-like neurodegeneration in mice.[35] Increased glucose 6-phosphatase function in the cerebral cortex indicates enhanced gluconeogenesis in that tissue trying to maintain glucose homeostasis in the hypoglycaemic situation as a metabolic adjustment (Figure 5).

Additionally, free amino nitrogen decreased at low doses of PS-MP exposure, and it might be utilized as an alternate source of the gluconeogenic substrate to provide energy. On the other hand, increased free amino nitrogen content at high doses in the cerebral cortex is indicative of enhanced amino acid mobilization via circulation due to polystyrene-mediated stress in animals or due to metabolic conversion of keto acids to amino acids by promoting transaminase function. However, there was mild increase in the glutamate-oxaloacetate transaminase function in moderate to high doses of PS-MP exposure indicating motivation of transamination process in polystyrene exposed cerebral cortex. On the other hand, the glutamate-pyruvate transaminase activity was decreased at a low dose of PS-MP exposure whereas increased at the highest dose of exposure. Change in GPT activity in rat cerebral cortex by PS-MP follows the same changing pattern as that of free amino nitrogen suggesting that substrate availability plays a key regulatory role to alter the enzyme function.

Conclusion

Polystyrene is one of the most common forms of microplastics, having the most adverse effects on marine living organisms. Humans are also getting microplastics in their bodies via environmental and occupational exposures. Most *in vitro* studies were carried out in mammalian cell lines, and *in vivo* effects were studied in marine creatures and murine models. Metabolic disintegration is one of the major causes of organ dysfunction. Accordingly, the impact of sub-acute exposure to three different doses of polystyrene is studied in terms of metabolic dysfunctions in discrete organs like the liver and brain in Wistar rats. Polystyrene disturbs glucose homeostasis by inducing hypoglycemia and modulating glycolytic and TCA cycle functions in a dose-dependent and tissue-specific manner. Gluconeogenesis is also affected differentially by metabolic adjustment in the studied organs. It is thus concluded that polystyrene contamination in mammals is detrimental in terms of metabolic perturbation, which may contribute to organ malfunctions if unlimited exposure to microplastics is not prohibited.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.