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Effects of Combined Arsenic and Polystyrene Microplastics on Epididymal Sperm Quality in Wistar Rats

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Abstract **Background:** Arsenic contamination and increasing environmental exposure to microplastics have emerged as important public health concerns due to their potential adverse effects on reproductive health. While both toxicants have been independently associated with male reproductive dysfunction, evidence concerning their combined impact on sperm quality remains inadequate. **Objective:** To investigate the effects of arsenic and polystyrene microplastics, alone and in combination, on epididymal sperm characteristics in adult Wistar rats and to evaluate the potential protective effect of imperatorin. **Methods:** Thirty adult male Wistar rats were randomly assigned to five groups (n = 6): G1 (control), G2-(arsenic), G3 (Microplastics), G4 (arsenic + Microplastics) and G5 (arsenic + Microplastics + imperatorin Epididymal sperm concentration, motility and morphology were measured after 28 days of exposure. Statistics were evaluated using the Kruskal-Wallis test, with $p<0.05$ considered significant. **Results:** Individual exposure to arsenic or polystyrene microplastics adversely affected epididymal sperm parameters compared with the control group. The combined exposure caused the most harm, as sperm concentration, motility and morphologically normal sperm decreased. Administration of imperatorin attenuated these detrimental effects, leading to a partial improvement in sperm quality when compared with the combined exposure group; however, the measured parameters remained below those observed in the control animals. **Conclusion:** Co-exposure to arsenic and polystyrene microplastics produced more severe adverse effects on epididymal sperm quality than individual exposures, indicating increased reproductive toxicity. Imperatorin partially protected against these alterations, indicating its potential as a treatment for environmental pollutant-induced male reproductive dysfunction.

Key Words Environmental Toxicology, Oxidative Stress, Reproductive Toxicity, Animal Model, Experimental Study

INTRODUCTION

Male reproductive health is a growing concern worldwide as there is increasing evidence that environmental pollutants have been significant contributors to declining semen quality and fertility potential [1]. Among the various contaminants in the environment, arsenic and microplastics because of their widespread distribution and potential toxic effects on biological systems have significant consideration [2]. Prolonged exposure to these pollutants through food, water and air has raised concerns regarding their effect on reproductive function and long-term health outcomes [3,4]. Arsenic is a metalloid found in groundwater, soil and industrial waste. Water and food contamination cause most human and animal exposure [5]. Chronic arsenic exposure has been associated with several adverse health effects, including carcinogenesis, neurotoxicity, cardiovascular disorders and reproductive dysfunction [6]. Experimental studies have demonstrated that arsenic can impair spermatogenesis, alter endocrine function, induce oxidative stress and reduce sperm quality [7]. The testes are particularly vulnerable to arsenic-induced damage because germ cells possess high metabolic activity and are highly sensitive to oxidative injury [8]. Excessive production of reactive oxygen species can disrupt cellular homeostasis, leading to membrane damage, DNA fragmentation and apoptosis of developing germ cells [9].

Microplastics have recently emerged as an important environmental contaminant. These particles, generally smaller than 5 mm in diameter, originate from the degradation of larger plastic materials or are manufactured directly for industrial and commercial applications [10]. Due to their persistence in the environment, microplastics found in drinking water, food products, marine organisms and even human biological samples [11]. Increasing evidence suggests that microplastics can cross biological barriers and accumulate in different organs, including reproductive tissues [12]. Experimental studies have reported that exposure to polystyrene microplastics may impair sperm production, reduce sperm motility, induce oxidative stress and disrupt normal testicular architecture [13].

Recent evidence indicates that microplastics can act as carriers of environmental pollutants in addition to being contaminants themselves. Because of their large surface area, they readily adsorb heavy metals and various chemical contaminants, which may increase the uptake and toxicity of these substances in living organisms [14,15]. Therefore, exposure to microplastics together with toxic metals may result in greater biological damage than exposure to either contaminant alone [16]. Although the individual reproductive effects of arsenic and microplastics have been extensively studied, relatively few investigations have examined their combined impact on epididymal sperm characteristics and male reproductive function.

Sperm quality is widely recognized as an important indicator of male reproductive health and fertility potential [17]. Sperm concentration, motility and morphology are among the most informative indicators of fertility and are widely used to assess the normal progression of spermatogenesis. Disturbances in these parameters may indicate impaired testicular or epididymal function resulting from cellular or molecular damage [18,19]. Therefore, examining epididymal sperm characteristics provides a sensitive and reliable means of determining the effects of environmental toxicants on the male reproductive system [20].

Imperatorin, a naturally occurring furanocoumarin isolated from several medicinal plants, possesses antioxidant, anti-inflammatory and cytoprotective properties [21]. Experimental studies have demonstrated its ability to attenuate oxidative stress-induced tissue injury and strengthen cellular defence systems [22]. Despite these promising pharmacological properties, its protective potential against male reproductive toxicity resulting from combined exposure to arsenic and microplastics remains largely unexplored.

This study aimed to investigate the effects of arsenic and polystyrene microplastics, administered individually or in combination, on epididymal sperm characteristics in Wistar rats. It also evaluated the potential protective effect of imperatorin against reproductive damage induced by combined exposure. The findings are expected to enhance the current understanding of the reproductive toxicity associated with co-exposure to environmental pollutants and provide preliminary evidence supporting the therapeutic potential of imperatorin in preventing pollutant-induced sperm damage.

Objective

To investigate the effects of arsenic and polystyrene microplastics, alone and in combination, on epididymal sperm characteristics in adult Wistar rats and to evaluate the potential protective effect of imperatorin.

METHODS

Study Design

This preliminary experimental study designed to study the impact of arsenic and polystyrene microplastics, administered individually or in combination, on epididymal sperm characteristics in adult male Wistar rats. The study also evaluated the protective potential of imperatorin against the reproductive toxicity associated with combined exposure.

Experimental Animals

The study used 30 healthy adult male Wistar rats weighing 180-220 g and aged 8-10 weeks. This experiment included animals from a CPCSEA-approved facility that were acclimatized for two weeks. Standard laboratory settings were maintained for rats, including a 22±2°C temperature, 50-60% relative humidity and a 12-hour light/dark cycle. Standard pellet feed and water were available ad libitum during the research.

Ethical Approval

Institutional Animal Ethics Committee (IAEC), Saveetha Medical College and Hospital, SIMATS, Chennai, India, accepted the experimental protocol. All protocols followed the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Chemicals

Sodium arsenite ($NaAsO_2$), polystyrene microplastics (10 µm particle size) and imperatorin (purity ≥98%) were procured from standard commercial suppliers. All chemicals and reagents used in the study were of analytical grade.

Experimental Design

The animals were randomly divided into five groups comprising six rats per group:

- **Group I (Control):** Received normal drinking water and standard diet
- **Group II (Arsenic):** Received sodium arsenite in drinking water
- **Group III (Microplastics):** Received polystyrene microplastics
- **Group IV (Arsenic + Microplastics):** Received both sodium arsenite and polystyrene microplastics
- **Group V (Arsenic + Microplastics + Imperatorin):** Received arsenic and microplastics along with imperatorin treatment

Sodium arsenite was administered through drinking water at a concentration of 10 mg/L for 28 consecutive days [23]. Polystyrene microplastics were administered orally by gavage at a dose of 0.1 mg/kg body weight/day [24]. Imperatorin was administered orally at a dose of 1 mg/kg body weight/day throughout the treatment period [25].

Collection of Epididymal Sperm

At the end of the experimental period, the animals were euthanized under appropriate anaesthesia and the cauda epididymis was carefully excised. The tissue was transferred into physiological saline, where small incisions were made to facilitate the release of spermatozoa. The resulting sperm suspension was gently mixed to ensure a uniform sample for subsequent evaluation.

Assessment of Sperm Concentration

The epididymal sperm suspension was diluted and analysed using a Neubauer haemocytometer. Sperm cells were counted under a light microscope and sperm concentration was expressed as million spermatozoa per millilitre of suspension.

Assessment of Sperm Motility

A drop of freshly prepared epididymal sperm suspension was placed on a clean glass slide and examined immediately under a light microscope. At least 200 spermatozoa were assessed per sample and motility was classified as progressive, non-progressive, or non-motile, with results expressed as percentages.

Assessment of Sperm Morphology

Sperm morphology was assessed using stained sperm smears prepared on clean glass slides. A minimum of 200 spermatozoa from each sample were examined under oil immersion microscopy. Sperm exhibiting normal head, midpiece and tail structures were recorded as morphologically normal, whereas those showing structural defects in any of these regions were classified as abnormal. The proportion of normal and abnormal spermatozoa was expressed as a percentage.

Statistical Analysis

IBM SPSS 29.0 was used for statistical analysis. Shown as mean ± Standard Deviation (SD). The Shapiro-Wilk test determined data normality. Kruskal-Wallis tested experimental group differences. A p-value <0.05 indicated statistical significance.

RESULTS

Sperm Concentration

Epididymal sperm concentration differed among the experimental groups. Animals exposed to arsenic or polystyrene microplastics individually showed lower sperm concentrations than the control group, with the greatest reduction observed following combined exposure. Imperatorin treatment resulted in a partial recovery of sperm concentration compared with the combined exposure group, although values remained below those of the control animals. Pairwise comparison showed a significant difference between the control and combined exposure groups ($p = 0.045$).

Sperm Motility

Progressive sperm motility was reduced in all exposure groups compared with the control group, with the greatest decline observed following exposure to polystyrene microplastics alone and in combination with arsenic. Treatment with imperatorin partially restored progressive motility relative to the combined exposure group. In contrast, non-progressive sperm motility increased following exposure to arsenic and polystyrene microplastics, with the highest proportion observed in the microplastic-exposed group. Imperatorin treatment was associated with a reduction in non-progressive motility compared with the combined exposure group.

Sperm Morphology

Exposure to arsenic and polystyrene microplastics was associated with a reduction in the proportion of morphologically normal spermatozoa compared with the control group. The greatest decrease in normal sperm morphology was observed following combined exposure, whereas imperatorin treatment resulted in a partial restoration of normal sperm morphology. On the other hand, the proportion of morphologically abnormal sperm increased across the exposure groups, with the highest incidence recorded in the combined exposure group. Pairwise comparison demonstrated a significant difference between the control and combined exposure groups ($p = 0.010$) (Table 1, Figure 1).

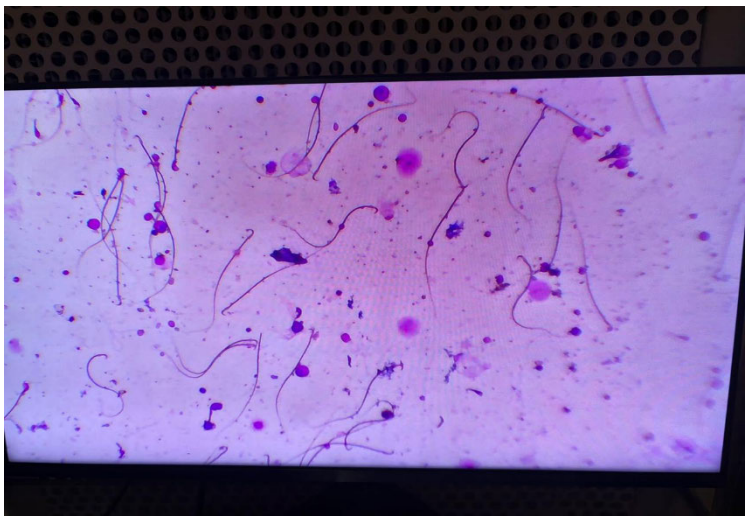

Figure 1: Representative Photomicrograph of Epididymal Spermatozoa used for Morphological Assessment

Table 1: Effects of Arsenic, Polystyrene Microplastics and Imperatorin on Epididymal Sperm Concentration, Progressive Motility, and Normal Sperm Morphology in Wistar Rats

Group	Treatment	Sperm concentration (million/mL)	Progressive motility (%)	Normal morphology (%)
G1	Control	18.33±7.23	84.00±2.00	95.00±1.00
G2	Arsenic	13.33±4.72	47.67±2.52	60.00±2.00
G3	Polystyrene microplastics	10.00±1.00	44.00±2.00	55.00±3.00
G4	Arsenic + Polystyrene microplastics	5.00±1.00	10.00±2.00	22.33±2.52
G5	Arsenic + Polystyrene microplastics + Imperatorin	7.00±2.00	65.00±3.00	72.33±2.51

Values are Presented as Mean±SD (n = 6 animals per group). Progressive Motility and Morphology were Assessed using Cauda Epididymal Sperm Collected after 28 days of Treatment

The combined exposure group exhibited the greatest impairment across all evaluated sperm parameters, including sperm concentration, progressive motility and morphology. In comparison, animals receiving imperatorin demonstrated improved sperm quality relative to the combined exposure group, although the values remained below those of the control group.

DISCUSSION

This study evaluated the effects of individual and combined exposure to arsenic and polystyrene microplastics on epididymal sperm characteristics in adult Wistar rats and examined the potential protective effect of imperatorin. Exposure to either toxicant reduced sperm concentration, progressive motility and normal morphology, while the combined exposure produced the greatest impairment across all evaluated parameters. Imperatorin treatment improved these sperm characteristics compared with the combined exposure group, although complete recovery was not observed. These findings suggest that imperatorin may help reduce reproductive damage associated with co-exposure to arsenic and polystyrene microplastics.

Sperm concentration is considered one of the most reliable indicators of normal spermatogenesis and male reproductive capacity, reflecting the overall functional status of the testes [17,20]. In the present study, arsenic exposure reduced epididymal sperm concentration compared with the control group, which is consistent with previous reports demonstrating the adverse effects of arsenic on male reproductive function [7,9]. The decline in sperm concentration has been attributed to arsenic-induced oxidative stress, mitochondrial dysfunction and apoptosis of developing germ cells, all of which can impair spermatogenesis and reduce sperm production [7,8]. Arsenic has also been shown to disturb the balance between oxidants and antioxidants within the testes, leading to oxidative damage of the seminiferous epithelium and impaired spermatogenic activity [7,8]. The decrease in sperm concentration observed in this study is therefore likely to be associated with these pathological changes.

Exposure to polystyrene microplastics alone also decreased sperm concentration and other sperm quality parameters [13]. Increasing evidence indicates that microplastics can penetrate biological barriers, accumulate within reproductive tissues and initiate oxidative stress and inflammatory responses that interfere with normal spermatogenesis [12]. Similar alterations in sperm count, motility and fertility have been documented in experimental models exposed to polystyrene microplastics, supporting the observations of the present investigation [13,24]. Considering the widespread environmental distribution of microplastics, these findings further emphasize their potential role as emerging reproductive toxicants.

The outcome from the current investigation showed deterioration of sperm quality followed by the simultaneous exposure to arsenic and polystyrene microplastics. Animals in the co-exposure group resulted in significantly lower sperm concentration, progressive motility and normal morphology than those receiving either toxicant alone, indicating that combined exposure produced more severe reproductive impairment thus the results suggests enhanced toxicity during co-exposure [15,16]. Microplastics possess physicochemical properties that enable adsorption of metals and metalloids onto their surface, thereby facilitating transport, tissue retention and polonged cellular exposure to environmental contaminants [14,15]. Co-exposure may therefore amplify oxidative stresrs and cellular injury within reproductive tissues beyond that produced by individual toxicants [15,16]. Similar findings have been observed in microplastic cumulative toxicity investigations with arsenic, supporting the biological plausibility of the present findings [26].

Sperm motility is one of the key determinants of male fertility, as the ability of spermatozoa to move progressively is essential for successful fertilization [18,19]. In the present study, both arsenic and polystyrene microplastic exposure resulted in reduced progressive sperm motility, with the most pronounced impairment observed in the combined exposure group. This decline may be associated with oxidative stress, which damages the sperm plasma membrane and disrupts mitochondrial function, leading to reduced ATP production and impaired flagellar movement [26]. The observed reduction in sperm motility is in agreement with previous studies reporting impaired sperm function following arsenic exposure and oxidative damage to the male reproductive system [7,9,20].

Evaluation of sperm morphology is widely used to assess the quality of spermatogenesis and the reproductive competence of spermatozoa [17,20]. In the present study, exposure to arsenic and polystyrene microplastics was associated with a progressive decline in the proportion of morphologically normal sperm, while the frequency of abnormal forms increased, with the most pronounced changes observed following combined exposure. Structural defects involving the sperm head, midpiece, or tail are often indicative of disrupted spermatogenesis or impaired epididymal maturation [20]. Previous studies have shown that damage to Sertoli cells and developing germ cells can

interfere with normal sperm differentiation, leading to morphological abnormalities and reduced reproductive capacity [8]. The marked increase in abnormal sperm morphology observed in the co-exposure group suggests that simultaneous exposure to arsenic and polystyrene microplastics has a greater detrimental effect on sperm development and maturation than either toxicant alone.

Treatment with imperatorin improved sperm concentration, progressive motility and normal morphology when compared with the untreated co-exposure group, although complete restoration to control values was not achieved. The beneficial effects of imperatorin may be attributed to its well-documented anti-inflammatory properties, which have been demonstrated in a range of experimental studies [21,22]. While the molecular mechanisms were not examined in the present investigation, the observed improvement in sperm quality suggests that imperatorin may help limit oxidative stress, maintain cellular integrity and reduce reproductive tissue damage caused by combined exposure to arsenic and polystyrene microplastics. These findings are consistent with earlier studies highlighting the cytoprotective potential of imperatorin and support its further evaluation as a promising therapeutic candidate for protecting against environmentally induced male reproductive toxicity [22].

The findings of this study provide preliminary evidence that combined exposure to arsenic and polystyrene microplastics adversely affects epididymal sperm quality, while imperatorin may offer partial protection against these changes. Further investigations integrating biochemical, molecular and histopathological approaches, together with functional fertility assessments, will contribute to a more comprehensive understanding of the underlying mechanisms and the protective role of imperatorin.

The present findings demonstrate that combined exposure to arsenic and polystyrene microplastics has a greater adverse effect on epididymal sperm quality than either toxicant alone. Partial restoration of sperm parameters following imperatorin treatment suggests its potential to mitigate reproductive damage associated with combined environmental toxicant exposure. Considering the widespread occurrence of arsenic contamination and microplastic pollution, further studies Further investigations integrating mechanistic and functional reproductive endpoints are needed to better understand the mechanisms underlying their combined toxicity and to further evaluate the therapeutic potential of imperatorin.

CONCLUSION

This study shows that exposure to arsenic and polystyrene microplastics adversely affects epididymal sperm quality in Wistar rats, with combined exposure producing greater impairment than either toxicant alone. Co-exposure was associated with marked reductions in sperm concentration, progressive motility and normal sperm morphology, indicating enhanced reproductive toxicity. Treatment with imperatorin partially improved these sperm parameters, suggesting its potential to mitigate reproductive damage induced by combined environmental contaminants. These findings highlight the reproductive risks associated with concurrent exposure to arsenic and microplastics and provide preliminary evidence supporting the protective role of imperatorin.

Limitations

This study was limited to adult Wistar rats, which may restrict the direct applicability of the findings to humans. Only epididymal sperm parameters were evaluated, while reproductive hormones, oxidative stress markers, histopathology and fertility outcomes were not assessed. Additionally, the study used a single dose and exposure duration, limiting the evaluation of dose-dependent and long-term effects of arsenic, polystyrene microplastics and imperatorin.

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