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RESEARCH ARTICLE

Ameliorative effects of Banana (*Musa acuminata*) on nephrotoxicity and neurotoxicity experimentally induced by Magnesium Oxide Nanoparticles in Rohu (*Labeo rohita*)

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ABSTRACT

The increasing use of magnesium oxide nanoparticles (MgO NPs) in various industries has raised significant concerns about their detrimental effects via oxidative stress and histopathological damage in aquatic species. This study evaluated the protective potential of banana (*Musa acuminata*) pulp and peel against MgO NPs-induced toxicity in Rohu (*Labeo rohita*). Fish were divided into seven groups: a control group, three groups (T1-T3) treated with varying doses of MgO NPs (100, 200, and 400mg/kg feed), and three groups (T4-T6) treated with MgO NPs and banana pulp. Visceral organs (kidneys and brain) of all the experimental fish were removed and processed for inquisition of oxidative stress and antioxidant enzymes at days 7, 14, 21 and 28th of trial. Fish exposed to MgO NPs (T1-T3) exhibited significant increase ($p < 0.05$) in oxidative stress biomarkers like reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), while significantly lower contents of antioxidant enzyme including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were recorded. Histopathological analysis revealed severe damage in kidneys (renal atrophy, glomerular deterioration, necrosis of renal tubular epithelial cells) and brain (neuronal degeneration, atrophy of neuron, eccentric neurons and necrosis of neurons) of MgO NPs treated fish. Conversely, fish reared in groups (T4-T6) supplemented with mixture of banana pulp and peel noticeably indicated improvement in the contents of antioxidant enzymes, reduced oxidative stress and lowered severity of tissue damage demonstrating the efficacy of banana derived compounds in alleviating MgO NPs-induced toxicity. This study underscores the potential of banana-based feed additives as a natural and sustainable solution to enhance the health and resilience of aquatic species in different environmental contaminated ecosystems.

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INTRODUCTION

Aquaculture plays a pivotal role in global food security by serving as a primary source of proteins supporting nutrition and contributing to health and well-being of human population (Hussain *et al.,* 2025). It addresses malnutrition challenges by fulfilling the growing demand for high-quality and nutrient-rich food (Vijayaram *et al.,* 2023; Nwafili *et al.,* 2023). Among all fish species, Rohu (*Labeo rohita*) is known as the major organism of aquaculture due to high nutritional value and a source of vital proteins. Antioxidants and bioactive compounds play important role by providing protective effects like anti-inflammatory, hepatoprotective responses and cardioprotective potential (Chen *et al.,* 2022;

Holman *et al.,* 2023; Elbehary *et al.,* 2023). However, the intensification of aquaculture practices has raised numerous problems of contamination of the environment with harmful toxins which threatens sustainability and health of aquatic species (Malik *et al.,* 2022; Hamza *et al.,* 2023; Maqsood *et al.,* 2023).

Contamination due to release of variety of frequently employed natural and synthetic compounds including nanoparticles (NPs) by industrial and agricultural activities is one of the major environmental concerns of aquaculture (Liu *et al.,* 2021; Ahmad *et al.,* 2025; Hussain *et al.,* 2025). The frequent and persistent use of magnesium oxide nanoparticles (MgO NPs) in many industries like electronics, water treatment and pharmaceuticals are increasingly poisoning aquatic ecosystems (Ali *et al.,* 2023; Hussain *et al.,* 2025). Over release of nanoparticles into water bodies and environment causes oxidative stress in different animals (Iqbal *et al.,* 2024; Younas *et al.,* 2024). It is speculated that abrupt and rapid generation of reactive oxygen species (ROS) due to environmental pollutants causes cellular damage and disruption in DNA resulting in compromised metabolic processes in exposed species (Gul *et al.,* 2017; Afzal *et al.,* 2022; Shafqat *et al.,* 2024). Moreover, MgO NPs have the potential to easily penetrate due to their nanoscale size in aquatic organisms via ingestion or absorption from gills and can accumulate in muscles, liver, brain and kidneys (Tortella *et al.,* 2020; Sajjad *et al.,* 2023; Kanwal *et al.,* 2024).

Natural antioxidants have been explored to minimize the toxicological effects of nanoparticles (Salman *et al.,* 2022; Anjum *et al.,* 2023). Different natural compounds with potential antioxidant properties like beta carotene, ascorbic acid, phenolic acids, dopamine and tocopherol are obtained from *Musaceae* (banana) family (*Musa acuminata*). These compounds are bioactive in nature and are known to 'neutralize' oxidative stress, enhance the immune response and prevent cellular damage (Someya *et al.,* 2002; Qusti *et al.,* 2010). Significant biological activities (anti-inflammatory, antitumor and anti-ulcer effects) were observed due to both banana pulp and peel during hypertension and cholesterol imbalance (Kanazawa and Sakakibara, 2000).

It is recorded that banana-based diet enhances the growth, health, and antioxidant profile of *Labeo rohita* fish along with different other aquatic species. The dried banana (*Musa acuminata*) pulp and peel contains high antioxidant capacity with high total flavonoid (3.05±0.12 mg/g), total phenolic (89.75±2.17mg/g) and DPPH value (83.95±2.55ug/ml) (Benzie and Strain, 1999). Studies have shown that the addition of banana ingredients in fish feed boosts disease resistance as well as provides physiologic natural defense against marine stressors (Giri *et al.,* 2016). Keeping in view the increasing concerns regarding the nanoparticle toxicity (Shahid *et al.,* 2023; Aslam *et al.,* 2023; El-Hamaky *et al.,* 2023), this study aims to contribute to the major efforts in making aquaculture practices more sustainable and promoting public health by reducing nanoparticle contamination in aquatic ecosystems using banana derived natural antioxidants.

MATERIALS AND METHODS

***Musa acuminata* pulps and peels collection and phytochemical analysis:** *Musa acuminata* was purchased from local fruit market and phytochemical analysis were carried out at the Institute of Botany, Bahauddin Zakariya University, Multan, Pakistan. After washing, bananas were separated into pulp and peel and were cut into small pieces (5cm^2 thickness) using a knife. Unpigmented and natural fresh yellow colored ripened banana pulp and peel were dried in shade for 2weeks. The dried pulps and peel were grinded separately in the electric juicer until powder formation. The banana pulps and peel powder were mixed in commercial fish feed at ratio 1:3. Phytochemical characteristics of banana pulps and peel were determined by standard techniques, AACC (2000). The phytochemical analysis of Banana pulps and peel exhibited high moisture contents (67.09%), proteins (3.03%), carbohydrates (12.59%), fibers (07.60%), fats (0.50%) and ash contents (04.18%). The phytochemical analysis indicated presence of different minerals contents such as phosphorus (31200ppm), potassium (312ppm), calcium (0.004ppm), magnesium (20.05ppm), iron (0.011ppm) and zinc (0.013ppm). (Moreno *et al.,* 2006). Quantitative analysis and radical scavenging assay were executed to determine the total phenolic and flavonoid contents.

Experimental protocol and management of fish: Various concentrations of previously synthesized and characterized MgO NPs (100, 200 and 400mg/kg feed) were utilized throughout the experiment (Hussain *et al.,* 2025). A total of 150 Rohu (*Labeo rohita*) fish were purchased and shifted to the laboratory in oxygenated plastic bags. All the fish were reared in cemented tanks, each with a capacity of 200liters of water at Bahauddin Zakariya University, Multan, Pakistan. Fish were randomly divided into seven groups as T0 (control), T1 (MgO NPs; 100mg/kg), T2 (MgO NPs; 200mg/kg), T3 (MgO NPs; 400mg/kg), T4 (MgO NPs 100mg/kg + 1/3 banana pulps and peel of commercial diet), T5 (MgO NPs 200mg/kg +1/3 banana pulps and peels of commercial diet), T6 (MgO NPs 400mg/kg + 1/3 banana pulps and peel of commercial diet). All the fish were acclimatized prior to conduct the experiment and fed with commercial diet for 28 days. Prior to administration, careful mixing of feed ingredients was carried out to feed fish twice daily at 3% of body weight during experimental period.

Analysis of oxidative stress and antioxidant enzymes: At days (7, 14, 21 and 28th) of experimental research, fish specimens were randomly picked and dissected for enzymatic investigation. After removing and the washing in chilled solution, kidneys and brain (visceral organs) were refrigerated (-20ºC) for ROS estimation (Hayashi and Su, 2007), GSH (Jollow *et al.,* 1974) and (Iqbal *et al.,* 1996). Kidneys and brain of treated fish were evaluated for various antioxidant enzymes that included SOD (Kakkar *et al.,* 1984; Wang *et al.,* 2022), POD (Chance and Maehly, 1955) and CAT (Chance and Maehly, 1955).

Histopathological examination: Kidneys and brain were removed from treated fish at the necropsy level for histopathological examination at day 7, 14, 21 and 28th of experiment and were fixed immediately in 10% solution of formaldehyde (Ghaffar *et al.,* 2019. Prior to necropsy, each fish was anaesthetized individually with 5.0mg/L of clove oil. (Ghaffar *et al.*, 2021). Both absolute and relative weight of brain and kidneys were also recorded. The collected tissues were dehydrated in alcoholic ascending grades after fixation and were cleared in xylene. Sections were cut by using automated microtome and then Eosin and Hematoxylin stains were used for staining (Hussain *et al.*, 2019; Hussain *et al.*, 2020). All the prepared sections were observed under light microscope (Nikon Eclipse 80i, Nikon Co., and Tokyo, Japan).

Statistical analysis: All the data regarding absolute and relative weight, oxidative stress and antioxidant enzymes were analyzed using ANOVA by IBM statistical software (SPSS). All collected data (means ± SE) of various parameters was compared by Tukey's test ($p<0.05$ significance level).

RESULTS

Absolute and relative weight of tissues: MgO nanoparticle dietary supplementation resulted in significant changes in absolute and relative weight of brain and kidneys (Figure 1). Absolute, and relative weight of brain showed significant increase at higher doses of nanoparticles (200 and 400mg/kg) implying a dose- and time dependent response. Results indicated a significant increase in both the absolute and relative weight of kidneys at day 28 of experiment in fish treated with nanoparticles (400mg/kg diet). The results exhibited that the supplementation of banana pulp and peel in fish diet noticeably ameliorated the adverse impacts of nanoparticle toxicity.

Analysis of antioxidant enzymes: In kidney tissues, the contents of superoxide dismutase (SOD) were non-significantly ($p > 0.05$) reduced in fish of group T1 (100mg/kg MgO NPs) at all sampling intervals (7, 14, 21 and 28th days). The SOD contents were significantly ($p<0.05$) decreased in fish reared in groups T2 (200mg/kg MgO NPs) and T3 (400mg/kg MgO NPs) on days 14, 21, and 28th of research trial. With the addition of mixture of banana pulp and peel in groups (T4, T5, and T6), SOD contents showed noticeably improvements towards normal in group T4 (100mg/kg MgO + pulp and peel), where no significant reduction was recorded in fish at all sampling intervals (Figure 2). Peroxidase (POD) contents followed a similar trend, with significant reduction ($p < 0.05$) in T3 at days 14 becoming more pronounced at days 21 and 28th of experimental trial. The effects of dietary supplementation of banana pulp and peel in fish of groups T4, T5, and T6 mitigated toxic effects of MgO NPs with improving the POD level in fish closer to control values at day 28th of study. Catalase (CAT) contents also exhibited dose and time-dependent declines in nanoparticles treated fish. CAT levels in T1 were non-significantly altered at day 14 but significantly reduced ($p<0.05$) in fish of groups T2 and T3 at days 21 and 28th of experiment. Supplementation with banana pulp and peel (T4–T6) significantly improved CAT contents especially in group T4, where level approached to those of the control group at day 28th of experiment.

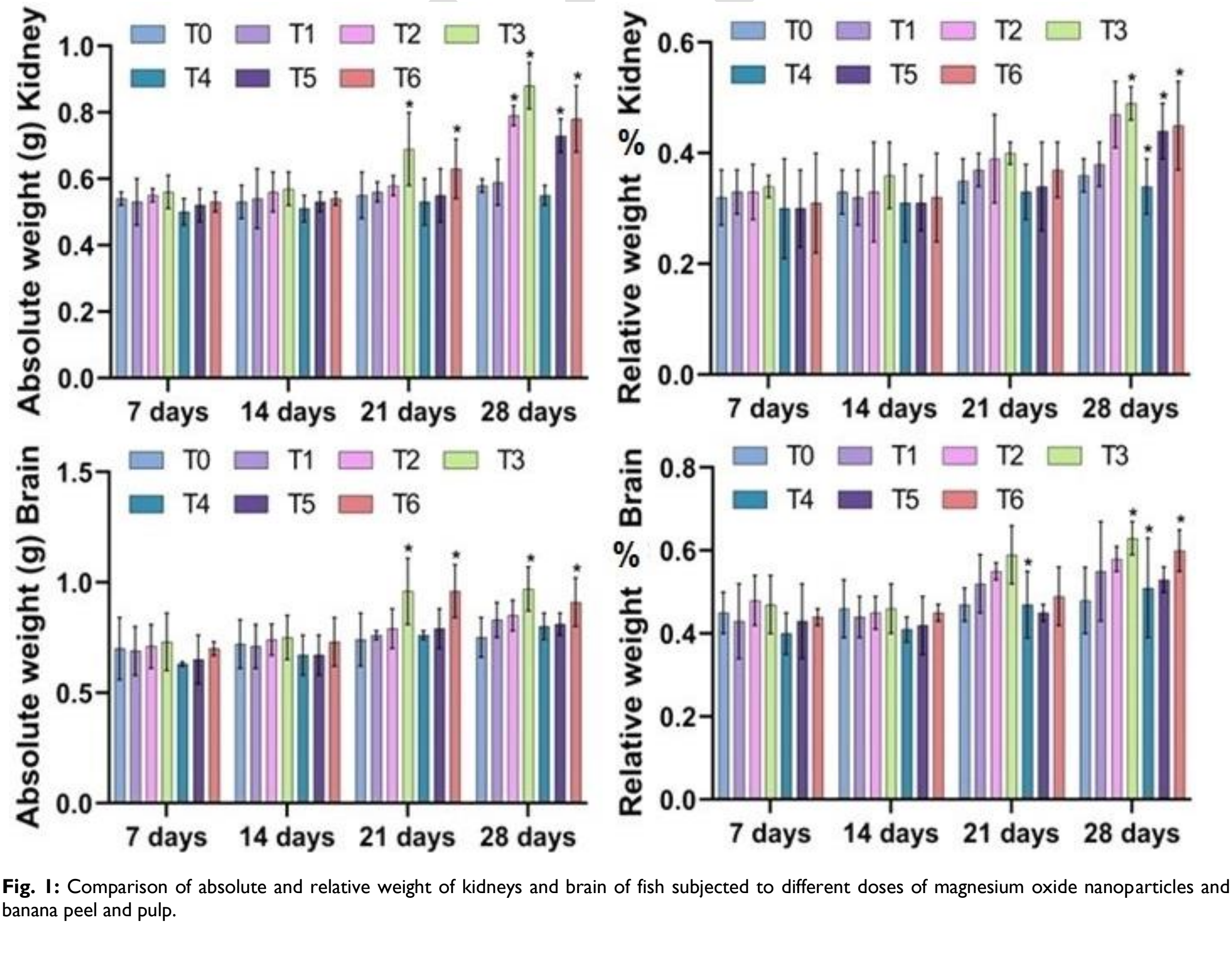

Fig. 1: Comparison of absolute and relative weight of kidneys and brain of fish subjected to different doses of magnesium oxide nanoparticles and banana peel and pulp.

In brain tissues, the contents of SOD showed a dose- and time-dependent reduction. Fish of group T1 showed non-significant reductions at all sampling points, while in fish of groups T2 and T3, the contents of SOD were significantly reduced ($p<0.05$) at days 14, 21 and 28th. Results exhibited that supplementation of banana pulp and peel in fish kept in groups (T4-T6) improved the contents of SOD (Figure 3). POD contents significantly ($p < 0.05$) decreased in fish of group T3 at day 14 and onward sampling intervals. The results on supplementations of banana pulp and peels in fish of groups T4 and T5 indicated moderate improvements with non-significant reductions. At day 28, the fish of group T4 showed substantial recovery in the contents of POD compared to the untreated groups. The values of CAT in group T1 were significantly reduced in T2 and T3 at days 14 and 28th of trial. The values of CAT in groups T4 and T5 exhibited significant improvement nearly approaching control fish.

The levels of reactive oxygen species (ROS) and thio-barbituric acid reactive substances (TBARS) increased significantly ($p<0.05$) in fish of groups T1, T2, and T3 indicating oxidative stress in both kidneys and brain tissues. ROS levels peaked in fish of group T3 at day 28. Antioxidant supplementation significantly reduced ROS levels in T4 and T5 treated fish maintaining near-normal levels. Similarly, TBARS levels were elevated in fish reared in group T3, with significant mitigation in fish of groups T4 and T5.

Histopathological examination: The results about the severity of various histopathological alterations in brain of *Labeo rohita* (Rohu) and kidneys of fish treated with MgO NPs (100 and 200mg/Kg) on days 7, 14 and 21th of experiment showed mild to moderate histopathological lesions in comparison to that of control fish. Moderate to severe histopathological changes in kidneys of fish exposed to MgO NPs (200 and 300mg/kg) including degeneration of renal tubules, deterioration of glomerulus, edema, melanomacrophage aggregates increased urinary space, necrosis of renal tubules, edema, hemorrhages, inflammatory cells and renal tubules disorganization were observed at day 28th of research trial (Fig. 4). Supplementation of the mixture of banana peel powder and pulp significantly reduced the intensity of these

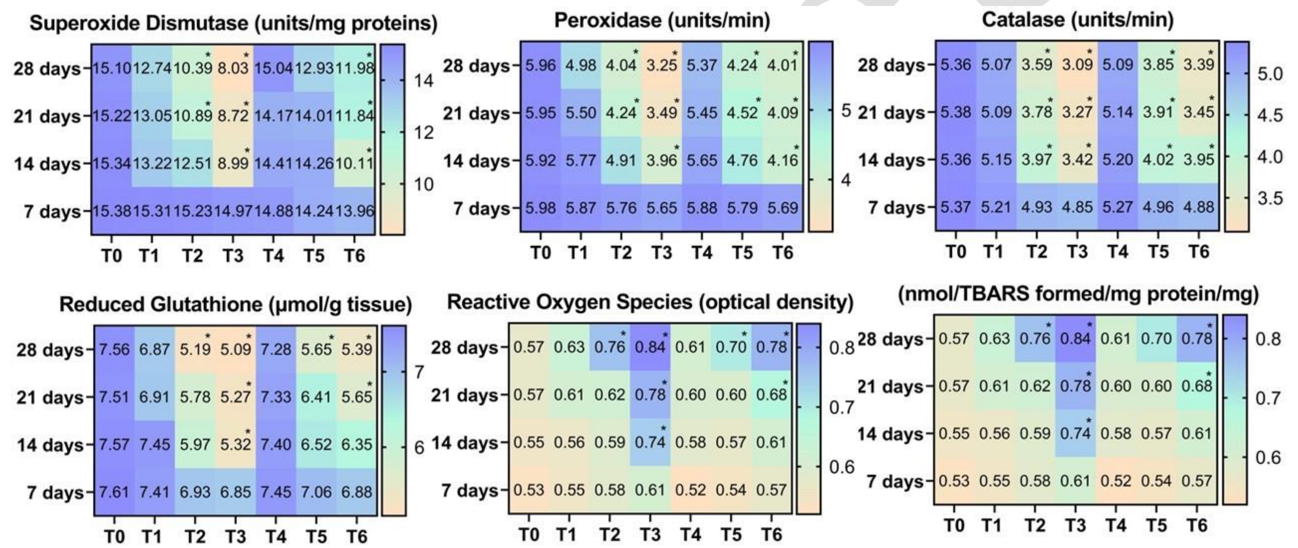

Fig. 2: Comparisons of oxidative stress and antioxidant enzyme levels in kidneys of *Labeo rohita* exposed to various doses of MgO NPs and banana pulp and peel.

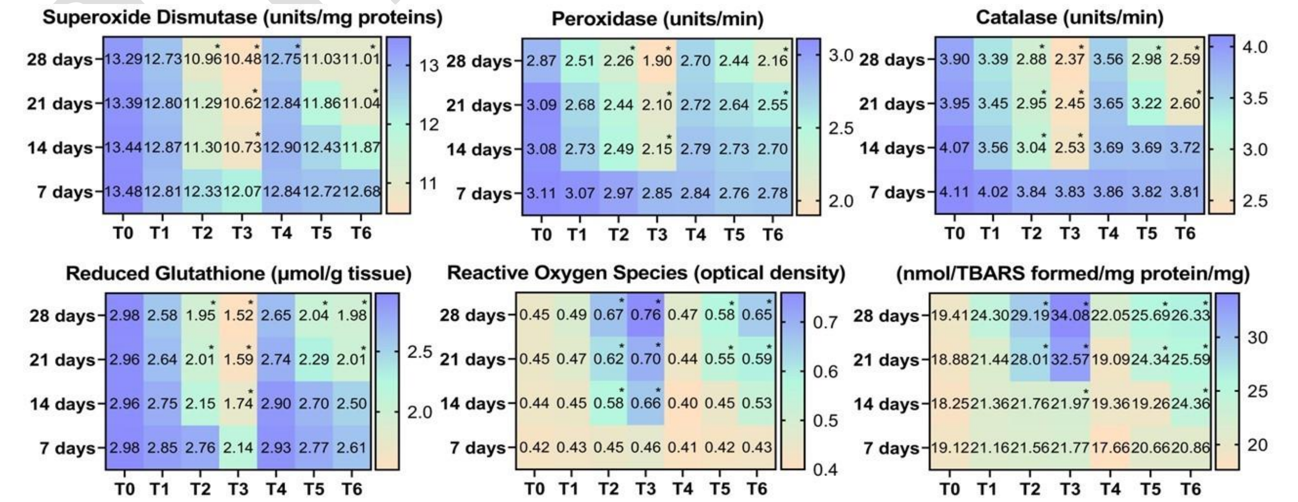

Fig. 3: Comparisons of oxidative stress and antioxidant enzyme levels in brain of *Labeo rohita* exposed to various doses of MgO NPs and banana pulp and peel.

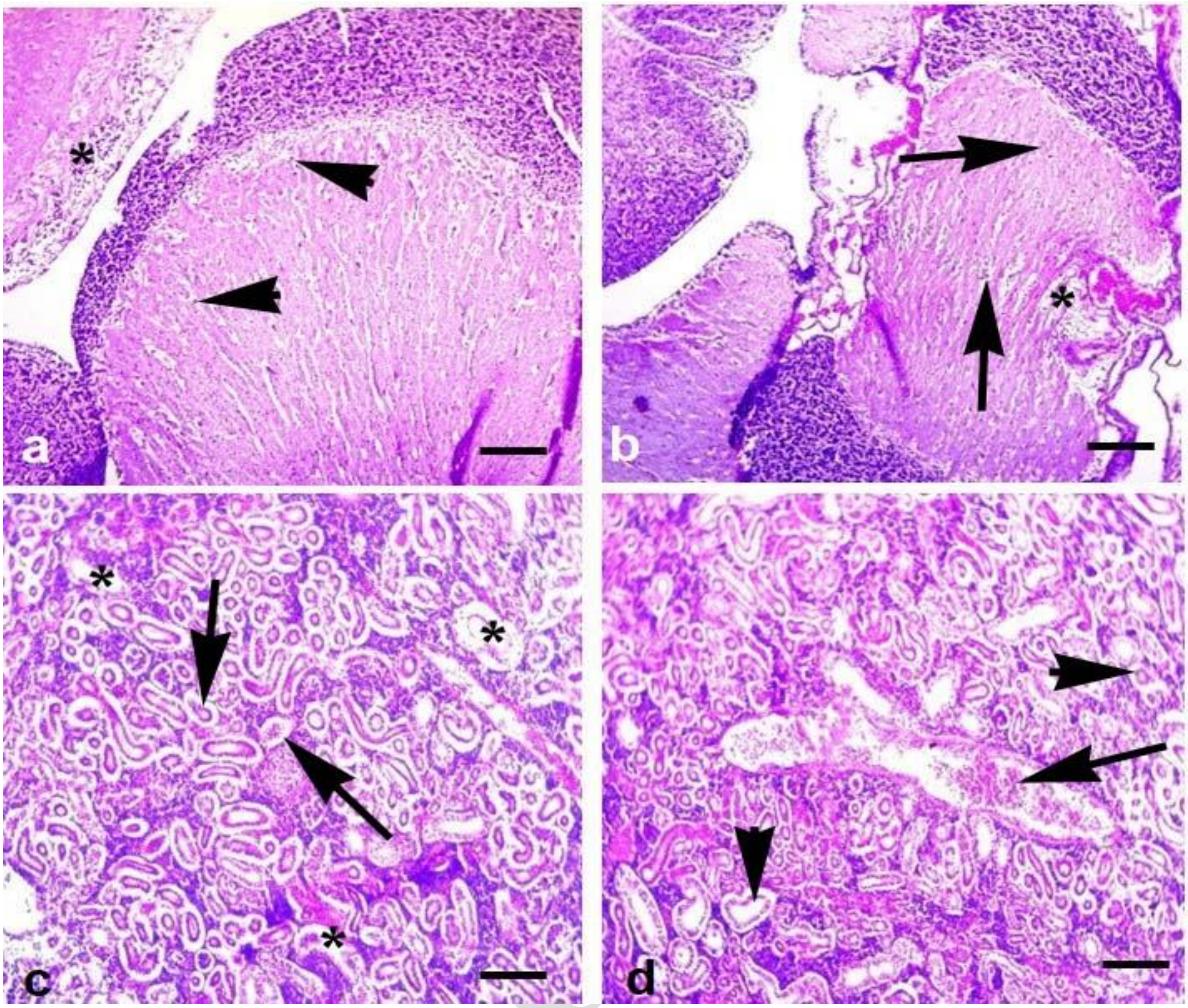

Fig. 4: Photomicrograph of brain and kidneys of *Labeo rohita* fish treated with different doses of MgO NPs at day 28 of experiment: a) degeneration of brain tissue, nuclear atrophy, neural necrosis (arrow heads), microgliosis (*) and hypertrophy of neural cytoplasm. b) brain tissue exhibiting microgliosis, inflammatory exudate (*), degeneration, atrophy of nuclei of neuron, necrosis of neuron (arrows), and hypertrophy of cytoplasm of neuron; c) kidneys showing degeneration of renal tubules, increased urinary space (arrows), necrosis of renal tubules (*) and edema; d) kidneys showing hemorrhages (arrow), inflammatory cells, increased urinary space, necrosis and disorganization of renal tubules (arrow heads). H&E; 400X

histopathological alterations in fish treated with nanoparticles. Various mild to very severe microscopic lesions in different sections of brain of treated fish were observed in the groups T2 and T3 at day 28th of trial such as necrosis of the neurons, degeneration of neurons, atrophy of nuclei, necrosis of neurons and hypertrophy of cytoplasm of neuron, microgliosis, inflammatory cells and hypertrophy of cytoplasm of neuron. On the days 21 and 28th of trial, mild histopathological changes were observed in kidneys of fish administered nanoparticles along with supplementation of a mixture of banana peel and pulp.

DISCUSSION

Aquaculture is rapidly intensifying and evolving across many regions of the globe. However, the presence of toxicants in aquatic ecosystems such as lakes, rivers, and seas has been linked to harmful effects on aquatic animals. The numerous environmental pollutants cause tissue damage in multiple visceral organs of aquatic organisms like kidneys (Mahmood *et al.*, 2022; Naseem *et al.,* 2022), brain (Iqbal *et al.,* 2024), liver (Naz *et al.,* 2021) and gills (Naz *et al.,* 2023). Moreover, nuclear and morphological changes in red blood cells (RBCs) are also caused by toxicants (Akram *et al.,* 2021), DNA damage (Akram *et al.,* 2021; Raza *et al.,* 2022), and growth retardation in freshwater fish (Akram *et al.,* 2022). Therefore, the monitoring of the xenobiotic hazards in the aquatic environments is essential for the avoidance of any possible toxic effects to the target or non-target organisms. (Shafqat *et al.,* 2023; Khan *et al.,* 2023). It is recorded that banana and its pulp and peel are rich in antioxidants and have significant medicinal value (Someya *et al.,* 2002; Wall, 2006; Lim *et al.,* 2007). The natural antioxidants act as a promising potential for controlling oxidative stress (Shahid *et al.,* 2023). The supplementation of dried banana pulp and peel and their potential to alleviate oxidative stress induced by magnesium oxide nanoparticles are investigated in current study. The plant extracts which are rich in phenolic compounds have been shown to remarkably retard lipid peroxidation because of their high antioxidant capacity

(Zhang *et al.*, 2010). It is observed that the increasing demand for alternates to synthetic antioxidant products from plants with natural antioxidants is regarded as sustainable synergies for health and environmental issues. MgO nanoparticles also cause variation in absolute as well as the relative weight of brain and kidneys of the treated fish which could be due to physiologic or metabolic disturbances induced by nanomaterials.

Magnesium oxide nanoparticle exposure caused significant increases in organ weight suggesting that nanoparticles induced oxidative stress in association with inflammation and hypertrophy of tissue (Manke *et al.*, 2013). These effects were dose dependent and are in consistent with the previous results suggesting that the exposure to toxicant induces oxidative damage and histopathological changes (Makhdoumi *et al.*, 2020; Younas *et al.*, 2024). However, these changes were partially ameliorated in fish supplemented with banana pulp and peel indicating the antioxidant potential of banana derived compounds. The results might be related to presence of bioactive phytochemicals and polyphenols and decrease oxidative damage due to presence of anthocyanins and catecholamines (Seymour *et al.*, 1993; Kanazawa and Sakakibara, 2000; Vergara-Valencia *et al.*, 2007). The non-significant variation in weight of visceral organs in fish treated with low doses of nanomaterials and supplemented with banana pulp and peel indicate that dietary antioxidants play an important role in countering the deleterious effects of MgO nanoparticles. The understanding of the mechanisms of induction of oxidative stress due to environmental contaminants and the ability of naturally occurring dietary supplements to reduce adverse effects is of great concerns to underscore the utility of integrative approaches to mitigate toxicity.

In this study, the fish exposed to higher doses of MgO nanoparticles unveiled significantly enhanced oxidative stress biomarkers (ROS and TBARS) in kidneys and in brain. The higher contents of oxidative stress profile might be due to nanoparticle exposure causing increased free radical production and damage to tissues (Akram *et al.*, 2021; Aslam *et al.*, 2023; Kanwal *et al.*, 2024). The inclusion of banana pulp and peel noticeably reversed these effects in fish exposed to nanomaterials. Result indicated that the presence of MgO nanoparticles caused a detrimental effect upon the antioxidant enzymes quantity and oxidative stress induction in the brain and kidneys of fish which led to problems in normal structural and physicochemical functions of cells (Qiao *et al.*, 2021). Banana derived- antioxidants considerably improved the enzymatic antioxidants (CAT, SOD, POD, and GSH) suggesting the protective role. The results may be linked to DPPH radical scavenging activity or the capacity to recover oxidative balance of banana peel and pulp (Bhutta *et al.*, 2022). Previously, it is also reported that over release of free radical and interaction between nanoparticles and cells may cause abnormal activation of an immune response leading to mitochondrial dysfunctions (Manke *et al.*, 2013; Makhdoumi *et al.*, 2020). The noticeable improvement in contents of antioxidant enzymes and reduction of oxidative stress in fish fed with banana peel and pulp suggested that antioxidants supplementation is a promising dietary intervention against the toxic effects of nanoparticles in aquatic organisms as the supplementation enhanced the enzymatic defense system and reduced oxidative biomarkers. The results suggested that therapeutic use of banana-based antioxidants provide reliable evidence for their use as a potent, sustainable, low-cost and natural solution for addressing the mitigation of environmental toxicological problems.

Various histopathological changes including neural cell degeneration, cytoplasmic hypertrophy, necrosis of neurons, microgliosis, edema, and neuronal necrosis in brains of nanomaterials treated fish could be related to depletion of antioxidant defense responses and over generation of free radicals. Similar previous histopathological alterations have also been reported in brain tissues of various fish species including *Oreochromus niloticus* (Ayoola and Ajani, 2008), *Oreochromus mossambicus* (Gobi *et al.*, 2018; Murali *et al.*, 2018) and *Catla catla* (Bose *et al.*, 2013) exposed to environmental contaminants.

The kidneys of MgO nanoparticles treated fish showed mild to severe microscopic lesions including congestion, glomerular deterioration, edema, increased Bowman's space, aggregates of melanomacrophages, necrosis of tubular cells, and nuclear hypertrophy. These changes may be linked to lipid peroxidation, resulting in the generation of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), which disrupt cellular membranes (Ghazanfar *et al.*, 2018; Kiran *et al.*, 2022). Previous studies have reported similar renal anomalies such as glomerular deterioration and destruction of renal tubules in tilapia (Faheem *et al.*, 2019) and *H. fossilis* (Pal and Reddy, 2018; Vasu *et al.*, 2019). The dietary supplementation with banana pulp and peel powder significantly mitigated the severity of histopathological anomalies in both kidneys and brain of fish. The observed reduction in tissue damage may be attributed to the antioxidant properties of the banana components which counteract oxidative stress and the elevated contents of cytochrome P450 enzymes, which play a critical role in detoxification processes (Villa-Cruz *et al.*, 2009).

Conclusions: Based on results of our study, it can be suggested that banana (*Musa acuminata*) pulps and peel powder possess vital compounds including minerals, antioxidants and carbohydrates. Thus, it is recommended that banana pulps and peels powder may be used as natural antioxidant in aquaculture practices to minimize the toxicity such as oxidative stress induction by magnesium oxide nanoparticles.

Authors contribution: RI and RH designed and supervised the trial; MK and SRK conducted the research and collected the data; BI**,** MA and MRA contributed to manuscript preparation; ZI, KA and SA involved in statistical analysis, interpretation of results and wrote the initial draft.

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