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Research article

Behavioral abnormalities in Labeo Rohita Under the Acute Exposure of Organophosphate Insecticide, Chlorpyrifos

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Abstract

Chlorpyrifos (CPF) is a widely used organophosphate pesticide which has an unfavorable impact on the aquatic ecosystem. This work was designed to compute the LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita*. The LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita* was determined as 10.39±0.03 and 15.31±0.05 μgL^{-1}, respectively. During acute toxicity test, behavioral changes in *Labeo rohita* were also observed i.e. rapid opercular movement, profuse mucus secretions, imbalance swimming, increased surface activity, loss of equilibrium, convulsion, body discoloration, and decreased hyperactivity. Jumping of fish out of exposed medium proved the avoidance behavior against pesticide toxicity.

Keywords: Acute toxicity, behavior, fish, pesticides

1. Introduction

Environmental pollution by different compounds including pesticides is a basic concern which may have an unfavorable impact on the sensitive ecosystem [1, 2]. In numerous zones, these delicate ecosystems are at a greater risk due to release of pesticides from agricultural and urban sources to waterbodies influencing the aquatic life [3, 4]. An organophosphate pesticide like chlorpyrifos (CPF) is widely applied due to its short term persistence and low amassing ability in the environment.

This insecticide, extensively supplied to kill pests but also has serious hazardous impact on non-target aquatic fauna [5]. Acute tests such asLC_{50} and lethal concentration are commonly applied to determine the tolerance limits of fish against different toxicants[6]. In these assays, data on mortality are use to measure the duration dependent response of different species of fish to chemicals that might be provide useful information regarding to sustainable conservation of major carps in Pakistan. These assays also determined the toxic impact of any test chemical on aquatic fauna in a short time of their life span[7-9] and permit us to evaluate the impacts of different toxicants on the biology of organisms [10] to check their capacities to adjust beneath certain toxicity levels and to estimate conceivable impacts of harmfulness on them [11, 12]. The sudden behavioral changes seen in fish make them perfect subjects for observation, and examination of fish behavior has been a wellknown approach to identify changes in the aquatic ecosystem[13]. Fish are perfect animal for behavioral tests of different stress factors and toxicants (like metals and insecticides) exposure due to their ecological relevance in various natural water bodies[14, 15].

Exposure of chlorpyrifos caused change in the behavior of fish such as erects swimming, vertical hanging, convulsions, equilibrium loss, convulsions [16].fish showed rapid gulping of water and increased opercular movement under acute exposure of chlorpyrifos.

Therefore, this work was performed to observe the behavioral abnormalities in *Labeo rohita* under the acute exposure of chlorpyrifos. Fish behavior is often a sensitive indicator of environmental stress, and alterations in behavior could indicate

shifts in the ecosystem's functioning and stability. This information could prompt policymakers and environmentalists to take action to prevent further degradation and promote ecosystem restoration.

2. Material and Methods

2.1 Fish and acclimatization

The experiment was conducted in limnology laboratory at Fisheries Research Farms, University of Agriculture, and Faisalabad. *Labeo rohita* (Average weight 8.12±0.11) were housed in tank to acclimatized laboratory environment for couple of weeks. No fish were died during this period. In acclimatization, 12-hr light and 12-hr dark photoperiod was maintained.

2.2 Acute Toxicity Assay

Technical grade chlorpyrifos was dissolved in methanol to prepare stock solutions while further dilutions were made by dissolving an appropriate volume of stock solution in deionized water. *Labeo rohita* was exposed to each of the 21 different concentrations (0.75-15 mg/L) of chlorpyrifos to estimate the LC_{50} and lethal concentration for 96-hr. The whole experiment was carried out with triplet in 100-liter water capacity glass aquaria each holding 10 juveniles. All the test and control mediums were supplied with continuous air with an air pump through capillary system.

2.3 Physico-chemistry of water

The total hardness, temperature and pH of water were maintained as 225 mL^{-1}, 30ºC and 7.0, respectively. Other physico-chemical variables such as electrical conductivity, total ammonia, calcium, sodium, magnesium, potassium and carbon dioxide were also determined (A.P.H.A., 2005).

3.3 Behavioral study

The behavioral changes like jumping, equilibrium status, opercula movement, fin movement, erratic swimming, convulsion, skin discoloration, hyperactivity, surfacing activity, mucus secretion, caudal bending in control and treated fish were observed.

3.4 Statistical Analyses

Probit Analysis method (at 95% confidence interval) was applied on mortality data to compute the LC_{50} and lethal concentrations (96-hr) of chlorpyrifos for *Labeo rohita*.

3. Results and Discussion

3.1 Acute toxicity Assay

LC_{50} and lethal concentration (96-hr) of chlorpyrifos for *Labeo rohita* was estimated as 10.39±0.03and 15.31±0.05μgL^{-1}, respectively (Table 1). The fish mortality was increased with increasing concentrations of chlorpyrifos and exposure duration. The chlorpyrifos-methyl toxicity (96-hr LC_{50}) to the tilapia was computed as 1.57 mg/L [18]. The tolerance limit (96-hr LC_{50}) of chlorpyrifos was estimated at 0.160 mg/L [19]. Chlorpyrifos toxicity to *Gambusia affinis* and *Oreochromis moss ambicus*was found to be 0.297 and 0.0259mg/L[20, 21]. The 96-hr LC_{50} of chlorpyrifos for *Puntius chola* was estimated as 0.219 ppm [22]. The 96-hr LC_{50} value of chlorpyrifos for *Labeo rohita* was found to be 0.01 mg/L [23].

3.2 Behavioral study

From both exposed and control groups behavior of fish was observed. Exposed fish showed rapid opercular movement, profuse mucus secretions and imbalance swimming, increased surface activity, loss of equilibrium, body discoloration and decreased hyperactivity and fin movement before the death were observed. Jumping of fish out of exposed medium, which proves the evasion behavior of fish against pesticide toxicity. In the control conditions, no behavioral changes were observed in fish (Table 2). Alterations in the behavior of organisms are the most important sensitive indicator of stress caused by toxicants [24]. In present observation, exposed fish showed rapid opercular movement, profuse mucus secretions and imbalance swimming, increased surface activity, loss of equilibrium, convulsion, body discoloration, caudal bending, increased hyperactivity, jumping and fin movement were observed. The fast opercular movements may be due to increase in mucous over gill surface due to the pollutants[25]. Loss of balance and erratic swimming in *Oreochromis mossambicus* exposed to chlorpyrifos was observed [26, 17] Recorded abnormal swimming, aggressive behaviour,

Table 1 96-hr acute toxicity of chlorpyrifos (μgL^{-1}) for *Labeo rohita*

Fish specie	Pesticide	LC_{50}	95% Confidence Interval	Lethal conc.	95% C.I LCL- UCL	Pearson Goodness of Fit Tests		
						χ^2	DF	p-value
Labeo rohita	CPF	10.39	9.63-11.07	15.31	14.27-16.87	5.16	18	0.999

CPF=Chlorpyrifos; Lethal Concentrations=Lethal Conc. (mgL^{-1}); Chi-Square=χ^2; Degree of Freedom =DF.

Table 2Effect of chlorpyrifos on behavior of *Labeo rohita*under the acute exposure

Concentrations μgL^{-1}	Erratic swimming	Jumping	Opercula movement	Fin Movement	Mucous secretion	Loss of Equilibrium	Skin discoloration	Hyper-activity	Surface activity
0.00	-	-	-	-	-	-	-	-	-
0.75	-	-	-	-	-	-	-	-	-
1.50	-	-	-	-	-	-	-	-	-
2.25	-	-	-	-	-	-	-	-	-
3.00	-	-	-	-	-	-	-	-	-
3.75	-	-	-	-	-	-	-	-	-
4.50	+	-	-	-	-	-	-	-	-
5.25	+	-	+	+	+	+	+	+	+
6.00	+	-	+	+	+	+	+	+	+
6.75	++	+	++	++	++	++	++	++	++
7.50	++	+	++	++	++	++	++	++	++
8.25	++	+	++	++	++	++	++	++	++
9.00	++	++	++	++	++	++	++	++	++
9.75	+++	++	+++	+++	+++	+++	+++	+++	+++
10.50	+++	++	+++	+++	+++	+++	+++	+++	+++
11.25	+++	+++	+++	+++	+++	+++	+++	+++	+++
12.00	++	+++	+++	+++	+++	+++	+++	+++	+++
12.75	+	+++	+++	+++	+++	+++	+++	++	+++
13.50	+	++	++	+	+++	+++	+++	+	+
14.25	-	-	+	+	+++	+++	+++	+	-
15.00	-	-	-	-	+++	+++	+++	-	-

Note: Normal (-); Mild(+); Moderate (++); Strong (+++)

increased opercular movement and hyperactivity in *Poecila reticulata* as a response to chlorpyrifos exposure. *Channa punctatus* exposed to chlorpyrifos showed loss of balance, reduction in swimming rate, reduced feed intake, and convulsions [27].

Exposure to chlorpyrifos increased secretion of mucusin [19]. Fast swimming activity, loss of equilibrium, jerky movement, profuse secretion of mucus and hypersensitivity in chlorpyrifos exposed fish was observed by Ramesh et al. [28]. Banaee et al. [29] reported unbalanced swimming and increased surface swimming in *Cyprinus carpio* exposed to chlorpyrifos. Behavioural changes such as swimming erratically, convulsions, vertical hanging, coughing, loss of balance and abnormal opercular movement in chlorpyrifos exposed *punctatus* was observed by Yogita et al. [16]. Similar behavioral changes were also observed by Verma et al. [22]. Prashanth et al. [30] recorded the increased opercular movement, secretion of mucus and surface activity, body discoloration, irregular swimming and rapid jerk movement of *C. mirgala* exposed to lethal level of cyper menthrin. Common carp exposed to chlorpyrifos showed irregular, erratic and darting swimming movements, hyper excitability, loss of equilibrium, sinking to the bottom and caudal bending [19, 31]. Irregular swimming of *Rutilus frisiiKutum* and *Rutilus caspicus*exposed to pesticide (Hinsosen) was observed by Naserabad et al. [32]. *Clarias gariepinus*showed erratic and

jerky swimming, attempt to jump out of water, increased surfacing and gulping of air, reduced opercula movement and secretion of mucus on the body and gills followed by exhaustion and death after acute exposure to chlorpyrifos[31].

4. Conclusions

Behavioural changes can be used as a beneficial approach in biomonitoring programme to check ecotoxicity threat of pesticides to the test animals. During this study, acute exposure of chlorpyrifos had clear negative effects on the behavior of *Labeo rohita*. Chlorpyrifos reduced instinctive behavioural response and also cause the morphological changes. So, to mitigate the effects of pesticides pollution on fish, it is crucial to implement strict regulations and practices to minimize the release of these contaminants into water bodies

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

Mubashra Ikram executed the research work; Sajid Abdullah planed this work. Huma Naz and Khalid Abbas help in writing article. Tanveer Ahmed assist in lab work. Iqra Zulfiqar and Nimra Zahid help in statistical analysis.

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REFERENCES

1. David, M., et al., Alterations in the levels of ions in tissues of freshwater fish Cirrhinus mrigala exposed to deltamethrin. 2010.
2. David, M. and R.J.O.V.J. Kartheek, Histopathological alterations in spleen of freshwater fish Cyprinus carpio exposed to sublethal concentration of sodium cyanide. 2015. 5(1): p. 1-5.
3. Austin, B.J.J.o.a.m., The effects of pollution on fish health. 1998. 85(S1): p. 234S-242S.
4. Srivastava, R.K., K.K. Yadav, and S.P.J.J.o.e.b. Trivedi, Devicyprin induced gonadal impairment in a freshwater food fish, Channa punctatus (Bloch). 2008. 29(2): p. 187.
5. Sparling, D. and G.J.E.P. Fellers, Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval Rana boylii. 2007. 147(3): p. 535-539.
6. Abdullah, S. and M. Javed, Studies on acute toxicity of metals to the fish, Catla catla. 2006.
7. Ebrahimpour, M., et al., Influence of water hardness on acute toxicity of copper and zinc on fish. 2010. 26(6): p. 361-365.
8. Javed, M.J.P.V.J., Effects of zinc and lead toxicity on the growth and their bioaccumulation in fish. 2012. 32(3): p. 357-362.
9. Javed, M.J.P.V.J., Tissue-specific bio-accumulation of metals in fish during chronic waterborne and dietary exposures. 2012. 32: p. 567-570.
10. Javed, M. and M.A.J.I.J.A.B. Saeed, Growth and bioaccumulation of iron in the body organs of Catla catla, Labeo rohita and Cirrhina mrigala during chronic exposures. 2010. 12: p. 881-886.
11. Abdul, R., et al., Assessment of heavy metals in sediments of the river Ravi, Pakistan. 2009. 11(2): p. 197-200.
12. Azmat, H., M. Javed, and G.J.P.V.J. Jabeen, Acute toxicity of aluminium to the fish (Catla catla, Labeo rohita and Cirrhina mrigala). 2012. 32(1): p. 85.
13. Habeeba, U., M.J.I.J.o.T. David, and A. Pharmacology, Studies on acute and behavioural toxicity of lambda-cyhalothrin on fresh water fish, Cyprinus carpio. 2016. 6(1): p. 1-6.
14. Fırat, Ö., et al., A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus. 2011. 37: p. 657-666.
15. Xin, Z., et al., Species sensitivity analysis of heavy metals to freshwater organisms. 2015. 24: p. 1621-1631.
16. Yogita, D., M.J.I.J.o.P. Abha, and B. Sciences, Study of behavioural and morphological anomalies of fry fish of fresh water teleost, Channa punctatus under chlorpyrifos intoxication. 2013. 4(1).
17. Sharbidre, A.A., et al., Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, Poecilia reticulata. 2011. 101(2): p. 132-141.
18. Gül, A.J.C., Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (Oreochromis niloticus L.) larvae. 2005. 59(2): p. 163-166.
19. Halappa, R., M.J.T.J.o.F. David, and A. Sciences, Behavioral responses of the freshwater fish, Cyprinus carpio (Linnaeus) following sublethal exposure to chlorpyrifos. 2009. 9(2).
20. Rao, J.V., et al., Toxicity of chlorpyrifos to the fish Oreochromis mossambicus. 2003. 70: p. 0985-0992.
21. Rao, J.V., et al., Changes in behavior and brain acetylcholinesterase activity in mosquito fish, Gambusia affinis in response to the sub-lethal exposure to chlorpyrifos. 2005. 2(3): p. 478-483.
22. Verma, V.K. and A.J.I.J.o.F. Saxena, Investigations on the acute toxicity and behavioural alterations induced

by the organophosphate pesticide, chlorpyrifos on Puntius chola (Hamilton-Buchanan). 2013. 60(3): p. 141-145.
23. Syed, A., Mazhar, S., Rafi, U., Hussain, D., & Hayee, S. Effects of Chlorpyrifos on biochemical characteristics of Labeorohitafish during acute and chronic exposure: Effects ofChlorpyrifos on biochemical characteristics of Labeorohita (2021). Pakistan BioMedical Journal, 4(2), 107-112.
24. Remyla, S.R., et al., Influence of zinc on cadmium induced haematological and biochemical responses in a freshwater teleost fish Catla catla. 2008. 34: p. 169-174.
25. Jagadeesan, G., S.J.I.J.O.E. Vijayalakshmi, and TOXICOLOGY, Alterations in the behaviour pattern in Labeo rohita (HAM) fingerlings induced by mercury. 1999. 9: p. 50-50.
26. Padmanabha, A., et al., Acute effects of chlorpyrifos on oxygen consumption and food consumption of freshwater fish, Oreochromis mossambicus (Peters). 2015. 6(4): p. 3380-3384.
27. Stalin, A., Suganthi, P., Mathivani, S., Paray, B. A., Al-Sadoon, M. K., Gokula, V., & Musthafa, M. S. (2019). Impact of chlorpyrifos on behavior and histopathological indices in different tissues of freshwater fish Channa punctatus (Bloch). Environmental Science and Pollution Research, 26, 17623-17631.
28. Ramesh, M. and M.J.I.j.o.i.b. Saravanan, Haematological and biochemical responses in a freshwater fish Cyprinus carpio exposed to chlorpyrifos. 2008. 3(1): p. 80-83.
29. Banaee, M., B.N. Haghi, and A.T.A.J.I.J.o.A.B. Ibrahim, Sub-lethal toxicity of chlorpyrifos on Common carp, Cyprinus carpio (Linnaeus, 1758): Biochemical response. 2013. 1(6): p. 281-288.
30. Prashanth, M., M. David, and S.J.J.o.E.B. Mathed, Behavioural changes in freshwater fish, Cirrhinus mrigala (Hamilton) exposed to cypermethrin. 2005. 26(1): p. 141-144.
31. Nwani, C., et al., Investigation on Acute toxicity and behavioral changes in Tilapia zillii due to glyphosate-based herbicide, forceup. 2013. 23(3): p. 888-892.
32. Naserabad, S.S., et al., Acute toxicity and behavioral changes of Caspian kutum (Rutilus frisii Kutum Kamensky, 1991) and Caspian roach (Rutilus rutilus caspicus Jakowlew, 1870) exposed to the fungicide hinosan. 2015. 14(20): p. 1737-1742.

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Research article

Acute Intoxication of Metals in Cirrhinus mrigala with Special Reference to the Physiological, Biochemical and Molecular Effects

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Abstract

An experiment to assess the changes in hematology, serum biochemistry and DNA integrity in *Cirrhinus mrigala* exposed to metals was conducted. Results showed that the copper exposure to the fish had more pronounced effects as it resulted in significantly lower RBCs, Hb, Hct and higher WBCs, while Zn exposure showed least toxic effect towards hematological parameters as compared to other metals. Among all the exposure durations of metals, the 96-hr exposure caused maximum negative effects on fish. Lower level of serum Na, Cl, Alb and TP were observed in fish under the exposure of Cu as compared to other metals while K, AST and ALT levels were higher. However, least toxic effect on all above-mentioned parameters were noticed in Zn exposed fish. It is also observed that the highest DNA damage in terms of percent genomic DNA template stability (%GTS) was observed in Cu exposed fish while the Zn exposure to fish resulted in lowest DNA damage. The results revealed maximum squared Euclidean distance between Cu treated fish and the control. This study proposed that the occurrence of toxic metals in aquatic environment has strong impact on hematology, serum biochemistry and DNA integrity of fish.

Keywords: Fish, hematology, serum biochemistry, metals, DNA damage

1. Introduction

Freshwater is highly susceptible to pollution since it acts as a direct sink for the consequences of anthropogenic activities which are always accompanied with the danger of criminal negligence or accidental discharges [1]. Currently, new technologies, the legacies of past contamination and the extensive usage of metals continue to intensify the concentration of metals into the aquatic ecosystems [2].

Survival rate of aquatic organisms is affected due to the cadmium exposure and leads to gradual extinction of their generations in polluted water [3]. Copper is one of the most disastrous metals to aquatic organisms and ecosystems. Its toxicity to aquatic organisms had previously been described by several researchers [4,5,6]. Nickel (Ni) is an essential element for living organisms but it is highly toxic at higher concentration [7]. Recently, several reports are available regarding the Ni toxicity in different animal species [8,9,10]. Zinc (Zn) is also an essential micronutrient and is important for various physiological processes of cells [11], and act as a co-factor of various enzymes. If its level exceeds the physiological requirements, it can act as a toxicant [12].

Fishes are the animals that cannot escape from the negative effects of these contaminants and prove as good bioindicators of aquatic pollution [13]. Fish blood is a pathophysiological indicator of the entire body functions, for that reason it is essential in diagnosing the functional and structural status of fish exposed to toxicants. Numerous studies have showed that metals, for instance cadmium, copper, nickel and zinc induce changes in blood parameters of fish [14-16]. Measurements of serum biochemical parameters is valuable to ascertain the toxicity of target organs along with the overall health status of

animals and provides initial warning of potentially detrimental alterations in stressed organisms [17].

Several studies of fish genotoxicity exhibit the role of metals in eliciting damage to DNA [18,19,20]. Fishes prove excellent material for the study of carcinogenic or mutagenic potential of water, as they can metabolize, concentrate and store waterborne contaminants [21]. In modern days, molecular genetics has provided several novel techniques for the measurement of genotoxicity. In recent times, the RAPD technique has been useful in noticing the genotoxic potential of metals in fish [22]. Pakistan is among the countries facing acute freshwater pollution problems where only 1% of industrial water is treated before its discharge into the rivers and streams [23]. Metals existing in aquatic habitats of Pakistan compete for the up taking routs in organisms, as well as lethal target sites, excretion routs within organism and transport mechanisms [24]. Thus, it is imperative to study the acute responses of fish to metallic ion toxicity that could affect the hemato-biochemical parameters and DNA integrity in *Cirrhinus mrigala*.

2. Material and Methods

The present experiment was conducted in the Wet Laboratory of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad in 2018. *Cirrhinus mrigala* of desired weight (30g) were obtained from the Fish Seed Hatchery, Faisalabad. They were brought to the Wet Laboratory and acclimatized to the laboratory conditions for 14 days. Pure chloride compounds of cadmium ($CdCl_2$. H_2O) and copper ($CuCl_2$. $2H_2O$) nickel ($NiCl_2$. $6H_2O$) and zinc ($ZnCl_2$. $2H_2O$) were used in this experiment and stock solutions were prepared for required metal dilution. Fish were exposed for 96-hr to waterborne lethal concentrations (LC_{50}) of metals which were already determined by [25,26]. The Physico-chemical parameters of the test media were monitored on daily basis by following the methods of [27].

2.1 Hematological parameters: The blood samples were taken at different time intervals from metals exposed and control fishes to study the hematological parameters. Specimens with an average body weight of 30 g were used for sample collection. After anaesthetizing the fish with MS-222 (100 mg/L), blood samples were collected under sterile conditions by the puncture of caudal vein with a heparin-coated 23-gauge needle attached to a 2.5 mL syringe. The hematological parameters i.e. red blood cells, hemoglobin, hematocrit and white blood cells were determined by using automated cell counter (Sysmex KX 21).

2.2 Serum biochemical parameters: Blood samples for serum biochemical analysis were collected without an anticoagulant. The blood samples were left for 1-hr on ice and then centrifuged at 3000 rpm for 10 minutes to isolate the serum. Samples were stored at -80°C before the further analysis. Serum biochemical parameters (sodium, potassium, chloride, total protein, aspartate aminotransferase, alanine aminotransferase) were estimated following standard methods using commercially available kits by BioMed Company.

2.3 RAPD Analysis: The fish liver tissues were taken from metal treated and control fish at specific time intervals during acute trial and stored at -20°C. Total genomic DNA was extracted from small amount (20 mg) of frozen tissues by using traditional Proteinase-K digestion and standard phenol/chloroform technique following the [28]. procedure with slight modifications and visualized on 0.8 % high melt agarose gel in TAE buffer. Amplification of genomic DNA was performed in a gradient thermal cycler (Multigene OptimaxLabNet, USA). The PCR reaction was carried out by using 20 ng of genomic DNA as template DNA. All optimized conditions were used to get reproducible and consistent banding pattern from RAPD (PCR). Initially, 20 random decamer primers were screened in order to test amplification profiles for polymorphism and reproducibility. Finally, the present study utilized 6 primers for RAPD-PCR analysis that provided good results, as shown in Table 1.

2.4 Band Scoring

Only RAPD fragments having high concentration and reproducibility were targeted for markers, estimation of size of such fragments being helped using the 200 bp DNA Ladder (TaKaRa). The presence or absence of fragments was scored as

1 or 0, respectively. RAPD patterns were visually analyzed and scored from photographs. For the analysis and comparison of the patterns a set of distinct well separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) of these bands.

Table 1. Primers used for the amplification of DNA.

Sr. #	Primer	Sequence
P1	OPC-01	TTCGAGCCAG
P2	OPC-11	AAAGCTGCGG
P3	OPB-06	TGCTCTGCCC
P4	OPB-15	GGAGGGTGTT
P5	OPAY-07	GACCGTCTGT
P6	OPAY-09	CCGATCCAAC

2.5 Estimation of genomic DNA template stability

The GTS (%) was calculated as:

GTS % = 1-a/n × 100 Savva *et al.* (1994)

Where a is the average number of the polymorphic bands detected in each treated sample and n is the total number of bands found in the control.

2.6 Statistical Analysis

Data were statistically analyzed through Factorial design under CRD. Means were compared for statistical differences through Tukey's student Newnan-Keul test [29]. Numerical analysis based on banding pattern obtained from metals exposed fish was compared with the untreated samples (control) via hierarchical cluster analysis. Dendrogram was constructed by the between-groups linkage method using squared Euclidean distance measurement. Genotoxicity judgments were made on the basis of the distance between the specimens. All the analyses were performed by using SPSS software.

3. Results and Discussion

Cirrhinus mrigala was exposed to 96-hr LC_{50} of metals. The physiological, biochemical and molecular changes in fish was determined after 24, 48, 72 and 96-hrs of metals exposure.

3.1 Physiological Changes

The hematological parameters studied during the experiment include red blood cells (RBCsC) count, hemoglobin (Hb), hematocrit (Hct) and white blood cells count (WBCsC). The control fish exhibited higher RBCsC, Hb and Hct than those exposed to different metals while higher WBCsC was noted in metals treated fish than control fish. Among metals, Cu exposure to the fish resulted in minimum RBCsC, Hb and Hct while the same was maximum in Zn exposed medium. Among metals, Cu exposure to the fish maximally increased the WBCsC while the same was minimum under the exposure of Zn as compared to other metals. Comparison of means revealed significantly lower RBCsC and Hb after 96-hr exposure duration as compared to 24-hr exposure duration. The Hct content decreased significantly with the progressive increase in exposure duration and maximum decline was noted after 96-hr exposure duration (Figure 1).

Similar results about hematological parameters have been reported by Hedayati et al. [30]. in silver carp exposed to different concentrations of copper sulfate. Al-Ghanim et al. [31] documented the same results as reduction in Hb, Hct and RBCs in the blood of *Cyprinus carpio* after the Ni exposure. Reduction in these indices was also detected in *Oreochromis mykiss* exposed to Pb and Cu [32]. For the Ni metal similar findings were given by the Ololade et al. [33]. in African catfish with the decrease in Hb, Hct and RBCs. Capkin et al. [34] reported decrease in Hct content of dogfish, *Scyhorhinus canicula* after 24-hr exposure to Cd. They attributed this decrease in Hct content to hemodilution. The high concentrations of metals for short-term exposure usually decline the above-mentioned parameters. Immunological activities and defense mechanisms are usually maintained by WBCsC as reported by Abhijith et al. [35]. According to Moraes et al. [36], one of the most basic ways to assess the immune system is to explore the changes in WBCsC. The Ni exposure resulted in progressive and significant increase in total leucocytes count with an increase in the exposure duration [37]. Increase in WBCsC indicates a defensive response to the metal's exposure [38]. High level of WBC count indicates damage due to infection of body tissues and severe physical stress [39].

3.2 Biochemical Changes

The sodium (Na), chloride (Cl) and total protein (TP) level in serum of metals treated fish was less as compared to their respective control. Among metals, comparatively more toxic effect on Na, Cl and TP level in fish serum was observed under the Cu exposure while least toxic effect was noted under the

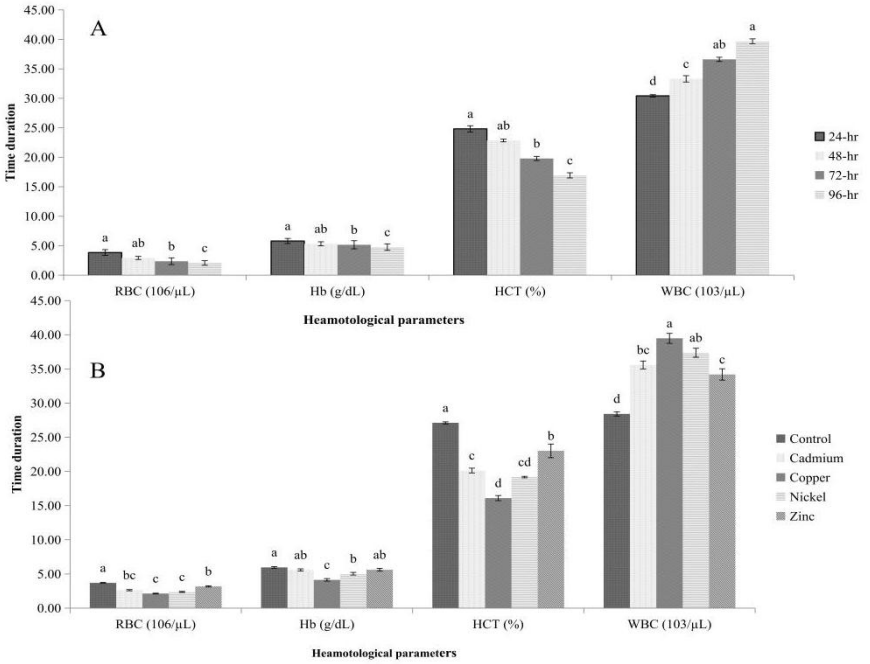

Figure 1: Changes in hematological parameters under the exposure of metals for different time durations

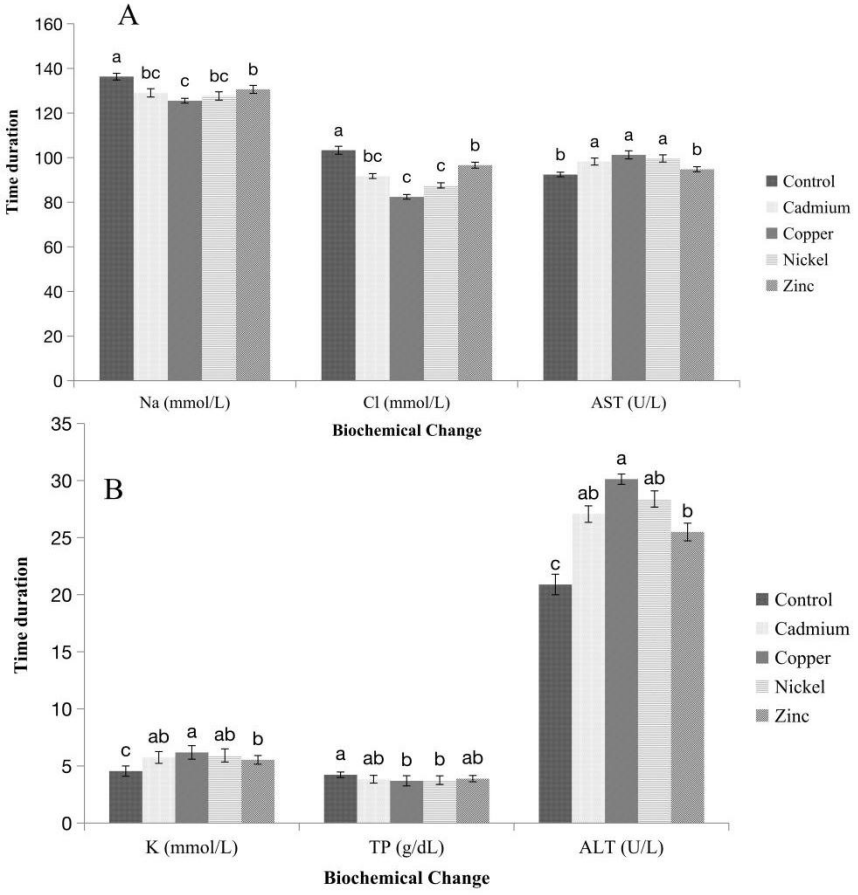

Figure 2: Changes in serum biochemical parameters under the exposure of metals for different time durations.

parameters were recorded after 24-hr of metals exposure and exposure of Zn. The Na, Cl and TP level in control fish was significantly higher than treated. Maximum level of all these *the* same was minimum after 96-hr of exposure (Figure 2).

The present results are in agreement with the findings of Oner et al. [40]. They found that Na and Cl levels decreased in serum of *Oreochromis niloticus* following metals exposure. Similarly, previous studies of Grosell et al. [41] and Firat et al. [42]. showed loss of Na ions after Cu exposure. Levels of Na and Cl decreased in *Oreochromis mossambicus* as studied by Pelgrom et al. [43]. and *Cyprinion maleness* [44]. after exposure to metals. Grosell et l. [45]. reported the toxic effect of metals on gills function which resulted in loss of Na ions. Hypoproteinemia observed in metals treated fishes could be due to liver and kidney damage. This is in agreement with [46]. who stated that every 2-hr analysis of serum total protein level of *Cyprinus carpio* fish showed an initial sharp increase for varying periods from 2- to 20-hr. After this period a steady decline in serum total protein level was observed over a period of 72-hr metals exposure.

The exposure of metals resulted in progressive increase in potassium (K), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level in fish with increasing exposure durations while it was consistent in control. The fish exposed to Cu and Zn had maximum and minimum K, AST and ALT level, respectively than other metals (Figure 2).

This is in agreement with Vaglio et al. [47]. who observed an increase in liver enzymes activities of fish *Sparus aurata* exposed to Cd. [48]. recorded an increase of liver enzyme activities in stressed *Epinephelus areolatus* fish due to hepatic cells injury or increased synthesis of these enzymes by the liver. Increases in liver enzyme activities in the serum of metals treated fish are assumed to be a result of liver damage by metals [49]. The Cd effect on *Oreochromis mykiss* caused an increase in level of K described by Chowdhury et al. [50].

3.3 Molecular Changes

A total of 59 bands with molecular sizes ranging from 469 to 2654 bp were amplified with six primers using the liver sample of control *C. mrigala*. After exposure to the metals, the amplified products of genomic DNA revealed some differences from fingerprinting patterns of control *C. mrigala*. Present findings are in agreement with those of Galindo et al. [51]. who studied the alterations in RAPD profiles, including appearance and disappearance of bands, after 6-hr, 24-hr and 15-day of Al exposure. These results are consistent with the observations of Zhou et al. [52], who validated DNA damage with RAPD analyses in marine ciliate *Euplotes vannus* exposed to nitrofurazone and found that the damage was dose and exposure time dependent. Oliveira et al. [53]. documented DNA damage in the fish under acute exposure of Cu. [54]. reported Al concentration dependent increase in DNA damage in the lymphocytes of *Cyprinus carpio*.

The percentage of genomic DNA template stability (GTS) in metals treated fish as compared to control at various exposure periods has been presented in Figure 3. It was observed that the percentage of GTS in the fish decreased concomitantly with increase in the exposure duration. However, the minimum of GTS (81.36 %) was observed after 96-hr Cu exposure. The decrease of GTS is considered as the first molecular response toward a toxicant and has been demonstrated being directly related to the extent of DNA damage and/or to the efficiency of DNA repair and replication [55]. The decrease in GTS may be the result of band disappearance and appearance of new bands. The data obtained in the present study on genomic stability are in agreement with the findings of Mohanty et al. [56] who examined *Labeo rohita* fingerlings exposed to furadan at 24-, 48-72- and 96-hr after exposure.

Dendrogram was constructed using “between-groups linkage” method to estimate the level of DNA polymorphism among control and metals treated fish. The squared Euclidean distance between control and metals treated fish at various exposure durations has been given in Figure 4. The farthest squared Euclidean distance (15) from control was recorded for fish exposed to Cu for 96-hr while the nearest squared Euclidean distance (1) was recorded for fish exposed to Ni and Zn for 24-hrs. In the present study, there is an obvious distance between the fingerprinting from fishes treated with metals and control fishes. In a previous study, Zhiyi et al. [57]. demonstrated an

obvious distance between the fingerprinting from *Danio rerio* exposed to the chemicals tested and control. The observed changes in these parameters may provide valuable information concerning the environmental conditions and risk assessment of aquatic organisms.

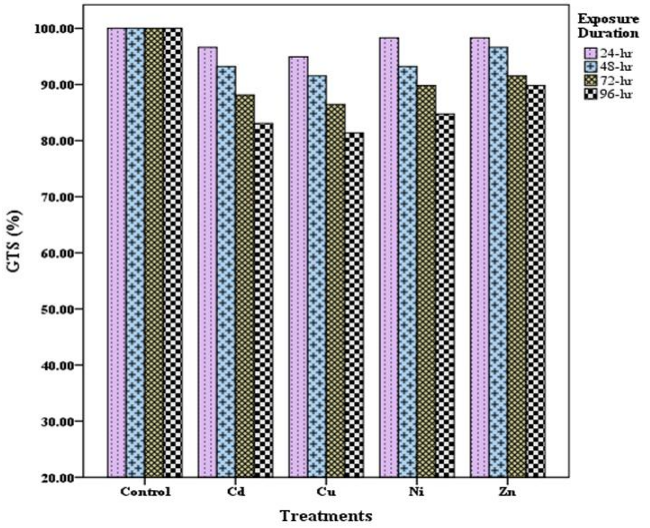

Figure 3: Percentage of genomic DNA template stability (GTS) in metals exposed fish.

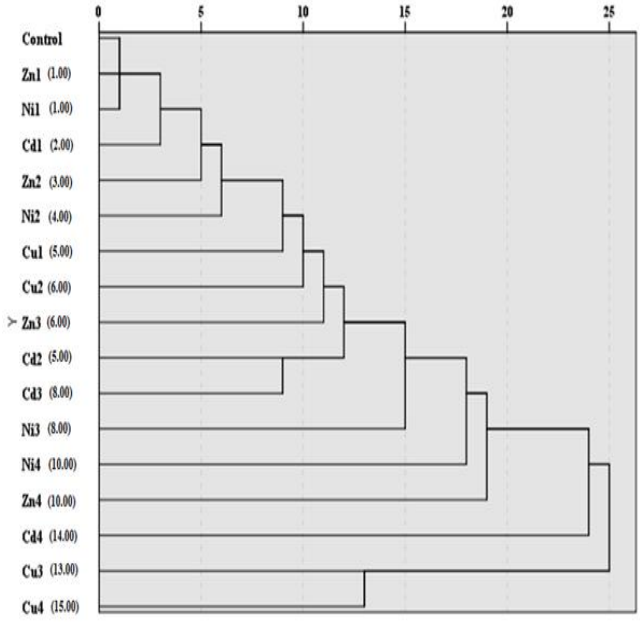

Figure 4: Dendrogram (using average linkage between groups) constructed with control and metals exposed fish for 24 (Cd1, Cu1, Ni1 and Zn1), 48 (Cd2, Cu2, Ni2 and Zn2), 72 (Cd3, Cu3, Ni3 and Zn3) and 96-hr (Cd4, Cu4, Ni4 and Zn4). Numerical values in parenthesis on Y axis denote the squared Euclidean distance from control

4. Conclusions

The present toxicity study on fish revealed the significant effects of metals on fish blood related parameters and DNA damage. This research work will contribute to the applied and basic research needs of aquatic toxicology. Based on results, it appears that human manipulation has a major impact on the fish and the studied parameters are useful tools for detecting this.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

Formal analysis, Warda Hassan; Investigations, Dr Sajid Abdullah; Software, Sana Ashraf; Writing – original draft, Warda Hassan; Review & Editing, Shaza Zaheer.

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REFERENCES

1. Sachar, A. and Raina, S., 2014. Hematological alterations induced by lindane in a fish, Aspidoparia morar. Global Journal of Biological Agriculture and Health Sciences, 3, pp. 38-42.
2. Luoma, S. M. and Rainbow, P. S., 2008. Metal contamination in aquatic environments: Science and lateral management. Cambridge Univ. Press: Cambridge, pp. 573.
3. Sridhara, C. N., Kamala, C., Samuel S. and Raj, D., 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicology and Environment Safety, 69, pp. 513-524.
4. Dhanapakiam, J., Ramasamy, V. R. and Joseph, J. M., 2006. Changes in the level of transaminases in Indian major carp, Labeo rohita exposed to sublethal concentration of tannery and distillery effluents. Journal of Environmental Biology, 27, pp. 567-570.
5. Ketpadung, R. and Tangkrock-Olan, N., 2006. Changes in oxygen consumption andheart rate of the blue swimming crab, Portunus pelagicus (Linnaeus, 1766) following exposure to sublethal concentrations of copper. Journal of Environmental Biology, 27, pp. 7-12.
6. Lodhi, H.S., Khan, M.A., Verma, R. S. and Sharma, U. D., 2006. Acute toxicity of coppersulphate to fresh water prawns. Journal of Environmental Biology, 27, pp. 585-588.
7. Magyarosy, A., Laidlaw, R. D., Kilaas, R., Echer, C. and Clark, D. S., 2002. Nickel accumulation and nickel

oxalate precipitation by Aspergillus niger. Applied Microbiology and Biotechnology, 59, pp. 382-388.
8. Doreswamy, K., Shrilatha, B., Rajeshkumar, T. and Muralidhara, T., 2004. Nickel-induced oxidative stress in testes of mice: Evidence of DNA damage and genotoxic effects. Journal of Andrology, 25, pp. 996-1003.
9. Hoang, T. C., Tomasso, J. R. and Klaine, S. J., 2004. Influence of water quality and age on nickel toxicity tofathead minnows (Pimephales promelas). Environmental Toxicology and Chemistry, 23, pp. 86-92.
10. Gupta, V. K., Rastogi, A., Saini V. K. and Neeraj, J., 2006. Biosorption of copper (II) from aqueous solutions by Spirogyra species. Journal of Colloid and Interface Sciences, 296, pp. 59-63.
11. Varin, A., Larbi, A., Dedoussis, G., Stavroula, V., Kanoni, S. and Jajte, J., 2008. In vitro and in vivo effects of zinc on cytokine signaling in human T cells. Experimental Gerontology, 4, pp. 72-82.
12. Gupta, P. and Srivastava, N., 2006. Effects of sub-lethal concentrations of zinc on histological changes and bioaccumulation of zinc by kidney of fish Channa punctatus (Bloch). Journal of Environmental Biology, 27, pp. 211-215.
13. Pandey, G., 2013. Overviews on diversity of fish. Res. Journal of Animal Veterinary and Fishery Sciences, 8, pp. 12-18.
14. Ajani, E. K. and Akpoilih, B. U., 2010. Effect of chronic dietary copper exposure on hematology and histology of common carp (Cyprinus carpio L.). Journal of Applied Sciences and Environmental Management, 14, pp. 39-45.
15. Al-Ghanim, K. A., 2011. Impact of nickel (Ni) on hematological parameters and behavioral changes in Cyprinus carpio (common carp). African Journal of Biotechnology 10, pp. 13860-13866.
16. Shokr, E. A. M., 2015. Effect of zinc on hematology and biochemistry of Nile tilapia. Journal of Chemical and Pharmaceutical Research, 7, pp. 1943-1950.
17. Jacobson-Kram, D. and Keller, K. A., 2001. Toxicology testing handbook. Marcel Dekker, New York.
18. Vargas, V. M., Migliavacca, S. B., DeMelo, A. C., Horn, R. C., Guidobono, R. R., Ferreira I. C. D. and Pestana, M. H., 2001. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. Mutatation Research, 490, pp. 141-158.
19. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. and Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico Biological Interactions, 160, pp. 1-40.
20. Barbosa, J. S., Cabral, T. M., Ferreira, D. N., Agnez-Lima, L. F. and De-Medeiros, S. R., 2010. Genotoxicity assessment in aquatic environment impacted by the presence of heavy metals. Ecotoxicology and Environmental Safety, 73, pp. 320-325.
21. Ates, B., Orun, I., Talas, Z. S., Durmaz, G. and Yilmaz, I., 2008. Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss Walbaum 1792) exposed to Pb2+ and Cu2+. Fish Physiology and Biochemistry, 34, pp. 53-59.
22. Osman, A. G. M., Abuel-Fadl, K. Y. and Kloas, W., 2012. In situ evaluation of the genotoxic potential of the river Nile: II. Detection of DNA strand-breakage and apoptosis in Oreochromis niloticus (Linnaeus, 1758) and Clarias gariepinus (Burchell,1822). Mutatation Research, 747, pp. 14-21.
23. Khan, B., Khan, H., Muhammad, S. and Khan, T., 2012. Heavy metals concentration trends in three fish species from shah alam river Pakhtunkhwa province, Pakistan.International Journal of Environmental Science and Technology, 3,1-8.
24. Eaton, D. L. and Gilbert, S. S., 2008. Principles of toxicology: In Casarett & Doull's toxicology: The basic science of poisons. 7th Ed. New York: McGraw-Hill. pp. 11-44.
25. Yaqub, S. and Javed, M., 2012. Acute toxicity of waterborne and dietary cadmium andcobalt for fish. International Journal of Agriculture and Biology, 14, pp. 276-280.
26. Kousar, S. and Javed, M., 2014. Assessment of DNA damage in peripheral blood erythrocytes of fish exposed to arsenic under laboratory conditions. International Journal of Current Microbiology and Applied Sciences, 3, pp. 877-888.
27. A.P.H.A, 2005. Standard methods for the examination of water and wastewater (21stEd.). Washington DC.
28. Yue, G. H. and Orban, L., 2005. A simple and affordable method for high-throughput DNA extraction from animal tissues for polymerase chain reaction. Electrophoresis. 26, pp. 3081-3083.
29. Steel, R.G.D., Torrie, J. H. and Dinkkey, D. A., 1996. Principles and Procedures of Statistics (3rd Ed.) McGraw Hill Book Co., Singapore.
30. Hedayati, A. and Ghaffari, Z., 2013. Effect of mercuric chloride on some hematological, biochemical parameters in silver carp (Hypophthalmichthys molitrix). International Journal of Veterinary Medicine and Research, 50, pp. 1-11.
31. Al-Ghanim, K. A, 2011. Impact of nickel (Ni) on hematological parameters and behavioral changes in Cyprinus carpio (common carp). African Journal of Biotechnology, 10, pp. 13860-13866.
32. Ateeq, B., Farah, M. A., Ali M. N. and Ahmad, W., 2005. Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2, 4-dichloro phenoxyacetic acid and butachlor. Mutation Research, 518, pp. 135-144.
33. Ololade, I. A. and Oginni, O., 2010. Toxic stress and hematological effects of nickel on African catfish, Clarias gariepinus, fingerlings. Journal of Environmental Chemistry and Ecotoxicology, 2, pp. 14-19.

34. Capkin, E., Kayis, S., Boran, H. and Altinok, I., 2010. Acute toxicity of some agriculture fertilizers to rain bow trout, Oncorhynchus mykiss. Turkish Journal of Fishries and Aquatic Sciences, 10, pp.19- 25.
35. Abhijith, B. D., Ramesh, M. and Poopal, R. K., 2012. Sub-lethal toxicological evaluation of methyl parathion on some hematological and biochemical parameters in an Indian major carp Catla catla. Comparative Clinical Pathology, 21, pp. 55-61.
36. Moraes, F. R., 2007. Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. Veterinary Clinical Pathology and Medicines, 36, pp. 49-54.
37. Mahananda, H. B., 2014. Alterations in some hemato-biochemical parameters of a fresh water, air breathing fish, Channa punctatus (Bloch) under the stress of chronic, sub-lethal dose of nickel. Biolife, 2, pp. 1392-1397.
38. Abhijith, B. D., Ramesh, M. and Poopal, R. K., 2012. Sub-lethal toxicological evaluation of methyl parathion on some hematological and biochemical parameters in an Indian major carp Catla catla. Comparative Clinical Pathology, 21, pp. 55-61.
39. Singh, D., Nath, K., Trivedi, S. P. and Sharma, Y. K., 2008. Impact of copper on hematological profile of freshwater fish, Channa punctatus. Journal of Environmental Biology, 29, pp. 253-257.
40. Oner, M., Atli G. and Canli, M., 2008. Changes in serum biochemical parameters of freshwater fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr,Cu, Zn) exposures. Environmental Toxicology and Chemistry, 27, pp. 360-366.
41. Grosell, M., Blanchard, J., Brix, B. V. and Gerdes, R., 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. Aquatic Toxicology, 84, pp.162-172.
42. Firat, O., Cogun, H., Yuzereroglu, T., Gok, G., Firat, O., Kargin, F. and Kotemen, Y., 2011. A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper and lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus. Fish Physiology and Biochemistry, 37, pp. 657-666.
43. Pelgrom, S. M. G. J., Lock, R. A. C., Balm, P. H. and Bonga, S. E. W., 1995. Effects of combined waterborne Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, Oreochromis mossambicus. Comparative Biochemistry and Physiology, 111, pp. 227-235.
44. Al-Attar, A. M., 2006. The physiological responses of the fish, Cyprinion mhalensis to mercury intoxication. Journal of Egyptan German Society of Zoology, 51, pp.123-137.
45. Grosell, M., Blanchard, J., Brix, B. V. and Gerdes, R., 2007. Physiology is pivotal for interactions between salinity and acute copper toxicity to fish and invertebrates. Aquatic Toxicology, 84, pp. 162-172.
46. Gopal, V., Parvathy, S. and Balasubramanian, P. R., 1997. Effect of heavy metals on the blood protein biochemistry of the fish Cyprinus carpio and its use as a big indicator of pollution stress. Environmental Monitorind and Assessment, 48, pp. 117-124.
47. Vaglio, A. and Landriscina, C., 1999. Changes in liver enzyme activity in the teleost Sparus aurata in response to cadmium intoxication. Ecotoxicology and Environmental Safety, 43, pp. 111-116.
48. Wu, R. S., Pollino, C. A., Au, D. W., Zheng, D. W., Yuen, B. and Lam, P. K., 2003. Evaluation of biomarkers of exposure and effect in juvenile areolated grouper (Epinephelus areolatus) on food-borne exposure to benzo-a-pyrene. Environmental Toxicology and Chemistry, 22, pp. 68-73.
49. Kim, S. G. and Kang, J. C., 2004. Effect of dietary copper exposure on accumulation, growth and hematological parameters of the juvenile rockfish, Sebastes schlegeli. Marine Environmental Research, 58, pp. 65-82.
50. Chowdhury, M. J., Pane, E. F. and Wood, C. M., 2004. Physiological effects of dietary cadmium acclimation and waterborn cadmium challenge in rainbow trout: respiratory, ionoregulatory and stress parameters. Comparative Biochemistry and Physiology, 139, pp. 163-173.
51. Galindo, B. A., Troilo, G., Colus, I. M. S., Martinez, C. B. R. and Sofia, S. H., 2010. Genotoxic effects of aluminium on the Neotropical fish Prochilodus lineatus. Water Air Soil Pollution, 212, pp. 419-428.
52. Zhou, L., Li, J., Lin, X. and Al-Rasheid, K. A. S., 2011. Use of RAPD to detect DNA damage induced by nitrofurazone in marine ciliate, Euplotes vannus (Protozoa, Ciliophora). Aquatic Toxicology, 103, pp. 225-232.
53. Oliveira, B. L., Fernandes, L. F. L., Bianchini, A., Gomes, A. R. C., Silva, B. F., Brandao, G. P. and Gomes, L. C., 2014. Acute copper toxicity in juvenile fat snook Centropomus parallelus (Teleostei: Centropomidae) in sea water. Neotropical Ichthyology, 12, pp. 167-175.
54. Garcia-Medina, S., Razo-Estrada, C., Galar-Martinez, M., Cortez-Barberena, E., Gomez- Olivan, L. M., Alvarez-Gonzalez, I. and Madrigal-Bujaidar, E., 2011. Genotoxic and cytotoxic effects induced by aluminium in the lymphocytes of the common carp (Cyprinus carpio). Comparative Biochemistry and Physiology, 153, pp.113-118.
55. Rocco, L., Santonastaso, M., Mottola, F., Costagliola, D., Suero, T., Pacifico, S. and Stingo, V., 2015. Genotoxicity assessment of TiO2 nanoparticles in the teleost Danio rerio. Ecotoxicology and Environmental Safety, 113, pp. 223-230.
56. Mohanty, G., Mohanty, J., Garnayak, S. K. and Dutta, S. K., 2009. PCR based detection of furadan genotoxicity effects in rohu (Labeo rohita) fingerlings. Veterinary Research and Communications, 33, pp. 771-780.
57. Zhiyi, R. and Haowen, Y., 2004. A method for genotoxicity detection using random amplified

polymorphism DNA with Danio rerio. Ecotoxicology and Environmental , Safety, 58, pp. 96-103.

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Research article

Effect of Bromelain-Fermented Diets on Digestive Enzyme Activities and Muscle Proximate Composition of *Labeo Rohita*

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Abstract

Plant proteins are considered most suitable to replace fish meal because they are cheap, readily available, and abundant. However, plant proteins are not digested efficiently in fish due to the presence of complex protein structures. Therefore, this preliminary study was conducted to evaluate the effect of bromelain-fermented plant diets on the digestive enzyme activities and muscle proximate composition of *Labeo rohita*. For this purpose, healthy fingerlings with an average initial weight of 10±0.2 g were procured and acclimatized under laboratory conditions. Then, 20 fingerlings were transferred to each of 20 glass aquaria (160 L capacity) in three replicates. Five diets were prepared using plant meals and were fermented using 10, 20, 30 and 40% bromelain powder for 48 hours at 55°C. The control diet was not fermented. Fermented diets were fed to fish for 90 days, and then digestive enzyme activities and muscle proximate composition were determined. Fish fed bromelain-fermented diets (10-40%) showed a significant increase in digestive enzyme activities (protease and lipase) compared with the control group. However, no significant effect was observed on the amylase activities in fish fed bromelain-fermented diets. Muscle proximate composition revealed that crude protein (CP) contents were increased while crude fat (CF) contents were decreased in fish fed fermented diets compared with the control group. However, no significant differences were observed in moisture and ash contents. In conclusion, fish fed fermented diets showed enhanced activities of digestive enzymes (protease and lipase) and crude protein contents in the muscle of *L. rohita*. Therefore, it is recommended to conduct a detailed trial on bromelain fermentation.

Keywords: Bromelain, digestive enzyme activities, fermentation, muscle proximate, plant protein

1. Introduction

Proteins constitute a crucial component of fish diets, serving as the primary source of essential amino acids required for growth, tissue repair, and metabolic activities. Traditionally, fish meal has been the predominant protein source in aquafeed due to its well-balanced amino acid profile and palatability [1]. However, the escalating costs and limited availability of fish meal, coupled with concerns over its sustainability and environmental implications, have driven researchers and feed manufacturers to explore alternative protein sources [2].

The quest for viable alternative protein sources has led to investigations into plant-based ingredients. Plant meals derived from various sources possess the potential to replace or supplement fish meal in aquafeed formulations [3]. However, the full substitution of fish meal with plant meals presents challenges, mainly related to differences in amino acid profiles, anti-nutritional factors, and digestibility. These factors can

potentially hinder the growth and performance of farmed fish when plant-based ingredients dominate the feed composition [2, 3].

One promising strategy to overcome the limitations of plant-based ingredients is fermentation. Fermentation has gained attention as a means to improve the nutritional quality and digestibility of plant protein sources in aquafeeds [4]. Exogenous proteases such as bromelain breakdown complex carbohydrates and enhance the availability of nutrients for fish digestion and absorption. The utilization of fermentation techniques in digesting plant protein sources holds the potential to create more balanced and sustainable aquafeeds, thereby contributing to the growth and well-being of farmed fish [5, 6].

Labeo rohita, commonly known as Rohu, is a notable freshwater species in the aquaculture sector that belongs to the *Cyprinidae* family. It is the most widely cultured fish species in the Indian subcontinent due to its fast growth rate. Furthermore, this species has a delicious taste, consumer preference and enormous demand in the local market [7]. This study was performed to evaluate the effects of bromelain fermentation on the digestive enzyme activities and proximate composition of *L. rohita*, shedding light on the potential benefits and implications of incorporating fermented plant protein sources in the diets of this commercially important fish.

2. Material and Methods

2.1 Feed formulation and fermentation process

Feed ingredients were taken from local stores and were processed to a fine powder. Then, ground feed ingredients were subjected to proximate composition analysis [8]. Then, the ingredients (other than vitamin premix, mineral mixture, and fish oil) were mixed with water (1:1) and heated to 55°C. Bromelain enzyme powder was added to the mixture at 1%, 2%, 3% and 4% concentrations and then incubated for 48 hours at 55°C. After the completion of the incubation period, the mixture was subjected to 90°C for 5 minutes for inactivation of the enzyme and then dried to formulate feed [9]. Control diet ingredients were not fermented. Fish oil, vitamin premix and mineral mixture were mixed with fermented ingredients in a mixer. Water was added to make a stiff dough that was pelleted through meat mincer (Anex, AG3060). The pellets were shade-dried, weighed and packed in self-sealing bags. Five experimental diets containing 0, 10, 20, 30 and 40 g/kg fermented bromelain were prepared. The experimental diet was given to fingerlings throughout the 90 days.

Table 1. Composition of the experimental diets.

Ingredients (%)	**Percentage**
Fish Meal	10
Soybean Meal	25
Sunflower Meal	25
Wheat Flour	10
Rice Polish	20
Fish Oil	7.0
Mineral Mixture*	1.0
Vitamin premix**	1.0
Choline Chloride	1.0
Proximate composition%	
Dry matter	91.23±0.15
Crude protein	30.45±0.34
Crude fat	12.04±0.12
Ash	4.54±0.05

*Mineral mixture contained the following per kilogram: selenium 100 mg, manganese 23750 mg, iodine 2750 mg, copper 5000 mg, zinc 75000 mg, magnesium 200000 mg, and cobalt 2000 mg.

**Vitamin premix contained the following per kilogram: 60000 mg inositol, 2400 mg vitamin E, 4000000 IU, 2400 mg vitamin K3, 4000 mg vitamin B1, 4000 mg vitamin B6, 1200 mg folic acid, 40000 mg vitamin C, 10 mg vitamin B12, vitamin A 10000 mg, 100 mg D-biotin, 4000 mg niacin, Cal. D. Pantothenate, vitamin D3 480000 IU.

2.2 Fish procurement and rearing conditions

L. rohita (10 ± 0.2 g) were obtained from the Department of Fisheries and Aquaculture, UVAS, Ravi Campus, Pattoki. Fish were acclimatized to laboratory conditions after treatment with $KMnO_4$. After acclimatization, the fish were distributed to glass aquaria at a stocking rate of 20 fish per aquarium in triplicate (a total of 45 aquaria). During the 3 months of the experimental trial, the fish were fed two times a day at 3% of

their body weight. During the trial, water quality parameters (pH, temperature, DO) were checked throughout the experiment, and their mean values were 28±0.7 ℃, 7.2±0.2 and 7.6±0.3 mg per liter, respectively.

2.3 Fish harvesting

At the end of the feeding trial, 5 fish were anaesthetized with clove oil (5 mg/L) and dissected. Hepatopancreases of these fish were collected and stored in distilled water. Another five fish from each treatment were sacrificed for muscle proximate analysis.

2.4 Digestive enzyme activities

Hepatopancreas samples (1 g) were homogenized with 5% sucrose solution (0.25 M) from each treatment. The homogenate was centrifuged at 5000 × g for 15 minutes, and the supernatant was collected for further analysis of digestive enzymes. Amylase activities were determined using the start solution following Rick and Stegbauer [10]. The casein digestion method of Kunitz [11] was used to calculate the protease enzyme activity. Lipase activities were determined using p-nitrophenylpalmitate (pNPP) following the method of Mahadik, Puntambekar [12].

2.5 Muscle proximate composition analysis

The muscle proximate composition was determined following the standard protocols of AOAC [8].

2.6 Statistical analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) using SPSS (version 23). Means of the parameters were compared using Tukey's HSD test. The results were considered significant at $p < 0.05$.

3. Results

3.1 Digestive enzyme activities

Fermented diets with different levels of bromelain showed significant improvements in digestive enzyme activities (Table 2). Diets fermented with all bromelain levels showed higher protease activities compared with the control group. However, all bromelain levels showed nonsignificant differences from each other. Similarly, lipase activities were enhanced in fish fed diets fermented with graded levels of bromelain compared to the control group. Amylase activities were not affected by different levels of bromelain in fermented feeds.

3.2 Muscle proximate composition

The proximate muscle composition of *L. rohita* fed fermented diets containing various levels of bromelain is presented in Table 3. Moisture contents were not affected in the muscle of fish. Significantly higher crude protein contents were observed in fish fed fermented diets containing 10-40% bromelain compared with the control group. Fish fed a fermented diet with 10-40% bromelain showed a significant reduction in crude lipid content compared with the control group. Ash contents were not affected by fermented diets.

4. Discussion

The main challenges in the aquaculture sector are the consistent availability and high cost of feeds [13]. The cost of feed is critical since it accounts for 30-70% of overall expenses and helps in determining the sustainability of aquaculture success [14]. To decrease the cost of feed, plant protein sources are excellent options and are available abundantly. However, plant proteins have lower digestibility than fishmeal due to the presence of complex protein structures, high carbohydrate levels and antinutritional factors [3]. Therefore, fermentation with exogenous enzymes can be useful to enhance the digestibility of plant proteins. The bromelain enzyme is present in natural compounds such as pineapple (*Ananas comosus*) fruit peels and stems and is a blend of proteolytic enzymes [15].

In the current study, digestive enzyme activities were enhanced significantly in fish fed fermented diets with bromelain, except amylase activities. This might be because bromelain primarily aids in protein digestion by partly hydrolysing protein molecules into smaller units and boosting their bioavailability [16]. Fermentation is an efficient method to enhance the quality of feed ingredients [17] by inactivating anti-nutritional components [18] and increasing the absorption of nutrients [19]. Digestive and brush border enzyme activities are closely related to fish digestive and metabolic capabilities [20]. The findings of our study are in line with the results of [21], who reported that dietary fermented bromelain supplementation had no effect on amylase activity in the mid-

Table 2. Effect of bromelain-fermented diets on the hepatopancreatic digestive enzyme activities of *Labeo rohita*.

Parameters	Diets fermented with different levels of bromelain				
	0%	10%	20%	30%	40%
Amylase	2.76±0.04	2.79±0.04	2.78±0.03	2.79±0.03	2.79±0.04
Protease	0.76±0.04^{b}	0.89±0.05^{a}	0.88±0.04^{a}	0.89±0.05^{a}	0.9±0.04^{a}
Lipase	0.49±0.03^{b}	0.71±0.04^{a}	0.7±0.04^{a}	0.69±0.06^{a}	0.68±0.05^{a}

The data presented are the mean ± SD of three replicates. Values with different superscripts in a row indicate significant differences ($p < 0.05$).

Table 3. Effect of bromelain-fermented diets on the muscle proximate composition of *Labeo rohita*.

Parameters	Diets fermented with different levels of bromelain				
	0%	10%	20%	30%	40%
Moisture	75.12±0.07	75.14±0.06	75.11±0.06	75.12±0.07	75.12±0.06
Crude protein	17.19±0.06^{b}	17.41±0.07^{a}	17.4±0.05^{a}	17.37±0.05^{a}	17.38±0.07^{a}
Crude fat	4.39±0.04^{b}	4.23±0.06^{a}	4.21±0.06^{a}	4.23±0.06^{a}	4.24±0.06^{a}
Ash	2.55±0.03	2.55±0.03	2.54±0.03	2.54±0.03	2.53±0.02

The data presented are the mean ± SD of three replicates. Values with different superscripts in a row indicate significant differences ($p < 0.05$).

gut and hindgut of Gibel carp. Similarly, protease and lipase activity increased in Nile tilapia intestines given a diet combined with exogenous enzymes compared to the control [22].

Proximate composition is an excellent indicator of the nutrient profile in the body [23]. In the current study, crude protein contents were significantly increased while crude fat contents were decreased in fish fed diets fermented by bromelain. The enhancement of protease activity indicates that fish effectively utilized proteins in the muscle compared with the control group, which is in line with the study of [24]. This can be attributed to the enhancement of protease activities, which can lead to better nutrient utilization. A decrease in crude fat content in the muscle of fish indicates a better nutrient profile, as the aim is to increase the protein and decrease the fat contents. This might be due to an increase in lipase activities, which enhanced the utilization of lipids in the body instead of their deposition. No differences were observed in moisture and ash contents, which is supported by [25].

5. Conclusion

In conclusion, *Labeo* rohita fed diets fermented by bromelain showed significantly enhanced digestive enzyme activities compared to the control group. Protease and lipase activities were enhanced, while amylase activities were not affected. A significant increase in crude protein and a decrease in crude lipid contents in muscles were observed. However, no alterations were noted in moisture and ash contents. This study is helpful for the aquafeed industry to solve the problem of expensive and scarce fishmeal using fermented plant-based diets. However, the current study is limited to fermentation of the whole diet, including fishmeal and other ingredients. Further studies need to be conducted on separate fermented plant ingredients and their replacement with fishmeal.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

TY: Investigation; MF: Conceptualization, Methodology, Supervision; SZHS: Conceptualization, Methodology, Writing - Review & Editing; WA: Writing - Original Draft, Formal analysis; SQ: Data Curation

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REFERENCES

1. Olsen, R.L. and M.R. Hasan, A limited supply of fishmeal: Impact on future increases in global aquaculture production. Trends in Food Science & Technology, 2012. 27(2): p. 120-128.

2. Mugwanya, M., et al., Replacement of fish meal with fermented plant proteins in the aquafeed industry: A systematic review and meta-analysis. Reviews in Aquaculture, 2022. 15(1): p. 62-88.
3. Hua, K., et al., The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. One Earth, 2019. 1(3): p. 316-329.
4. Dawood, M.A.O. and S. Koshio, Application of fermentation strategy in aquafeed for sustainable aquaculture. Reviews in Aquaculture, 2019. 12(2): p. 987-1002.
5. Gopalraaj, J., et al., The effect of dietary supplementation of proteases on growth, digestive enzymes, oxidative stress, and intestinal morphology in fishes – A review. Aquaculture International, 2023.
6. Zheng, C.c., et al., Exogenous enzymes as functional additives in finfish aquaculture. Aquaculture Nutrition, 2019. 26(2): p. 213-224.
7. Fatima, M., M. Afzal, and S.Z.H. Shah, Effect of dietary oxidized oil and vitamin E on growth performance, lipid peroxidation and fatty acid profile ofLabeo rohitafingerlings. Aquaculture Nutrition, 2019. 25(2): p. 281-291.
8. AOAC, Official methods of analysis of AOAC International. Twentieth edition. ed. 2016, Rockville, MD: AOAC International. 2 volumes : illustrations.
9. Singh, T.A., P. K. Sarangi, and N.J. Singh, Tenderisation of Meat by Bromelain Enzyme Extracted from Pineapple Wastes. International Journal of Current Microbiology and Applied Sciences, 2018. 7(09): p. 3256-3264.
10. Rick, W. and H.P. Stegbauer, α-Amylase Measurement of Reducing Groups, in Methods of Enzymatic Analysis. 1974. p. 885-890.
11. Kunitz, M., Isolation of a Crystalline Protein Compound of Trypsin and of Soybean Trypsin-Inhibitor. Journal of General Physiology, 1947. 30(4): p. 311-320.
12. Mahadik, N.D., et al., Production of acidic lipase by Aspergillus niger in solid state fermentation. Process Biochemistry, 2002. 38(5): p. 715-721.
13. Dawood, M.A.O., Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Reviews in Aquaculture, 2020. 13(1): p. 642-663.
14. Kumar, P., et al., Alternate feeding strategies for optimum nutrient utilization and reducing feed cost for semi-intensive practices in aquaculture system-A review. Agricultural Reviews, 2017. 38(02).
15. Azizan, A., et al., Potentially Bioactive Metabolites from Pineapple Waste Extracts and Their Antioxidant and α-Glucosidase Inhibitory Activities by 1H NMR. Foods, 2020. 9(2).
16. Barbosa Abreu, G., et al., Estimation of Genetic Parameters of Turiaçu Pineapple Clones and Genetic Correlation between Traits. Agricultural Sciences, 2017. 08(11): p. 1253-1262.
17. Han, B.-Z., et al., Mucoraceous moulds involved in the commercial fermentation of Sufu Pehtze. Antonie van Leeuwenhoek, 2004. 85(3): p. 253-257.
18. Song, Y.S., et al., Immunoreactivity reduction of soybean meal by fermentation, effect on amino acid composition and antigenicity of commercial soy products. Food Chemistry, 2008. 108(2): p. 571-581.
19. Hotz, C. and R.S. Gibson, Traditional Food-Processing and Preparation Practices to Enhance the Bioavailability of Micronutrients in Plant-Based Diets1. The Journal of Nutrition, 2007. 137(4): p. 1097-1100.
20. Hakim, Y., et al., Relationship between intestinal brush border enzymatic activity and growth rate in tilapias fed diets containing 30% or 48% protein. Aquaculture, 2006. 257(1-4): p. 420-428.
21. Feng, X., et al., Bromelain Kinetics and Mechanism on Myofibril from Golden Pomfret (Trachinotus blochii). Journal of Food Science, 2018. 83(8): p. 2148-2158.
22. Lin, S., K. Mai, and B. Tan, Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, Oreochromis niloticus x O. aureus. Aquaculture Research, 2007. 38(15): p. 1645-1653.
23. Ahmed, I., et al., Muscle proximate composition of various food fish species and their nutritional significance: A review. Journal of Animal Physiology and Animal Nutrition, 2022. 106(3): p. 690-719.
24. Wiszniewski, G., et al., The use of bromelain as a feed additive in fish diets: Growth performance, intestinal morphology, digestive enzyme and immune response of juvenile Sterlet (Acipenser ruthenus). Aquaculture Nutrition, 2019. 25(6): p. 1289-1299.
25. Seong, M., et al., The effects of different levels of dietary fermented plant-based protein concentrate on growth, hematology and non-specific immune responses in juvenile olive flounder, Paralichthys olivaceus. Aquaculture, 2018. 483: p. 196-202.

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Review article

Effects of Microplastic Pollution on Marine Environment: a Mini Review

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Abstract

Global awareness of microplastic contamination and its effects on the environment has grown. Plastics are resistant to breakdown and penetrate aquatic environments and are ultimately easily accessible to a wide range of aquatic animals and ultimately transported along the food web. Microplastics in cells and tissues have long-term consequences for marine organisms. A major factor in the spread of microplastics to the environment is their high adsorption capacity on the water surface. Microplastics and persistent organic pollutants interact to make the pollutants even more dangerous to living things. Microplastic pollution and its impact on the ecological environment have attracted worldwide attention. To effectively control microplastic pollution, there is a need to understand how Microplastics affect the ecological environment. This review discusses the formation, transfer and distribution of Micro plastics and the current physical, chemical and biological impacts on the environment. It is crucial to prevent plastic additives' overuse and enact laws and regulations to control plastic waste on account of the current threats posed by Microplastics to marine life and human health. We can eliminate marine litter by establishing plastic recycling schemes in the future or by promoting plastic awareness programs through both social and informational media.

Keywords: MP Micro plastic pollution, Marine animals, polyethylene (PE), POPs, plastic degradation

1. Introduction

The first reports of the plastic waste structure appeared in the early 1970s. In the mid-20th century, notice an increase in plastic manufacture. Global plastics demand has risen to 245 million tonnes [1-3]. Plastics have a wide range of applications since they are strong, lightweight, and adaptable. The book Marine Pollution by Plastics examines how plastics affect the marine environment toxicologically. Pathogens, metals, and organic contaminants in the environment can all be absorbed by Microplastics. The presence of Micro plastics in the marine environment is extremely harmful to marine ecosystems. Pesticides, POPs (persistent organic pollutants), hydrocarbons, heavy metals, plastics, and Microplastics are among the contaminants in the marine environment. Marine life can consume small particles that are high in POPS (Persistent Organic Pollutants). Food webs can be thrown off if marine animals ingest these POPs [4, 5].

Around 60-80% of the world's litter is in the form of plastic, with almost 10% of annual plastic production ending up into oceans. Plastic pollution is now highly visible in oceans across the planet and it can take several hundred years to degrade in the environment. Surface to seafloor and pole to pole, micro plastic waste can move, reproduce, and accumulate in the ecosystem. This kind of pollution threatens marine life since it is pervasive and persistent in waters all around the world. Degradation of the plastic on the beach results in surface

fractures that produce tiny particles that are blown into the ocean by the wind or waves. It is reported that 44% of seabird species in the world consume plastic [6, 7]. Micro plastic pollution has an adverse effect on a large variety of marine creatures. Plastic contamination has an impact on 267 marine species, according to estimates. The environment, human health, food quality, and other factors are threatened by plastic pollution. Ingesting plastics results in intestinal blockage, illness, mortality, and damage to the intestinal mucosa in marine creatures and mammals. People do not directly consume organs since Micro plastics are discovered in the intestines of living things [8]. The dangers of consuming Micro plastics by humans include tissue injury, displacement, redistribution, and retention with other body tissues. Micro plastics contaminate the most aquatic ecosystems on Earth. Micro plastics may enter the food chain by being directly eaten by marine animals but can also adhere to the surface of micro-organisms that form the prey for higher levels of the food chain, such as fish [7, 9].

Humans who consume Micro plastics may have biological impacts include intestinal obstruction or injury, decreased energy absorption, and food chain disruption. Another method of introducing micro plastic into the ocean is the breakdown of micro plastic waste [10-12]. There are an increasing number of and demand for small marine plastic particles worldwide. Zooplankton waste is another way that Micro plastics might infiltrate the marine environment. Studies have shown that different marine creatures may consume various forms of Micro plastics. Once the body can consume Micro plastics, it can expel them in the feces and produce pseudo-feces, which are harmless to the body. Around 65 million Micro plastics per day are released into rivers by wastewater treatment facilities (WWTW). The efficiency of removing Micro plastics can be raised from 72 to 99.4% [13]. Wastewater contains nitrogen and phosphorus from human waste, food, certain soaps, and detergents. Once the water is cleaned to standards set and monitored by state and federal officials, it is typically released into a local water body, where it can become a source of nitrogen and phosphorus pollution. Global demand for plastic can be change over time. As plastic is made from a range of petrochemical products, largely derived from crude oil, increased consumption of plastic is set to propel demand for raw materials like naphtha that are needed to make petrochemicals — in other words, spurring the need for more oil. For that reason plastic demand is increasing day by day.[11, 12, 14].

1.1 Types/sources of micro plastics

There are numerous different types of Micro plastics that can be found in the marine environment, and they range in size, shape, chemical makeup, and other characteristics.

Micro plastics are the main source of micro-pollution in the marine environment.

Primary Micro plastics are those produced in industrial and home settings. It is made up of synthetic raw plastic ingredients. The size of primary Micro plastics is tiny. Scrubs, infant creams, toothpaste, cleansers, and other products contain the majority of micro plastic particles. Size ranges from around 0.5 mm in diameter to about 0.1 mm [15].

Secondary Micro plastics: These are produced when macro plastics break down during specific spatial processes like ageing and weathering.

Table 1. Micro plastic types and their potential sources

Micro plastic type	Definition	Potential sources
Fragment	Hard, jagged plastic Particle	Bottles; hard, sturdy plastics
Fiber	Thin or fibrous, straight Plastic	Fishing line/nets; clothing or textiles
Pellet	Hard, rounded plastic Particle	Virgin resin pellets; facial Cleansers
Foam	Thin plane of flimsy plastic	Plastics bags, wrappers, or sheeting
Film	Lightweight, sponge-like plastic	Foam floats, Styrofoam, cushioning

Table 2. Impact of micro plastics on marine organism

Species name	Effects	References
Blue mussel (*Mytilus edilus*)	Decreased feeding activity Formations of granulocytoma in digestive glands and lysosome membranes' destabilization	[37, 38]
Mytilus galloprovincialis	Ingestion of resin pellets	[39]
Zooplankton	Zooplankton ingested and accumulated phthalic acid esters, organophosphate ester flame retardants, and plasticizers.	[38, 40]
Blue mussels (*Mytilus galloprovincialis*)	PCBs absorbed at higher amounts, which had harmful effects. The blue mussels' increased desorption of pyrene caused anomalies and fatal effects on their DNA, which suggested neurotoxic effects.	[3, 41]
Pelagic fishes and holothurians	A pelagic fish named Boops absorbed 70% of the fibers from Micro plastics. Holothurians ingesting plastic pellets through the food chain	[3, 42]
Copepod (*Calanus helgolandicus, C. cristatus*, *Euphasiapacifa*)	Consumption, decreased eating, lowered reproductive success, and decreased egg output	[43, 44]
European flat oysters (*Ostreaedulis*)	Ingestion and abnormal respiration rates	[45]
Mussel	Cytotoxicity, decrease in phagocytic activity, and increase in lysozyme activity	[46]
Sea turtles (Chelonioidea)	Ingestion	[47]
Mussel, amphipods (*Allorchestes compressa*)	Consumption, the development of Granulocytom, and lysosome membrane destabilization/accumulation of POP	[48]
Lugworm (*Arenicola marina*)	Ingestion may result in enhanced metabolic rates, a reduction in the development of fecal casts, and fitness effects.	[38, 49]
Oyster	Significant decrease in fertilization and embryo–larval growth deformities	[38, 50]
Marine fish (*Pomatoschistus microps*, *Artemia nauplii*, *Danio rerio*, *Oryzias latipes*)	Ingestion, liver inflammation, pathological and oxidative stress, lipid accumulation in liver	[51]
Mussels	Superoxide dismutase (SOD) activity was temporarily increased after exposure to Micro plastics (for 24 hours and for seven days), whereas exposure to Nano plastics resulted in an innate immunological reaction.	[52]
Commercial fish	Gastro intestinal system and fish gills. Micro plastics have Cr and Fe found on them.	[38, 40]

They are widely distributed in marine and coastal habitats worldwide. Secondary pollutants are the term for larger particles found in the soil and water. Larger plastic debris that may wash up on beaches and in the sea might cause secondary contamination [12, 16]. Optically decomposing plastic results from larger plastic waste or particles being exposed to ultraviolet (ultraviolet radiation) from the sun. Both forms of Micro plastics can be observed in marine habitats.[15, 17] (Table 1)

1.2 Occurrence of microplastic pollution in terrestrial ecosystem

Soil, the ecosystem's foundation, is under severe stress from anthropogenic pollution. Plastic breaks down relatively gradually in soil. According to several studies, there is a little breakdown of synthetic polymers in soil [12, 18].

Some research indicates that after 800 days in soil, PE only loses weight by 0.1% to 0.4%. Some claim that polyvinyl chloride (PVC) does not disintegrate in soil after 10-35 years, but polypropylene loses 0.4% of its weight after a year of incubation. Soil texture is a significant component that influences polymer breakdown [19]. By influencing soil structure, bulk density, water holding capacity, and microbiological activity, particulate matter disturbs the interactions between water and soil. There are three ways to describe soil particle matter. The first technique uses pressure fluid extraction (PEF) to detect particle matter in soil samples. However, this technique can't measure all of the MP size [18, 20].

Using this technique, the Sydney region's topsoil near industrial areas includes between 0.03% and 0.67% particle matter. Show a 0.5–5mm range for PM particle size. Another straightforward and affordable approach is adopted to extract, quantify, and differentiate the luminance of PM in soil. With a recovery rate of about 90%, this technique uses distilled water to remove soil particles [20, 21].When the sample was subjected to higher temperatures, the soil-related particles melted and transformed into rounded clear particles. This technique typically determines the particle's light-limited density. FT-IR microscopy is the most recent technique for examining soil particles' size, concentration, distribution, and composition. The amount of particles retained, deposited, and transported is influenced by a variety of factors, including particle qualities (such as size, shape, and density), human activity, weather (precipitation), water, and environmental topography [22].

2. Transport of MPs in aquatic ecosytem

Waste has a greater ecological impact now than it did in previous decades due to increased exposure to the marine environment. Dispersion and movement of PM in the ocean, including sediments in shallow and deep waters, beaches etc [23]. There is a lot of MP, PP, and PE content on the water's surface. The following are some sources of PM in the Southern Ocean: (a) wastewater discharged to research facilities 52% of research facilities lack wastewater treatment equipment. High quantities of ultraviolet light in the Southern Ocean cause PM to be produced there through bleaching of synthetic fibers (d) deterioration of floating garbage. % found in marine sediments from the Arctic. Due to this high PM concentration, sea ice creatures and seabirds are at risk [24]. Marine plastic is thought to originate between 75 and 90 percent from land and between 10 and 25 percent from oceanic sources. In the marine environment, the primary source of PM is wastewater treatment plants (WWTPS)[20, 25].Higher daily concentrations of PM are discharged into WWPTS. The treatment facility is thought to discharge 1.76 trillion PM, of which 1.28 trillion are deposited in the primary tank, 0.36 trillion are deposited in the secondary tank, and 0.03 trillion are released into the receiving marine environment. It is anticipated that 13 billion particles per day will be discharged to the wastewater treatment facility annually such as hurricanes, choppy seas, and tsunamis etc [26, 27].

3. Plastics used in marine environment

Plastics are advantageous for a variety of applications because they are strong, flexible, transparent, and lightweight. Its low cost and superior resistance to oxygen and moisture make it a great packing material. Plastic packaging or more modern designs replace metal, paper, and glass materials. Plastics of all kinds, including polystyrene (PS), polyethylene terephthalate

(PET), polyethylene (PE), polypropylene (PP), and polyvinyl chloride, are utilized in packing materials (PVC). [28].Future marine uses, overfishing, and recreational activities may increase the amount of plastic garbage entering the oceans. Around 80% of plastic garbage is generated on land, including beach trash. Polyolefin (PE and PP) and nylon are used in fishing gear and applications. Fishing is responsible for about 18% of the plastic garbage in the oceans.[29, 30]. The accumulation of plastic garbage in the oceans is also facilitated by aquaculture. As a result of excluding plastic in sediments and other bodies of water, the amount of floating plastic trash is significantly understated. Sea water weights approximately 1.025. In the maritime environment, various plastics are utilized that have densities comparable to those of saltwater. Nylon and other polymers frequently melt in the water column and in coastal sediments [30, 31].

4. Microplastic pollution in ocean

Over the past forty years, so-called micro plastic debris has accumulated in the world's waters. There are various definitions for Micro plastics and micro waste. Visible particles larger than 500 microns, stopping at 67 microns, and measuring 0.67 to 0.5 mm in diameter are referred to as microliters; otherwise, these larger particles are known as Mesolithic. The size of the other micro particles ranges from about 5 mm. The size of the plastic particles in seawater ranges from a few millimetres to 500 microns (5mm). Mesolithic are larger particles, such as primary plastics. Even when combined with sand, the minuscule particles visible to the unaided eye do not have as large chunks as plastic particles. Some of the suggested techniques are [32]based on the author's personal experience. To eliminate Mesolithic, a coarse filter was used on the water sample. Brine was added to sand and sediment samples to help the micro particles float to the top. To make the water denser and enable the plastic flakes to float, mineral salts are dissolved in the mud or water sample that was taken [28, 33].

Surface water samples and floating micro particle samples were collected for testing. Micro plastics may build on the surface of saltwater samples due to concentration and evaporation. These samples can be seen under a microscope by staining them with a lipophilic dye. Although the water samples contain plankton and other microbes, lipophilic dyes cannot be used to stain them [34, 35]. Since the treatment does not affect the micro plastic fraction, pollutants can be removed from biomass by diluting with hot, diluted mineral acid. Additionally, FTIR Spectroscopy, Raman Microscopy, Light Microscopy, and Electron Microscopy can all be used to identify micro plastic suspensions [35, 36]

Table 3 . Toxicological effect of plastic on fish species

Fish species	Particle size	Micro plastic type	Effects
Acanthochormis Polyacanathus	1-2 mm	Polyethylene terephthalate	Decrease the growth
Pomatoshistos microps	1-5micrometer	Polyethylene	AChE activity decrease
Dictrarchus labrax	1-5micrometer	Polymer	swimming speed decrease
Carrassius Carassius	(24.7±0.2) nm	Polystyrene	vitality decrease
Daniorerio	70μm	Polyethylene polyvinyl Chloride	intestinal injury

5. Plastic degradation under marine conditions

A chemical reaction called degradation lowers a polymer's average molecular weight. Because the high average molecular weight and level of material weakening determine the plastic's integrity. Plastic has suffered considerable degradation, which makes it brittle and hard enough to crumble into dust when handled. Microbial biodegradation further breaks down these invisible fragments, which turns the polymer's carbon content into CO2 and incorporates it into the biomass [53]. Complete mineralization is the term used when this process is finished and all of the macromolecular organic carbon has been transformed. The organism that caused the deterioration is typically used to classify it.(a) Biodegradation - the result of living things (usually bacteria), (a) Photo degradation caused by light exposure (outdoors, usually sunlight), (b) Slow oxidative thermal oxidative degradation mild temperature decomposition, (c) High-temperature service and thermal deterioration and (d) Hydrolysis is a water-based reaction. Common polymers exposed to the marine environment include LDPE, PP, HDPE, and nylon. Sunlight's UV-B photons are primarily what start photo-oxidative damage [54]

Once decomposition has begun, thermal oxidation can continue without additional UV exposure for a while. As long as the system has access to O2, automated catalytic decomposition reaction sequences can continue to operate. The polymer's molecular weight drops and oxygen-rich functional groups are generated following decomposition. Other types of decay happen more slowly than light-induced oxidation [55]. All biological materials, including plastics, harm the marine environment when they are hydrolyzed, and bottom sediments degrade plastics more slowly than light-induced oxidative degradation does. The plastic is exposed to the air and even the beach surface through an efficient method called solar UV radiation-induced deterioration. However, degradation is greatly postponed if the same plastic material is exposed to the sun in the same location [56]. Other polymers also degrade when they come into touch with water or sand. For instance, sunlight damage to fishing equipment has a variety of effects. Plastic gear exposed to air in marine environments, such as nylon liners and polyethene textiles, weather. The slow pace of deterioration may cause blockage. The energy of the resin affects the initial rate of color. The physicochemical polymeric properties of the PWs, as shown in Fig. 1, as well as several environmental factors, define the plastic degradation process [39].

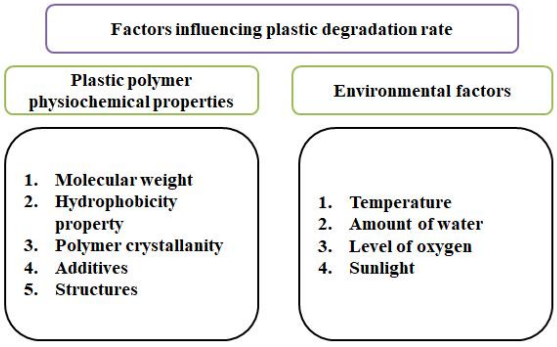

Figure 1 . Factors influencing plastic degradation rate

6. Toxicological effect of micro plastics

Micro plastics can harm the ability of marine species to reproduce. The amount of eggs laid by Crassostrea gigas is significantly decreased when it is exposed to Styrofoam Micro plastics, for example. The amount of sperm motility decreased, indicating that Micro plastics would severely impair sperm motility [37].

After Micro plastics, biological tissues and organs engage a variety of immune responses in the direction of marine fishing. For instance, white blood cells from Sparus aurata and Dicentrarchus labrax can be oxidative damaged by polyvinyl chloride (PVC) and polyethylene (PE) Micro plastics with a particle size of 40–150 m, leading to immune toxicity [57] (table 3).

Conclusions and recommendations

Micro plastics are prevalent, common, and persistent on a global scale [58]. When paired with increased amounts of chemical pollutants in the water that are easily absorbed and condensed into Micro plastics, which can be consumed indiscriminately by aquatic organisms, they offer a serious threat that calls for worldwide action. Global contamination occurrences due to micro plastic pollution of the oceans are rising; however, no viable solutions are available. We must

begin to purge other toxins using a variety of techniques. Future research should study size, shape, and associated impurities to better evaluate Micro plastics. It is essential for all parties involved to raise awareness of the harmful impacts of Micro plastics and the incorrect treatment of plastic waste. Strict regulations are required at the local, national, regional, and worldwide levels to limit the use and consumption of plastics and to provide incentives for the prevention of plastic pollution and garbage reduction.

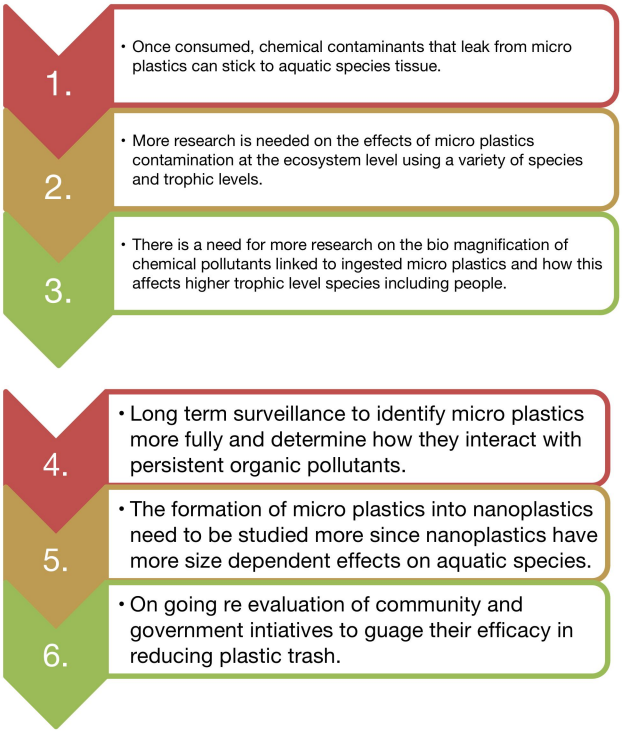

Figure 2 . Future directions for Micro plastics mitigation.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

Formal analysis, Zainab Riaz; Investigation, Dr. Shakeela Parveen, Urwa Ishaq, and Mehwish Sultana; Software, Muhammad Tyyab; Writing – original draft, Zainab Riaz, Dr Shakeela Parveen; Writing – review & editing, Zainab Riaz, Zainab Shafqat, Saman Shabbir and Zunaira Faiz.

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REFERENCES

1. Auta, H.S., C.U. Emenike, and S.H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environment International, 2017. 102: p. 165-176.
2. Alimba, C.G. and C. Faggio, Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. Environ Toxicol Pharmacol, 2019. 68: p. 61-74.
3. Casillas, G., et al., Microplastics Scoping Review of Environmental and Human Exposure Data. Microplastics, 2023. 2(1): p. 78-92.
4. Oberbeckmann, S. and M. Labrenz, Marine Microbial Assemblages on Microplastics: Diversity, Adaptation, and Role in Degradation. Annual Review of Marine Science, 2020. 12(1): p. 209-232.
5. Bhuyan, M.S., Effects of Microplastics on Fish and in Human Health. Frontiers in Environmental Science, 2022. 10.
6. ter Halle, A., et al., Understanding the Fragmentation Pattern of Marine Plastic Debris. Environmental Science & Technology, 2016. 50(11): p. 5668-5675.
7. Ledieu, L., et al., May a Former Municipal Landfill Contaminate Groundwater in Microplastics? First Investigations from the “Prairie de Mauves Site” (Nantes, France). Microplastics, 2023. 2(1): p. 93-106.
8. Belzagui, F., et al., Microplastics' emissions: Microfibers' detachment from textile garments. Environmental Pollution, 2019. 248: p. 1028-1035.
9. Borrelle, S.B. and J. Ringma, Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. 2020. 369(6510): p. 1515-1518.
10. Free, C., et al., High-levels of microplastic pollution in a large, remote, mountain lake. Marine Pollution Bulletin, 2014. 85: p. 156-163.
11. Assas, M., et al., Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. Mar Pollut Bull, 2020. 158: p. 111446.
12. Wan, Y., et al., Informal landfill contributes to the pollution of microplastics in the surrounding environment. Environmental Pollution, 2022. 293: p. 118586.
13. Lozano, Y.M., et al., Microplastic Shape, Polymer Type, and Concentration Affect Soil Properties and Plant Biomass. Frontiers in Plant Science, 2021. 12.
14. Auta, H.S., C.U. Emenike, and S.H. Fauziah, Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environ Int, 2017. 102: p. 165-176.
15. Amelia, T.S.M., et al., Marine microplastics as vectors of major ocean pollutants and its hazards to the marine

ecosystem and humans. Progress in Earth and Planetary Science, 2021. 8(1): p. 12.
16. Alfaro-Núñez, A., et al., Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. Scientific Reports, 2021. 11(1): p. 6424.
17. Yu, Q., et al., Distribution, abundance and risks of microplastics in the environment. Chemosphere, 2020. 249: p. 126059.
18. Rillig, M.C. and A. Lehmann, Microplastic in terrestrial ecosystems. Science, 2020. 368(6498): p. 1430-1431.
19. Brahney, J. and M. Hallerud, Plastic rain in protected areas of the United States. 2020. 368(6496): p. 1257-1260.
20. Mohammadi, A., et al., Occurrence, seasonal distribution, and ecological risk assessment of microplastics and phthalate esters in leachates of a landfill site located near the marine environment: Bushehr port, Iran as a case. Sci Total Environ, 2022. 842: p. 156838.
21. Rillig, M.C., Microplastic in Terrestrial Ecosystems and the Soil? Environmental Science & Technology, 2012. 46(12): p. 6453-6454.
22. Dissanayake, P.D., et al., Effects of microplastics on the terrestrial environment: A critical review. Environmental Research, 2022. 209: p. 112734.
23. Khatmullina, L. and I. Chubarenko, Transport of marine microplastic particles: why is it so difficult to predict? Anthropocene Coasts, 2019. 2(1): p. 293-305.
24. Shamskhany, A., et al., Evidence of Microplastic Size Impact on Mobility and Transport in the Marine Environment: A Review and Synthesis of Recent Research. Frontiers in Marine Science, 2021. 8.
25. Evangeliou, N., et al., Atmospheric transport is a major pathway of microplastics to remote regions. Nature Communications, 2020. 11(1): p. 3381.
26. Pohl, F., et al., Transport and Burial of Microplastics in Deep-Marine Sediments by Turbidity Currents. Environmental Science & Technology, 2020. 54(7): p. 4180-4189.
27. Zeenat, et al., Plastics degradation by microbes: A sustainable approach. Journal of King Saud University - Science, 2021. 33(6): p. 101538.
28. Thushari, G.G.N. and J.D.M. Senevirathna, Plastic pollution in the marine environment. Heliyon, 2020. 6(8): p. e04709.
29. Oliveira, J., et al., Marine Environmental Plastic Pollution: Mitigation by Microorganism Degradation and Recycling Valorization. Frontiers in Marine Science, 2020. 7.
30. Bordoloi, S., C.B. Gupt, and A.K. Sarmah, Exploring the theoretical effects of landfill based microplastic accumulation on the hydro-mechanical properties of porous soil media. Current Opinion in Environmental Science & Health, 2022. 26: p. 100332.
31. Yang, H. and G. Chen, Microplastics in the Marine Environment: Sources, Fates, Impacts and Microbial Degradation. 2021. 9(2).
32. Stenger, K.S., et al., Microplastics pollution in the ocean: Potential carrier of resistant bacteria and resistance genes. Environmental Pollution, 2021. 291: p. 118130.
33. Treilles, R., et al., Microplastic and microfiber fluxes in the Seine River: Flood events versus dry periods. Science of The Total Environment, 2022. 805: p. 150123.
34. Sharma, S. and S. Chatterjee, Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental science and pollution research international, 2017. 24(27): p. 21530-21547.
35. K, M.B., et al., Spatial distribution of microplastic concentration around landfill sites and its potential risk on groundwater. Chemosphere, 2021. 277: p. 130263.
36. Sheela, A.M., B. Manimekalai, and G. Dhinagaran, Review on the distribution of microplastics in the oceans and its impacts: Need for modeling-based approach to investigate the transport and risk of microplastic pollution. Environmental Engineering Research, 2022. 27(4): p. 210243-0.
37. Sharma, S. and S. Chatterjee, Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environ Sci Pollut Res Int, 2017. 24(27): p. 21530-21547.
38. Samandra, S., et al., Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia. Science of The Total Environment, 2022. 802: p. 149727.
39. Talbot, R. and H. Chang, Microplastics in freshwater: A global review of factors affecting spatial and temporal variations. Environmental Pollution, 2022. 292: p. 118393.
40. Ory, N., et al., Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. Marine Pollution Bulletin, 2018. 127: p. 211-216.
41. Guzzetti, E., et al., Microplastic in marine organism: Environmental and toxicological effects. Environmental Toxicology and Pharmacology, 2018. 64: p. 164-171.
42. Guzzetti, E., et al., Microplastic in marine organism: Environmental and toxicological effects. Environ Toxicol Pharmacol, 2018. 64: p. 164-171.
43. Devriese, L.I., et al., Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Marine Pollution Bulletin, 2015. 98(1): p. 179-187.
44. Su, Q., et al., Gut microbiome signatures reflect different subtypes of irritable bowel syndrome. Gut Microbes, 2023. 15(1): p. 2157697.
45. Gola, D., et al., The impact of microplastics on marine environment: A review. Environmental Nanotechnology, Monitoring & Management, 2021. 16: p. 100552.

46. Shang, Y., et al., The Effect of Microplastics on the Bioenergetics of the Mussel Mytilus coruscus Assessed by Cellular Energy Allocation Approach. Frontiers in Marine Science, 2021. 8.
47. Meaza, I., J.H. Toyoda, and J.P. Wise Sr, Microplastics in Sea Turtles, Marine Mammals and Humans: A One Environmental Health Perspective. Frontiers in Environmental Science, 2021. 8.
48. Pittura, L., et al., Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus galloprovincialis. Frontiers in Marine Science, 2018. 5.
49. Besseling, E., et al., Effects of microplastic on fitness and PCB bioaccumulation by the lugworm Arenicola marina (L.). Environ Sci Technol, 2013. 47(1): p. 593-600.
50. Cressey, D., Microplastics damage oyster fertility. Nature, 2016.
51. Nanthini devi, K., et al., Impacts of microplastics on marine organisms: Present perspectives and the way forward. The Egyptian Journal of Aquatic Research, 2022. 48(3): p. 205-209.
52. Naji, A., et al., Small microplastic particles (S-MPPs) in sediments of mangrove ecosystem on the northern coast of the Persian Gulf. Marine Pollution Bulletin, 2019. 146: p. 305-311.
53. Corami, F., et al., A novel method for purification, quantitative analysis and characterization of microplastic fibers using Micro-FTIR. Chemosphere, 2020. 238: p. 124564.
54. Onyena, A.P., et al., Governance Strategies for Mitigating Microplastic Pollution in the Marine Environment: A Review. Microplastics, 2022. 1(1): p. 15-46.
55. Arpia, A.A., et al., Microplastic degradation as a sustainable concurrent approach for producing biofuel and obliterating hazardous environmental effects: A state-of-the-art review. Journal of Hazardous Materials, 2021. 418: p. 126381.
56. Klein, S., et al., Analysis, Occurrence, and Degradation of Microplastics in the Aqueous Environment, in Freshwater Microplastics : Emerging Environmental Contaminants?, M. Wagner and S. Lambert, Editors. 2018, Springer International Publishing: Cham. p. 51-67.
57. Mao, X., et al., The impact of microplastic pollution on ecological environment: a review. FBL, 2022. 27(2).
58. Khan, Q., Kashif, M., & Shah, S. J. Comprehensive analysis of the mechanism underlying plastic microbiome and plants interaction, with future perspectives. Journal of Soil, Plant and Environment, 2022. 1(2), 31–43.

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Research article

Insights Into Green Synthesized and Chemical Synthesized Nanoparticles for Biomedical Applications

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Abstract

Nanotechnology currently garners substantial attention due to its capacity to alter metals' chemical, physical, and optical attributes through nanosizing. Consequently, a significant emphasis exists on devising novel approaches that utilize biological sources to synthesize diverse nanoparticles with specific sizes and compositions. Most current approaches are costly, environmentally harmful, and inefficient in using materials and energy. The properties of nanoparticles are affected by a range of factors such as time, temperature, pH, and ambient conditions. The potential of eco-friendly nanoparticles is also evident in agriculture, which can safeguard the environment and enhance agricultural productivity. Moreover, the thorough characterization of synthesized nanoparticles is paramount, especially in their potential applications in drug delivery and biomedicine. Green-synthesized nanoparticles excel in biocompatibility and sustainability, while chemically-synthesized nanoparticles provide precise control and functional versatility. The choice between these approaches depends on specific biomedical demands, cost factors, and the desire for sustainable healthcare solutions. Harnessing the strengths of both synthesis methods holds the potential to revolutionize biomedical applications, advancing healthcare accessibility and efficacy. This review paper mainly focuses on green synthesis, chemical synthesis, economic impact and biomedical application.

Keywords: Green synthesis, chemical synthesis, economic impact, biomedical applications

1. Introduction

Nanotechnology is a branch of science concerned with nanometer-sized particles, also known as nanoparticles (NPs). Nanomaterials are solid entities with dimensions between 1 and 100 nanometers. Nanomaterials show promise in antibacterial therapy as a result of their enhanced and distinct physicochemical properties, such as their extremely small dimensions, large surface area relative to their mass, and increased reactivity [1, 2]. El-Belely et al. [3] say that nanoparticles are parts of nanometers widely used in medicine, environmental defense, sunscreen, and cosmetics. Researchers in material science fields face problems when they try to study biomaterials. Researchers waste a lot of time developing new ideas, especially regarding plastics used in medicine and microorganisms resistant to antibiotics [1]. Physical and chemical differences can be seen in nanocomposite materials' mechanical and biological characteristics (Figure 1)[4]. A nanoparticle has better qualities than bulk materials and could be used in many ways in the real world. It has a smaller ratio of surface area to volume and important properties like being flexible, strong, and able to carry electricity. This material is stronger and sticks together better than bulk materials that are made of the same chemicals [5].

Nanoparticles made using biological means or green technology have different properties and are more stable and the right size because they are made in a single step. Many bad

processing conditions are handled by letting the synthesis happen at average temperatures, pH, and pressure at a negligible cost [5, 6]. Specific characterization methods can determine how synthesized nanoparticles might be used in the drug delivery and biomedical fields. NPs are used as supercapacitors because they have a high energy density, are electrochemically active, are suitable for the environment, are easy to find, and are cheap. ZnO nanoparticles have been used to remove arsenic and sulfur from water because they have a lot of surface area. Compared to green synthetic ways, these have problems, like being hard to use, expensive, giving off radiation, needing very high pressure, and being toxic [7]. The buildup of dangerous chemicals on the surface of some technologies makes it harder for them to be used in medicine. This review paper looks at how green and chemical syntheses compare their economic effects and medical uses.

2. Green Synthesis/Methods of Extractions

Green-synthesized nanoparticles, which are made using eco-friendly processes, have several benefits. They are ideally suited for biomedical functions because of their innate biocompatibility, which lowers the possibility of negative reactions when interacting with biological systems [7]. These nanoparticles are a perfect fit for the expanding need in healthcare for environmentally friendly and sustainable solutions. When natural or renewable materials are used in their synthesis, the environmental impact is reduced and a more conscientious approach to nanoparticle synthesis is encouraged. Additionally, some green synthesis techniques can be economical, which could result in more reasonably priced medical diagnoses and therapies. Furthermore, biomolecules can be easily functionalized onto these nanoparticles to enable targeted drug administration, molecular imaging, and the development of customized medicine strategies [1,7,8].

Green synthesis is the process of making materials, chemicals, or nanoparticles in a way that is good for the environment and has a small amount of environmental damage. This method often involves using resources that can be replenished, things that break down, and reaction conditions that do not harm the environment. Upadhyay et al. [8] say that the sizes of avocado fruits were cut and washed six times by double distillation and three times by ethanol to make them smaller. A magnetic mixer was used to keep heating 16 g of avocado and 170 mL of double-distilled water for 30 minutes. The watery solution made from avocado extracts was filtered with Whatman paper and kept at room temperature until needed. Using a triple bean balance, 10 g of $Zn(NO_3)$, $2.6H_2O$ was weighed and dissolved in 30 mL of double-distilled water for 30 minutes using a high-speed magnetic stirrer at a constant temperature. Then, 1.75 M of avocado extract was added to the $Zn(NO_3)_2.6H_2O$ mixture, which was mixed all the time for 1 hour. Now, the solutions had sat for 24 hours at room temperature. Papaya and mango were handled in the same way.

3. Chemical Synthesis Methods of Nanoparticles

Nanoparticles that are chemically manufactured offer exact control over their dimensions, form, and surface characteristics. Because of their accuracy, nanoparticles may be customized to fit certain biological needs, which makes them useful for applications like cancer treatment, drug administration, and imaging [9]. Chemical synthesis's consistency and reproducibility guarantee the dependability of medical interventions and diagnostics, which is essential for regulatory approval and clinical application. Furthermore, the chemically produced nanoparticles' functional diversity allows for the attachment of diverse functional groups, increasing their versatility for a range of biomedical applications, such as the targeted delivery of medicines. These approaches for chemical synthesis remain the focus of much study, which is pushing the frontiers of biomedical innovation with the discovery of new materials and processes [9].

As salt precursors, $Zn(NO_3)_2.6H_2O$, $Zn(CH_3COO)_2.2H_2O$, and $ZnSO_4.7H_2O$ were used in the chemical synthesis. NaOH, PVA, and KOH were used as reducing agents. Zinc oxide nanoparticles were made using the method described in Brar et al. [9]. By using a beam balance, all of the zinc salts and reducing agents were analyzed. Twelve grams of the sodium hydroxide (NaOH) solution were mixed with 70 milliliters of

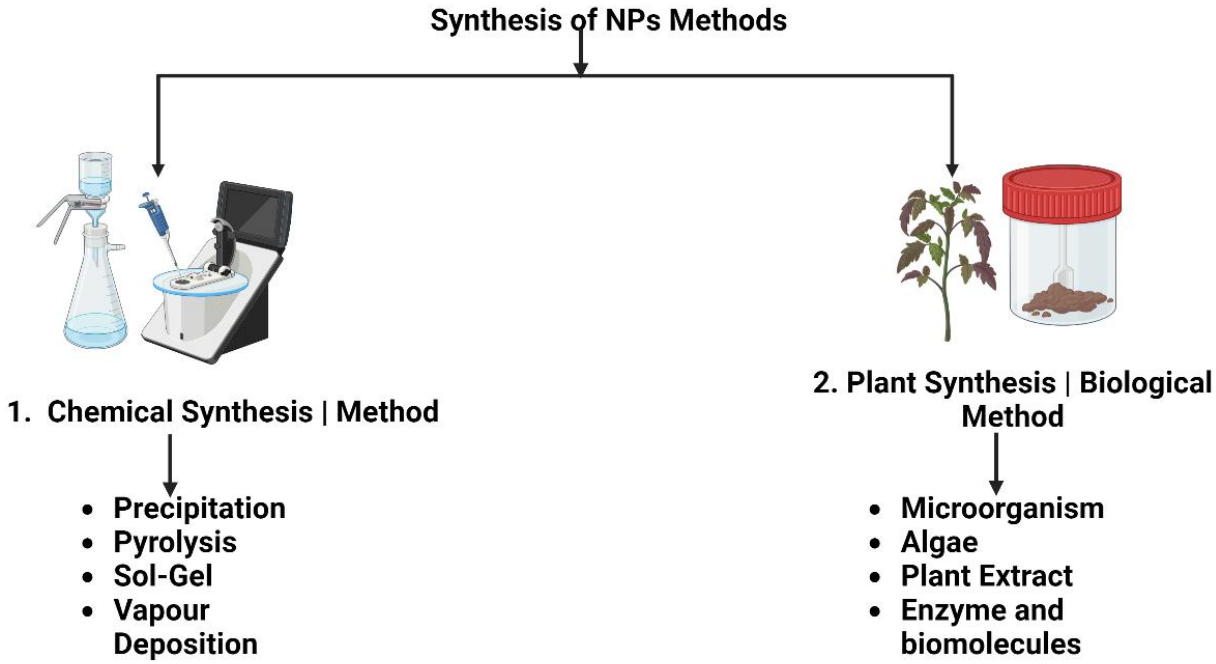

Figure 1 Chemical and plant methods for the synthesis of nanoparticles.

water distilled twice—thirty minutes under a soft magnetic mixer. Again, 4 g of $Zn(NO_3)_2.6H_2O$ was mixed into 30 mL of double-distilled water and stirred for 20 minutes. Drop by drop, the $Zn(NO_3)_2.6H_2O$ solution was added to the NaOH solution, which was constantly mixed for 2 hours at 60°C. At this point, gel-like solutions were made and left to cure in a 160°C oven for 10 hours overnight. Then, the sample was put in a kiln (Model: MC2-5/5/10-12, BIOBASE, China) and calcined at 300°C for 6 hours. Upadhyay et al., [8], did the same thing with zinc acetate ($Zn(CH_3COO)_2.2H_2O$) and zinc sulphate hydrate ($ZnSO_4.7H_2O$).

4. Economic Impact Synthesis of Green and Chemical Nanoparticles

The economic impact of green and chemical nanoparticles is multifaceted. Green nanoparticles, crafted through environmentally friendly processes, hold the promise of reducing environmental costs, opening up novel market opportunities, fostering innovation, and potentially offering cost efficiencies. As global sustainability gains prominence, demand for green nanoparticles in agriculture, energy, and healthcare is growing, stimulating economic growth. Moreover, these eco-friendly materials can navigate regulatory pathways more smoothly, further contributing to economic benefits [10]. On the other hand, chemical nanoparticles, renowned for their diverse applications, advanced materials, and research-driven innovations, play a pivotal role in industries ranging from electronics to pharmaceuticals. This versatility generates economic impacts through enhanced product quality, job creation, and participation in a global market. Ultimately, the economic impact of both green and chemical nanoparticles is influenced by market dynamics, governmental policies, and the quest for sustainable solutions. Balancing economic gains with environmental and health considerations is imperative for ensuring the long-term viability of the nanoparticle industry [9]. Certain conditions must be met when making nanoparticles to get the best results and shape you want. Some of these conditions and factors are the ratio of the volume of the

extracting solvent to the amount of plant material, the temperature, the concentration of the precursor solution, the pH of the solution, the reaction, and the time it is left to sit. Iron oxide NPs are one of the most common metal oxide NPs. Because ferrous oxide nanoparticles (IONPs) have a unique property, they can be used in medicine and many other fields, like gas monitors, electrochemical, magnetic, and energy storage [10, 11].

4.1. Cost of nanoparticles

Facilitating the widespread utilization of nanoparticles in contemporary applications necessitates regulating and controlling their production costs. Hence, a pivotal factor influencing nanoparticle manufacturing is the cost-effectiveness of the production process. While the chemical synthesis method offers rapid returns, it aligns poorly with cost-saving endeavours. Consequently, the feasibility of producing nanoparticles through chemical and physical approaches may be constrained, whereas the biological approach presents a more economical and scalable alternative [11].

The properties of nanoparticles are intricately linked to their size. Akbari et al. [11] established that their melting points decrease as nanoparticles reach the nanometer scale. Moreover, nanoparticles exhibiting different shapes possess comparable energy levels, rendering them amenable to shape alteration. The energy typically employed for nanoparticle investigation plays a role in inducing shape changes. Baer et al. [12] demonstrated that the morphology and mobility of synthesized nanoparticles significantly impact their chemical properties, underlining the significance of such aspects.

4.2. Time

The duration the reaction medium can remain undisturbed significantly influences the quality and characteristics of nanoparticles produced through green technology. Baer et al. [12] observed that the attributes of the synthesized nanoparticles also underwent alterations over time, with substantial sensitivity to the synthesis method, light exposure, storage conditions, and similar factors. Temporal variations can manifest in various ways, such as particle aggregation due to prolonged storage, size alteration due to extended storage duration, or even due to inherent shelf-life effects, all collectively impacting their potential [11].

4.3. pH

The pH level becomes a crucial factor when employing green technology approaches to synthesize nanoparticles. Studies have revealed that the size and morphology of the resulting nanoparticles are significantly influenced by the pH of the solution medium [13]. Consequently, adjusting the pH of the fluid can lead to modifications in the size of nanoparticles. Specifically, in the case of silver nanoparticles, altering the pH can directly impact their shape and size during the synthesis process [14].

It has been found that the pH, which is a measure of how acidic or basic the reaction medium is, is an important factor in making IONPs and other metal oxide NPs from plant materials. Jacob et al. [15] have written about how the pH of the solution medium affects the size and shape of NPs made from plant extract. So, Huang et al. [16] found that changing the pH of the fluid was a good way to control and change the shape and size of the NPs that were made. Lenders et al. [17] found that the best way for Aeromonas hydrophile to make IONPs was when the pH of the basic medium was between 7 and 9. It has been said that the biosynthesis that happens at pH 12 and 4 totally slows down the making of IONPs. This showed that conditions that are too acidic or too basic are not good for making IONPs from plant material [17].

4.4. Temperatures

Temperature stands out as one of the paramount factors influencing the physical, chemical, and biological methods of nanoparticle (NP) synthesis. According to Patra et al. [18], the green production of iron oxide nanoparticles (IONPs) via plant extracts necessitates a temperature range spanning 25 to 100 °C. However, many researchers lean towards room-temperature synthesis for IONPs due to the instability of secondary molecules within plant extracts required to bio-reduct iron ions at elevated temperatures. Notably, the shape of NPs is influenced by the temperature of the reaction solution, as highlighted by results from Patra et al. [18].

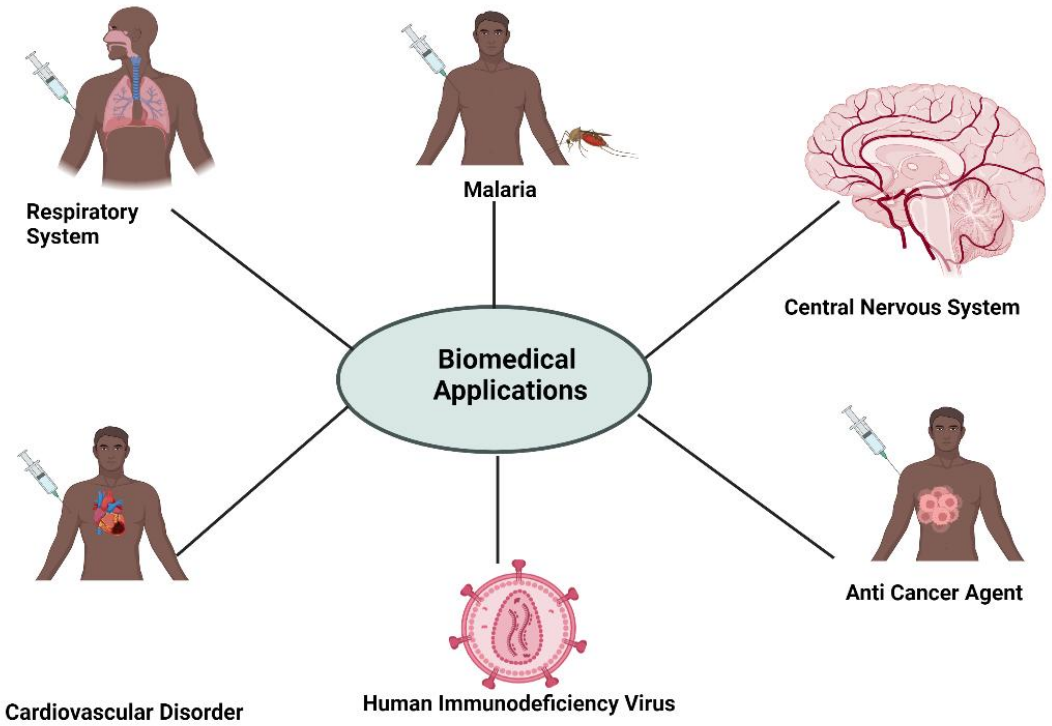

Figure 2 Green synthesis of metal nanoparticles and their applications

UV-visible studies of IONPs produced from various plant components within the temperature range of 30 to 40 °C demonstrated that completion of the synthesis occurred at 48 to 72 hours, indicating accelerated synthesis at higher temperatures. Conversely, the study revealed suboptimal IONP synthesis above 40°C due to the deactivation of biomolecules responsible for reducing the iron precursor, as elucidated by Rajendran & Sen [19].

4.5. Environment

The surrounding environment profoundly shapes the properties of nanoparticles, emerging as a pivotal determinant. Frequently, the transformation of a solitary nanoparticle into a core-shell nanoparticle occurs due to its interaction with the external milieu. This interaction involves absorbing materials from the surroundings or engaging with other substances, a process often facilitated by factors such as oxidation. This phenomenon underscores the intricate interplay between nanoparticles and their environment in sculpting their characteristics and behavior [21]. In biological systems, manufactured nanoparticles tend to develop a thicker and larger coating, altering their characteristics [22]. Additionally, the environment significantly impacts the resulting nanoparticles' physical shape and chemical composition. While limited instances demonstrate how the environment influences nanoparticle synthesis, specific observations stand out. For instance, their crystallinity immediately changed when zinc sulfide nanoparticles were transferred from a wet environment to a dry one. Similarly, the chemical properties of cerium nitrate nanoparticles vary according to the concentration of peroxide present in the surrounding fluid [13].

5. Biomedical Applications

Nanoparticles find extensive applications across diverse industries, the realm of biomedical sciences, the electronics sector various markets, the energy domain, and notably in the field of chemistry [23]. This multifaceted utility has sparked an escalating commercial demand for nanoparticles. Mainly, nanoparticles such as silver and gold, which rank among the most prevalent, have garnered significant attention due to their utilization in biomedical applications, the emergent

nanotechnology field, and beyond [9].

Researchers have identified that naturally synthesized nanoparticles exhibit superior efficacy in treating diseases than nanoparticles produced through other physicochemical techniques. Plant-extracted metal nanoparticles manifest stability and easy degradability into distinct types, achieved by controlling variables like pH, temperature, retention time, and mixing proportions. Noteworthy examples of green metal nanoparticles stem from plant sources such as neem (Azadirachta indica) leaves, tulsi (Ocimum tenuiflorum) leaves, curry (Murraya koenigii) leaves, guava (Psidium guajava) leaves, and mango (Mangifera indica) leaves [25].

Metallic nanoparticles synthesized from various medicinal plants have demonstrated significant therapeutic attributes encompassing antimicrobial, insecticidal, antioxidant, wound-healing, antidiabetic, immunomodulatory, hepatoprotective, and anticancer activities (Fig. 2) [23]. Notably, Muhammad et al. [22] established that metallic nanoparticles sourced from medicinal plants offer substantial benefits within the biomedicine domain. The core concept driving the integration of green nanotechnology into agriculture revolves around its potential to mitigate environmental harm and reduce the expense associated with chemical applications. Green nanoparticles (GNPs) derived from diverse plant sources have additionally shown efficacy in curbing harmful emissions such as carbon dioxide, nitrous oxide, and methane. Furthermore, these nanoparticles augment agricultural productivity and mitigate health concerns among farmers.

The inherent phytochemical constituents within plants make them a valuable resource for this approach. Such constituents are cost-effective and environmentally benign. Green nanoparticles play a pivotal role in addressing the imperative of eliminating heavy metal pollutants from the environment. Notably, Jadoun et al. [23] demonstrated green nanoparticles' role in alleviating environmental toxicity, especially concerning heavy metal contamination in soil and water.

Given that various phytochemicals are distributed across different plant parts—roots, stems, leaves, seeds, and fruits—the method of synthesizing metallic nanoparticles through green synthesis is not only cost-effective but also less environmentally detrimental, proving more efficacious than other biological methods [9]. Generating green nanoparticles involves washing specific plant parts with tap or distilled water following extraction, filtration, and introducing specific salt solutions. Observable changes in solution colour signify successful nanoparticle production. Throughout the metallic nanoparticle synthesis process, phenolic acids such as ellagic acid, caffeic acid, protocatechuic acid, and gallic acid play a crucial role. Ali et al. [24] elaborate on the role of phytochemical agents that facilitate the reduction and stabilization of laboratory-produced metal nanoparticles.

6. Conclusion

Nanoparticles are being used more and more in the medical, food, pharmaceutical, and agricultural industries. There is much interest in developing more manageable ways to make eco-friendly, non-toxic, and harmless nanoparticles using tools from green biotechnology. The use of plants for green production of nanoparticles is an exciting and growing part of nanotechnology. It significantly impacts the environment and the field of nanoscience, making it more sustainable and allowing for more progress. Green nanotechnology research going on now and, in future, will give us a better understanding of the different factors that affect the green synthesis of nanoparticles and the most advanced technology that can be used to characterize the synthesized nanoparticles so that they can be used more effectively in the biomedical and pharmaceutical industries in the future.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

Mahreen Fatima - Methodology, original draft preparation ; Maham Fatima - Helped in revision, give suggestion and correction. Both authors read and granted to the published this version of manuscript.

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REFERENCES

1. Sangeetha, G., Rajeshwari, S., & Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. Materials Research Bulletin, 46(12), 2560-2566.
2. Rambabu, K., Bharath, G., Banat, F., & Show, P. L. (2021). Green synthesis of zinc oxide nanoparticles using Phoenix dactylifera waste as bioreductant for effective dye degradation and antibacterial performance in wastewater treatment. Journal of hazardous materials, 402, 123560.
3. El-Belely, E. F., Farag, M. M., Said, H. A., Amin, A. S., Azab, E., Gobouri, A. A., & Fouda, A. (2021). Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Arthrospira platensis (Class: Cyanophyceae) and evaluation of their biomedical activities. Nanomaterials, 11(1), 95.
4. Akpomie, K. G., Ghosh, S., Gryzenhout, M., & Conradie, J. (2021). One-pot synthesis of zinc oxide nanoparticles via chemical precipitation for bromophenol blue adsorption and the antifungal activity against filamentous fungi. Scientific reports, 11(1), 8305.
5. Kolahalam, L. A., Prasad, K. R. S., Krishna, P. M., & Supraja, N. (2021). Saussurea lappa plant rhizome extract-based zinc oxide nanoparticles: synthesis, characterization and its antibacterial, antifungal activities and cytotoxic studies against Chinese Hamster Ovary (CHO) cell lines. Heliyon, 7(6).
6. Rana, A., Yadav, K., & Jagadevan, S. (2020). A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. Journal of Cleaner Production, 272, 122880.
7. Awwad, A. M., Amer, M. W., Salem, N. M., & Abdeen, A. O. (2020). Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Ailanthus altissima fruit extracts and antibacterial activity. Chem. Int, 6(3), 151-159.
8. Upadhyay, P. K., Jain, V. K., Sharma, S., Shrivastav, A. K., & Sharma, R. (2020, March). Green and chemically synthesized ZnO nanoparticles: A comparative study. In IOP Conference Series: Materials Science and Engineering (Vol. 798, No. 1, p. 012025). IOP Publishing.
9. Brar, K. K., Magdouli, S., Othmani, A., Ghanei, J., Narisetty, V., Sindhu, R., ... & Pandey, A. (2022). Green route for recycling of low-cost waste resources for the biosynthesis of nanoparticles (NPs) and nanomaterials (NMs)-A review. Environmental Research, 207, 112202.
10. Vallabani, N. S., & Singh, S. (2018). Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics. 3 Biotech, 8(6), 279.
11. Akbari, B., Tavandashti, M. P., & Zandrahimi, M. (2011). Particle size characterization of nanoparticles–a practical approach. Iranian Journal of Materials Science and Engineering, 8(2), 48-56.
12. Baer, D. R., Engelhard, M. H., Johnson, G. E., Laskin, J., Lai, J., Mueller, K., ... & Moon, D. (2013). Surface characterization of nanomaterials and nanoparticles: Important needs and challenging opportunities. Journal of Vacuum Science & Technology A, 31(5).
13. Armendariz, V., Herrera, I., Peralta-Videa, J. R., Jose-Yacaman, M., Troiani, H., Santiago, P., & Gardea-Torresdey, J. L. (2004). Size controlled gold nanoparticle formation by Avena sativa biomass: use of plants in nanobiotechnology. Journal of nanoparticle research, 6, 377-382.
14. Soni, N., & Prakash, S. (2011). Factors affecting the geometry of silver nanoparticles synthesis in Chrysosporium tropicum and Fusarium oxysporum. Am J Nanotechnol, 2(1), 112-121.
15. Jacob, P. J., Masarudin, M. J., Hussein, M. Z., & Rahim, R. A. (2019). Optimization of process parameters influencing the sustainable construction of iron oxide nanoparticles by a novel tropical wetlands Streptomyces spp. Journal of Cleaner Production, 232, 193-202.
16. Huang, L., Luo, F., Chen, Z., Megharaj, M., & Naidu, R. (2015). Green synthesized conditions impacting on the reactivity of Fe NPs for the degradation of malachite green. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 137, 154-159.
17. Lenders, J. J., Mirabello, G., & Sommerdijk, N. A. (2016). Bioinspired magnetite synthesis via solid precursor phases. Chemical science, 7(9), 5624-5634.
18. Patra, J. K., & Baek, K. H. (2015). Green nanobiotechnology: factors affecting synthesis and characterization techniques. Journal of Nanomaterials, 2014, 219-219.
19. Rajendran, K., & Sen, S. (2016). Optimization of process parameters for the rapid biosynthesis of hematite nanoparticles. Journal of Photochemistry and Photobiology B: Biology, 159, 82-87.
20. Sarathy, V., Tratnyek, P. G., Nurmi, J. T., Baer, D. R., Amonette, J. E., Chun, C. L., ... & Reardon, E. J. (2008). Aging of iron nanoparticles in aqueous solution: effects on structure and reactivity. The Journal of Physical Chemistry C, 112(7), 2286-2293.
21. Lynch, I., Cedervall, T., Lundqvist, M., Cabaleiro-Lago, C., Linse, S., & Dawson, K. A. (2007).
22. Muhammad Mailafiya, M., Abubakar, K., Danmaigoro, A., Musa Chiroma, S., Bin Abdul Rahim, E., Aris Mohd Moklas, M., & Abu Bakar Zakaria, Z. (2019). Cockle shell-derived calcium carbonate (aragonite) nanoparticles: a dynamite to nanomedicine. Applied Sciences, 9(14), 2897.
23. Jadoun, S., Chauhan, N. P. S., Zarrintaj, P., Barani, M., Varma, R. S., Chinnam, S., & Rahdar, A. (2022). Synthesis of nanoparticles using microorganisms and their applications: A review. Environmental Chemistry Letters, 20(5), 3153-3197.
24. Ali, M., Khan, T., Fatima, K., Ali, Q. U. A., Ovais, M., Khalil, A. T., ... & Idrees, M. (2018). Selected hepatoprotective herbal medicines: Evidence from

ethnomedicinal applications, animal models, and possible mechanism of actions. Phytotherapy research, 32(2), 199-215.
25. Devatha, C. P., & Thalla, A. K. (2018). Green synthesis of nanomaterials. In Synthesis of inorganic nanomaterials (pp. 169-184). Woodhead Publishing.

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Review article

ZnO Nanoparticles Impact on Organ Systems in Rats: A Comprehensive Exploration of Diverse Exposure Pathways

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Abstract

The synthesis and utilization of nanomaterials with precise spatial dimensions on the nanoscale are pivotal in the field of nanotechnology. In recent years, metal oxide nanoparticles have become increasingly common, raising concerns in both scientific community and the general public about their potential harm to the environment and living organisms. Despite this, there are still significant debates and misconceptions regarding the adverse effects and mechanisms of these nanoparticles. To facilitate their safe and responsible use, it is imperative to gain a comprehensive understanding of their adverse effects. This review aims to provide an overview of the biological fate of zinc oxide (ZnO) nanoparticles in rats through various exposure routes, shedding light on their toxicological consequences and the underlying mechanisms of toxicity. Despite the fact that ZnO nanoparticles have a propensity to target organs such as the liver, kidneys, and lungs, it is noteworthy that higher concentrations of zinc are detected in these tissues following exposure via various routes. The liver plays a central role in the metabolism of ZnO nanoparticles. Multiple exposure routes, including oral, intraperitoneal, intravenous, and intratracheal routes, have been shown to induce liver damage, along with adverse effects on the kidneys and lungs when exposure occurs via airways. A significant toxicological mechanism associated with ZnO nanoparticles involves the generation of reactive oxygen species (ROS) and the subsequent initiation of oxidative stress. ROS production can result from both excessive release of Zn^{+2} ions and the particulate effect stemming from the semiconductor or electronic properties of ZnO nanoparticles. The potential for surface coatings and modifications holds the promise of further expanding the range of biomedical applications for ZnO nanoparticles, opening up exciting possibilities for futuristic medical treatments, including targeted drug delivery, advanced imaging techniques, and diagnostics.

Keywords: Albino rats, intravenous route, airway exposure, potential infection, hepatic toxicity.

1. Introduction

Nanotechnology is a term used to define areas of science and engineering in which phenomena occurring at nanoscale dimensions are used in the design, characterization, manufacture, and applications of materials, structures, devices, and systems [1]. It has the potential to revolutionize the medical research industry and open up new fields for the betterment of humanity [1,2,3]. The three main categories of nanotechnology are nanotools, nanodevices, and nanostructured materials. Nanotechnology relies on a wide range of techniques, such as computer modeling, surface science, supramolecular chemistry, nanolithography, synthetic approach, and analytical tools. Nanoelectronics, nanospintronics, nanosensors, nano-optical electronics, and nano-drug delivery systems are all examples of nanodevices.

Figure 1. Risks associated with pharmaceutical ZnO utilization [4].

Some examples of nanostructured materials are nanowires, nanoparticles, fullerene, carbon nanotubes, graphene, nanocomposites, thin solid films, nano-patterned surfaces, and supramolecular systems [5]. Different morphologies of ZnO nanoparticles, such as nanoflake, nanoflower, nanobelt, nanorod, and nanowire, have been reported [6, 7, 8].

ZnO is a white inorganic substance which is soluble in acidic or alkaline solutions but insoluble in water. It doesn't naturally occur in large amounts [9]. There are numerous ways to make ZnO nanoparticles, including thermal evaporation, gas evaporation, hydrothermal, the vapor-liquid-solid process, self-combustion, simple thermal sublimation, and green synthesis [10]. Moreover, the simplicity, cost-effectiveness, and eco-friendly nature of green synthesizing ZnO nanoparticle synthesis using extracts from different plants and fruits have garnered significantly attention and interest [11]. Several issues have been highlighted by the extensive and extended use of ZnO at pharmaceutical levels in pig husbandry, including ZnO nanoparticle's advantages are lost with excessive or prolonged ZnO intake, which also increases the risk of harmful consequences [12, 13]. Due to the excessive Zn buildup in animal organs such the pancreas, liver, and kidney [14], which is susceptible to Zn excess [13].

ZnO nanoparticles their increasing application has prompted safety concerns [15]. Size, surface properties, solubility, and mode of exposure are the most important factors in determining the toxicity of metal oxide nanoparticles. The biological fate and toxicity of ZnO nanoparticles upon exposure via various mechanisms must be understood.Workers are most likely to be exposed to ZnO nanoparticles since they are used as food additives and packaging materials ingestion, in addition to the more common routes of exposure (inhalation and skin contact) [16]. If you consume ZnO nanoparticles orally, they will dissolve in your stomach's acidic environment (pH 1.5-2.0) and Zn^{+2} will be absorbed into your blood [17]. ZnO nanoparticles are likely absorbed in both ionic and particle forms after oral administration to rats [18]. After being injected intraperitoneally, ZnO nanoparticles are taken up by the body as ions rather than particles. After oral and intraperitoneal administration, Zn^{+2} is taken up by the liver via the first-pass effect and subsequently redistributed. By dissolving in the acidic lining fluid of the lungs, ZnO

nanoparticles are able to cross the alveolar membrane and enter the circulation after being inhaled [19, 20]. Inhaled ZnO nanoparticles pass the alveolar barrier and enter the circulation because they dissolve in the acidic lining fluid of the lungs. Skin has a pH that varies from the surface to the stratum corneum, which may lead to the dissolution of topically administered ZnO nanoparticles and the dermal absorption of Zn [21].

ZnO nanoparticles are particularly useful in biomedical applications due to their unique properties, such as their size similarity to biomolecules, availability of functionality over wide surfaces, and quantum size impact. ZnO quantum dots can be used for medicine delivery and bioimaging thanks to their high biocompatibility, low toxicity, and good stability [22]. The potential health benefits of ZnO nanoparticles as an antibiotic, nutritional supplement, and food additive are also the subject of ongoing study [23] characteristics are present in ZnO Nanoparticles [24, 25]. They are employed to get rid of aquatic vegetation Applications like medication delivery, cancer prevention, diabetes prevention, anti-diabetic, anti-bacterial, and agronomic properties [26]. Although ZnO is employed for targeted drug delivery, its cytotoxicity limitation has not yet been overcome [27]. They exhibit stronger antibacterial effects than chemically produced ZnO nanoparticles [28, 29, 30]. Additionally, they have been used in the production of rubber, paint, water purification, protein adsorption, and dentistry applications. piezoelectric and pyroelectric that is immune to all types of eradication methods, including physical, chemical, and mechanical ones [31].

2. Different Exposure Routes

Emerging pollutants having ecological and toxicological consequences on individuals, communities, and diverse ecosystems, manufactured nanoparticles, and more especially metal oxide nanoparticles, are finding ever-increasing uses in industrial and consumer products [32, 33].

2.1 Intraperitoneal Administrations

According to Li et al. [34] mice were injected intraperitoneally with ZnO nanoparticles (average size 93 nm) at a dose of 2.5 g/kg, and the findings revealed that Zn accumulated in all organs except the brain (blood-brain barrier). Zn concentrations were highest in the liver, then in the spleen, the lungs, the kidneys, and finally the heart cardiac vascular dysfunction as shown in Figure (2). According to Lin et al. [35] ZnO nanoparticles (size 47.8 nm, dosage 10 mg kg^{-1}) accumulated in the liver, lung, kidneys, spleen, and heart 6 hours after a single intraperitoneal injection. Amara et al, studied the effect of intraperitoneal injection of ZnO nanoparticles (size 20-30 nm, dosage 25 mg kg^{-1}) and they were found no accumulation of Zn in the liver or kidneys of the rats [36]. Elshama et al. [36] found that long-term intra-peritoneal injection of ZnO nanoparticles generated histological and ultrastructural abnormalities in the brains and spinal cords of rats, with the severity of these changes dependent on the dosage and the generation of reactive oxygen species [37].

2.2 Oral Administrations

Baek et al. [18] studied the ZnO Nanoparticles (20 and 70 nm) accumulated in the kidneys, liver, and lungs of rats after a single oral treatment Zn levels in all these organs were were significantly increased 6-24 hours after a low dosage (50 and 300 mg kg^{-1}) of ZnO nanoparticles . After two days of receiving a substantial dosage (2000 mg kg^{-1}), considerable buildup occurred in the liver and kidneys; however, by day seven, levels had returned to normal (about 20 g/g in the liver and around 10 g/g in the kidneys). Earlier research has demonstrated that, upon acute oral exposure, nano-forms of certain particles are more hazardous than their micro-counterparts. Liu N et al. [17] found the level of Zn content accumulation in the heart, liver, spleen, lungs, kidneys, and brain of exposed mice, after receiving a single oral dosage of 45 mg kg^{-1} of ZnO nanoparticles (size 27.54.1 nm), The tissue distribution pattern of ZnO nanoparticles was found to be different from that of $ZnCl_2$ with a greater concentration in the lungs and a lower concentration in the kidneys and liver which leads to oxidative stress as will as DNA damage shown in Figure (3). ZnO nanoparticles (size 40 nm, dosage 134.2-536.8 mg $kg^{-1} day^{-1}$) were distributed to the liver and kidneys in rats

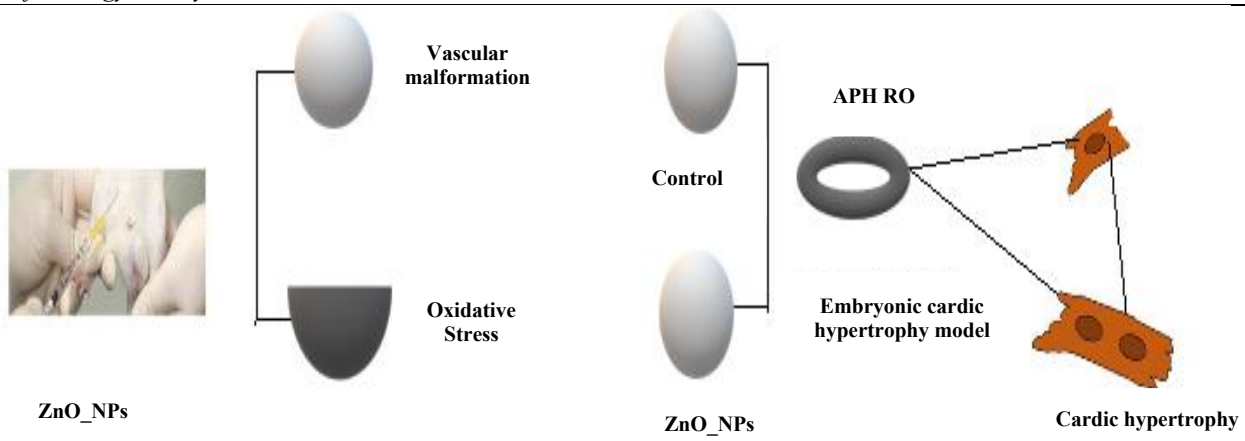

Figure 2. Intraperitoneal ZnO nanoparticles induces vascular malformation and oxidative stress.

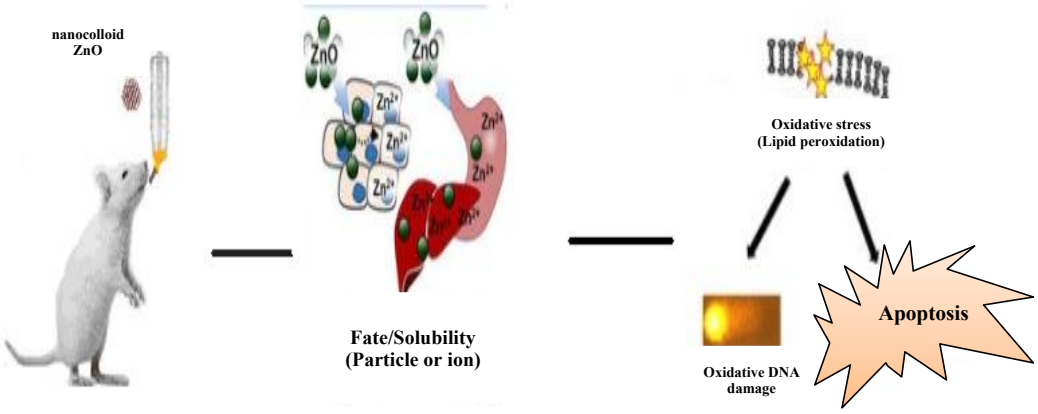

Figure 3. Oral ingestion leading to stomach digestion-induced apoptosis and DNA damage.

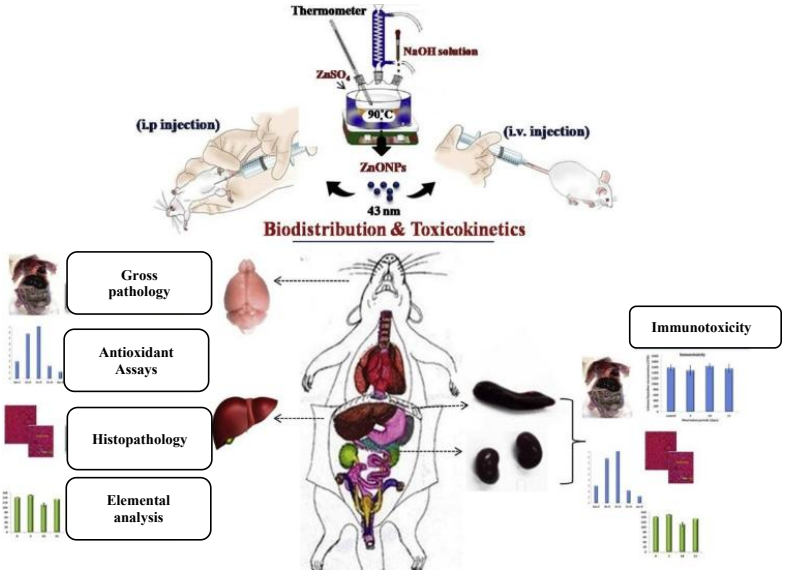

Figure 4. Intravenous and intraperitoneal injection of ZnO nanoparticles induces acute toxicity in vital organs including liver, lungs, kidneys, and other body systems [38].

given repeated oral doses for 13 weeks, although lung distribution was not mentioned.

After being given ZnO nanoparticles (size 30 nm, dose 300 mg kg^{-1}) orally for 14 days in a row, mice saw their liver Zn content significantly increase and their kidney Zn content somewhat increase [16]. According to Choi et al. [15] a single oral dose of either 3 or 30 mg kg^{-1} of ZnO nanoparticles showed that these particles were predominantly distributed in the liver, kidneys, and lungs. Following oral administration, it was observed that ZnO nanoparticles had limited absorption in the gastrointestinal tract (GIT) and were primarily excreted in feces. [39].

Pasupuleti et al. [40] considered that ZnO nanoparticles accumulated in the liver of mice after 14 days of oral treatment of 30 nm ZnO nanoparticles, and they also caused oxidative stress. Sprague Dawley (SD) rats were also given oral administration of ZnO nanoparticles for 14 days. They discovered that rats treated with low doses of nanoparticles had higher rates of lesions in the liver, pancreas, heart, and stomach than rats treated with high doses; however, high dosages of the micro-sized nanoparticles produced more lesions than the low one.

2.3 Intravenous Administrations

Lee J et al. [41] were used rats for checking the effects of intravenous injections of 5, 10, and 20 mg kg^{-1} of ZnO nanoparticles on dams and fetuses from gestation day 6 to day 20. Twenty dams in the 20 mg kg^{-1} treatment group lost two of them while under treatment. In treated dams, hematological analysis and serum biochemistry revealed dose-dependent damage. Tubular dilatation in the kidneys, extremely hemopoiesis in the liver, and multifocal mixed cell infiltration and thrombosis in the lung were all discovered by histopathological study of treated dams.

Yeh et al. [38] when injected intravenously into mice, radioactive ZnO nanoparticles that emit gamma rays were mostly localized in the lungs, with some also found in the organs responsible for digestion and detoxification. The distribution of ZnO nanoparticles at 24 hours after injection showed the largest amounts in the lungs and liver. ZnO nanoparticles (size 10 and 70 nm, dosage 120 g $mouse^{-1}$) were injected intravenously and studied for their effects on mice over time (days, weeks, and months).

Radioactive ZnO nanoparticles that release gamma rays were predominantly located in the lungs after being injected intravenously into mice, with some also identified in the organs responsible for digesting and detoxification. After being administered intravenously into mice, radioactive ZnO nanoparticles that produce gamma rays were found mostly in the lungs, with some being found in the digestive and detoxifying organs as shown in Figure 4 were given a single intravenous injection, and by the next day, nanoparticles could be found throughout the mice's bodies, including their blood, liver, spleen, lungs, brain, and heart [38].

Fujihara J et al. [42] were administered intravenously ZnO nanoparticles (size 58.5 nm, 0.2 mg kg^{-1}) to mice, and their short-term tissue distribution in the lungs, liver, kidneys, and spleen was evaluated for up to 1 hour after administration Liver and lung Zn levels peaked 5 minutes after the treatment, kidney, and spleen Zn levels peaked 15 minutes after the administration, and tissue Zn levels peaked 1 hour after the dose. They also reported that Zn tissue accumulation over time 6 days following intravenous injection of ZnO nanoparticles (0.05 or 0.2 mg kg^{-1}). Zn levels were only substantially higher in the kidneys after one day at 0.05 mg kg^{-1} compared to the control group. Zn content was considerably elevated in the liver and spleen after just one day and six days at a level of 0.2 mg kg^{-1}.

2.4 Inhalation Exposure

Vysloužil et al., [43] were reported that ZnO nanoparticles (size 374.2 nm, dosage 6.46104 and 1.93106 particles/cm^3) to enter rats organs from ambient air at two different doses, with the lower dose considerably increasing Zn content in the liver and at higher dose significantly increasing Zn content in the lungs. Konduru et al., were injected intratracheally ZnO nanoparticles (size: 4.62.5 nm, dose: 1 mg kg^{-1}) into rats and their tissue distribution was mapped out. The transfer of 65Zn to the skeletal muscle, bone, kidneys, liver, and skin occurred on day 2. It was injected into the skin, bones, and muscles on

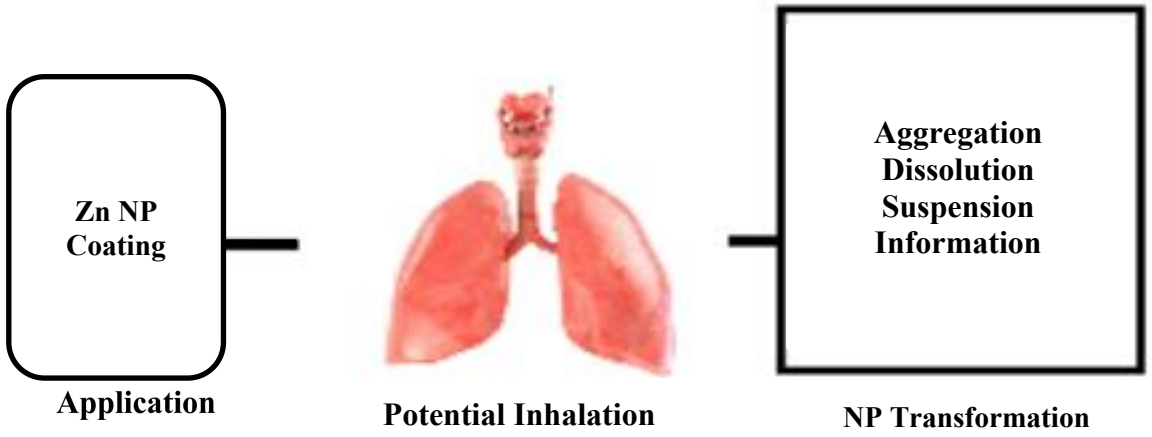

Figure 5. Inhaled ZnO nanoparticles directly induce lung injury.

days 7 and 28 [44]. Wang et al., were injected intratracheally ZnO nanoparticles (size 4218 nm, dosage 2.5 mg kg^{-1}) in the lungs and liver of mice [19]. Depending on the particle size and solubility, ZnO nanoparticles induced inflammatory and fibrotic responses in the tracheobronchial and alveolar tissues after inhalation. Lung fluid is acidic, so when ZnO nanoparticles were dissolved in it, their concentration increased and they were hazardous to the lungs [45]. Fujihara J., ZnO nanoparticles inhalation experiments revealed negligible lung cytotoxicity, histopathologic alterations, or pulmonary inflammation. ZnO nanoparticles have likely been dissolved in the respiratory system following inhalation if there is a higher Zn content in the BAL fluid and lungs. The toxicity of ZnO nanoparticles was significantly influenced by the exposure concentration, exposure mode, and time post-exposure. To conclude that ZnO nanoparticles had low sub-chronic toxicity via inhalation, exposure for 13 weeks at a cumulative dose of 10.9 mg kg^{-1} resulted in increased lung cellularity, but other markers of toxicity did not differ from sham-exposed animals [46].

3. Toxicological effects ZnO nanoparticles on various organs of Rats

ZnO nanoparticles have been shown to exert harmful effects on different cells, including membrane damage, an inflammatory response, DNA damage, and apoptosis. According to recent research, the release of Zn^{+2} ions is what causes ZnO nanoparticles to be poisonous.

3.1 Effects on Body Weight and Organ Weight

Treatment of rats orally with 536.8 mg kg^{-1} day^{-1} for 13 weeks resulted in a reduction in body weight [47] Rats (size 12-90 nm) and mice (size 205 nm) were given 30.3 mg kg^{-1} intraperitoneally on a daily basis for 28 days, and 1, 10, and 100 mg kg^{-1} intraperitoneally daily for 14 days [48]. Rats and mice have been used as examples. After a single oral dosage of 2000 mg kg^{-1} (size 20 and 70 nm), rats lost a small amount of weight. At 14 days post-exposure, mice given a single intragastric dose (size205 nm, 100 mg kg^{-1}) and rats given repeated intraperitoneal and intravenous exposure (size1290 nm, dose 30.3 mg kg^{-1} for 14 and 28 days) showed decreased organ weight of the heart, liver, spleen, lungs, kidneys, and brain [48]. After 400 mg kg^{-1}/day of nanoparticles were administered to the dams, their body weight dramatically fell. They also ate less after receiving 200 and 400 mg kg^{-1} /day of nanoparticles, and after 400 mg kg^{-1} /day of nanoparticles, their liver and adrenal gland weights also rose [49].

Jo et al. [49] were exposed rats to ZnO nanoparticles (500 mg kg^{-1} bw) with a size smaller than 100 nm. Additionally, the zinc concentration in dams and offspring's bodies was assessed. Rats given nano-ZnO treatment had lower pup weights, fewer live births, and higher fetal resorption rates. The liver and kidney of puppies as well as the mammary tissue of mothers were given ZnO nanoparticles. These findings suggest that nanoscale ZnO nanoparticles [50]. Wang et al. [50] found that 50 and 500 mg kg^{-1} nano ZnO illustrated increases in body weight while 5000 mg kg^{-1} showed declines in body weight, indicating that high dosages of ZnO nanoparticles in the diet

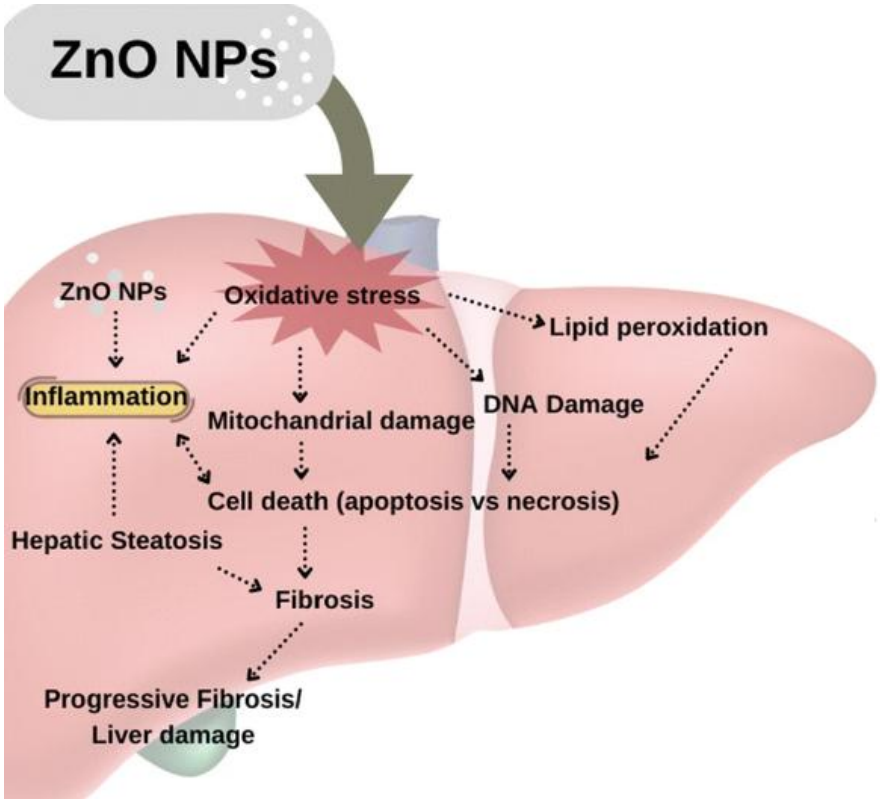

Figure 6. Graphical representation of the effects of ZnO nanoparticles on the liver [51].

could have toxicological effects [52]. The rise in the relative organ weights of the pancreas, brain, and lung at 5000 mg kg^{-1} ZnO nanoparticles may be largely attributed to the decrease in body weight [53].

3.2 Effects on Liver Tissues

After entering the body through any of the various methods, ZnO nanoparticles may function as a key target organ for the liver, which is the main organ of metabolism. The ability of ZnO nanoparticles to induce apoptosis and genotoxicity in human liver cells (HepG2), as well as the underlying molecular mechanisms of its cellular toxicity. Investigations were done on the part that dissolution plays in the toxicity of ZnO nanoparticles [54]. Given that inhalation is the primary method of exposure to ZnO nanoparticles in the workplace, pulmonary toxicity caused by ZnO nanoparticles has come under increased scrutiny. Acute pulmonary inflammation, chronic inflammation, altered metabolisms, histological abnormalities in the lungs, and airway irritation were among the toxicity outcomes caused by ZnO nanoparticles that were previously documented in vivo investigations [55].

ZnO nanoparticles, regardless of how they enter the body, preferentially accumulate in the liver. ZnO exposure has been linked to hepatic damage, suggesting that the liver, the body's biggest detoxifying organ, is vulnerable to xenobiotic-mediated damage which is shown in Figure (6). ZnO nanoparticles caused increased levels of the enzymes aspartate transaminase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH) in rats and mice after a single oral dosage [56]. Alkaline phosphatase (ALT) levels were shown to be increased in studies using a single intraperitoneal dosage of ZnO nanoparticles. When a single dosage of ZnO nanoparticles was given orally to mice [23]. Histopathological changes in the liver have been reported after a single oral dosage of ZnO nanoparticles in mice, including extensive hepatic edema, vacuolization, cellular necrosis, congestion, and fibrosis. glycogen buildup [34].

Table 1. Toxicological effects ZnO nanoparticles on the livers, kidneys, and lungs of rats.

Organs	Routes	Effects	References
Livers	Oral, IM, IV, IP	Abnormal rise of blood liver enzymes • AST, ALT, ALP, and LDH and Histopathological changes	[19,23,34,48,57,58]
	Oral, IV	causes significant liver enlargement, vacuolization, cellular necrosis, congestion, and glycogen buildup.	[34,58]
	Oral	Low-dose apoptotic liver alterations and single-dose IP localized inflammation	[23]
	IM	The hepatic sinusoid can be partially dilated	[42]
Kidneys	IP	Elevated levels of kidney injury marker BUN and creatine phosphokinase	[35, 59]
	Oral	Histopathological alterations were seen that decreased total kidney glutathione levels.	[58]
	IP	Resulting in necrosis, edema, and hydropic degeneration	[16]
	SC, IP	causes tubular dilation, Focal interstitial edema, and inflammation	[23]
Lungs	IP	Markers for oxidative stress and inflammation were found to be elevated, including lipid peroxide, heme oxygenase-1 (HO-1), and -tocopherol in the lungs	[60]
	IT	Extreme alveolar desquamation caused by massive acute lung inflammation caused	[61]
	IT	lung and systemic inflammation, dyslipidemia, and elevated blood HO-1 levels.	[20]
	IP, IT	Induces edema, lymphoid cell infiltration, and increased bronchiole epithelial cell proliferation and hypertrophy, induced pulmonary fibrosis and inflammation	[19]
	IV, IT	can cause aortic damage; cause serious inflammation, significant hyperemia in the alveoli, and edema;	[20,58]
	IP	causes mild interstitial inflammation.	[23]
	IV		[42]

System localized infarction at high dosages and early apoptotic changes in the liver were seen after a single intraperitoneal injection of ZnO nanoparticles in rats. We found that 1 day after a single intravenous injection (0.2 mg kg^{-1} of ZnO nanoparticles in mice, the hepatic sinusoid was slightly dilated and the Zn concentration in liver was 10.1-8.6 g/g [39].

3.3 Effects on Kidney Tissues

Yan G et al. [62] examined the biochemical compositions of urine and kidney samples from rats that received doses of 100, 300, and 1000 mg kg^{-1} of ZnO nanoparticles over a 14-day period, using a 1H nuclear magnetic resonance (NMR) technique. The results show that ZnO nanoparticles can disrupt energy metabolism and result in mitochondrial and cell membrane impairment in the rat kidney, which may contribute to ZnO nanoparticles-induced nephrotoxicity.

Rani V et al. [63] treated the rats with ZnO nanoparticles (50 mg kg^{-1}). Their histopathological studies also showed that the morphology of the liver cells had improved. ZnO nanoparticles may provide protection by selectively intoxicating proliferating

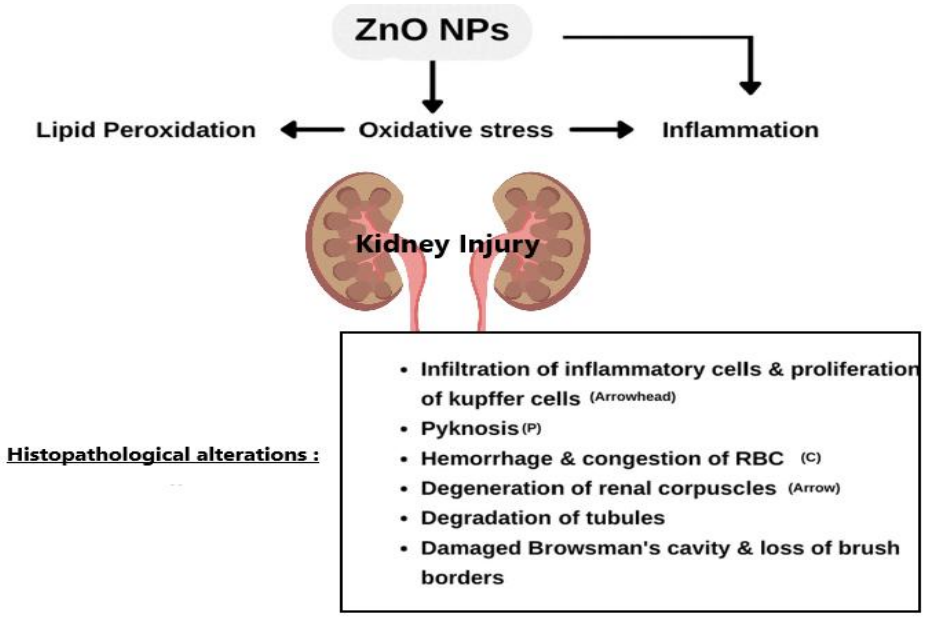

Figure 7. Flowchart depicting kidney damage caused by ZnO nanoparticles [51].

tissue, such as the adenomatous islands developed in the liver. The amelioration of DMN-induced toxic effects may also involve zinc metallothionein (Zn-MT), which is induced by ZnO nanoparticles. The major mechanism underlying ZnO nanoparticles' protective properties is still their ability to reduce oxidative stress. ZnO nanoparticles accumulated in the tested organs including kidneys, suggesting that the kidney could be one of the major target organs for the ZnO nanoparticles induced toxicity [35]. The most recent research on the nephrotoxicity caused by ZnO nanoparticles in several animal models. Bowman's gap increases, the distal convoluted tubule is destroyed, there is intratubular protein deposition, inflammatory cells are infiltrated, and there are capillaries clogged between the tubules, among other histological alterations in the kidney. Histological examination of the kidney and serum biochemical analysis [64]. The kidneys, due to their high blood supply and ability to concentrate toxins, are especially susceptible to xenobiotics and are the preferred accumulation site for ZnO nanoparticles following oral ZnO exposure (600 milligrams or 1 gram per kilogram of body weight every day for 5 days) total glutathione in the kidneys is significantly reduced, suggesting functional impairment to kidney tissue [59].

The rats had elevated levels of blood urea nitrogen (BUN) and creatinine (Cre) 6 hours after receiving a single intraperitoneal injection of ZnO nanoparticles (dosage 10 mg kg^{-1}), These are biochemical markers of kidney damage and a Zn level of around 70 g/g in the kidneys. In contrast, we found that mice given a single intravenous injection of ZnO nanoparticles (0.2 mg kg^{-1}) did not develop any pathological alterations to the kidneys, including an increase in BUN or Cre levels. In comparison, the Zn content in kidneys was 8.6 1.0 g/g [57]. ZnO nanoparticles (size 30 nm, dosage 300 mg kg^{-1}) were given orally to mice over the course of 14 days, and Zn content was measured in kidneys to be about 40 g/g, indicating that the tubules had enlarged as a result. After receiving a single intraperitoneal dose of ZnO nanoparticles (size 20 nm, dose 100 g/mL daily for 14 days), the kidneys of mice were inflamed and developed focal interstitial edema, as determined by a pathological investigation. Khorsandi et al. [65] reported that inhaled ZnO nanoparticles cause renal irritation to last for a long time. Malonaldehyde, H_2O_2, and NO concentrations in the kidney were reduced in DMN (2 l/100 g body weight/rat)-treated rats when ZnO nanoparticles (50 mg kg^{-1} body weight/rat) were administered. The healing of oxidative DNA damage and less apparent histological abnormalities in the

kidney lend weight to these findings. ZnO nanoparticles are thought to be harmful to renal tissue, yet their high therapeutic and antioxidative properties aid in lessening the rat kidney damage caused by dimethylnitrosamine (DMN) [66].

3.4 Effects on Lung Tissues

Inhalation is the most common route of ZnO nanoparticles exposure on the job. Studies on the toxicity and injury caused by ZnO nanoparticles to the lungs have mostly involved exposure by inhalation, intratracheal instillation, or intranasal administration. Researchers have shown that inhaling ZnO nanoparticles is the most lethal route of exposure [67]. Due to the acidic nature of lung lining fluid, ZnO nanoparticles degrade and release Zn^{+2}, leading to ROS-induced inflammation, necrosis, and cell death [68]. High levels of oxidative stress were seen in rats after receiving a single intratracheal injection of ZnO nanoparticles (size 21 nm, dosage 70 g mL^{-1}), as evidenced by an increase in lung lipid peroxide, heme oxygenase-1 (HO^{-1}), and -tocopherol. The ZnO nanoparticles also stayed there in the lungs and continued to release Zn^{+2} [60]. Jacobsen et al. [61] were injected ZnO nanoparticles (size 12–3 nm, low dose–0.3 mg kg^{-1}) into the trachea of mice, and the animals afterward experienced massive acute pulmonary infarction, excessive desquamation of alveolar barrier epithelial cells, and death, and histological alterations (including edema, eosinophilic granuloma, lymphoid cell infiltration, and enhanced proliferation and hypertrophy of bronchiole epithelial cells). An increase in the lung weight to body weight ratio, as well as histological abnormalities such as pulmonary fibrosis and inflammation, were seen 7 days after intratracheal injection of ZnO nanoparticles in mice. Huang et al., studied the effects of ZnO nanoparticles inhalation in mice (dosage 2.5 mg/m3, 5 hours/day for 5 days) and hypothesized that ZnO nanoparticles inhalation may lead to the development of allergic airway inflammation [69]. Figure 5 shows the toxicity effect of in lungs.

Saptarshi et al. [70] investigated the 24 hours inhalation of ZnO nanoparticles (size 30 nm, dose 5 mg kg^{-1}), mice showed signs of pulmonary inflammation as evidenced by an increase in eotaxin mRNA expression in lung tissue and the release of pro-inflammatory cytokines in their blood. Histopathological abnormalities in the lungs of mice were seen after a single oral exposure to ZnO nanoparticles. These abnormalities included severe inflammation, vascular damage figure (8) severe hyperemia in the alveoli, and edema [71]. Cho W-S et al. [72] administered ZnO nanoparticles intratracheally to rats at two different dosages (50 and 150 cm2/rat). They conducted assessments at 24 hours, one week, and four weeks to evaluate the dose-dependent and time-dependent responses. Eosinophilia, airway epithelial cell proliferation, goblet cell hyperplasia, and lung fibrosis were all brought on by ZnO nanoparticles. Chronic bronchocentric interstitial lung fibrosis was linked to elevated myofibroblast accumulation and positive transforming growth factor. The fundamental source of ZnO nanoparticles-induced various progressive severe lung damage is a pH-dependent breakdown of ZnO nanoparticles inside phagosomes. Wang, D et al. [19] were given varied doses of ZnO nanoparticles (200, 400, 800 g/kg) to mice. Animal mortality, organ/body weight ratios, hematological, blood biochemistry, and histopathology were used to determine the acute toxicity of the substance. Malondialdehyde levels in the lung homogenates also rose. In addition, it was found that the lungs had undergone inflammatory and hyperplastic alterations.

4. Therapeutic Mechanism of ZnO Nanoparticles

The exact toxicological mechanisms of ZnO nanoparticles are yet unclear. Nanoparticles have various physical and chemical characteristics, which may be related to their potential toxicity because their surface area is proportionately bigger than that of larger particles [73].When ingested, ZnO nanoparticles break down and release Zn^{+2}. Some research has put forward the theory that Zn^{+2} is responsible for toxicity effects of ZnO nanoparticles [74]. Fukui et al., injected intratracheally ZnO nanoparticles stayed in the lungs of rats, where they constantly produced Zn^{+2} and caused severe oxidative stress. ZnO nanoparticles induced a rise in 8-hydroxydeoxyguanosine (8-OHdG), a major ROS product and a widely used marker for

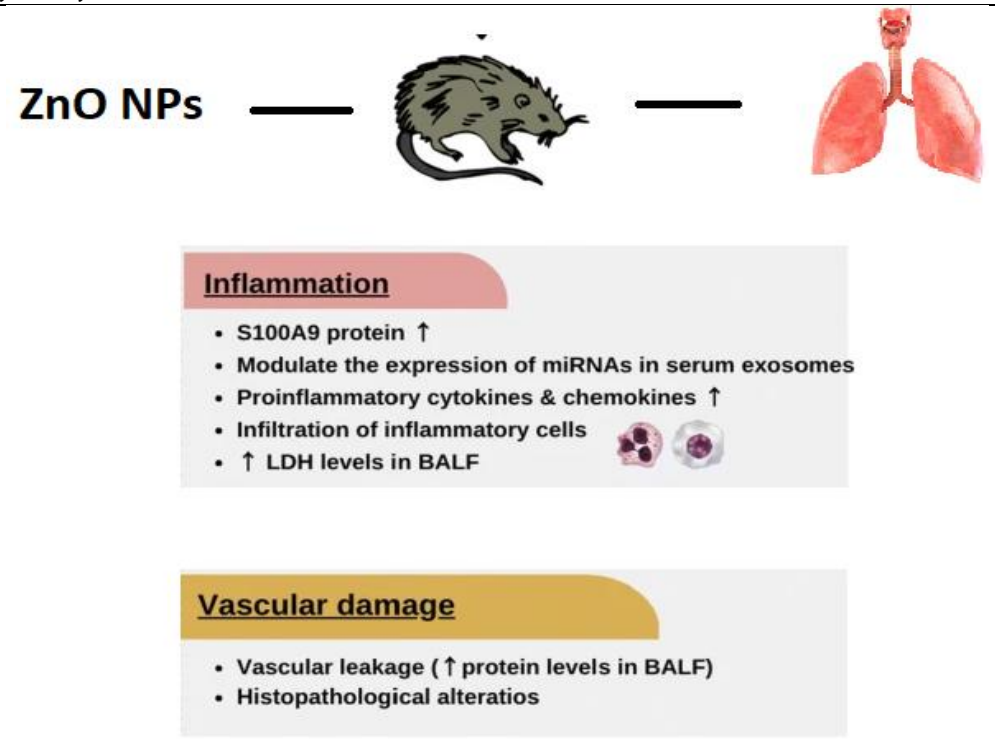

Figure 8. Lung, vascular damage, and histopathological alterations induced by ZnO nanoparticles [51].

oxidative DNA damage, whether injected intratracheally or breathed. ZnO nanoparticles are capable of producing high amounts of free radicals, which can lead to oxidative damage [60]. Li YS et al. [75] found that 8-OHdG was highly accumulated in the lungs after intratracheal installation of ZnO nanoparticles containing lipopolysaccharides. Possible involvement of oxidative stress in inflammation; this can lead to DNA damage and cell death, or apoptosis. They postulated further that ZnO nanoparticles may cross the blood-air barrier and harm the liver in this way. Previous research examined how much 8-OHdG was excreted in the urine following a single intravenous dose of ZnO nanoparticles. The concentration was significantly higher after day one and decreased dose-dependently over the next six days. Serum superoxide dismutase levels were considerably elevated at 24 and 48 hours after intravenous injection of 0.2 mg kg^{-1} ZnO nanoparticles. An in vitro study found that both ZnO nanoparticles and Zn^{+2} entered cells. Zn^{+2} impacts enzyme balance, transcription factors, and signaling pathways, whereas nanoparticles induce cell inactivation, oxidative stress, mitochondrial damage, and intracellular Ca^{+2} excess. Comparing the effects of ZnO nanoparticles and bulk ZnO on astrocytes revealed that both were toxic, but that astrocytes exposed to ZnO nanoparticles had more ROS production and caspase activity than those exposed to bulk ZnO [76]. Tang et al. [77] found that after a week of oral treatment of ZnO Nanoparticles at 100, 300, and 600 mg kg^{-1}, mRNA expression for cytochrome P450 1A2 (CYP1A2) was downregulated, whereas expression for cytochrome P450 2C11 and CYP3A4 was upregulated, and pathological abnormalities were observed in liver and kidney tissues.

5. Conclusion

ZnO nanoparticles are rapidly distributed throughout the body and are efficiently eliminated. They are primarily absorbed in ionic form, with some in particle form. Importantly, these nanoparticles do not tend to accumulate in tissues over an extended period. Regardless of the exposure method, higher concentrations of Zn were detected in the key target organs for ZnO nanoparticles, including the liver, kidneys, and lungs. The liver is the primary site of accumulation for ZnO nanoparticles, and exposure through various routes led to histological changes and liver damage. Following a single oral or intraperitoneal

dose, kidney injury was observed. Notably, Lung damage was assessed using intratracheal instillation and inhalation exposure methods. The primary toxicological mechanism associated with ZnO nanoparticles involves the generation of substantial oxidative stress, characterized by the production of significant levels of reactive oxygen species (ROS). ROS generation is attributed to two main factors: the release of Zn^{+2} ions from ZnO nanoparticles and the particulate effect resulting from the semiconductor or electronic properties of ZnO nanoparticles. One effective strategy to mitigate the toxicity of these particles is by coating the surface of ZnO nanoparticles with silica, which effectively suppresses ROS production and Zn^{+2} ion release.

Data Availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

All authors participated in the initial draft creation, reviewed the manuscript, and contributed to the editing process.

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REFERENCES

1. Haleem, A., Javaid, M., Singh, R. P., Rab, S. and Suman, R., 2023. Applications of nanotechnology in medical field. Global Health Journal, 7, pp. 70-77
2. Moshed, A., Sarkar, M. K. I. and Khaleque, M. A., 2017. The application of nanotechnology in medical sciences: New horizon of treatment. Am. J. Biomed. Sci, 9, pp. 1-14.
3. Klymchenko, A. S., Liu, F., Collot, M. and Anton, N., 2021. Dye-loaded nanoemulsions: Biomimetic fluorescent nanocarriers for bioimaging and nanomedicine. Advanced healthcare materials, 10, pp. 2001289.
4. Kumar, R., Kumar, M. and Luthra, G., 2023. Fundamental approaches and applications of nanotechnology: A mini review. Materials Today: Proceedings.
5. Paulkumar, K., Rajeshkumar, S., Gnanajobitha, G., Vanaja, M., Malarkodi, C. and Annadurai, G., 2013. Biosynthesis of silver chloride nanoparticles using bacillus subtilis MTCC 3053 and assessment of its antifungal activity. International Scholarly Research Notices, pp. 1-8.
6. Paulkumar, K., Gnanajobitha, G., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Pandian, K. and Annadurai, G., 2014. Piper nigrum leaf and stem assisted green synthesis of silver nanoparticles and evaluation of its antibacterial activity against agricultural plant pathogens. The Scientific World Journal, pp. 829-894.
7. Rajeshkumar, S., Malarkodi, C., Paulkumar, K., Vanaja, M., Gnanajobitha, G. and Annadurai, G., 2014. Algae mediated green fabrication of silver nanoparticles and examination of its antifungal activity against clinical pathogens. International journal of Metals, pp. 1-8.
8. Subramaniam, V. D., Prasad, S. V., Banerjee, A., Gopinath, M., Murugesan, R., Marotta, F., Sun, X.-F. and Pathak, S., 2019. Health hazards of nanoparticles: Understanding the toxicity mechanism of nanosized zno in cosmetic products. Drug and chemical toxicology, 42, pp. 84-93.
9. Senthilkumar, K., Senthilkumar, O., Yamauchi, K., Sato, M., Morito, S., Ohba, T., Nakamura, M. and Fujita, Y., 2009. Preparation of ZnO nanoparticles for bio-imaging applications. Physica status solidi (b), 246, pp. 885-888.
10. Madhumitha, G., Elango, G. and Roopan, S. M., 2016. Biotechnological aspects of ZnO nanoparticles: Overview on synthesis and its applications. Applied microbiology and biotechnology, 100, pp. 571-581.
11. Poulsen, H. D., 1995. Zinc oxide for weanling piglets. Acta Agriculturae Scandinavica A-Animal Sciences, 45, pp. 159-167.
12. Burrough, E. R., De Mille, C. and Gabler, N. K., 2019. Zinc overload in weaned pigs: Tissue accumulation, pathology, and growth impacts. Journal of Veterinary Diagnostic Investigation, 31, pp. 537-545.
13. Komatsu, T., Sugie, K., Inukai, N., Eguchi, O., Oyamada, T., Sawada, H., Yamanaka, N. and Shibahara, T., 2020. Chronic pancreatitis in farmed pigs fed excessive zinc oxide. Journal of Veterinary Diagnostic Investigation, 32, pp. 689-694.
14. Bonetti, A., Tugnoli, B., Piva, A. and Grilli, E., 2021. Towards zero zinc oxide: Feeding strategies to manage post-weaning diarrhea in piglets. Animals, 11, pp. 642.
15. Choi, S.-J. and Choy, J.-H., 2014. Biokinetics of zinc oxide nanoparticles: Toxicokinetics, biological fates, and protein interaction. International journal of nanomedicine, 9, pp. 261-269.
16. Sharma, V., Singh, P., Pandey, A. K. and Dhawan, A., 2012. Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 745, pp. 84-91.

17. Liu, N., Mu, Y., Chen, Y., Sun, H., Han, S., Wang, M., Wang, H., Li, Y., Xu, Q. and Huang, P., 2013. Degradation of aqueous synthesized cdte/zns quantum dots in mice: Differential blood kinetics and biodistribution of cadmium and tellurium. Particle and fibre toxicology, 10, pp. 1-9.
18. Baek, M., Chung, H.E., Yu, J., Lee, J.A., Kim, T.H., OH, J.M., Lee, W.J., Paek, S.M., Lee, J. K. and Jeong, J., 2012. Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. International journal of nanomedicine, pp. 3081-3097.
19. Wang, D., Li, H., Liu, Z., Zhou, J. and Zhang, T., 2017. Acute toxicological effects of zinc oxide nanoparticles in mice after intratracheal instillation. International journal of occupational and environmental health, 23, pp. 11-19.
20. Yan, Z., Wang, W., Wu, Y., Wang, W., Li, B., Liang, N. and Wu, W., 2017. Zinc oxide nanoparticle-induced atherosclerotic alterations in vitro and in vivo. International journal of nanomedicine, 12, pp. 4433-4442.
21. Holmes, A. M., Kempson, I., Turnbull, T., Paterson, D. and Roberts, M. S., 2020. Penetration of zinc into human skin after topical application of nano zinc oxide used in commercial sunscreen formulations. ACS Applied Bio Materials, 3, pp. 3640-3647.
22. Xiong, H. M., 2013. ZnO nanoparticles applied to bioimaging and drug delivery. Advanced Materials, 25, pp. 5329-5335.
23. Hong, T.-K., Tripathy, N., Son, H.-J., Ha, K.-T., Jeong, H.-S. and Hahn, Y.-B., 2013. A comprehensive in vitro and in vivo study of ZnO nanoparticles toxicity. Journal of Materials Chemistry B, 1, pp. 2985-2992.
24. Jha, A. K., Prasad, K. and Kulkarni, A., 2007. Microbe-mediated nanotransformation: Cadmium. Nano, 2, pp. 239-242.
25. Nagajyothi, P., Sreekanth, T., Tettey, C. O., Jun, Y. I. and Mook, S. H., 2014. Characterization, antibacterial, antioxidant, and cytotoxic activities of ZnO nanoparticles using coptidis rhizoma. Bioorganic & medicinal chemistry letters, 24, pp. 4298-4303.
26. Hameed, A. S. H., Karthikeyan, C., Ahamed, A. P., Thajuddin, N., Alharbi, N. S., Alharbi, S. A. and Ravi, G., 2016. In vitro antibacterial activity of ZnO and nd doped zno nanoparticles against esbl producing escherichia coli and klebsiella pneumoniae. Scientific reports, 6, pp. 24312.
27. Ma, H., Williams, P. L. and Diamond, S. A., 2013. Ecotoxicity of manufactured ZnO nanoparticles–a review. Environmental pollution, 172, pp. 76-85.
28. Vimala, K., Sundarraj, S., Paulpandi, M., Vengatesan, S. and Kannan, S., 2014. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the bax and BCl-2 expression in breast and colon carcinoma. Process biochemistry, 49, pp. 160-172.
29. Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E. R., Sharma, N. and Sahi, S., 2017. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in leucaena leucocephala seedlings: A physiochemical analysis. Plant Physiology and Biochemistry, 110, pp. 59-69.
30. Hazra, C., Kundu, D., Chaudhari, A. and Jana, T., 2013. Biogenic synthesis, characterization, toxicity and photocatalysis of zinc sulfide nanoparticles using rhamnolipids from pseudomonas aeruginosa BS01 as capping and stabilizing agent. Journal of Chemical Technology & Biotechnology, 88, pp. 1039-1048.
31. Rajeshkumar, S., 2016. Synthesis of silver nanoparticles using fresh bark of pongamia pinnata and characterization of its antibacterial activity against gram positive and gram negative pathogens. Resource-Efficient Technologies, 2, pp. 30-35.
32. Viswanath, B. and Kim, S., 2017. Influence of nanotoxicity on human health and environment: The alternative strategies. Reviews of Environmental Contamination and Toxicology Volume 242, pp. 61-104.
33. Amde, M., Liu, J.-f., Tan, Z.-Q. and Bekana, D., 2017. Transformation and bioavailability of metal oxide nanoparticles in aquatic and terrestrial environments. A review. Environmental pollution, 230, pp. 250-267.
34. Li, C.H., Shen, C.C., Cheng, Y.W., Huang, S.H., Wu, C.C., Kao, C.C., Liao, J.W. and Kang, J.J., 2012. Organ biodistribution, clearance, and genotoxicity of orally administered zinc oxide nanoparticles in mice. Nanotoxicology, 6, pp. 746-756.
35. Lin, Y.-F., Chiu, I.-J., Cheng, F.-Y., Lee, Y.-H., Wang, Y.- J., Hsu, Y.-H. and Chiu, H.-W., 2015. The role of hypoxia- inducible factor-1α in zinc oxide nanoparticle-induced nephrotoxicity in vitro and in vivo. Particle and fibre toxicology, 13, pp. 1-14.
36. Amara, S., Slama, I. B., Mrad, I., Rihane, N., Khemissi, W., El Mir, L., Rhouma, K. B., Abdelmelek, H. and Sakly, M., 2014. Effects of zinc oxide nanoparticles and/or zinc chloride on biochemical parameters and mineral levels in rat liver and kidney. Human & experimental toxicology, 33, pp. 1150-1157.
37. Elshama, S. S., El-Kenawy, A. E.-M. and Osman, H.-E. H., 2017. Histopathological study of zinc oxide nanoparticle-induced neurotoxicity in rats. Toxicology, 13, pp. 95-103.
38. Choi, J., Kim, H., Kim, P., Jo, E., Kim, H.-M., Lee, M.-Y., Jin, S. M. and Park, K., 2015. Toxicity of zinc oxide nanoparticles in rats treated by two different routes: Single intravenous injection and single oral administration. Journal of Toxicology and Environmental Health, Part A, 78, pp. 226-243.
39. Pasupuleti, S., Alapati, S., Ganapathy, S., Anumolu, G., Pully, N. R. and Prakhya, B. M., 2012. Toxicity of zinc oxide nanoparticles through oral route. Toxicology and Industrial Health, 28, pp. 675-686.
40. Lee, J., Yu, W.-J., Song, J., Sung, C., Jeong, E. J., Han, J.-S., Kim, P., Jo, E., Eom, I. and Kim, H.-M., 2016. Developmental toxicity of intravenously injected zinc oxide nanoparticles in rats. Archives of pharmacal research, 39, pp. 1682-1692.
41. Yeh, T.K., Chen, J.-K., Lin, C.-H., Yang, M.-H., Yang, C. S., Chou, F.I., Peir, J.J., Wang, M.Y., Chang, W.H. and Tsai, M.H., 2012. Kinetics and tissue distribution of

neutron-activated zinc oxide nanoparticles and zinc nitrate in mice: Effects of size and particulate nature. Nanotechnology, 23, pp. 085102.
42. Fujihara, J., Tongu, M., Hashimoto, H., Yamada, T., Kimura-Kataoka, K., Yasuda, T., Fujita, Y. and Takeshita, H., 2015. Distribution and toxicity evaluation of ZnO dispersion nanoparticles in single intravenously exposed mice. The Journal of Medical Investigation, 62, pp. 45-50.
43. Vysloužil, J., Kulich, P., Zeman, T., Vaculovič, T., Tvrdoňová, M., Mikuška, P., Večeřa, Z., Stráská, J., Moravec, P. and Balcar, V. J., 2020. Subchronic continuous inhalation exposure to zinc oxide nanoparticles induces pulmonary cell response in mice. Journal of Trace Elements in Medicine and Biology, 61, pp. 126511.
44. Konduru, N. V., Murdaugh, K. M., Sotiriou, G. A., Donaghey, T. C., Demokritou, P., Brain, J. D. and Molina, R. M., 2014. Bioavailability, distribution and clearance of tracheally-instilled and gavaged uncoated or silica-coated zinc oxide nanoparticles. Particle and fibre toxicology, 11, pp. 1-13.
45. Condello, M., De Berardis, B., Ammendolia, M. G., Barone, F., Condello, G., Degan, P. and Meschini, S., 2016. ZnO nanoparticle tracking from uptake to genotoxic damage in human colon carcinoma cells. Toxicology in Vitro, 35, pp. 169-179.
46. Fujihara, J. and Nishimoto, N., 2023. Review of zinc oxide nanoparticles: Toxicokinetics, tissue distribution for various exposure routes, toxicological effects, toxicity mechanism in mammals, and an approach for toxicity reduction. Biological trace element research, 1, pp. 1-15.
47. Cho, W.S., Kang, B.C., Lee, J. K., Jeong, J., Che, J.H. and Seok, S. H., 2013. Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Particle and fibre toxicology, 10, pp. 1-9.
48. K Handral, H. and Kelmani R, C., 2018. A comparative in vivo scrutiny of biosynthesized copper and zinc oxide nanoparticles by intraperitoneal and intravenous administration routes in rats. Nanoscale research letters, 13, pp. 1-15.
49. Hong, J.S., Park, M.K., Kim, M.S., Lim, J.H., Park, G.J., Maeng, E.H., Shin, J.H., Kim, M.K., Jeong, J. and Park, J.A., 2014. Prenatal development toxicity study of zinc oxide nanoparticles in rats. International journal of nanomedicine, 9, pp. 159-171.
50. Jo, E., Seo, G., Kwon, J.-T., Lee, M., cheun Lee, B., Eom, I., Kim, P. and Choi, K., 2013. Exposure to zinc oxide nanoparticles affects reproductive development and biodistribution in offspring rats. The Journal of toxicological sciences, 38, pp. 525-530.
51. Wang, C., Lu, J., Zhou, L., Li, J., Xu, J., Li, W., Zhang, L., Zhong, X. and Wang, T., 2016. Effects of long-term exposure to zinc oxide nanoparticles on development, zinc metabolism and biodistribution of minerals (Zn, Fe, Cu, Mn) in mice. PloS one, 11, pp. e0164434.
52. Valdiglesias, V., Costa, C., Kiliç, G., Costa, S., Pásaro, E., Laffon, B. and Teixeira, J. P., 2013. Neuronal cytotoxicity and genotoxicity induced by zinc oxide nanoparticles. Environment international, 55, pp. 92-100.
53. Sharma, V., Anderson, D. and Dhawan, A., 2012. Zinc oxide nanoparticles induce oxidative DNA damage and ros-triggered mitochondria mediated apoptosis in human liver cells (hepG2). Apoptosis, 17, pp. 852-870.
54. Larsen, S. T., Jackson, P., Poulsen, S. S., Levin, M., Jensen, K. A., Wallin, H., Nielsen, G. D. and Koponen, I. K., 2016. Airway irritation, inflammation, and toxicity in mice following inhalation of metal oxide nanoparticles. Nanotoxicology, 10, pp. 1254-1262.
55. Smeets, M. A. and Dalton, P. H., 2005. Evaluating the human response to chemicals: Odor, irritation and non-sensory factors. Environmental Toxicology and Pharmacology, 19, pp. 581-588.
56. Chong, C. L., Fang, C. M., Pung, S. Y., Ong, C. E., Pung, Y. F., Kong, C. and Pan, Y., 2021. Current updates on the in vivo assessment of zinc oxide nanoparticles toxicity using animal models. BioNanoScience, 11, pp. 590-620.
57. Yan, G., Huang, Y., Bu, Q., Lv, L., Deng, P., Zhou, J., Wang, Y., Yang, Y., Liu, Q. and Cen, X., 2012. Zinc oxide nanoparticles cause nephrotoxicity and kidney metabolism alterations in rats. Journal of Environmental Science and Health, Part A, 47, pp. 577-588.
58. Fujihara, J., Tongu, M., Hashimoto, H., Fujita, Y., Nishimoto, N., Yasuda, T. and Takeshita, H., 2015. Pro-inflammatory responses and oxidative stress induced by ZnO nanoparticles in vivo following intravenous injection. Eur Rev Med Pharmacol Sci, 19, pp. 4920-4926.
59. Esmaeillou, M., Moharamnejad, M., Hsankhani, R., Tehrani, A. A. and Maadi, H., 2013. Toxicity of ZnO nanoparticles in healthy adult mice. Environmental Toxicology and Pharmacology, 35, pp. 67-71.
60. Lin, Y.-F., Chiu, I.J., Cheng, F.Y., Lee, Y.H., Wang, Y.J., Hsu, Y.H. and Chiu, H.W., 2015. The role of hypoxia-inducible factor-1α in zinc oxide nanoparticle-induced nephrotoxicity in vitro and in vivo. Particle and fibre toxicology, 13, pp. 1-14.
61. Faddah, L. M., Baky, N. A. A., Al-Rasheed, N. M., Al-Rasheed, N. M., Fatani, A. J. and Atteya, M., 2012. Role of quercetin and arginine in ameliorating nano zinc oxide-induced nephrotoxicity in rats. BMC complementary and alternative medicine, 12, pp. 1-14.
62. Fukui, H., Horie, M., Endoh, S., Kato, H., Fujita, K., Nishio, K., Komaba, L. K., Maru, J., Miyauhi, A. and Nakamura, A., 2012. Association of zinc ion release and oxidative stress induced by intratracheal instillation of zno nanoparticles to rat lung. Chemico-biological interactions, 198, pp. 29-37.
63. Jacobsen, N. R., Stoeger, T., Van Den Brûle, S., Saber, A. T., Beyerle, A., Vietti, G., Mortensen, A., Szarek, J., Budtz, H. C. and Kermanizadeh, A., 2015. Acute and subacute pulmonary toxicity and mortality in mice after intratracheal instillation of ZnO nanoparticles in three laboratories. Food and Chemical Toxicology, 85, pp. 84-95.

64. Rani, V., Verma, Y., Rana, K. and Rana, S. V. S., 2018. Zinc oxide nanoparticles inhibit dimethylnitrosamine induced liver injury in rat. Chemico-biological interactions, 295, pp. 84-92.
65. Abdel-Aziz, H. O., Hamdan, H. M. and Ragab, E. E., 2018. The histological effects of zinc oxide nanoparticles on the kidney of adult male rabbits. Sohag Medical Journal, 22, pp. 297-301.
66. Khorsandi, L., Heidari-Moghadam, A. and Jozi, Z., 2018. Nephrotoxic effects of low-dose zinc oxide nanoparticles in rats. Journal of Nephropathology, 7, pp. 158-165.
67. Rani, V., Verma, Y. and Rana, S., 2022. Zinc oxide nanoparticles ameliorate dimethylnitrosamine-induced renal toxicity in rat. Applied Biochemistry and Biotechnology, 194, pp. 1-17.
68. Vanderiel, R. and Jong, W., 2012. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol. Sci. Appl, 5, pp. 61-71.
69. Osmond, M. J. and Mccall, M. J., 2010. Zinc oxide nanoparticles in modern sunscreens: An analysis of potential exposure and hazard. Nanotoxicology, 4, pp. 15-41.
70. Huang, K.-L., Chang, H.-L., Tsai, F.-M., Lee, Y.-H., Wang, C.-H. and Cheng, T.-J., 2019. The effect of the inhalation of and topical exposure to zinc oxide nanoparticles on airway inflammation in mice. Toxicology and Applied Pharmacology, 384, pp. 114787.
71. Liu, M., Yu, X., Chen, Z., Yang, T., Yang, D., Liu, Q., Du, K., Li, B., Wang, Z. and Li, S., 2017. Aptamer selection and applications for breast cancer diagnostics and therapy. Journal of nanobiotechnology, 15, pp. 1-16.
72. Cho, W.S., Duffin, R., Howie, S. E., Scotton, C. J., Wallace, W. A., MacNee, W., Bradley, M., Megson, I. L. and Donaldson, K., 2011. Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn2+ dissolution inside lysosomes. Particle and fibre toxicology, 8, pp. 1-16.
73. Meng, Q., Wang, A., Hua, H., Jiang, Y., Wang, Y., Mu, H., Wu, Z. and Sun, K., 2018. Intranasal delivery of huperzine a to the brain using lactoferrin-conjugated n-trimethylated chitosan surface-modified plga nanoparticles for treatment of alzheimer's disease. International journal of nanomedicine, 13, pp. 705-718.
74. Camaioni, A., Massimiani, M., Lacconi, V., Magrini, A., Salustri, A., Sotiriou, G. A., Singh, D., Bitounis, D., Bocca, B. and Pino, A., 2021. Silica encapsulation of ZnO nanoparticles reduces their toxicity for cumulus cell-oocyte-complex expansion. Particle and fibre toxicology, 18, pp. 1-15.
75. Li, Y.S., Ootsuyama, Y., Kawasaki, Y., Morimoto, Y., Higashi, T. and Kawai, K., 2018. Oxidative DNA damage in the rat lung induced by intratracheal instillation and inhalation of nanoparticles. Journal of Clinical Biochemistry and Nutrition, 62, pp. 238-241.
76. Sudhakaran, S., Athira, S. and Mohanan, P., 2019. Zinc oxide nanoparticle induced neurotoxic potential upon interaction with primary astrocytes. Neurotoxicology, 73, pp. 213-227.
77. Tang, H.Q., Xu, M., Rong, Q., Jin, R.W., Liu, Q.J. and Li, Y.L., 2016. The effect of ZnO nanoparticles on liver function in rats. International journal of nanomedicine, 11, pp. 4275-4285.

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