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Genotoxic effect of manganese and nickel doped zinc ferrite ($Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$) nanoparticle in Swiss albino mouse *Mus musculus*

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Manganese and Nickel doped Zinc Ferrite (MNZF) nanoparticle $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ is used in fabrication of room temperature (25-30ºC) NH_3 gas sensor in large scale industries. However, there are no studies available on its toxic effects. Hence, in the present study, we assessed the genotoxic effect of various doses (125, 250 and 500 mg/kg) of the MNZF nanoparticle (NP) in Swiss albino mice *Mus musculus* employing the chromosomal aberration test, micronucleus test and single cell gel electrophoresis assay (comet assay). The NP was orally gavaged for 15 consecutive days. Dose-dependent study was conducted at 24 h after the last dose of gavage and time-dependent response was studied for 250 mg/kg at 24, 48 and 72 h of treatment. All the parameters employed showed a statistically significant dose-dependent increase of genetic damage indicating the genotoxic effect of this NP in Swiss albino mice. Proper precautions should be undertaken on handling this NP to avoid contact with it either through respiration or ingestion.

Keywords: Comet assay, Chromosomal aberrations, Micronuclei, MNZF

Nanoparticles (NPs) have numerous applications in the modern world where they may be an integral part of electronic gadgets, cosmetics, industrial products and also in medical treatment. NPs are also engineered using a mixture of compounds with unique properties and may potentially have more efficient functionality than non-engineered NPs. Engineered nanoparticles synthesized as nanoscale polymers, oxides, nanocomposites and quantum dots have a number of applications in electronics, pharmaceuticals, construction and medicine[1,2]. With such diverse applications, it is highly essential to know the possible toxic effects that are associated with NPs. Due to their small size, they can easily enter into the human body via respiratory route where they can be distributed to various parts of the body thereby causing a state of toxicity[3,4]. Engineered nanomaterials have been reported to induce genotoxicity by interacting directly with DNA and inducing damage in the form of chromosomal fragmentation, DNA strand breakages, point mutations, oxidative DNA adducts and alterations in gene expression profiles which will ultimately lead to carcinogenesis, tissue degradation and other diseases[5,6]. Engineered nanomaterials also indirectly affect the genetic material by generating reactive oxygen species (ROS) which can induce double stranded breaks[5] or by inhibiting the DNA repair pathway[7], disturbances in the cell cycle checkpoint functions, inhibition of antioxidant defenses and ultimately cell death[8].

However, few scientists have reported contradicting observations where in metal NPs did not induce genotoxicity. Landsiedel *et al*.[9] did not observe significant increase of MN in the bone marrow cells of mice which were administered intraperitoneally with ZnO nanoparticles. Significant DNA damage was also not observed in human peripheral lymphocytes exposed to Titanium dioxide nanoparticles[10].

Spinel ferrites exhibit a great property of detecting gaseous species such as NH_3 in the environment apart from being used as semi-conducting materials[11-13]. These ferrites are technologically important materials extensively used in diverse applications such as transformer core, noise filters and recording heads. The present test agent, manganese and nickel doped zinc ferrite (MNZF), $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ is used as a gas sensor for the detection of ammonia[14] which can potentially be used for detection of leaks or spills in industries such as the ones which manufacture

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fertilizers, chemicals and metal treating operations. MNZF also finds application in transformer cores used in power supplies which are an integral part of most of the electronic equipment[15]. Despite all these studies, no reports are available on the genotoxic effect of MNZF on biological systems. Therefore, in the present study, we investigated the potential genotoxic effect of the manganese and nickel doped zinc ferrite (MNZF) nanoparticle to understand if safety precautions are required for handling it.

Material and Methods

Nanoparticle (NP) synthesis

Sodium fumarate in aqueous medium was stirred with hydrazine hydrate (99-100%) in an inert atmosphere for 2 h. A stoichiometric amount of freshly prepared ferrous chloride solution mixed with manganous chloride, nickel chloride and zinc chloride was added drop wise to the above solution with constant stirring in an inert atmosphere. The yellow coloured precursor thus obtained was filtered, washed with ethanol and dried with diethyl ether using suction technique. This dried precursor was then auto-catalytically decomposed (reaction product acts as a catalyst on the reaction course) to yield nano-size $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ ferrite powder[14,15]. The average particle size of MNZF as measured by Transmission Electron Microscopy (TEM) was found to be 10 nm (Fig. 1).

Chemicals

All chemicals used were of analytical grade and were procured from HiMedia Laboratories, India unless specified otherwise.

Assay animals

Prior permission of the Institutional Animal Ethics Committee was taken to use the Swiss albino mice *Mus musculus* for experimentation, and were maintained strictly according to the guidelines of the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Male Swiss albino mice (2n=40), 4-6 weeks old, were procured from M/S Sri Venkateshwara Enterprises (Reg. No. CPC-237), Bangalore, India and were acclimatized for two weeks in the Animal House of Zoology Department, Goa University with prior permission of the Institutional Animal Ethics Committee (Reg. No. 446/CPCSEA). They were reared and maintained in polypropylene cages (290×220×140 mm) bedded with paddy husk, at a temperature of 28°C and 50% humidity. The mice were fed with standard mouse pellets and water *ad libitum*. Healthy males (average body wt. of 25 g) were selected for the present study. Five individuals were used for each of the experimental and control groups.

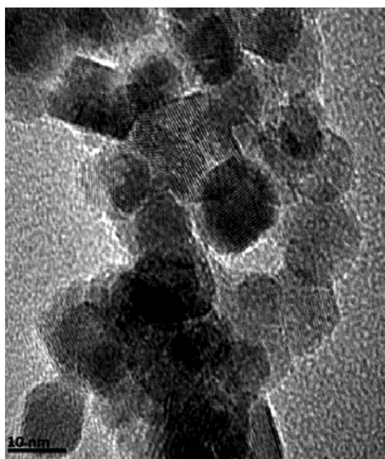

Fig. 1 — TEM micrograph of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$

Dose of NP

Since the LD_{50} value of MNZF NP is not available, the LD_{50} values of a similar NP, $Mn_{0.4}Zn_{0.6}Fe_2O_4$ was taken for dose selection (6.026 g/kg in mice)[16]. Based on this value, we selected three different sub-lethal doses viz. 500, 250 and 125 mg/kg (high, medium and low, respectively) of the $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ NP were selected for the present study.

Cyclophosphamide (CP)

Cyclophosphamide (Sigma, India) was selected as a positive mutagen because of its well established genotoxic effect[17,18]. A dose of 50 mg/kg body wt. of CP was used for the present study.

Preparation of NP suspension and administration

Precise quantity of nanoparticles (NP) was mixed with a known volume of distilled water and a homogenous suspension was prepared using a sonicator. As and when needed for feeding, this suspension was again made homogenous and was orally administered (gavage) to the mice using an oral catheter.

Treatment protocols

Dose response assay

Precise quantity of the suspension of NP was orally administered to three groups (Low, Medium and High) of experimental animals for 15 consecutive days at 24 h time interval.

Time response assay

Time dependent genotoxic responses were studied using the medium dose of the NP at 24, 48 and 72 h of the last dose of gavage.

Negative and Positive controls

Mice orally administered with equal amount of distilled water, for 15 days consecutively served as negative controls. A group of animals administered with 50 mg/kg of CP intraperitoneally (i.p.) served as positive controls, to confirm the sensitivity of mice to genotoxic agents.

Parameters

Genotoxic effects of the nanoparticle were studied in mice by employing the following parameters.

Chromosomal aberration assay

Bone marrow chromosomal preparations were made following the method of Tjio & Whang[19] with modifications from OECD[20]. Mice were injected intraperitoneally with 0.2 mL of 0.025% colchicine, maintained under laboratory conditions as cited earlier and were sacrificed by cervical dislocation after one hour. The marrow cells were collected from the femur bone by flushing it with 2.5 mL of 0.56% KCl, mixed well, incubated at 37°C for 20 min for hypotonic treatment and centrifuged at 1000 rpm for 8 min. The supernatant was discarded and the pellet was fixed gently with 2-3 mL of pre-chilled Carnoy's fixative. The suspension was centrifuged after one hour and the pellet with cells was re-suspended in fresh fixative for half an hour and again centrifuged. The process of fixation and centrifugation was repeated 2-3 times and the pellet got after the last centrifugation was suspended in about 0.5 mL of fixative to get a thick suspension. Two drops of this thick suspension were dropped with a Pasteur pipette on a pre-chilled slide and flame-dried. These slides were coded and stained with buffered 10% Giemsa (pH 6.8). As much as 100 metaphase spreads of each animal were analyzed. The mitotic index (MI) was calculated by screening 2000 cells per animal for mitosis.

Micronucleus test

The micronucleus (MN) test was performed by employing the method of Schmid[21] with slight modifications according to Chauhan *et al*.[22]. Smears were stained with May-Grünwald and Giemsa. 2000 polychromatic erythrocytes (PCEs) and the corresponding number of normochromatic erythrocytes (NCEs) per animal were analysed for the presence of MN. The ratio of PCEs to NCEs (P/N) was calculated for all the treated and control groups.

Single cell gel electrophoresis (Comet assay)

The alkaline comet assay was performed as per Singh *et al*.[23], with slight modifications[24]. Care was taken to prevent additional DNA damage resulting from direct exposure to visible light by performing all steps in the dark at 4°C. Fully frosted microscope slides (Fisher Scientific, Cat. no.: 12-544-5CY, USA) were coated with a thin layer of 1% normal melting-point agarose and allowed it to solidify. Subsequently, 20 μL of bone marrow suspension (in PBS) was mixed with 80 μL of 0.5% low melting agarose, pipetted onto the pre-coated slides and covered with a cover slip. The agarose layer on the slide was chilled for 5 min and after its solidification, the cover slip was removed. A final layer of 0.5% low melting agarose was placed over the second layer and allowed to solidify.

Slides with bone marrow cells embedded in agarose were submersed in a cold, alkaline (4°C, pH 10) lysis solution [2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris base (pH 10), 1% Triton X-100, 10% DMSO] and maintained for 4h. They were then placed in an alkaline electrophoresis buffer of pH 13 (1 mM Na_2EDTA/300 mM NaOH) for 25 min to induce unwinding of DNA strands. The slides were then transferred to an electrophoresis tank with fresh alkaline electrophoresis buffer and electrophoresis was performed at field strength of 20V/125 mA for 25 min at 4°C. The DNA in the agarose was neutralized by incubation in 0.4 M Tris (pH 7.4) at room temperature (26ºC) for 5 min.

DNA was stained by placing 20 μL/mL of ethidium bromide on the agarose, covered with a cover slip and incubated for 5 min in dark. The DNA damage was quantified by observing the cells under 20X objective of a fluorescence microscope (Olympus BX 53, Japan) equipped with an excitation filter of 510-560 nm and an emission filter of 590 nm. One hundred comet images were recorded for each sample (2 slides, 50 images from each slide). The comets were analysed by the image analysis software CASP[25].

Statistical analysis

The statistical significance of data of chromosomal aberration test, micronucleus test and comet assay were analysed employing one-way ANOVA with a post hoc Bonferroni's test to check the significance between a value and its corresponding control. The results were presented as the Mean ± SD and the values of P <0.001, 0.01 and 0.05 were regarded as statistically significant at 0.1, 1 and 5% level, respectively.

Results

The bone marrow cells showed statistically significant increase of genotoxicity in all the

parameters employed. The increase was dose dependent. However, in time dependent studies, genotoxicity increased after the 24 h time period.

Chromosomal aberration test

Dose response assay

Table 1A summarizes the various chromosomal aberrations induced by the three doses of the NP in the bone marrow cells and their frequency. A significant increase of aberrations (F = 125.62, *P* <0.001) was observed in a dose dependent manner from Low (13.41±1.26%) to Medium (30.7±4.25%) to High (56.91±4.75%). A significant decline was observed in the mitotic index with increasing dose. Different types of chromosomal aberrations such as centric fusions, fragmentations, chromatid exchange, ring chromosomes (Fig. 2A), breaks and gaps were observed in the bone marrow metaphase plates of mice.

Time response assay

Data of the time response assay is represented in Table 1B. In the time response assay, significant aberrations were seen at all the time intervals (F = 75.42, *P* <0.001) of the study. The total aberrations (TA) were highest at the 48 hour time interval which subsequently decreased by 72 h of exposure. Significant decrease in the mitotic index values were also found in the NP treated mice in comparison to the negative control. Various chromosomal aberrations were observed to be highest at the 48 h time interval compared to the other time intervals studied.

Micronucleus test

Dose response assay

Table 2A indicates the frequency of micronuclei induced by various doses of MNZF in the bone marrow erythrocytes. A dose dependent increase of the micronuclei was observed (F = 164.6, *P* <0.001)

Table 1 — Frequency (%) of various chromosomal aberrations [a] in the bone marrow cells of *Mus musculus* (A) treated with various doses of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at 24 h of treatment; and (B) treated with 250 mg/kg of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at different time intervals

Time (h)	Treatment	Dose	MI[b]	BS	F	CF	EX	R	MA	GS	TA±SD	% CA/ Cell
(A) treated with various doses of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at 24 h of treatment												
	Control	DW	7.4 ±0.12	1.6 ±0.3	0	2.2 ±0.06	1.2 ±0.11	0.8 ±0.16	0	1.28 ±0.12	7.08 ±0.81	0.07 ±0.02
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	125 mg/kg	7.13 ±0.09	3.24 ±0.22*	0	4.14 ±0.21**	3.22 ±0.4*	2.35 ±0.17*	0	0.46 ±0.13	13.41 ±1.26*	0.13 ±0.04
		250 mg/kg	5.62 ±0.24*	4.45 ±0.14**	0	14.23 ±0.13***	5.84 ±0.25**	3.52 ±0.22**	0.8 ±0.23*	1.86 ±0.32	30.7 ±4.25*	0.3 ±0.08*
		500 mg/kg	4.83 ±0.44*	6.56 ±0.26**	15.74 ±0.44***	17.44 ±0.33***	6.55 ±0.31**	6.42 ±0.27**	1.92 ±0.47*	2.28 ±0.14*	56.91 ±4.75**	0.56 ±0.05**
	Cyclo-phosphamide	50 mg/kg	3.85 ±0.32**	10.8 ±0.45**	7.6 ±0.32***	23.2 ±0.26***	9.6 ±0.21***	8.4 ±0.15***	3.2 ±0.32**	4.64 ±0.33**	67.44 ±6.81**	0.67 ±0.09**
(B) treated with 250 mg/kg of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at different time intervals												
24	Control	DW	7.4 ±0.12	1.6 ±0.3	0	2.2 ±0.06	1.2 ±0.11	0.8 ±0.16	0	1.28 ±0.12	7.08 ±0.81	0.09 ±0.02
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250	5.62 ±0.24*	4.45 ±0.14**	0	14.23 ±0.13***	5.84 ±0.25**	3.5 ±0.22**	0.8 ±0.23*	1.86 ±0.32	30.7 ±4.25*	0.30 ±0.08*
	Cyclo-phosphamide	50 mg/kg	3.85 ±0.32*	10.8 ±0.45**	7.6 ±0.32***	23.2 ±0.26***	9.6 ±0.21***	8.4 ±0.15***	3.2 ±0.32**	4.64 ±0.33**	67.44 ±6.81**	0.67 ±0.09**
48	Control	DW	6.52 ±0.2	1.8 ±0.18	0	2.0 ±0.08	1.4 ±0.06	0.6 ±0.07	0	1.31 ±0.14	7.36 ±0.83	0.07 ±0.03
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	5.23 ±0.31*	4.98 ±0.21**	0	18.62 ±0.37***	6.33 ±0.22**	3.9 ±0.13**	0.8 ±0.04*	2.04 ±0.1*	36.67 ±4.89*	0.37 ±0.04*
	Cyclo-phosphamide	50 mg/kg	3.14 ±0.19**	10.8 ±0.18***	7.3 ±0.22***	23.2 ±0.29***	9.6 ±0.18***	8.4 ±0.19***	2.97 ±0.1**	4.72 ±0.24**	66.58 ±6.27**	0.66 ±0.07**
72	Control	DW	6.78 ±0.15	1.7 ±0.09	0	2.1 ±0.11	1.5 ±0.14	0.7 ±0.09	0	1.26 ±0.08	6.81 ±0.81	0.06 ±0.02
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	5.32 ±0.27*	4.46 ±0.24**	0	15.1 ±0.17***	6.2 ±0.33**	3.2 ±0.28**	0.6 ±0.02*	1.76 ±0.13*	31.32 ±4.25*	0.31 ±0.06*
	Cyclo-phosphamide	50 mg/kg	3.15 ±0.22**	10.5 ±0.36***	7.9 ±0.41***	22.5 ±0.2***	9.4 ±0.25***	8.2 ±0.21***	3.34 ±0.14**	4.61 ±0.27**	69.41 ±6.28**	0.69 ±0.08**

[[a] from 100 metaphases; [b] from 2000 cells/animal; MI, mitotic index; BS, breaks; F, fragmentation; CF, centric fusion; EX, exchange; R, ring chromosome; MA, multiple aberration; GS, gaps TA, total aberrations; CA, chromosomal aberrations; SD, standard deviation of mean. * *P* <0.05, ** *P* <0.01, *** *P* <0.001]

Table 2 — Frequency (%) of micronuclei[a] in the bone marrow cells of *Mus musculus* (A) treated with various doses of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at 24 h of treatment; and (B) treated with 250 mg/kg of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at various time intervals

Time (h)	Treatment	Dose	MN in PCE ± SD	MN in NCE ± SD	Total MN ± SD	P/N RATIO
(A) treated with various doses of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at 24 h of treatment						
	Control	DW	0.46±0.24	0.43±0.3	0.89±0.29	1.02±0.02
		125 mg/kg	1.26±0.21	0.98±0.17	2.24±0.19*	0.88±0.04*
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	2.22±0.15*	1.72±0.16*	3.29±0.15*	0.84±0.04*
		500 mg/kg	2.47±0.16*	1.67±0.14*	4.64±0.14**	0.74±0.07*
	Cyclophosphamide	50 mg/kg	3.81±0.22**	2.94±0.27*	6.75±0.26**	0.61±0.09*
(B) treated with 250 mg/kg of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at various time intervals						
24	Control	DW	0.46±0.24	0.43±0.3	0.89±0.09	1.026±0.07
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	2.22±0.15*	1.72±0.16*	3.29±0.15**	0.849±0.06
	Cyclophosphamide	50 mg/kg	2.56±0.06**	2.37±0.07*	4.93±0.05**	0.614±0.04*
48	Control	DW	0.47±0.12	0.37±0.08	0.84±0.04	1.011±0.04
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	2.31±0.16**	1.71±0.09**	3.54±0.13**	0.784±0.03*
	Cyclophosphamide	50 mg/kg	2.48±0.07**	2.42±0.11**	4.9±0.04**	0.660±0.06*
72	Control	DW	0.48±0.13	0.25±0.06	0.73±0.03	1.005±0.05
	$Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$	250 mg/kg	1.72±0.14*	1.57±0.08**	4.02±0.13**	0.807±0.08
	Cyclophosphamide	50 mg/kg	2.5±0.08**	2.48±0.09**	4.98±0.02***	0.599±0.07*

[[a] from 2000 cells per animal. MN, micronucleus; PCE, polychromatic erythrocyte; NCE, normochromatic erythrocyte. * P <0.05, ** P <0.01, *** P <0.001]

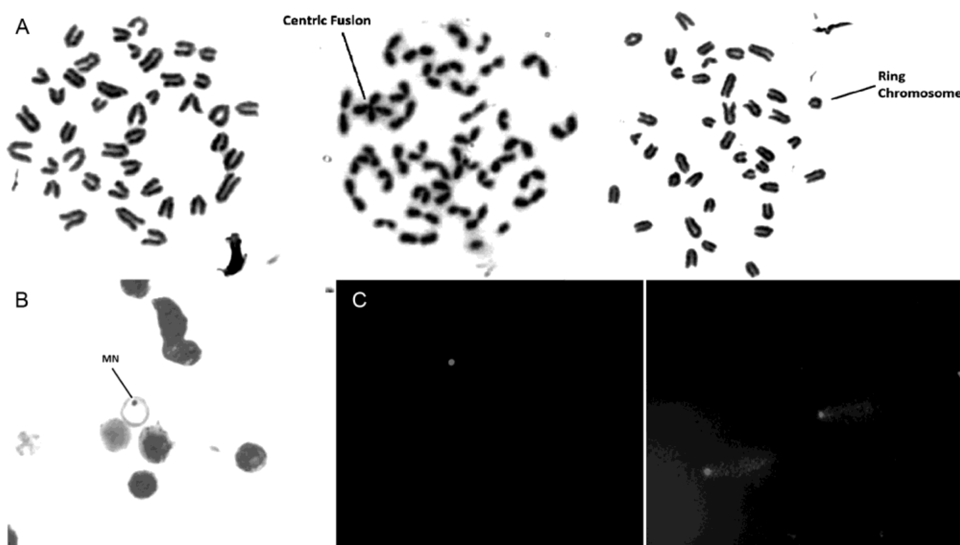

Fig. 2 — Genotoxicity parameters (A) metaphase plates showing normal chromosomes, centric fusion and ring chromosome; (B) micronucleus (MN) in polychromatic erythrocyte (PCE); and (C) bone marrow cells with intact DNA and DNA damage

(Fig. 2B). Highest frequency of MN (4.64±0.14) occurred in the mice treated with the high dose of the NP. A significant decline in the P/N ratio was also evident with increasing dose.

Time response assay

The frequency of MN induced by medium dose of MNZF (250mg/kg) at different time intervals (24, 48 and 72 h) is presented in Table 2B. A gradual increase in the MN frequency was observed from the 24 to 48 h time interval and this increased further by 72 h time period (F = 73.96, P <0.001). A significant decrease in the P/N ratio was also observed in the NP treated mice at all the time intervals.

Single cell gel electrophoresis / comet assay

Dose response assay

Fig. 3A represents the DNA damage (% Tail DNA) induced by various doses of the NP. The length of the tail indicates the extent of DNA damage (Fig. 2C). A significant increase of DNA damage was observed in all the cells treated with MNZF (F = 256.63, P <0.001). Further, a dose dependent increase of the percentage tail DNA was observed, with the high dose of the NP inducing the highest damage (% tail DNA= 80.87±2.35).

Time response assay

DNA damage induced by MNZF (250 mg/kg) at various time intervals is presented in Fig. 2B. The tail

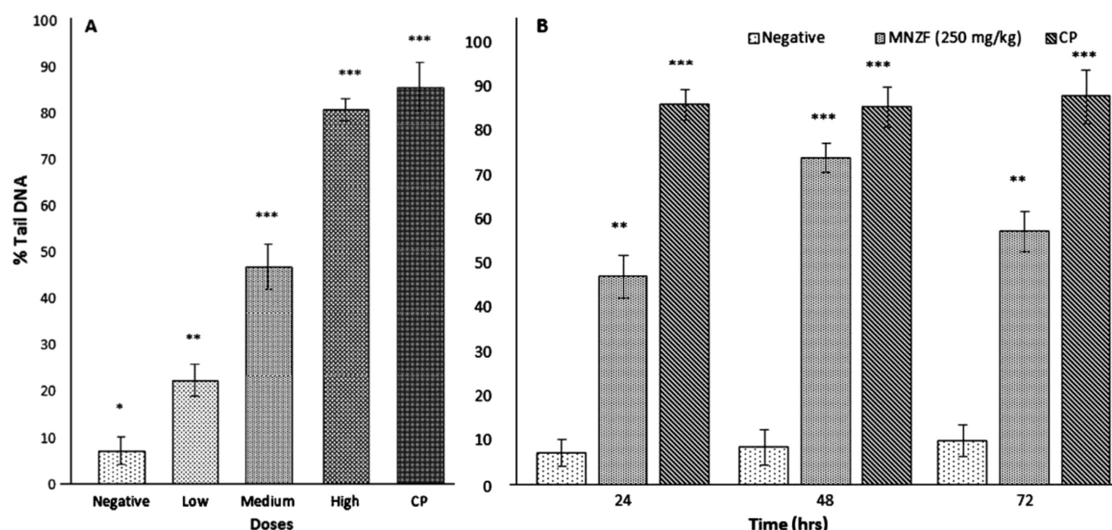

Fig. 3 — Tail DNA (%) of comets in the bone marrow cells of *Mus musculus* (A) treated with various doses of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at 24 h of treatment; and (B) treated with 250 mg/kg of $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ at various time intervals. [CP, cyclophosphamide. * P <0.05, ** P <0.01, *** P <0.001]

DNA (%) increased significantly from 24 to 48 h and this gradually decreased by 72 h (F = 174, P <0.001).

Discussion

The increased genetic damage observed in all the three parameters employed in the present investigation viz. chromosomal aberration test, micronucleus test and comet assay indicate the mutagenicity of MNZF in Swiss albino mice *Mus musculus*, *in vivo*.

Chromosomal aberration test

Significant chromosomal aberrations were induced by MNZF indicating its clastogenic effect in the bone marrow cells of *Mus musculus*, *in vivo*. These results are on par with that of Balasubramanyam et al.[26] in which they observed increased frequencies of chromosomal aberrations and micronuclei induced by aluminium oxide nanoparticles in the bone marrow cells of Wistar rats. The chromosomal damage that occurs as a result of exposure to NPs could lead to a delay in the cell cycle or could completely arrest cell division and induce apoptosis[27]. This is also evident in the significant decrease of 'mitotic index' observed by us in the present study. This indicates a drastic reduction of dividing cells induced by MNZF in the bone marrow cells of exposed mice. Srivastav *et al*.[28] also observed a decrease the mitotic index of mice exposed to zinc oxide NPs and reported that the damage to chromosomes was due to the production of ROS induced by the NPs. Additionally, they also reported that NPs are able to reach the chromosomes when the cells undergo division and can induce clastogenesis.

Micronucleus test

Clastogenicity of test agents is more often studied employing the micronucleus test as this affords quick and simple testing of genotoxic agents. Significant increase in the frequency of MN observed in the present study with increased dose of MNZF is in agreement with the studies of Sadeghiani *et al*.[29], where they observed an increased incidence of micronuclei in polychromatic erythrocytes of mice exposed to magnetic nanoparticles, *in vivo*. Song *et al*.[30] also reported a significant increase of MN in the reticulocytes of mice exposed to various doses (0, 1, 3 mg/mouse) of metal oxides (CuO, Fe_2O_3, Fe_3O_4, TiO_2 and Ag) NPs. An increase of oxidative stress coupled by the decrease of antioxidant enzymes induced by NPs in the body could cause significant MN formation[31]. The significant decrease of P/N ratio in the MNZF exposed mice suggests the suppression of the proliferative activity by MNZF in the bone marrow. Suzuki *et al*.[32] are of the opinion that the reduction in P/N ratio is caused by the rapid differentiation and multiplication, including the denucleation of erythroblasts which stayed in the bone marrow instead of entering the peripheral blood.

Single cell gel electrophoresis (Comet assay)

The comet assay is the most preferred test for assessment of DNA damage as it is quick, simple and can be analysed visually or with the help of computer

generated software and can be used reliably to assess the genotoxicity of various NPs[33]. The significant increase of the tail DNA (%) observed in the present study indicates the mutagenic potential of MNZF. Our results are in agreement with the studies of Singh *et al*.[34] in which they observed significant DNA damage (% Tail DNA) in the blood leucocytes of Wistar rats, orally exposed to MnO_2. Sharma *et al*.[35] also observed an increase in the % tail DNA in the liver and kidney cells of mice exposed to ZnO NPs which is in par with our study. NP-induced ROS could be responsible for the induction of DNA strand breaks in cells and could also induce DNA strand breaks via a pathway that releases cytochrome c from mitochondria and induces DNA fragmentation[36,37].

In the time-dependent study, the frequency of chromosomal aberrations and DNA damage increased gradually from 24 to 48 h of treatment which decreased subsequently by 72 h of treatment. It suggests that the NP MNZF took 48 h to be active and exhibit maximum genotoxic effect which reduced later on may be due to varying reasons. According to Shyama & Rahiman[38], the decline in the frequency of aberrations observed at the later time interval may be due to any one or a few or all of the following reasons: (i) repair of damaged genetic material; (ii) elimination of the toxic agent and its metabolites from the body; (iii) elimination of the cells/chromosomes with genetic material; and (iv) inactivation of the toxic agent and/ or its metabolites. However, the frequency of micronuclei was observed to increase further from the 48 to 72 h time interval. This may be due to the longer time taken for the aberrant chromosomes / chromosomal acentric fragments to form micronuclei. Additionally, the bone marrow, being one of the main organs of the reticuloendothelial system (RES), is involved in the removal and neutralization of potential pathogens that may enter the body from the gastrointestinal tract. The RES cells (macrophages, monocytes and specialized endothelial cells) have the capacity to take-up NPs and may cause indirect genotoxic effect to the bone marrow by inflammation or formation of ROS[39,40].

Conclusion

The results of the present study reveals that MNZF nanoparticle, $Mn_{0.3}Ni_{0.3}Zn_{0.4}Fe_2O_4$ was able to induce genotoxicity in mice *in vivo*. Significant dose-dependent and time-dependent chromosomal aberrations and micronuclei were observed in the bone marrow cells of mice indicating the clastogenic effect of this NP. Further, the results of the comet assay reveal that the MNZF nanoparticle could also induce DNA strand breaks in a dose- and time-dependent manner. The damage induced by this NP could possibly be due to the production of ROS which in turn affects the DNA. Although the MNZF nanoparticle has potential applications, the results suggest the need for proper handling of this nanoparticle so as to avoid contact with it by occupational exposure either through respiration or ingestion. Further studies are required to understand the exact mechanism of toxicity of the MNZF nanoparticle.

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Conflict of Interest

The authors report no conflict of interests.

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