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Research Article

In vivo Toxicity Investigation of Magnesium Oxide Nanoparticles in Rat for Environmental and Biomedical Applications

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Background: Magnesium oxide nanoparticles are characterized with a wide variety of applications and are mass-produced throughout the world. However, questions remain regarding their safety. There has been paucity of toxicology research on their side effects, especially under *in vivo* conditions.
Objectives: The present paper aims at evaluating the toxicity of administering 10-15 nm magnesium oxide nanoparticles to Wistar rat under *in vivo* conditions. In addition, hematology, biochemistry, and histopathology of the rats are examined at various concentrations (62.5-125-250-500 μg.mL^{-1}) over 28-days period.
Materials and Methods: In this study, 35 male Wistar rats were randomly divided into five groups, comprising one control group and four experimental groups, assigned to various doses of MgO nanoparticles by intraperitoneal injection. Eventually, blood samples were collected, and all animals were sacrificed for liver and kidney tissue investigation.
Results: The findings showed that high concentrations of Magnesium oxide nanoparticles (250 and 500 μg.mL^{-1}) significantly increased white blood cells, red blood cells, hemoglobin, and hematocrit compared with the control group ($P < 0.05$). Moreover, the nanoparticles elevated the levels of aspartate aminotransferase and alkaline phosphatase, whereas no significant difference in levels of alanine aminotransferase, gamma-glutamyl transpeptidase, urea, and creatinine were recorded in comparison with the control group ($P < 0.05$). Histopathological examinations in the rat's liver showed proliferation of bile ductules, congestion in some regions of the liver sinusoids, and apoptotic cells (probably) in high-dose groups, but no histological changes were found in the kidney functions.
Conclusions: The results from the present study showed that the magnesium oxide nanoparticles in concentrations lower than 250 μg.mL^{-1} are safe for desired applications.

Keywords: in vivo; Magnesium Oxide; Toxicity

1. Background

Nanoparticles are miniscule materials (with at least one dimension less than 100 nm) with unique properties, which make them suitable for novel applications. These attributes make them very attractive for commercial and medical developments (1). In recent years, the breakthroughs in nanotechnology have been accompanied with inorganic and organic nano-sized particles with growing applications to be used as modifier in industrial, medicine, therapeutics, synthetic textiles, and food packaging (2). Moreover, nanoparticles are expected to play a crucial role in water purification (3). Now, the rapid increase in world's population and shortage in fresh water demand appropriate, cost-effective, and rapid wastewater treatment techniques (4). By nanotechnology, water and wastewater can be treated not only to deal with main challenges of present treatment technology, but also to provide new treatment potentials, which in turn allows economic exploitations of unconventional water sources as a water supply (5).

As Sawai *et al.* pointed out, the moisture absorptions on the MgO nanoparticle surfaces, which forms a thin water layer around the particles, is a possible antibacterial mechanism. The local pH of the mentioned water layer can be greater than its equilibrium value under solution sets. When the nanoparticles are faced with bacteria, the high pH in that water layer could damage the membrane, resulting in cells death (6).

Given the unique properties of these particles, nanotechnology is also being applied in medical

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sciences (7). Recently, the initiative methods as per development of nanoparticle drugs have emerged in cancer treatment. Such particles offer controlled drug delivery, enhance permeability, and tumor specific targeting. In addition, nano-drugs cope with carcinogenic cells, enter them in an easy manner and, at the same time, have little side impacts on normal cells (8). The most important characteristics of these drugs are particle size, molecular weight, pH, ionic strength, monomer concentration, and surface charge among others (9). At the same time, potentially nanoparticles have great contribution in diagnosis and imaging of brain tumors (10).

In medicine, MgO is used for the alleviation of heartburn, stomach sore, and for bone regeneration (11, 12). Nowadays, MgO nanoparticles are applied in tumor inhibition (13) and additionally, have remarkable potential as an antimicrobial agent (14). Other experimental results revealed the possible utility of MgO nanoparticles in the treatment of cancer, including Nano-cryosurgery and hyperthermia (15).

The applications of MgO nanoparticles and also the issues on its feasible toxicity are increasing (16). Unfortunately, there is a paucity of knowledge about the effect of the prolonged exposure to nanoparticles on human health and the environment. Before their large scale production and application in diverse fields, the impact of nanoparticles on health and environment merits more assessment (17, 18). Hence, estimating the cost/benefits ratio for utilizing nanoparticles in technological or medical procedures is of great importance.

The nanomaterial toxicity such as nanoparticles, quantum dots, nanotubes, and nanowires has been declared in the past few years (19-24). In this respect, various studies dealt with the toxicity of MgO nanoparticles. Lai *et al*. showed that treatment of human astrocytoma (astrocytes-like) U87 cells with MgO nanoparticles for 48 h did not significantly decrease their survival until the concentrations were higher than 50 $\mu g.mL^{-1}$(25). Additionally, the cytotoxicity and neurotoxicity of MgO nanoparticles on SH-SY5Y cell line have also been investigated, and the results showed that MgO nanoparticles are not toxic in concentration ranges from 1nM to 1mM for 24, 48, and 72 h to both undifferentiated and differentiated SH-SY5Y cells. MTS method was used for cell viability (16).

These reports were mainly based on *in vitro* studies. It seems that *in vitro* cultures cannot provide significant data on the response of the physiological system under study (26). Consequently, studying the *in vivo* toxicity of MgO nanoparticles is very useful.

2. Objectives

There are few toxicological investigations of MgO nanoparticles in animal models. On this basis, the present research attempts to shed lights on *in vivo* toxicity of 10-15 nm MgO nanoparticles under various concentrations (62.5-500 $\mu g.mL^{-1}$) for 28 days. Here, animals' hematology, biochemistry, and histopathology were characterized.

The outcome of this *in vivo* research will determine which concentration of MgO nanoparticles has no poisonous effect and would be appropriate for various applications

3. Materials and Methods

3.1. Nanoparticles Characterization

A stock suspension of MgO nanoparticles (1000 $\mu g.mL^{-1}$) was obtained from Neutrino Co. At first, characterization of the synthesized MgO nanoparticles was performed using transmission electron microscopy and X-ray diffraction analysis. Then, the MgO suspension was diluted by the distilled water. The various concentrations of these particles were obtained through a serial reducing of 1000 $\mu g.mL^{-1}$ by 1×Time, 2×Time, 3×Time, and final 4×Time to get 500, 250, 125, and 62.5 $\mu g.mL^{-1}$, respectively.

3.2. Experimental Design

The study was conducted on 10 weeks age male Wistar rat, weighing 200-250 g, housed in polycarbonate cages an ambient temperature of 22±2°C with 12h-light and 12h-dark cycle. Animals were obtained from Darou Pakhsh Co. and given food and water *ad libitum*. All the experiments on animals were performed based on the guidelines of the institutional animal ethics committee. In this study, 35 rat were randomly divided into five groups (seven in each group) comprising one control group and four experimental groups assigned to various doses of MgO nanoparticles. Administration of the rats was performed using intraperitoneal injection. Each rat of group 2 to 5 received 1mL of MgO nanoparticles solution at doses of 62.5 $\mu g.mL^{-1}$, 125 $\mu g.mL^{-1}$, 250 $\mu g.mL^{-1}$, and 500 $\mu g.mL^{-1}$, respectively (every other day). The control group rats were treated with citrate buffer alone. At the end of 28 days treatment, all the rats of the five groups were starved overnight and sacrificed after injection of MgO nanoparticles to determine the toxicity through examination of hematological, biochemical and histological analysis.

3.3. Hematological and Biochemical Analysis

28 days after treatment, blood samples were collected by intra-cardiac puncture following anesthesia with ketamine 100 $mg.kg^{-1}$ (Rotexmedica Co., Germany) and xylazine 10 $mg.kg^{-1}$ (Alfasan Co., Holland) (27).

For hematological analysis, blood was immediately collected in EDTA coated vials and hematologic toxicity was determined by the use of automated hematological analyzer (Sysmex Cell Counter Model K-1000). Hematological parameters examined in this study included white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), platelet count (PLT), hemoglobin (Hb) levels, mean corpuscular hemoglobin

(MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV).
For estimation of serum biochemical analysis, blood was placed in a clotted vial. The serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min to an automated analyzer (Hitachi 912 Chemistry Analyzer). Liver function was dealt with based on the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT).
Nephrotoxicity was determined by urea (UREA) and creatinine (CREA). The level of these biochemical parameters was measured by means of quantitative diagnostic kits with photometric method (Pars Azmun Co., Tehran, Iran).

3.4. Histopathology

After the treatment for 28 days, the histological analysis was performed by examining the morphological changes induced by MgO nanoparticles, over the liver and kidney. These organs were collected and fixed with a 10% formalin, embedded in paraffin, and cut into 5-µm-thick sections.
The fixed sections were stained for analysis using hematoxylin and eosin (H and E) staining. The sections were examined under binocular microscope (Olympus CH-2, Tokyo, Japan), and photomicrographs of the fixed organs were obtained.

3.5. Statistical Analyses of Data

Results are presented as mean ± standard deviation. Data analysis was performed by one-way analysis of variance (ANOVA), followed by Tukey HSD test for multiple comparisons. The level of significance was set at $P < 0.05$.

4. Results

4.1. Characterization of MgO Nanoparticles

The morphology and size of the biologically synthesized MgO nanoparticles were determined using Transmission electron microscopy (model EM 208 Philips). The images clearly illustrated that the average size of the particles was found to be approximately 10-15 nm and spherical in shape, as shown in **Figure 1**.
The XRD pattern obtained presented 5 intense peaks in the whole spectrum of 2θ values ranging from 30 to 80. The diffraction peaks at 2θ values of 36.94°, 42.90°, 62.30°, 74.67° and 78.61°, corresponding to 111, 200, 220, 311 and 222 planes, respectively, for MgO based on the X-ray diffraction (XRD) analysis using a Philips PW 1710 X-ray powder diffractometer, as shown in **Figure 2**.

4.2. Hematology Results

The important step for toxicity detection is assessment of standard hematological parameters, including determination of white blood cells (WBC), red blood cells (RBC), hematocrit (HCT), platele count (PLT), hemoglobin (Hb) levels, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV). Concentration-dependent hematology results are presented in **Table 1**.

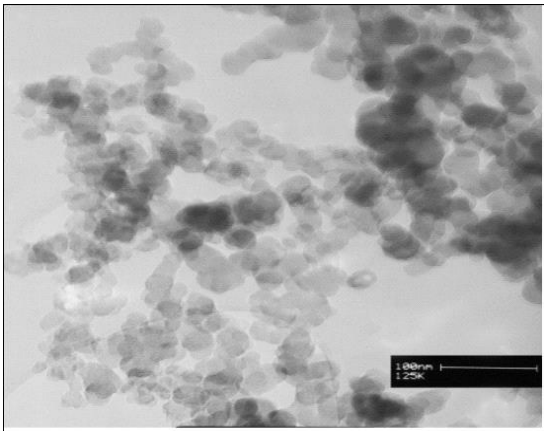

Figure 1. TEM images of the resulting MgO nanoparticles. The images clearly illustrated that the average size of the particles was found to be approximately 10-15 nm and spherical in shape.

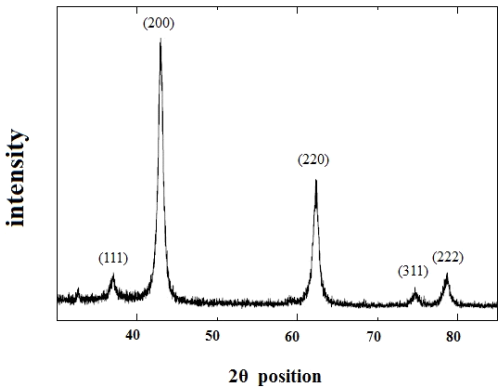

Figure 2. The XRD pattern of the resulting MgO nanoparticles presented 5 intense peaks in the whole spectrum of 2θ Values ranging from 30 to 80. The diffraction peaks at 2θ values of 36.94 °, 42.90 °, 62.30 °, 74.67 ° and 78.61 °, corresponding to 111, 200, 220, 311 and 222 planes, respectively.

We observed that white blood cells significantly increase at MgO nanoparticles doses of 250 and 500 µg.mL^{-1}, respectively ($P < 0.05$).
This information demonstrated a concentration-dependent trend correlated with treatment. Meanwhile, amounts of hemoglobin, red blood cells, and hematocrit were significantly increased with the injection of MgO nanoparticles at a dosage of 500 µg.mL^{-1} ($P < 0.05$).

4.3. Serum Analysis

The biochemical effects of various doses of MgO nanoparticles, as shown in **Table 2** and **Figure 3**, including on aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, urea, and creatinine were investigated. It was found that the level of AST and ALP are significantly increased in the higher than

125 and 250 μg.mL^{-1}, respectively, but the level of ALT, GGT, urea, and creatinine, showed no significant change for any dose of particles.

4.4. Histopathological Research

In order to assess the toxicity of MgO nanoparticles, histological analysis was done on liver and kidney. The results are shown in **Figure 4**. The liver of the control rats showed normal hepatic architecture, portal triad, and central vein (**Fig. 4A, C, and E**). The MgO nanoparticles treated rats revealed some changes in the liver mainly at the highest concentration.

The histopathological changes of the livers are proliferation of bile ductules (**Fig. 4B, D**). In some regions of the liver sinusoids, a congestion was found (**Fig. 4F**), which showed that MgO nanoparticles induced inflammation in liver tissues. Exposure to these particles also caused apoptotic cells (probably) around the portal triad (**Fig. 4D**).

The MgO nanoparticles treated kidney by various doses did not exhibit any degenerative effects in the cells (**Fig. 4G, H**).

Table 1. Hematological analysis revealing the toxic effect of MgO nanoparticles in rats based on μg.mL^{-1}.

Parameters	Control	62.5	125	250	500
Hb (g/dl)	13.96 ± 0.38	13.96 ± 0.26	14.07 ± 0.41	14.01 ± 0.41	15.40 ± 0.81*
RBC (×10^6/μl)	7.93 ± 0.39	8.02 ± 0.44	8.22 ± 0.28	8.10 ± 0.23	9.10 ± 0.77*
MCV (fl)	51.02 ± 1.68	50.37 ± 0.74	50.30 ± 0.80	50.58 ± 3.41	51.20 ± 3.04
MCH (pg)	17.03 ± 0.60	16.86 ± 0.38	16.90 ± 0.27	17.11 ± 0.65	17.44 ± 0.85
MCHC (g/dl)	33.37 ± 0.89	32.79 ± 1.20	33.71 ± 1.11	32.60 ± 0.87	32.91 ± 1.28
PLT (×10^3/μl)	627.43 ± 47.55	619.86 ± 70.16	617.71 ± 27.75	591.43 ± 80.90	604.71 ± 92.46
HCT (%)	41.24 ± 0.83	41.27 ± 0.39	41.80 ± 0.82	42.74 ± 1.63	46.20 ± 1.80*
WBC (×10^3/μl)	6.8 ± 0.47	6.97 ± 0.38	7.54 ± 0.73	9.84 ± 1.72*	12.46 ± 2.10*

Bars represent mean ± standard deviation of n = 7; Hb, Hemoglobin; RBC, Red Blood Cells; MCV, mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; HCT, hematocrit; WBC, white blood cells. Data were analyzed using One Way ANOVA followed by Tukey HSD Test. * Represents significant difference from the Control Group ($P < 0.05$).

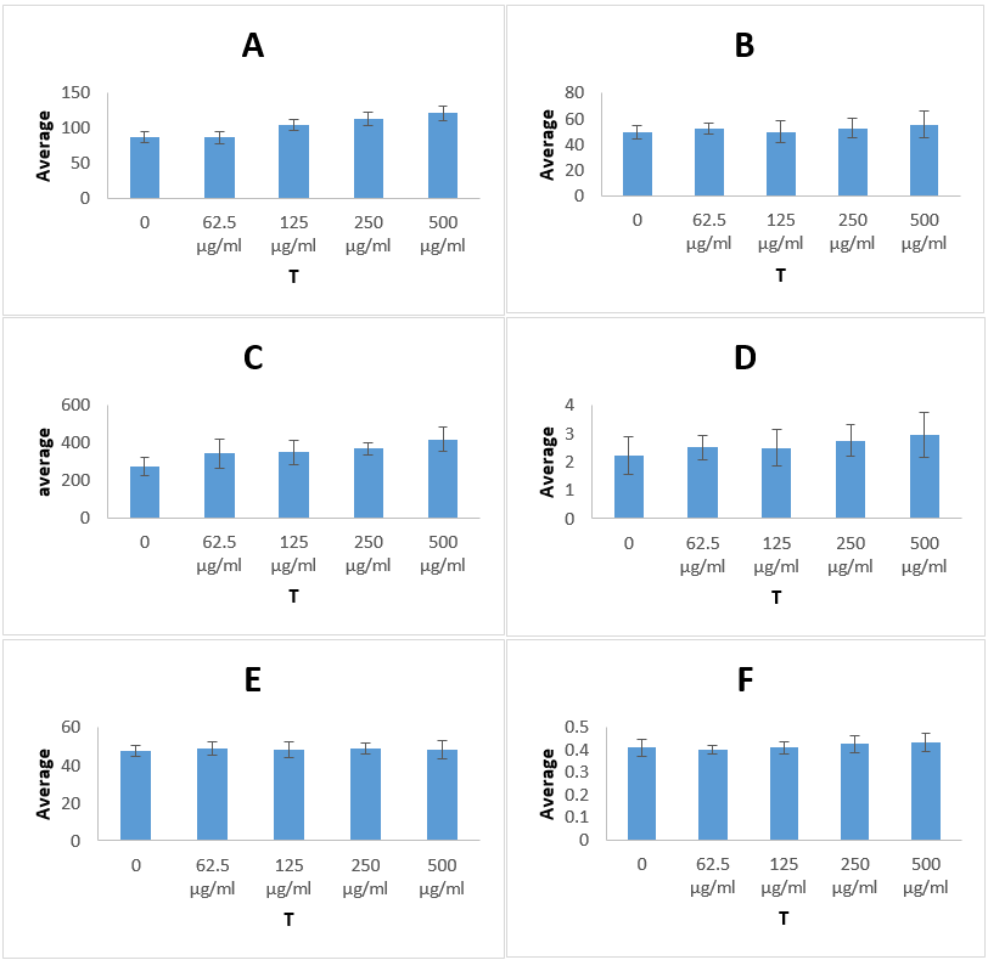

Figure 3. The biochemical results for rats treated with MgO nanoparticles 28 days after intraperitoneal injection at a different concentrations (62.5-500 μg. mL^{-1}). These results show mean and standard diviations of: (A) aspartate transaminase; (B) alanine transaminase; (C) alkalin phosphatase; (D) γ-glutamyl transpeptidase; (E) urea; (F) creatinine. Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; UREA, urea; CREA, creatinine

Table 2. Biochemical analysis metabolic enzymes and renal function after intraperitoneal injection of different doses (62.5-500 µg.mL-1) for 28 days (every other day).

Groups	AST	ALT	ALP	GGT	UREA	CREA
Control	86.79 ± 8.27	49.93 ± 5.51	274.71 ± 49.35	2.23 ± 0.71	47.68 ± 3.29	0.41 ± 0.38
62.5 (µg/ml)	86.29 ± 9.79	52.29 ± 4.78	342.71 ± 79.32	2.52 ± 0.45	48.94 ± 3.77	0.40 ± 0.19
125 (µg/ml)	105.00 ± 7.80*	49.79 ± 8.74	351.14 ± 68.22	2.50 ± 0.69	48.10 ± 4.34	0.41 ± 0.26
250 (µg/ml)	113.71 ± 9.66*	52.50 ± 7.97	396.43 ± 33.04*	2.76 ± 0.58	49.03 ± 3.15	0.43 ± 0.41
500(µg/ml)	121.14 ± 10.82*	55.71 ± 10.84	419.14 ± 65.71*	2.96 ± 0.81	48.46 ± 5.17	0.43 ± 0.42

Bar represent mean ± standard deviation of n=7. Data were analyzed using one way ANOVA followed by Tukey HSD test. *Represents significant difference from the control group ($P < 0.05$). All the values of AST, ALT, ALP and GGT are expressed as $U.L^{-1}$, also Urea and Creatinine in $mg.dL^{-1}$.

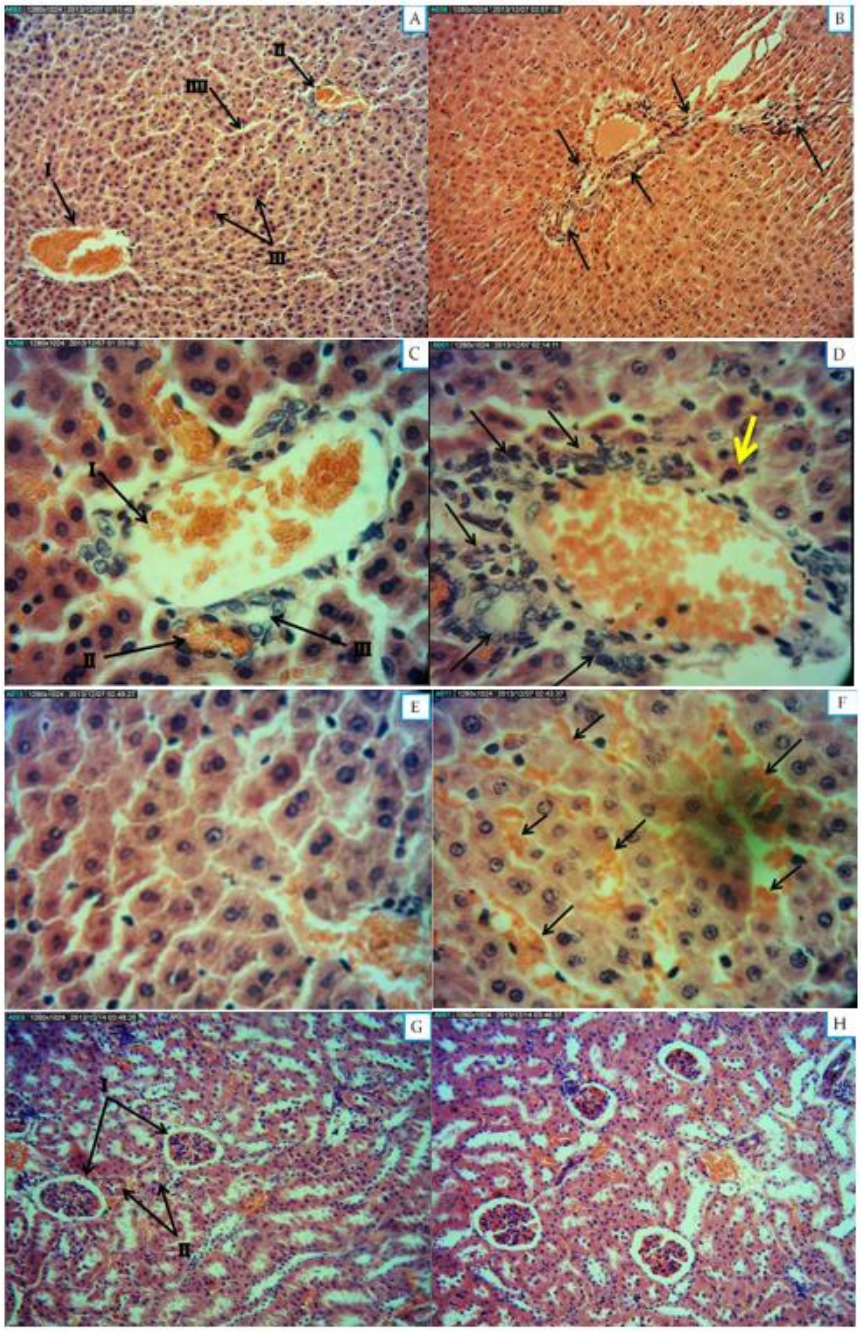

Figure 4. Toxicity studies of MgO nanoparticles in rat organs. Histological specimens of rat tissues (liver and kidney) collected from rats euthanized on day 28, stained with hematoxylin and eosin (H and E) showed normal histology, n=7 for each group. **A.** Control animal liver section showing normal central vein (I), portal triad (II), hepatocytic architecture (III) and liver sinusoids (IIII); **B.** MgO nanoparticles at a dose of 500 $\mu g.mL^{-1}$ treated liver showing proliferation of bile ductules; **C.** Control liver section showing normal hepatic artery (I), portal vein (II), and bile duct (III); **D.** MgO nanoparticles at a dose of 500 $\mu g.mL^{-1}$ treated liver showing proliferation of bile ductules and apoptotic cell (probably); **E.** Control liver section showing normal liver sinusoids; **F.** MgO nanoparticles at a dose of 500 $\mu g.mL^{-1}$ treated liver showing Congestion in some regions of the liver sinusoids; **G.** Control kidney section showing normal corpuscle (I), and tubules structure (II); **H.** MgO nanoparticles at a dose of 500 $\mu g.mL^{-1}$ treated kidney also showed normal structure similar with control group.

5. Discussion

The aim of this study was to survey the toxicity effect of MgO nanoparticles on animal model. For this purpose, estimation of hematology and biochemistry is very useful (28). The results of the present study indicated that white blood cells, hemoglobin, red blood cells, and hematocrit were increased with the injection of MgO nanoparticles doses of 250 and 500 $\mu g.mL^{-1}$.

As for rats, their white blood cells are found to be susceptible to physiological responses. Hauck *et al.*, suggested that rising white blood cells in the rats treated with quantum-dot can be recognized as an inflammatory response (29). Additionally, red blood cells are obtained from hematopoietic stem cells mostly in the marrow bones. After a series of puberty steps and mainly upon hormone erythropoietin, red blood cells enucleate and enter through circulation system. Thus, all changes in red blood cell levels may be associated with the hematopoietic system (26). Any elevation in red blood cells as it can be seen in rats treated with MgO nanoparticles suggesting that size particles (10-15 nm) and dose (500 $\mu g.mL^{-1}$) affect the hematopoietic system. Thus, nanoparticles are able to trigger an inflammatory response, and in addition increases or decreases immune system function, and simultaneously alters related hematologic factors including blood cell counts (29, 30). It can be noted, the higher dose of MgO nanoparticles, the more hematologic factors will be affected.

In this research, findings demonstrated that injection of MgO nanoparticles to Wistar rats induces an increase in the level of markers of liver function such as AST and ALP. In return, injected with MgO nanoparticles in rats no change was observed on serum levels of ALT and GGT as well as renal function. Aspartate aminotransferase and alanine aminotransferase activities have been considered as criteria for hepatocellular damage since 1955. Eighty percent of AST in hepatocytes is in mitochondria, whereas ALT is located elsewhere in the cytoplasm. The various intracellular locations of these enzymes have led to observations and speculations about their role in the diagnosis and prognosis of liver disease (31). Results showed that injection of MgO nanoparticles induces concentration-related increases in AST, but does not alter the levels of ALT. Previous research has reported that the ratio of AST: ALT, or De Ritis quotient, which normally is 1 or more, is at 3-4:1 in alcoholic liver disease, while the ALT level hovers around normal. The exact reason for this is unknown, though two possible explanations are available: 1) alcohol is a mitochondrial toxin, and 2) pyridoxine is deficient in alcoholics. Pyridoxal 5'-phosphate deficiency is rate limiting in the assay for ALT (32). As shown above, it seems that MgO nanoparticles are mitochondrial toxins like alcohol and because of the further presence of these enzymes in the mitochondria, with increasing the dose-dependent concentration of MgO nanoparticles, the amount of AST in serum will also increase.

ALP is also called cholestatic liver enzymes. Cholestasis is a condition that causes partial or thorough occlusion of the bile ducts (33). If the bile duct is fervent or defective, ALP can get backed up and spill out from the liver into the bloodstream. (34). Therefore, the release of ALP in serum by administration of MgO nanoparticles at doses of 250 and 500 $\mu g.mL^{-1}$ in connection with inflammation of bile ductules, may be predicted. In addition, the levels of urea and creatinine in rat's blood serum were tested as a measure of kidney function. When the serum creatinine and urea are significantly higher than normal, kidney function is seriously damaged (35).

A detailed analysis of these metabolites in serum of animals treated with various doses of MgO nanoparticles as compared to the controls showed no statistically significant differences in any of the tested parameters. Histopathological examinations in the rat's liver showed proliferation of bile ductules, congestion in some regions of the liver sinusoids, and apoptotic cells (probably) in high-dose groups, but no histological changes were found in the kidney functions. New studies on cholestatic liver disorders showed the contributions of resident macrophages in the liver, kupffer cells, as well as pathogenesis of a sustained inflammatory response following apoptotic cell death (36).

On the basis of the histological results, it seems that variations in liver tissue including proliferation of bile ductules, sinusoidal dilatation and congestion (SDC), accompanied by elevated ALP in the long term, may lead to chronic biliary disease. The authors proved that venous outflow impairment leads to sinusoidal dilatation and congestion (SDC) in the liver biopsy (37, 38). Liver biopsies of patients with venous outflow impairment exhibited bile ductular proliferation, portal inflammations and portal-based fibrosis as well as elevated ALP and GGT this ultimately will have resulted in suspicion of chronic biliary disease (39).

The results of Gelli *et al.*, showed that a dose-dependent pulmonary toxicity in rats and various tissue damage markers, like alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), in Broncho alveolar lavage (BAL) fluid and histopathology of lungs at 1, 7, and 30 days of post-exposure intervals was generated by MgO nanoparticles exposed via intra-tracheal instillation at the doses of 1 mg/kg or 5 mg/kg into rat lung (40). The results of Mangalampalli *et al.*, indicated that the induced significant DNA damage and aberrations in chromosomes after 28 day repeated oral administration in Wistar rats was induced by the MgO NPs with three different doses (250, 500, and 1000 mg/kg). Biochemical and genotoxic parameters in dose-

dependent and gender-independent manner were improved by the oral administration of MgO NPs (41). In a research performed by Lasagna-Reeves *et al.* on bioaccumulation and toxicity of gold nanoparticles, it was found that exposing to repeated injection of gold nanoparticles was led to significant increase in the amount of gold in kidneys. Substantially, the higher gold nanoparticles dose, the smaller the percentage of gold accumulated was found, showing effective removal of gold nanoparticles from the body (42). Experimental results of Chen *et al.* also showed that injection of titanium dioxide (TiO_2) nanoparticles with various doses (0, 324, 648, 972, 1296, 1944 or 2592 $mg.kg^{-1}$) induced relatively higher ALT and AST levels in treated mice in comparison to the control group, whereas the differences in BUN value between experimental groups and the control group were not evident. It can suggest that TiO_2 nanoparticles had a stronger toxicity to liver than to kidney (43). As a whole, considering her disability of the experimental results, it can be seen that administration of 10-15 nm MgO nanoparticles at doses of 250 and 500 $\mu g.mL^{-1}$ can cause obvious adverse effects on liver function and indicates an inflammatory response.

Subsequently, treatment with low concentrations of MgO nanoparticles (62.5 and 125 $\mu g.mL^{-1}$) did not induce any apparent toxicity in the rats during the study period. The present results are in good agreements with the results of Ge *et al.*, showed low concentration (below 200 $\mu g.mL^{-1}$) of MgO nanoparticles suspension no cytotoxicity on human umbilical vein endothelial cells (HUVECs) *in vitro*. Nonetheless, when these particles concentrations go up to 500 $\mu g.mL^{-1}$, the relative growth rate will be lower than the control (44). Investigation of antioxidants in rat serum after exposure to magnesium oxide (MgO) nanoparticles revealed that instillations of MgO (at a dose of 1 and 5 $mg.kg^{-1}$ of body weight) induces oxidative stress by reducing the total antioxidant capacity in rats. Findings of recent studies suggest potentially undesirable effects of these particles in chronic exposures (45).

Experimental data in association with influence on cell viability and oxidative stress together with physical and chemical characterizations of industrial metal oxide nanoparticles showed the major cytotoxic agent of these particles such as MgO was metal ion release (46). Patel Manoj K *et al.*, indicated that MgO nanoparticles are appropriate for anti-cancer activities. In this paper, human intestinal cell lines (INT 407) and human cervical cancer cell lines (SiHa) were examined for cytotoxic assay of MgO nanoparticles in the range of 0–350 $\mu g.mL^{-1}$. Results showed that in low concentrations (below 300 $\mu g.mL^{-1}$). No significant cytotoxicity was found in Human intestinal and cancerous cells but when the concentration of MgO nanoparticles was increased to above 300 $\mu g.mL^{-1}$, the comparative growth rate in cancerous cells was lower than the control (47). In accordance with previous *in vitro* findings and *in vivo* toxicity investigation, authors declare that MgO nanoparticles at concentrations lower than 250 $\mu g.mL^{-1}$ are found to be safe. Therefore, it is viable to use these particles for water purification, encapsulate drugs for utilization in the clinical setting, and other desired applications.

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