

TOXICOLOGICAL EVALUATION OF MAGNESIUM HYDROXIDE NANOPARTICLES IN RATS FOLLOWING 28 DAYS OF REPEATED ORAL EXPOSURE

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Abstract

The rapidly growing field of nanotechnology has offered innovative discoveries in medical and industrial fields. The potential risks of these nanoparticles have also been identified via environmental and occupational exposure. Magnesium nanoparticles have gained commercial interest in the areas of waste remediation, water treatment and drug delivery. However, the potential toxic effect of magnesium hydroxide nanoparticles (MgOHNPs) is yet to be unraveled. The present study elucidated the effect of repeated doses of MgOHNPs in rats. Adult Wistar rats were exposed to 0, 50, 100, 200 and 1000 mg/kg body weight of the nanoparticle for 28 days. Exposure to MgOHNPs impaired the functionality of liver and kidney as evidenced by significant elevation in plasma aspartate aminotransferase (AST), alkaline phosphatase (ALP), magnesium, calcium, potassium and creatinine levels compared with control. Also significant reduction in the plasma concentrations of albumin, total protein, globulin and chloride was observed at the tested doses. The altered integrity of these organs was corroborated by a significant elevation in the levels of total protein, AST and ALP and reduction in alanine aminotransferase (ALT), was observed in the kidney. Also, there was a significant reduction in the hepatic total protein. Whereas, a dose dependent significant ($p < 0.05$) increase was observed in the activities ALP, ALT and AST in the liver. The exposure induced a marked dose-dependent decrease in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol. However the blood levels of glucose, ALT and hematological parameters remained unaltered throughout the experimental period. These findings suggest that repeated exposure to MgOHNPs may have consequential effects on the liver and kidney functions.

Keywords: *Nanoparticles, Magnesium hydroxide, Exposure, Repeated doses, Rats*

Introduction

Engineered nanomaterials are employed in various fields of medicine, pharmaceuticals, biotechnology, energy production, environmental sciences, crop protection, and waste remediation [1-3]. The biomedical application of nanoparticles has been attributed to their unique physicochemical characteristics such as good dispersibility, colloidal stability in biological media, effective delivery to target sites and low toxicity [4]. Nanotechnology has been used to alter the pharmacokinetics of drugs thereby prolonging their bioavailability [5]. A variety of methods for synthesizing high-quality metal oxide nanoparticles in organic solvents have been reported [6 - 10]. Engineered nanomaterials are manufactured from metals, metal oxides, sulphides and biological molecules such as lipids, carbohydrates, peptides, proteins, and nucleic acid [11]. Notable among metal nanoparticles are those of zinc, silver, nickel, gold, copper and magnesium. Among the metal oxide nanomaterials, magnesium nanoparticles have attracted the interest of researchers due to its low cost and usefulness in waste remediation processes. Their toxicity has been found to be lower than other metal oxide nanoparticles [12]. Magnesium nanoparticles have been shown to exhibit anticonvulsive effects in diabetic mice [13]. They are also known to have *in vitro* cytotoxic effects on human cancer cell lines [4]. Despite the wide application of nanoparticles in the fields of life science and biomedicine, there is paucity of information concerning the health and environmental implications of manufactured nanomaterials [14 - 15]. Studies have shown that inhalational and dermal absorption of some nanomaterials may have adverse effects on health [16]. Research has shown that exposure of rats and mice to copper nanoparticles provoked the proliferation of endothelial cells of brain capillaries [17]. Toxicological risk assessment of nanoparticles involves hazard identification, dose-response assessment and exposure assessment [18]. Safety assessments of nanoparticles include organ toxicity that can result from acute or sub chronic exposure. Serum enzymes and metabolites serve as good markers for hepatotoxicity and nephrotoxicity [19]. The goal of this study was to evaluate the possible adverse effects of magnesium hydroxide

nanoparticles following 28 days of repeated oral exposure in wistar rats. Little is known about the influence of repeated doses of magnesium nanoparticles, thus different doses were assessed in this study. Levels of plasma enzymes and other biochemical parameters were measured to assess if repeated dosing caused any organ damage.

Methods

Experimental Animals

Four week old female Wistar rats with specific pathogen-free health status were purchased Central Animal House, College of Medicine, Ekiti State University, Ado Ekiti, Nigeria for the study. Upon arrival the rats were allowed to acclimatize for two weeks before the start of the experiment. The body weight of the animals at the beginning of the study was 160 ± 20 g (N = 28). The rats were housed standard wooden cages with a 12:12-h reversed light/dark cycle from 7 p.m. to 7 a.m. The rats were fed with normal rat pellets and water *ad libitum*. The animal study was performed under conditions outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science.

Assay Kits

The assay kits for creatinine, calcium, sodium, potassium, chloride, magnesium, albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), acid phosphatase (ACP), Low density lipoprotein (LDL), high density lipoprotein (HDL), Triglycerides (TG) and total cholesterol (TCHOL) were obtained from Randox Laboratories United Kingdom.

Acute Toxicity Assessment

Acute oral toxicity was evaluated in rats in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines [20]. with some modifications. Twenty five (25) female animals were divided into five groups (A-E) consisting of five animals each. Group A served as control and received 1ml distilled water while groups B to E received a single dose of 500, 1000, 2000, 4000, and 5000mg/kg body weight (b/w) of the nanoparticle. The rats were deprived of food except water for 15 h prior to dosing. The general behavior of the rats was continuously monitored for 1 h after dosing. Thereafter, all animals were observed for clinical signs including injury, pain,

signs of illness and mortality daily for 14 days. On the 14th day, all animals were sacrificed. Since there was no death or any physically observed sign of toxicity, 50, 100, 200 and 1000mg/kg b/w of the extract were selected for the sub chronic toxicity study.

Repeated Dose 28-Day Oral Toxicity Study

The rats were randomly divided in five groups and received 50, 100, 200 and 1000 mg/kg, p.o. of the nanoparticle by oral gavage a day for 28 days. The rats were weighed on a weekly basis and visual observations for mortality, behavioral pattern and physical appearances were conducted once daily during the experimental period. After 28 days of repeated dosing, the animals were fasted for 18 h and sacrificed. Blood samples were collected via cardiac puncture into lithium heparinized and EDTA-containing tubes for biochemical and hematological analyses respectively. The selected organs were excised, blotted, weighed, and examined macroscopically. They were then homogenized in ice-cold 0.25M sucrose solution (1:10 weight/volume) and centrifuged at 4000 x g for 15 min. The homogenates were kept frozen overnight to ensure maximum release of the enzymes located in the cells of the tissues before being used for the various biochemical assays [21].

Hematological Analysis

The anti coagulated blood samples were subjected to hematological analysis according to the method described by Alkaladi *et al.* [22]. The following parameters were determined: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Biochemical Parameters

The parameters were determined colorimetrically by employing the standard ready-to-use Randox kits. The parameters assayed for include aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), acid phosphatase (ACP), albumin, total bilirubin, creatinine, and electrolytes (sodium, potassium, magnesium, and chloride). The manufacturer's instructions for each biochemical parameter were strictly adhered to in the course of the investigations. The protein concentration of the various samples was determined by the Biuret method described by

Gornalet *et al.* [23]. using bovine serum albumin (BSA) as the standard. The serum globulin level was calculated by subtracting the albumin from the obtained total protein as described by [22]. Low density lipoprotein (LDL), high density lipoprotein (HDL), Triglycerides (TG) and total cholesterol (TCHOL) were determined by enzymatic methods according to Diniz *et al.* [24] using commercial diagnostic kits (Randox, UK). The blood glucose levels were determined using glucose reagent strips [25].

Statistical analysis

Data were expressed as mean \pm standard deviation (SD) and subjected to one-way analysis of variance. The means were separated by Duncan's Multiple Range Test. Values were considered statistically significant at $P < 0.05$.

Results

General observations

The rats in all the experimental groups remained active and vigorous throughout the experimental period. The daily repeated oral dose treatment with the nanoparticle for 28 days did not induce any evident sign of toxicity in the treated animals, including those administered 1000 mg/kg body weight. No mortality or obvious adverse clinical signs were observed in any of the test groups throughout the treatment period.

Body Weight and Relative Organ Weights

The body weight and relative organ weights of control rats and those exposed to MgOHNP are presented in Figures 1 and 2. There was significant ($P < 0.05$) weight gain in the nanoparticle treated groups and the control. Following exposure period, the relative liver weight increased significantly ($P < 0.05$) in the 100, 200 and 1000mg/kg administered groups. However, the administration of MgOHNP had no significant effects on the relative weights of the kidney heart and brain when compared with the control.

Haematological Parameters

Table 1 showed that the nanoparticle at all the doses investigated did not significantly alter the red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cell count (WBC) throughout the period of administration.

Lipid Profile

At all the doses tested, there were significant reductions ($P < 0.05$) in the total cholesterol, triglyceride, LDL and HDL cholesterol levels of the rats compared with the control group (Table 2).

Effect of Repeated Doses of MgOHNPs on Plasma Biochemical parameters

To investigate the impact of MgOHNPs treatment on some organs function, the concentrations of some biomarkers were determined in the serum of control and treated rats after 28 days (Table 3). The results indicated a significant, non dose dependent elevation in ALP, AST, magnesium, calcium, potassium and creatinine levels in treated rats when compared with the control. Also a significant reduction was observed in the concentrations of albumin, total protein, globulin and chloride at the tested doses. However the levels of glucose and ALT remained unaltered throughout the experimental period.

Effect of Repeated Doses of MgOHNPs on Some Markers of the Liver and Kidney

The effects of oral administration of MgOHNP on the levels of total protein, ALP, ALT and AST in the liver and kidney are shown on Tables 4 and 5. The administration of the nanoparticle resulted in significant reduction in the activities of ALT, AST and ALP in the liver. Whereas, the hepatic total protein concentration remain unaltered throughout the experimental period (Table 4). Also, a non-dose dependent significant ($p < 0.05$) increase was observed in the levels of total protein, AST and ALP in the kidney. In contrast, the administration of the nanoparticle resulted weight resulted in significant decrease ($p < 0.05$) in ALT activities in the kidney at a dose of 200 and 1000 mg/kg (Table 5).

Discussion

The rapidly expanding development of NPs for biomedical applications and drug delivery requires a nanotoxicological evaluation of these particles to ensure their biological safety prior to commercialization [19]. It is a known fact that any therapeutic agent is not totally free from harmful effects [26]. Nanoparticles are also not excluded as they can only be beneficial after a careful measurement of the *pros and cons* associated with their usage. In the present study, the mean body weights of animals in all experimental groups

increased throughout the duration of the experiment. The weight gained by the animals during the experimental period may be an indication that the nanoparticle did not hinder the growth of the animals [27]. The relative organ weight is a veritable index of assessing swelling, atrophy, or hypertrophy [28]. An increase in this parameter is associated with inflammation [29]. Therefore, the significant change observed in the relative weight of the liver (200 to 1000mg/kg) could be as a result of inflammation of the hepatocytes.

Hematological profile can be used to diagnose the actual physiological or pathophysiological status of an organism [30]. It is a veritable tool for monitoring animal health and the reaction of the body to injury [31]. The major components of the blood are the erythrocytes, leucocytes, and indices that relates to both of them [32]. The major function of the erythrocyte is the distribution of oxygen to peripheral tissues. Hemoglobin (Hb), RBC, PCV are directly linked to the total population of red blood cells. The non-effect of the nanoparticle on these indices may indicate that neither the incorporation of haemoglobin into red blood cells nor the oxygen-carrying capacity of the red blood cells was affected [33]. In addition, MCV and MCH which are diagnostic indices of anaemia remained unaltered throughout the duration of the experiment. This suggests that repeated administration of MgOHNPs may not affect the average size of RBC as well as the weight of haemoglobin per RBC [30]. The white blood cell and its differential are the main cells of the immune system that provide innate and specific adaptive immunity [32]. The non significant effect of repeated doses of MgOHNPs on this parameter suggests that the nanoparticle may not pose any challenge on the immune system.

The result of the lipid profile revealed that repeated administration of MgOHNPs produced remarkable reductions in the concentration of TC, TG, LDL and HDL cholesterol at all the tested doses. The observed hypocholesterolemia in all the treated groups may be associated with impairment in the β -oxidation of fatty acids [33]. Also the reduction in triglyceride levels may be an indication of depletion of the energy store in the animals [34]. The reduced concentrations of LDL - C and HDL - C may be due to alterations in the transport of cholesterol in the tissues of the animals.

The liver is involved in the detoxification of xenobiotics and drugs in biological systems. Liver damage is associated with the elevation of blood levels of cellular enzymes (AST, ALT and ALP) and decreased levels of albumin, globulins and total proteins. Elevated levels of enzymes in the extracellular fluid result from leakage from tissues [35]. The significant increase in the activities of AST, ALT and ALP which was corroborated by their reductions in the liver may be attributed to altered permeability of the hepatic membrane [36]. Also, decreased levels of albumin, globulins and total proteins in the plasma are an indication of impaired function of the liver [37].

Kidney damage is associated with decline in renal function which could eventually lead to renal failure. The significant increase in plasma level of creatinine in rats treated is an evidence of decreased renal function. Elevated level serum creatinine has been attributed to impairment in the kidneys, particularly for glomerular filtration rate [29]. The significant elevation in the plasma concentrations of magnesium, calcium, potassium coupled with the reduction in chloride levels may indicate a consequential effect of MgOHNPs on the ion-dependent processes in the treated animals [36]. Furthermore, the decrease observed in the activity of renal level of total protein could also be an indication of impairment. Also the reduction of ALT at a dose of 200 and 1000 mg/kg could be as a result of leakage into the extracellular compartment. However, the significant increase in the activities of ALP and AST activity of rat kidney following the administration of the nanoparticle may be adduced to induction of the enzymes probably by de novo synthesis [38].

Conclusion

This study obviously demonstrated that repeated exposure of rats to magnesium hydroxide nanoparticles for 28 days is capable of inflicting damage on the liver and kidney at the tested doses. The data on the hepatic and renal function parameters observed in this study following the subchronic exposure of rats to MgOHNPs further corroborated the dose dependent adverse effect of the nanoparticle. The data presented here are novel and show, for the first time, the hepatorenal toxicity of magnesium nanoparticles in experimental rats.

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Table 1: Effect of repeated doses of magnesium hydroxide nanoparticles on hematological parameters of rats

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg	1000 mg/kg
PCV (%)	43.75±3.69 ^a	39.75±3.30 ^a	46.25±2.06 ^a	44.75±2.22 ^a	43.05±3.50 ^a
RBC (x10 ⁶ /L)	8.79±2.57 ^a	7.58±1.62 ^a	7.44±0.78 ^a	8.17±1.19 ^a	7.69±1.02 ^a
WBC (x10 ³ μ/L)	9.63±0.63 ^a	8.64±0.63 ^a	9.97±0.27 ^a	9.87±0.48 ^a	9.00±0.43 ^a
Hb (g/dL)	16.86±3.52 ^a	18.94±1.36 ^a	17.38±4.81 ^a	18.08±2.23 ^a	18.00±1.49 ^a
MCV (fL)	53.08±15.11 ^a	53.59±7.26 ^a	52.50±3.88 ^a	55.93±10.57 ^a	63.82±16.45 ^a
MCH (pg)	22.52±6.77 ^a	25.63±5.04 ^a	26.32±4.68 ^a	21.55±3.75 ^a	22.54±4.08 ^a

Results are expressed as means ± SD (n=6). Test values carrying superscripts (a) are not significantly different (P < 0.05) from the control (a) for each parameter.

Table 2: Lipid profile in rats exposed to magnesium hydroxide nanoparticles for 28 days

Dose	Total cholesterol mmol/L	Triglyceride mmol/L	LDL-C mmol/L	HDL-C mmol/L
Control	127.53±4.30 ^a	156.01±5.76 ^a	65.16±8.21 ^a	47.50±5.1 ^a
50 mg/kg	111.28±3.72 ^b	146.53±4.42 ^{ab}	62.08±8.28 ^{ab}	47.42±3.38 ^a
100 mg/kg	107.15±3.33 ^b	140.67±8.91 ^b	54.22±5.46 ^b	44.31±4.80 ^{ab}
200 mg/kg	94.36±8.30 ^c	129.88±8.85 ^c	54.07±6.66 ^b	38.98±3.69 ^{bc}
1000 mg/kg	88.76±8.32 ^c	119.88±3.17 ^c	42.89±3.78 ^c	34.02±3.76 ^c

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P < 0.05) from the control (a) for each parameter.

Table 3: Effect of repeated doses of magnesium hydroxide nanoparticles on some biochemical parameters in the plasma of rats

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg	1000 mg/kg
Albumin (mmol/L)	51.96±2.44 ^a	42.95±3.53 ^b	36.40±2.84 ^c	21.43±1.25 ^d	22.95±2.78 ^d
Total protein (g/L)	155.86±12.96 ^a	89.35±9.27 ^b	66.56±7.20 ^c	98.25±13.35 ^b	89.62±15.62 ^b
Globulin (g/L)	103.90 ± 9.52 ^a	46.40±6.06 ^d	30.16±5.64 ^e	76.82±12.05 ^b	66.67±12.84 ^c
Glucose (mmol/L)	6.56±0.59 ^a	6.10±0.86 ^a	6.40±0.96 ^a	6.78±0.98 ^a	6.26±0.26 ^a
ALP(U/L)	37.46±3.75 ^a	41.83±13.93 ^a	52.61±14.64 ^{ab}	70.92±13.70 ^b	69.78±12.41 ^b
ALT(U/L)	5.05±2.79 ^a	4.79±1.92 ^a	4.36±1.77 ^a	4.10±1.90 ^a	4.23±1.71 ^a
AST(U/L)	4.20±0.47 ^a	4.61±1.97 ^{ab}	3.14±1.12 ^a	6.56±3.07 ^{ab}	4.20±1.23 ^{ab}
Magnesium	1.58±0.47 ^a	1.55±0.27 ^a	2.39±1.07 ^{bc}	2.54±0.48 ^{bc}	3.23±1.16 ^c
Calcium (mmol/L)	3.75±0.68 ^a	4.20±1.12 ^a	5.31±1.34 ^b	7.28±1.70 ^b	9.57±1.68 ^c
Potassium (mmol/L)	2.75±0.51 ^a	7.97±1.37 ^b	10.07±1.37 ^c	7.02±2.15 ^b	6.10±1.90 ^b
Chloride (mmol/L)	16.87±1.39 ^a	15.32±0.59 ^{ab}	13.87±0.90 ^c	13.43±1.46 ^c	11.22±1.37 ^c
Creatinine (mmol/L)	1.95±0.46 ^a	3.23±0.99 ^a	5.41±0.57 ^b	6.35±0.79 ^b	8.26±1.68 ^c

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P < 0.05) from the control (a) for each parameter.

Table 4: Effect of repeated doses of magnesium hydroxide nanoparticles on some markers in the liver of rats

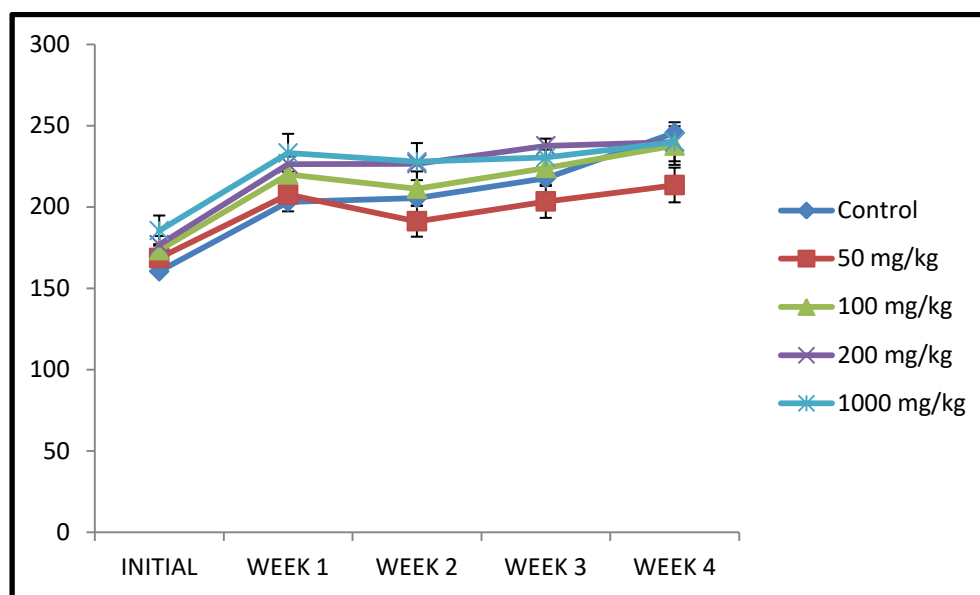
Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg	1000 mg/kg
Total Protein (g/L)	35.10±11.89 ^a	20.45±3.61 ^a	19.35±1.01 ^a	22.30±4.40 ^a	29.95±3.71 ^a
ALT (U/L)	6.83±0.24 ^a	6.88±1.26 ^a	6.62±0.64 ^a	4.83±0.98 ^b	3.14±1.38 ^b
AST (U/L)	6.83±0.24 ^a	6.79±1.26 ^a	6.62±0.64 ^a	3.63±1.98 ^b	4.77±2.38 ^b
ALP (U/L)	110.59±7.08 ^a	94.56±8.48 ^b	83.62±4.94 ^b	82.40±5.89 ^b	80.57±15.49 ^b

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b) are significantly different (P < 0.05) from the control (a) for each parameter.

Table 5: Effect of repeated doses of magnesium hydroxide nanoparticles on some markers in the kidney of rats

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg	1000 mg/kg
Total Protein (g/L)	2.58 ± 1.03 ^a	12.42 ± 4.36 ^b	9.16 ± 1.53 ^b	23.06 ± 6.29 ^c	20.57 ± 5.51 ^c
ALT (U/L)	2.15 ± 0.29 ^a	2.86 ± 0.72 ^a	2.3.8 ± 0.47 ^a	1.81 ± 0.82 ^b	1.63 ± 0.23 ^b
AST (U/L)	2.70 ± 0.529 ^a	2.84 ± 0.72 ^a	4.64 ± 0.40 ^c	3.14 ± 0.11 ^b	3.20 ± 0.14 ^b
ALP (U/L)	2.75 ± 0.82 ^a	2.39 ± 1.38 ^a	2.85 ± 0.73 ^a	2.94 ± 1.45 ^{ab}	3.87 ± 0.45 ^b

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P < 0.05) from the control (a) for each parameter.

**Figure 1:** Body weights of rats exposed to MgOHNP for 28 days (N = 6).

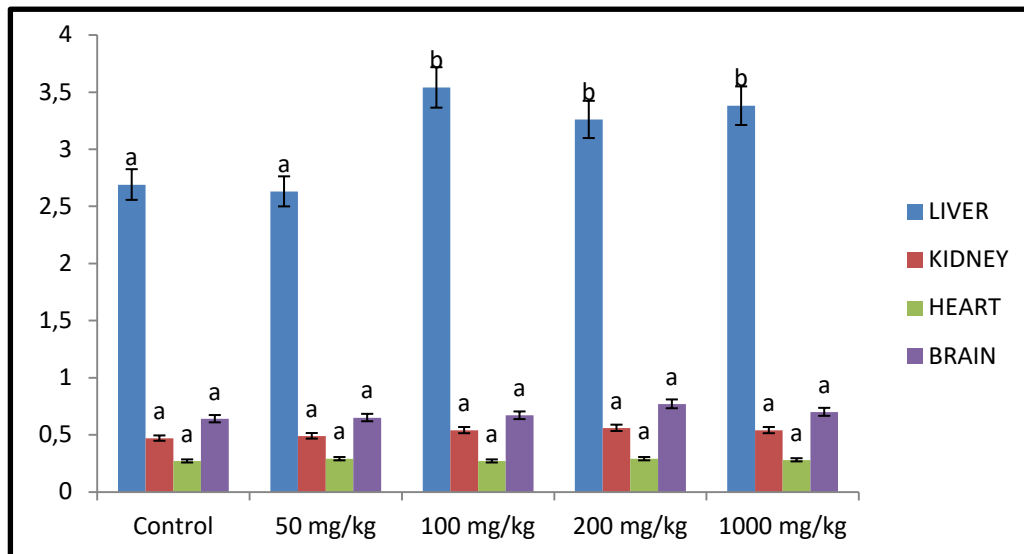


Figure 2: Relative organ weights of rats exposed to MgOHNPs for 28 days (N = 6). Values represent mean \pm SD. Test values carrying ^b indicates statistical difference from control ^a ($P < 0.05$).