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RESEARCH

Toxicological insight of magnetite nanogel: neuro-ethological, hepato-renal, antioxidant, and histopathological traits in *Clarias gariepinus*

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Abstract
Assessment of acute toxicity of magnetic nanogel (MNG) is crucial to conclude the safe applicable dose and to warrant its application in aquaculture. Therefore, the current study is a novel step to assess behavior, neuro-stress response, hepato-renal, oxidative, and histopathological variations produced by MNG' acute toxicity in *Clarias gariepinus*. Two experiments were conducted: the first was a determination of the 96-h lethal concentration 50 (LC_{50}) of MNG in *C. gariepinus*. Meanwhile, the second was an assessment of the toxicological impacts of three different concentrations of MNG in *C. gariepinus* following a 10-day exposure period and a subsequent 10-day depuration trial. One hundred and eighty fish were allotted to four groups exposed to 0, 1/10, 1/8, or 1/5 96-h LC_{50} of MNG. The outcomes exhibited that 96-h LC_{50} of MNG for *C. gariepinus* was 44 mg/L. The subjected group to MNG induced a concentration-dependent elevation in the serum values of cortisol, alanine transaminase, aspartate transaminase, urea, and creatinine following MNG exposure. Marked elevation in the oxidative stress indicators (catalase (CAT), glutathione S-transferase activity (GST), and superoxide dismutase (SOD)) was also evident. Meanwhile, the value of the neurological biomarker, acetylcholinesterase (AChE), was markedly reduced in a concentration-dependent way. These biochemical changes were complemented by pathological alterations in the hepato-renal architecture. Interestingly, in response to the 10-day depuration period, most of the tested parameters were eliminated in *C. gariepinus* exposed to 1/10 of LC_{50}. Conclusively, MNG can induce numerous adverse effects only at higher doses (1/5 and 1/8 of LC_{50}). Meanwhile, the lowest tested concentration of MNG (1/10 of LC_{50}) was safe for application in aquaculture practices with only mild disruptions in the bio-indices. In addition, a retrieval period of 10 days was sufficient to renovate these alterations only in fish exposed to the same concentration.

Keywords Toxicity · Magnetite nanogel · Behavior and oxidative stress indices · Histopathological alterations · *Clarias gariepinus*

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Introduction

Utilization of nanomaterials originating from various materials has been used broadly in different applications (Neeti et al. 2023; Paul et al. 2023). Magnetite-based nanomaterials are one of the most successfully utilized nanoparticles which own magnetic characteristics (Kumari and Parashara 2018; Jv et al. 2019). The functionalized magnetic nanoparticles are beneficial in the treatment of wastewater because of their magnetic properties (Shahid et al. 2018). In spite of the reported safety of magnetite nanoparticles in aquaculture practices (Mahboub et al. 2021a), their toxicity has recently verified in zebrafish for inducing physiological and morphological alterations (Guillén et al. 2022). It could be returned to that the aquatic environment receives various pollutants which in turn enhances growth of nanomaterials (Halpern et al. 2015). Magnetite nanogel is another form of nanoparticle that is tiny and swollen and comprised of polymeric chains in nanosize (Duan et al. 2023). Currently, they are successfully practical in many applications due to their wide surface area and the power to load high percentage of water (Pinelli et al. 2023).

Nile catfish models are a fundamental principle of animal research in Egypt and have been extensively cultured and utilized to investigate the toxicity in the aquatic environment (Mahboub et al. 2021b; Abd El-Rahman et al. 2019).

Although the outcomes for using nanogels, either magnetite or titanium, are promising as an antioxidant agent and against bacterial infection (Rahman et al. 2023; Mahboub et al. 2024), their security in the aqueous environment must be assured prior their use. Therefore, the present work is a pioneer trial to investigate the acute toxicity exhibited by higher concentrations of MNG to assess the accurate safe dose for application. Further, we decided to investigate the efficacy of various concentrations of MNG on stress-neuro function, hepato-renal activity, hepatic antioxidant capacity, and histopathological alterations in Nile catfish.

Material and methods

Synthesis and characterization of MNG

At first, Fe3O4 NPs were synthesized as follows: an additional 0.4 g of the hematite ore (Fe3O4) was performed drop by drop to 40 mL of H_2O_2. Then, the mixture was exposed to ultrasound at 60 kHz for 2.5 h using an ultrasonic device (Sonica 4200 EPS3, Milano, Italy) till the formation of black particles of Fe3O4. After 1.5 h, the black-colored Fe3O4 NPs precipitated from the supernatant which is red in color. The separation of Fe3O4 NPs was carried out from the solution via centrifugation at 4000 rpm, and, lastly, the Fe3O4 NPs were rinsed four times using methanol. Characterization procedures were classified into three groups: morphology, identification, and index following the approach of Hassan et al. [15].

Fish and cultural condition

A total of 290 apparently healthy *C. gariepinus* (average body weight, 95.14 ± 0.45 g) were procured from a private fish farm in Sharkia province, Egypt. Prior to the initiation of the experiment, a 14-day acclimatization period was adopted for the fish to adapt to the laboratory conditions. During that duration, fish were reared in constantly aerated

120-L glass aquaria filled with dechlorinated tap water. The water in each aquarium was exchanged twice a week; meanwhile, the fish waste was siphoned daily. The water quality criteria were maintained within the optimum values along the course of the experiment, following Apha (1992): temperature, 25 ± 1.5 °C; dissolved oxygen, 6.55 ± 0.7 mg/L; pH, 6.70 ± 0.3; ammonia, 0.02 ± 0.001 mg/L; and nitrite, 0.015 ± 0.001 mg/L. The photoperiod was 12 h of light:12 h of darkness. During the adaptation period, fish received a basal diet (37% crud protein), formulated following the earlier protocol of Council (2011). To fulfill the nutritional requirements of *C. gariepinus*, they were fed at a rate of 3% of body weight, three times per day (8:00, 12:00, and 16:00 h).

Experimental design

Firstly, an acute experiment was conducted to monitor the 96-h LC_{50} of Magnetite nanogel (MNG) in *C. gariepinus*. For this purpose, 110 *C. gariepinus* were assigned into 11 groups each with 10 fish. The first group was maintained in clean dechlorinated water for 96 h and served as a control. The other ten groups were exposed to ten various levels of MNG (15, 20, 25, 30, 35, 40, 45, 50, 55, 60 mg/L). During the experimental period, neither the water was exchanged nor were the fish groups fed. The mortalities in all groups were recorded at 24, 48, 72, and 96 h. The behavioral, clinical symptoms, and post-mortem alterations were documented throughout the investigational period. The 96-h LC_{50} value was computed using Finney's probit analysis (Finney 1971).

The second experiment (sub-acute exposure and recovery), included 180 *C. gariepinus* which were distributed into four equal groups, each group consisted of three replicates (15 fish/replicate, 45 fish/group). The first group was considered a control without any exposure. The other three groups were exposed to MNG at levels of 1/5, 1/8, and 1/10 of 96-h LC_{50}, respectively. Fish were exposed to various concentrations of MNG for 10 days followed by a subsequent recovery period for another 10 days. Throughout this period, fish were kept in clean dechlorinated tap water.

Sampling

At the end of the two investigational periods, 15 fish samples were randomly selected from each group (5 fish per replicate) and anesthetized in ice-cold water. The blood was then collected from the caudal blood vessels using tubes without anticoagulants to isolate serum. Such serum was used for biochemical analysis including stress-related hormone, hepato-renal function, and the neurological indicator, AChE. In addition, the gills, liver, kidney, and intestine specimens were gathered and fixed for 48 h in 10% neutral buffered formalin for histopathological investigations. The liver homogenate was further processed to estimate oxidative stress indicators. Furthermore, spleen specimens were transported instantly in liquid nitrogen to be kept at −80 °C for the gene expression analysis of immune-related and apoptosis-related genes.

Hepatic oxidative stress indicators

Oxidative stress indices including catalase (CAT), glutathione S-transferase activity (GST), and superoxide dismutase (SOD) were assessed using colorimetric commercial kits

(Biodiagnostic Co., Cairo, Egypt) following Aebi (1984), Habig et al. (1974), and Velkova-Jordanoska et al. (2008), respectively.

Serum biochemical parameters

The anti-stress indicator (cortisol level) was monitored using the spectrophotometry assay according to the previous method of Burtis and Ashwood (1994). The liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferases (AST)) and renal-damaged products (urea and creatinine) were computed in serum using the Spinreact kits (Esteve De Bas, Girona, Spain) following the protocol of Burtis and Ashwood (1994), Murray et al. (1984), Kaplan (1984), and Fossati et al. (1983), respectively. The activity of the neurological indicator, AChE, was spectrophotometrically determined depending on the assay of Ellman et al. (1961).

Histopathological investigation

Nine representative specimens from hepatic and renal tissues were freshly collected from the sacrificed *C. gariepinus* from each group. Preservation of these specimens was carried out by using 10% neutral-buffered formalin solution. Then, they were dehydrated via gradual emersion in ascending concentrations of ethanol, cleared in xylene, and fixed in paraffin. A microtome (Leica RM 2155, England) was used for sectioning of the samples to obtain a 5-micron thick sample, then stained with hematoxylin and eosin (Suvarna et al. 2018).

Statistical analysis

Firstly, the value of 96-h LC50 of MNG was calculated via using Finney's probit analysis (Finney 1971). The data was confirmed for both normality and homogeneity of the variance by using by Levene's tests. The outcomes of the impact of the grading concentrations of MNG during sub-acute exposure trial and after the depuration trial were scrutinized by using a two-way analysis of variance (two-way ANOVA) in SPSS version 18 (SPSS, Chicago, IL, USA). Tukey's multiple range tests were used to evaluate the differences among means, and the results are displayed as means ± standard error (SE), and they were statistically significant at $P < 0.05$.

Results

Results of MNG characterization

Analysis of X-ray diffraction (XRD) revealed the fingerprint curve and data for magnetite based on the Brucker Database library, which aimed to confirm our synthesis protocol without any inferior phases. Zeta potential data and dynamic light scattering (DLS) revealed a regular size (one peak) of 60 nm (Fig. 1A). Because of a significant degree of zeta potential (−35 mV), the findings showed a greater colloidal structure in aqueous suspension (Fig. 1B).

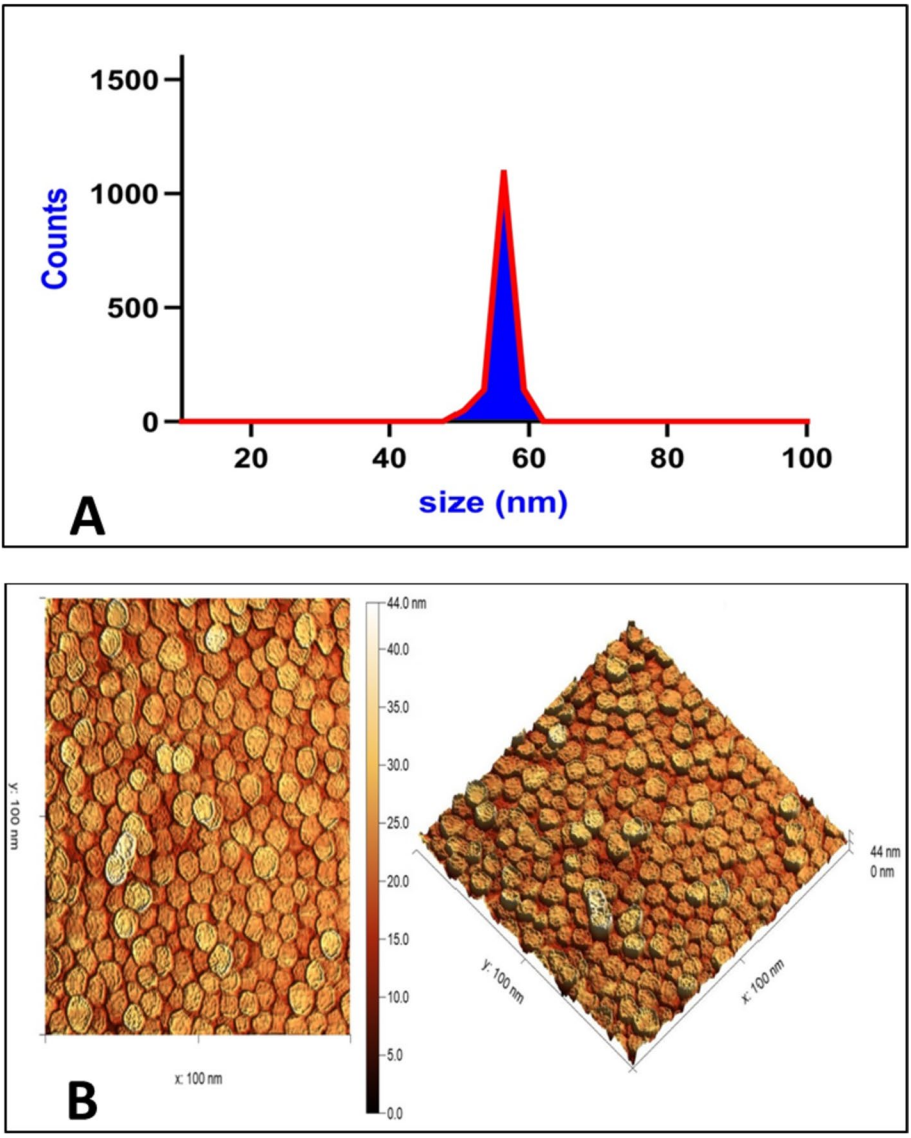

Fig. 1 **A** Zeta potential data and dynamic light scattering (DLS) and showed a regular size (one peak) of 60 nm. **B** MNG appeared as a greater colloidal structure in aqueous suspension

Results of the acute toxicity experiment (MNG-96-h LC_{50} assays)

As illustrated in Fig. 2, fish exposed to different levels of MNG exhibited significant elevation in mortality rates in a concentration-reliant pattern, in comparison to the control group that recorded a 0% mortality rate. *C. gariepinus* exposed to the lower concentrations of MNG (15, 20, 25, 30, 35, and 40) for 96 h showed normal swimming movement and active response to escape reflex and other external stimuli. Nevertheless, fish exposed to higher

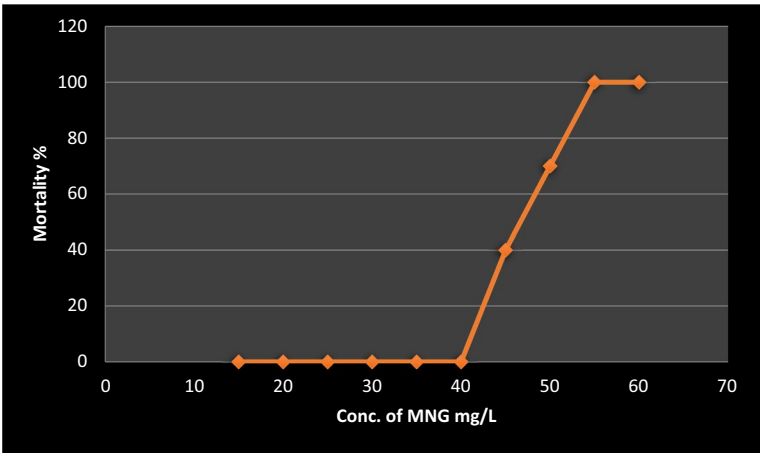

Fig. 2 The percentage of *C. gariepinus* mortalities relative to various MNG concentrations during the acute 96-h toxicity experiment

concentrations of MNG for the same experimental period (96 h) were lethargic, anorexic, sluggish swimming movement, and weak response to the external stimuli. Moreover, hemorrhages and skin erosions were also evident. The mean 96-h LC_{50} MNG value for *C. gariepinus* was computed as 44 mg/L.

Results of sub-acute toxicity experiment and the recovery trial

Behavioral, clinical alterations, and post-mortem findings

Following the 10-day sub-acute exposure to different concentrations of MNG, the reaction to external stimuli was markedly and moderately decreased in fish subjected to 1/5 and 1/8 LC_{50} concentrations of MNG, respectively. However, *C. gariepinus* exposed to 1/10 LC_{50} MNG strongly reacted to external stimuli. Throughout the experimental duration, the exposed fish showed multiple clinical symptoms. Herein, the major detectable lesions were dark skin coloration, fin rot, and superficial hemorrhage at the base of the fins and ventral part of the abdomen (Table 1). The severity of the aforementioned signs was concentration related, where fish belonging to the 1/5 LC_{50} and 1/8 LC_{50} groups possessed the most announced signs, followed by those of the 1/10 LC_{50} group. Necropsy of freshly dead fish revealed congestion and enlargement of the liver and spleen, in addition to distention of the gall bladder.

Oxidative stress mediators

Concerning the effect of the two investigational trials on the serum cortisol level, the results indicated that after the sub-acute exposure experiment, *C. gariepinus* showed a significant enhancement in the activity of oxidative stress mediators (CAT, SOD, and GST) in comparison to their activity after the 10-day recovery trial ($P < 0.01$). Regarding the effect of the two investigational periods, the ascending increase in MNG concentration resulted in a remarkable elevation in the level of CAT, SOD, and GST,

Table 1 Survivability percentage, clinical signs, and post-mortem lesions in *C. gariepinus* following a 10-day period of sub-acute exposure to various concentrations of 96-h LC_5 of MNG

Items	Experimental groups			
		1/5 LC50	1/8 LC50	1/10 LC50
No. of survived fish		30/45	34/45	36/45
Survival percentage		66.66%	75.55%	80%
Sluggish swimming	Number	3/30	3/34	2/36
	Score	++	++	+
Response to escape reflex	Number	2/30	20/34	33/36
	Score	+	++	+++
Hemorrhage	Number	12/30	5/34	3/36
	Score	++	++	+
Fin rot	Number	4/30	3/34	2/36
	Score	++	+	+
Skin erosions	Number	4/30	3/34	0/36
	Score	+	+	−
Postmortem alterations	Number	13/15	5/11	3/9
	Score	+++	++	+

The score of symptoms was recorded as follows: (−) no, (+) weak, (++) moderate, (+++) severe

in a concentration-associated pattern ($P < 0.01$). The interaction between the effect of the investigational periods and MNG concentration yielded a significant increase in the serum levels of CAT, SOD, and GST. Such elevation was obvious especially following the sub-acute exposure to LC_{50}, 1/8 LC_{50}, followed by 1/10 LC_{50}, then the control group. Interestingly, following the 10-day recovery trial, the 1/10 LC_{50} group's level of these parameters displayed a remarkable improvement with a non-significant variation ($P > 0.01$) when compared with the control group. On the other hand, the hepatic levels of the antioxidant indicators revealed significantly boosted values in the 1/5 LC_{50} and 1/8 LC_{50} groups, in comparison to the 1/10 LC_{50} group and the control group ($P < 0.01$) (Table 2).

Stress-related corticosteroid hormone

The sub-acute exposure to MNG induced a marked rise in the cortisol level compared to its levels following the subsequent depuration experiment ($P < 0.01$). With regard to the effect of MNG concentration, the cortisol level was highly raised in a concentration-dependent manner ($P < 0.01$). The interaction between the two experimental periods and MNG concentration resulted in a significant reduction in the serum cortisol level in the 1/10 LC_{50} group compared with the 1/5 LC_{50} and 1/8 LC_{50} groups following the sub-acute exposure trial and the recovery trial ($P < 0.01$). Post the 10-day recovery period, the cortisol level was not significantly varied between the 1/10 LC_{50} group and the control one ($P > 0.05$) (Table 2). Additionally, the 1/5 LC_{50} and 1/8 LC_{50} groups maintained higher serum cortisol levels than the control group even after the 10-day recovery period.

Table 2 The effect of the investigational trials (sub-acute exposure and recovery trial), MNG concentration, and their interaction on oxidative stress mediators, plus the cortisol level in *C. gariepinus*

		CAT (U/g)	SOD (U/g)	GST (ng/mg)	Cortisol
Investigational trials effect					
Sub-acute exposure trial		53.25 ± 5.144^{a}	105.5 ± 3.821^{a}	33.43 ± 4.161^{a}	42.65 ± 4.813^{a}
Recovery trial		46.50 ± 6.058^{b}	98.83 ± 2.510^{b}	14.65 ± 1.436^{b}	22.11 ± 1.780^{b}
MNG concentration effect					
Control		26.00 ± 0.632^{d}	90.50 ± 0.428^{d}	10.00 ± 0.224^{d}	16.68 ± 0.230^{d}
1/5 LC_{50}		69.00 ± 0.365^{a}	115.2 ± 2.212^{a}	32.83 ± 5.223^{a}	44.30 ± 6.434^{a}
1/8 LC_{50}		64.33 ± 0.494^{b}	109.3 ± 2.871^{b}	29.02 ± 5.299^{b}	38.92 ± 5.992^{b}
1/10 LC_{50}		40.17 ± 6.199^{c}	93.67 ± 1.333^{c}	24.31 ± 6.366^{c}	29.61 ± 5.923^{c}
Interaction					
Sub-acute exposure trial	Control	25.33 ± 1.202^{d}	90.00 ± 0.577^{f}	9.833 ± 0.441^{f}	16.92 ± 0.098^{f}
	1/5 LC_{50}	69.00 ± 0.577^{a}	120.0 ± 0.577^{a}	44.50 ± 0.289^{a}	58.63 ± 1.190^{a}
	1/8 LC_{50}	64.67 ± 0.882^{b}	115.7 ± 0.882^{b}	40.85 ± 0.454^{b}	52.26 ± 1.308^{b}
	1/10 LC_{50}	54.00 ± 0.577^{c}	96.33 ± 0.882^{e}	38.54 ± 0.344^{c}	42.78 ± 1.336^{c}
Recovery trial	Control	26.67 ± 0.333^{d}	91.00 ± 0.577^{f}	10.17 ± 0.167^{f}	16.43 ± 0.440^{f}
	1/5 LC_{50}	69.00 ± 0.577^{a}	110.3 ± 0.882^{c}	21.17 ± 0.441^{d}	29.96 ± 0.317^{d}
	1/8 LC_{50}	64.00 ± 0.577^{b}	103.0 ± 0.577^{d}	17.18 ± 0.428^{e}	25.59 ± 0.221^{e}
	1/10 LC_{50}	26.33 ± 0.667^{d}	91.00 ± 1.000^{f}	10.08 ± 0.189^{f}	16.44 ± 0.522^{f}
Two-way ANOVA *P*-value					
Investigational trial effect		< 0.001	< 0.001	< 0.001	< 0.001
MNG concentration		< 0.001	< 0.001	< 0.001	< 0.001
Interaction		< 0.001	< 0.001	< 0.001	< 0.001

MNG magnetite nanogel, *CAT* catalase, *SOD* superoxide dismutase, *GST* glutathione S-transferase

[a–f]Means in the same column carrying different superscripts are significantly different ($P \leq 0.05$)

Hepato-renal injury indices

The serum levels of ALT, AST, urea, and creatinine revealed a remarkable rise ($P < 0.01$), following the 10-day exposure trial compared to their levels after the subsequent recovery trial (Table 3). Regarding the investigational trial effect, a concentration-dependent elevation in the levels of the hepato-renal indices was detected concurrently with increasing concentration of MNG surpassing the control group ($P < 0.01$). The interaction between the investigational trial and MNG concentration showed that AST, ALT, urea, and creatinine levels were significantly increased in fish exposed to various concentrations of MNG respective to their values in the control group ($P < 0.05$). In response to the recovery trial, the serum values of AST, urea, and creatinine exhibited a non-significant difference ($P > 0.05$) between fish exposed to 1/10 LC_{50} and the control group. In contrast, the ALT level possessed a significant rise in this group relative to the control one ($P < 0.05$). Furthermore, the level of hepato-renal damage indices was still higher in the 1/5 and 1/8 LC_{50} groups than in the control group ($P < 0.05$) and the 1/10 LC_{50} group.

Table 3 The effect of the investigational trials (sub-acute exposure and recovery trial), MNG concentration, and their interaction on the hepato-renal injury indices, plus the AChE level of *C. gariepinus*

		AST (U/L)	ALT (U/L)	Urea (mg/dL)	Creatinine mg/dL	AChE (pg/mg)
Investigational trial effect						
Sub-acute exposure trial		52.00 ± 3.360^{a}	24.81 ± 3.235^{a}	13.76 ± 2.464^{a}	0.707 ± 0.078^{a}	0.316 ± 0.017^{b}
Recovery trial		40.58 ± 1.948^{b}	17.50 ± 1.881^{b}	7.961 ± 1.639^{b}	0.436 ± 0.067^{b}	0.382 ± 0.011^{a}
MNG concentration effect						
Control		34.33 ± 0.494^{d}	10.17 ± 0.477^{d}	2.760 ± 0.187^{d}	0.250 ± 0.015^{d}	0.412 ± 0.009^{a}
1/5 LC_{50}		55.50 ± 3.128^{a}	32.30 ± 2.584^{a}	19.17 ± 1.586^{a}	0.822 ± 0.044^{a}	0.295 ± 0.017^{d}
1/8 LC_{50}		52.67 ± 3.480^{b}	25.17 ± 2.798^{b}	15.28 ± 2.348^{b}	0.738 ± 0.076^{b}	0.325 ± 0.021^{c}
1/10 LC_{50}		42.67 ± 3.739^{c}	17.00 ± 1.390^{c}	6.237 ± 1.443^{c}	0.475 ± 0.111^{c}	0.363 ± 0.020^{b}
Interaction						
Sub-acute exposure trial	Control	34.33 ± 0.667^{d}	10.00 ± 0.577^{f}	2.693 ± 0.296^{e}	0.280 ± 0.012^{d}	0.403 ± 0.015^{a}
	1/5 LC_{5}	62.33 ± 1.202^{a}	37.92 ± 0.512^{a}	22.50 ± 1.171^{a}	0.920 ± 0.012^{a}	0.260 ± 0.012^{d}
	1/8 LC_{5}	60.33 ± 1.202^{a}	31.33 ± 0.882^{b}	20.44 ± 0.802^{b}	0.903 ± 0.009^{a}	0.280 ± 0.012^{d}
	1/10 LC_{50}	51.00 ± 0.577^{b}	20.00 ± 0.577^{d}	9.403 ± 0.440^{d}	0.723 ± 0.015^{b}	0.320 ± 0.012^{c}
Recovery trial	Control	34.33 ± 0.882^{d}	10.33 ± 0.882^{f}	2.827 ± 0.288^{e}	0.220 ± 0.012^{e}	0.420 ± 0.012^{a}
	1/5 LC_{5}	48.67 ± 0.882^{b}	26.67 ± 1.202^{c}	15.84 ± 0.303^{c}	0.723 ± 0.009^{b}	0.330 ± 0.012^{c}
	1/8 LC_{5}	45.00 ± 0.577^{c}	19.00 ± 0.577^{d}	10.11 ± 0.485^{d}	0.573 ± 0.035^{c}	0.370 ± 0.006^{b}
	1/10 LC_{50}	34.33 ± 0.333^{d}	14.00 ± 0.577^{e}	3.070 ± 0.443^{e}	0.227 ± 0.012^{e}	0.407 ± 0.009^{a}
Two-way ANOVA *P*-value						
Investigational trial effect		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
MNG concentration		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Interaction		< 0.001	< 0.001	< 0.001	< 0.001	0.016

MNG magnetite nanogel, *AST* aspartate aminotransferases, *ALT* alanine aminotransferase, *AChE* acetylcholinesterase

[a–f]Means in the same column carrying different superscripts are significantly different ($P \leq 0.05$)

Acetylcholinesterase (AChE) level

Considering the MNG concentration, the serum values of the neurotransmitter, AChE, were markedly declined in all groups following the sub-acute exposure period compared to its level post-recovery ($P < 0.01$). With regard to the effect of the investigational trial, the level of AChE exhibited a significant concentration-related reduction in MNG-exposed fish compared to the control group ($P < 0.01$). Moreover, the interaction between the investigational trials and MNG concentration indicated that the sub-acute exposure of *C. gariepinus* to higher concentrations of MNG (1/5 and 1/8 LC_{50}) resulted in a significant depletion in AChE activity compared to the control group. But, this effect was recorded in fish exposed to 1/10 LC_{50} to a lesser extent. Nevertheless, after the recovery trial, the serum values of AChE were not significantly varied between the 1/10 LC_{50} group and the control group ($P > 0.05$). The level of AChE was higher in both the 1/5 LC_{50} and 1/8 LC_{50} groups relative to the 1/10 LC_{50} and the control groups ($P < 0.05$) (Table 3).

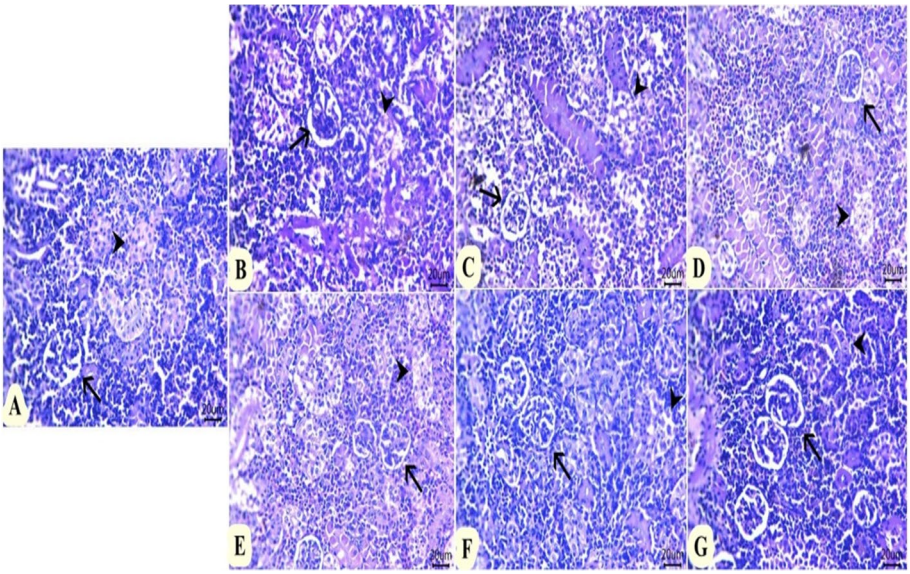

Fig. 3 Representative photomicrograph of H&E stained sections from the kidneys (scale bar 20 µm) showing **A** normal morphology of glomerular corpuscle (arrow), renal tubule (arrowhead), and abundant of interstitial hematopoietic series in control group. **B** Necrosis with pyknotic nuclei in most renal tubular epithelium (arrowhead) accompanied with atrophic glomerular tufts (arrow) in *C. gariepinus* sub-acutely exposed to 1/5 LC_{50} MNG. **C** Moderate number of necrotic renal tubules (arrowhead) with normal morphology of glomeruli (arrow) in *C. gariepinus* sub-acutely exposed to 1/8 LC_{50} MNG. **D** Necrotic changes in mild number of renal tubules (arrowhead) beside normal morphology of glomeruli (arrow) in *C. gariepinus* sub-acutely exposed to 1/10 LC_{50} MNG. **E** Degenerative and necrotic changes in few renal tubular epitheliums (arrowhead) with normal glomerular tufts (arrow) in 1/5 LC_{50} group after recovery trial. **F** Focal dissociation of renal epithelium in some renal tubules (arrowhead) with preserved structures of glomerular tufts (arrow) in in 1/8 LC_{50} group after recovery trial. **G** Prominent improvement in architectures of glomeruli (arrow) and renal tubule (arrowhead) in 1/10 LC_{50} group after recovery trial

Histopathological investigations

Considering the histopathological outcomes of the renal tissue, the control group showed normal morphology of glomerular corpuscles, renal tubules, and abundant interstitial hematopoietic series (Fig. 3A), while fish exposed to 1/5 96-h LC_{50} revealed necrosis with pyknotic nuclei in most renal tubular epithelium accompanied with atrophic glomerular tufts (Fig. 3B). Moreover, moderate number of necrotic renal tubules were seen in fish exposed to 1/8 96-h LC_{50} (Fig. 3C) with normal morphology of glomeruli. But, necrotic changes in mild number of renal tubules besides normal morphology of glomeruli were seen in exposed to 1/10 96-h LC_{50} (Fig. 3D). Ten days after cessation of exposure to MNG, the renal tissue of fish exposed to 1/5 96-h LC_{50} revealed degenerative and necrotic alterations in few renal tubular epitheliums with normal glomerular tufts (Fig. 3E). On the other hand, focal dissociation of renal epithelium in some renal tubules with preserved structures of glomerular tufts was observed in fish exposed to 1/8 96-h LC_{50} (Fig. 3F). Surprisingly, prominent improvement in architectures of glomeruli and renal tubules was observed in fish exposed to 1/10 96-h LC_{50} (Fig. 3G).

Regarding the histopathological findings of the hepatic tissue following the 10-day exposure period to MNG, the control group revealed normal hepatic acini, sinusoids, and central veins (Fig. 4A). As shown in Fig. 4B, fish exposed to 1/5 LC_{50} displayed a large number of vacuolated hepatocytes and congested vasculatures. Fish exposed to 1/8 LC_{50} revealed scattered round cell infiltrates and a moderate number of vacuolated hepatocytes

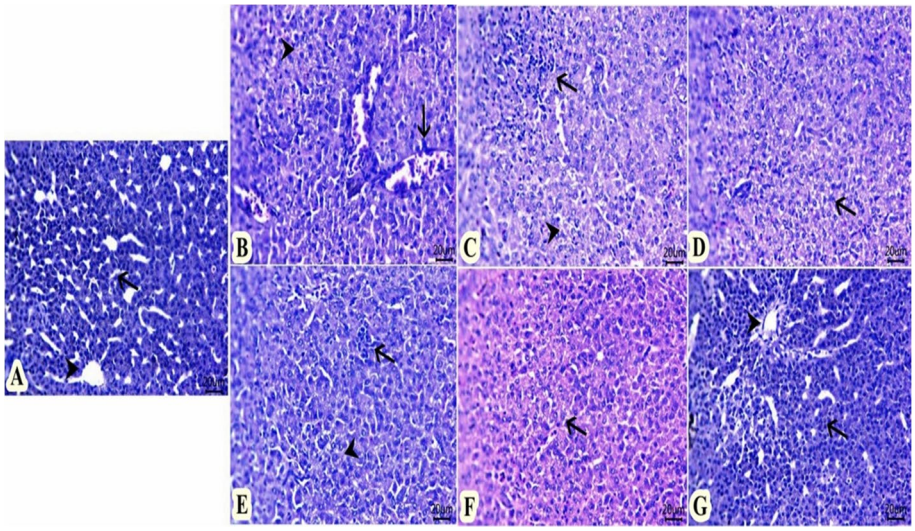

Fig. 4 Representative photomicrograph of H&E stained sections from the liver (scale bar 20 μm) showing **A** normal hepatic acini (arrow), sinusoids, and central vein (arrowhead) in control group. **B** Large number of vacuolated hepatocytes (arrowhead) and congested vasculatures (arrow) in *C. gariepinus* sub-acutely exposed to 1/5 LC_{50} MNG. **C** Scattered round cells infiltrates (arrow) and moderate number of vacuolated hepatocytes (arrowhead) in *C. gariepinus* sub-acutely exposed to 1/8 LC_{50} MNG. **D** Mild degenerative changes in some hepatic cells (arrow) in *C. gariepinus* sub-acutely exposed to 1/10 LC_{50} MNG. **E** Degenerative changes in few hepatocytes (arrowhead) and minute number of round cells aggregates (arrow) in 1/5 LC_{50} group after recovery trial. **F** Mild degenerative changes in few hepatocytes (arrow) in 1/8 LC_{50} group after recovery trial. **G** Improvement of architectures of hepatic acini (arrow) and vasculature tissue (arrowhead) in 1/10 LC_{50} group after recovery trial

(Fig. 4C), whereas mild degenerative changes in some hepatic cells were the most characteristic findings shown by those exposed to 1/10 LC_{50} (Fig. 4D). On the other hand, at the end of the 10-day recovery period, there was still the presence of degenerative changes in few hepatocytes beside minute numbers of round cells aggregates were noticed in fish previously exposed to 1/5 LC_{50} (Fig. 4E). Moreover, as displayed in Fig. 4F, fish exposed to 1/8 LC_{50} exhibited mild degenerative changes in few hepatocytes. However, a remarkable improvement in the architectures of hepatic acini and their vasculatures was evident in fish exposed to 1/8 LC_{50} (Fig. 4G).

Discussion

Nowadays, the field of nanotechnology has played a crucial role in aquaculture practices for effectively utilizing nanoparticles as a dietary supplement (Ahmed et al. 2023), in spite of its reported toxicity (Kakakhel et al. 2023). Exposure of fish to metal oxide nanoparticles can induce malformation of different organs, abnormal behavior, immune dysfunction, genotoxicity, and higher mortalities (Cazenave et al. 2019; Bai and Tang 2020). Therefore, the current report is pioneering approach for the assessment of acute toxicity elicited by MNG via testing various concentrations and studying its influence on the stress response, hepato-renal indices, antioxidant mechanism, and histopathological changes in hepato-renal organs.

Considering clinical signs and mortality records, the present study monitored the mortalities throughout the experimental period and revealed the occurrence of mortalities with increasing the concentration of MNG. In addition, fish demonstrated clinical manifestations including dark coloration of the skin, severe fin rot, and some hemorrhages in the skin. Internally, the examined fish revealed congestion and enlargement of the liver and distention of the gall bladder, especially at higher doses of MNG. The severity of mortalities and clinical signs was associated with augmenting the dose of MNG implying MNG toxicity at higher doses (1/5 and 1/8 of LC_{50}), while the exposure to 1/10 LC_{50} demonstrated the lowest death rate and clinical signs. The signs of toxicity and severity of mortalities provoked by magnetite nanocomposite depend on the period of exposure and its concentration as reported by Zhu et al. (2012). Likewise, a recent study by Guillén et al. (2022) supported our findings and revealed that the exposure of zebrafish to a high concentration (1000 µg/mL) of magnetite-based nanocomposites induced morphological alterations. In line with an earlier study, Zhu et al. (2012) mentioned the occurrence of ulcerations, pericardial edema, and spinal curvature post-acute exposure of zebrafish embryos to magnetite nanocomposites for 168 h.

Assessment of oxidative stress is crucial to reflect the adverse effect of nanoparticles in catfish which resulted in a reduction of antioxidant enzymes including CAT, GST, and SOD (Iheanacho and Odo 2020; Iheanacho et al. 2021). Oxidative stress is raised because of an imbalance between the antioxidant resistance of the host and the release of ROS after exposure to stressors including nanomaterials (Song et al. 2023; Alzahrani et al. 2022; El-Houseiny et al. 2023). The enzymatic antioxidant defense mechanism is responsible for mitigating the production of ROS involving CAT, SOD, and GST enzymes and antagonizing the toxicity in *C. gariepinus* (El-Houseiny et al. 2023). To tackle the oxidative damage induced by MNG on the antioxidant defense system, the present perspective monitored different concentrations of acute exposure to MNG. Additionally, it determined their impact on the antioxidant enzymes (SOD, CAT, and GST) and established a fact of the existence of oxidative disruption

indicated by an increase in the antioxidant biomarkers in a dose-based manner. Astonishingly, after a 10-day recovery period, the 1/10 LC_{50} group's level of these indicators exhibited a clear improvement reflecting a potent antioxidant activity of MNG at lower concentration. It is opined that MNG has cytotoxic effects via the occurrence of inflammatory responses and oxidative damage or could be returned to the ability of magnetite nanocomposites to suppress the Na+/K+-ATPase activity in a concentration-reliant array as clarified by Suganya et al. (2018). Similar report by Kaloyianni et al. (2020) was conducted on zebrafish, *Danio rerio*, and revealed the occurrence of oxidative stress in the gills and liver indicated by elevation of lipid peroxidation and protein oxidation.

Valuation of acute toxicity of freshwater fish to various nanoparticles is crucial to evaluate the accurate safe dose for application, and in turn, avoid the occurrence of stress condition and hepato-renal dysfunction (Rashidian et al. 2021). Cortisol is a vital stress indicator in fish and is a precise marker for evaluation of acute stress (Sadoul and Geffroy 2019). Herein, we reveal elevation in cortisol, liver enzymes (ALT, AST), and kidney biomarkers (urea, creatinine). Such elevations were concurrent with the increased concentration of MNG representing a persuasive stress response and hepato-renal dysfunction. Nonetheless, the serum values of AST, urea, and creatinine exhibited a non-significant difference ($P > 0.05$) in the exposed group to the 1/10 LC_{50} group and the control implying the MNG safety at a lower dose. These findings were synchronized with a recent study by Rahman et al. (2022), who reported the existence of stress status symbolized by an elevation in the cortisol upon exposure to the acute toxicity by silica nanoparticles in *C. gariepinus*.

Acetylcholinesterase (AChE) has an essential role in the nervous system representing in breaking down acetylcholine into acetic acid and choline (Ibrahim et al. 2022; Kim and Kang 2015). Consequently, AChE conserves correct levels of acetylcholine and inhibits AChE resulting in accretion of acetylcholine at the synaptic junctions, indicating neurotoxicity (Kais et al. 2015). In the present investigation, it was perceived that the exposure of *C. gariepinus* to MNG lessened the brain biomarkers (AChE) in a dose-dependent pattern, implying neurological dysfunction and oxidative damage. On the other hand, an obvious restoration of the level of this neurotransmitter activity was evident after the 10-day depuration period, especially at a lower dose of exposure to MNG (1/10 LC_{50}). It is assumed that the MNG can translocate straight away from the olfactory nerve to reach the brain inducing neurotoxic impacts as previously described by Wu et al. (2013). Likewise, Yousef et al. (2019) revealed that the exposure to magnetite nanocomposite resulted in neurotoxicity and inflammatory response in rats, indicated by a reduction in the AChE.

Regarding the ecotoxicological effect of MNG on the architecture of hepato-renal organs, we reported the existence of pathological changes in the liver and kidneys especially in the exposed groups to higher concentrations of MNG (1/5 and 1/8 of LC_{50}). Surprisingly, a noticeable regeneration in the renal architectures was observed in the fish exposed to 1/10 96-h LC_{50} implying the safety of MNG when applied at low concentration. Concurrent with previous reports, Qualhato et al. (2018) recorded the occurrence of hepatic alterations in guppy (*Poecilia reticulata*) throughout 3 weeks of exposure to magnetite nanosized particles. Moreover, Rezende et al. (2018) noted histopathological alterations in *Oreochromis niloticus* upon exposure to titanium dioxide nanoparticles.

Conclusion

Overall, the sub-acute toxicity with MNG induces adverse impacts, only at higher doses, 1/5 LC_{50} and 1/8 LC_{50}. The ethological alteration, hepato-renal dysfunction, oxidative stress, neurological disorders, and histopathological changes represented the major negative impacts provoked by MNG toxicity in *C. gariepinus*. Nevertheless, the lowest applied concentration of MNG, 1/10 of LC_{50}, was less toxic, so it could be safely applied in aquaculture practices with only minor disruptions in the bio-indicators. Future studies are required to test the influence of MNG on the immune system and gene expression.

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Data availability The datasets generated or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Declarations

Ethics approval All experimental procedures with live fish were approved by the animal welfare and ethical review committee of Faculty of Veterinary Medicine, Zagazig University, Egypt (ZU-IACUC/2/F/435/2023). All experimental procedures were directed in compliance with the ethical guidelines agreed by the National Institutes of Health for Use and Treatment of Laboratory Animals.

Informed consent Not applicable.

Conflict of interest The authors declare no conflict of interest.

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