Original Research

Gallic acid and omega-3 fatty acids decrease inflammatory and oxidative stress in manganese-treated rats

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Impact statement

Humans and animals are regularly exposed to toxic chemicals with subsequent adverse effects. Manganese exposure occurs via contaminated sources; over-exposure is associated with neuronal, hepatorenal dysfunction, etc. This work advances the field of natural chemopreventive agents by reporting evidence lacking in the literature on GA and ω-3-FA obtained primarily from the diet in protecting biological beings against toxic chemicals. Individually, GA and ω-3-FA exhibit various pharmacological effects. Our findings confirm the previous reports; however, we demonstrate the additional evidence for GA and ω-3-FA in abating toxic response incumbent on oxidative damage associated with manganese exposure. These findings further underscore the relevance of GA usage in food, cosmetics–pharmaceutical industries, and ω-3-FA as a safe supplement. Dietary supplements with GA and fish oil-rich in ω-3FA may be the potential natural therapy against hepatorenal injury in individuals inadvertently or occupationally exposed to manganese, thereby, promoting human and veterinary health outcomes.

Abstract

Evidence abounds—epidemiological and experimental—linking overexposure to manganese with hepatic and renal dysfunction. We investigated the beneficial effects of gallic acid or omega-3 fatty acids (ω-3-FA) on hepatorenal function in rats exposed to manganese (Mn). Rats were exposed to manganese (15 mg/kg) only or in the presence of ω-3-FA (30 mg/kg) or gallic acid (20 mg/kg) continuously for 14 days. Gallic acid or ω-3-FA co-treatment significantly ($P < 0.05$) suppressed manganese-mediated increases in the biomarkers of hepatorenal toxicity. Furthermore, gallic acid or ω-3-FA relieved manganese-induced oxidative stress, lipid peroxidation, and glutathione depletion.In addition to decreases in nitric oxide, interleukin-1β, tumor necrosis factor-α levels, and myeloperoxidase concentration in treated rats, biochemical data on hepatorenal protection were buttressed by our histological findings. Gallic acid or ω-3-FA ameliorated manganese-induced hepatorenal toxicity by reducing the oxidative/inflammatory stress and preserved tissue integrity in rats.

Keywords: Manganese, gallic acid, omega-3 fatty acids, hepatorenal toxicity, oxido-inflammation, rats

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Introduction

Toxicity resulting from undue exposure to metals is a significant concern to the general public and researchers globally.[1] Manganese (Mn) exists naturally in the soil, water, air, and food.[2].However, environmental pollution by Mn has been related to certain anthropogenic activities such as agriculture, mining, and welding.[3] Adverse health effects of Mn reportedly occur via the consumption of contaminated food, water, and air. Epidemiological data confirmed that over-exposure to Mn is correlated with neuronal, hepatic, renal, and reproductive dysfunction.[4,5] Previous human studies on the influence of excessive exposure to Mn on renal impairment showed tubular impairment and glomerular injury characterized by increases in *N*-acetyl-β-D-glucosaminidase activity and microalbuminuria in Mn-exposed children.[6] A vital relationship between Mn exposure renal dysfunction in children has been reported.[7] Experimental studies using rodents also

demonstrated that Mn-mediated hepatorenal damage in rodent model is associated with induction of oxidative stress and initiation of apoptosis.[8,9] Therefore, viable chemotherapeutic or chemo-preventive approaches to alleviate the harmful effects of Mn in the exposed populace are warranted.

ω-3-FA is a polyunsaturated acid, which is mainly obtained from the diet because they cannot be synthesized in adequate quantities by the body. Previous investigations demonstrated that ω-3-FA is well-known to elicit beneficial health effects on several diseases associated with metabolism, cardiovascular, and inflammation.[10] Numerous clinical evidence revealed that the administration of ω-3-FA via fish oil supplements is safe and exhibited anti-inflammatory and antioxidant effects by suppressing proinflammatory cytokines and oxidative stress.[11–13] The phenolic GA (3,4,5-trihydroxy benzoic acid) is of plant origin with several pharmacological activities. Gallic acid is well documented to elicit antioxidant, anti-carcinogenic, anti-microbial, anti-inflammatory effects.[14] Industrially, owing to its antioxidant activity, GA is used as a food additive to prevent oxidation and rancidity of oils and fats. GA is reportedly used in several therapeutic and pharmaceutical applications due to its potent and diverse mechanisms of action.[15,16]

There is a shortage of scientific reports in the literature for either GA or ω-3-FA protective effect on hepatic and renal injury associated with Mn exposure. Here we probed, originally, the beneficial effects of GA or ω-3-FA on Mn-induced hepatic and renal dysfunction in Wistar rats. We evaluated liver and kidney antioxidant status, oxidative, and inflammatory stress indices together with histological analyses to realize this aim. Furthermore, we delineate the modulatory mechanisms of GA or ω-3-FA on hepatorenal toxicity in rats intoxicated with Mn.

Materials and methods

Chemicals

The following reagents and chemicals used for these experiments were procured mainly from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA): Manganese chloride ($\geq$99.9%), gallic acid ($\geq$95%), trichloroacetic acid (TCA), epinephrine, 1-chloro-2 4-dinitrobenzene (CDNB), glutathione, 5′,5′-dithio-bis-2-nitrobenzoic acid (DNTB), and thiobarbituric acid. Spectrophotometric Plate reader compactible enzyme-linked immunosorbent assay (ELISA) 96-well plate kits used in the assessment of interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α) levels (E-labscience Biotechnology Beijing, China), and other reagents of analytic purity were procured from the BDH Chemicals (Dorset, U.K).

Experimental model and animal husbandry

The Wistar rats (adult; sex: male) utilized in this study were sourced from Laboratory Experimental Animal Colony, Veterinary Medicine Department, University of Ibadan, Nigeria. Experimental rats were safely quarantined in polycarbonate cages located in an amply aired rodent experimental house and maintained under light/dark 12-h cycle. Rats were feed and adequately supplied with rodent chow, had unrestricted access to clean drinking water, and were allowed to acclimatize for one week before the experiment started. The experimental animal was satisfactorily cared for and humanely treated. Experimental procedures were performed following accepted recommendations established by the University of Ibadan Laboratory Animal Committee on the Ethical Use of Experimental Laboratory Animals and the National Institute of Health published Guidelines for the Care and Use of Laboratory Animals.

Design of experiment

The experimental rats were randomly distributed into six groups ($n = 10$ each), and all animals were treated by gavage for 14 consecutive days as described below:

- **Group I (Control):** corn oil alone (2 mL/kg body weight).
- **Group II (ω-3 FA alone):** ω-3 FA alone (20 mg/kg body weight).
- **Group III (GA alone):** GA alone (30 mg/kg).
- **Group IV (Mn alone):** Mn (15 mg/kg)
- **Group V (Mn + ω-3 FA):** co-treated with Mn + ω-3 FA (15 and 20 mg/kg, respectively).
- **Group V (Mn + GA):** co-treated with Mn + GA (15 and 30 mg/kg, respectively).

Stock solutions (100 mg/mL) of GA and ω-3 FA were freshly prepared every daily for treating experimental rats. Mn: 15 mg/kg; ω-3 FA:20 mg/kg; and GA:30 mg/kg, doses used for this study were based on our pilot studies and data that were previously published.[17–19]

On day 15, after the last treatments were administered, the experimental rat weights were obtained, and blood samples collected from retro-orbital venous plexus into both uncoated and ethylenediaminetetraacetic acid (EDTA)-coated sample bottles before animal were sacrificed through cervical dislocation. Subsequently, the blood samples were allowed to clot and centrifuged (3000*g*; 10 min) to get the serum. The liver and kidney were rapidly extracted, weighed, and treated for biochemical and histological analyses.

Evaluation of antioxidant status of hepato-renal tissues post experimentation

Separately, the liver and kidney sections in Tris–HCl buffer (50 mM; pH:7.4) were homogenized to obtain the homogenates which were subsequently subjected to cold centrifugation (12,000*g*; 15 min at 4°C). The tissue supernatant obtained post centrifugation was then utilized for further biochemical analyses. The Bradford method was employed for the determination of protein concentration.[20] Assessment of lipid peroxidation was performed by quantifying malondialdehyde (MDA) levels according to the recognized method of Farombi *et al.*[21] and the values were expressed as μmole MDA equivalents/mg protein.

Quantification of endogenous activity of superoxide dismutase (SOD) was performed by Misra and Fridovich technique[22] and the values were expressed in nmoles epinephrine oxidized/min/mg protein. Concentration of catalase (CAT) was calculated following Clairborne's procedure[23] and the values were expressed in μmole H_2O_2 consumed/min/mg protein. Level of glutathione (GSH) was estimated following accepted protocol[24] and the values were expressed as units/mg protein. Glutathione peroxidase (GPx) activity was determined by the approved method[25] and the values were expressed in μmole of residual GSH/mg protein. Glutathione-S-transferase (GST) activity was measured using the standard method[26] and the values were expressed in μmole CDNB–GSH complex formed/min/mg protein. Assessment of CAT and SOD activities was performed utilizing a UV-VIS Spectrophotometer (752S), whereas other assays were performed using a Molecular Devices M3 SpectraMax-384 plate reader (San Jose, CA, USA).

Determination of levels of RONS – Reactive oxygen and nitrogen species

Determination of hepatorenal RONS levels were performed following recognized procedure.[27,28] Briefly, the hepatic or renal homogenate (10 μL) was reacted with 2′, 7′-dichlorodihydrofluorescin diacetate (DCFH-DA (5 μL) in a reaction environment containing phosphate buffer (0.1 M; pH 7.4; (150 μL) and distilled water (35 μL). The oxidation of DCFH-DA to DCF by RONS in the samples was probed at the following wavelengths: excitation (488 nm) and emission (525 nm) for 10 min; at an interval of 30 s using a 96-well-format SpectraMax-M3^{TM} plate reader. Production of DCF under these conditions is depicted as a percentage (%) folds over control.

Inflammatory biomarkers determination

MPO—myeloperoxidase—activity in (Units/mg protein) was determined using the standard procedure of Granell *et al.*,[29] as previously expounded by Trush, while the levels of nitric oxide (NO: μmol nitrite/mg protein) were estimated following the established protocol.[30] ELISA-based assessment of TNF-α (pg/mg protein) and IL-1β (pg/mg protein) levels was performed and readout with the multimodal SpectraMax-M3^{TM} plate reader following the manufacturer's manual.

Analysis of hematological parameters

Whole blood samples obtained using EDTA-coated sample tubes were analyzed by the automated hematology analyzer (Sysmex XK-21, Kobe, Japan). Packed cell volume (PCV), eosinophils, neutrophils, monocytes, and lymphocytes were expressed in percentages (%), while white blood cells (WBCs) were expressed as counts/1000 cells.

Light microscopic analysis

Sectioned samples of the liver and kidney excised from experimental animals were fixed in formalin (10% phosphate-buffered) for three consecutive days and prepared for histological examination using the established protocol,[31] and scored following the OECD standard degree of lesion: None (0); mild (1); mild-moderate (2); moderate (3); moderate to severe (4); and severe (5) system.[32,33] Hematoxylin and eosin (H&E) dye was used to stain the representative tissue (liver and kidney) slides under appropriate conditions. Subsequently, the slides were coded before examination by a pathologist utilizing a Leica DM light microscopic (Wetzler, Germany). Representing images of coded-slides were taken using Leica ICC50E digital camera (Wetzler, Germany).

Statistical analysis

Experimental data were analyzed by ANOVA—one-way analysis of variance—and Bonferroni's *post hoc* test with the aid of GraphPad PRISM 8 software (San Diego, CA, USA); www.graphpad.com. $P < 0.05$ was considered statistically significant.

Results

GA and ω-3-FA improved final body weight gain and relative organ weights in rats treated with Mn

GA and ω-3-FA alone caused no significant ($P < 0.05$) treatment-related changes, while treatment with Mn alone decreased body weight gain (Table 1). Rats co-treated with Mn and either GA and ω-3-FA showed improvement in body weight gain and corresponding reduction in organo-somatic weight compared to Mn alone treated rats.

Table 1. Effect of gallic acid and ω3-FA on the body weight gain and relative organ weight in manganese-treated rats for 14 consecutive days.

	Control	ω3-FA alone	GA alone	Mn only	Mn + ω3-FA	Mn + GA
Final body weight (g)	193.50 ± 16.04	188.88 ± 11.79[a]	193.63 ± 26.48	184.20 ± 19.19	197.00 ± 11.73[b]	186.70 ± 21.87[b]
Initial body weight (g)	171.63 ± 13.45	166.50 ± 15.78	171.13 ± 25.11	182.4 ± 17.21	177.00 ± 16.57	167.90 ± 17.50
Weight gain (g)	21.88 ± 1.26	22.38 ± 1.08	22.50 ± 1.25	1.80 ± 1.14[a]	20.00 ± 1.07[b]	18.80 ± 1.02[b]
Liver (g)	6.46 ± 0.21	6.63 ± 0.15	6.13 ± 0.47	7.67 ± 0.06[a]	6.78 ± 0.46[b]	6.35 ± 0.24[b]
Kidney (g)	1.03 ± 0.04	0.97 ± 0.05[a]	0.98 ± 0.05	1.20 ± 0.08[a]	1.04 ± 0.07[b]	1.00 ± 0.09[b]
RLW (%)	3.34± 0.04	3.51± 0.06	3.17± 0.09	4.16± 0.12[a]	3.44± 0.11[b]	3.40 ± 0.07[b]
RKW (%)	0.53± 0.14	0.51± 0.03	0.51± 0.04	0.65± 0.17[a]	0.53± 0.05[b]	0.54± 0.04[b]

Note: Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean ± SD; $n = 10$ rats.
[a]$P < 0.05$ versus Control.
[b]$P < 0.05$ versus Mn alone.

GA and ω-3-FA protected against depletion of antioxidants in the hepato-renal system of rats treated with Mn

Hepatic and renal antioxidant enzymes activities of experimental rats are presented in Table 2. CAT, SOD, GPx, and GST activities were not significantly ($P < 0.05$) different from the control and in rats treated with GA and ω-3-FA only. Administration of Mn only to rats decreased ($P < 0.05$) hepatic and renal SOD, CAT, GST, and GPx activities when matched with control. However, co-administration of GA or ω-3-FA increases ($P < 0.05$) the antioxidant enzyme activities to standard levels in the treated rats when compared with the Mn alone rats.

GA and ω-3-FA reduced biomarkers of hepato-renal injury in Mn-treated rats

Figure 1 shows the modulatory effects of GA and ω-3-FA co-treatment on marker enzymes of liver function and biomarkers of kidney injury in Mn-treated rats. Exposure of rats to Mn only markedly increased the serum levels of renal toxicity indices, specifically creatinine and urea, as well as biomarkers of hepatic injury, namely AST, ALP, ALT, GGT, and LDH were increased ($P < 0.05$) in Mn-treated rats in comparison with control. Nevertheless, co-treatment of GA and ω-3-FA decreased ($P < 0.05$) these biomarkers of hepato-renal injury in the serum of treated rats.

Table 2. Effects of gallic acid and ω3-FA on hepatic and renal antioxidant enzymes activities in manganese-treated rats for 14 consecutive days.

	Control	ω3-FA only	GA only	Mn only	Mn + ω3-FA	Mn + GA
SOD						
Liver	1.32 ± 0.03	1.33 ± 0.01	1.46 ± 0.06^a	0.71 ± 0.05^a	1.21 ± 0.06^b	1.31 ± 0.09^b
Kidney	1.73 ± 0.07	1.83 ± 0.04^a	1.86 ± 0.06^a	1.07 ± 0.05^a	1.58 ± 0.05^b	1.68 ± 0.06^b
CAT						
Liver	7.52 ± 0.62	8.91 ± 0.76^a	8.41 ± 0.19^a	3.09 ± 0.38^a	5.24 ± 0.72^b	5.44 ± 0.64^b
Kidney	11.62 ± 0.25	12.48 ± 0.39	14.25 ± 0.77^a	6.41 ± 0.80^a	9.08 ± 0.29^b	11.16 ± 0.52^b
GST						
Liver	0.68 ± 0.03	0.72 ± 0.04^a	0.77 ± 0.02^a	0.24 ± 0.07^a	0.57 ± 0.01^b	0.63 ± 0.04^b
Kidney	0.16 ± 0.03	0.19 ± 0.02	0.21 ± 0.04^a	0.10 ± 0.01^a	0.15 ± 0.02^b	0.16 ± 0.01^b
GPx						
Liver	53.37 ± 3.08	59.39 ± 1.02^a	60.16 ± 2.89^a	45.74 ± 0.91^a	53.18 ± 0.45^b	52.62 ± 2.07^b
Kidney	74.11 ± 1.62	81.29 ± 4.15^a	77.59 ± 0.96	60.51 ± 2.98^a	72.18 ± 8.05^b	69.52 ± 0.76^b
GSH						
Liver	2.01 ± 0.38	2.05 ± 0.08	2.03 ± 0.24	1.28 ± 0.03^a	1.68 ± 0.02^b	1.70 ± 0.02^b
Kidney	2.41 ± 0.15	2.15 ± 0.53	2.38 ± 0.15	1.32 ± 0.04^a	2.11 ± 0.26^b	2.05 ± 0.08^b

Note: SOD activity (nmoles epinephrine oxidized/min/mg protein); CAT activity (μmole H_2O_2 consumed/min/mg protein), GST activity (μmole CDNB–GSH complex formed/min/mg protein), GPx activity (μmole of residual GSH/mg protein), GSH level (Units/mg protein). Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean ± SD; $n = 10$ rats.
[a] $P < 0.05$ versus Control.
[b] $P < 0.05$ versus Mn alone.

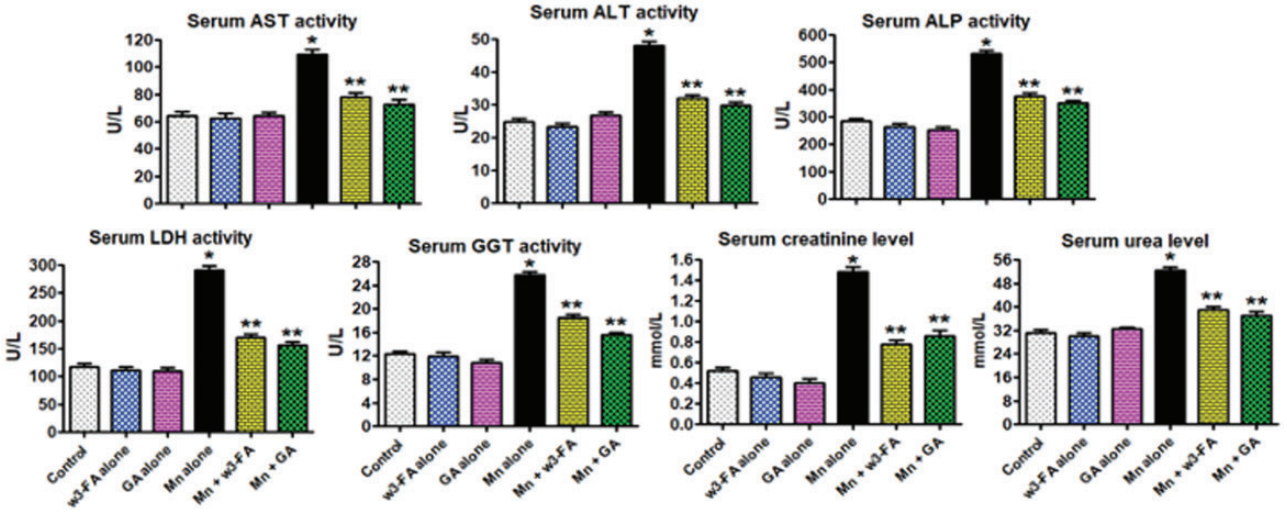

Figure 1. Effect of gallic acid and ω-3 FA on biomarkers of hepatorenal toxicity in rats treated with manganese for 14 successive days. Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean ± SD; $n = 10$ rats. *$P < 0.05$ versus Control; **$P < 0.05$ versus Mn alone. (A color version of this figure is available in the online journal.)

GA and ω-3-FA reduced hepato-renal oxidative stress and inflammatory responses in Mn-treated rats

Figures 2 to 4 show the modulatory effects of GA and ω-3-FA co-treatment on the oxidative stress (RONS and LPO) and inflammatory (NO, IL-1β, MPO, and TNF-α) biomarkers in Mn-treated rats. Alone, GA or ω-3-FA did not alter biomarkers of oxidative stress and inflammation in treated rats compared to the control. But Mn only treatment increased ($P < 0.05$) MPO activity as well as the levels of RONS, LPO, TNF-α, IL-1β, and NO in hepatorenal tissues of treated rats in relation to control. Conversely, co-treatment with GA and ω-3-FA decreased ($P < 0.05$) the oxidative stress and inflammatory biomarkers of treated rats compared with rats treated with Mn only.

GA and ω-3-FA improved hepato-renal lesions in Mn-treated rats

Representative sections of the hepatorenal tissues and frequency of lesion identified semi quantitative from rats are shown in Figure 5 and Table 4, respectively. The liver and kidney histology from control (a) and rats treated with ω-3-FA only (b) and GA only (c) appear normal. Rat liver, treated with Mn only (d) showed severe portal and sinusoidal congestion (red arrows) with severe periportal cellular infiltration and proliferation of periportal connective tissue (black arrows). The kidney lesions (d) in rats exposed to Mn only were characterized with mild interstitial congestion at the renal cortex (red arrows), tubular degeneration, necrosis, and pink staining material in their lumen (black arrows). The hepatic and renal lesions were partially ameliorated in rats co-treated with Mn and ω-3-FA (E), whereas the tissues in rats co-administered with Mn and GA (F) appeared structurally and functionally normal.

GA and ω-3-FA ameliorated Mn-induced changes in hematological parameters

Table 3 shows the modulatory effects of GA and ω-3-FA co-treatment on the PCV, WBC, and differential leukocyte count, namely monocytes, eosinophils, lymphocytes, and neutrophils in Mn-treated rats. PCV, WBC levels, and differential leukocyte count in rats treated with GA or ω-3-FA only were not altered ($P < 0.05$) from the control. Exposure of Mn only caused a decrease ($P < 0.05$) in the PCV level but increased WBC and differential leukocyte count in rats compared with t control group. Co-administration with GA or ω-3-FA increased ($P < 0.05$) the level of PCV, decreased WBC and differential leukocyte count in the rats when compared with rats treated with Mn only.

Discussion

The present study showed the protective effects of gallic acid and ω-3FA on hepatorenal oxidative injury and inflammation related to manganese intoxication in rats. Manganese toxicity is associated with exposure to contaminated food, water, and air, and has been implicated with the pathophysiology of several disorders including neuronal, hepatic, renal, and reproductive dysfunction,[4,5] hence the need to explore novel strategies in mitigating manganese toxic outcome upon inadvertent exposure. Pharmacokinetically, gallic acid appears to be rapidly

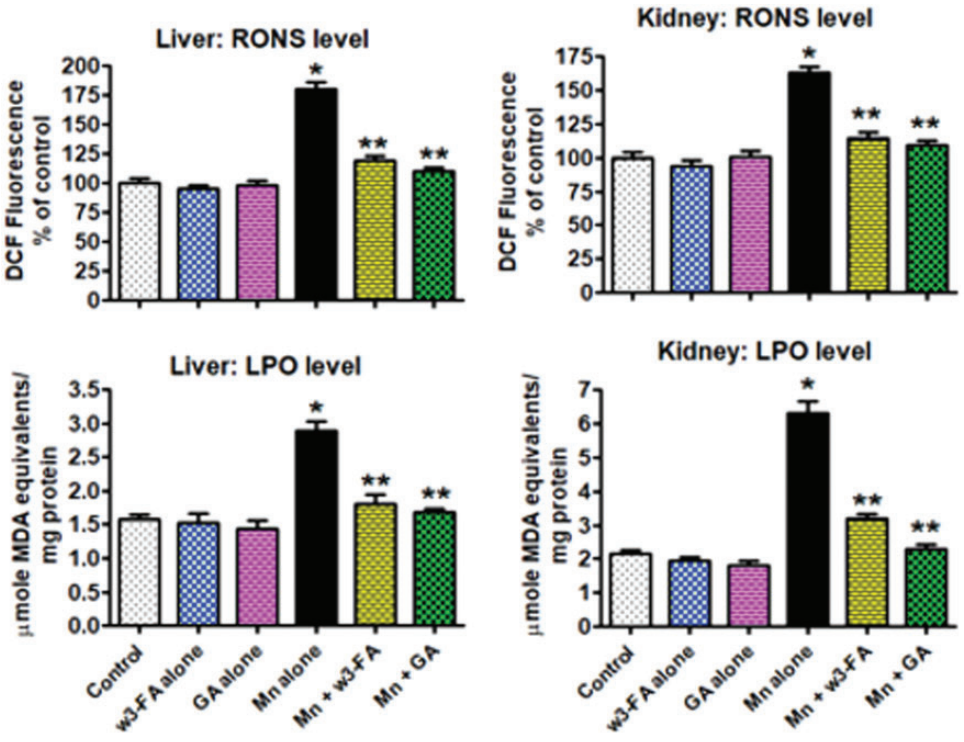

Figure 2. Effect of gallic acid and ω-3 FA on RONS and LPO levels in hepatorenal tissues of rats treated with manganese for 14 successive days. Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean ± SD; $n = 10$ rats. *$P < 0.05$ versus Control; **$P < 0.05$ versus Mn alone. (A color version of this figure is available in the online journal.)

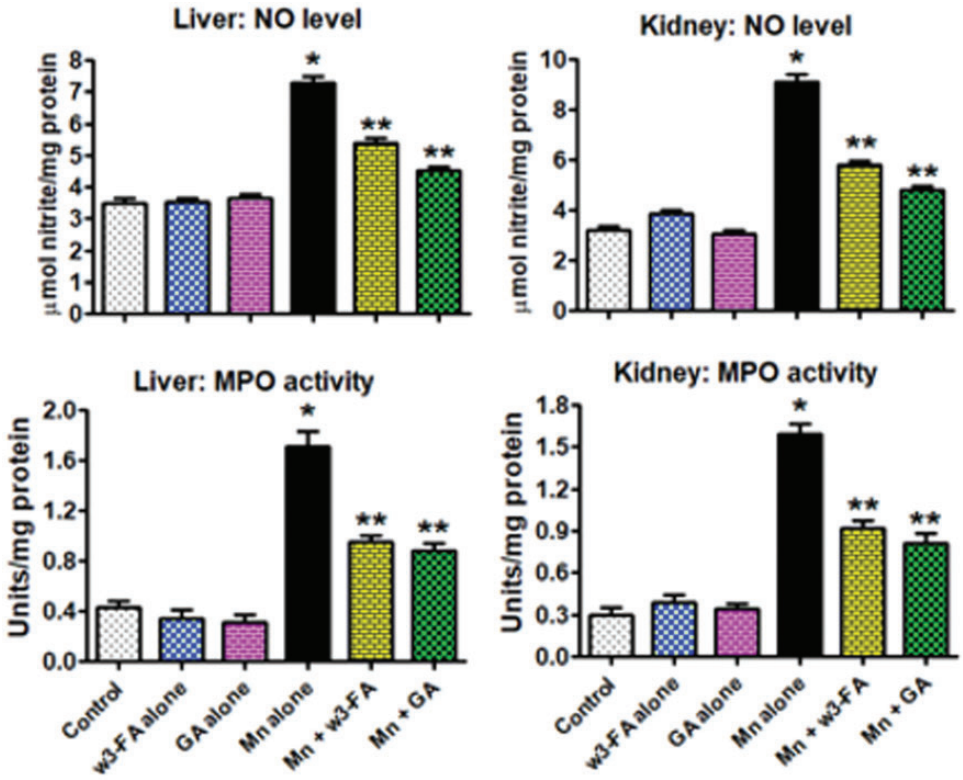

Figure 3. Effect of gallic acid and ω-3 FA on MPO activity and NO level in hepatorenal tissues of rats treated with manganese for 14 successive days. Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean $\pm$ SD; $n = 10$ rats. *$P < 0.05$ versus Control; **$P < 0.05$ versus Mn alone. (A color version of this figure is available in the online journal.)

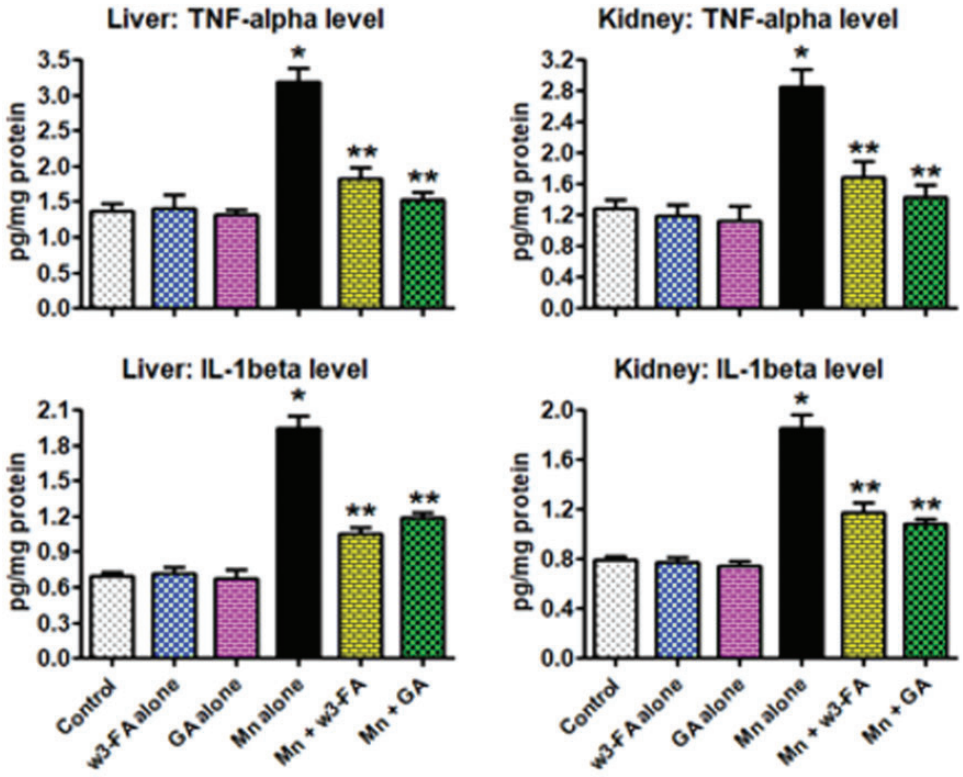

Figure 4. Effect of gallic acid and ω-3 FA on TNF-α and IL-1β level in in hepatorenal tissues of rats treated with manganese for 14 successive days. Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean $\pm$ SD; $n = 10$ rats. *$P < 0.05$ versus Control; **$P < 0.05$ versus Mn alone. (A color version of this figure is available in the online journal.)

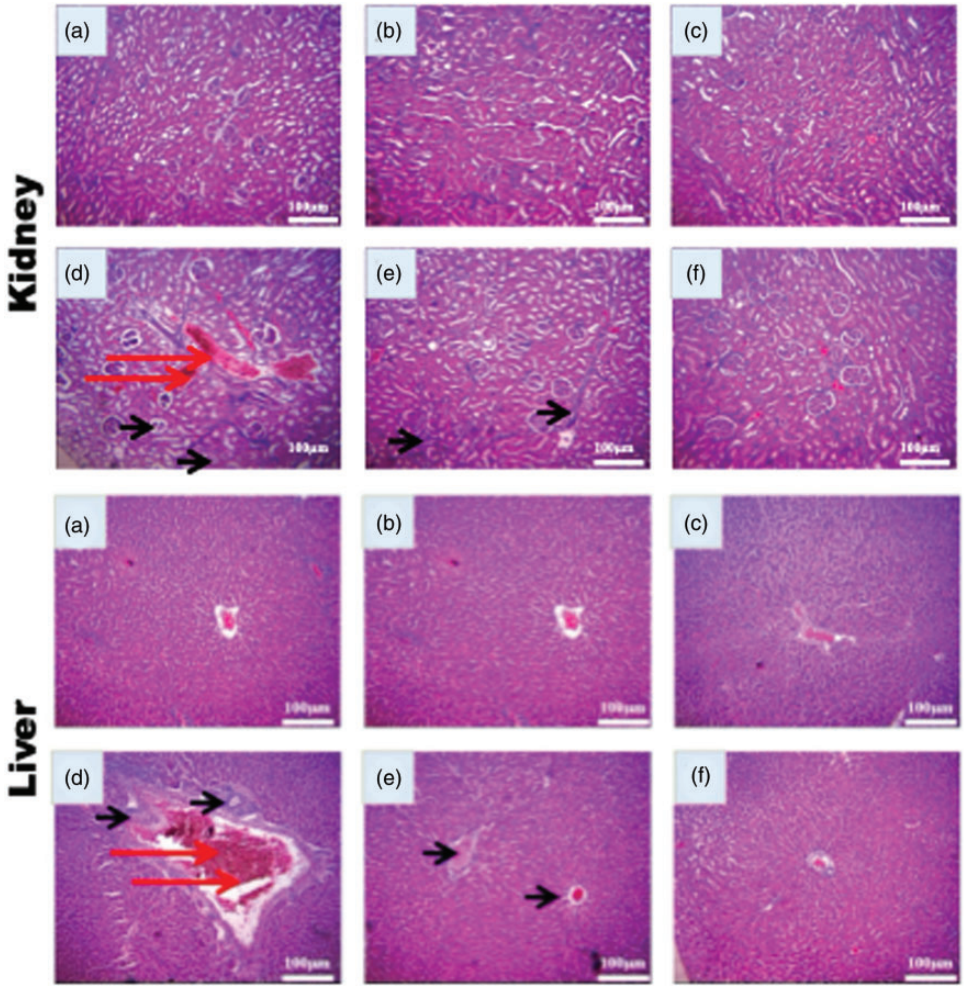

Figure 5. Representative photomicrographs of the kidney and liver. Upper panel: The kidney of control (a), ω-3-FA (b), and gallic acid (c) alone rats showing normal histology. The kidney of rats exposed to manganese alone (d) showing severe interstitial congestion at the renal cortex (black arrows) and tubule degeneration (red arrows). The kidney of rats treated with manganese and ω-3-FA (e), showing very mild interstitial congestion. The kidney of rats co-treated with manganese and gallic acid seems comparable to control. Lower panel: the liver of control (a), ω-3-FA only (b), and gallic acid alone (c) treated rats showing normal histology. Liver of rats exposed to manganese only (d) displaying severe portal congestion (red arrows) and the proliferation of periportal connective tissue (black arrows). The liver of rats treated with manganese and ω-3-FA (e), showing mild portal and sinusoidal congestion. The rat liver treated with manganese and gallic acid seems comparable to control. The magnification: $\times$100. (A color version of this figure is available in the online journal.)

Table 3. Effects of gallic acid and ω-3-FA on hematological parameters in manganese-treated rats.

	Control	ω3-FA only	GA only	Mn only	Mn + ω3-FA	Mn + GA
WBC count/1000	6.47 ± 0.21	5.40 ± 0.57	5.70 ± 0.28	13.60 ± 0.28[a]	6.06 ±0.91[b]	6.50 ± 0.14[b]
PCV (%)	51.50 ± 1.00	53.00 ± 1.41	52.33 ± 1.15	40.83 ± 1.15[a]	51.00 ± 1.02[b]	51.25 ± 2.06[b]
Neutrophils (%)	36.50 ± 2.12	36.50 ± 2.12	38.00 ± 1.41	55.50 ± 0.71[a]	39.00 ± 1.83[b]	39.50 ± 0.71[b]
Lymphocytes (%)	47.00 ± 1.41	46.50 ± 2.12	46.00 ± 2.83	68.33 ± 1.53[a]	51.33 ± 2.31[b]	49.50 ± 0.71[b]
Eosinophils (%)	1.67 ± 0.58	1.67 ± 0.58	1.50 ± 0.58	1.50 ± 0.58	2.00 ± 0.01[b]	2.00 ± 0.01[b]
Monocytes (%)	2.75 ± 0.50	2.75 ± 0.50	2.50 ± 0.71	5.67 ± 0.58[a]	3.00 ± 0.01[b]	3.00 ± 0.03[b]

Note: Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Data bar denotes a mean ± SD; $n = 10$ rats.
[a]$P < 0.05$ versus Control.
[b]$P < 0.05$ versus Mn alone.

taken up and slowly eliminated from studies in rats,[34,35] hence its bioavailability to serve as a protective agents against toxic chemical exposure; on the other hand, ω-3FA bioavalibity tends to be impacted by storage condition and oxidation[36,37] despite its essential role in cell membrane function and its use as supplements in mitigating pathologic condition[38] toxicity and enhancing normal cellular function. The hepatic functional integrity is usually evaluated by measuring the standard biochemical parameters, precisely the serum level of hepatic aminotransferases (ALT and AST), ALP, LDH, and GGT activities coupled with hepatic histological analysis.[39] The marked upsurge in serum aminotransferases in rats exposed to manganese only unveiled alteration in the membrane

Table 4. Effects of gallic acid and ω3-FA on hepatic and renal lesions frequencies, identified in manganese-treated rats for 14 consecutive days.

Parameters	Control	ω3-FA only	GA only	Mn only	Mn + ω3-FA	Mn + GA
Liver						
Portal/sinusoidal congestion	0	0	0	5	2	1
Focal area of necrosis	0	0	0	5	1	0
Cellular infiltration	0	0	1	5	1	0
Hepatic degeneration	0	0	0	3	1	0
Kidney						
Congestion of renal cortex	0	0	3	2	1	0
Tubular degeneration	0	0	5	2	1	0
Necrotic tissues	0	0	5	2	1	0

Note: Manganese (Mn:15 mg/kg); Gallic acid (GA:20 mg/kg); Omega-3 fatty acids (ω-3 FA:30 mg/kg). Values are the number of rats with observed lesions in their tissues. Degree of lesion: 0–5; None (0); mild (1); mild-moderate (2); moderate (3); moderate to severe (4); and severe (5).

integrity due to hepatic injury leading to their release from the periportal hepatocytes into the blood. The protective role of gallic acid or ω-3FA in manganese-mediated liver injury was evidenced by a reduction of the serum AST and ALT activities in the treated rats.

Also, estimation of an ectoenzyme ALP is a well-known indicator for hepatobiliary health and bile movement to the intestine. At the same time, LDH, which is situated in the cytosolic part of the plasma membrane, is a recognized index for acute liver toxicity.[40,41] GGT, which is responsible for glutathione metabolism and amino acid reabsorption from the glomerular filtrate and intestinal lumen[42] is well-documented to be a usual sensitive enzymatic indicator of hepatobiliary diseases.[43] The noticeable increase in serum LDH, ALP, and GGT concentration in rats treated with manganese only reveals liver injury and cholestasis in experimental rats. Co-administered with manganese and gallic acid or ω-3FA significantly decreases serum concentrations of ALP, LDH, and GGT and connotes hepatoprotective effects of gallic acid or ω-3FA.

Urea is formed during protein metabolism, while creatinine is formed from the breakdown of creatinine phosphate in the muscle. The kidney is responsible for the clearance of both urea and creatinine from the bloodstream. Therefore, an upsurge in the serum or plasma creatinine and urea levels is well known to indicate renal damage.[44,45] The present study revealed that exposure to manganese is associated with impairment in the kidney function, as shown by the increase in serum levels of urea and creatinine. Co-administration of gallic acid or ω-3FA significantly diminished these biomarkers of renal injury in the treated rats, thus signifying the beneficial role of gallic acid or ω-3FA on the manganese-mediated renal injury. Histopathological data verified that manganese intoxication occasioned hepatic and renal toxicity in rats, hence supporting the clinical biochemistry data. Gallic acid or ω-3FA effectively abrogated hepatorenal injury associated with manganese exposure in the treated rats.

Exposure to excessive environmental contaminants including metals is connected with free radicals generation which reportedly has made organisms to develop defense mechanisms comprising of enzymatic antioxidants including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST) and non-enzymatic antioxidants such as GSH, vitamin C, and vitamin E.[46] Endogenous hepatorenal antioxidant defense enzymes and oxidative stress indices were assayed to delineate the hepatorenal protective mechanisms of gallic acid or ω-3FA in manganese-exposed rats. This study demonstrated that manganese exposure caused marked decreases in the activities of antioxidant enzymes, namely CAT, GPx, SOD, and GST, as well as GSH level in rats, thus connoting impairment in their antioxidant protective function in the hepatorenal tissues of treated rats. Besides, the upsurge in RONS and LPO levels indicates that exposure to manganese triggered a damaging impact on cellular membrane integrity and further reduces mechanism of endogenous antioxidant defenses in experimental animals. Rats administered manganese, and gallic acid or ω-3FA exhibited a significant increase in the antioxidant status with a marked decrease in the RONS and LPO levels. This observation implies that gallic acid or ω-3FA shows antioxidant and anti-lipid peroxidative mechanisms against manganese-mediated hepatorenal toxicity.

Inflammatory cytokines specifically IL-1β and TNF-α are vital mediators of inflammation. TNF-α controls cytokine generation and inflammatory response progress, whereas an increase in NO level or MPO concentration is linked with oxido-inflammatory stress.[47–49] The marked increase in MPO concentration and levels of TNF-α, NO, and IL-1β in manganese exposed rats implies a state of hepatic and renal inflammation in the exposed rats. Moreover, the marked increase in hepatic and renal NO levels may result in nitration of proteins and modifications in signal transduction pathways. Gallic acid or ω-3FA adequately protected against hepatorenal toxicity due to manganese exposure via anti-inflammatory mechanism relating to decreasing renal and hepatic MPO activity similarly as in the levels of NO, IL-1β, and TNF-α in the treated rats.

Accumulation of manganese in bones,[50] liver, and kidneys[51] suppresses the hematopoietic function by the secretion of defective erythropoietin that affects the RBC formation.[52] The present investigation showed that manganese treatment caused a severe decreased in packed cell volume (PCV), clinically relevant in detecting anaemia.[53] Metal toxicity may precipitate the production of abnormal and reduced RBC count.[54] Furthermore, excessive generation of ROS due to manganese exposure can lead to anemia from ROS-mediated erythrocyte damage,[51] reduced quantity/quality of RBC, and tissue hypoxia.[55] GA and ω-3FA

significantly increased the PCV level compared to manganese-treated group, by averting the impact of manganese-induced hepatic-secretion of defective erythropoietin-/renal toxicity,[55] and oxidative damage. GA and ω-3FA exhibited similar potential in improving white blood cells (WBC) levels in treated rats, and similar to previous report,[55] WBC increased significantly in rats exposed to manganese compared with control. Conversely, Stookey *et al*.,[56] reported decrease in WBC count after manganese exposure. These discrepancies might in part be due to the route and duration of manganese administration. Increases in WBC count observed in manganese-treated rats maybe indicative of rat immune response to manganese load and tissue damage,[57] since WBCs play important function in organismic defense.

Taken together, the protective effects of gallic acid or ω-3FA on hepatorenal damage due to manganese exposure are related to the restoration of antioxidant status, the inhibition of RONS, LPO, and inflammation in the treated rats. Therefore, dietary supplements with GA or fish oil, which is rich in ω-3FA, can serve as nutriceutical presently available as non-prescriptive/prescriptive supplements[58]; despite conflicting report on ω-3FAefficacies, the additive effect of polyphenolic compounds in combination with ω-3FA in modulating oxidative damage has been reported.[59] Strategies to combine polyphenols such as GA or its esters with stable ω-3FA with limited opportunities for oxidation maybe potential therapeutic drug candidates against target organs injuries, mediated by oxido-inflammatory responses in individuals environmentally or occupationally exposed to manganese or relevant toxic chemicals.

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DECLARATION OF CONFLICTING INTERESTS

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