



Manganese Inhibits Indomethacin-Induced Hepatorenal Oxidative Stress in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TSA and OOD performed animals' experiments, data acquisition, the decision to publish and manuscript preparation. Author FEO provided some of the chemicals and reagents used and revised the manuscript and provided valuable advices. Author TSA designed the experiments and interpreted the results. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Manganese (Mn) is an essential trace element in many cellular processes. However, there is dearth of literature on its influence on indomethacin-induced hepatorenal damage. Therefore, this study was conducted to investigate the effect of manganese on indomethacin-induced hepatorenal damage in rats.

Methods: Rats were divided into four groups of eight rats consisting of control group, indomethacin (IND) alone (20 mg/kg), Mn alone (10 mg/kg) and co-treated group that were treated orally for 14 consecutive days. Twenty four hours after treatment, under pentobarbital anesthesia, blood was collected and liver was excised to prepare homogenate and histology staining. Liver and kidney function tests aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glutamine dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G6PD), bilirubin (BIL), urea, creatinine, cholesterol (CHOL),

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triglycerides (TG), low and high density lipoprotein (LDL and HDL), electrolytes and oxidative stress superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and lipid peroxidation (LPO) biomarkers were assessed.

Results: The results showed that indomethacin caused hepatorenal damage in rats manifested with increase in serum hepatic and renal function biomarkers. But co-administration of IND with Mn significantly ($p < 0.05$) decreased the level of hepatorenal biomarkers. Additionally, co-administration of IND with Mn improved the antioxidant status with concomitant reduction of LPO and restored the integrity of the liver and kidney histologically.

Conclusion: The results of this study emphasize that co-administration of IND with Mn to rats alleviated IND-induced hepatorenal toxicities and oxidative stress in rats.

Keywords: Hepatorenal; indomethacin; manganese; lipid peroxidation; rats.

1. INTRODUCTION

Indomethacin is a methylated indole member of acetic acid derivative class of non-steroidal anti-inflammatory drugs (NSAIDs). Indomethacin is a non-selective potent inhibitor of the cyclooxygenases (COX) enzyme responsible for the conversion of arachidonic acid to prostaglandins [1,2] and has antipyretic, analgesic and anti-inflammatory activities. Despite its clinical uses, indomethacin has been associated with many adverse effects including gastrointestinal toxicity, liver, kidney, brain, lung, blood vessels and spleen injury and subsequent deterioration and cell death [3-5]. Mitochondrial injury and necrosis have been reported in liver of experimental animals following indomethacin administration [6,7] and in the kidney, indomethacin caused mitochondrial dysfunction, reduced sodium and water retention, hyperkalemia, tubulointerstitial nephritis accompanying with interstitial nephropathy and papillary cell death resulting in acute renal failure [8,9]. Mechanisms of indomethacin toxicity on prolonged use include generation of reactive oxygen species (ROS) leading to oxidative stress, DNA damage, direct inhibition of prostaglandin synthesis and cellular respiration [10].

Due to generation of ROS, indomethacin exposure induces oxidative stress as a result of cell membrane damage and disturbances in antioxidant status resulting in alterations in cellular metabolic functions in experimental animal tissues.

Manganese (Mn) is a critical trace element in numerous physiological processes in plant, animals and humans at low doses but at high concentration due to chronic occupational exposure is toxic [11]. Mn is essential for proper functioning of the immune system, nervous

system, reproductive hormone, bone and connective tissue growth and development and regulation of cellular energy metabolism [12,13]. Manganese also acts as a cofactor for many enzymes in the Krebs cycle, particularly in the decarboxylation process and antioxidant enzyme manganese superoxide dismutase, arginase, pyruvate decarboxylase and glutamine synthase [14,15]. Route of exposure of manganese to human is mainly from diet, environmental and occupation locale. Though a very essential element in many cellular processes, prolonged exposure has been linked to some pathophysiological events in humans and animals [14,16]. For example, exposure occurs in occupational locations like welding, battery manufacture and mining inhale toxic levels daily and those living in close proximity to industrial and high traffic areas where Mn containing exhaust from methylcyclopentadienyl manganese tricarbonyl (MMT) gasoline are discharged into the air [17,18]. Furthermore excessive exposure to Mn has been reported to cause kidney, reproductive and hepatic dysfunction as well as neurotoxicity due to accumulation in the basal ganglia of humans and animals [19,20].

Liver is a vital organ responsible for detoxification, metabolism, storage, secretion and homeostatic regulation of the body [21] and is susceptible to injury from xenobiotics. Liver injuries due to exposure to xenobiotics result in many pathophysiologicals via various mechanisms including induction of oxidative injury [22]. Since human can be exposed to drugs and metals concurrently, their influence on the organs responsible for their metabolism and excretion is important.

There is dearth of evidence in the literature on the effect of co-administration of indomethacin and Mn on liver and kidney functions despite the

beneficial and toxic role of Mn. Therefore, this study, investigated the influence of oral administration of indomethacin and Mn on biochemical and histological profiles of hepatic and renal functions and oxidative stress markers in adult male rats.

2. MATERIALS AND METHODS

2.1 Chemicals and Drug

Indomethacin (Fabrique par: YangzhouNo.3 pharmaceutical Co. Ltd, Jiangsu, China). The reagent kits for alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin (BIL), total cholesterol (CHOL), gamma glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), triglyceride (TRIGS), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), lactate dehydrogenase (LDH), and triglycerides (TRIGS) were purchased from Randox Laboratories Limited, UK. Sorbitol dehydrogenase (SDH), glutathione (GSH), thiobarbituric acid (TBA), trichloroacetic acid (TCA) 5', 5'-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), hydrogen peroxide (H₂O₂), manganese chloride (MnCl₂.4H₂O) ≥ 99% (Sigma Aldrich Chemical Co. St. Louis, MO, USA). All other chemicals were of analytical grade and were obtained from the British Drug Houses (Poole, Dorset, UK) and other standard suppliers.

2.2 Experimental Animals

Thirty two adult healthy male Wistar rats (10 weeks old, 180 ± 5 g) were purchased from the Veterinary Anatomy Department animal house of the University of Ibadan, Nigeria, and were kept in the Biochemistry Department animal house throughout the period of this experiment in polyethylene-walled cages. The rats were kept on a 12-h light: 12-h dark regime at 28 °C prior to the experiments and were fed with standard rats chow with free access to water. They were acclimatized for 2 weeks.

2.3 Experimental Design

Rats were randomly divided into four groups of eight rats each and treated once daily for 14 days consecutively as follows:

Control group: The rats received normal saline

Indomethacin alone group: The rats received 20 mg/kg indomethacin alone

Indomethacin and Mn group: The rats co-administered with 20 mg/kg indomethacin + 10 mg/kg Mn.

Mn alone group: The rats received 10 mg/kg manganese alone.

Doses of indomethacin and Mn used in this study were chosen from our previous preliminary studies and previous pilot studies [23]. The control group received the same volume as the treatment groups. The initial and final body weights and relative organ weights of each group were recorded for the calculation of the weight gain and relative organ weights.

2.4 Sample Preparation for Biochemical Analyses

Weights of all the rats were taken before blood sample collection via retro-orbital venous plexus into plain tubes and the animals were sacrificed by cervical dislocation 24 hours after the experiment. Serum was obtained by centrifuging the clotted blood samples at 3000 g for 10 min at 4°C and kept at -20°C in the freezer for hepatic and renal function tests.

Liver and kidney samples of the rats were homogenized in ice cold 50 mM Tris-HCl buffer (containing 1.15% of potassium chloride; pH 7.4) using a Teflon glass homogenizer. The homogenate was then centrifuged at 14,000 g for 15 min at 4°C. The liver and kidney supernatant obtained was used for evaluating the antioxidant status/oxidative stress markers and histological analysis.

2.5 Determination of Liver and Kidney Function Markers

The serum activities of sorbitol dehydrogenase (SDH) (Sigma Chemical Co., St Louis, MO, USA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), glucose-6-phosphate dehydrogenase, glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and concentrations of direct and total bilirubin (dBIL and tBIL), creatinine and urea levels were estimated using commercially available kits according to the manufacturer's instructions (Randox Laboratories Limited, UK). Serum electrolytes (sodium, potassium, chloride, and bicarbonate) were determined by flame photometry.

2.6 Assessment of Serum Lipid Profile

Levels of serum total cholesterol (CHOL), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined according to the manufacturer's instructions using commercially available diagnostic kits (Randox Laboratories Limited, UK).

2.7 Estimation of Oxidative Stress Markers

Superoxide dismutase (SOD) activity was estimated as described by Misra and Fridovich [24], catalase (CAT) activity was determined using H_2O_2 as a substrate according to the method described by Clairborne [25]. Reduced GSH level was determined at 412 nm according to the method described by Rahman et al. [26], lipid peroxidation was quantified as malondialdehyde (MDA) according to method of Sahreen et al. [27] and the result was expressed as micromoles of MDA per milligram protein. Protein concentration was determined according to the method described by Popovic et al. [28].

2.8 Histopathological Analysis

The liver and kidney tissues were processed for histology according to Songur et al. [29] method. Briefly, liver and kidney specimens were fixed in 10% neutral phosphate-buffered formalin solution for at least 24 h. After dehydration procedures, the samples were blocked in paraffin. Sections of 4 - 5 μm were cut by a microtome, deparaffinized, hydrated and stained with hematoxylin and eosin (H & E). All slides were coded before examination with light microscope (Olympus BX-41 microscope, Hamburg, Germany) and photographed using a digital camera by pathologists who were blinded to control and drug-treated groups.

2.9 Statistical Analysis

Data were expressed as mean \pm standard error of mean (SEM) for 8 rats. Statistical analysis was carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Duncan's test to identify significantly different groups (SPSS for Windows, version 22, SPSS Inc., Chicago, Illinois). Graphical construction was performed using Graphpad® Prism 7 (Graphpad Software, La Jolla, CA) and $p < 0.05$ is considered statistically significant.

3. RESULTS

3.1 Effect of Manganese on Relative Organ Weight and Body Weight in IND-Treated Rats

The results on the body weight gain and the relative liver and kidney weights of rats treated for 14 days are depicted in Table 1. Rats treated with IND alone showed a significant ($p < 0.05$) decrease in the body weight but had increase in liver and kidney weights compared to the control rats, but co-administration of IND and Mn showed an increased in body weight of the rats with reduced liver and kidney weights compared to IND alone treated rats. However, rats treated with Mn alone did not show any changes in both the body weight and relative organ weights of the rats.

3.2 Manganese Improves Liver and Kidney Function Markers in Indomethacin-Treated Rats

The serum activities and levels of liver and kidney function biomarkers of the control, IND alone, IND + Mn and Mn alone groups were shown in Table 2. The serum activities of ALT, ALP, AST, GGT, G6PDH, GLDH, LDH, MDH, SDH and levels of dBIL, tBIL were significantly ($p < 0.05$) elevated in the IND alone rats compared to the control group. Rats treated with Mn concomitantly with IND showed a significant ($p < 0.05$) reduction in all these markers. Rats treated with Mn alone showed activities and levels that were similar to that of control except for GGT. Also, renal function indices were significantly ($p < 0.05$) increased in IND alone rats compared to the control group but co-treatment with Mn alleviated this effect. The enzyme activities and levels of other markers obtained from rats given Mn alone showed values similar to the control group.

3.3 Effect of Manganese on Indomethacin-Induced Lipid Profile

The effect of Mn on serum lipid profile is represented in Table 3. Oral administration of IND caused a marked increase in the levels of CHOL, TRIGS and LDL with simultaneous decrease in HDL level as evidenced in the IND alone treated rats compared to the control rats. But co-treatment of IND with Mn decreased significantly ($p < 0.05$) the levels of CHOL, TG and LDL and concomitantly increased the HDL level. The rats given Mn alone showed values similar to that of control group.

Table 1. Body weight gain and relative organ weights of Control, IND alone, IND+ Mn and Mn alone rats for 14 days

	Control	IND alone	IND + Mn	Mn alone
Body weight gain (g)	18.65 ± 2.04	8.23 ± 0.98 ^a	16.58 ± 1.21 ^{ab}	17.24 ± 2.07 ^b
Relative kidney weight	1.98 ± 0.16	2.85 ± 0.02 ^a	2.45 ± 0.13 ^{ab}	2.01 ± 0.23 ^b
Relative liver weight	4.68 ± 0.21	6.20 ± 0.18 ^a	5.78 ± 0.02 ^{ab}	4.65 ± 0.25 ^b

IND: 20 mg/kg indomethacin, Mn: 10 mg/kg manganese. n = 8. Values are expressed as mean ± SEM of 8 rats.
^ap < 0.05 versus control; ^bp < 0.05 versus IND alone; ^cp < 0.05 versus Mn alone.

Table 2. Liver and kidney function biomarkers in rats treated with IND alone, IND with Mn and Mn for 14 days

Group	Control	IND alone	IND + Mn	Mn alone
ALT (U/L)	23.42 ± 1.12	98.86 ± 3.06 ^a	94.32 ± 2.78 ^{abc}	23.31 ± 5.87 ^b
ALP (U/L)	58.45 ± 3.67	104.34 ± 9.67 ^a	86.46 ± 7.32 ^{abc}	57.65 ± 2.67 ^b
AST (U/L)	34.18 ± 3.43	76.42 ± 6.89 ^a	56.78 ± 5.45 ^{abc}	33.91 ± 6.21 ^b
GGT (U/L)	52.34 ± 5.63	123.68 ± 12.54 ^a	97.56 ± 2.23 ^{abc}	55.89 ± 4.45 ^{ab}
G6PDH (U/L)	2.34 ± 0.07	5.32 ± 0.94 ^a	3.24 ± 0.68 ^{abc}	2.18 ± 0.45 ^b
GLDH (U/L)	48.12 ± 3.24	86.97 ± 6.32 ^a	67.26 ± 2.45 ^{abc}	48.32 ± 4.11 ^b
LDH (U/L)	12.24 ± 2.16	31.97 ± 4.12 ^a	26.31 ± 3.76 ^{abc}	12.26 ± 2.27 ^b
MDH (U/L)	98.16 ± 2.13	188.93 ± 27.78 ^a	124.92 ± 24.56 ^{abc}	97.89 ± 2.22 ^b
SDH (U/L)	34.08 ± 1.67	123.46 ± 24.25 ^a	87.68 ± 18.95 ^{abc}	34.14 ± 1.25 ^b
dBIL (mg/dL)	0.68 ± 0.03	2.34 ± 0.23 ^a	1.18 ± 0.06 ^{abc}	0.72 ± 0.05 ^b
tBIL (mg/dL)	1.79 ± 0.04	3.86 ± 0.08 ^a	2.31 ± 0.03 ^{abc}	1.72 ± 0.06 ^b
BUN (mg/dL)	32.66 ± 1.34	58.52 ± 1.56 ^a	45.34 ± 1.22 ^{abc}	31.95 ± 1.46 ^b
Creatinine (mg/dL)	1.02 ± 0.02	3.86 ± 0.95 ^a	2.88 ± 0.09 ^{abc}	0.98 ± 0.06 ^b
Na ⁺ (mmo/L)	92.08 ± 2.32	126.62 ± 24.96 ^a	98.06 ± 12.58 ^{abc}	93.06 ± 1.98 ^b
K ⁺ (mmo/L)	3.25 ± 0.06	8.05 ± 0.46 ^a	6.93 ± 0.04 ^{abc}	3.33 ± 0.09 ^b
HCO ₃ ⁻ (mmo/L)	10.20 ± 0.35	24.38 ± 0.47 ^a	19.67 ± 0.96 ^{abc}	10.01 ± 0.14 ^b
Cl ⁻ (mmo/L)	45.22 ± 0.36	68.67 ± 0.43 ^a	52.34 ± 0.67 ^{abc}	44.97 ± 0.76 ^b

IND: 20 mg/kg indomethacin, Mn: 10 mg/kg manganese. n = 8. Values are expressed as mean ± SEM of 8 rats.
^ap < 0.05 versus control; ^bp < 0.05 versus IND alone; ^cp < 0.05 versus Mn alone.

Table 3. Serum lipid profile of rats treated with IND alone, IND with Mn and Mn for 14 days

Group	Control	IND alone	IND + Mn	Mn alone
CHOL (mg/dL)	14.22 ± 2.18	58.34 ± 2.98 ^a	41.67 ± 2.04 ^{abc}	13.98 ± 1.36 ^b
TG (mg/dL)	24.04 ± 1.26	67.86 ± 2.78 ^a	65.45 ± 3.02 ^{abc}	23.45 ± 1.42 ^b
HDL (mg/dL)	40.25 ± 1.38	32.37 ± 1.68 ^a	38.04 ± 1.68 ^{abc}	39.68 ± 1.32 ^b
LDL (mg/dL)	36.17 ± 0.27	75.34 ± 3.07 ^a	58.79 ± 2.67 ^{abc}	35.93 ± 1.65 ^b

IND: 20 mg/kg indomethacin, Mn: 10 mg/kg manganese. n = 8. Values are expressed as mean ± SEM of 8 rats.
^ap < 0.05 versus control; ^bp < 0.05 versus IND alone; ^cp < 0.05 versus Mn alone

3.4 Manganese Enhances Hepatic and Renal Antioxidant Status in IND-Treated Rats

The results in Figs. 1 and 2 showed the effect of IND, IND with Mn and Mn alone on antioxidant biomarkers in the liver and kidney of rats. Administration of IND alone for 14 consecutive days produced a significant ($p < 0.05$) decrease in SOD, CAT activities and GSH level with concomitant increase in LPO level in the liver and kidney of rats compared to the control rats. Rats given Mn alone on the other hand

significantly ($p < 0.05$) increased both the liver and kidney activities of SOD, CAT and GSH level with values similar to that of control rats. Co-administration of IND with Mn however, improved the antioxidant status compared with the IND alone rats.

3.5 Manganese Prevents Histological Damage in the Liver and Kidney of IND-Treated Rats

The photomicrographs of the representative liver and kidney of control and rats treated with IND

alone and IND co-treated with Mn and Mn alone are shown in Fig. 3. Histological examination of the tissues showed an intact structural arrangement of the liver and the kidney of the control group. But, the liver of rats treated with IND alone revealed degeneration, necrosis in the focal area and infiltration of inflammatory cells

(red arrows), whereas the liver of Mn alone treated rats showed mild congestion. Also, kidney of rats treated with IND alone revealed noticeable degeneration and inflammatory cell infiltration in the proximal tubules with congestion of vessels and rats treated with Mn alone showed no lesions.

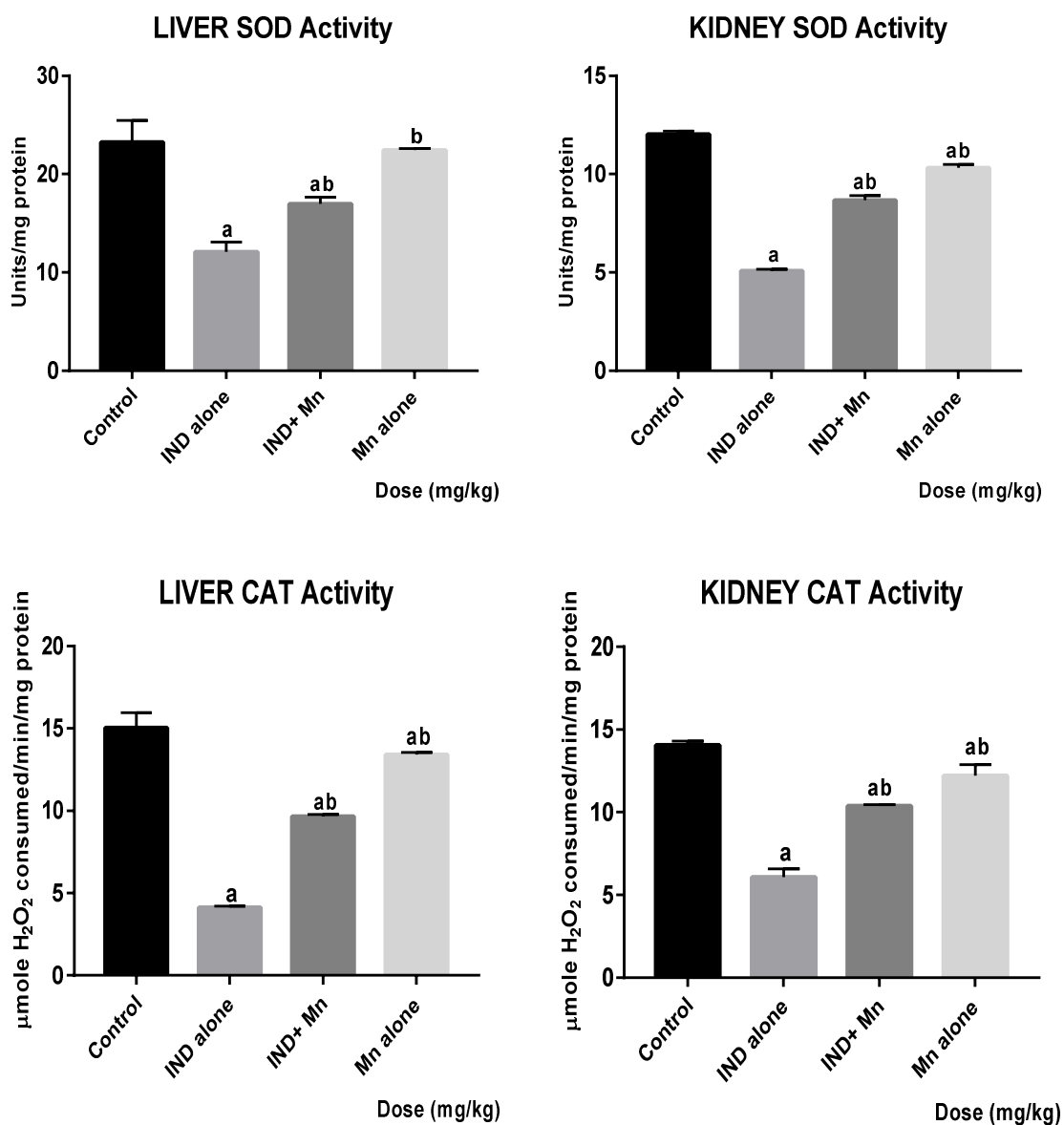


Fig. 1. Activities of SOD and CAT in liver and kidney of rats following oral exposure to IND alone, IND + Mn and Mn alone for 14 consecutive days. IND: 20 mg/kg indomethacin; Mn, 10 mg/kg Manganese. n=8. Each bar represents mean \pm SEM of 8 rats. ^ap < 0.05 versus Control and ^bp < 0.05 versus IND alone

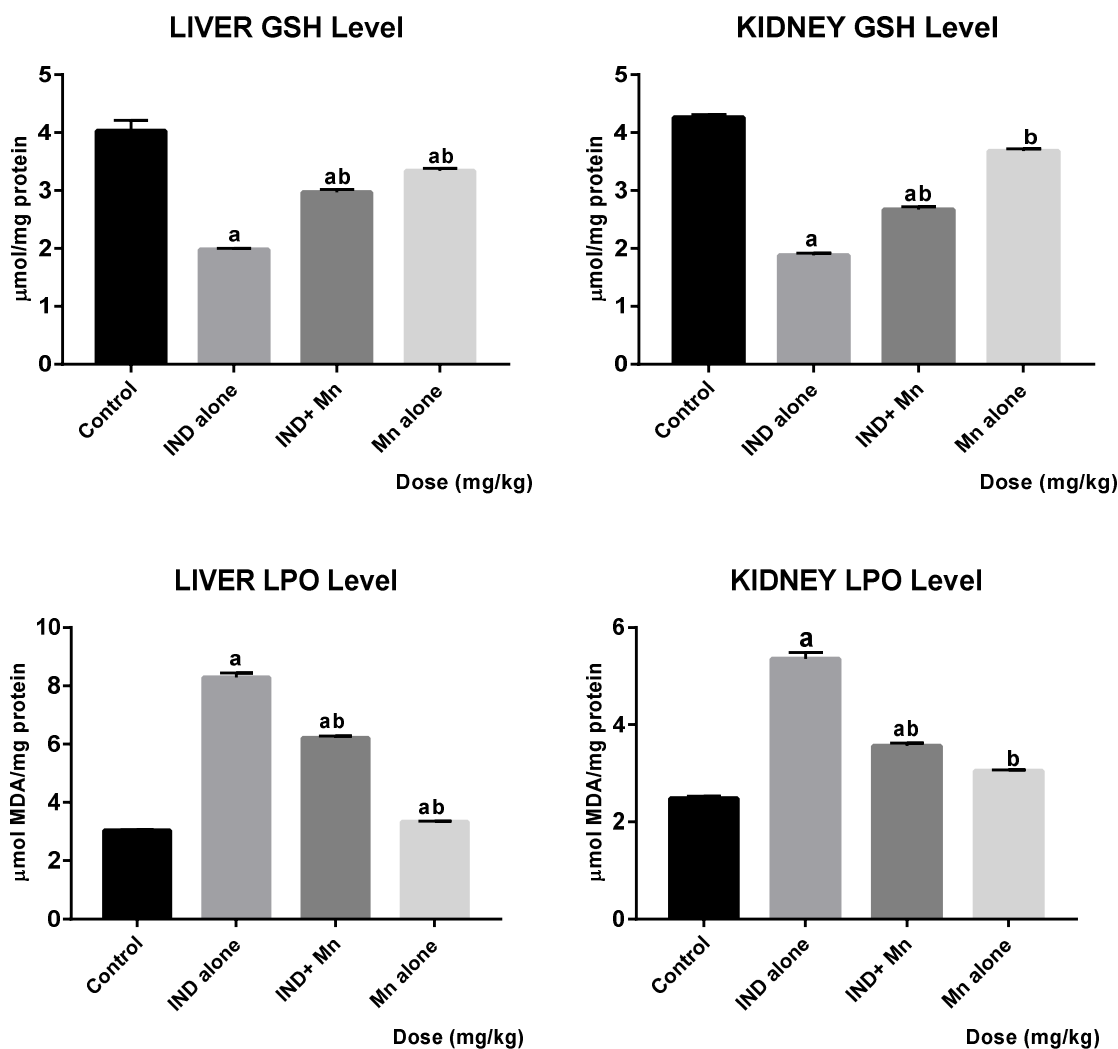


Fig. 2. Levels of GSH and LPO in liver and kidney of rats following oral exposure to IND alone, IND + Mn and Mn alone for 14 consecutive days. IND: 20 mg/kg indomethacin; Mn, 10 mg/kg Manganese. n=8. Each bar represents mean \pm SEM of 8 rats. ^a $p < 0.05$ versus Control and ^b $p < 0.05$ versus IND alone

4. DISCUSSION

Indomethacin is thought to be safe at low doses and is a commonly used antipyretic and analgesic drug in clinical practice. However, persistent use of indomethacin for a long time in both humans and animals causes multi-organ damage particularly hepatorenal toxicity [6,30,31]. Currently compounds particularly of plant origin are used as hepato and renoprotective agents in indomethacin-induced toxicities. Despite the reported beneficial uses of manganese, there is paucity of data on its

influence on indomethacin-induced toxicities. This study therefore, sought to investigate its effect on ameliorating indomethacin-induced toxicities on the liver and kidney of rats. In this study, rats treated with IND alone showed a significant decrease in the body weight but increase liver and kidney weights compared to the control rats, co-administration of IND and Mn showed an increase in body weight of the rats with concomitant decreases liver and kidney weights when compared with the IND alone treated rats. However, rats treated with Mn alone did not reveal any changes in both the body

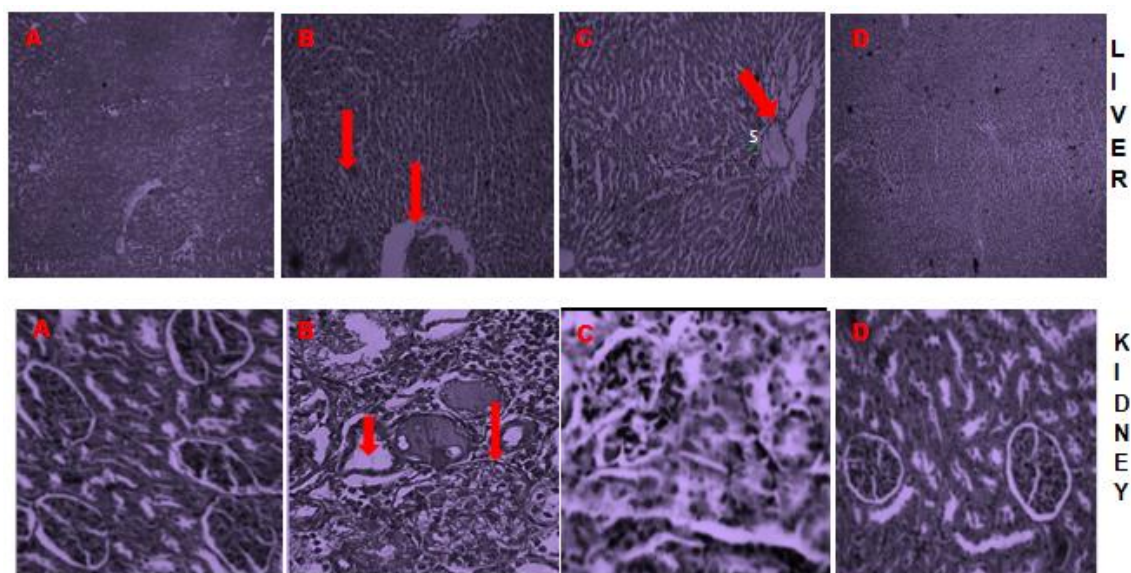


Fig. 3. Photomicrograph of representative liver and kidney of (A) control, (B) IND alone, (C) IND + Mn and (D) Mn alone. Liver and kidney of control showed intact morphology. Liver of IND alone rats showed infiltration of inflammatory cells, disruption of hepatocytes arrangement and dilated blood sinusoids; Liver of rat co-treated with IND + Mn showing few inflammatory cells and sinusoids dilatation while Mn alone treated group showed near normal architecture. The kidney of IND alone group showing advanced disintegration of the proximal tubules characterized by mild edema (arrow) at interstitium, inflammatory cellular infiltration and congestion of the glomeruli. The kidney of rats co-treated with IND + Mn had mild congestion of the glomeruli with inflammatory cells infiltrate.

weight and relative organ weights of the rats. The present study showed that indomethacin caused marked hepatorenal damage and co-administration with Mn alleviates this damage via various mechanisms.

Alanine aminotransferase is a cytoplasmic enzyme while AST is both cytoplasmic and mitochondrial are usually released into the circulation after hepatocyte disruption [32,33]. Likewise, ALP is localized in the cells lining the biliary duct and is commonly used as a marker of hepatobiliary damage [34]. GGT is a specific marker for cholestasis and biliary effects [35] of bile duct lesions in the rat liver. In our study, oral administration of IND caused significant elevation in the serum ALT, ALP, AST and GGT activities in rats indicating liver injury especially looking at the elevated activities of ALT, ALP and GGT after IND administration alone to rats suggesting hepatobiliary and cholestatic events in the rats. This is in agreement with the reports of Sarhat et al., and Khan et al., [36,37]. However, co-administration with Mn reduced these indices of hepatotoxicity showing that Mn abrogated the

toxic effects of IND-induced hepatic damage in the rats.

Additionally, to establish the extent of hepatic damage following administration of IND to rats, we evaluated the activities of some of the enzymes involved in various metabolic pathways as well as some metabolites found in the liver. Lactate dehydrogenase, a cytoplasmic enzyme found in anaerobic glycolysis is a useful biomarker of hepatocellular necrosis [38]. MDH is a tricarboxylic acid cycle enzyme that exists in the cytoplasm of the periportal hepatocytes and GLDH an enzyme involved in deamination of amino acids and is found in the mitochondrial matrix; primarily in the centrilobular region of the liver and its presence in the blood reflects loss of mitochondrial integrity [39], whereas SDH is both cytoplasmic and mitochondria enzyme found in the hepatocytes and a precise biomarker of the acute hepatocellular injury and necrosis in rats [40]. Glucose-6-phosphate dehydrogenase an enzyme of the pentose phosphate pathway that catalyzes the conversion of glucose-6-phosphate to produce NADPH used in lipogenesis and as an antioxidant. The results of our study showed

that IND alone significantly increased the activities of these enzymes compared to the control group; this is in accordance with the reports of Khan et al., and Sheli et al., [37,41]. These observations were attenuated by co-administration with Mn. Bilirubin, a byproduct of heme degradation was observed to be high in the IND alone rats, the elevated level may be due to inhibition of bilirubin conjugation to glucuronides or obstruction to its flow from the liver to the duodenum resulting in its accumulation [42], moreover with the elevated ALP and GGT activities. This result conformed to the previous report [43]

The effect of IND alone on kidney showed elevated levels of metabolic wastes creatinine and urea which are markers of kidney dysfunction. This observation is significant, because increase in serum urea indicates decrease in reabsorption at the renal tubular epithelium, an increase in serum creatinine reflects impaired glomerular filtration rate. This result is similar to the report of Khan et al., [37]. The levels of Na^+ , K^+ , Cl^- and HCO_3^- which are major electrolytes used to assess the homeostatic functionality and ion-based balance of the kidney were also elevated. This observation may indicate a consequential effect on the ion-dependent processes in the IND alone rats. Based on the serum biochemical parameters obtained in the present study, IND alone exposure induced renal damage in the treated rats. Remarkably, co-administration of IND with Mn reduced the serum creatinine, urea and electrolytes levels, showing that Mn mitigates IND induced renal dysfunction in rats.

Liver plays a critical role in lipid metabolism, thus liver damage affects lipid metabolism markedly [44]. In this study, IND alone caused elevated serum levels of total cholesterol, triglycerides, low density lipoprotein and decreased the high density lipoprotein level in rats. This is significant as it reveals altered metabolic and transport functions of the liver. For instance, HDL a key lipid constituent of the serum that aids in cholesterol transport to various sites in the organism, especially to the liver and serve as building block for cell membranes and as energy supply [45]. This result agreed with the previous report of Akinlolu et al. [46]. Co-administration of IND with Mn decreased the levels of CHOL, TG and LDL and simultaneously increased the HDL level.

One of the mechanisms by which IND is toxic to various organs is by generation of ROS and

decreasing the endogenous antioxidant mechanisms comprising SOD, CAT and GSH by causing oxidative stress [47]. An imbalance between ROS generation and the activity of antioxidant defense systems in an organism results in oxidative stress which has been reported to be one of the major contributor to liver and kidney damage [35]. IND has been reported to increase the generation of ROS in the hepatic and renal tissues and decreased antioxidant defense status [38]. In our present study IND alone diminished the activities of SOD, CAT and GSH level significantly in the liver and the kidney of the rats. The reduction in this antioxidant status in both the liver and the kidney of rats exposed to IND alone suggests inhibition of these endogenous antioxidants biomarkers or overutilization especially GSH and thus buildup of ROS. This result conformed to the report of Khan et al., [37]. However, co-administration of IND with Mn increase the activities and level of the antioxidant system in rats, probably because Mn as a cofactor for SOD acts to prevent the damaging effect of super oxide radicals that would have initiated the cascade of events that would subsequently lead to oxidative stress [48,49]. Also, Mn-binding proteins (manganoproteins) when combine with small non-proteinaceous biomolecules like phosphates or carboxylates can prompts antioxidant action particularly in medium where there is low level of iron. Increased lipid peroxidation in IND alone rats in our study implies induction of oxidative damage in the hepatic and renal tissues. However, co-treatment of IND with Mn decreased the level of the LPO an indication that Mn mitigates against IND-induced lipid peroxidation in both the liver and kidney of the rats. This observation implies that IND-induced oxidative stress is due to increased LPO and inhibition of the antioxidant system in the liver and kidney of rats while the attenuation of these observations by Mn could be associated with the enhanced antioxidant capacity of these metalloenzymes. The histological analysis of the liver and kidney of rats co-treated with IND and Mn in this study corroborated with the biochemical data obtained in this present study.

5. CONCLUSION

Overall, indomethacin exposure triggers hepatorenal damage in rats via induction and upregulation of oxidative stress. But co-treatment of indomethacin with manganese alleviates the hepatorenal injury caused by indomethacin in rats by improving the antioxidant status thereby

restoring the morphology of the liver and kidney of rats.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

Animal Ethic Committee approval has been taken to carry out this study. All procedures in this study conformed to the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health and the study carried out according to the US NAS guidelines was approved by the University Ethical Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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