ISSN: 0973-3469, Vol.19, No.(1) 2022, Pg.17-25

Material Science Research India

www.materialsciencejournal.org

Zinc Chloride Ameliorates the Adverse Effects of Silver Nitrates Compared to Silver Nanoparticle in Post-Natal Model of Toxicity.

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Abstract

Silver nanoparticles have been shown to increase postnatal toxicity in breastfeeding female rats, with negative consequences for their offspring. We wanted to investigate more about the differences in toxicity between silver nitrates and silver nanoparticles, as well as the impact of zinc chloride treatment on the silver nitrates induced toxicity on female albino rats. For 21 days, 30 breastfeeding female albino rats were divided into 4 groups; the first three group received orally 0, 50,100 mg/kg of silver nitrate. While the fourth group received 100 mg/kg of silver nitrate plus 300 ppm of zinc chloride in drinking water and fifth group received only AG-NPs at dose of 100 ppm The results demonstrated that silver nitrates were more hazardous than nano-silver, as evidenced by higher free radical release, increased MDA levels, and decreased antioxidant enzyme levels (SOD). In addition, the silver ions-treated group had higher levels of liver enzymes and creatinine. Zinc chloride treatment, in particular, had a protective impact and mitigated the negative effects of silver nitrates, as seen by the restoration of baseline levels of liver enzyme, creatinine, and antioxidant enzyme. In addition, zinc chloride therapies reduced the harmful effects of silver nitrates on liver and kidney tissues but not lung tissue.

Article History
Received: 22 March 2022
Accepted: 19 April 2022

Keywords
Hepatorenal Functions; Mda; Silver Nitrates; Silver Ions; Sod; Zinc Chloride.

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Doi: http://dx.doi.org/10.13005/msri/190102

Introduction

Since the early centuries, silver ions have been used as a biocide, antibacterial, disinfectant, and antiseptic against pandemic diseases such as cholera, eye infection, and burn wounds.[1] Silver-based products have recently been employed in a variety of food industries, including food packaging materials, medical healthcare, and hard surface materials and textiles.[2,3]

There is a lot of evidence that silver nitrates or nano-silver are present in the food chain.[4] and its toxicity to animals and humans could be increased as residues and in the ecology around us. The yearly release of silver into the ecosystem through industrial wastes and emissions was estimated to be around 2,500 tonnes, with 80 tonnes contaminating surface waters.[5,6] Silver ions could be released into the environment as a result of silver nitrate pollution or silver nanoparticles.[4] Silver ion residues have been found in milk, meat, and water sources.[6,7]

In a prior post-natal toxicity model, the hepatorenal toxicity of silver nanoparticles in feti development and dams of rats was documented.[8] and according to many research, silver nanoparticles caused reproductive, respiratory, and cutaneous toxicity.[9,10]

More than 300 enzymes need zinc as a cofactor in the majority of metabolic processes and a wide range of cellular processes.[11] In addition, zinc supplementation improved the effects of nanosilver on brain cells[12] and the use of hazardous dosages of silver nitrates resulted in a decrease in intracellular zinc and iron levels.[13]

Notably, in rats, zinc had a protective effect against silverinduced toxicity.[14] Similarly, zinc increased the antioxidant state of nickel treated rats' livers.[15] Also, zinc protected the liver and kidneys from mercury induced injuries.[16,17] Zinc level is critical for tissue regeneration following tissue injury because zinc regulates cytokine expression, suppresses inflammation, and is essential to activate antioxidant enzymes that scavenge reactive oxygen species and lowering oxidative stress.[18]

With aspecial comparison to silver nanoparticles, this study postulated that zinc chloride would create a protective effect against silver nitrate-induced toxicity in breastfeeding female rats and their puppies.

Materials and Methods

Laboratory Animals

Female albino rats weighing 180 to 200 grammes were purchased from the experimental section of Mansoura University's Faculty of Pharmacy. The animals appeared to be in good health and were kept in plastic cages with wood shavings as bedding. All animals were kept in plastic cages in groups of four in a regulated environment[25] (3 °C temperature and 45–65 percent humidity) and in illuminated rooms with a 12-hour light/dark cycle. Animals were housed for two weeks prior to the experiment and fed a balanced ration with free access to food and water. We observe all animal ethics rules at the college of veterinary medicine in Mansoura, as well as all experiments conducted in the forensic medicine and toxicology department of the faculty of veterinary medicine in Mansoura, Egypt, under code number (R/68).

Tested Chemicals

Silver nitrates and zinc chloride were purshased from Sigma Aldrich (USA).

Experimental Grouping and Design

Thirty pregnant female rats divided into three groups each one contained six rats weighed 150 to 200 g and kept until delivery. In the current study, Silver nitrates (Sigma Aldrich, USA) given orally, dissolved in di-ionized water, at dose level 0, 50, 100 ppm (equivalent to 1/50 and 1/100 of the LD_{50} recorded earlier on first day of lactation till 21 days and control group intubated with di-ionized water as control. The fourth group received daily 100 ppm of silver nitrates plus zinc chloride as water supplement at dose of 300 ppm/L. Dams were weighted prior to dosing to provide a consistent dose throughout the trial. On the 21st day of lactation, all rats were slaughtered with an overdose of thiopental, blood was collected from dams and puppies for serum separation, and different tissue samples such as liver, kidney, and lung were taken. Puppies' liver, kidney, and lung tissues were isolated and fixed in buffered formalin for histological analysis. The bodies of the other euthanized

rats were discarded using the technique outlined before.[19] For comparison with silver ion, we also give data from a previously published article on nano-silver treatments of postnatal models at a dose of 1/50 of LD50 equivalent to 100 mg/kg (group 5).[8]

Histopathological Examination

Different tissues from dams and puppies were fixed in 10% neutral buffered formalin, including liver, kidney, and lung. For pathology detection, fixed tissue was stained with traditional eosin and hematoxylin staining, imaging, and reading.[20]

Biochemical Analysis

Sera from all dams and puppies in the treated and control groups were examined for AST, ALT, cholesterol, BUN, and creatinine levels to assess the postnatal toxicity of silver nitrates and compare it to zinc therapies.

Oxidant/Antioxidants Index

To investigate the toxicity of silver ions and the protection of zinc by kits, we evaluated the oxidant/antioxidant MDA (oxidant), sod1, and GSH (antioxidants) in dams' liver tissue homogenates.

Statistical Analysis

For homogeneity, sample size and statistical difference between groups, all experimental data was evaluated using One-way Annova and the turkey test. $P<0.05$ was considered significant (SPSS version 20).

Results

When compared to the control group, silver nitrates had no significant effects on body weight. Furthermore, silver nitrates caused hepatomegaly, particularly at higher doses of 100 mg/kg, while zinc kept the same liver weight as the control group.

Table 1: In postnatal toxicity in rat dams, oral administration of silver nitrate treatment had hepatorenal consequences.

	ALT	AST	UREA	Creatinine	cholesterol
Group1 Control	9.3937±1.88508	40.1906± 5.977	40.8418±1.9	0.3333± 0.04	23.3333± 4.3
Group 2	14.86 ±0.9184	42.0167±0.95	44.0633± 1.8	0.5123±0.003	55.6667±4.9
Group 3 (ttt name should be mentioned)	25.6±0.55076	74.3933± 8.2	101.31±1.5	0.855±0.04	103.31±6.7
Group 4	9.1660. ±. 0.33	40 ±.0.57735	40.333± 0.33	0.3547±0.05	23.87± 4.7
Group 5 (AG-NPS)	24.73889±0.9	45.29567±0.04	45.65889±0.3	0.755±0.04	153.9027±0.3.1

Furthermore, the weight of the liver was unaffected by silver nitrates. In particular, when compared to the control group, oral treatment of silver nitrate at a dose of 100 mg/kg resulted in significant increases in liver enzyme, urea, creatinine, and cholesterol levels in dams and puppies. While zinc treatment at a dose of 300 mg/L restored hepatorenal parameters to their baseline levels, hepatorenal parameters remained identical to control. At a dose of 100 mg/kg, silver nitrate was found to be more hazardous than AG-NPs. (see table 1, table 2).

Group 1 served as control, Group2 received 1/20 of LD50 of silver nitrates (equivalent to 50 mg/kg), group 3 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg), group 4 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg) + 300 mg of zinc chloride and group 5 received 1/10 of silver nanoparticle (equivalent to 100 mg/kg). p value at ≤ 0.05 considered significant between groups.

Table 2 shows that silver nitrates treatments increased liver toxicity as seen by higher levels

of ALT and AST, as well as produced renal damage as evidenced by increased levels of creatinine and urea, particularly in the feti of rat dams given a dose of 100 mg/kg silver nitrates. Zinc chloride improved the hepatorenal functions of rat dams treated with silver nitrates at a level of 100 mg/kg. Furthermore, nano-silver had a lower harmful effect in feti than silver nitrates, as evidenced by lower levels of ALT, urea, creatinine, and cholesterol in nano-silver treated dams compared to silver nitrates treated dams, with the exception of the level of AST.

Table 2: Silver nitrates have hepatorenal consequences in rat puppies, as demonstrated by serum biochemical markers.

	ALT	AST	UREA	Creatinine	cholesterol
Group 1 Control	7.2010±1.03	44.2329±3.3	33.7310±2.4	0.2433±0.01	29.6667±4.3
Group 2	10.3433±1.04	36.3333±1.8	76.5610±2.9	0.3563±0.02	45.8733±3.7
Group 3	19.7800±1.03	50.0867±3	119.5553±6.1	0.5957±0.09	62.0500±2.2
Group 4	7.2333±0.08	33.3333±1.2	36.3333±2.02	0.2467±0.08	29.6667±4.8
Group 5 (AG-NPS)	16.72±1.83a	81.13±7.37a	80.27±6.48ab	0.3849±1.86	11.64±0.73a

Group 1 served as control, Group2 received 1/20 of LD50 of silver nitrates (equivalent to 50 mg/kg), group 3 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg), group 4 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg) + 300 mg of zinc chloride and group 5 received 1/10 of silver nanoparticle (equivalent to 100 mg/kg). p value at ≤ 0.05 considered significant between groups.

Table 3 shows that silver nitrates increased oxidant as MDA in a dose-dependent manner as compared to the control group and dramatically reduced GSH and SOD1 levels. Silver nanoparticles were shown to be less hazardous than silver ions, with lower MDA induction and lower GSH and SOD1 reductions than silver nitrate-treated groups. MDA, SOD1, and GSH levels in the zinc chloride group were similar to those in the control group.

Table 3: Oxidant/antioxidant effects of silver nitrates on liver homogenates of dams.

Groups	MDA	GSH	SOD1
Group 1	614.1171±32.70247	6.5143±0.32617	399.7194±35.7
Group 2	712.6250±13.45752	5.6625±0.29395	355.0000±30.7
Group 3	800.5000±33.46925	4.8750±0.12500	286.3750±28
Gropup4	614.0764±32.73509	6.4845±0.37571	399.7194±35
Group 5 (AG-NPS)	625.2097±31.707	6.796657±0.125	407.0506±35.2

Group 1 served as control, Group2 received 1/20 of LD50 of silver nitrates (equivalent to 50 mg/kg), group 3 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg), group 4 received 1/10 of LD50 of silver nitrates (equivalent to 100 mg/kg) + 300 mg of zinc chloride and group 5 received 1/10 of silver nanoparticle (equivalent to 100 mg/kg). p value at ≤ 0.05 considered significant between groups.

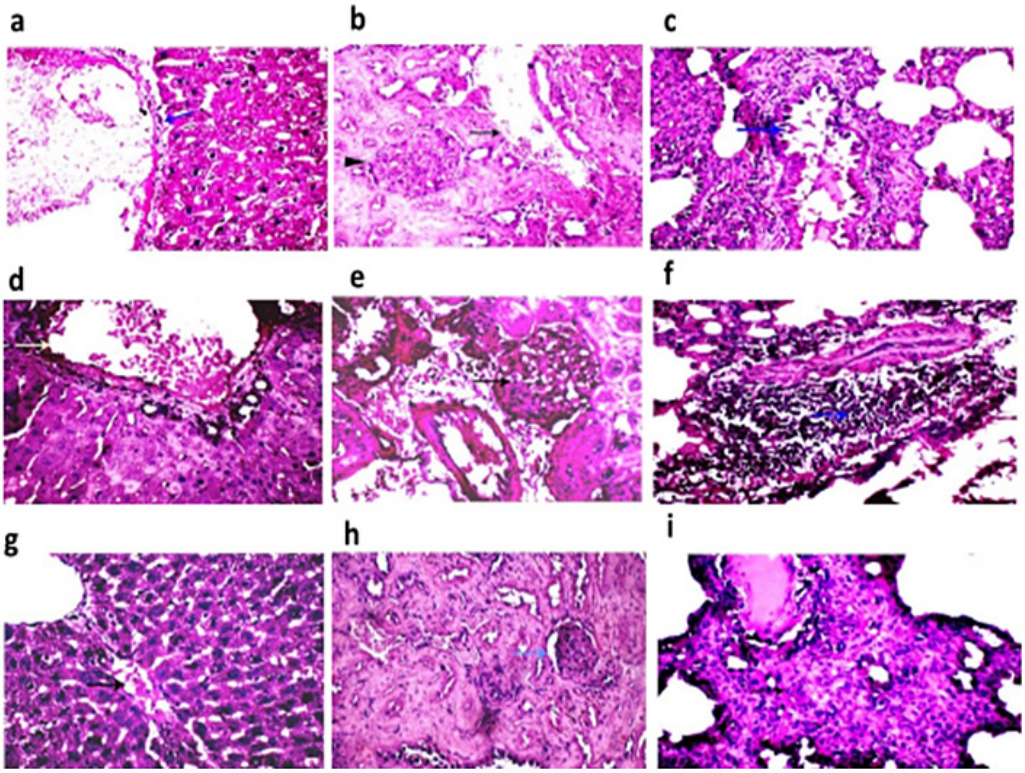

Fig.1: Dams of rats treated with AGNO3 1/10 LD50 (a), proliferation of the renal glomeruli and bleeding in interstitial renal tissue (HE, 400x) (b), and necrosis and desquamation of the bronchiolar epithelium (HE, 400x) (c). While dams of rats treated with AGNO3 1/20 LD50 display hepatic blood vessel congestion (HE, 400x) (d), proliferation of renal glomeruli and neutrophilic recruitment (HE, 400x) (e), and proliferation of interstitial tissue, with pulmonary atelectasis. (400x, HE) (f). Finally, dams of rats given AGNO3 1/10 LD50 + zinc chloride therapy showed minor congestion of hepatic sinusoids (HE, 400x) (g), lymphocytic infiltration in interstitial renal tissue (HE, 400x) (h), and proliferation in interstitial pulmonary tissue (HE, 400x) (i)

Pathological Findings

Hemorrhage, necrosis, and leucocytic infiltration in the liver, alveolar atelectasis, and renal degeneration were all caused by silver nitrates. Furthermore, silver nitrates were more harmful than nano-silver since their nanoparticle only caused intralobular fibroblastic growth in the liver, bleeding in the lungs, glomerular capillary congestion, and renal glomeruli proliferation. In addition, zinc therapies reduced the negative effects of silver nitrates on liver and kidney tissue but did not restore lung tissue to its original state (fig 2).

Moreover, feti of dams received silver nitrates displayed a signet ring appearance of the hepatocytes where sharp clear vacuoles pushing nucleus of hepatocytes to the periphery, kidney showed a proliferation of the mesangial cells in the renal glomeruli and degenerative changes in the renal tubular epithelium lining renal tubules and lung shown an atelectasis of pulmonary alveoli and compensatory emphysema in neighboring alveoli (fig 3 a-d). While the feti of dams treated with silver nanoparticles showed a local necrosis of hepatocytes, the kidney of a suckling feti (21 day old age) from dam received 100 mg/kg (1/50 LD50) AG-NPs from delivery until weaning, showing a congestion of interstitial blood capillaries, and the lung of a suckling feti (21 day old age) from dam received 100 mg/kg (1/50 LD50 (fig 3 d-f)

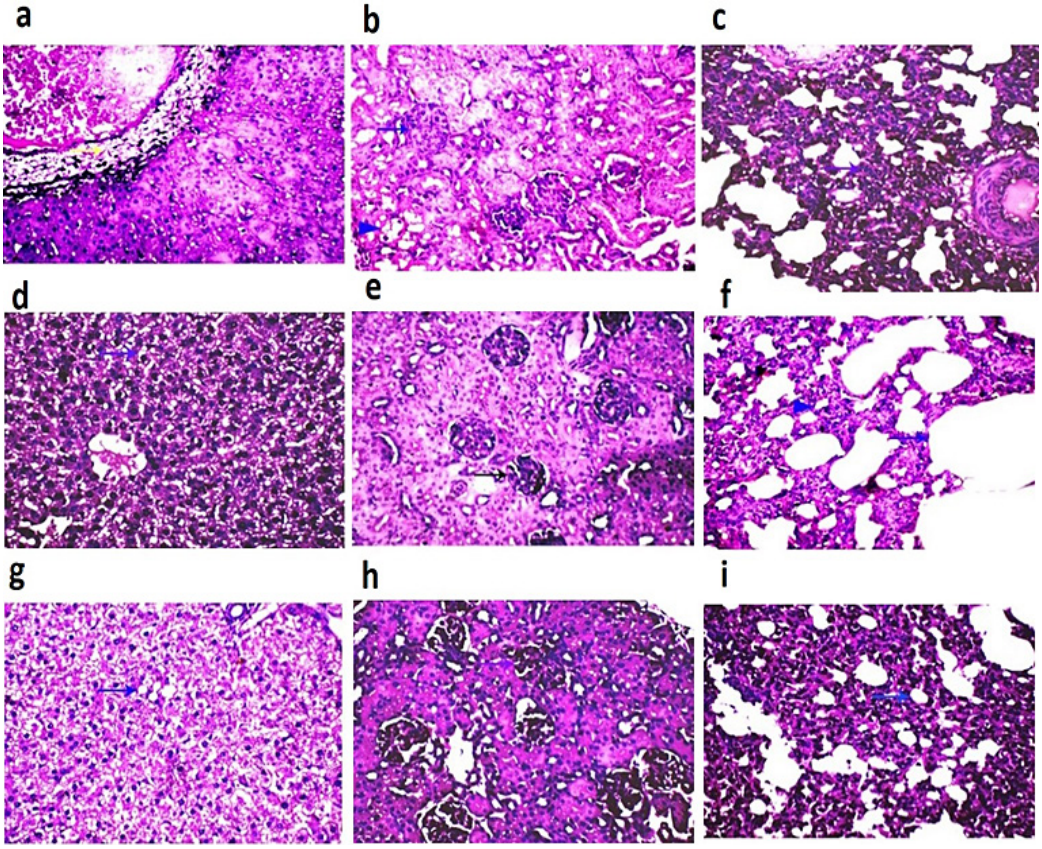

Fig. 2: The liver of feti of dams treated with 1/10 of LD50 shows signet ring appearance of hepatocytes with sharp clear vacuoles pushing the nucleus of hepatocytes to the periphery (HE, 400x) (a), and the kidney shows proliferation of mesangial cells in the renal glomeruli (arrow) and degenerative changes in the renal tubular epithelium lining renal tubules (HE, 400x) (b) (b). Atelectasis of pulmonary alveoli and compensatory emphysema in surrounding alveoli (HE, 400x) are visible in the lung (c). While feti of dams treated with 1/20 of LD50 showed congestion of hepatic blood vessels (HE, 400x) (d), proliferation of renal glomeruli and neutrophilic recruitment (HE, 400x) (e), and proliferation of interstitial tissue with pulmonary atelectasis (HE, 400x) (f). (400x, HE) (f).Finally, the feti of zinc treated groups displayed little degenerative changes of hepatocytes (HE, 400x) (g), proliferation of the renal glomeruli (HE, 400x) (h) and proliferation of interstitial tissue, with collapsed pulmonary alveoli. (HE, 400x) (i).

Discussion

Toxicological studies on silver nitrates and its nanoparticle on animal, human, and environmental health have increased because of the increased usage of silver nanoparticle in animal feed and human medicinal resources.[21] We previously reported on silver nanoparticles' maternal toxicity in lactating female albino rats and their puppies.[8] When compared to the control group, silver nitrates or nano-silver treatments had no influence on body weight, liver to body weight ratio, or puppies after 21 days. Similarly, after 28 days of repeated oral administration, both silver nitrates and its nano form had no influence on the body weights of male and female rats, according to Qin *et al*. [21] and kim *et al*.[22]

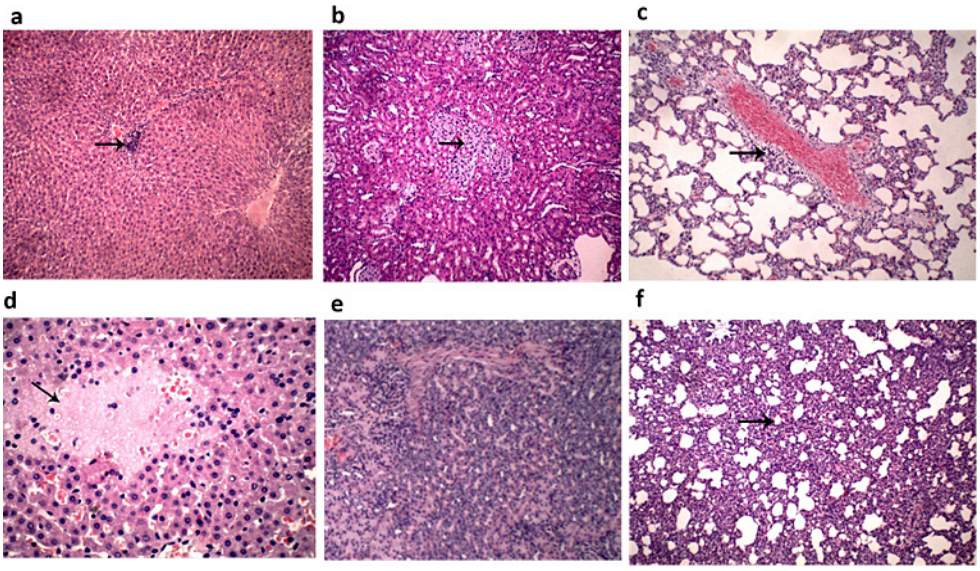

Fig. 3.Dam's liver was exposed to 100 mg/kg (1/50 LD50) AG-NPs from the time of birth till weaning, resulting in intralobular fibroblastic proliferation (arrow). (400x, HE) (a). The dam's kidney received 100 mg/kg (1/50 LD50) AG-NPs from the time of parturition until weaning, resulting in glomerular capillary congestion and renal glomeruli growth (arrow). (100x, HE) (b). The dam's lungs were exposed to 100 mg/kg (1/50 LD50) AG-NPs from the time of birth until weaning, resulting in interlobular blood capillary congestion and neutrophilic recruitment into the pulmonary tissue (arrow). (100x, HE) (C). From delivery to weaning, the liver of a suckling feti (21 days old) of dams received 50 mg/kg (1/100 LD50) AG-NPs displaying local hepatocyte necrosis (arrow). (400x, HE) (D).From delivery until weaning, kidneys of suckling feti (21 days old) from dam received 100 mg/kg (1/50 LD50) AG-NPs, demonstrating constriction of interstitial blood capillaries (arrow). (100x, HE) (e). From delivery to weaning, the lungs of suckling feti (21 days old) from the mother were exposed to 100 mg/kg (1/50 LD50) AG-NPs, resulting in interstitial tissue growth and pulmonary atelectasis (arrow). (100x, HE) (f).

When compared to the control group, silver nitrates' cytotoxicity was more evident than its nano-form in nursing female albino rats after 21 days of treatment. This toxicity was revealed by an increase in liver enzymes, creatinine, blood urea nitrogen, cholesterol, and a decrease in total protein and albumin levels, despite the fact that our previous study found that silver nanoparticles caused only minor non-significant changes in liver and kidney function tests.[8,23] Furthermore, silver nanoparticles caused less pathogenic changes than silver ions, as demonstrated by Qin *et al*.[21] kim *et al*.[22] and Adeyemi *et al*.[23] While Silver nitrates caused liver, kidney, and lung damage in both the dams and their offspring. Notably, zinc chloride treatment reduced silver nitrate cytotoxicity, as demonstrated by biochemical and histological changes in the liver and kidneys that were identical to the control group. In rats, zinc showed a protective effect against silver-induced damage.[14] Similarly, zinc increased the antioxidant state of nickel-treated rats' livers[15] Zinc also protected the liver and kidneys from mercury-induced damage.[16,17]

Acknowledgments

We grateful thanks for Eman Abdulrahim for help during carrying out the experimental research in forensic medicine and toxicology department, faculty of veterinary medicine, Mansoura university.

Funding

The authors declare that there is no funding source.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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