Zinc Phosphide-Induced Hepato-Nephrotoxicity in Wistar Rats: The Ameliorative Role of Curcumin from *Curcuma longa* Rhizome

Kemi F. Akinwunmi[1*], Emmanuel B. Ofeniforo[2] and Kehinde H. Fasunle[1]

[1]*Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.*
[2]*Department of Biochemistry, University of Ilorin. Kwara State, Nigeria.*

Authors' contributions

Original Research Article

ABSTRACT

Globally used pesticides contains zinc phosphide (ZnP) which are toxic. This present study was carried out to investigate the potency of bioactive curcumin in ameliorating the toxicity of zinc phosphide on biochemical enzymes present in kidney and liver of Wistar rats. A total of 30 (120–150 g) male adult Wistar rats were used. Experimental animals were divided into five groups and treated as follows for a period of 21 days: Group I rats, serving as the control, orally received 1 ml/kg body weight of corn oil with administration of same volume of saline. Group II rats were orally administered Zinc phosphide at a dose of 4.57 mg/kg body weight (one-tenth LD50) in corn oil. Group III rats orally received curcumin at a dose of 100 mg/kg body weight. Groups IV and V rats were orally administered curcumin at graded doses of 100 and 200 mg/kg body weight respectively, 2 hours before administration of Zinc phosphide. At the end of the time interval, experimental animals were anesthesized with diethylether and organs (kidney and liver) were

Corresponding author: E-mail: kfarinde@yahoo.com, kakinwunmi@oauife.edu.ng;

harvested for biochemical assays. The oral administration of Zinc phosphide at 4.57 mg/kg body weight for 21 days resulted in a significant increase in hepatic and nephridial malondialdehyde. This index of lipid peroxidation, was accompanied by decreased activity of the antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) followed by a drastic reduction in the non-enzymatic antioxidant indices of reduced glutathione when compared to control. Pre-administration of Curcumin significantly ameliorated zinc phosphide-induced hepatic and nephrotic effects by subduing oxidative stress indices and improving antioxidant status. The result of the present study shows that curcumin has a protective effect against zinc phosphide induced liver and kidney damage in male Wistar rats.

1. INTRODUCTION

The standard of human health has improved by controlling vector-borne diseases using pesticides. However, indiscriminate use has resulted in serious health defects. Most commonly accessible pesticides contain zinc phosphide (ZnP) as its active substance. Zinc phosphide (ZnP) is a dark grey crystalline compound commonly used as a rodenticide against rats, mice and field mice [1]. Life-threatening complications occur from intentional intake of pesticides for suicidal purposes as highly prevalent in Asian countries [2]. Other fatal exposures are unintentional. Zinc phosphide poisoning is a significant cause of morbidity and mortality in developing African countries [3]. Exposure to this chemical is either through inhalation, ingestion, or penetration via skin [4]. Toxic effects are produced when the concentration of pesticide in the body increases far more than its initial concentration in the environment [5].

The use of pesticides has increased over the past few decades thus the likelihood of exposure to these chemicals and its toxic effects has also increased considerably. The effects of pesticides on human health may be acute or chronic. Which include headache, stinging of the eyes and skin, skin itching, irritation of the nose and throat, appearance of the rash and blisters on the skin, dizziness, diarrhea, abdominal pain, nausea and vomiting, blurred vision, blindness, damage to multiple body organs such as liver, lungs, kidneys [6].

ZnP exerts its toxic effects through the liberation of highly toxic gas phosphine (PH_3), which is released under aqueous conditions [7]. The deadly gas phosphine is a potent respiratory chain enzyme inhibitor that inhibits cellular respiration and induces oxidative stress in insects and mammalian cells [8]. Its most important effect is the inhibition of cytochrome c oxidase. The inhibition of cytochrome c oxidase and other enzymes leads to the generation of superoxide radicals and cellular peroxides and subsequent cellular injury through lipid peroxidation and other oxidant mechanisms [9].

Turmeric (*Curcuma longa* Linn) belongs to the family of Zingiberaceae. It is valued as a functional food because of its health promoting potentials [10]. Turmeric comprised of a group of three curcuminoids: curcumin (diferuloylmethane), demethoxycurcumin and bisdemethoxycurcumin which are found to be natural antioxidants [11]. Curcumin has been reported to be effective in protecting the liver by reducing oxidative stress in carbon tetrachloride (CCl_4) induced liver injury [12]. Alp et al. [13] reported the effect of curcumin against oxidative stress induced by malathion (MAL) an organophosphorus insecticide (OPI).

Information on the protective effect of curcumin on zinc phosphide-induced toxicity is sparse in literature. Additionally, the effect of curcumin on Zinc phosphide-induced toxicity has not been established. Therefore, the present work was carried out to investigate the effect of zinc phosphide exposure on the rat liver and kidney and the potential of curcumin as a protective agent against zinc phosphide induced oxidative damage in Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Zinc Phosphide, Trichloroacetic acid (TCA), and Adrenaline (Epinephrine) were procured from Sigma-Aldrich (Munich, Germany), while trichloroacetic acid and thiobarbituric acid were by Qualigens Fine Chemicals (Mumbai, Maharashtra, India). All other chemicals were of analytical grade.

2.2 Plant Collection and Extraction of Curcumin from Curcuma Longa

Rhizomes of turmeric (*Curcuma longa*) were collected from Akinola market, Ile-Ife Nigeria in March, 2019. The rhizome was authenticated at Ife herbarium Obafemi Awolowo University. They were washed, sliced and allowed to dry under shade and ground into uniform powder using a warring blender. Dried and powdered rhizome of *C. longa* was extracted with ethylacetate using Soxhlet extraction method [14]. The ethylacetate extract was concentrated using rotary evaporator. The dried extract was re-dissolved in n-Hexane and the solution was centrifuged and the pellets were collected, dried and broken into curcumin powder.

2.3 Animal Treatment

Thirty male Wistar rats (120–150 g) aged between 10 and 12 weeks were procured from the animal house, Faculty of Pharmacy, Obafemi Awolowo University Ile-Ife. Rats were kept in plastic cages in a ventilated room under controlled laboratory conditions of normal light–dark cycle (12 h light/dark) and temperature (25 + 2°C). The animals had access to food and water *ad libitum.* Experimental design and animal handling were executed according to the guidelines in Guide for the Care and Use of Laboratory Animals published by the National Institute of Health [15].

2.3.1 Experimental design

Rats were randomly assigned to five different groups of five animals each after 2-weeks of acclimatization period. Group I rats, served as the control, orally received 1 ml/kg body weight (bwt) of corn oil, 2 hours before administration of same volume of saline. Group II rats were orally administered ZnP at a dose of 4.57 mg/kg bwt (one-tenth LD50) in corn oil. Animals in group III orally received curcumin dissolved in saline, at a dose of 100 mg/kg bwt. Groups IV and V were orally administered curcumin at doses of 100 and 200 mg/kg bwt, respectively, 2 hours before treatment with ZnP. All treatments were given for 21 days. The dose of one-tenth LD50 for ZnP was chosen to produce a sub-lethal toxic effect in the animal. Twenty-four hours after the last treatment, the rats were anaesthetized using diethyl ether and killed by cervical dislocation.

The liver and kidney were excised and processed for biochemical assays after been cleared from other adhering tissues.

2.4 Biochemical Assays

2.4.1 Determination of malondialdehyde (MDA) concentrations

The level of lipid peroxidation was quantified by MDA content in the liver and kidney. Hepatic and Nephridial MDA was determined using the thiobarbituric acid reactive substance assay, as described by [16]. Briefly, 10% of homogenate (liver and kidney) was prepared in 0.15 M KCl. One volume of homogenate was mixed thoroughly with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 M hydrochloric acid. The mixture was heated for 15 min in a boiling water bath. After cooling, the mixture was centrifuged at 1000 rpm for 10 min to remove the precipitate. The absorbance of the clear supernatant was determined at 535 nm and MDA concentration calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$. The MDA concentration was expressed as nmol per gram of wet tissue.

2.4.2 Determination of catalase activity

The activity of CAT was measured by the method of [17]. In brief, the assay mixture maintained at 20°C consisted of 0.1 ml of homogenate (liver /kidney), 1.9 ml of 50 mM phosphate buffer and 1 ml of 30 mM H_2O_2. The decomposition of H_2O_2 was continuously monitored spectrophotometrically at 240 nm for 60 s. The change in absorbance was the measure of CAT activity and was expressed as nmol/mg protein.

2.4.3 Determination of Superoxide Dismutase (SOD) activity

The method of Misra and Fridovich [18] was used in determining SOD activity in the rat homogenates (liver/kidney). The reaction was initiated with the addition of 0.3 ml of epinephrine (0.01%) to the mixture of 2.5 ml carbonate buffer (0.05 M) and 0.2 ml sample. Change in absorbance was measured at 480 nm, and the activity was expressed as unit per milligram of protein. One unit was defined as the amount of enzyme required to inhibit the rate of autooxidation by 50%.

2.4.4 Determination Glutathione Peroxidase (GPx) activity

The assay of GPx activity was carried out according to the method of Rotruck [19]. Liver/kidney homogenate (0.1 ml) was mixed

with 0.5 ml of 0.2 M phosphate buffer, pH 8.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of 4 mM GSH, 0.1 ml of 2.5 mM H_2O_2 and 1 ml of distilled water. The mixture was incubated at room temperature for 3 min and 0.5ml of 10% (w/v) TCA was added. The mixture was centrifuged at 3000 rpm for 10 min. About 0.1 ml of the supernatant was mixed with 0.9 ml of 0.3 M disodium hydrogen phosphate and 1 ml of freshly prepared 0.6 mM 5,5' –dithiobis-2-nitrobenzoic acid (DTNB) also known as Ellman's reagent in 0.2 M sodium phosphate buffer, pH 8.0. The absorbance was read at 412 nm against the blank which contained distilled water.

2.4.5 Determination of Reduced Glutathione (GSH)

The reduced glutathione level in the homogenate (liver/kidney) was estimated according to the method of Moron [20]. Typically, 1 ml of liver/kidney homogenate was mixed with 5% trichloroacetic acid (TCA, 4 ml), followed by centrifugation at 4000 rpm for 10 min. The supernatant was collected and the residue discarded. The reaction mixture consisted of supernatant (0.1 ml), 0.9 ml of 0.2 M sodium phosphate buffer, pH 8.0 and 2ml of freshly prepared 0.6 mM 5,5'- dithiobios (2-nitrobenzoic acid). The reaction mixture was mixed properly and the absorbance was read at 412 nm after 10 min against the reagent blank. The GSH level was expressed as µg GSH/g of sample.

2.5 Statistical Analysis

One-way analysis of variance was used for the statistical analysis after which multiple comparisons were carried out by Tukey's test. The results were expressed as the mean ± standard deviation (SD) and values with p < 0.05 were considered statistically significant. Data were analysed using GraphPad Prism version 6.49.

3. RESULTS

The changes in the levels of lipid peroxidation products in control and treated rats are presented in Table 1. The levels of hepatic and nephrotic MDA were significantly increased in ZnP-treated rats compared with the control group (p < 0.05). Conversely, pre-administration of curcumin significantly decreased the levels of lipid peroxidation products (MDA) (p < 0.05), almost to the levels found in the control group. There was no significant change in lipid peroxidation product levels in liver/kidney of rats administered curcumin alone in comparison to the control group.

Data are expressed as mean ± SD of five animals per group [b]Significant difference compared to normal control at p<0.05. [c]Significant difference compared to Zinc Phosphide group at p< 0.05.

The enzymatic antioxidant status in control rats and those treated with zinc phosphide or in combination with curcumin is presented in Table 2. There was no significant difference in the activities of CAT, GPx, GSH and SOD in rats treated with curcumin when compared with the control. Treatment with zinc phosphide alone resulted in decrease in activities of CAT, GPx and GSH level when compared with the control (p<0.05). Pretreatment with curcumin significantly reverse the reduction in activities by stimulating the activities to near control in curcumin pretreated rats. The SOD activity was significantly reduced in rats treated with zinc phosphide alone when compared with the control (p<0.05). Curcumin administration restore SOD activity to near normalcy in rats pretreated with curcumin when compared with the control (p<0.05).

The administration of Zinc phosphide significantly reduced CAT and GPx activities in rat kidney when compared with the control (p<0.05) Table 3. It also significantly decreased SOD activities

Table 1. Hepatic and nephridial MDA levels in rats after zinc phosphide exposure and pretreatment with curcumin

Groups	MDA Concentration (nmol/mg) in the Liver	MDA Concentration (nmol/mg) in the Kidney
Control	19.19 ± 0.460	2.28 ±0.02
Zinc Phosphide	24.72 ± 0,057[b]	3.78 ±0.03[b]
Curcumin	17.78 ± 1.102[c]	2,68 ±0.17[c]
Curcumin (100mg) + Zinc Phosphide	21.11 ± 0.476[c]	2.59 ±0.07[bc]
Curcumin (200mg) + Zinc Phosphide	19.59 ±0.679[c]	2.43 ±0.03[c]

Table 2. Hepatic antioxidant status in rats after zinc phosphide exposure and treatment with curcumin

Groups	CAT (nmole/mg protein)	GPx (U/mg protein)	GSH (µmol/ mg tissue)	SOD (U/mg protein)
Control	1.339±0.029	0.305 ±0.002	216.7 ±0.260	16.24 ±0.120
Zinc Phosphide	0.658 ±0.007[b]	0.267 ±0.005[b]	110.0 ±0.550[bc]	9.06 ±0.140
Curcumin	1.449 ±0.023[c]	0.308 ±0.002[c]	233.3 ±0.720[c]	16.56 ±0.080
Curcumin(100 mg) + Zinc phosphide	1.153 ±0.256[c]	0.284 ±0.005	190 ± 0.090[c]	15.54 ±0.100
Curcumin(200 mg) + Zinc Phosphide	1.277 ±0.132[c]	0.298 ±0.012[c]	230 ±0.550[c]	15.92 ±0.200

CAT: catalase; GPx: Glutathione peroxidase; GSH: reduced glutathione; SOD: superoxide dismutase. Data are expressed as means ± SD of five rats per group. [b]Significant difference compared to control at p <0.05. [c]Significant difference compared to zinc phosphide group at p<0.05

Table 3. Nephrotic antioxidant status in rats after zinc phosphide exposure and treatment with curcumin

Groups	CAT (nmole/mg protein)	GPx (U/mg protein)	GSH (µmol/ mg tissue)	SOD (U/mg protein)
Control	101.20±0.71	0.73±0.01	3.37±0.06	0.74±0.04
ZnP	74.42±0.21[b]	0.59±0.01[b]	0.95±0.04[b]	0.48±0.03[b]
Curcumin	100.79±0.06[c]	0.67±0.02[c]	3.16±0.15[c]	0.77±0.05[c]
Curcumin (100mg) +ZnP	105.03±0.02[c]	0.58±0.04[c]	2.86±0,35[c]	0.87±0.02[c]
Curcumin (200) +ZnP	103.53±0.81[c]	0.60±0.01[c]	3.48±0.03[c]	0.88±0.03[c]

CAT: catalase; GPx: Glutathione peroxidase; GSH: reduced glutathione; SOD: superoxide dismutase. Data are expressed as means ± SD of five rats per group. [b]Significant difference compared to control at p <0.05. [c]Significant difference compared to zinc phosphide group at p<0.05

and lowers the level of GSH in the kidney of rats treated with Zinc phosphide when compared with the control (p<0.05). Administration of curcumin ameliorated these above changes induced by zinc phosphide which was evident from the restoration to near normal activities of these enzymes in the group administered curcumin.

4. DISCUSSION

Oral administration of zinc phosphide at 4.5 mg/kg bwt induced marked kidney and liver damage in Wistar rats. The kidney carries out vital essential excretory activities in animals making it one of the primary target organ for different circulating toxins that can lead to nephrotoxicity [21]. Liver is one of the important target organ of phosphine poisoning in animals [22]. After ingestion of Zinc phosphide, it liberates the highly toxic gas, phosphine (PH_3) on exposure to moisture. PH_3 gas is rapidly absorbed through the gastrointestinal tract and it is partly carried to the liver by the portal vein. Phosphine can cause liver and kidney

dysfunction especially after the first day of poisoning [23]. Nephrotoxicity is a renal dysfunction that arises due to exposure to chemicals, drugs and toxicants. Oxidative stress is one of the main pathological pathways involved in its etiology [21].

Oxidative degradation of lipid known as lipid peroxidation causes a decrease in membrane fluidity and in the barrier functions of the membrane [24]. Malondialdehyde (MDA) and lipid hydroperoxide (LOOH) are indicators of lipid peroxidation which signifies damage to tissues due to oxidative stress [25]. The exposure of liver and kidney to zinc phosphide in this study significantly increased hepatic and nephrotic MDA levels (Table 1). PH_3 increases the generation of free radicals with subsequent lipid peroxidation [26]. The result obtained in this study is similar to [27] who reported significant increase in MDA levels in liver and kidney of aluminum phosphide treated rats. The mechanism of phosphine induced lipid peroxidation could involve ROS generated from

inhibition of cellular respiration or a direct reaction between phosphine and H_2O_2 [28]. However, the increased MDA levels induced by zinc phosphide administration was significantly reversed by pre-administration of curcumin. Curcumin have been reported to reduce MDA levels [13]. Also, [29] have also demonstrated that curcumin possesses the ability to attenuate lipid peroxidation in rats exposed to Cadmium industrial pollutant.

In tissues, SOD and CAT are considered the first line of defense against the toxic effects of reactive oxygen species [30]. Superoxide radicals are converted to H_2O_2 by SOD which is subsequently converted into water and oxygen by the action of CAT. In this study, decrease in the activities of both SOD and CAT were observed in the liver and kidney of rats administered zinc phosphide. The observed decrease in both SOD and CAT activities indicated a breakdown of enzymatic system due to Zn-P-induced oxidative stress. A reduction in SOD activity usually leads to an increased level of superoxide anion, which can subsequently inactivate CAT activity [31]. Conversely the inability of CAT to prevent H_2O_2 accumulation in the cell could lead to inactivation of SOD. The reduction in CAT activity is therefore suggestive of inactivation of the enzyme and its inability to eliminate H_2O_2 produced by SOD resulting to the accumulation of this free radical in these organs [32]. However, the administration of curcumin increased the activities of both SOD and CAT.

In biological systems GSH and GPx act as detoxifying agents by converting H_2O_2 and lipid peroxides to non-toxic product [33]. In our study, ZnP significantly depleted GSH concentration and caused decline in GPx activity in the liver and kidney of rats. The decline in GSH status could be attributed to an increased oxidative stress triggered by the production of ROS by phosphine gas that induced lipid and protein oxidation [34]. Increased production of lipid peroxide leads to diminished lipid soluble antioxidant system such as GSH. The decreased concentration of GSH increases the sensitivity of organs to oxidative and chemical injury. Depletion of GSH also seems to be a prime factor that permits lipid peroxidation in ZnP treated animals. Pre-administration of curcumin however rendered protection by preventing the oxidation of reduced glutathione and protected its related enzyme from oxidative attack. The enhanced GPx activity by curcumin administration could partly explain the protection of biomembrane from oxidative attack. The potential of curcumin to protect against oxidative stress - induced tissue injury observed in this study is similar to reports by [35,36].

5. CONCLUSION

Based on the data obtained from this study, Curcumin produced its protective effect by preventing increase in lipid peroxidation and by restoring the inhibited activities of enzymatic antioxidants (SOD, *GPx,* GSH and catalase). However, there is neither an antidote nor specific treatment for zinc phosphide toxicity therefore care must be taken to avoid exposure to zinc phosphide in food as well as occupational sources.

CONSENT

It is not applicable.

.
ETHICAL APPROVAL

All animal experiments were conducted according to the institutional principles on the use of laboratory animals and in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

ACKNOWLEDGEMENT

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1.   Bumbrah GS, Krishan K, Kanchan T, Sharma M, Sodhi GS. Phosphide poisoning: A review of literature. Forensic Sci. Int. 2012;214(1-3):1-6.
2.   Bilics Gergely, Júlia Héger, Éva Pozsgai, Gábor Bajzik, Csaba Nagy, Csilla Somoskövi and Csaba Varga. Successful management of zinc phosphide poisoning—a Hungarian case. Int J of Emergency Med. 2020;13:48-57.
3.   Doğan E, Güzel A, Çiftçi T, Aycan İ, Çelik F, Çetin B, Kavak GÖ. Zinc Phosphide

Poisoning. Case Reports in Critical Care. 2014;1–3..
DOI:10.1155/2014/589712

4. Spear R. Recognized and possible exposure to pesticides. In: Handbook of Pesticide Toxicology (Hayes WJ, Laws E, eds.). General Principles. Academic Press, San Diego. 1991;I:245–274.

5. Hayo MG, Werf VD. Assessing the impact of pesticides on the environment. Agric and Ecosystem of Environment. 1996;60: 81-96.

6. Anand R, Kunari P, Kaushal A, Bal A, Wani WY, Sunkaria A, et al. Effect of acute aluminum phosphide exposure on rats: A biochemical and histological correlation. Toxicology Letters. 2012; 215(1):62-9.
DOI: 10.1016/j.toxlet.2012.09.020

7. Kariman H, Heydari K, Fakhri M, Shahrami A, Dolatabadi AA, Mohammadi HA, et al. Aluminium phosphide poisoning and oxidative stress. J of Med Toxicology. 2012;8(3):281–284.
DOI: 10.1007/s13181-012-0219-1

8. Repetto MG, Ferrarotti NF, Boveris A. The involvement of transition metal ions on iron- dependent lipid peroxidation. Archives of Toxicology. 2010a;84:255-262.
DOI: 10.1007/s00204-009-0487-y

9. Chugh SN, Arora V, Sharma A, Chugh K. Free radical scavengers and lipid peroxidation in acute aluminium phosphide poisoning. Indian J Med Research. 1996;104:190-193.

10. Jayaprakasha GK, Rao LJM, Sakariah KK. Chemistry and biological activities of *C. longa*. Trends in Food Sci and Tech. 2005;16(12):533-548.
DOI: 10.1016/J.TIFS.2005.08.006

11. Ruby AJ, Kuttan G, Dinesh Babu K, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Letters. 1995;94(1):79–83.
DOI: 10.1016/0304-3835(95)03827-j.

12. Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Molecular Pharmacology. 2008;73:399-409.
DOI: 10.1124/mol.107.039818

13. Alp H, Aytekin I, Hatipoglu NK, Alp A, Ogun M. Effects of sulforophane and curcumin on oxidative stress created by acute malathion toxicity in rats. European

Review for Med and Pharmacological Sci. 2012;16:144-148

14. Pawar HA, Gavasane AJ, Choudhary PD. A novel and simple approach for extraction and isolation of curcuminoids from Tumeric Rhizomes. Nat Prod Chem Res. 2018;6(1).
DOI: 10.4172/2329-68361000300.

15. NRC. National research council committee for the update of the guide for the care and use of laboratory animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.

16. Buege JA, Aust SD. Microsomal lipid peroxidation. Biomembranes - Part C: Biological Oxidations. 1978;302–310.
DOI: 10.1016/s0076-6879(78)52032-6

17. Aebi H. Catalase. In: Bergmeyer HU., Ed., Methods of enzymatic analysis, verlag chemie/academic Press Inc., Weinheim/NewYork. 1974;673-680.
DOI: 10.1016/b978-0-12-091302-2.50032-3

18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972;247:3170–3175.

19. Rotruck JT, Pope AL, Ganther HE. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179:588–590.
DOI: 10.1126/science.179.4073.588

20. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta1979;582:67–78.
DOI: 10.1016/0304-4165(79)90289-7

21. Sharma S, Sanjula B, Saima A, Showkat RM. Ameliorative effect of a standardized polyherbal combination in methotrexate-induced nephrotoxicity in the rat, Pharmaceutical Biology. 2020;58(1):184-199.

22. Sudakin DL. Occcupational exposure to aluminium phosphide and phosphine gas? A suspected case report and review of the literature. Hum Exp Toxicol. 2005;24(1):27-33.
DOI: 10.1191/0960327105ht496oa

23. Saleki S, Ardalan FA, Javidan-Nejad A. Liver histopathology of fatal phosphine poisoning. Forensic Sci Int. 2007;166:190-193.
DOI: 10.1016/j.forsciint.2006.05.033

24. Dianzani M, Barrera G. Pathology andphysiology of lipid peroxidation and its carbonyl products, inAlvarez S and Evelson P (ed), Free Radical pathophysiology. Transworld Research Network, Kerala, India. 2008;19-38.
   ISBN: 978-81-7895-311-3.

25. Repetto MG, Boveris A. Transition metals: Bioinorganic and redox reactions in biological systems. *In:* Transition metals: uses and characteristics. Nova Science Publishers Inc (ed.)*:* New York, USA 349-370;2012.
   ISBN: 978-1-61761-110-0.

26. Mashali AA, Salama N, Elsobky HA, Sobh ZK. Prediction of zinc phosphide-induced hepatotoxicity and cardiotoxicity from clinical, laboratory, and radiological indicators. Environmental Science and Pollution Research. 2020;10:1007-10020.

27. Yousef MI, Soliman NF, El-Demerdash FM. Aluminium phosphide-induced hepato-nephrotoxicity and oxidative damage in rats: The protective effect of α-lipoic acid. Open Conf Proc J. 2005;6:18–23.
   DOI: 10.2174/2210289201506010018

28. Quistad GB, Sparks SE, Casida JE. Chemical model for phosphine induced lipid peroxidation. Pest Manage Sci. 2000;56:779-83.
   DOI: 10.1002/15264998(200009)56:9<779::aid-

29. Eybl V, Kotyzova D, Bludovska M. The effect of curcumin on cadmium-induced oxidative damage and trace elements level in the liver of rats and mice. Toxicology Letter. 2004;151:79-85.
   DOI: 10.1016/j.toxlet.2004.02.019

30. Farombi EO, Adedara IA, Abolaji AO, Anamelechi JP, Sangodele JO. Sperm characteristics, antioxidant status and hormonal profile in rats treated with artemisinin. Andrologia. 2003;46(8):893–901.
   DOI: 10.1111/and.12170

31. Afolabi OK, Wusu AD, Ugbaja R, Fatoki JO. Aluminium phosphide- induced testicular toxicity through oxidative stress in Wistar rats: Ameliorative role of hesperidin. Toxicology Research and Application. 2018;2:1-11.
   DOI: 10.1177/2397847318812794

32. Sinet PM, Garber P. Inactivation of the human CuZn superoxide dismutase during exposure to O_2_ and H_2O_2. Arch Biochem Biophys. 1981;212: 411–416.
   DOI: 10.1016/0003-9861(81)90382-9

33. Turner TT, Lysiak JJ. Oxidative stress: A common factor in testicular dysfunction. J Androl. 2008;29:488–498.
   DOI: 10.2164/jandrol.108.005132

34. El-Desoky GE, Bashandy SA, Alhazza IM. Improvement of mercuric chloride-induced testis injuries and sperm quality deteriorations by Spirulina platensis in rats. PLOS One. 2013;8:e59177.
   DOI: 10.1371/journal.pone.0059177

35. Sankar P, Telang AG, Manimaran A. Protective effect of curcumin on cypermethrin-induced oxidative stress in Wistar rats. Experimental Toxicology and Pathology. 2012;64(5):487-493. DOI: 10.1016/j.etp.2010.11.003.

36. Al-Harbi MS, Hamza RZ, Daway AA. Ameliorative effect and curcumin on sodium fluoride induced hepatotoxicity and oxidative stress in male mice. Journal of Chemical and Pharmaceutical Research. 2014;6(4):984-998.