



Hepatic damage associated with fatal zinc phosphide poisoning in broiler chicks



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Abstract Zinc phosphide (Zn_3P_2) is a widely used rodenticide which has the potential to cause high mortality if ingested. The present study was designed in order to explore the hepatic injury in broiler chicks that were acutely intoxicated with Zn_3P_2 . For this purpose, a total number of 12 broiler *Saso* chicks were divided into two equal groups. Birds of the first group were exposed to 300 ppm Zn_3P_2 via food. Hepatic damage of intoxicated birds was evaluated biochemically and histologically using the transmission electron microscope and subsequently compared with another healthy non-treated controls (second group). The serum activity of aspartate aminotransferase (AST) was significantly higher in those poisoned with Zn_3P_2 . While, activities of both Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), as well as, zinc concentration of hepatic tissue did not represented a significant difference between treated and control birds. Histological examination revealed presence of numerous heterogenic shaped mitochondria in hepatocytes of non-treated birds. Glycogen deposits were also scattered in the form of large electron dense deposits. Kupffer cell was irregular in shape and had numerous pseudopods often projected into sinusoidal lumen. In hepatic cells of intoxicated birds, mitochondrial swelling with cristolysis, few glycogen deposits, vacuoles in the cytoplasm and shrunken darkly stained nuclei are the major ultra-structural changes which were detected. It was concluded that the mitochondria could be one of the main target in hepatocytes for the toxic effect of Zn_3P_2 in broiler chicks.

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1. Introduction

Zinc phosphide (Zn_3P_2) is a dull greyish black powder which has been used as rodenticide since the early 1930s [1]. Accidental

toxicity by Zn_3P_2 has been reported in non-target animal species including birds [2]. Zn_3P_2 has a disagreeable odor resembling acetylene or rotten fish. It is hydrolyzed in the acidic environment of the stomach, liberating phosphine gas and free radicals [3]. Phosphine acts as a respiratory poison. It blocks the enzyme cytochrome C oxidase as a result of which mitochondrial oxidative phosphorylation is inhibited, causing, in turn, the cells to die rapidly. The inhibition of mitochondrial cytochrome C oxidase leads to pulmonary and cardiac toxicity.

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This can lead to a blockage of mitochondrial electron transport chain [4].

Although lungs, heart, gastrointestinal tract and kidney are considered the major targets for Zn_3P_2 in chickens [5], it is also known that phosphine can induce hepatic dysfunction, especially after the first day of poisoning. The most frequently observed histopathological lesions in the liver were sinusoidal congestion, cytoplasmic vacuolization, centrilobular necrosis, dilated central vein and nuclear fragmentations [6–8]. According to Gupta [9], phosphine causes CNS depression, irritation of the lungs, and damage to the liver, kidney, heart, and CNS. Death occurs as a result of heart failure and/or pulmonary edema. Following a large dose, death usually occurs within an hour, while with smaller doses, death can occur between 4 and 72 h.

However, there are insufficient data about the ultrastructural changes of chicken hepatocytes after the exposure to lethal dose of Zn_3P_2 .

As described in the previous studies carried out by Purton, Ohata and Ito [10–12] the ultrastructure of chicken liver parenchyma was fundamentally similar to that of mammalian liver; however, there were a few differences. The inter anastomosing hepatocyte plates as recognized and termed in mammalian liver were more like cords or tubules in the chicken. They were two cells thick, limiting the contact between surface of hepatocytes and blood to only one side of the cell. In the past this has given rise to the suggestion that the mammalian liver structure as sheets of single cell thickness provided a better constructional stability compared to that of the avian species. Also, it is essential for the greater physiological efficiency by increasing the contact surface between hepatocytes and blood [13].

In this study, the ultrastructural modification in chick's hepatocytes after acute experimental Zn_3P_2 intoxication as well as the associated serum biochemical alterations with special attention to hepatic zinc concentration as a possible marker element in cases of suspected Zn_3P_2 poisoning are scrutinized.

2. Materials and methods

All procedures conducted in this experiment were approved by the local authorities (Faculty of Veterinary Medicine, Alexandria University, Egypt).

2.1. Birds and experimental design

Twelve broiler *Saso* chicks were obtained from a commercial hatchery on their first day of life and were housed in cages. They were provided by incandescent light for heating ($30 \pm 2^\circ\text{C}$) and with free access to food and water. Birds were kept under supervision for 2 weeks without any medication for detection of any abnormal signs or behavior, and then were divided into two groups of 6 birds in each. The chicks of the first group were exposed to a commercial diet (yellow corn, soya bean meal, gluten, minerals and vitamins with 23% protein) supplemented with commercial product of Zn_3P_2 (RAT KILL 80%) at dose of 300 ppm, while the birds of the second group served as control and not subjected to any type of treat-

ment. Chicks were clinically observed for signs of toxicity and a particular attention was paid to time of death.

2.2. Sampling

At the moment of death, which guided by gasping (stop of respiration), blood samples were collected from wing vein in sterile test tubes without anticoagulant to obtain serum for liver functions assay. In control birds, blood was collected from wing vein and then euthanized by slaughtering. Quickly after death, liver was rapidly extracted from birds and divided into two parts: The first part was kept frozen at -20°C until the assessment of zinc concentration. The second part was rapidly submersed in cold 4FIG fluid which consists of 4% formaldehyde and 1% glutaraldehyde for transmission electron microscopic examination.

2.3. Liver function assay

Serum activity of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were measured spectrophotometrically using commercial diagnostic kits obtained from Vitro Scient Co. (Egypt) according to the manufacture instructions.

2.4. Zinc concentration

Zinc concentration in liver samples was measured using 4100 MPAES Spectrometer (Microwave Plasma-Atomic emission Spectrometry: Agilent Tech., Santa Clara, CA, USA) at wave length 213.857 nm according to the method described by Jorhem and Engman [14]. Metal concentration is expressed on a wet weight basis as ppm.

2.5. Examination of hepatic tissue using transmission electron microscope

Pieces of 1 mm were cut from the liver samples using sharp blade and quickly fixed in 6% solution of phosphate buffered glutaraldehyde pH 7.4 for 6 h at 4°C [15]. After initial fixation, the tissues were washed several times in cold (4°C) 0.1 M phosphate buffer every 15 min for 2 h. The tissues were post fixed in 1% solution of cold osmium tetroxide (OsO_4) 0.1 M buffer pH 7.2 for 2 h. Then, rapidly dehydrated through ascending grades of ethyl alcohol and transferred to a 1:1 mixture of propylene oxide and epoxy araldite. Semi-thin sections ($1\ \mu\text{m}$) were cut firstly and stained with toluidine blue and viewed with light microscope to select the suitable areas for the electron microscope examination. The ultrathin sections (60–100 nm) were cut by a glass knife with LKB microtome, and stained with uranyl acetate followed by lead citrate [16]. These sections were examined under the transmission electron microscope (JEOL 100cx, USA) operating at 80 kV.

2.6. Statistical analysis

Data were expressed as mean \pm standard error. The significance of the difference between treated and control parameters

was analyzed using student's T test by computerized CoStat statistical software program, version 6.4.

3. Results

3.1. Clinical observation

Within 2–4 h after food intake, depression, anorexia, ruffled feather, inability to raise and tachypnea have been seen in all intoxicated birds. All exposed birds died within 6–8 h after the initial ingestion of intoxicated food. Gross postmortem examination revealed dark reddish and enlarged livers, from which blood was oozing. Kidneys were pale in most intoxicated birds. Congestion of the proventriculus, gizzard and intestine has also been observed.

3.2. Liver function assay

As data presented in Table 1, activity of AST is significantly increased ($p < 0.05$) in the serum of Zn_3P_2 intoxicated birds. While, the activities of both ALT and ALP showed a non significant deviation between treated and control birds.

3.3. Concentration of zinc

As shown in the Table 1, there is no significant difference in the hepatic zinc concentration between treated and control birds.

3.4. Ultra structural alterations of hepatocytes

Concerning control group, hepatic cells were polygonal in shape with large spherical nucleus (Fig. 1). Mitochondria were the most numerous detected organelles; they exhibited considerable heterogeneity of shape, varying from oval, round to pear shaped. Flattened cisternae of the rough endoplasmic reticulum, lysosomes and free ribosomes were scattered among mitochondria. Glycogen deposits were scattered in the cytoplasm in the form of large electron dense deposits (Figs. 2 and 3). Nucleus of hepatocytes was large and spherical in shape, the nuclear envelope was almost smooth in outline with few irregularities, and it had some nuclear pore (Fig. 1). The cell membrane at base of the cell modified to form many microvilli projecting into sinusoidal space (Fig. 1). In the sinusoids, Kupffer cells and flat endothelial cells were observed (Fig. 3).

In birds intoxicated by Zn_3P_2 , the hepatocytes showed pronounced changes. Mitochondrial swelling with cristolysis is the main lesion was noticed in all examined hepatic cells of intoxicated birds (Figs. 4–6). They had flocculated material; glycogen deposits were few and disappeared from

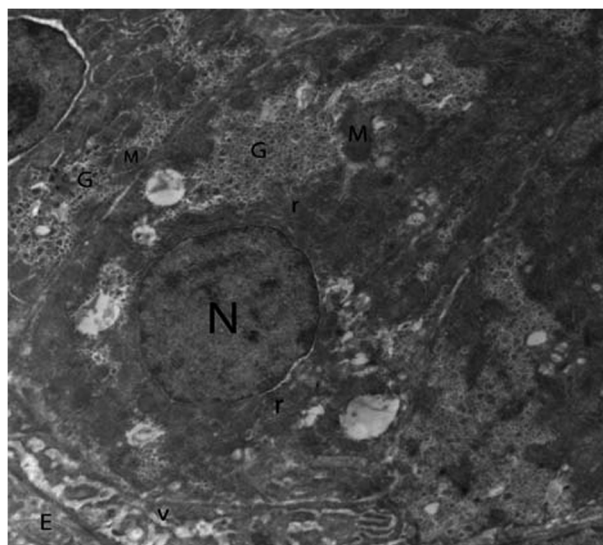


Fig. 1 Transmission electron micrograph of the chick's hepatocyte in control group showing mitochondria (M), rough endoplasmic reticulum (r), glycogen (G), nucleus (N), microvilli (v) and endothelial cell (E). (x2000).

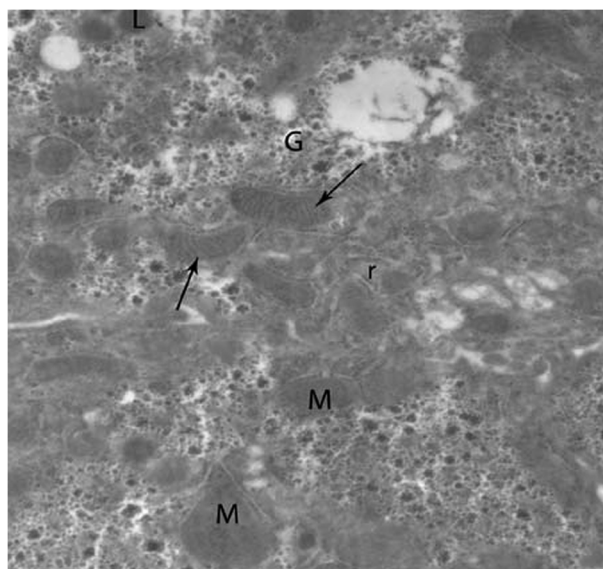


Fig. 2 Higher magnification of control hepatocytes showing several shapes of mitochondria (M), cristae (arrows) rough endoplasmic reticulum (r) and glycogen (G). (x5000).

Table 1 Effect of acute Zn_3P_2 poisoning on liver function parameters and hepatic zinc concentration.

Group	AST (U/l)	ALT (U/l)	ALP (U/l)	Zinc level ($\mu\text{g/g}$ wet weight)
Treated	174.00 \pm 3.46*	14.66 \pm 5.03	39.67 \pm 14.50	41.53 \pm 6.97
Control	93.00 \pm 12.77	9.00 \pm 1.00	30.67 \pm 9.02	47.17 \pm 8.65

All values are expressed as means \pm SE ($n = 6$).

* Significant difference between Zn_3P_2 -exposed group and the corresponding control at $p < 0.05$.

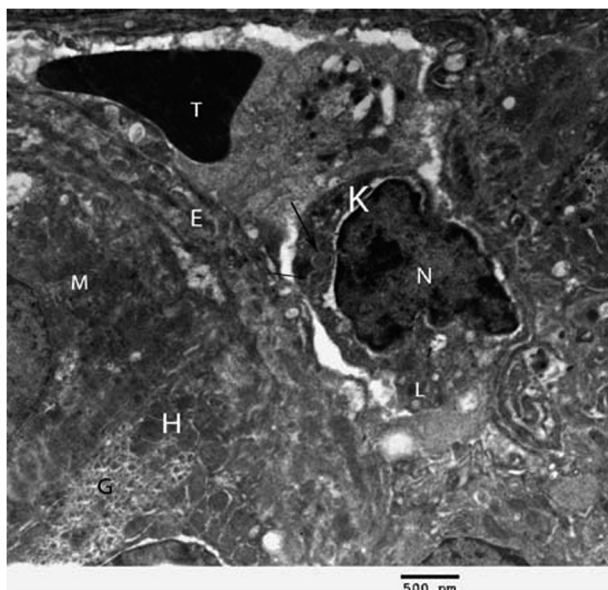


Fig. 3 Electron micrograph of chick's liver in control group showing hepatocytes (H), mitochondria (M), glycogen (G), kupffer cell (K), erythrocyte (T), endothelial cell (E), lysosomes (L) and lysosomes with electron dense content (arrows) ($\times 2500$).

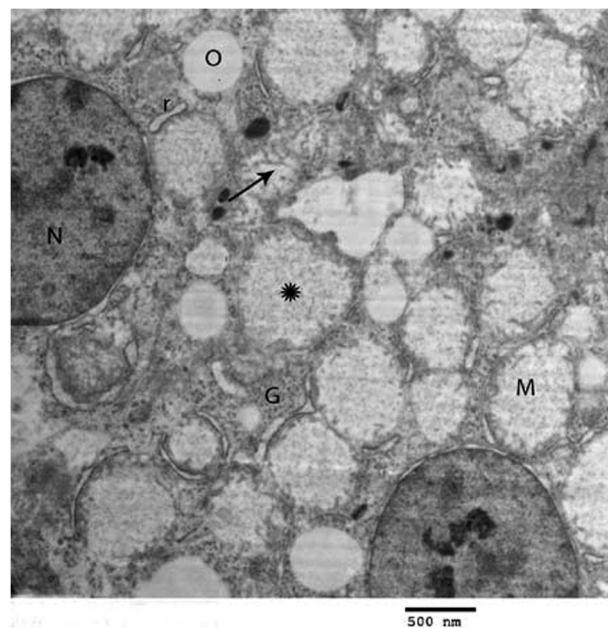


Fig. 5 Higher magnification of intoxicated hepatocyte showing swollen mitochondria (M), cristolysis (arrow), flocculant material (asterisk), dilated rough endoplasmic reticulum (r), glycogen (G), vacuoles (O) and nucleus (N) ($\times 3000$).

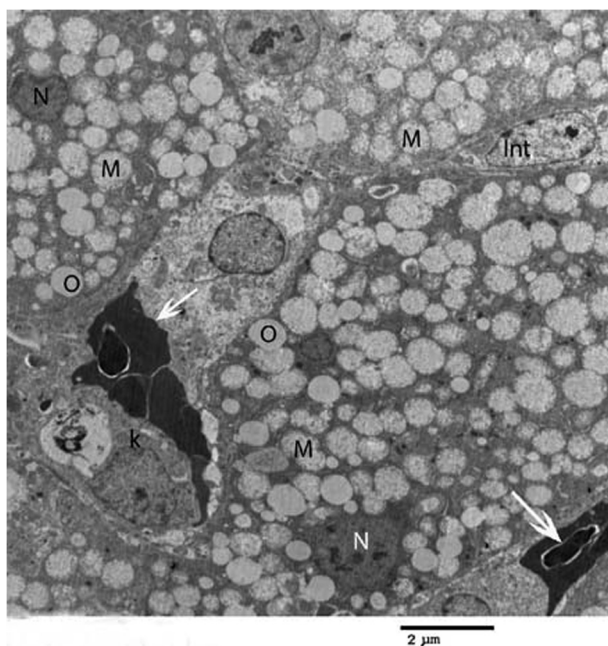


Fig. 4 Transmission electron micrograph of the hepatocyte of chicks intoxicated by zinc phosphide showing swollen mitochondria (M), vacuoles (O), nucleus (N), erythrocytes (arrow), intercalated cell (Int) and kupffer cell (K) ($\times 1000$).

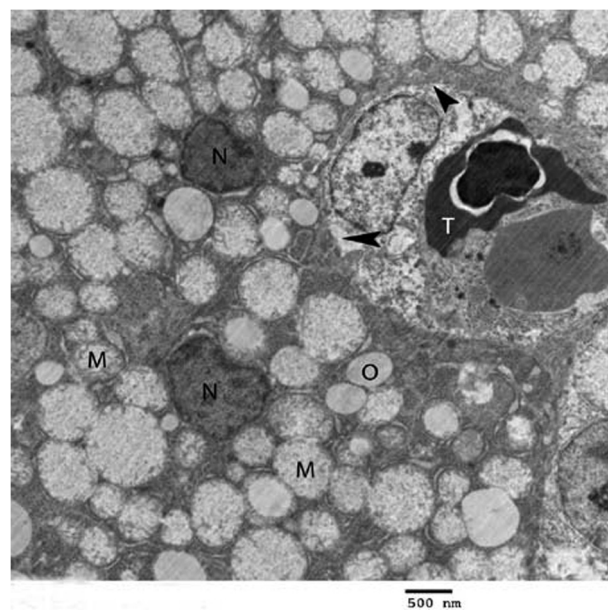


Fig. 6 Another electron micrograph of the hepatocyte of chicks treated with zinc phosphide showing swollen mitochondria (M), vacuoles (O), erythrocyte (T) and shrunken nucleus (N) and absence of microvilli in sinusoids (arrow head) ($\times 2000$).

some area and vacuoles were observed in the cytoplasm along with distended rough endoplasmic reticulum (Fig. 5). Some of the nuclei were shrunken, irregular in shape and darkly stained. Absence of microvilli in sinusoidal lumen was also observed (Fig. 6).

4. Discussion

Metal phosphides (aluminum and zinc phosphides) are highly effective insecticides and rodenticides. Zn_3P_2 is toxic to birds, non-target mammalian species, and freshwater fish. There are several reported cases of wildlife and domestic animal

intoxication following accidental ingestion of Zn_3P_2 [3,17,18]. It has been reported that phosphine poisoning able to induce acute liver failure or a degree of hepatic damage associated with the presence of a kind of histopathological and biochemical alterations [8,19–21]. Nevertheless, the ultrastructural changes in avian hepatocytes in relation to biochemical alterations after the exposure to fatal phosphine poisoning not yet extensively investigated. On the other hand, there is evidence to the elevation of zinc concentration in the content of crop or viscera after the exposure to lethal doses of Zn_3P_2 [3,18,22,23], while the analysis of hepatic zinc concentration, to author's knowledge; had been studied in very limited case report studies. Therefore, we have tried to measure zinc concentration in the liver of broiler chicks experimentally intoxicated with Zn_3P_2 .

Based on the clinical observation in the present study, experimentally poisoned birds exhibited depression, anorexia, ruffled feather, inability to raise and tachypnea. Onset of clinical signs is most often after 2–4 h following ingestion. These findings are consistent with the earlier reports about Zn_3P_2 poisoning in birds [2,23,24]. Likewise, gross postmortem lesions were nonspecific and were limited to dark red livers, pale kidneys in most intoxicated birds and the congested proventriculus, gizzard and intestine. Relatively similar lesions have been described previously by Shivaprasad and Galey [17].

Considering hepatic biochemical parameters, obtained data could be explained in the light of that AST enzyme is quite widely distributed in the body; in particular it is found in skeletal muscle, heart, liver and erythrocytes. It is used to investigate muscular, cardiac and hepatic damage. Thus, the elevation of the activity of AST in Zn_3P_2 -treated birds may be attributed to the myocardial damage beside the deleterious effect on the liver [25]. It has been established that the major lethal consequence of phosphide ingestion is the circulatory collapse due to the direct effect on cardiac myocytes [26]. Due to the extent of damage to the heart and the lungs following the ingestion of phosphide, the patients are lost in the early stages of toxicity as reported by Doğan et al. [27]. In the same context, Bildfell et al. [18] stated that, the elevation in the activity of creatine kinase and AST was the only remarkable biochemical alterations detected in Zn_3P_2 -intoxicated geese. Another explanation is that AST is located in high concentration in mitochondria, while ALT is located mainly in the cytosol [28]. This is in accordance with the obtained result of electron microscopic examination of hepatocytes which revealed marked disruption of mitochondrial morphology. The short course of the toxicity in the present study may be responsible for the non-significant elevation in the activity of both ALT and ALP.

Analysis of hepatic zinc concentration revealed non-significant alteration in comparing with control birds. This result is in agreement with data obtained by Shivaprasad and Galey [17] who stated that, zinc level was elevated only in crop contents of Zn_3P_2 -intoxicated peafowl, while the analysis of liver and kidney for zinc did not reveal any toxic levels. We can suggest that, the analysis of hepatic zinc concentration couldn't be included in the diagnostic procedure of Zn_3P_2 poisoning.

Histological investigation of hepatocytes by transmission electron microscope revealed that the major ultrastructural alteration was the mitochondrial swelling with cristolysis. This swelling is thought to be related to change in osmolarity that

leads to influx of salts and water through the inner mitochondrial membrane which becomes distended. Consequently, the outer membrane eventually ruptures as a result of water and ion accumulation [29]. Also mitochondrial swelling may be attributed to the severe drop in mitochondrial membrane potential [30]. The obtained data also are in parallel to those stated by Bumbrah et al. [4] who reported that phosphide poisoning disturbs the mitochondrial morphology, inhibits oxidative respiration by 70%, causing, in turn, the cells to die rapidly. Phosphine is also known to inhibit mitochondrial cytochrome C oxidase and protein synthesis, particularly in the mitochondria of lung and heart cells. This can lead to a blockage of mitochondrial electron transport chain. In aluminum phosphide-intoxicated rats, there was a significant decrease in the activities of mitochondrial complexes I, II, IV and catalase in liver tissue with a significant increase in lipid peroxidation. Mitochondrial injury is the main ultrastructural change which was observed in heart, liver and kidney tissues indicating that the toxicity by aluminum phosphide occurs as a consequence of both energy insufficiency and oxidative stress [31]. Vacuoles were also detected in the cytoplasm, as well as, distended rough endoplasmic reticulum; some of the nuclei were shrunken, irregular in shape and darkly stained. These changes may be resulted from failure of calcium homeostasis and accumulation of unfolded proteins in the endoplasmic reticulum [32].

5. Conclusion

The present study highlights the significance of hepatic injury after acute zinc phosphide toxicity. We can conclude that the mitochondria could be one of the main target organelles in hepatocytes for the toxic effect of zinc phosphide in broiler chicks.

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