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SOME BIOCHEMICAL STUDIES ON THE RODENTICIDE ZINC PHOSPHIDE

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ABSTRACT

The present investigation is an attempt to study the hazardous effects associated with the use of the rodenticide zinc phosphide (Zn_3P_2) in the albino rats as a useful model for man. The LD_{50} of Zn_3P_2 205 mg/kg b.wt. caused a significant decrease ($P < 0.05$) of serum glucose and a highly significant decreases ($P < 0.01$) in liver and muscle glycogen. Total lipid contents, total protein contents, Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) activities showed a great alterations. The sublethal dose of Zn_3P_2 (102.5 mg/kd b.wt.) elicited a great deteriorations in all the above mentioned parameters in serum, liver and muscles. These deteriorating effects of Zn_3P_2 may extend to the sixth day after its administration. Thus, the toxic effect of the rodenticide is not limited to the target animals, so it should be empolyed with a great care in rodent control.

INTRODUCTION

The use of zinc phosphide (Zn_3P_2) as a rodenticide in Egypt may cause several toxic effects. Inhalation of phosphin gas (pH_3) during the preparation of wet baits, inhalation of the dust during the preparation of the dry baits or its accidental ingestion during manufacturing and handling leads to some hazardous effects. On ingestion of Zn_3P_2, it reacts with the stomach acid/water and beside the release of pH_3, $ZnCl_2$ is also formed. The latter is then hydrolysed to Zn^{2+} and Cl^{-1} ions. Zn^{2+} ion is also highly toxic[1].

Many studies have been carried out to evaluate Zn_3P_2 baits as a rodenticide[1-5] zinc phosphide, like many other pesticides is toxic to non-target species including man and other desirable forms of life[6] but little studies have been done to evaluate this toxicity[7,8]. So, the present study is an attempt to further elucidate the effect of acute and subacute exposure to Zn_3P_2, on some physiological and biochemical aspects in the serum, liver and skeletal muscles of the albino rat, as a useful model for man, to evaluate the possible hazardous effects resulting from exposure to such rodenticide.

MATERIALS AND METHODS

Experimental Animals :

Healthy, mature male albino rats (*Rattus norvegicus*) weighing 120 ± 20 gm obtained from the breeding unit of Egyptian organization for biological and vaccine production were used for this study. They were fed on standard pelleted diet with free access to water.

Zinc phosphide (Zn_3P_2) of technical grade, 19% purity was obtained from pesticide committee (Ministry of Agriculture and Land Reclamation, Egypt). It is produced by El-Nasr for Intermediate Chemicals Company. The LD_{50} was determined[4]. Two doses were applied in the present study, LD_{50} and half the LD_{50} which corresponds to 205 and 102.5 mg/kg body weight, respectively.

Experimental :-

Rats were grouped as follows:

I Control group (10 animals).

II LD_{50} treated group 205 mg/kg.b.wt. (10 animals).

III Half LD_{50} treated group 102.5 mg/kg.b.wt. (50 animals) divided into five equal subgroups, 10 rats, each. Rats were sacrificed after 24, 48, 72, 120 and 144 hours of treatment zinc phosphide was given orally in a single dose to group II & III using a metallic stomach tube for rats.

Samples :

Blood samples were collected from each group in centrifuge tubes, kept for 3 hours at 4°C for clotting and then the serum was separated by centrifugation for 15 min. at 3000 rpm for biochemical analysis. Sera samples were kept at-20°C until used. Liver and skeletal muscles were immediatly excised after slaughtering and were rapidlly frozen in deefreezer at-20°C until used for biochemical analysis.

Biochemical analysis :

The following biochemical variables were determined using commercial kits supplied by bioMerieux (France) according to the following methods. Blood glucose[9], liver and skeletal muscle glycogen[10], serum and tissue total lipids[11], serum and tissue total protein[12], and serum and tissue transaminases (AST + ALT) activities[13].

Statistical Analysis :

The mean ± standard deviation was computed for each parameter. The Student's "t" test [14] was used to analyze the data from control and treated groups. A significant difference was considered at $P < 0.05$; While at $P < 0.01$ it was considered as a highly significant.

RESULTS AND DISCUSSION

The effects of LD_{50} of Zn_3P_2 (205 mg/kgm.b.wt.) is illustrated in Table (1). Four rats out of the ten were died within the experiment, they were immediately dissected out and examined for post mortem changes. The P.M. lesions showed generalized congestion all over the alimentary tract and liver. These findings were in accordance with[15]. It was clear that the biochemical parameters of rat serum, liver and skeletal muscles were greatly altered after 24 hours of administration. These effects appears to be most probably due to ingestion of Zn_3P_2 which reacts with the acid of the stomach or its water content leading to the release of phosphine gas. $Zn_3P_2 + 6HCl \text{ ----> } 3\ ZnCl_2 + 2pH_3$ which is absorbed, and in the presence of water, $ZnCl_2$ is hydrolysed to Zn_2+ and Cl- ions. Phosphine gas and zinc ions are toxic[4,7,16], zinc ions are readily absorbed leading to toxic effects[1, 17]. It was shown from Table (1) that serum glucose level decreased significantly after 24 hours of Zn_3P_2 (LD_{50}) administration. This might be attributed to the decreased food inatke recorded in our study and previously reported[1] and/or inhibition of gluconeogenesis in the rat liver[18,19] by Zn_3P_2 LD_{50}. Phosphine gas act as a local irritant[3-5] causing cellular infiltration to the mucosa of the gut[7,8], which may affect glucose absorption.

Both liver and muscle glycogen content showed a high significant decrease $P < 0.01$ (Table 1). This might be most probably due to the enhancement of glycolysis and/or the inhibition of glycogenesis by the toxic effect of Zn_3P_2 on the enzymes affecting these processes, i.e., a marked exhaustion of the hepatic glycogen is accompanied by depressed synthesis. Also, the total lipid content of the liver are highly significant decreased $P < 0.01$ (Table 1) due to suppression of fatty acid synthesizing enzymes activities. Alterations of liver weight and function were also previously reported[1,6] as a result of Zn_3P_2 toxicity.

The serum and muscles total protein contents were markedly increased ($P < 0.01$), while that of the liver are highly significantly decreased, suggesting that the toxic effect of Zn_3P_2 may inhibit protein synthesis[20] in the liver and destruction of the hepatic cells by the effect of Zn^{2+}. It was reported that phosphine causes wide spread cellular toxicity with necrosis of the gastrointestinal tract and injury to other organs such as the liver and kidneys. Moreover, these effects appear to be most probably due to haemoconcentration resulting from severe diarrhaea, as it has been reported that inhalation of zinc phosphide dust by children from treated grains results in vomiting, diarrhaea[20]. Also, Zn_3P_2 may alter the rate of protein synthesis and/or catabolism in the muscles. The AST activity are highly significantly increased in serum, liver and muscle (Table 1) this might be due to tissue destruction[17,18] that affected by Zn^{2+}[1,16]. The ALT activity showed similar elevations in the muscle only. Our results were in accordance with[21] they reported that serum AST activity was early found to be elevated in hepatic necrosis in the horse, cow, pig and cat. Moreover, significant elevation in serum ALT has been reported in fatty change of the dog liver[22].

The effect of sublethal dose of Zn_3P_2 are represented in Tables (2,3, 4) in serum, liver and skeletal muscles, respectively. Zinc phosphide (102.5 mg/kg) caused a gradual elevation of serum glucose (hyperglycemia), which was highly significant after 48, 72 and 120 hours of administration and it was significant at the first and sixth days of the experiment (Table 2). This indicates that the toxic effect of Zn_3P_2 on blood glucose control systems began to be cured after six days of administration. Zinc ion counteract the pancreatic secretion effect and/or it may cause beta cells destruction[1]. At the same time, liver and muscle glycogen contents were mostly highly significantly increased all over the experiment, except after 24 hours of administration which showed a highly significant decrease (Table 3 & 4). It may be the first reaction of sublethal dose of Zn_3P_2 toxic effect that enhance glycogenolysis in the tissues, then it is counteracted by the blockage of this pathway enzymes. The muscle glycogen contents returned to the control value after 144

hours of administrtaion. This means that the toxic effect of Zn_3P_2, is more efficient on the liver[1], and the hepatocellular injury would therefore affect carbohydrate metabolism and store in the liver[17,18].

The serum total lipids content were increased significantly after 24 hours of Zn_3P_2 (sublethal dose) administration, followed by a highly significant decrease ($P < 0.01$) till the end of experiment (144 hours) (Table 2). This decrease of serum lipids may indicate atrophy of the liver that caused by Zn_3P_2. liver cirrhosis may be a cause of low serum lipid content[18]. On the other hand, both liver and muscle total lipid contents were higher than the control values allover the experimental period (Table 3 & 4). This indicates that both lipid metabolism and liver function were deteriorated by the toxic effect of Zn_3P_2. It has been reported that the liver is a target organ of the toxic action of Zn_3P_2[1, 6]. Desposition of fat in the liver may occur from the action of toxic substances[17].

The serum total protein content increased significantly after 24 hours of Zn_3P_2 (102.5 mg/kg) administration, but the percent of increase was higher after 24 hr than that after 48 hours, then it nearly returned to the control value till the end of the experiment (Table 2). This could be explained on the same basis as with the LD_{50} of Zn_3P_2. This indicates that Zn_3P_2 toxic effect on the serum total protein content not exceeds more than 48 hours. In the liver this effect was extended to 120 hours after administration (Table 3), where the liver total protein content are highly significantly decreased and then significantly increased after 24 hours and 48 hours of Zn_3P_2 administration, respectively. Then it was transitionally decreased to the control value after 72 hours and reincreased significantly after 120 hours, and began to return to the control value after 144 hours of Zn_3P_2 (102.5 mg/kg) administration. This may be a compensatory hyperatrophy of the hepatic cells[19]. The muscle protein content increased significantly for 72 hours, and began to return to the control value, with a non significant increase, after 120 hours of Zn_3P_2 (sublethal dose) adminsitration (Table 4). This indicates that Zn_3P_2 is able to alter the rate of protein synthesis or/and catabolism in the muscles by altering the activities of transaminases and the enzymes concerned with gluconeogenesis.

Transaminases (AST & ALT) activities showed a general and highly significant decreases in the serum and liver (Table 2 & 3) and increases in the skeletal muscles (Table 4) at the different experimental periods after Zn_3P_2 (sublethal dose) adminsitration. This indicates atrophy of the hepatic cells or blockage of the hepatoceullar secretion of the enzymes and demands by the tissues to the enzymes[18,19]. This might be related to the toxic effect of zinc ions which was previously reported[15] as a depressing growth effect or/and decreased food intake and heavy breathing that were recorded throughout the experimental period. The recorded increases in AST and ALT activites in the muscle compensate the decrease of the enzymes secretion from the liver cells and its atrophy by the toxic effect of Zn_3P_2. It may be also due to different affinities and responses of the body organ to detoxify Zn^{+2}. The increase in muscle AST and ALT activities may also be a result of increased protein catabolism to induce hepatic uptake of amino acids by increased activities of transaminases and other enzymes concerned with gluconeogenesis. The activity of the enzymes may also be increased by formation of coordination complex between the enzymes and Zn^{+2} in the muscles, that confirms a catalytic activity. The increased serum glucose levels (Table 2) is an indication to the increased gluconeogenesis to increase the availability of glucose for energy and glycogen storage[18]. Thus far, it could be concluded that Zn_3P_2, beside being an effective rodenticide, has also untoward effects and should be handled with a great care in its manufacturing, preparation and in rodent control.

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Table (1): Changes in serum, liver and muscles biochemical parameters of rats treated with Zn_3P_2 (24 hrs. LD_{50}).

Variable Mean ± S.D.	Control	24 hs. after treatment
Serum :		
Glucose mg/100 ml	94.856 ± 3.440	85.00 ± 5.400*
Total lipids gm/100 ml	0.358 ± 0.007	0.581 ± 0.085**
Total proteins gm/100 ml	4.717 ± 0.310	7.580 ± 0.33**
AST unit/ml	123.622 ± 3.664	137.081 ± 4.211**
ALT unit/ml	93.09 ± 3.622	96.105 ± 5.012
Liver :		
Glycogen (mg/100 gm) freh tissue	318.698 ± 43.505	204.327 ± 24.170**
Total lipids (gm/100 gm) fresh tissue	8.854 ± 1.700	5.456 ± 0.471**
Total proteins (gm/100 gm) fresh tissue	73.036 ± 6.304	42.050 ± 3.645**
AST (unit/gm) fresh tissue	275.482 ± 4.922	299.928 ± 11.604**
ALT (unit/gm) fresh tissue	575.465 ± 19.079	583.980 ± 10.467
Muscles :		
Glycogen (mg/100 gm) fresh tissue	45.586 ± 4.447	14.940 ± 2.460**
Total lipids (gm/100 gm) fresh tissue	5.968 ± 1.222	6.846 ± 0.626
Total proteins (gm/100 gm) fresh tissue	19.045 ± 2.348	41.706 ± 10.676**
AST (unit/gm) fresh tissue	234.080 ± 10.261	303.82 ± 12.202**
ALT (unit/gm) fresh tissue	210.812 ± 10.460	309.225 ± 21.399**

S.D. = Standard deviation. hs = hours.
* = Significantly different from the control value at $P < 0.05$.
** = Highly significantly different from the control value at $P < 0.01$.

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Table (2): Changes in serum biochemical parameters of rats treated with Zn_3P_2 (1/2. 24 hrs. LD_{50}).

Parameters Mean ± S.D.	Control	After administration of Zn_3P_2 by				
		24 hs	48 hs	72 hs	120 hs	144 hs
Glucose (mg/100 ml)	94.856 ± 3.440	101.225* ± 3.128	121.600** ± 2.600	732.975** ± 58.027	446.060** ± 25.990	107.676* ± 10.097
Total lipids (gm/100 ml)	0.358 ± 0.007	0.546** ± 0.011	0.273** ± 0.018	0.168** ± 0.008	0.145** ± 0.017	0.294** ± 0.015
Total proteins (gm/100)	4.717 ± 0.310	7.320** ± 0.526	5.774** ± 0.114	4.904 ± 0.055	4.960 ± 0.430	4.487 ± 0.552
AST (unit/ml)	123.622 ± 3.664	119.801 ± 5.907	101.330** ± 3.370	113.905** ± 2.710	116.312** ± 1.181	104.613** ± 2.143
ALT (unit/ml)	93.090 ± 3.622	79.360** ± 2.570	64.804** ± 7.119	97.390 ± 2.340	79.250** ± 0.840	97.008 ± 3.559

S.D. = Standard deviation. hs = hours.
* = Significantly different from the control value at $P < 0.05$.
** = Highly significantly different from the control value at $P < 0.01$.

Table (3): Changes in liver biochemical parameters of rats treated with Zn_3P_2 (1/2.24 hrs. LD_{50}).

Parameters Mean ± S.D.	Control	After administration of Zn_3P_2 by				
		24 hs	48 hs	72 hs	120 hs	144 hs
Glycogen (mg/100 gm) fresh tissue	318.698 ± 43.505	214.376** ± 16.416	474.874** ± 27.765	204.102** ± 28.134	507.439** ± 17.671	563.997** ± 20.639
Total lipids (gm/100 gm) fresh tissue	8.854 ± 6.305	13.645** ± 0.655	11.037 ± 1.961	21.062** ± 3.214	28.664** ± 1.435	11.080 ± 1.523
Total proteins (gm/100 gm) fresh tissue	73.036 ± 6.305	44.056** ± 3.067	84.906* ± 6.229	73.216 ± 2.751	82.160* ± 4.627	75.246 ± 9.628
AST (unit/gm) fresh tissue	275.482 ± 4.922	243.203** ± 3.057	262.740* ± 8.190	253.550** ± 8.780	281.266 ± 9.66	249.280** ± 7.630
ALT (unit/gm) fresh tissue	575.466 ± 19.079	468.820** ± 4.700	460.176** ± 20.633	528.174** ± 19.242	483.414** ± 11.597	476.587** ± 17.225

S.D. = Standard deviation. hs = hours.
* = Significantly different from the control value at $P < 0.05$.
** = Highly significantly different from the control value at $P < 0.01$.

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Table (4): Changes in muscles biochemical parameters of rats treated with Zn_3P_2 (1/2.24 hrs. LD_{50}).

Parameters Mean ± S.D.	Control	After administration of Zn_3P_2 by				
		24 hs	48 hs	72 hs	120 hs	144 hs
Glycogen (mg/100 gm) fresh tissue	54.586 ± 4.447	16.978** ± 3.041	71.830** ± 11.033	173.084** ± 11.050	91.002** ± 14.803	44.651 ± 7.955
Total lipids (gm/100 gm) fresh tissue	5.968 ± 1.222	7.550 ± 1.026	9.448** ± 0.258	12.785** ± 1.643	8.472 ± 2.059	10.772** ± 2.057
Total proteins (gm/100 gm) fresh tissue	19.045 ± 2.348	36.190** ± 7.959	78.386** ± 10.264	27.742** ± 4.360	23.450 ± 3.400	23.165 ± 3.408
AST (unit/gm) fresh tissue	234.080 ± 10.261	241.630 ± 2.940	236.479 ± 12.260	251.578* ± 6.330	284.697** ± 16.636	248.274 ± 8.479
ALT (unit/gm) fresh tissue	210.812 ± 10.960	227.172* ± 3.083	249.139* ± 21.953	283.810** ± 7.710	252.490** ± 4.630	270.009** ± 22.095

S.D. = Standard deviation. hs = hours.
* = Significantly different from the control value at $P < 0.05$.
** = Highly significantly different from the control value at $P < 0.01$.

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بعض الدراسات الكيمائية الحيوية علي مبيد القوارض فوسفيد الزنك

محمد السيد عبد الحليم - عزيزه عبد الصمد الشافعى و مشيرة محمد عزت سليم

قسم علم الحيوان - كلية العلوم - جامعة الزقازيق - فرع بنها - بنها

تم فى هذا البحث دراسة تأثير مبيد القوارض فوسفيد الزنك على فأر التجارب الأبيض وقد أوضحت هذه الدراسة أن :- الجرعة ٢٠٥ مجم/كجم من وزن الفأر تسبب نقص فى معدل كل من جلوكوز الدم ومحتوى الجليكوجين فى الكبد و العضلات. أما بالنسبة لمحتوى البروتين والدهون ونشاط أنزيمى ALT, AST، فقد أظهرت النتائج تغير واضح فى كل من المصل والكبد والعضلات أما بالنسبة للجرعة ٥ر١٠٢ مجم/كجم من وزن الفأر فقد أظهرت النتائج تغيرات مختلفة فى كل القياسات السابقة فى المصل والكبد والعضلات وهذه التغيرات الناتجة من تأثير فوسفيد الزنك قد تمتد إلى اليوم السادس بعد تعاطى المبيد، ومن هنا يجب أتخاذ إجراءات الوقاية اللازمة عند إستخدام فوسفيد الزنك كمبيد للقوارض.