Kafr El-Sheikh Vet. Med. J. Vol. 2 No. 1 (2004) (45-56)

BIOCHEMICAL CHANGES IN SERUM OF CHICKENS AFTER PROLONGED ADMINISTRATION OF A FUNGICIDE PROPINE

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ABSTRACT

This study investigated the effect of propineb "antracol" (a fungicide of propylene bisdithiocarbamate group) on some enzymatic activities and biochemical constituents in serum of chickens. Ninety-one day old chicks were divided into two groups (45 each). Birds of the first group were fed on balanced ration for ten weeks and kept as control group. The chicks of the second group were kept on a ration containing the drug in a rate of 1 g/kg ration for six weeks (first phase) followed by four weeks where the ration was free from the drug (second phase).

Blood samples were collected by slaughtering five chickens from each group weekly. The study revealed that antracol increased serum AST and ALT activities and bilirubin content while significant decrease in cholinesterase activity was recorded. Blood glucose and cholesterol levels were increased during 3^{rd} and 4^{th} weeks then began to decrease. Serum total protein content was increased, as a result of elevation in the albumin level. Creatinine level was increased from the 7^{th} to the 12^{th} weeks of the experiment, also potassium content was increased. On the other hand, sodium level was decreased.

INTRODUCTION

The fungi differ so greatly from other forms of life as they are distinct not only in form but in physiology,so fungi may be combated successfully by compounds that have only extremely low toxicities to other organisms, notably mammals ***(Klaassen et al., 1996)***.

The fungicides, like other classes of pesticides comprise a heterogenous group of chemical compounds with a few exceptions, the fungicides have not attracted the detailed toxicological research as have insecticides. Although many of the compounds used to control fungus diseases on plants, seeds and products are rather non-toxic acutely, there are some notable exceptions ***(Hassall, 1990)***.

Dithiocarbamates form a part of the large group of synthetic organic fungicides that have been developed and produced on a large scale in the last 40-50 years. They are effective against a broad spectrum of fungi and plant diseases caused by them. Additional uses are as biocides for industrial or other commercial applications, and in household products, some are used for vector control in public health ***(Samia, 1991)***.

As chickens are an important source of human proteins, the aim of the present study is to throw light on some toxicological aspects of these fungicides on blood biochemical constituents of the treated chickens.

MATERIAL AND METHODS

The fungicide used in this study is propineb(antracol). It is produced by Bayer AG (West Germany). It is an organic fungicide with a common name propineb and chemical name polymeric zinc propylene bis (dithiocarbamate). Its empirical formula is $C_5H_8N_2S_4Zn$.

Ninety apparently normal one day old chicks(Harbourd breed)of both sexes were used in this study. The birds were housed in constant environmental and hygienic condition, fed on a balanced ration. They were kept under observation for two weeks before starting the experiment. The chicks were vaccinated against Newcastle disease at the 3rd week using F strain by head dipping and at 6th week with Lasota strain in drinking water. Birds were classified into two equal groups each of 50 chickens, the first one was served as control (group I). The second (group II) received ½ LD_{50} of antracol in their diet (1 g/kg ration) for studying the biochemical effect of this fungicide. This experiment passed through two phases. First phase began at the end of second week of age and lasted for six weeks till the end of the 8th week of age where the birds received antracol throughout this phase. Second phase began at the 9th week and lasted for four weeks till the end of 12th week of age where antracol was withdrawn from the ration throughout this phase.

Five chickens from each group were randomly taken and sacrificed weekly from the 3rd week of age. Blood samples were collected in clean, dry and labeled centrifuge tubes and left at room temperature for clotting, then centrifuged at 3000 rpm for 15 minutes. Clear supernatant was collected in clean labeled vials for the following determinations.

Transaminase activity ***(Reitman and Frankel, 1957)***, pseudocholinesterase activity ***(Szasz,1968)***,total bilirubin ***(Monnet,1963)***,cholesterol ***(Schettler and Nussel, 1975)***, glucose ***(Schmidt, 1971)***, total protein ***(Weichselbaum, 1946)***, albumin ***(Drupt, 1974)***, creatinine ***(Brod and Sirota, 1948)*** and sodium and potassium ***(Burriel and Ramirez, 1957)***.

Results were statistically analyzed using t-student test according to ***Petrie and Watson (1999)***.

RESULTS

Table (1) showed that ½ LD_{50} of antracol (1 g/kg feeding) caused significant alterations in aspartate aminotransferase (AST), alanine amino transferase (ALT) and cholinesterase activities, total bilirubin and cholesterol content in blood of chickens. The obtained data revealed that the activity of AST showed initial significant increase at the 7^{th} week and at the 5^{th} week in ALT and this significant increase continued till the end of experiment.

The mean value of cholinesterase activity showed significant decrease at 4^{th}, 5^{th} and 6^{th} weeks of experiment. The mean value of total bilirubin showed significant increase from the 3^{rd} week to the 9^{th} week. Also initial significant increase of cholesterol concentration was recorded at 3^{rd} week, then it showed significant decrease at the 4^{th} and the 5^{th} week of experiment.

Table (2) illustrated mean values of total protein, albumin, globulin, A/G ratio and glucose concentration in control(I) and antracol intoxicated chicks(II). Significant increase in total protein, albumin and globulin were recorded from the beginning of experiment and continue till the 10^{th} week. Initial hyperglycaemic effect was recorded at the beginning of 3^{rd} and 4^{th} weeks of experiment then hypoglycaemia was noted at the 5^{th} week of experiment.

Table (3) summarized the changes obtained in creatinine, sodium and potassium concentrations in serum of antracol intoxicated chicks (group II) in comparison with that of normal chicks (group I). It was shown that creatinine level was increased significantly from 7^{th} week till the end of experiment, while potassium content was increased at 3^{rd}, 4^{th}, 5^{th}, 7^{th}, 9^{th} and 10^{th} weeks of experiment. On the other hand, sodium level was decreased at the 3^{rd} week.

Table(1): The estimated values of serum AST,ALT and cholinesterase activities, total bilirubin and cholesterol concentration in control (I) and antracol intoxicated chicks (II).

Age (weeks)	AST IU/l		ALT IU/l		Cholinestlrase IU/l		Bilirubin (mg/dl)		Cholesterol (mg/dl)	
	I	II	I	II	I	II	I	II	I	II
3	266.6 ±1.89	262.4 ±2.79	218.6 ±7.89	214.4 ±2.79	1360.92 ±82.31	1142.43 ±101.01	4.86 ±0.53	15.38** ±0.31	161.46 ±1.06	173.88** ±0.96
4	270.4 ±1.66	262.0 ±5.02	223.9 ±1.72	220.0 ±4.16	1285.73 ±48.43	700.96** ±10.94	5.04 ±0.54	12.70** ±1.03	148.76 ±4.18	109.90* ±10.67
5	274.4 ±2.48	269.2 ±2.35	219.2 ±1.77	238.6** ±0.75	1299.42 ±70.15	683.48** ±22.75	4.48 ±0.81	12.43** ±1.11	160.60 6.52	107.80** ±11.41
6	273.0 ±1.41	268.6 ±3.49	212.8 ±1.56	246.0** ±3.81	1328.08 ±156.67	421.06** ±27.88	4.48 ±0.70	11.12** ±0.81	166.68 ±1.68	133.80** 0.81
7	251.2 ±6.96	289.4** ±1.78	202.4 ±5.78	237.8** ±1.11	1409.85 ±211.55	1314.67 ±76.17	4.86 ±0.74	10.23** ±0.88	130.02 ±7.39	137.52 ±8.46
8	250.8 ±2.23	285.4** ±2.48	192.2 ±0.68	236.8** 0.86	1550.05 ±154.35	1413.53 ±55.23	4.72 ±0.83	8.95** ±0.79	128.83 ±6.61	129.08 ±14.02
9	230.5 ±1.80	265.6** ±1.83	158.2 ±0.99	193.8** ±0.66	1231.32 ±90.62	1180.47 ±52.99	4.50 ±0.66	9.36** ±0.36	133.84 ±5.95	136.38 ±4.17
10	225.2 ±2.11	252.2** ±4.69	180.4 ±1.94	221.4** ±2.96	1093.59 ±163.05	1082.96 ±38.92	4.56 ±0.61	6.90 ±0.56	131.16 ±3.18	146.82 ±13.49
12	226.4 ±1.33	254.8** ±4.10	175.2 ±0.58	219.2** ±0.37	1349.29 ±239.08	1292.66 ±44.24	4.50 ±0.59	5.54 ±0.24	120.96 ±11.02	125.80 ±7.57

Each value represents mean and standard error of five determinations.

*** Significant at P<0.05** **** Significant at P<0.01**

Table (2): The estimated values of serum total protein, albumin, globulin, A/G ratio and glucose concentration in control(I)and antracol intoxicated chicks (II).

Age (weeks)	Total protein (g/l)		Albumin (g/l)		Globulin (g/l)		A/G		Glucose (mg/dl)	
	I	II	I	II	I	II	I	II	I	II
3	14.50 ±1.06	22.0** ±1.02	7.26 ±0.65	10.38** ±0.31	7.24 ±0.41	11.62** ±0.71	1.00 ±0.16	0.89 ±0.44	335.12 ±1.79	365.22** ±1.57
4	15.32 ±0.51	24.22** ±1.38	8.92 ±0.38	12.90** ±0.52	6.4 ±0.13	11.32** ±0.86	1.39 ±0.29	1.14 ±0.60	336.02 ±2.77	368.90** ±0.88
5	16.20 ±0.4	18.46** ±0.70	8.16 ±0.59	10.24** ±0.33	8.04 ±0.31	8.22** ±0.37	1.01 ±0.19	1.25 ±0.89	361.16 ±1.01	319.88* ±0.93
6	16.65 ±0.42	20.02** ±0.93	8.56 ±0.36	10.52** ±0.04	8.09 ±0.06	9.5** ±0.53	1.06 ±0.6	1.11 ±0.11	372.20 ±0.49	367.44 ±0.64
7	17.10 ±0.79	25.52** ±1.05	9.14 ±0.36	12.96** ±0.20	7.96 ±0.61	12.56** ±0.85	1.15 ±0.59	1.03 ±0.24	368.54 ±0.87	367.68 ±0.92
8	16.28 ±0.65	20.82 ±0.81**	10.33 ±0.56	12.86** ±0.75	5.95 ±0.30	7.96** ±0.42	1.74 ±0.17	1.62 ±0.13	351.86 ±5.92	362.76 ±0.56
9	15.46 ±0.67	20.06** ±1.04	8.26 ±0.37	11.70** ±0.95	7.2 ±0.3	8..36** ±0.09	1.15 ±0.12	1.40 ±0.11	356.26 ±6.61	364.64 ±2.19
10	15.40 ±0.97	21.86** ±1.16	9.02 ±0.37	12.42** ±0.91	6.38 ±0.66	9.44** ±0.25	1.41 ±0.62	1.32 ±0.36	373.64 ±1.92	263.02 ±7.76
12	14.30 ±2.28	16.10 ±1.77	8.08 ±1.02	9.13 ±1.33	6.22 ±1.26	6.79 ±0.44	1.30 ±0.81	1.31 ±0.30	364.74 ±4.14	353.42 ±6.49

Each value represents mean and standard error of five determinations.

*** Significant at P<0.05** **** Significant at P<0.01**

Table(3): The estimated values of serum creatinine,sodium and potassium levels in control (I) and antracol intoxicated chicks (II).

Age (weeks)	Creatinine (g/l)		Sodium (ml eq/l)		Potassium (ml eq/l)	
	I	II	I	II	I	II
3	0.012 ±0.0001	0.009 ±0.0001	39.01 ±0.63	31.77** ±0.35	0.83 ±0.01	1.79** ±0.06
4	0.013 ±0.001	0.009 ±0.0004	39.20 ±0.29	36.99 ±1.40	0.84 ±0.01	0.98** ±0.02
5	0.014 ±0.0022	0.009 ±0.0001	39.39 ±0.27	38.50 ±0.15	0.86 ±0.01	1.10** ±0.02
6	0.014 ±0.0024	0.011 ±0.0011	39.34 ±0.49	41.04 ±1.49	1.30 ±0.13	1.30 ±0.07
7	0.007 ±0.0007	0.714** ±0.1886	39.32 ±1.36	41.36 ±2.31	1.06 ±0.06	1.55* ±0.17
8	0.009 ±0.0012	1.040** ±0.0509	39.19 ±1.66	41.88 ±0.89	1.10 ±0.06	1.02 ±0.01
9	0.0100 ±0.0015	0.910** 0.033	39.28 ±2.21	42.00 ±1.15	1.11 ±0.08	1.43* ±0.05
10	0.0120 ±0.0020	1.040** ±0.0509	39.24 ±2.85	44.51 ±2.82	1.13 ±0.11	1.91* ±0.07
12	0.0142 ±0.0023	0.730** ±0.0374	40.11 ±1.03	42.37 ±0.81	1.22 ±0.04	0.78 ±0.02

Each value represents mean and standard error of five determinations.

*** Significant at P<0.05** **** Significant at P<0.01**

DISCUSSION

Various metallic salts of ethylene bisdithiocarbamic acid are widely used as fungicides. These products are used in great quantities and have a strong effect on biological systems,their presence as well as their chemical products or metabolic degradation, is therefore a potential danger in the food chain and environment, for both animals and man.

Antracol was chosen for our study as an example of dithiocarbamates group. It was examined for its toxicological effects as well as its effect on biochemical constituents.

Regarding the estimation of transaminases in the treated chickens it was found that antracol caused a significant increase in the serum level of AST and ALT.Serum AST showed significant increase from the 7^{th} week, while serum ALT from the 5^{th} week and continued throughout the second phase.

The elevation of transaminases in serum may be due to tissue damage particularly liver,kidney and heart and increased permeability of cell membrane ***(Plaa and Hewitt, 1982)***. ***Murraay et al. (2000)*** noted that serum aspartate aminotransferase was elevated in case of acute hepatitis, cirrhosis of the livers and metastatic or primary liver neoplasm.These findings were in agree with that recorded previously by***El-Hawari and Plaa(1979)***who found elevation in serum transaminases in rats and hamsters after administration of β-naphthylisothiocyanate (BNIT) in different doses. ***Plaa and Hewitt (1982)*** noted elevation in serum AST and ALT activities in mice due to administration of thiobendazole (TBZ) in the diet at levels of 0.8 and 1.6% for 13 weeks.Also our results were in agreement with ***Attahirus et al. (1991)***; ***Tamano et al. (1991) and Elia et al. (1995)***.

Several investigators reported the effect of dithiocarbamate fungicides on the liver of different animals ***(Hunter and Neal, 1975)*** who revealed the presence of varying degrees of centrilobular hepatic necrosis in rats that I/P injected with thino-sulfur containing compounds (dithiocarbamates).

Regarding the effect of the tested fungicides on the activity of cholinesterase in the treated chickens, it was noted that the activity of serum cholinesterase showed initial inhibition at the 4^{th}, 5^{th} and 6^{th} weeks of experiment. The initial inhibition in cholinesterase activity may be attributed to the toxic effect of the tested fungicide on the liver cells and this was supported by ***Varley et al. (1975)*** who mentioned that cholinesterase enzyme is formed in the liver and its serum activity is reduced in case of liver cell damage.

The mean value of total bilirubin in intoxicated chickens showed marked and significant elevation. Concerning the effect of dithiocarbamate fungicide on the level of total bilirubin, ***El-Hawari and Plaa (1977)*** found that A-naphthyl isothiocyanate(ANIT)caused elevation in bilirubin in rats and hamster when administered I/P in dose level 150 mg/kg B.W. Also ***Mihail and Luckhaus (1985)*** reported that serum bilirubin levels were increased in rats given 300 mg/kg B.W.from the fungicide bitertamol. These finding revealed that antracol has toxic effects upon liver tissue and bile duct.

The mean values of cholesterol level in serum of intoxicated chicken showed initial significant increase at the 3^{rd} week of experiment, after which the level was significantly decreased at the 4^{th}, 5^{th} and 6^{th} weeks of experiment. Our results of hypercholesteraemia which was evident at the initial period of experiment was similar to those recorded by ***Szepvolgyi et al. (1989)***, who reported significant increase in cholesterol level in rats fed dithame in their diet at dose level of 75 mg/kg B.W. while, the hypocholesteraemia noted at the terminal period of the experiment was similar to the results of ***Hunter et al. (1982)*** who reported that cholesterol level was lowered in male mice that fed diet containing propiconazole.

The mean values of total protein, albumin and globulin levels in sera of intoxicated chickens showed marked significant increase.In this respect our results are in agreement with those reported by ***Sachsse et al. (1979)*** who mentioned that significant increase in total protein was observed in groups of rats fed on propiconazole in their diet. Contrary to our results those are recorded by ***Watanabe et al. (1981)*** who recorded hypoproteneamia in rats treated with fungicide alprazolam.

The mean value of blood glucose showed initial significant increase (hyperglycaemia) at the 3rd and 4th weeks of experiment. After this, hypoglycaemia was noticed at the 5th week.The initial hyperglycaemia may be due to liberation of catecholamines from the adrenal medulla as a result of the accumulation of acetylcholine and subsequent glycogenolytic effect of catecholamines may cause hyperglycaemia ***(Gupta et al., 1981)***. The followed hypoglycaemic phase may be resulted from the impairment of carbohydrate metabolism due to the toxic effect of fungicide on liver and thyroid gland ***(Steinhoff et al., 1983).Basler (1980)*** recorded an increase in blood glucose level in rats given propiconaizole for 26 days while ***Hunter et al.(1982)*** mentioned that blood glucose concentration was decreased in rats fed on diet containing the same drug.

The study revealed that serum creatinine level was increased significantly from the 7th week and continued throughout the experimental period.Also sodium level was decreased significantly at the 3rdweek while serum potassium showed significant hyperkalaemia at 3rd, 4th, 5th, 7th, 9th and 10th weeks.The significant elevation in creatinine level resemble those recorded by ***Bomhard and Loser (1981)***, who found significant increase in creatinine level in rats administered propylene thiourea. Significant elevation of creatinine level may be attributed to damage that occur in the kidney, which was confirmed by ***Bomhard and Loser (1978)***, who found that rats fed on a diet contained triadimefon showed signs of damage of the proximal tubules cells of the kidney. Also, the changes which occurred in sodium and potassium levels may be due to nephritis or renal failure ***(Varley et al., 1975)***.

CONCLUSION

This study concluded that the fungicide antracol has a toxic effect upon chicks through its influence on liver and kidney. This toxic effects were manifested by significant changes of some serum enzymatic and biochemical changes which remained after the withdrawal of antracol from ration indicating its toxic effect on hepatic and renal tissue.

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