Experimental Studies in Japanese Quail Exposed to a recently used pesticide

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Abstract: Thirty quails (80 – 100 gm B.wt) were divided into 2 equal groups to determine the nutritional and clinicopathological changes induced by 1% copper oxychloride. Blood samples were collected for determination of hemoglobin, PCV and some biochemical blood parameters. Liver and kidney weight increased and were associated with clinicopathological changes. A significant decrease was detected in the value of lib, PCV and iron. On the other hand, there was significant increase in the levels of ALT. AST, urea, creatinine, sodium, potassium. glucose, copper. cortisol hormone and insulin in treated groups in comparison with control ingroup untreated. Microbiological examination, revealed presence of strept pyogens, klebsiella, E.coli and anthracoids. Post Mortem examination of scarified quails revealed hi peremia of internal organs (kidney and liver).

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1. Introduction

Organic chemicals and pesticides are capable of depressing the immune, rig to increased susceptibility to diseases **(Asztalos et al., 1990).**

The depression emphasizes dysfunction of adaptive immunity which differs from compromise of innate defenses that serves as the first line of defence against invading microorganisms. Also, it can behave as stress factor, acting through the neuro-endocrine immune axis (hypothalamus-hypophysisadrenal system) on the immune response. Chronic stress is responsible for the

Copper is an essential trace element for many biological processes. However, at high concentrations it is highly toxic. The use of copper in industry and agriculture polutes natural water and may cause significant tissue damage in fish **(Barham et al., 1972)** . This tissue damage can be demonstrated by changes in activity of the cellular enzymes in sera. The increase in activity of these enzymes depends primarily on the magnitude and severity of cell damage **(Bartels et al., 1972).** The rise in blood glucose level has been used for detection of metabolic stress **(Blaxhall, 1972)** and hemogram as an index of health status in quails **(Blaxhall, 1972).**

In recent years pesticide has been widely used in agriculture and their residue in the environment have increased. These chemical compound have been found in many natural products as green food, water, even man, animal and birds. Many clinicopathalogical disturbances in man and animal were observed. The important toxicological, aspects of pesticide effect on animal cells remain unknown **(EI-Hennawy et al., 1980; Jacobs and Gerstein, 1982; Mostafa et al., 1992 and Nebbia et al., 1993).**

In addition, pesticides such as copper oxychloride have not been investigated in birds as experimental animals. So the present investigation is conducted to study the effect of new pesticides, such as copper oxychloride on some nutritional and clinicopathalogical changes in experimental birds such as Japanese quails.

2. Material and Methods

Experimental Design:

Copper oxychloride was obtained from the pesticide Central laboratory. Cairo, Egypt.

Thirty quails weight 80-100gm used in this study being obtained from the National Research Center farm of El-Noubaria. The birds were divided into equal groups. The first group (15 quails) were fed on ration containg I% copper oxychloride, for 30 days as shown in Table (1). The 2nd group (15 quails) were kept as untreated control.

Blood samples from quails (at 3, 15 and 30 days) were taken on heparinized tube to study P.C.V, hamoglobin and non-heparinized tubes were centrifuged at 3000 r.p.m for 10 min, serum was separated and stored at - 20°C until analysed. Determination of serum Alanin aminotransferase (ALT), and Aspartate aminotransferase (AST) were done by commercial kits from Alkan Company **(Reitman and Frankel, 1957).**

Serum glucose. was assessed according to **Trinder, (1969).** Blood hemoglobin was assessed by

cyanmethemoglobin method. Haematocrit value was carried out by using capillary microhaematocrit method **(Coles, 1986 and Drabkin, 1946).** Serum cortisone level was determined using radiommunoassay technique according to the method of **Pickering and Pottinger, (1983).**

Serum copper was determined using atomic absorption according to **Joseph and Roger, (1976).** Values of sodium and potassium in serum were determined by flame photometer according to the method described by **Silversmit, (1965).** Serum creatinine was carried out according to **Bartels et al., (1972).** Enzymatic determination of urea was done according to Patton and **Bauman, (1983).** Insulin wasestimated by radioimmunoassy method using coat A. count insulin kits was obtained from Diagnostic products corporation (DPC) and done according to Pickering & **Pottinger, (1983).**

Microbiological examination of liver and kideny:

1. **Isolation of micro-organisms:** by sterile swabs obtained from internal organ e.g. liver and kideny from sacrified quails. The swabs were directly transferred to nutrient broth and incubated aerobically at 37°C for 24 hours.

The main media used for sub-culturing micro-organisms isolated from internal organs were transformed into nutrient agar media, 5% sheep-blood agar, MacConkeys agar and Saborauds agar.

2. **Identification of the isolates:** the nutrient broth cultures of the swabs were sawn into the surface of the a fore mentioned media in standard plates, incubated at 37°C aeirobecally for 3 days during which they were examined every 24 hours and selected colonies were picked up for further identification. The isolates were classified according to **Jacob and Gerstein, (1980)** into different groups.

Statistical analysis:

The obtained data were statistically subject to the students T test according to **(Gad & Weil, 1983).**

3. Results

As shown in Tables (3 and 4), there is a nonsignificant decrease in body weight of quails throughout the course of the experiment. The Copper oxychloride induced a significant increase in the liver and kidney weights throughout the course of experiment as compared with control group. The Copper oxychloride in quails of group (2) has a significant decrease on Hb level, PCV% and iron.

The bioassay of serum AST and ALT activities revealed a remarkable elevation in treated group with copper oxychloride. Serum, urea, creatinine, sodium and potassium was significantly elevated in the treated group. Serum cortisol, insulin and copper were significantly elevated in group (2) during the course of experiment. Hyperglycaemia was a constant finding from the beginning until the end of experiment in treated group. Micorbiological examination revealed the presence of Strept pyogens, E.coli, kelebsiella and B. anthrocaids in treated birds.

Table (1): Diet composition for Japanese Quiel

Ingredient	%	Calculate Nutrient composition	%
Yellow corn	64.2	Crude Protein	21.4
Soya bean	22.1	ME/K.cal.(Energy)	2970
Wheat bran	2.2	Ether Extract	3.54
Broiler Concentrate*	10.0	Crude Fiber	3.42
Line stone	1.0	Calcium	0.9
Vit. & Min. Mix	0.5	Phosphorus	0.72
		Lysine	1.12
		Methionine	0.43

Table (2): Broiler Concentrate.

Ingredient	% Composition
- Meat and Bone meal	49.5%
- Fish meal	34.4%
- Lime stone	10.0%
- Sodium Chloride	2.5%
- Methionline	2.4%
- Lysine	1.2%

Table (3): Vitamin and Mineral Mixture.

Ingredient			
- Vit. A	10.000 I.U.	- Vit B_6	30 mg
- Vit. D_3	20.000 I.U.	- Vit B_{12}	100mg
- Vit. E.	150mg	- Biotin	750mg
- Vit. K_3	30mg	- Iron	600mg
- Vit.B_1	15mg	- Iodine	30mg
- Pautothinete	100mg	-Manganese	1250mg
- Folic acid	10mg	- Copper	125 mg
- Niacin	30mg	- Selenium	3mg
- Calcium chloride	5000mg		

Table (4): Bacteriological findings from kidneys and livers of Japanese quails 1% treated with copper oxychloride in ration.

Organisms	
Strept. pyogens	++++
E.coil	+++
Kebesiella	++
B.anthrocoid	++

Table (3): Changes in body weight & Liver & kidney weight in quail exposed to Copper Oxychloride 1%.

Weight	3 days		15 days		30 days	
	Control	Treatment	Control	Treatment	Control	Treatment
Initial body weight	80.4 ± 0.34	64 ± 0.04	84 ± 0.01	74 ± 0.09*	83 ± 0.08	70 ± 0.08 *
Absolute liver weight/gm	3.09 ± 0.02	64.4 ± 0.74	3.72 ±	3.82 ± 0.14 *	3.84 ± 0.76	4.02 ± 0.74 *
Absolute kidney weight (gm).	0.99 ± 0.14	1.2 ± 0.18	0.98 ± 0.23	1.6 ± 0.72 *	0.95 ± 0.55	1.9 ± 0.94 *

N=15 * Data are significant different (P < 0.01) Data are means ± DS

Table (4): Some Clinicopathological Changes In Quails Treated With Copper Oxychloride 1%

	3 days		15 days		30 days	
	Control	Treatment	Control	Treatment	Control	Treatment
Hemoglobin g/dl	7.12 ± 0.13	6.3 ± 0.04	7.0 ± 1.02	5.8 ± 0.43 *	7.0 ± 0.34	5.4 ± 0.23 *
P.C.V %	25 ± 0.10	23 ± 0.16	24 ± 0.07	21 ± 0.38 *	23 ± 0.05	18.5 ± 0.20*
AST U/L	80 ± 0.20	90 ± 0.16	82 ± 0.27	95 ± 0.23 *	84 ± 0.24	110 ± 0.73 *
ALT U/L	18.4 ± 0.42	22.6 ± 0.52	19.0 ± 0.42	29.7 ± 0.17*	20.4 ± 0.30	39.6 ± 0.69*
Urea mg/dl	3.2 ± 0.017	3.9 ± 0.34	3.7 ± 0.10	4.2 ± 0.04*	3.00 ± 0.84	5.2 ± 0.07*
Creatinine mg/dl	0.64 ± 0.10	0.92 ± 0.23	0.72 ± 0.56	1.2 ± 0.23 *	0.78 ± 0.17	1.9 ± 0.44 *
Na MEq/L	110 ± 3.0	11.5 ± 4.2	112 ± 3.4	125 ± 5.3*	116 ± 0.72	150 ± 6.4*
K MEq/L	3.27 ± 0.11	3.6 ± 0.72	3.6 ± 0.23	4.2 ± 0.62*	3.8 ± 0.13	5.4 ± 0.71 *
Cortisol ng/dl	0.70 ± 0.24	0.82 ± 0.13	0.76 ± 0.28	0.92 ± 0.14 *	0.80 ± 0.23	1.5 ± 0.74 *
Glucose md/dl	54.2 ± 0.73	62 ± 0.64	56 ± 0.62	72 ± 0.78*	60 ± 0.08	89 ± 0.62 *
Insulin ng/dl	9.2 ± 0.07	10.4 ± 0.72	9.6 ± 0.43	11.3 ± 0.48*	9.3 ± 0.07	12.4 ± 0.94*
Copper mg%	160 ± 0.68	169 ± 0.99	159 ± 0.73	179 ± 0.23*	162 ± 0.04	182 ± 0.74*
Iron mg %	190 ± 0.43	196 ± 0.10	180 ± 0.36	160 ± 0.54*	198 ± 0.72	140 ± 0.52*

N=15 * Data are significant different (P < 0.01) Data are means ± DS

4. Discussion

Data presented in Table (3) revealed the effect of I% copper oxychloride on quail body weight. It could be noticed that the concentration of copper oxychloride (1%) decreases body weight but the decrease is not significant. It also could be noticed that quails receiving copper oxychloride in diet exhibted some response, to some enzymes which effects organ weight, the maximum ratio observed in kidney and liver wight in day 30, (P < 0.01).

As shown in Table (2) Strept. pyogens, E.coli, Kelebsiella and B. anthrocoid were isolated from kidney and liver from sacrified quail. Similar finding were reported by **Mestecky and McGhee, (1987) and Jacob and Gerstein, (1980),** they observed that Staph. and Strept. pyogens are present in animals suffering from pollution or exposed to pesticide.

Results of the present work revealed that serum glucose was elevated during the experimental period. Rise of glucose level indicates the presence a stressful stimuli eliciting rapid secretion of both gtucocorticoros and catecholamines from adrenal tissues (11). The hyperglycaemia during pesticide poising may be attributed to activation of phosphorylase enzyme system.

Response to administration of copper oxychloride in diet led temporary changes in serum insulin concentration.. This may be attributed ui high sensitivity to glucose of pancreatic cells producing somatostatin which in turn inhibits insulin during the initial period after administration of copper oxychloride in the ration **(Hassan et al., 1990; Hayes, 1989 and Ibrahim 1992).**

The significant increase of cortisol hormone level may be due to activation of hypothalamus (pituitary axis) due to stress These result coincides with **Ibrahim, (1992).** Who found the consistent effect of cortisol lead to reduction in the hemoglobin, PCV, and iron levels as a result of decrease appetite in quails or more likely to be the direct result of a catabolic effect of cortisol **(Coles, 1986; Hayes, .1989 and Joseph and Roger, 1976)**. In this experiment, sodium and potassium concentrations were significautlh increased in treated birds, which nay be attributed to kidney function impairment where the kidney is the normal pass way fo, Na, K and this ma% explain the main cause for elevation of serum creatinine and urea in treated group. This result confirmed by the results of **Mostafa *et al.*, (1992)**[4,7,12] **Coles (1986),** who stated that pesticide affects kidney functions **(Ibrahimm 1992, Sheridan et al., 1991).**

Concerning the copper level in serum, it was noticed that the administration of copper oxchloride pesticide in ration increased significantly serum copper levels in blood **(Nebbia et al., 1993 and Weeks et al., 1986).**

The measured activity of serum enzyme aspartate and alanine aminotransferase for the treated

and control quail during the whole experiment showed a noticable increase of ALT & AST of quails treated with the pesticides. Such finding most probably denotes liver cell affection (toxic hepatitis). Also this increase was attributed to the distruction of the cells of liver, since the liver is the primary organ of detoxification as well as a major site for detoxifications reactions. Therefore, a significant increase in liver enzymes suggest an explanation for the presence of toxin in the liver cells **(Hennawy, 1980).** Our results agree with **Nebbia et al., (1993),** who observed the damage of liver and skeletal muscle in rabbits exposed to pesticide that caused liver and kideny disfunction. The significant increase in urea and creatinine, sodium and potassium could be attributed to the observed degeneration within some renal tubules in quails recieving 1 % copper oxychloride. Data agree with **(Blaxhall, 1972; Husain et al., 1987 and Neskovi et al., 1992)**

In conclusion 1% pesticide copper oxychloride is not safe sine affects liver and kidney dysfunctions and its use should be restricted.

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References

1. **Asztalos B.; Nemesok .1.; Benedeezky L; Gabriel R.: Szabo A. and Refaie O.J. (1990):** Arch. Environ. Contarn. Toxicol., 19: 275-282.
2. **Barham W. T.; Smit, G.J. and Shoobee, H. J. J. (1972):** Fish. Biology., 17: 275.
3. **Bartels, H.; Bohnzer, M. and Heierli, C. (1972):** Clinical chemistry Acta, 37: 193.
4. **Blaxhall (1972):** Journal of Bio. Chem.,4: 593-605.
5. **Coles, E.T. (1986):** Veterinary Clinical Pathology.4th Ed. W.S. saunders. Company, Philadelphia, London, Retman S. and Frankel S. Am. J. Clin.Pathol. 28,56 (1997)
6. **Drabkin, D.J. (1946):** Biol. Chem., 164, 703,.
7. **El-Hennawy, S.L.; Shama, A.I.; Geabah, N.E. and Sadek, M. (1980):** Research Bull, (1274) 1-15, Faculty of Agriculture Ain Shams University.
8. **Gad, S.C. and Well, C.S. (1983):** Raven Press, New York, pp. 237-320.
9. **Hassan, A.A.M.; El-Sewedy , S. M.; Minatogawa , Y. and kido, R. (1990):** Journal of Environment of Science and Health Part B, B 25: 333-346. Hayes, WA. (1989): principles and techniches 2nd Edition, Haper and Row. P. 525.
10. **Husain, R.; Ashari, R. A. and Gupta, P.C. (1987):** Journal or Environmental Biology, 8: 137-142.
11. **Ibrahim, Th. A. (1992):** Proc. Of 2'd Cong Faculty Verterinary Medicine Cairo University, 47 : 51.
12. **Jacobs, M.B. and Gerstein M.J. (1982):** Hand-book of Microbiology. B. Van Nastrand company Inc. Toronto, New York & London.
13. **Joseph, A. and Roger, W. G. (1976): Clinical** chemistry principles and procedures forth ed. Boston , pp 186-197.
14. **Linman, J. W. (1975):** Haematology, Physiologyic, publish Company, Inc, New york 119 pp.
15. **Mostafa, I. Y.; Zayed, S.M.A.D. and Farghaly, M. (1992 a):** Journal of Environmental Science and Health, B., (27)4:399-405.
16. **Mestecky, J. and McGhee, J.R. (1987):** Advanced Immunology, 40:153-245 (510 ref.).
17. **Nebbia, C.; Dacasto, M.; Gennaro Soffietti, M.; Rasero, R. and Copenhag (1993):** 73:233-239.
18. **Neskovic, N.K.; Karan, V.; Budimir, M.; Vojionvic, V.; Gasic, S. and Silij-Vitorivic. (1992):** Journal of Environmental Science and Health, B (27) 4: 387-397.
19. **Patton, CJ. and Bauman, WA. (1983):** Rifking Excerpta Medica, NewYork , pp. 119-150.
20. **Pickering, A.D. and Pottinger, P. (1983):** Gen. Corn. Endocrinol. 49, 232.
21. **Reitman, S. and Frankel, S. (1957):** Am. J. Clin. Pathol., 28: 56.
22. **Sheridan, M.A.; Eilertson, C.D. and Pisetskaya, E.M. (1991):** Endocrinol. 81, 365.
23. **Silversmit, A.B. (1965):** Med 45, 175.
24. **Trinder, P. (1969):** Ann, Clim biochem. 6, 24.
25. **Weeks, B.A.; Warinn2r, J.E.; Mason, P.L. and Mc Ginnis, D.S. (1986):** Journal of Fish Biol., 28: 635.

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