

ZETAMETHRIN POST-EXPOSURE HAEMATO-IMMUNO MODULATIONS IN NON-VACCINATED CHICKS (*GALLUS DOMESTICUS*)

NAJMA ARSHAD, ANWAR MURTAZA, SAIRA MAHMOOD, ZAHEER IQBAL AND MOHAMMAD ARSHAD

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore (NA, AM, SM), Poultry Research Institute (ZI), Rawalpindi and Department of Zoology, Government College (MA), Lahore

Abstract: Study was designed to find Zetamethrin post-exposure haemato-immuno modulations in non-vaccinated broiler chicks. Zetamethrin was administered @ 1.265 ± 0.198 mg/kg body weight / day for six weeks. It induced persistent anaemia, but reversible changes in haematological parameters and serum proteins.

A significant ($P < 0.05$) depletion in haemoglobin level (Hb), total leukocyte count (TLC), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was recorded after six weeks of intoxication. Whereas, elevated serum protein levels were observed at the same time. Hemoglobin contents and mean cell haemoglobin concentration remained significantly low after six weeks of removal of stress ($P < 0.05$). Total serum proteins and total leukocyte contents were found in normal range following 15 days of removal of stress, whereas mean corpuscular haemoglobin was restored to normal level after 15 days of removal of stress.

Key words: Zetamethrin, haemato-immunomodulation, chicks

INTRODUCTION

It is now an established fact that human being, livestock, poultry and wildlife are continuously facing a threat from the extensive use of biocides in the agriculture (Repetto and Baliga, 1996). Biocides are applied for the elimination of pests. Different insecticides have been found to bioaccumulate in primary consumers and contaminate food chain (O'Brien and Hamilton, 1976; George and Sundararaj, 1995).

A substantial data is available on the deleterious effects of all major groups of biocides including organo-phosphates, organo-chlorine and pyrethroids on various haematological and biochemical parameters (Thaker *et al.*, 1996; Kakkar, *et al.*, 1996; Fauzia, *et al.*, 1995; Rustamov, *et al.*, 1994).

However, there are some reports, which show that few insecticides are safe and do not exert measurable effects on non-target organisms (Mlynarcikova *et al.*, 1995).

The available literature gives information on exposure to response relationship but least is known about the post-exposure responses. Therefore, we planned to find out

any reversible or irreversible alteration in various haematological and immunological parameters after the removal of insecticidal stress.

MATERIALS AND METHODS

Experimental animals:

Broiler chicks (*Gallus domesticus*) were obtained from local hatchery (H₁-Tech. Shadman, Lahore) and maintained under recommended conditions, in the animal house of Zoology Department, University of the Punjab, Lahore, Chick feed and water were provided *ad libitum*.

Animal grouping and treatment:

Animals were divided into two groups, i.e. control and experimental. All of the animals were left un-vaccinated. Animals of experimental group were exposed to a sublethal dose of Zetamethrin (1.265 ± 0.198 mg / kg body weight / day) continuously for 6 weeks. This intoxication was stopped and its effects were monitored in following 6 weeks.

Various haematological and some biochemical parameters were recorded fortnightly from the age of 6 weeks to 12 weeks. Haemoglobin (Hb) contents were estimated according to Vankampen and Zijlstra (1961). Total erythrocyte (TEC) and leukocyte counts (TLC) were observed according to Natt and Harriek (1952), whereas, packed cell volume and haematological indices were calculated following Swarup *et al.* (1986). Total serum proteins were estimated by Biuret Method (Henry *et al.*, 1974). Serum albumin was determined following Doumas *et al.*, 1971. Total globulin contents and albumin / globulin ratio was calculated following Varley *et al.* (1987).

RESULTS

Zetamethrin adversely affected haemoglobin concentration. It was found significantly ($P < 0.05$) low throughout experimental period. Total leukocyte count was found to be low after 6 weeks intoxication but it was reverted to normal range in post-exposure period.

Mean cell haemoglobin (MCH) and mean cell haemoglobin concentrations (MCHC) were found to be significantly low at the age of 6 & 10 weeks and 6 & 12 week respectively. Six week intoxication resulted in a significant increase in serum protein contents (20.54%) but it declined slowly to normal level during post-exposure times. Serum albumin was found to be high (19.15%) after intoxication; it also reverted to normal level in the following 15 days. Serum globulin levels and albumin to globulin ratio remained statistically unaltered throughout the experimental period (Table-1).

DISCUSSION

Blood is a complex suspension of specialized cells, most of which are swept along throughout their limiting lives by the streaming plasma. As they plunge through the blood stream blood cells supply most of the basic energy, nourishment, host defense and custodial services needed to sustain the life of organisms. This sagging cell filled liquid is a metaphor for life itself (Janes and Jandl, 1996).

During present studies a significant reduction in haemoglobin was recorded in intoxicated chicks. This decrease in haemoglobin level is possible due to (i) Decrease in number of RBCs (ii) Decrease in concentration of Hb/cell (iii) Decrease in the rate of synthesis of haemoglobin.

The number of RBCs were found to be undisturbed so we can conclude that in our study the depletion in Hb may be due to inhibition in hemoglobin synthesis. This view is further supported by decrease in MHC at subsequent intervals. Our findings are in consistence with Rustamov (1994), he reported the fall in haematological parameters following exposure to cypermethrin in chicks. White blood cells in the animal body constitute a special system combating the different infectious and toxic agents. These cells work in two different ways to prevent body from various pathogens. In the present studies after 6 weeks of intoxication a significant depletion of white blood cells was observed, but during the post-exposure period WBCs were found in the normal ranges. Depletion in leukocyte count following exposure to Pyrethroids has also been reported by various authors (Kakkar *et al.*, 1996; Rustamov *et al.*, 1994).

No alteration in PCV and erythrocyte count could be found in experimental chicks throughout the experimental period. Low level of haematological indices suggest that Zetamethrin exposure results in inhibition in haemoglobin synthesis and induces anaemia, which may persist for 2-4 weeks of post-exposure time. Our findings are in consistent with Mohiuddin *et al.* (1986), Majumder *et al.* (1994) and Sobhhy *et al.* (1994) who reported a decline in haematological values following Aflatoxin Cypermethrin and Fenvalerate exposure in broiler chicks.

Plasma proteins are released in blood mainly from liver. High level of plasma protein is indicating abnormal rate of protein metabolism. Pande *et al.* (1995) have reported similar changes after carbamate intoxication in chicks. During post-exposure studies no significant difference could be observed between protein level of control and experimental groups. Therefore, we can conclude that Zetamethrin @ 1.208 ± 0.135 mg / kg body weight / day for 6 weeks exerts reversible changes in various parameters and thus is safe for use at this concentration. Our results are in agreement with Kowalzyk *et al.*, 1990; Zukowicz and Krechniak, 1992 and Mlynarcikova *et al.*, 1995).

Table I: Zetamethrin (@ 1.2±0.2 mg/ kg b.w / day) Post-exposure Changes in Various Parameters in non vaccinated broiler chicks (values given as mean ±St. Essor & mean).

Parameters	6 week		8 week		10 week		12 week	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Hb (g/dl)	13.60 ± 0.22	11.37 ± 0.09*	11.37 ± 0.50	11.24 ± 0.23	14.46 ± 0.16	11.95 ± 0.17*	14.42 ± 0.16	11.08 ± 0.95*
RBCs (x10 ⁶ /µl)	2.62 ± 0.14	2.66 ± 0.16	3.40 ± 0.93	2.94 ± 0.23	2.71 ± 0.26	2.60 ± 0.07	2.39 ± 0.15	2.04 ± 0.13
WBCs (x10 ³ /µl)	18.12 ± 0.50	14.12 ± 1.45	15.65 ± 0.81	13.30 ± 0.30	20.49 ± 0.83	21.28 ± 2.48	17.12 ± 0.81	18.56 ± 1.43
PCV (%age)	30.46 ± 0.64	33.32 ± 0.30	35.39 ± 1.79	33.41 ± 1.03	37.78 ± 1.52	32.84 ± 2.77	37.77 ± 1.81	39.59 ± 1.03
MCV (fl)	116.25 ± 4.70	125.23 ± 4.83	104.08 ± 1.92	113.64 ± 4.89	137.61 ± 5.80	126.31 ± 39.46	156.35 ± 12.36	194.06 ± 5.53
MCH (pg)	51.90 ± 0.29	44.09 ± 0.15*	33.44 ± 0.46	38.23 ± 0.53	52.65 ± 2.40	45.96 ± 1.90*	60.33 ± 4.92	54.31 ± 2.85
MCHC (pg)	44.66 ± 3.38*	35.20 ± 4.06*	32.12 ± 0.28	33.64 ± 0.23	38.27 ± 0.11	36.38 ± 0.06	38.58 ± 3.39	27.98 ± 5.89*
Protein (g/dl)	3.66 ± 0.07	4.41 ± 0.01	2.98 ± 0.35	3.01 ± 0.14	3.99 ± 0.19	4.54 ± 0.22	3.11 ± 0.50	2.54 ± 0.37
Albumin (g/dl)	1.74 ± 0.89	2.07 ± 0.01	1.74 ± 0.06	1.71 ± 0.01	1.84 ± 0.08	1.54 ± 0.21	1.37 ± 0.19	1.53 ± 0.13
Globulin (g/dl)	1.92 ± 0.19	2.34 ± 0.21	1.24 ± 0.12	1.31 ± 0.13	2.15 ± 0.18	2.90 ± 0.21	1.74 ± 0.34	1.61 ± 0.24
Albumin / Globulin (ratio)	0.91 ± 4.65	0.87 ± 0.05	1.41 ± 0.51	1.30 ± 1.10	0.85 ± 0.43	0.53 ± 0.99	0.79 ± 0.55	0.95 ± 0.53

*=P<0.05; **=P<0.01; ***=P<0.001

REFERENCES

- DOUMAS, B.T., WATSON, W.A. AND BIGGS, H.G., 1971. *Clin. Chem. Asta.*, pp.31-87.
- FAUZIA, S.Y., BASHANDY, M.M. HASSAN, S.G. AND MOHAMMAD, A.H., 1995. Experimental studies on the effects of organo-phosphorous insecticide Nequvon on cellular and biochemical constituents of blood and fertility in the poultry. *Egyp. J. Comp. Path and Clin. Path.*, **8**:71-78.
- GEORGE, V.T. AND SUNDARARAJ A., 1995. Studies on residue of DDT in poultry. *Ind. Vet. J.*, **72**:17-20.
- HENRY, R.J., CANON, D.C. AND WINKELMAN, J.W., 1974. *Clinical Chemistry, Principles and Techniques*. 2nd Ed. Harper and Row. New York, pp.96-98.
- JANES, H. AND JANDL, M.D., 1996. *Text Book of Haematology*. 2nd ed. Little brown and Company Boston New York Toronto London, pp.46-51.
- KAKKAR, B.B. MAHIPAI, S.K. AND CHAUHAN, R.S. 1996. Effects of carbaryl and Malathion on cell mediated immune response in chickens. *Int. J. Anim. Sci.*, **11**:231-233.
- KOWALZYK, B., GUELDANOWSKI, J. AND BUBAK, B., 1990. Immunological profile of animals exposed to pesticide deltamethrin. *Arch. Immunol. Ther. Exp. Warsz.*, **32**:229-238.
- MAJUMDER, S., CHAKRABORTY, A.K., MANDAL, T.K., BHATTACHARYA, A. AND BASAK, D.K., 1994. Subacute toxicity of Fenvalerate in Broiler chicks; concentration, cytotoxicity and biochemical profiles. *Indian J. Exp. Bio.*, **32**:752-756.
- MLYNARCIKOVA, H., LEGATH, J. AND SOKOL, J., 1995. Chronic influence of pyrethroid supermethrin on some reproductive features in Japanese quail (140 days long avian reproduction test): *C. Euro. J. Pub. Hlth.*, **3**:132-133.
- MOHIUDDIN, S.M., REDDY, M.V., REDDY, M.M. AND RAMAKRISHINA, K., 1986. Studies on phagocytic activity and haematological changes of aflatoxicosis in poultry. *Indian Vet. J.*, **63**:442-443.
- NATT, M.P. AND HARRICK, C.A., 1952. A new blood diluent for counting the erythrocytes and leukocytes of chicken. *Poult. Sci.*, **31**:735-738.
- O'BRIEN, R.D. AND HAMILTON, B.D., 1979. The effects of DDT in mice. *Tohoku J. Exp. Med.*, **122**:143-153.
- PANDE, H.B., MOREQAONKAR, S.D., DEQLOORAKAR, N.M., VADLAMUDI, V.P. AND RAJURKAR, S.R., 1995. Pathology of subacute oncol (Benefuracarb) toxicity in chickens. *Ind. J. Vet. Path.*, **18**:151-153.
- REPETTO, R. AND BALIGA, S.S., 1996. *Pesticides and immune system: A public health risk*. World resource institute, 1st ed. pp. 1-102.

- RUSTAMOV, Yu. M., 1994. Effects of feeding cypermethrin to hens on hatchability and chick viability. *Problemy Vet. Sani-I-Ekol.*, **94**:67-68.
- SOBBHY, H.M., BASHANDY, M.M. AND IBRAHIM, Y.S., 1994 Effects of long term administration of Fenvalerate (Synthetic pyrethroid) insecticide on chickens. *Egypt. J. Comp. Path. And Clin. Path.*, **7**:99-107.
- SWARUP, H., ARORA, S. AND PATHAK, S.C., 1986. *Laboratory Techniques in Modern Biology*. 2nd Ed., pp. 177-277.
- THAKER, A.M., SUSHMA, R., GARO, B.D. AND RISHI, S., 1996. Effects of long term exposure of Malathion and endosulfan on immune response in poultry. *Haryana Vet.*, **35**:23-30.
- VANKAMPEN, E.J. AND ZIJLISTRA, W.G., 1961. Standardization of haemoglobinometry, II. The haemoglobin cyanide method. *Clin. Chem. Acta.*, **6**:538-544.
- VARLEY, H., GEONLOCK, A.H. AND BELL, M., 1987. *Practical Clinical Chemistry*. 2nd Ed., pp.535-574. William Heinemann Medical Ltd. U.K.
- ZUKOWICZ, R.J. AND KRECHNIAK, J., 1992. Effects of deltamethrin on immune system in mice. *Env. Res.* **59**:467-475.