

## TOXIC EFFECTS OF DELTAMETHRIN AND FLUORIDE ON HEMATOLOGICAL PARAMETERS IN RATS

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**SUMMARY:** As part of our study on the toxic effects of deltamethrin and fluoride (F) on antioxidant parameters in rats, hematological effects of these chemicals were evaluated in the same animals. Deltamethrin and F produced a marked decrease in the hematological parameters including total erythrocyte count, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total leukocyte count. Among alterations in the leukocytes, lymphopenia, neutrophilia, and eosinopenia were observed. Exposure of the rats to both deltamethrin and F together gave the greatest changes.

Keywords: Deltamethrin in rats; Fluoride intoxication; Hematological parameters; Leukocyte alterations.

### INTRODUCTION

Our recent study on deltamethrin and fluoride (F) in rats has shown they augment the toxicity of one another by the extensive oxidative stress they induce in erythrocytes.<sup>1</sup> The neurotoxic mechanisms of deltamethrin include prolonging the opening of the voltage-sensitive sodium channels and inhibition of voltage-gated chloride channels and GABA<sub>A</sub> (gamma amino butyric acid) receptors.<sup>2</sup> In the body, F can cross the cell membrane and affect various soft tissues leading to the impaired tissue functions.<sup>3</sup> Studies on these chemicals have also been found to produce hematological alterations.<sup>4,5</sup> Besides animals, humans are also exposed concurrently to a wide array of chemicals in the environment. However, relatively few studies have assessed the degree of hazard posed by simultaneous exposure to certain toxic chemicals, especially, at lower doses.<sup>1,6</sup> In the present study, the interactive effect of deltamethrin and F on the hematological parameters in rats, following their sub-acute oral exposure, was investigated.

### MATERIALS AND METHODS

The same Wistar rats involved in our recent study<sup>1</sup> were used in this research: a control group (left untreated) and three treatment groups receiving deltamethrin (1.28 mg/kg bw/day), F (20 ppm in their drinking water), and deltamethrin plus F co-exposure. The experimental design with the animals was approved by the Institutional Animal Ethical Committee. After 28 days of daily treatment, the rats were fatally anaesthetized with diethyl ether. Blood samples were collected from retro-orbital fossa using capillary tubes in separate aliquots containing heparin and di-potassium salt of EDTA at the concentration of 10 IU/mL and 2 mg/mL of blood, respectively. Heparinized blood was used for the analysis of hemoglobin

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(Hb),<sup>7</sup> whereas, total erythrocyte counts (TEC), packed cell volume (PCV), total leukocyte count (TLC) and differential leukocyte count (DLC) were analyzed in the EDTA treated blood.<sup>8</sup> The mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated mathematically.<sup>8</sup>

*Statistical analysis:* The results were subjected to analysis of variance (ANOVA) in completely randomized design (CRD) with statistical significance being tested using the Duncan Multiple Range Test.<sup>9</sup>

## RESULTS

*Changes in TEC, Hb, and PCV:* Results on the effect of deltamethrin and F on the erythrocyte indices are presented in Table 1. A significant decrease in the TEC, when compared with the control group, was observed in the rats exposed to F alone and to deltamethrin plus F, with lowest values being observed in the deltamethrin-F group. A significant decrease in the Hb and PCV values was observed in all the treated groups. However, in contrast to the TEC values, Hb and PCV results from the combined group were significantly different from both the F and deltamethrin groups.

**Table 1.** Effect of repeated oral administration of deltamethrin, fluoride, and their combination on erythrocytic indices in rats. (Values given are mean  $\pm$  SEM, n=6)\*

	Groups			
	Control	Deltamethrin	Fluoride	Deltamethrin+Fluoride
TEC ( $10^6/\text{mm}^3$ )	7.83 $\pm$ 0.32 <sup>a</sup>	7.13 $\pm$ 0.25 <sup>ab</sup>	6.92 $\pm$ 0.14 <sup>bc</sup>	6.22 $\pm$ 0.24 <sup>c</sup>
Hb (g/dL)	17.41 $\pm$ 0.35 <sup>a</sup>	15.09 $\pm$ 0.36 <sup>b</sup>	14.19 $\pm$ 0.26 <sup>c</sup>	12.10 $\pm$ 0.2 <sup>d</sup>
PCV (%)	54.07 $\pm$ 1.75 <sup>a</sup>	49.06 $\pm$ 1.74 <sup>b</sup>	48.27 $\pm$ 1.06 <sup>b</sup>	41.68 $\pm$ 0.86 <sup>c</sup>
MCH (pg)	22.32 $\pm$ 0.50 <sup>a</sup>	21.29 $\pm$ 0.88 <sup>ab</sup>	20.52 $\pm$ 0.30 <sup>ab</sup>	19.58 $\pm$ 0.67 <sup>b</sup>
MCHC (g/dL)	32.37 $\pm$ 1.22 <sup>a</sup>	30.84 $\pm$ 0.43 <sup>ab</sup>	29.41 $\pm$ 0.36 <sup>b</sup>	29.04 $\pm$ 0.24 <sup>b</sup>
MCV (femtoL)	69.48 $\pm$ 3.30 <sup>a</sup>	69.12 $\pm$ 2.95 <sup>a</sup>	69.80 $\pm$ 0.85 <sup>a</sup>	67.41 $\pm$ 2.18 <sup>a</sup>

\*Means with at least one common superscript do not differ significantly ( $p < 0.05$ ).

*Changes in MCH, MCHC, and MCV:* As seen in Table 1, MCH values of the treatment groups were lower than the control group. However, only in case of the combined group were these values significantly different from the control values. A statistically significant decrease in the MCHC values occurred in the F and combined groups, though it decreased non-significantly in the deltamethrin group. In comparison with the control group, the values of MCV were non-significantly lower in the treatment groups.

*Changes in TLC and DLC:* As shown in Table 2, a statistically significant decrease in the TLC was observed in the F and combined groups with DLC

marked by significant lymphopenia, eosinopenia, and neutrophilia, in the combined group. Similar, though non-significant alterations, were observed in the deltamethrin and F groups, when compared with the control group. The other DLC values in the treated groups were *at par* with the control group.

**Table 2.** Effect of repeated oral administration of deltamethrin, fluoride, and their combination on leukogram in rats. (Values given are mean  $\pm$  SEM, n=6)\*

	Groups			
	Control	Deltamethrin	Fluoride	Deltamethrin+Fluoride
TLC ( $10^3/\text{mm}^3$ )	9.65 $\pm$ 0.23 <sup>a</sup>	8.56 $\pm$ 0.41 <sup>ab</sup>	7.98 $\pm$ 0.56 <sup>bc</sup>	6.85 $\pm$ 0.68 <sup>c</sup>
Lymphocytes (%)	50.98 $\pm$ 1.23 <sup>a</sup>	50.00 $\pm$ 1.71 <sup>a</sup>	50.08 $\pm$ 1.75 <sup>a</sup>	43.65 $\pm$ 2.07 <sup>b</sup>
Neutrophils (%)	38.97 $\pm$ 1.06 <sup>a</sup>	42.63 $\pm$ 1.85 <sup>ab</sup>	42.05 $\pm$ 2.03 <sup>ab</sup>	48.00 $\pm$ 2.14 <sup>b</sup>
Eosinophils (%)	1.43 $\pm$ 0.23 <sup>a</sup>	1.07 $\pm$ 0.18 <sup>ab</sup>	0.92 $\pm$ 0.22 <sup>ab</sup>	0.77 $\pm$ 0.11 <sup>b</sup>
Basophils (%)	0.28 $\pm$ 0.05 <sup>a</sup>	0.32 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.17 <sup>a</sup>
Monocytes (%)	6.27 $\pm$ 0.23 <sup>a</sup>	6.04 $\pm$ 0.35 <sup>a</sup>	5.88 $\pm$ 0.56 <sup>a</sup>	6.45 $\pm$ 0.59 <sup>a</sup>

\*Means with at least one common superscript do not differ significantly ( $p < 0.05$ ).

## DISCUSSION

Decreased total erythrocyte counts, hemoglobin levels, and packed cell volume have been reported in laboratory animals with their exposure to pyrethroids<sup>10-14</sup> and to F.<sup>15-19</sup> Decreased heme synthesis in bone marrow,<sup>20</sup> increased rate of destruction or reduction in the rate of formation of RBCs,<sup>12</sup> and increased erythrocyte lipid peroxidation<sup>21</sup> could be the possible reasons for such reduced hematological levels.<sup>22</sup> In our study dealing with the toxic effects of deltamethrin and F on antioxidant parameters in rats, these two chemicals, especially when co-administered, were found to induce oxidative stress in the erythrocytes.<sup>1</sup> Damaging effects of free radicals on RBCs are suggestive for the decrease in PCV values.<sup>23</sup> In male mice, Mittal and Flora reported that NaF produces a decrease in hematocrit and a significant depletion in blood delta-aminolevulinic acid dehydratase activity, glutathione level, and white blood cells.<sup>17</sup> Thus, the decrease in these hematological values suggests that deltamethrin and F can produce oxidative stress that induces anemia, which is aggravated on co-exposure to these chemicals. This interpretation is supported by a study correlating oxidative stress and hematological alterations in goats following their exposure to bifenthrin.<sup>22</sup> A significant decrease of MCH and MCHC in combined group is suggestive of increased destruction of hemoglobin in this group. Since the MCV values between all the groups were *at par*, the anemia produced is normocytic and hypochromic.

Decrease in the leukocytic count has been reported with pyrethroids<sup>11,24,25</sup> and by F<sup>15,26</sup> in several studies. In mice poisoned with cypermethrin, a decrease in the

number of T-lymphocytes in the spleen and blood was observed, which suggests the disturbing effect of cypermethrin directly on the process of lymphopoiesis.<sup>27</sup> Non-specific tissue irritation due to the toxicant and/or its metabolites and resulting free radicals might induce production and release of inflammatory mediators like prostaglandins that produce neutrophilia and lymphopenia.<sup>28</sup> Stress induced by the toxicity of chemicals causes release of corticosteroids, which are responsible for neutrophilia. This can be supported by neutrophilia induced by dexamethasone, leading to enhanced release of mature neutrophils from the bone marrow and the consequent demargination, with the latter being the largest contributor to the expanded circulating pool.<sup>29</sup> The stress-altered leukogram of the deltamethrin and/or F treated rats is characterized by differential leukocyte count (DLC) consisting of neutrophilia, lymphopenia, and eosinopenia.<sup>30</sup>

In summary, deltamethrin and F toxicity produce hematological alterations characterized by anemia and stress leukogram. With the combined exposure to these chemicals, the alterations are more severe, indicating a positive hemato-toxic interaction between these two chemicals.

#### REFERENCES

- 1 Dubey N, Raina R, Khan AM. Toxic effects of deltamethrin and fluoride on antioxidant parameters in rats. *Fluoride* 2012;45:242-6.
- 2 Imamura L, Yasuda M, Kuramitsu K, Hara D, Tabuchi A, Tsuda M. Deltamethrin, a pyrethroid insecticide, is a potent inducer for the activity-dependent gene expression of brain-derived neurotrophic factor in neurons. *J Pharmacol Exp Ther* 2006;316:136-43.
- 3 Kant V, Srivastava AK, Raina R, Verma PK, Singh P. Negligible amelioration by aluminium sulphate on subacute fluoride-induced enzymatic alterations in goats. *Fluoride* 2010;43:246-52.
- 4 Mongi S, Mahfoud M, Amel B, Kamel J, Abdelfattah el F. Protective effects of vitamin C against haematological and biochemical toxicity induced by deltamethrin in male Wistar rats. *Ecotoxicol Environ Saf* 2011;74(6):1765-9.
- 5 Singh JL, Swarup G. Fluorosis in buffaloes. *Vet Rec* 1994;135:260-1.
- 6 Wade MG, Foster WG, Younglal EV, McMahon A, Leingartner K, Yagminas A, et al. Effects of subchronic exposure to a complex mixture of persistent chemicals in male rats; systemic, immune and reproductive effects. *Toxicol Sci* 2002;67:131-43.
- 7 Drabkin DL, Austin JH. Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *J Biol Chem* 1935;112:51-65.
- 8 Schalm OW, Jain NC, Carroll EJ. *Veterinary hematology*. 3rd ed. Philadelphia: Lea and Febiger; 1975.
- 9 Duncan DB. Multiple range and multiple F tests. *Biometrics* 1955;11:1-42.
- 10 Mohamed ZA. Changes in rat blood profile and blood chemistry after repeated dermal application of fenvalerate and decamethrin. *Egypt J Food Sci* 1988;16:79-86.
- 11 Manna S, Bhattacharyya D, Mandal TK, Das S. Repeated dose toxicity of deltamethrin in rats. *Indian J Pharmacol* 2005;37:160-4.
- 12 Shah MK, Khan A, Rizvi F, Siddique M, Sadeeq-Ur-Rehman. Effect of cypermethrin on clinico-haematological parameters in rabbits. *Pakistan Vet J* 2007;27:171-5.
- 13 Ahmad LA, Khan MZ, Khan I, Hussain. Cypermethrin induced anaemia in male rabbits. *Pakistan Vet J* 2009;29(4):191-5.
- 14 Sharaf S, Khan A, Khan MZ, Aslam F, Saleemi MK, Mahmood F. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: their attenuation with vitamin E and selenium. *Exp Toxicol Pathol* 2010;62:333-41.
- 15 Eren E, Ozturk M, Mumcu EF, Canatan D. Fluorosis and its hematological effects. *Toxicol Ind Health* 2005;21(10):255-8.
- 16 Bouaziz H, Fetoui H, Ketata S, Jammoussi K, Ellouze F, Zeghal N. Effects of sodium fluoride ingested by lactating mice on some haematological parameters in suckling pups and dams. *Fluoride* 2006;39(3):211-9.

- 17 Mittal M, Flora SJ. Vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice. *Drug Chem Toxicol* 2007;30(3):263-81.
- 18 Karadeniz A, Altintas L. Effects of *Panax ginseng* on fluoride-induced haematological pattern changes in mice. *Fluoride* 2008;41(1):67-71.
- 19 Vijaya Bhaskara Rao A, Vidyunmala S. Cumulative effect of fluoride on hematological indices of mice, *Mus norvegicus albinus*. *American-Eurasian J Toxicol Sci* 2009;1(2):81-3.
- 20 Shakoori AR, Aslam F, Sabir M. Effect of prolonged administration of insecticide (cyhalothrin/karate) on the blood and liver of rabbits. *Folia Biol (Krakow)* 1992;40:91-9.
- 21 Tung HT, Cook FW, Wyatt RD, Hamilton PB. The anemia caused by aflatoxin. *Poult Sci* 1975;54:1962-9.
- 22 Khan AM, Sultana M, Raina R, Dubey N, Dar SA. Effect of sub-acute toxicity of bifenthrin on antioxidant status and hematology after its oral exposure in goats. *Proc Natl Acad Sci India Sect B Biol Sci* [DOI 10.1007/s40011-013-0157-y]. 2013 Jan [cited 2013 Jan 19]. Available from: <http://link.springer.com/article/10.1007/s40011-013-0157-y#page-1>.
- 23 Gossett KA. Anemias associated with drugs and chemicals. In: Feldman BF, Zinkl JG, Jain NC, editors. *Schalm's veterinary hematology*. 5th ed. Philadelphia, PA, USA: Lippincott, Williams and Wilkins; 2000. p. 185-9.
- 24 Varshneya C, Singh T, Sharma LD, Bahga HS, Garg SK. Immunotoxic responses of cypermethrin, a synthetic pyrethroid insecticide in rats. *Indian J Physiol Pharmacol* 1992;36:123-6.
- 25 Dési I, Dobronyi I, Varga L. Immuno-, neuro-, and general toxicologic animal studies on a synthetic pyrethroid: cypermethrin. *Ecotoxicol Environ Safety* 1986;12:220-32.
- 26 Kant V, Verma PK, Pankaj NK, Kumar J, Kusum, Raina R, et al. Haematological profile of subacute oral toxicity of fluoride and ameliorative efficacy of aluminium sulphate in goats. *Toxicol Int* 2009;16:31-5.
- 27 Haratym-Maj A. Hematological alternations after pyrethroids poisoning in mice. *Ann Agric Environ Med* 2002;9:199-206.
- 28 Ulich TR, Dakay EB, Williams JH, Ni RX. *In vivo* induction of neutrophilia, lymphopenia, and diminution of neutrophil adhesion by stable analogs of prostaglandins E1, E2, and F2 alpha. *Am J Pathol* 1986;124(1):53-8.
- 29 Nakagawa M, Terashima T, D'yachkova Y, Bondy GP, Hogg JC, van Eeden SF. Glucocorticoid-induced granulocytosis: contribution of marrow release and demargination of intravascular granulocytes. *Circulation* 1998;98:2307-13.
- 30 Kahn CM, Line S, editors. *The Merck Veterinary Manual*. 10th ed. Whitehouse Station, NJ, USA: Merck and Co. Inc; 2010.