Available online at www.sciencedirect.com
ScienceDirect
Experimental and Toxicologic Pathology 62 (2010) 333–341
EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY
www.elsevier.de/etp

Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium

Summaira Sharaf, Ahrar Khan*, Muhammad Zargham Khan, Faiza Aslam, Muhammad Kashif Saleemi, Fazal Mahmood

Department of Veterinary Pathology, University of Agriculture, Faisalabad 38040, Pakistan

Received 8 November 2008; accepted 7 May 2009

Abstract

This study was carried out on 90 one-day-old broiler chicks to know clinico-hematological alterations, DNA damage caused by cypermethrin (CY), and attenuation of toxic effects by vitamin E (Vit E) and selenium (Se). Birds were randomly divided into five equal groups. Groups 1–4 received CY (600 ml kg^{-1} b.wt) daily for 30 days by crop tubing. In addition to CY, groups 2, 3 and 4 received Vit E (150 mg kg^{-1} b.wt), Se (0.25 mg kg^{-1} b.wt), and Vit E (150 mg kg^{-1} b.wt) + Se (0.25 mg kg^{-1} b.wt), respectively. Group 5 served as control. Birds were monitored twice daily for clinical signs. They were weighed and blood samples were collected at experimental days 10, 20 and 30 for hematological studies. CY-treated birds showed more prominent signs of toxicity compared to CY + Vit E, CY + Se and CY + Vit E + Se birds. Body weight in groups 1–3 was significantly ($P<0.05$) smaller at days 20 and 30 when compared with the control group. Significantly ($P<0.001$) higher numbers of micronuclei appeared in chicks treated with CY compared to CY + Vit E- and CY + Se-treated birds. Significantly decreased total erythrocyte counts (TEC), hemoglobin (Hb) concentration and packed cell volume (PCV) in all treated groups were recorded. Treated birds suffered from macrocytic hypochromic anemia. Leukocytosis in early stage and later leucopenia was seen in treated birds. It can be concluded that CY induces toxic effects in broilers chicks; however, these toxic effects can be ameliorated by Vit E or Se. Combination of Vit E and Se was more effective to ameliorate toxic effects of cypermethrin.

Keywords: Cypermethrin; Broiler chicks; Clinical signs; Weight loss; Anemia; Micronucleus; Vitamin E; Selenium

Introduction

Pesticides are major contaminants of our environment and many persist in the environment including various feeds and foodstuffs (Garg et al., 2004). These pesticides constitute the key control chemicals for management of pests and diseases (Das et al., 2007). Pyrethroids are used preferably over organochlorines, organophosphates and carbamates due to their high effectiveness against a wide range of insects, low toxicity to non-target organisms (mammals) and easy biodegradability (Dorman and Beasley, 1991). They are used to control ectoparasites infecting poultry, sheep, cattle and companion animals. This class of insecticides has also been

*Corresponding author. Tel.: +92 41 9200161 170x3119/ +92 3336517844; fax: +92 41 9200764.
E-mail address: ahrar1122@yahoo.com (A. Khan).

0940-2993/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.etp.2009.05.002

used in agricultural and home formulations for more than 30 years and account for approximately 25% of the worldwide insecticide market (Shafer et al., 2005). Pyrethroids derived from natural compounds (the pyrethrins) of Chrysanthemum plant (Casida, 1980) are more hydrophobic than other classes of insecticides (Michelangeli et al., 1990). Their selective toxicity seems to be based mainly on the responses of neuronal sodium channels and partly on metabolic degradation (Narahashi, 1996). In spite of a wide margin of safety, cypermethrin (CY), type II pyrethroid, is hazardous for mammals.

Due to their lipophilicity, pyrethroid insecticides favor absorption through the gastrointestinal and respiratory tracts and also confer preferential distribution into lipid-rich internal tissues (Fetoui et al., 2009), thus leading to hemodynamic changes in the body. Several studies have shown that CY induces alterations in hematology (Yousef et al., 2003; Khan et al., 2009). Signs like muscular tremors, ataxia, and weakness of limbs, convulsions, incoordination, coma and death from respiratory depression have been reported after ingesting high dose of CY. CY given intraperetoneally in the rats caused muscular weakness, swaying gait, respiratory distress, pallor and prostration; convulsions preceded death apparently due to respiratory failure (Iyaniwura and Okonwo, 2004).

Micronuclei (MN) in cells are considered as a biomarker of damage to the DNA (Saleh and Sarhan, 2007). Micronuclei are cytoplasmic chromatin-containing bodies that appear in the cell like a small satellite nucleus around the cell nucleus, due to chromosome fragments or entire chromosomes that are not incorporated in the main nucleus after cell division. Micronucleus test has been well established in several systems, i.e. ovary, bone marrow, peripheral blood, liver and fetus cells of several laboratory animals or human (Heddle, 1990). CY is known to cause DNA damage, thereby leading to micronucleus formation (Campana et al., 1999; Çelik et al., 2005).

Estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. Oxidative damage caused by CY in cells has been reported to be alleviated by antioxidant vitamins, such as Vit E, Vit C, and β-carotene, and antioxidant minerals, such as Se and zinc (Pregiosi et al., 1998).

Though enough information is available about CY toxicity in mammals, the information about CY-induced micronuclear changes, hematological alterations and its attenuation by vitamin E and selenium in broiler chicks is sparse. The present study describes clinico-hematological alterations induced by CY and the role of vitamin E and selenium in alleviating the harmful effects of cypermethrin in broiler chicks.

Materials and methods

Synopsis of this experiment was designed considering all the national legislation regarding protection of animal welfare and following the guidelines of the Advanced Studies and Research Board (ASRB) of the University. Before execution, the experimental proposal was approved by the ASRB.

Chemicals

Cypermethrin (92% technical grade) was donated by Ali Akbar Group of Companies, Pakistan. Vitamin E (Vit E-50), sodium chloride and selenium (sodium selenite) were purchased from Roche (Germany), KKWRiedel (Germany) and Applichem GmbH (Germany), respectively. Disodium hydrogen phosphate 12-hydrate, disodium phosphate, potassium ferricyanide, disodium ethylene diamine tetra acetic acid (Na_2EDTA), potassium cyanide and methyl alcohol (absolute) were the products of Merck, Germany. Potassium dihydrogen phosphate and sodium bicarbonate were procured from Fisher Scientific (USA). Giemsa stain and methyl violet 2B were purchased from BDH and Wright stain was obtained from Eyer (China).

Experimental birds and management

After procurement, all birds were kept in wire cages under similar management and housing conditions. Basal diet, i.e. chick starter crumbs and clean water, was offered *ad libitum*. Chicks were vaccinated against Newcastle disease (ND) at the age of 2 and 23 days, infectious bursal disease (IBD) on days 8 and 21 and hydropericardium syndrome (HPS) on day 19 of age using live vaccines.

Experimental procedure

This study was carried out on 90 one-day-old broiler chicks procured from a local hatchery. After six days of acclimatization, chicks were randomly divided into five equal groups. The treatments started at the age of 7 days (it was day 1 of the experiment) and continued till the age of 37 days (end of experiment). All the treatments were given orally through crop tubing. Groups 1–4 received CY @ ($600\,mg\,kg^{-1}$ b.wt) in 2 ml corn oil daily for 30 days. Groups 2, 3 and 4 also received Vit E @ ($150\,mg\,kg^{-1}$ b.wt), Se @ ($0.25\,mg\,kg^{-1}$ b.wt), and Vit E + Se ($150 + 0.25\,mg\,kg^{-1}$ b.wt), respectively, and these groups were treated with the respective treatment for 30 days on alternate days. Group 5 served as control and received $2\,ml\,kg^{-1}$ b.wt physiologic saline for 30 days. All the birds were monitored twice daily for clinical