EFFECT OF ALPHA-TOCOPHEROL ON HEMATO-BIOCHEMICAL PROFILES OF BROILERS IN DELTAMETHRIN INDUCED TOXICITY

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Abstract: Deltamethrin is a broad-spectrum insecticide mainly used to protect crops, fruit and vegetables from mites, ants, weevils, beetle etc. Thus deltamethrin contaminated food of commercial poultry diet may cause toxic effects in broilers and indirect adverse effects on human through food chain. Therefore, hematological and biochemical alterations in White Leghorn birds due to deltamethrin induced toxicity along with protective effect of alpha-tocopherol were evaluated. A dose dependent decrease in hemoglobin, packed cell volume, total erythrocyte count and increase in serum ALT and AST levels were noticed in deltamethrin exposed birds after 42 days in comparison to control. Reduction in ALT and AST levels with alpha-tocopherol were observed, however serum level of total protein, creatinine and BUN remained unaltered. Exposure of birds to deltamethrin at 100 to 150 mg kg\(^{-1}\) was found capable of inducing hematological and biochemical alterations which could be effectively ameliorated by alpha tocopherol at 300 mg kg\(^{-1}\).

Key words: Alpha-tocopherol, Deltamethrin, Broilers chicks

INTRODUCTION

Poultry farming made rapid strides during last two decades and today it is considered as one of the fastest growing agro-based businesses in the world. Consequently, there has been tremendous pressure on the availability and selection of quality feed ingredient free from toxins. Adverse effects of pesticides have been recognized as a serious health concern during the past decades [1-6]. Deltamethrin \(\{(1R,3R)\text{-cyano\,(3-phenoxyphenyl) methyl\text{-3\-(2,2-dibromo-ethenyl\text{-2,2-dimethyl cyclopropane carboxylate)}}\}\) (Fig. 1) is a broad-spectrum insecticide [7]. It is registered for use in various crops including cotton, corn, cereals, soybeans and vegetables for pests such as mites, ants, weevils and beetles [8]. Deltamethrin has an alpha-cyano group that induces long-lasting inhibition of sodium channel activation gate. This results in prolonged permeability of nerve to sodium and produces a series of repetitive nerve signals in sensory nerves and muscles [9]. Deltamethrin may also affect ion channels in the nervous system other than sodium channels, possibly due to their phosphorylation state [10]. Deltamethrin produces characteristic effects of choreoathetosis (sinuous writhing) and salivation, also known as CS Syndrome [11]. In rats, this causes pawing and burrowing behavior followed by salivation. Deltamethrin produces characteristic effects of motor incoordination, respiratory defects, and spasms involving the limbs and tail, and clonic seizures when administered orally in rats. It
causes vomit, hyper excitability, stiffness in hind legs and impaired body movement in dogs [9]. Under normal conditions excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by alpha-tocopherol, which is a naturally occurring antioxidant nutrient that plays important roles in animal health by inactivating harmful free radicals produced through normal cellular activity from various stressors. Alpha tocopherol enhances immunity by maintaining functional and structural integrity of important immune cells [12]. It also protects polyunsaturated fatty acid [PUFA] within membrane phospholipids and within circulating lipoproteins. Peroxy radicals reacts 1000 fold faster with alpha-tocopherol than with PUFA, forming corresponding hydro peroxide and tocopherol radical. Tocopherol radical, in turn, interacts with other antioxidant compounds, such as ascorbic acid, which regenerate tocopherol [1,13,14] Several studies have shown that pyrethroid caused alterations in biochemistry, hematology and reproduction [12] in bird and other animal.

Studies describing oxidative stress mechanisms in pyrethroid-induced toxicity are limited. Few reports have demonstrated induction of oxidative stress by pyrethroids such as cypermethrin and fenvalerate [15,16]. Because of health problems induced by many environmental pollutants, efforts have been made to evaluate antioxidant potential of alpha tocopherol [15,17]. Information on deltamethrin induced toxicity and protective effects of alpha tocopherol on hematological and biochemical profile in White Leghorn (WLH) bird [broiler] are lacking, hence present study was conducted to explore deltamethrin induced toxicity and protective effect of alpha-tocopherol in broiler birds.

**MATERIALS AND METHODS**

One week old broilers chicks (White Leghorn) in 48 numbers were procured from Phoenix Poultry, India and used for the experiment. Commercial formulation of deltamethrin (2.8% E.C.) DecisR, Batch 73 No. PH 00876, manufactured by Punjab Pesticides Industrial Cooperation Society Ltd. and marketed by Bayer Crop Science Limited, Mumbai, India was procured from local market. EVION capsules of 400 mg (Merck Limited, Usugaon, Ponda, Goa – 403407) were used as the source of alpha-tocopherol at the dose of 100-300 mg kg-1 feed, daily for 42 days.

Experimental design: Chicks were divided into six groups with eight chicks in each group. Chicks of different groups were kept separately and maintained under similar hygienic conditions and fed the standard poultry food supplied by All India Coordinated Research Project on Poultry, MPPCVV, Jabalpur. Drinking water was provided ad libitum. Experiment was approved by institutional animal ethics committee. A summary of various treatments is provided in Table 1. Deltamethrin based commercial formulation and alpha-tocopherol were given orally in feed for 6 weeks (42 days). Blood and organs were collected from chicken on day 42 and hematological and serum biochemical estimation was done.

Haematological studies: The blood was collected during decapitation of chicken into sterile vials with sodium citrate (1:9 v/v with blood) as anticoagulant for estimations of haematological parameters such as haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and erythrocyte indices (MCV, MCH and MCHC) by standard procedures [18].

Diluting fluid: Diluting fluid was prepared as recommended by Natt and Herrick [19]. The fluid contained the following ingredients (NaCl- 3.88 gm, Na2SO4- 2.50 gm, Na2HPO4 12H2O- 2.91 gm, KH2PO4 - 0.25 gm, Formalin 37% - 7.50 ml, Methyl violet (2B) - 0.10 gm, distilled water to make1000 ml.

Biochemical parameters: Blood was collected from chicken of respective group in dry test tubes and serum was separated after eight hours [20]. Serum biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total protein, Blood urea nitrogen
(BUN) and creatinine were estimated by using eppendorf ECOM–F 6124 and Erba CHEM-5 Plus V2 semi auto analyzer (Mannheim, Transasia Bio-medicals Limited). The methodology and the standardized reagents used in respect of each parameter were as per the recommendation of the manufacturer of analyzer system.

Statistical analysis: Data were calculated as mean ± SEM (standard error of mean) and analyzed using analysis of variance (ANOVA). Statistical significance between two means was assessed by Student–Newman Keuls’s multiple comparison test at (P= 0.05). All statistical analyses were done with GENSTAT. Probability of 0.05 or less was considered insignificant.

RESULTS AND DISCUSSION

Total erythrocyte count: Lysis of RBC usually occurs in microangiopathic anemia, infectious diseases, oxidative haemolysis and nonoxidative chemical-induced anemia [21]. In the present study, a dose dependent decrease in total erythrocyte count was observed in deltamethrin treated chicken (Table 2). Significant difference (P=0.05) in total erythrocyte count (TEC) was observed after 42 days among all groups. TEC value under group I and II was in range of 3.89 to 3.44 millions µL-1. However difference in MCV, MCH and MCHC were not significant (P=0.05) in comparison to control (Table 1). Similar findings have also been reported by [22] in rats treated with cypermethrin a type II pyrethroid but such information was not found in chicken. Similarly dose dependent decreasing trend was also observed in buffalo calf [23], swiss mice [24,25] and rats [26]. Decrease in total erythrocyte count may be due to oxidative haemolysis [27]. In addition, erythrocytes are the most susceptible to oxidative stress because of presence of relatively high oxygen and polyunsaturated lipid-rich plasma membranes [28]. Deltamethrin exposure attenuated antioxidant defense system, decreased activities of glutathione reductase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, enzymes and induced expression of heat shock protein 70 in a dose dependent manner [29]. In the present study, significant increase in total erythrocyte count was observed upon supplementation of alpha-tocopherol in feed along with deltamethrin as comparison to other groups.

Hemoglobin concentration: Synthesis of haeme involves a series of reactions that occur in cytoplasm and mitochondria of erythroblasts. In initial step mitochondria synthesize delta-aminolevulinic acid, which is affected by xenobiotics [23]. Delta-aminolevulinic acid synthetase (ALAS) enzyme catalyses condensation of glycine and succinyl-CoA to form ALA and has been accepted as a rate-limiting enzyme in haeme biosynthesis. Among the groups, haemoglobin (Hb) was higher in group I and II (10.13 and 10.30) in comparison to group III, IV, V and VI [8.78, 9.30, 8.05 and 8.20%, respectively. DM significantly reduced Hb in groups III, IV, V and VI. After 42 d, significant difference (P=0.05) of hemoglobin count (Hb) between II and II, II and IV and IV and V were observed (Table 2). Significant reduction in hemoglobin concentration levels in broilers were due to deltamethrin induced toxicity. Little improvement (non significant at P=0.05) on blood urea concentration was seen in alpha-tocopherol treated groups compared to other groups treated only with deltamethrin (Table 2). Higher hemoglobin concentration of alpha-tocopherol treated chicken as compared to toxic group indicated antioxidative and haematinic effect of alpha-tocopherol. Alpha-tocopherol is a major

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of chicken</th>
<th>Treatment</th>
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<tbody>
<tr>
<td>I</td>
<td>8</td>
<td>Standard feed &amp; water ad-libitum</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>Standard feed &amp; water ad-libitum + α- tocopherol @ 300 mg/kg of feed for 42 days</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>Same as in group I + Oral administration of deltamethrin @ 100 mg/kg of feed daily for 42 days</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>Same as in group I + Oral administration of deltamethrin @ 100 mg/kg of feed + α- tocopherol @ 300 mg/kg of feed for 42 days</td>
</tr>
<tr>
<td>V</td>
<td>8</td>
<td>Same as in group I + Oral administration of deltamethrin @ 150 mg/kg of feed daily for 42 days</td>
</tr>
<tr>
<td>VI</td>
<td>8</td>
<td>Same as in group I + Oral administration of deltamethrin @ 150 mg/kg of feed + α- tocopherol @ 300 mg/kg of feed for 42 days</td>
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Table 1: Experimental design of deltamethrin induced toxicity in broilers
antioxidant found in the lipid phase of membranes and acts as a powerful terminator of lipid peroxidation [14,30,31]. This might be one of the reasons for protective effect of alpha-tocopherol.

Reduction in Hb contents in only deltamethrin treated groups could be due to the impaired biosynthesis of haeme in bone marrow [14] and increased rate of destruction instead of formation of RBCs. Decrease in hemoglobin concentration in deltamethrin treated chicken might also be due to intravascular haemolysis and anemia as a result of depressed erythropoiesis and erythrolysis by free radicals. Hepatopathy and renal damage by any chemical or biological process resulted in decreased formation of liver globulin, renal erythropoietin and ultimately decreased erythropoiesis. Similar affects of deltamethrin have been reported in rats [26], buffalo calf [23], chicken [24,32] and Swiss mice [24], dogs [33] and crossbred calf [34].

Packed cell volume: Among the groups, PCV was higher in group I and II (29.75 and 30.75 %) in comparison to group III, IV, V and VI [26.25, 27.75, 24.13 and 24.50%, respectively] (Table 2). DM significantly reduced PCV in groups III, IV, V and VI. After 42 d, significant difference (P=0.05) of PCV between groups I and II, II and IV and IV and V were observed. Reduction in PCV levels in broilers was due to deltamethrin induced toxicity, which may lead to decreased haematocrit values. In addition, reduction in blood parameters may be attributed to hyperactivity of bone marrow [35] leading to the production of red blood cells with impaired integrity which was easily destroyed in circulation by reticular-endothelial system [25,36]. Improvement in packed cell volume in alpha-tocopherol treated group indicated that alphatocopherol was effective in reducing oxidative stress caused by deltamethrin on haemopoietic system of chicks.

Alanine aminotransferase (ALT) and Aspartate aminotransferase ([AST): It is reported that ALT is
found in high concentration in liver and to a lesser extent in kidney, heart, skeletal muscle, pancreas, spleen and lungs. ALT levels increase as a result of primary liver diseases such as cirrhosis, carcinoma, viral or toxic hepatitis and obstructive jaundice. In this study, there was a dose dependent rise in ALT and AST levels in chickens in entire groups exposed to deltamethrin. Significant amelioration was found in chickens treated with deltamethrin along with alpha-tocopherol in comparison to deltamethrin alone. Higher ALT and AST (12.18 and 301.66 I.U. L-1) were found in chickens of group V after 42 days followed by group VI (9.23 and 254.36 I.U. L-1), III (8.19 and 291.10 I.U. L-1) and IV (8.79 and 223.13 I.U. L-1) (Table 3). Activity of ALT and AST was indicative of degree of damage to liver cells, as level of these enzymes was elevated by subsequent hepatocellular injury [1,2,36,37,38]. Similar observations have also been reported in rats [25] and catfish [17,39].

Serum protein: Total serum protein is useful for monitoring gross changes in protein levels caused by various disease states. Increased protein could be due to dehydration, multiple myeloma and liver disease. Reduction in total protein could be due to inhibition of protein synthesis. Among the groups, total serum protein (TP) was found less (3.54 gm dL-1) in group V in comparison to group I, II, III, IV and VI (Table 3). Effects of deltamethrin induced toxicity on serum protein were not significant in all the groups fed with various levels of deltamethrin in comparison to the groups co treated with deltamethrin along with alpha-tocopherol (2,31,37,38,40).

Creatinine and blood urea nitrogen [BUN]: Creatinine concentration and BUN is considered as insensitive and poorly 251 diagnostic test in chicken [41]. Creatinine is an end product of creatine metabolism which is a part of energy utilization mechanism in muscles [42]. Creatinine quantity depends marginally on the dietary protein intake. Serum creatinine level depends directly on body muscle mass and is affected by intense muscular stress. Urea is a major end product of protein metabolism. It constitutes largest fraction of non-protein nitrogenous component of the blood. Consequently, circulating levels of urea depend upon protein intake, protein catabolism and kidney function along with infections, disease or toxic insults.

After 42 days of study, serum creatinine and BUN levels were not altered significantly (P=0.05) among the deltamethrin exposed chicken with or without alpha-tocopherol in comparison to control (Table 3). These results are contradictory with [17] and [39] and however, it can be envisage that difference in animal species might have resulted in disparity.

CONCLUSIONS

Supplementation of alpha-tocopherol in groups IV and VI during 6 weeks significantly increased hematological variables as compared to groups III and V, which confirmed the protective effects of alpha tocopherol. It can be concluded that exposure of deltamethrin to White Leghorn (WLH) birds is capable of inducing alterations in hematological and biochemical parameters which can be mitigated by use of alpha tocopherol as an antioxidant.

REFERENCES


