Research Article

Bromadiolone Induced Oxidative Stress and Cytological Damage in Layer Birds

Sandeep Sodhi[1], Rajinder Singh Brar[2], Harmanjit Singh Banga[2]

[1]Department of Vety. Biochemistry, Guru Angad Dev Veterinary & Animal Science University, Ludhiana, Punjab
[2]Department of Vety. Pathology, Guru Angad Dev Veterinary & Animal Science University, Ludhiana, Punjab

Abstract This study was undertaken to evaluate the oxidative stress induced by bromadiolone toxicity in layers. Birds were divided into two groups. Control group (I) received bromadiolone free feed and group (II) was exposed to bromadiolone (5 mg/kg feed) for seven weeks. Blood samples and organs were collected at the end of the experiment. Bromadiolone exposure resulted in significant rise in lipid peroxidation (LPO) levels in erythrocyte and liver. In addition decreased ($P<0.05$) level of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities were observed in erythrocyte and liver. Histopathological results revealed mild to severe type of necrosis and degenerative changes in heart and liver of bromadiolone intoxicated animals. In addition histopathological findings of tissues ovary, liver, heart and pancreas also showed generalized haemorrhage. It is therefore concluded that feeding of bromadiolone admixed feed lead to depletion of Vitamin K causing multifocal haemorrhages and oxidative stress and cellular damage in different tissues.

Keywords *Histopathology; oxidative stress; layer birds; bromadiolone*

1. Introduction

Bromadiolone, a second generation rodenticide is used as a preferred rodenticide in the urban and farm rodent control and acts by disrupting the normal blood clotting mechanism (Kocher and Parsad, 2003). As whole grain baits are highly attractive and palatable, therefore are readily consumed by animals and birds. Non-target species are potentially at risk from direct consumption of bait and also through eating poisoned rodents (Berny et al., 1997). Recently, ten cases of rodenticide poisoning in dogs were observed in Texas Vet Medical Diagnostic Laboratory and two neonatal puppies (Munday and Thompson, 2003). Eason and Spurr (1995) reported brodifacoum rodenticide toxicity in 200, one week old chicks, in which hundred percent mortality was observed. The source of rodenticide was found to be wood straw mats in the chick boxes. Toxicity data for a variety of animals suggests that anticoagulant rodenticides are no doubt a potential hazard to many wild mammals and birds (Berny et al., 1997; Stone et al., 1999). Thus, the present study was contemplated to study the effect of Bromadiolone induced Oxidative Stress and Cytological Damage in layer birds.

2. Materials and Methods

2.1. Chemicals

Bromadiolone (3 – [3 – [4' – bromophenyl – 4 – yl) – 3 hydroxy – 1 – phenyl propyl] – 4 – hydroxyl coumarin) and all other chemicals were of the analytical grade and were purchased from Merck.

2.2. Animals and experimental design

Twenty egg laying birds, 30 week old were maintained in the poultry sheds of Department of Livestock Production and Management, GADVASU, Ludhiana where proper hygienic conditions were maintained in the cages. These birds were provided with fresh water and feed ad libitum. The birds were randomly divided into two groups of ten birds, each. Control group received bromadiolone free feed and experimental group (II) was exposed to bromadiolone (5 mg/kg feed) for seven weeks. The conduct of experiment was done as per approval of IAEC and as per accordance with the guide lines for animal experimentation.

2.3. Sample collection

At the end of seventh week, blood samples were collected from 5 birds in each group by cardiac puncture for biochemical estimations. The plasma was separated and immediately stored at -5°C till further use. Blood was collected by cardiac puncture before sacrifice and tissue samples were collected for further analysis. The Liver, heart, pancreas and ovaries were removed and in a part were fixed in 10% buffered formalin for histopathological examination (Luna, 1968). These were processed, microtomed at 5 µ and stained with hematoxylin and eosin (H and E) stain. Half portion of the liver from each bird was processed immediately for biochemical estimation.

2.4. Biochemical assay

Lipid peroxide level in 10% RBC hemolysate was determined as per Placer et al. (1966) and was expressed as nmol malondialdehyde (MDA)/mg of hemoglobin (Hb) using 1.56×10^5 as extinction coefficient (Utley et al., 1967). For the preparation of 10% RBC hemolysate, blood samples were centrifuged at 2000 rpm for 10 min and supernatant plasma were separated out. The sedimented cells were washed with sterile 0.85% NaCl solution three times. Washed erythrocytes were hemolyzed with ninefold volume of distilled water to prepare 10% RBC hemolysate. Hemoglobin in the hemolysate was estimated by the cyano-methaemoglobin method (Van Kampen and Ziglstra, 1961). Lipid peroxides in 10% crude tissue homogenate of liver from birds was estimated following Okhawa et al. (1979) and was expressed in nmol of MDA/ mg of protein. Tissues were immediately perfused with cooled buffer that contained 0.9% KCl, pH 7.2. They were homogenized in 9 volume of 1.15% KCl, 125 mM sucrose, pH 7.2. The homogenates were employed for the assays. The protein in 10% tissue homogenate was measured by the method of Lowry et al. (1951). Superoxide dismutase activity in 10% supernatant tissues and RBC hemolysate was estimated as per Menami, M., Yoshikawa, H. (1979). The catalase activity in tissues and RBC hemolysate was estimated as per Cohen et al. (1970).

2.5. Statistical analysis

Data are expressed as means±SEM and were analyzed statistically using analysis of variance to compare the means of different treatment groups with that of the negative and positive control groups.

3. Results

3.1. Effect on LPO

The role of lipid peroxidation was assessed by studying the level of formation of malondialdehyde, an indicator of lipid peroxidation (Table 1). Bromadiolone exposure for 7 weeks resulted in significantly (P<0.05) increased LPO levels in erythrocytes and liver tissues as compared to control birds.

Table 1: Effect of bromadiolone on malondialdehyde (MDA) concentrations (erythrocyte and liver), superoxide dismutase (SOD), glutathion and Catalase in layers

Enzymes	Control	Bromadiolone
MDA nM/gm Hb	7.2 ± 0.3^b	16.7 ± 3.5^a
MDA nmol of MDA/ gm protein	18.7 ± 1.24^b	31.4 ± 1.3^a
SOD(U/gm tissue	803.5 ± 0.43^c	362.2 ± 2.6^a
GSH(mM /gm tissue)	6.8 ± 0.4^b	2.5 ± 1.2^a
Catalase(U/gm Tissue)	116.5 ± 0.4^b	72.6 ± 1.8^a

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P > 0.05$) between different groups in the same row.

3.2. Effect on nonenzymatic antioxidant

Result presented in Table 1 represented the reduced glutathione (GSH) levels of studied tissue in experimental animals. Administration of bromadiolone caused significant decrease (P<0.05) in the GSH level in erythrocyte and liver tissues.

3.3. Effect on antioxidant enzymes

Table 1 showed decreased SOD and CAT activities in bromadiolone exposed birds. Significantly (P<0.05) decreased activities of SOD and CAT enzymes were reported in erythrocyte and liver tissue.

3.4. Histopathological alterations

Histopathological findings

Bromadiolone feeding resulted in pathological alterations in various organs. In Liver, vascular compromise viz., congestion to hemorrhage, besides varying fatty changes of mild to severe intensity (Figure 1) were discernible. In ovary, marked hemorrhages were observed, replacing the ovarian stroma (Figure 2). In myocardium of heart, there was evidence of congestion, hemorrhage and edema in myofilness of birds which received bromadiolone (Figure 3).

4. Discussion

Present results showed that bromadiolone causes lipid peroxidation in liver. Increased lipid peroxidation is thought to be consequences of oxidative stress which occurs when the dynamic balance between peroxidant and antioxidant mechanism is impaired (Flora et al., 2008). A marginal decrease in GSH correlated well with an increase in LPO in the respective tissues. Adequate levels of the cellular GSH pool required not only for maintaining the cellular redox status by keeping sulfhydryl groups of cytosolic proteins in their reduced form but also because numerous toxic or potentially toxic compounds are either taken up by or removed from the cells by GSH-mediated pathways

(Chouchane and Snow, 2001). A decrease in cellular GSH concentration has been inversely correlated with lipid peroxidation in the liver (Maiti and Chatterji, 2000, 2001).
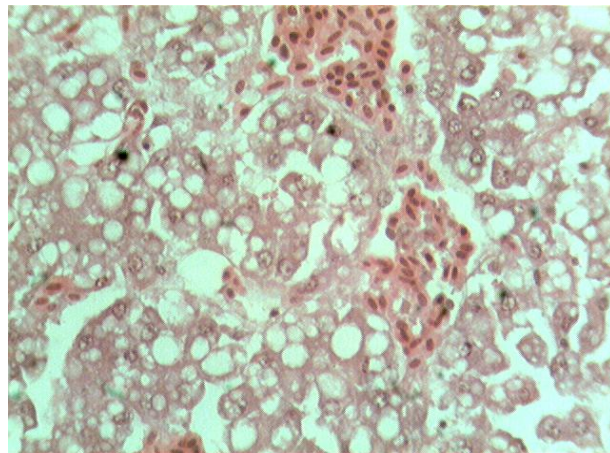

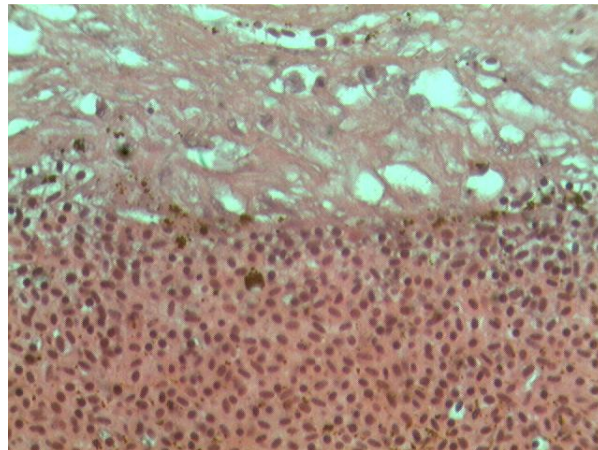*Figure 1: Liver: Section of liver, with evidence of fatty change in liver. H & E x 150*

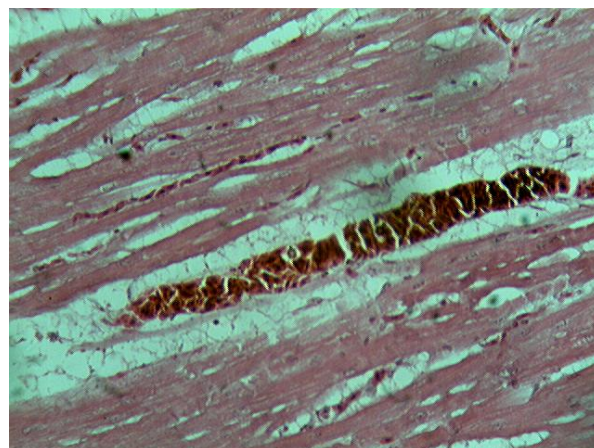*Figure 2: Ovary: Marked hemorrhages are seen in ovarian stroma. H & E x 150*

Figure 3: Heart: Exhibits congestion and edema in myocardium. H & E x 150

Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. SOD is an antioxidant metalloenzyme that reduces superoxide radicals to water and molecular oxygen (McCord, 1976). CAT is a haemoprotein, which reduces hydrogen peroxide to molecular oxygen and water (Gutteridge, 1995). In support to our results observed decreased SOD and CAT enzyme activities in the tissues of experimental birds against bromadiolone intoxication. Santra et al. (2007) conducted a study on arsenicosis and their results are parallel to this study and they concluded the changes in liver cells were due to oxidative stress in mitochondria that plays an important role in the pathogenesis of apoptotic cell injury. A decrease in GSH triggers the activation of neuronal 12-lipoxygenase, which leads to the production of peroxides, and, ultimately, cell death (Schulz et al., 2000). Histopathological observation in birds receiving bromadiolone exhibited extensive cytological alterations as compared to the normal tissue architecture. These gross postmortem lesions of subcutaneous hemorrhage, multifocal hemorrhages in visceral organs and presence of blood clots were evident, which are in accordance with other studies (Kocher et al., 2004).

Bromadiolone is an anticoagulant of high toxic potential for most mammals, as it interferes with the vitamin K dependent clotting factors, when a lethal or sub-lethal dose is ingested (Svendsen et al., 2002). The degree of hazard would be expected to vary by compound ingested, species involved and type of application, as poisoning can occur by direct ingestion of bait (primary poisoning) or via consumption of poisoned rodents causing secondary poisoning (Newton et al., 1990). Based upon the results of this experiment it may be concluded that bromadiolone exposure leads to depletion of antioxidant defense mechanism and varying degree of changes in tissue architecture in layer birds.

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