EFFECT OF ANTIOXIDANTS ON BODY WEIGHT AND BLOOD PLASMA OF BROILERS IN ENROFLOXACIN INDUCED OXIDATIVE STRESS

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ABSTRACT

The experiment was designed to evaluate the effect of antioxidants on body weight and blood plasma in enrofloxacin induced oxidative stress. Total fifty broiler chickens divided into five groups at the age of day 38 were used for this study. Group I was treated as control, group II was treated with enrofloxacin alone, group III was treated with enrofloxacin and alpha-lipoic acid, group IV was treated with enrofloxacin and vitamin E, group V was treated with enrofloxacin and co-treatment of alpha-lipoic acid vitamin E. The results of this study suggest that enrofloxacin induced oxidative stress in broilers at the therapeutic doses can be effectively ameliorated by treating with vitamin E and alpha-lipoic acid combination than treating with either of these drugs alone.

Keywords: Enrofloxacin, Antioxidants, Alpha lipoic acid, Vitamin E.

INTRODUCTION

In the intensive system of poultry production birds are exposed to various kind of stresses. Conventionally oxidative stress is defined as a "disturbance in the prooxidant - antioxidant balance in favour of former, leading to potential damage". The rapid growth in broilers makes the birds vulnerable to stress condition, which disturbs several physiological functions due to increased generation of reactive oxygen species (ROS) and the resultant oxidative stress.

Enrofloxacin is indicated for the control of mortality associated with Escherichia coli, Salmonella sp., and Pasteurella multocida in broiler chickens. Enrofloxacin are oxidized by liver microsomal enzymes of the cytochrome P450 family (Stratton, 1998), which induce oxidative damage through formation of free radicals. In order to increase the efficacy of enrofloxacin it is essential to counter its oxidative stress. Hence, in this study antioxidant potential of alpha-lipoic acid and vitamin E were evaluated against enrofloxacin induced oxidative stress.

MATERIALS AND METHODS

Fifty, day-old straight run commercial broiler chicks were utilized in this study. The birds were reared in cages under standard managemental practices from day old to six weeks of age. This experimental trial was approved by the institutional animal ethical committee of Madras Veterinary College (Approval no: 1831/DFBS/2011).

From 38th day to 42nd day, the birds were administered with enrofloxacin, vitamin E and alpha-lipoic acid as indicated in table-1. The dose for

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enrofloxacin is as regular therapeutic dose (10mg/kg body weight) and the dose of Alpha lipoic acid and Vitamin E were decided based on the previous studies (Srilatha et al., 2010) as 100mg/kg body weight each. The body weights of the all broilers were recorded on day 43. Blood was collected in sterile heparinised tubes from the recurrent tarsal vein and plasma was separated by centrifugation at 1000 rpm for 10 min at 4oC and stored at -20oC for the estimation of antioxidant assays. Antioxidant assays viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) and lipid peroxidation (MDA) were carried out by standard procedures.

RESULTS AND DISCUSSION

Body weight: In the present study, there was a marked reduction in the body weight of enrofloxacin treated group when compared to normal control group. The groups treated with vitamin E alone and alpha-lipoic acid alone showed better body weights than enrofloxacin alone treated group. Whereas, the group treated with the combination of both vitamin E and alpha-lipoic acid showed body weights as good as the control group. This study showed that exposure to enrofloxacin caused a marked increase in lipid peroxidation and reduction in free radical scavenging enzymes. Hence, this could have resulted in the reduction of body weights (Table-1). Osfor et al. (2010) also stated that alpha-lipoic acid (ALA) and vitamin E could improve daily food intake, body weight gain and feed efficiency ratio in lead and copper intoxicated rats. Supplementation of ALA resulted in increased body weight gains than in control broilers (Srilatha et al., 2010).

Blood Plasma: Effect of enrofloxacin on the enzymatic and non-enzymatic antioxidant and its amelioration with alpha-lipoic acid and vitamin E and in plasma of broilers was shown in Table – 2.

The group treated with the enrofloxacin resulted in drastic reduction in the levels of antioxidant parameters such as SOD, CAT, GPX and GSH when compared to the control group. Groups treated with alpha-lipoic acid alone and vitamin E alone showed marginal increase in the levels of antioxidant parameters such as SOD, CAT, GPX and GSH when compared to the enrofloxacin alone treated group. Whereas, the group treated with the combination of both vitamin E and alpha-lipoic acid showed significant restoration in the levels of SOD, CAT , GPX and GSH.

Similarly MDA levels were drastically increased in enrofloxacin treated group indicating its high lipid peroxidation activity. Groups treated with alpha-lipoic acid alone and vitamin E alone showed marginal decrease in the levels MDA when compared to the enrofloxacin alone treated group. Whereas, the group treated with the combination of both vitamin E and alpha-lipoic acid showed reduction in the levels of MDA indicating its protective nature.

Several types of ROS are generated in the body as a result of metabolite reactions in the form of free radicals or non-radicals. These species may be either oxygen derived or nitrogen derived and are called as pro-oxidants. They attack macromolecules including protein, DNA and lipid etc. causing oxidative modification of lipids which results in lipid peroxidation (Kurien and Scofield, 2006). Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids, primarily in cell membranes resulting in membrane damage (Kawamura et al., 1992). Free radical damage to protein can result in loss of enzyme activity and damage caused to DNA can result in mutagenesis and carcinogenesis (Devasagayam et al., 2004).

In broilers, infections of the alimentary and respiratory tract are quite common. Enrofloxacin is

a molecule of second generation fluoroquinolones, which is commonly used as "in water" preparation for the treatment of alimentary and respiratory tract infections in poultry. Fluoroquinolones such as enrofloxacin are oxidized by liver microsomal enzymes of the cytochrome P450 family (Stratton, 1998), which results in formation of free radical intermediates and induces oxidative damage (Carreras et al., 2004). Because of this there will be reduction in growth rate and meat quality leading to financial loss to the farmer. Thus, enrofloxacin further adds oxidative stress to birds which are already vulnerable to stress because of their rapid growth rate.

Combination of alpha-lipoic acid and vitamin E was able to effectively restore the antioxidant activity similar to control birds. This activity might be attributed to direct scavenging of free radicals. Srilatha et al. (2010) suggested that lipoic acid and vitamin E, when used together had their antioxidant capabilities improved as lipoic acid helps in recycling of vitamin E by exhibiting synergistic activity and effectively restores the antioxidant level and decreases oxidative stress in broilers.

Free radicals generated by enrofloxacin initiate the peroxidation of membrane polyunsaturated fatty acids of membrane and covalently bind to microsomal lipids and proteins (Carreras et al., 2004). The increase in MDA level of enrofloxacin treated group indicates enhanced lipid peroxidation due to tissue injury and failure of antioxidant defence mechanism, which prevents the formation of excess free radicals. Treatment of alpha-lipoic acid alone or vitamin E alone or combination of both with enrofloxacin, reduced the lipid peroxidation which leads to the reduction of MDA levels. This finds support from the findings of Carreras et al. (2004) in broilers.

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Table-1

Experimental trial and Effect of enrofloxacin on body weight and its restoration with alpha-lipoic acid and vitamin E in broilers

Group	Treatment (From 38th day to 42nd day)	No. of Birds	Body Weight (Kg)
I	Control	10	2.35c ± 0.01
II	Enrofloxacin (10mg/kg body weight)as oral administration	10	1.70a ± 0.01
III	Enrofloxacin (10mg/kg body weight) + alpha-lipoic acid (100mg/ kg body weight) as oral administration	10	1.94b ± 0.02
IV	Enrofloxacin (10mg/kg body weight) + vitamin E (100mg/kg body weight) as oral administration	10	1.97b ± 0.01
V	Enrofloxacin (10mg/kg body weight) + alpha-lipoic acid (100mg/ kg body weight) + vitamin E (100mg/kg body weight) as oral administration	10	2.32c ± 0.01

All values are Mean ± S.E of 10 birds
Means bearing different superscripts in a column differ significantly between groups

Table-2

Effect of enrofloxacin on enzymayic, non-enzymatic antioxidants and lipid peroxidation and its restoration with alpha-lipoic acid and vitamin E in plasma of broilers

Groups	Treatment (From 38th day to 42nd day)	Enzymatic antioxidants			Non enzymatic antioxidants	MDA (TBARS)
		SOD	CAT	GPX	GSH	
I	Control	$2.40^{c} \pm 0.01$	$42.40^{c} \pm 0.77$	$2.74^{d} \pm 0.06$	$1.16^{c} \pm 0.00$	$6.01^{a} \pm 0.02$
II	Enrofloxacin (10mg/kg body weight) as oral administration	$1.97^{a} \pm 0.01$	$32.70^{a} \pm 0.41$	$2.18^{a} \pm 0.15$	$1.03^{a} \pm 0.00$	$10.04^{e} \pm 0.07$
III	Enrofloxacin (10mg/kg body weight) + Alpha-lipoic acid (100mg/kg body weight) as oral administration	$2.18^{b} \pm 0.02$	$37.71^{b} \pm 0.38$	$2.43^{c} \pm 0.06$	$1.07^{b} \pm 0.00$	$7.97^{d} \pm 0.02$
IV	Enrofloxacin (10mg/kg body weight) + Vitamin E (100mg/kg body weight) as oral administration	$2.22^{b} \pm 0.02$	$38.12^{b} \pm 0.33$	$2.54^{b} \pm 0.08$	$1.09^{b} \pm 0.00$	$7.82^{c} \pm 0.04$
V	Enrofloxacin (10mg/kg Body weight) + Alpha-lipoic acid (100mg/kg body weight) + Vitamin E (100mg/kg body weight) as oral administration	$2.40^{c} \pm 0.02$	$42.17^{c} \pm 0.71$	$2.75^{d} \pm 0.05$	$1.14^{c} \pm 0.00$	$6.25^{b} \pm 0.03$

All values are Mean ± S.E of 10 birds
Means bearing different superscripts in a column differ significantly between groups
SOD - enzyme required to inhibit 50% pyrogallol autoxidation / min / mg protein, CAT - µM of H_2O_2 decomposed / min / mg protein, GSH-PX - µm of glutathione utilized/min/mg protein, GSH- mg / ml, TBARs- nm of MDA/ml.