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Amelioration of enrofloxacin induced oxidative stress in heart homogenates of broilers with alpha lipoic acid and Vitamin E

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Abstract
An investigation was carried out in the heart homogenates of broilers to study the effect of enrofloxacin induced oxidative stress and its amelioration by alpha-lipoic acid (ALA) and its comparison with the conventional antioxidant vitamin E, by analyzing the effect of them on oxidative biomarkers. Treatment with either ALA (100 mg/kg b.w) or vitamin E (100 mg/kg b.w) significantly restored the antioxidant status in the enrofloxacin (10 mg/kg b.w) induced oxidative stress in heart homogenates of broilers. Whereas, treatment combined with vitamin E and ALA treated group had significantly restored the antioxidant parameters which are comparable with the normal control birds. Thus the results of this study suggest that the combination of ALA and vitamin E with enrofloxacin administration effectively restored the normal antioxidant status which was comparable to normal control than either of these drugs alone.

Keywords: Enrofloxacin, alpha lipoic acid, vitamin- E, heart homogenates

Introduction
In poultry, infections of alimentary and respiratory tract are quite common. Enrofloxacin is a molecule of second generation fluoroquinolone, which is commonly used as "in water" preparation for the treatment of alimentary and respiratory tract infections in poultry. Hence, enrofloxacin is indicated for the control of mortality associated with *Escherichia coli* (*E. coli*), *Salmonella sp., Pasteurella multocida, Haemophilus sp. and Yersinia sp.* in poultry [1]. The recommended therapeutic dosage of enrofloxacin is 10mg/Kg body weight per day for 5 to 7 days in chickens and turkeys. This also contributes to an increase in oxidative stress, promoting inhibition of cellular enzymes [2].
Alpha-lipoic acid is soluble in both fat and water and it promotes rapid passive transport across cellular membranes. In the interior of the cell, dihydrolipoic acid, which is the oxidized form of alpha-lipoic acid, regenerates vitamin E, vitamin C and glutathione peroxidase [3]. Additionally, alpha-lipoic acid functions as a cofactor in several mitochondrial multi-enzyme complexes involved in energy production [4]. Vitamin E is a potent biological antioxidant. It prevents oxidation of unsaturated lipid materials within the cells, thus protecting the cell membrane from oxidative damage [5].
In recent decades, poultry farming has been transformed into poultry industry and this has been achieved largely due to the successful adoption of the high yielding strains of broilers with provision of nutritionally balanced feed. However, during rapid growth there is increased metabolic activity that offsets the antioxidant-pro-oxidant balance in the system. Enrofloxacin are oxidized through cytochrome P450, free radical intermediates are generated and these subsequently can cause tissue oxidative damage. Hence, it is essential to minimize the oxidative stress caused by enrofloxacin [6].
In this study, an effort has been made to evaluate the oxidative stress caused by enrofloxacin and its amelioration by alpha-lipoic acid and its comparison with conventional antioxidant vitamin E in broilers.

Materials and Methods
Fifty day-old straight run commercial broiler chicks were utilized in this study. The birds were reared in cages under standard management practices from day old to six weeks of age. The birds were fed with standard broiler ration of Post Graduate Research Institute of Animal Sciences, Kattupakkam and water *ad libitum*.

This experimental trial was approved by the institutional animal ethical committee of Madras Veterinary College (Approval no: 1831/DFBS/2011).

The birds were weighed and randomly divided into five groups on day 21, each group comprising of ten birds and the treatment was started on day 38. The treatments given to different groups of birds are listed in the table I. Drugs were administered to the birds individually, daily orally from day 38 to day 42 using an oral catheter. On day 43, the birds were sacrificed and heart tissues were collected from the sacrificed birds and rinsed in ice-cold saline for the estimation of antioxidant assays. Lipid peroxidation was estimated by the formation of Thiobarbituric acid reactive substances (TBARs) [7], Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Catalase (CAT) were assayed [8, 9, 10]. Reduced glutathione (GSH) and Total protein (TP) estimation also carried out [11, 12].

Results

The results are presented in table II. In the present study, enrofloxacin treated group showed significant reduction of the antioxidant parameters of the heart homogenates like SOD, CAT, GPX and GSH content with marked elevation in the level of MDA. The groups treated with alpha-lipoic acid and vitamin E alone, restored the SOD, CAT, GPX and GSH activity towards normal, but it was still less than normal control. However the combination of alpha-lipoic acid and vitamin E was able to effectively restore the SOD, CAT, GPX and GSH levels similar to control birds. Similarly, treatment of alpha-lipoic acid or vitamin E with enrofloxacin reduced the lipid peroxidation which leads to the reduction of MDA levels. Group treated with vitamin E and alpha-lipoic acid combination showed significant reduction in MDA activity which was almost nearer to normal control group.

The results of this study elucidated that enrofloxacin at normal recommended dose (10 mg/kg body weight) significantly affected both enzymatic, non-enzymatic antioxidants and lipid peroxidation in the heart and there by induced oxidative stress in broilers. Treatment with either alpha-lipoic acid (100 mg/kg body weight) or vitamin E (100 mg/kg body weight) significantly restored the antioxidant status of heart. However, it was lesser than that of control birds. Co-treatment of alpha-lipoic acid (100 mg/kg body weight) and vitamin E (100 mg/kg body weight) restored antioxidant status of heart, which are almost equal or comparable with the normal control birds.

Discussion

Enrofloxacin is used in veterinary medicine for the treatment of infections of the bacterial and mycoplasmal diseases in poultry. Free radicals generated by enrofloxacin administration initiate the peroxidation of polyunsaturated fatty acids of membrane and covalently bind to microsomal lipids and proteins [13]. Enrofloxacin is extensively metabolized to ciprofloxacin in chickens, and so the market residue is considered as the sum of enrofloxacin and its metabolite ciprofloxacin [14]. Fluoroquinolones such as enrofloxacin are oxidized by microsomal enzymes of the cytochrome P450 family resulting in the formation of free radical which induce oxidative damage [15]. Free radicals generated by enrofloxacin administration initiate the peroxidation of polyunsaturated fatty acids of membrane and covalently bind to microsomal lipids and proteins. This results in the generation of reactive oxygen species (ROS) like the superoxide anion, H_2O_2 and OH^-. Because of these free radical development there will be reduction in growth rate and meat quality leading to financial loss to the farmer [16].

The present study indicated a significant increase in MDA levels of heart in enrofloxacin treated group when compared to control. Free radicals generated by enrofloxacin initiate the peroxidation of membrane polyunsaturated fatty acids of membrane and covalently bind to microsomal lipids and proteins [15]. The increase in MDA level indicated enhanced lipid peroxidation due to tissue injury and failure of antioxidant defense mechanism. However, treatment of alpha-lipoic acid or vitamin E with enrofloxacin reduced the lipid peroxidation which leads to the reduction of MDA levels. Combination of vitamin E and alpha-lipoic acid with enrofloxacin treated group showed significant reduction in MDA activity which was almost nearer to normal control group. This was coherent with the previous observations in broilers [5]. They showed that co-supplementation of alpha-lipoic acid and vitamin E improved the antioxidant status and brought down the levels of TBARS.

SOD, CAT, GPX and GSH are the first line of cellular defense against oxidative stress by scavenging the free radicals. SOD catalyzes the conversion of superoxide (O_2^-) to H_2O_2 which further can be rapidly converted to water by CAT and GPX [17]. The reduction in the activities of SOD, CAT, GPX and GSH may be due to using up of free radical defense system against oxidative stress [18]. However, treatment with alpha-lipoic acid and treatment with vitamin E restored the SOD, CAT, GPX and GSH activity towards normal control, which is in agreement with the observations [5]& [19]. But it was still less than normal control. Combination of alpha-lipoic acid and vitamin E was able to effectively restore the SOD activity similar to control birds.

Table 1: Experimental design (n = 10)

Group	Treatment	Period of administration
I	Control	---
II	Enrofloxacin (10mg/kg b.w)as oral administration	From 38th day to 42 nd day
III	Enrofloxacin (10mg/kg b.w) + alpha-lipoic acid (100mg/kg b.w) as oral administration	-do-
IV	Enrofloxacin (10mg/kg b.w) + vitamin E (100mg/kg b.w) as oral administration	-do-
V	Enrofloxacin (10mg/kg B.w) + alpha-lipoic acid (100mg/kg b.w) + vitamin E (100mg/kg b.w) as oral administration	-do-

Table 2: Effect of enrofloxacin on the enzymatic and non-enzymatic antioxidant and its amelioration with alpha-lipoic acid and vitamin E in heart homogenates of broilers

Groups	Treatment	Enzymatic antioxidants			Non enzymatic antioxidants	MDA (TBARS)
		SOD	CAT	GSH-Px	GSH	
I	Control	$61.56^{c} \pm 0.540$	$19.59^{d} \pm 0.23$	$15.60^{c} \pm 0.30$	$1.90^{e} \pm 0.01$	$1.51^{a} \pm 0.01$
II	Enrofloxacin (10 mg /Kg b.w, P.O)	$51.32^{a} \pm 0.536$	$12.82^{a} \pm 0.15$	$11.69^{a} \pm 0.22$	$1.32^{a} \pm 0.01$	$2.09^{c} \pm 0.01$
III	Enrofloxacin (10 mg /Kg b.w, P.O) + Alpha-lipoic acid (100 mg /Kg b.w, P.O)	$54.74^{b} \pm 0.58$	$16.71^{b} \pm 0.16$	$12.86^{b} \pm 0.27$	$1.58^{b} \pm 0.01$	$1.82^{b} \pm 0.00$
IV	Enrofloxacin (10 mg /Kg b.w, P.O) + Vitamin E (100 mg/Kg b.w, P.O)	$55.37^{b} \pm 0.42$	$17.34^{c} \pm 0.23$	$13.24^{b} \pm 0.23$	$1.63^{c} \pm 0.01$	$1.79^{b} \pm 0.01$
V	Enrofloxacin (10 mg /Kg b.w, P.O) + Vitamin E (100 mg /Kg b.w, P.O) + Alpha-lipoic acid (100mg /Kg b.w, P.O)	$61.02^{c} \pm 0.49$	$19.21^{d} \pm 0.25$	$15.31^{c} \pm 0.26$	$1.85^{d} \pm 0.01$	$1.54^{a} \pm 0.01$
	F value	71.61^{**}	161.51^{**}	40.05^{**}	219.94^{**}	413.54^{**}

All values are Mean ± S.E of 10 birds

**- Highly significant ($P< 0.01$)

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/g of tissue, TBARs-nm of MDA/g of tissue.

Conclusion

The present study revealed that Enrofloxacin, commonly used antimicrobial drug in poultry could induce oxidative stress and significantly affected both enzymatic, non-enzymatic antioxidants and lipid peroxidation indicating oxidative stress in broilers. Vitamin E or alpha-lipoic acid were able to reverse the oxidative parameters partially, thus indicating their utility in combating oxidative stress. Combination of vitamin E and alpha-lipoic acid proved effective in ameliorating oxidative stress when compared to either of these drugs alone in enrofloxacin treated birds and they were able to restore the oxidative parameters to almost normal control values. Though the exact mechanism underlying the enrofloxacin induced oxidative stress and the protective effect of alpha-lipoic acid and vitamin E against it is not known, further experimental studies are to be conducted to confirm such effects.

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