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## Enrofloxacin induced oxidative stress in kidney homogenates of broilers and its amelioration with antioxidants vitamin e and alpha lipoic acid

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#### Abstract

The experiment was conducted to evaluate the effect of enrofloxacin induced oxidative stress in kidney homogenates of broiler chickens. Total fifty broiler chickens divided into five groups at the age of day 38 were used for this study. Oxidative stress was produced in the chickens with Enrofloxacin at an oral dose of 10 mg/kg b.w. In Enrofloxacin (10 mg/kg b.w) treated group, there was a significant elevation in the oxidative biomarkers like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) and marked increase in lipid peroxidation (MDA) of kidney homogenates. Treatment with either alpha-lipoic acid (100 mg/kg b.w) or vitamin E (100 mg/kg b.w) significantly restored the antioxidant status in Kidney homogenates. Whereas, co-treatment of alpha-lipoic acid (100 mg/kg b.w) and vitamin E (100 mg/kg b.w) had significantly restored the antioxidant parameters which are comparable with the normal control birds. Thus 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*, alpha lipoic acid, vitamin- e, kidney homogenates

#### Introduction

Poultry farming has been transformed into one of the most dynamic sector and it is possible 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 <sup>[1]</sup>. In a healthy body pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress.

Enrofloxacin is commonly used as “in water” preparation for the treatment of alimentary and respiratory tract infections in poultry. Enrofloxacin are oxidized through cytochrome P450, free radical intermediates are generated and these subsequently can cause tissue oxidative damage <sup>[2]</sup>. Hence, it is essential to minimize the oxidative stress caused by enrofloxacin. 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 managerial practices from day old to six weeks of age. This experimental trial was approved by the institutional animal ethical committee of Madras Veterinary College. The experiment was carried out as indicated in 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 kidney 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) <sup>[3]</sup>. Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Catalase (CAT) were assayed <sup>[4]</sup>, <sup>[5, 6]</sup>. Reduced glutathione (GSH) and Total protein (TP) estimation also carried out <sup>[7, 8]</sup>.

#### Results

The results are presented in table II. In the present study, enrofloxacin administration caused a

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drastic reduction in the levels of SOD, CAT, GPX and GSH activity. 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.

The present study also indicated a significant increase in

MDA levels of kidney in enrofloxacin treated group when compared to control. However, 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.

**Table 1:** Experimental design (n = 10)

Group	Treatment	Period of administration
I	Control	---
II	Enrofloxacin (10mg/kg b.w) as oral administration	From 38 <sup>th</sup> day to 42 <sup>nd</sup> 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 kidney homogenates of broilers

Groups	Treatment	Enzymatic antioxidants			Non enzymatic antioxidants	MDA (TBARS)
		SOD	CAT	GSH-P <sub>x</sub>	GSH	
I	Control	71.46 <sup>c</sup> ± 0.52	25.59 <sup>d</sup> ± 0.23	21.03 <sup>c</sup> ± 0.25	2.25 <sup>c</sup> ± 0.04	2.04 <sup>a</sup> ± 0.02
II	Enrofloxacin (10 mg /Kg b.w, P.O)	54.66 <sup>a</sup> ± 2.87	17.36 <sup>a</sup> ± 0.024	14.90 <sup>a</sup> ± 0.45	1.64 <sup>a</sup> ± 0.02	2.67 <sup>d</sup> ± 0.01
III	Enrofloxacin (10 mg /Kg b.w, P.O) + Alpha-lipoic acid (100 mg /Kg b.w, P.O)	63.29 <sup>b</sup> ± 0.50	20.06 <sup>b</sup> ± 0.24	17.22 <sup>b</sup> ± 0.15	1.84 <sup>b</sup> ± 0.02	2.38 <sup>c</sup> ± 0.01
IV	Enrofloxacin (10 mg /Kg b.w, P.O) + Vitamin E (100 mg /Kg b.w, P.O)	64.17 <sup>b</sup> ± 0.84	20.27 <sup>b</sup> ± 0.25	17.59 <sup>b</sup> ± 0.14	1.89 <sup>b</sup> ± 0.02	2.35 <sup>c</sup> ± 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)	70.24 <sup>c</sup> ± 0.55	23.83 <sup>c</sup> ± 0.31	20.49 <sup>c</sup> ± 0.23	2.18 <sup>c</sup> ± 0.02	2.11 <sup>b</sup> ± 0.01
	F value	22.82 <sup>**</sup>	158.82 <sup>**</sup>	85.57 <sup>**</sup>	100.81 <sup>**</sup>	239.11 <sup>**</sup>

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- $\mu$ m of H<sub>2</sub>O<sub>2</sub> decomposed/ min/ mg protein, GSH-P<sub>x</sub> -  $\mu$ m of glutathione utilized/min/mg protein, GSH- mg/g of tissue, TBARS-nm of MDA/g of tissue.

## Discussion

Enrofloxacin is extensively metabolized to ciprofloxacin in chickens, and so the market residue is considered as the sum of enrofloxacin and its metabolite ciprofloxacin [9]. Fluoroquinolones such as enrofloxacin are oxidized by kidney microsomal enzymes of the cytochrome P450 family resulting in the formation of free radical which induce oxidative damage [10]. 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<sub>2</sub>O<sub>2</sub> and OH<sup>-</sup>. Because of these free radical development there will be reduction in growth rate and meat quality leading to financial loss to the farmer [11].

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<sub>2</sub><sup>-</sup>) to H<sub>2</sub>O<sub>2</sub> which further can be rapidly converted to water by CAT and GPX [12]. 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 [13]. 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 [14, 15]. 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.

The present study also indicated a significant increase in MDA levels of kidney 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 [11]. The increase in MDA level indicated enhanced lipid peroxidation due to tissue injury and failure of antioxidant defence 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 [14]. They showed that co-supplementation of alpha-lipoic acid and vitamin E improved the antioxidant status and brought down the levels of TBARS.

## Conclusion

The present study revealed that Enrofloxacin can significantly affect both enzymatic, nonenzymatic antioxidants and lipid peroxidation causing oxidative stress in broilers. Vitamin E or alpha-lipoic acid when administered alone as antioxidant, is able to reverse the enrofloxacin induced changes partially,

indicating their utility in combating oxidative stress. Combination of vitamin E and alpha-lipoic acid has proved to be effective in ameliorating oxidative stress completely, when compared to either of these drugs used alone. Nevertheless, these results suggest that alpha-lipoic acid and vitamin E are useful antioxidants and their combination nullifies the tissue damage or oxidative stress caused by enrofloxacin in broilers.

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