ENROFLOXACIN INDUCED OXIDATIVE STRESS AND ITS AMELIORATION WITH ANTIOXIDANTS IN LIVER HOMOGENATES OF BROILERS

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ABSTRACT

The experiment was conducted to evaluate the effect of enrofloxacin induced oxidative stress in liver 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 liver 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 liver 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.

INTRODUCTION

In recent times, poultry farming has been transformed into one of the most dynamic sector of animal production. 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 (Dirain et al., 2005). 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, a second generation fluoroquinolone 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. Hence, it is essential to minimize the oxidative stress caused by enrofloxacin. In this study, an attempt has been made to study the oxidative stress caused by enrofloxacin and its amelioration by alpha-lipoic acid and its comparison with conventional antioxidant vitamin E in broilers.

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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). 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 liver 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) by the method of Yagi (1976). Superoxide dismutase (SOD) was measured by the method of Marklund and Marklund (1974). Glutathione peroxidase (GPX) was measured by the method of Rotruck et al. (1973). Catalase (CAT) was assayed by the method of Caliborne (1985). Reduced glutathione (GSH) was estimated by the method of Meron et al. (1979). Total protein (TP) estimation was carried out by the method of Markwell et al. (1978).

RESULTS

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

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 (Anadon et al., 1995). Fluoroquinolones such as enrofloxacin are oxidized by liver microsomal enzymes of the cytochrome P450 family (Stratton, 1998) resulting in the formation of free radical which induce oxidative damage. 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, H2O2 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 (Carreras et al., 2004).

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 (Bulger et al., 2001). 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 (Gurbay et al., 2001). 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 of Srilatha et al. (2010) and Acikgoz et al. (2011). 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 liver 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 (Carreras et al., 2004). 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. This finds support from the findings of Carreras et al. (2004) in broilers. 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 observations of Srilatha et al. (2010) in broilers. They showed that co-supplementation of alpha-lipoic acid and vitamin E improved the antioxidant status and brought down the levels of TBARS.

SUMMARY

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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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 42nd 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 liver homogenates of broilers

Groups	Treatment	Enzymatic antioxidants			Non enzymatic antioxidants	MDA (TBARS)
		SOD	CAT	GSH-P_X	GSH	
I	Control	$64.35^{c} \pm 0.54$	$21.59^{c} \pm 0.23$	$17.03^{c} \pm 0.28$	$2.03^{d} \pm 0.02$	$1.62^{a} \pm 0.01$
II	Enrofloxacin (10 mg /Kg, P.O)	$54.07^{a} \pm 0.52$	$14.80^{a} \pm 0.14$	$13.16^{a} \pm 0.26$	$1.48^{a} \pm 0.02$	$2.18^{c} \pm 0.01$
III	Enrofloxacin (10 mg / Kg, P.O) + Alpha-lipoic acid (100 mg /Kg, P.O)	$59.99^{b} \pm 0.39$	$18.72^{b} \pm 0.26$	$15.47^{b} \pm 0.18$	$1.71^{b} \pm 0.01$	$1.89^{b} \pm 0.01$
IV	Enrofloxacin (10 mg /Kg, P.O) + Vitamin E (100 mg /Kg, P.O)	$60.63^{b} \pm 0.26$	$18.99^{b} \pm 0.27$	$15.66^{b} \pm 0.19$	$1.75^{b} \pm 0.02$	$1.87^{b} \pm 0.01$
V	Enrofloxacin (10 mg /Kg, P.O) + Vitamin E (100 mg /Kg, P.O) + Alpha-lipoic acid (100mg /Kg, P.O)	$63.85^{c} \pm 0.54$	$21.39^{c} \pm 0.21$	$16.38^{c} \pm 0.23$	$1.97^{c} \pm 0.02$	$1.65^{a} \pm 0.01$
	F value	77.08**	140.27**	38.27**	94.35**	304.48**

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-P_X - µm of glutathione utilized/min/mg protein, GSH- mg/g of tissue,
TBARs-nm of MDA/g of tissue.