HISTOPATHOLOGICAL EVALUATION OF ENROFLOXACIN INDUCED OXIDATIVE STRESS AND ITS AMELIORATION WITH ANTIOXIDANTS

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ABSTRACT

In this research work, an attempt has been made to study the histopathological changes of oxidative stress caused by enrofloxacin at therapeutic doses and its restoration by alpha-lipoic acid and its comparison with conventional antioxidant vitamin E in tissue homogenates like liver, kidney and heart of broilers. Enrofloxacin was administered to the broiler birds orally at the dose of 10 mg/kg body weight once daily from day 38 to day 42. Histopathological examination revealed marked congestion and bile duct hyperplasia of liver, degeneration and swelling of tubular epithelial cells in kidney. Interestingly, there was no appreciable lesion in the heart. Treatment with either alpha-lipoic acid (100 mg/kg body weight) or vitamin E (100 mg/kg body weight) showed moderate restoration of changes observed in liver and kidney compared to normal control birds. Co-treatment of alpha-lipoic acid (100 mg/kg body weight) and vitamin E (100 mg/kg body weight) showed restoration of liver and kidney architecture towards normal.

Key words: Enrofloxacin, Alpha lipoic acid, Vitamin- E, Histopathology.

INTRODUCTION

Poultry farming in India has transformed into a techno-commercial industry from the status of backyard farming. This has come largely due to successful adoption of the high yielding strains of egg and meat type chicken, with provision of nutritionally balanced feed. 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 (Pamok et al., 2009). The antioxidant defense mechanisms existing in the system effectively combats the generation of ROS thereby minimizing oxidative stress at cellular and sub cellular levels. However, during rapid growth there is increased metabolic activity that offsets the antioxidant-prooxidant balance in the system. Therefore, it is essential to reestablish the altered antioxidant potential of broilers facilitating increased growth rate with little oxidative stress.

Enrofloxacin is indicated in the recommended therapeutic dosage of 10mg/Kg body weight per day for 3 to 10 days for the control of mortality associated with Escherichia coli (E.coli), Salmonella sp., and Pasterella multocida in broiler chickens. Enrofloxacin is extensively metabolized to ciprofloxacin in chickens, and so the market residue is considered as the sum of enrofloxacin and ciprofloxacin. Fluoroquinolones such as enrofloxacin are oxidized by liver microsomal enzymes of the cytochrome P450 family. As a result of fluoroquinolone metabolism through cytochrome P450, free radical intermediates

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are formed, which induce oxidative damage (Carreras et al., 2004). Thus, enrofloxacin further adds oxidative stress to birds which are already vulnerable to stress because of their rapid growth rate.

Alpha-lipoic acid (ALA) is described as an universal antioxidant because it can scavenge a wide range of free radicals, directly and indirectly, regenerates antioxidants, chelate a wide variety of metals that are associated with increased production of free radicals and inhibit gene over-expression (Packer et al., 1995). 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 (Srilatha et al., 2010).

The present research work was designed to estimate the histopathological changes of 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 birds were reared in cages and maintained under standard managemental practices from day old to six weeks of age. On day 21, birds were weighed and randomly divided into five groups comprising of ten birds in each group and they were subjected to treatment from day 38 to day 42. Group I was treated as control, group II was treated with enrofloxacin (10mg/kg body weight), group III was treated with enrofloxacin (10mg/kg body weight) + alpha-lipoic acid (100mg/kg body weight), group IV was treated with enrofloxacin (10mg/kg body weight) + vitamin E (100mg/kg body weight) and group V was treated with enrofloxacin (10mg/kg Body weight) + alpha-lipoic acid (100mg/kg body weight) + vitamin E (100mg/kg Body weight). All the above drugs were administered orally. Then, the birds were immediately sacrificed on day 43. Tissues like liver, kidney and heart were collected and the sections of above tissues were fixed in 10% formalin for histopathological examination. Tissues such as liver, kidney and heart were collected and fixed in 10% formalin, routinely processed, paraffin embedded and sectioned to 5 µm thickness for histopathological examination.

RESULTS AND DISCUSSION

Liver & Kidney: The histopathological features of liver and kidney in treatment groups are furnished in Plates (I-IV). The architecture of liver section from the control group of birds were appeared to be normal. In enrofloxacin administered group birds, there was marked congestion and bile duct hyperplasia (Plate I). However, Alpha-lipoic acid with enrofloxacin treated group showed moderate vacuolar degeneration and vitamin E with enrofloxacin treated group exhibited mild vacuolar degeneration when compared to control (Plate II). Interestingly,combination of alpha-lipoic acid and vitamin E with enrofloxacin treated group revealed near normal arrangement of hepatocytes as in control group.

The renal architecture appeared to be normal in the control group of birds. In enrofloxacin treated group, kidney showed degeneration and swelling of tubular epithelial cells (Plate III). However, Alpha-lipoic acid with enrofloxacin treated group showed mild degeneration of tubules and vitamin E with enrofloxacin treated group exhibited moderate degeneration with swelling of tubular epithelial cells (Plate IV). In combination of alpha-lipoic acid and vitamin E with enrofloxacin treated group, no appreciable lesion was observed and the tissues looked similar as that of the control.

These oxidative stress changes are in accordance with the observations of Sarkar et al. (1998), who observed that the toxicopathology induced by enrofloxacin in layer chicks and found that the histopathology revealed highly congested vessels with necrotic and degenerative changes in the liver and kidneys. Similarly, Niyogi and Bhowmik (2003) found that ciprofloxacin at its therapeutic level (5mg/kg body weight)

has produced histopathological lesions which comprised of toxic hepatitis, necrotic enteritis and toxic nephrosis and/or nephritis in birds.

There are previous reports in which vitamin E or alpha-lipoic acid has effectively reversed the oxidative stress induced histopathological changes. Aboul-Soud et al. (2011) found that the administration of vitamin E (100 mg/kg body weight) protected the liver of rats exposed to malathion as evidenced by the appearance of normal histological structures. Al-Attar (2011) observed that the administration of vitamin E improved the observed biochemical and histopathological changes induced by those heavy metals intoxication.

Similarly, Takaoka et al. (2001) observed that alpha-lipoic acid effectively reversed the toxicopathological changes induced by deoxycorticosterone in the kidney of experimental rats. Furthermore, Dulundu et al. (2007) found that alpha-lipoic acid has protected against the damage induced by hepatic ischemia-reperfusion injury in rats and reversed the histopathological changes in the liver.

Heart: In histopathological picture of heart, there were no marked changes in enrofloxacin treated group and other groups when compared to control indicating no damage to myocardial architecture by enrofloxacin. It is reported that residues of enrofloxacin and the major metabolite ciprofloxacin are mainly found in liver, kidney and muscles, but not probably in heart (Anadon et al., 1995).

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Plate I&II: Histopathology of Liver

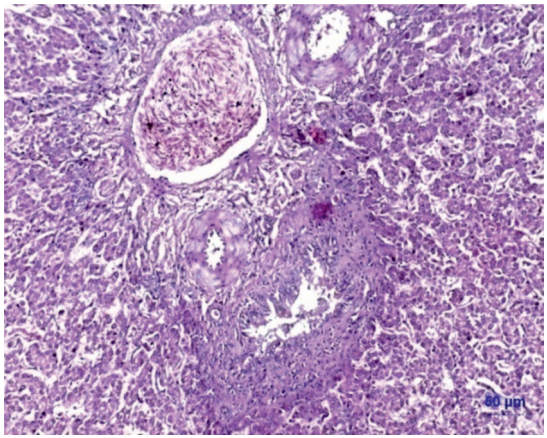

Plate I : Enrofloxacin treated group showing marked congestion and bile duct hyperplasia

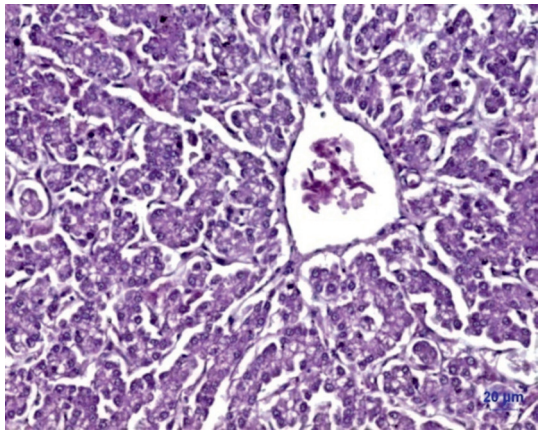

Plate II : ALA or Vitamin E's amelioration in oxidative stress with moderate vacuolar degeneration

Plate III&IV: Histopathology of Kidney

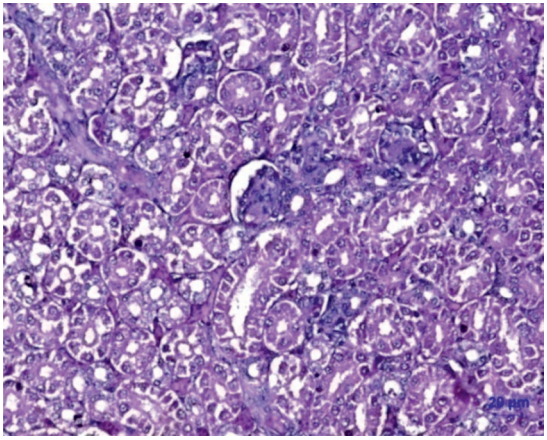

Plate III : Enrofloxacin treated group showing degeneration and swelling of tubular epithelial cells

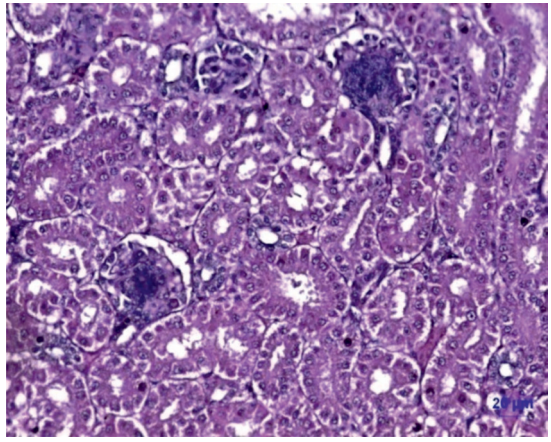

Plate IV : ALA or Vitamin E's amelioration in oxidative stress with moderate degeneration with swelling of tubular epithelial cells