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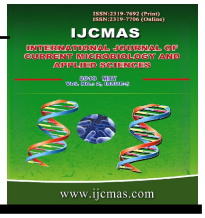

Original Research Article

Veterinary pharmacovigilance evaluation on impact of enrofloxacin administration on antioxidant status in broiler chicken

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Keywords

Veterinary pharmaco-vigilance; enrofloxacin; antioxidant status; broiler chicken.

A B S T R A C T

A veterinary pharmacovigilance study has been instigated in broiler chicken to explore the impact of enrofloxacin water medication on various antioxidants thereby to shed more light on the mechanism of oxidative stress produced by enrofloxacin. Day old broiler chicks were randomly divided into 4 groups each comprising 6 birds. Group I, control birds received non medicated water, while group II, III and IV were medicated with enrofloxacin @ recommended therapeutic dose 10mg/Kg body weight in drinking water for 5 successive days from 43rd to 47th day of age. On 1, 5 and 9 days after the last dose, respective treatment groups were sacrificed ethically and control birds were sacrificed at the end of the experiment. Serum, liver and muscle samples were collected and subjected to antioxidant analysis following standard procedures. Glutathione S-transferase, catalase and glutathione (GSH) levels were found to be significantly decreased in serum, muscle and liver in 1st and 5th day post treatment groups in comparison to control. Indeed, the antioxidant status showed restoration in tendency towards control values as evidenced by a non existence of significant difference between control and day 9 post treatments or day 5 and day 9 post treatment group. Despite significant fall in antioxidant status in serum, muscle and liver after enrofloxacin administration, the refurbishment of the same on 9th day of withdrawal period suggests that enrofloxacin is safe if medicated at recommended therapeutic dose and stipulated withdrawal period is stringently adhered.

Introduction

Fluoroquinolones stand for major class of antimicrobial agents advocated in food animals and poultry for the treatment of a wide range of infectious diseases. Enrofloxacin, a synthetic fluoroquinolone chronic respiratory diseases, Colibacillosis, Salmonellosis and Fowl cholera in poultry (Anderson *et al.*, 2003, Martinez *et al.*, 2006, Papich, M.G. and Riviere, 2009). Gurbay *et al.*, (2001)

postulated that during metabolic transformation of enrofloxacin into pharmacologically active metabolite ciprofloxacin in liver (Prescott *et al.,* 2000; Taccetti *et al.,* 2008; Papich, M.G. and Riviere, 2009), free radical intermediates are generated and resulted in lipid peroxidation. Further, Martinez-Cayuela (1998) opined that enrofloxacin residues may occur in meat, milk and eggs, generate free radicals owing to its metabolism and interact with other medicated drugs (Ershov *et al.,* 2001; Carrreras *et al.,* 2004; Sureshkumar *et al.,* 2004).

Fluoroquinolones are considered relatively well tolerated than the other commonly used antimicrobial agents. However, it has been reported that they are also associated with a low incidence of adverse effects related to gastrointestinal, skin, hepatic, and central nervous system functions, and phototoxicity (Hooper, *et al.,* 1985). Further, enrofloxacin administration in broiler chicken resulted in significant fall in lymphocyte count (Sureshkumar *et al.,* 2012) and reduction in haemagglutination inhibition (HI) titre and associated histopathological changes in lymphoid organs (Sureshkumar *et al.,* 2013). Literature evidences indicated that these adverse effects might be attributed to free radical formation (Wagai and Tawara. 1991; Hayem *et al.,* 1994) and very little reports have elucidated the mechanism of toxicity of fluoroquinolones (Gurbay *et al.,* 2001).

Glutathione S-transferase (GST) is the key antioxidant defends cells against toxicants by conjugating the thiol group of the glutathione to electrophilic xenobiotics, thus GST levels divulge the antioxidant ability of the body. Glutathione (GSH) detoxifies the xenobiotics by removing hydroperoxides, and maintaining the oxidation state of protein sulfhydryls (Akerboom and Sies, 1981). Oxidative stress arises when free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxides react with cellular constituents such as thiols and lipids and alter the antioxidant defense systems (Keyse and Tyrell, 1989; Wang and Huang, 1994; Liu *et al.,* 2001). In spite of the literature evidences available on the incidence of antibiotics to cause oxidative stress in other animal species (Salyi *et al.,* 1990; Mezes *et al.,* 1992), data in poultry is scanty (Altinordulu and Eraslan, 2009).

Hence a veterinary pharmacovigilance study has been instigated in broiler chicken to explore the impact of enrofloxacin water medication on various antioxidants thereby to shed more light on the mechanism of oxidative stress produced by enrofloxacin.

Materials and Methods

Enrofloxacin water medication

Institutional Animal Ethics Committee, Madras Veterinary College, TANUVAS has accorded permission for the biological trial. Twenty four one-day-old broiler chicks (Broiler strain B_1) were randomly grouped into control (I) and treatment (II, III and IV) and maintained under standard management conditions. Treatment groups were medicated with enrofloxacin @ recommended therapeutic dose 10mg/Kg body weight, in drinking water for 5 successive days, while control birds received non medicated water (Knoll *et al.,* 1999, Sureshkumar *et al.,* 2013). The pulsed water medication was given as described by Charleston *et al*., (1998).

Antioxidant enzyme analysis

On 1, 5 and 9 days after the last dose, respective treatment groups were sacrificed ethically and control birds were sacrificed at the end of the experiment. Serum, liver and muscle samples were collected and subjected to antioxidant enzyme analysis. Tissue sample of 100mg was homogenized in 1 mL of phosphate buffer (pH 8) and centrifuged at 10000rpm for 5min at 4˚C. The supernatant was used as enzyme/protein sample for further analysis. Glutathione S-transferase (GST) assay was carried out following the methodology of Habig et al. (1974); Mannervik and Danielson, (1988); Wilce and Parker, (1994). Glutathione **(**GSH) assay was carried out as described by Akerboom and Sies, (1981). Catalase (CAT) activity was estimated by the method of Sinha (1972). Statistical analysis was carried out as per Snedecor and Cochran (1994).

Results and Discussion

In liver, a significant decrease ($p<0.01$) in GST and GSH level was noticed at all the time points evaluated during the withdrawal period when compared to that of control. On the other hand, a statistically insignificant difference existed between day 1 and day 5 post treatment.

Muscle GST level was significantly ($p<0.05$) decreased on day 1 and day 5 post treatment. However, on day 9 post treatment it was statistically insignificant when compared to that of control. Muscle GSH level was significantly decreased ($p<0.01$) at all the time points evaluated during the withdrawal period, except on day 9 post treatment as indicated by insignificant difference between day 5 and day 9 post treatment.

Serum GSH activity was significantly decreased ($p<0.01$) on day 1 and day 5 post treatment. However, on 9th day post treatment the GSH level was statistically insignificant in comparison to control. A significant decrease ($p<0.01$) in serum GST level was noticed on day 5 post treatment. However, similar to that of muscle statistically insignificant difference was noticed between day 9 post treatment and control.

Catalase activity in liver and muscle was significantly decreased ($p<0.01$) on day 1 and day 5 post treatment from that of control. However, a trend in increase in the CAT level was noticed on day 9 post treatment as indicated by a statistically insignificant difference between day 9 post treatment and control. Serum CAT activity was significantly reduced ($p<0.01$) at all the time points evaluated during the withdrawal period. However, a trend in increase in the CAT level was noticed on day 9 post treatment as evidenced by a insignificant difference from that of day 1 post treatment(Table 1).

In the present study, a significant decrease in GST and GSH level in liver, muscle and serum is suggestive of depletion of glutathione store, which could be attributed due to free radical generation by enrofloxacin and its metabolite ciprofloxacin (Gurbay *et al.*, 2001; Altinordulu and Eraslan, 2009). The free radicals thus generated are scavenged by GSH by donating electrons, thereby the balance between GSH-reduced glutathione and GSH-oxidized glutathione is disturbed (Sies, 1993).

Evaluation of CAT activity in liver and muscle showed a significant difference on day 1 and day 5 post treatment, which is in agreement with Benzer et al. (2009).

Table.1 Effect of enrofloxacin administration (10mg/Kg body weight, in drinking water for 5 successive days) on antioxidants status (Mean±SE, n=6)

Parameters	Tissues	Control	Day 1 Post Treatment	Day 5 Post Treatment	Day 9 Post Treatment
GST (nmoles/mL/min)	Liver	$5.87^{a}\pm 0.26$	$2.82^{c}\pm 0.14$	$3.02^{c}\pm 0.12$	$4.36^{b}\pm 0.13$
	Muscle	$2.08^{x}\pm 0.14$	$1.70^{yz}\pm 0.06$	$1.62^{z}\pm 0.14$	$1.96^{xy}\pm 0.06$
	Serum	$1.96^{a}\pm 0.24$	$1.47^{abc}\pm 0.19$	$0.64^{c}\pm 0.04$	$1.62^{ab}\pm 0.31$
GSH (nmoles/mL)	Liver	$39.71^{a}\pm 1.18$	$8.36^{c}\pm 0.94$	$5.34^{c}\pm 0.25$	$16.70^{b}\pm 0.90$
	Muscle	$50.16^{a}\pm 0.99$	$23.15^{c}\pm 1.23$	$39.23^{b}\pm 0.57$	$42.12^{b}\pm 0.58$
	Serum	$45.31^{a}\pm 1.48$	$11.58^{c}\pm 0.51$	$35.14^{b}\pm 1.16$	$41.16^{a}\pm 1.20$
Catalase (µmoles of H_2O_2 consumed/min/g protein)	Liver	$49.46^{a}\pm 0.96$	$44.09^{b}\pm 0.47$	$33.35^{c}\pm 0.87$	$47.99^{a}\pm 0.55$
	Muscle	$43.40^{a}\pm 0.96$	$21.12^{c}\pm 0.73$	$27.50^{b}\pm 0.80$	$46.46^{a}\pm 0.60$
	Serum	$59.84^{a}\pm 1.03$	$38.66^{b}\pm 0.79$	$28.37^{c}\pm 0.86$	$36.77^{b}\pm 0.67$

Means bearing different superscript (x,y,z / a,b,c) within the row differ significantly ($p<0.05$ / $p<0.01$)

Decrease in CAT activity is suggestive of free radicals formation following the enrofloxacin administration. This is in accordance with Altinordulu and Eraslan (2009), who demonstrated that CAT activity is diminished in the event of hydrogen peroxide radical formation. However, a trend in restoration of CAT level was noticed on day 9 post treatment as represented by a statistically insignificant difference between day 9 post treatment and control.

In vivo, enrofloxacin is de-ethylated into its primary metabolite ciprofloxacin by liver microsomal enzymes of the cytochrome P450 family (Stratton, 1998, Taccetti *et al.,* 2008, Altinordulu and Eraslan, 2009). Further, ciprofloxacin is metabolized by alteration to the piperazine side chain (Sorgel, 1989). Several authors have speculated that consequent to the metabolism of enrofloxacin and ciprofloxacin, free radicals are generated, and resulted in lipid peroxidation (Martinez-Cayuela, 1998; Gurbay *et al.,* 2001; Altinordulu and Eraslan, 2009). These hypotheses are very well portrayed in the present findings as evidenced by significant decrease in GST, GSH and CAT level in liver, muscle and serum of broiler chicken administered with enrofloxacin. Vaccaro et al. (2003) reported that oxidation of ciprofloxacin by CYP450 leads to formation of reactive intermediates. As a sequel, a series of subsequent deleterious reactions occurred (Nie *et al.,* 2008).

The significant fall in antioxidants enzymes after enrofloxacin medication followed by its restoration during the withdrawal period could be attributed to the depletion of the enrofloxacin residues from liver and muscle during the withdrawal period. This proposition is substantiated by Chattha et al. (2008), who observed that enrofloxacin residues were washed out in 9 days whilst its major metabolite ciprofloxacin was washed out in 8 days in chicken meat. Further, Petrovic et al. (2006) showed that a 4 days of withdrawal period after enrofloxacin administration resulted in decrease in enrofloxacin residues to a tolerable level in the broiler meat and liver. While, San

Martin et al. (2010) demonstrated that based on the European Union maximum residue limits (EU MRLs) of 100 µg⁄Kg (muscle) and 200 µg⁄ Kg (liver), the withdrawal time was 5 days, and when Japan MRL was considered (10 µg⁄Kg,), the withdrawal time was found to be 8 days in broiler chicken. From these reports it is evident that enrofloxacin residues were high during the earlier days of the withdrawal period, whereas on day 9 post treatment it was below 10 µg⁄Kg. Same trend was reflected in the antioxidant status of the liver, muscle and serum as evidenced by corresponding restoration in the antioxidants level on 9th day of the withdrawal period.

Despite significant fall in antioxidant status in serum, muscle and liver after enrofloxacin administration, the refurbishment of the same on 9th day of withdrawal period suggests that enrofloxacin is safe if administered at recommended therapeutic dose and stipulated withdrawal period is adhered. Thereby it avoids untoward adverse drug reactions and residues.

Author's contributions

Component of the Ph.D. thesis work of the first author V. Sureshkumar, submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai-51. G. Sarathchandra was the Chairman and planned the trial. V. Sureshkumar conducted the experimental trial. J.Ramesh involved in sampling. G.Sarathchandra and V. Sureshkumar processed the data, arranged and corrected the article. All authors proof read and accepted the final article.

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Competing interests

Authors declare that they have no competing interest.

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