CHAPTER I
INTRODUCTION

Indian economy is the fastest growing and third largest economy in the world. Large proportion of Indian population still largely depends on agriculture and related fields for income. Poultry industry is one of the fastest growing sectors of Indian agriculture with annual growth rates of 5.57 % and 11.44 % in egg and broiler production, respectively (Anonymous, 2012). The global chicken meat and egg markets have grown over the last 5-year period by 19 % and 9.52 %, respectively (FAO Statistical Yearbook, 2013). Devastating threat to poultry industry is the bacterial infection like colibacillosis, salmonellosis, pasteurellosis, campylobacterosis, mycoplasmosis, tuberculosis, psittacosis, infectious coryza and coccidiosis (Hasan et al., 2010). Therapeutic and prophylactic use of antibiotics has allowed poultry production to achieve significant improvements by enhancing growth rate, feed efficiency and reduce mortality.

Target achieving activity of antimicrobial agents is largely governed by optimal dosage regimen and route of administration, which yields therapeutic effective concentration in plasma and body compartments for desired period of time. The dosage regimen of drugs can be optimized by their pharmacokinetic studies. Pharmacokinetics of a drug could be affected by a number of physiological, pathological and pharmacological processes which leads to enhanced or reduced therapeutic response (Chikhani and Hardman, 2016).

A vast array of fluoroquinolones having excellent broad-spectrum activity forms an invaluable part of the present antibacterial therapy in poultry. Fluoroquinolone inhibits the bacterial topoisomerase enzyme which is concentration-dependent and maximum killing rates achieved at the optimum bactericidal concentration which explains the concept of "rapid killing" with these agents (Morrissey, 1997). The development of floroquinolones strengthened antimicrobial therapeutics due to its broad spectrum of action and low toxicity. The first quinolone produced was nalidixic acid, which is a naphthyridine. The addition both of a fluorine molecule at the 6-position of the basic quinolone structure and a piperazine substitution at the 7-position was found to enhance quinolone antibacterial activity against Pseudomonas aeruginosa and gram-positive cocci and to increase the extent
of oral drug absorption and tissue distribution (Ball, 2000). Continuous efforts were directed to further modification of quinolone pharmacophore with more complex newer fluoroquinolones viz. danofloxacin, trovafoxacin, paziufloxacin, moxifloxacin, clinafoxacin and marbofloxacin etc. that had revolutionized the clinical importance of fluoroquinolones (Andriole, 1990).

Fluoroquinolones are considered to have a concentration dependent effect; although a time-dependent bactericidal effect against some gram-positive bacteria has also been described (Spreng et al., 1995; Cester et al., 1996). They also have some ideal characteristics such as a wide spectrum of bactericidal activity, a large volume of distribution, low plasma protein binding and relatively low MICs against susceptible target microorganisms (Spreng et al., 1995). General pharmacokinetic properties of fluoroquinolones includes variable but good oral absorption (except; ruminants and horses), complete parenteral absorption, good tissue distribution, good volume of distribution, renal excretion by glomerular filtrations, hepatic metabolism by oxidation and long half-life (Brown, 1996).

Marbofloxacin is a third generation, fluorinated quinolone for exclusive use in veterinary medicine (Brown, 1996). It exhibits bactericidal action by targeting the bacterial DNA topoisomerases II (gyrase) and IV, which are responsible for supercoiling of DNA around RNA core to provide a suitable spatial arrangement of DNA within the bacterial cell (Drlica and Zhao, 1997). Marbofloxacin having broad spectrum of antimicrobial activity against gram-negative, some gram-positive bacteria and mycoplasma spp. In vivo and in vitro efficacy against Staphylococcus intermedius, Escherichia coli, Proteus mirabilis, Pseudomonas spp., Pasteurella multocida, and Mannheima haemolytica have been reported (Spreng et al., 1995; Shojaee and Lees, 1997).

Marbofloxacin differs from other fluoroquinolones in chemical structure as presence of an oxadiazine ring, which may provide some pharmacokinetic advantages such as long elimination half-life and high bioavailability (Fitton, 1992). Important pharmacokinetic and pharmacodynamic properties of marbofloxacin like larger volume of distribution, greater tissue penetration and optimum AUC/MIC or C<sub>max</sub>/MIC ratio make it a potentially useful drug for the treatment of genital tract, respiratory tract, gastro-intestinal tract, skin and soft tissue infection in domestic animals (Brown, 1996; Ihrke et al., 1999). Marbofloxacin is mainly eliminated in
urine and its active form making it a suitable alternative for the treatment of urinary tract infections (Schneider et al., 1996).

Fluoroquinolones are intensively used in poultry production and facilitated better treatment of several infectious bacterial diseases; however, their prudent, ethical, and judicious use is essential. The inherent risks of the inadequate use of antimicrobials in poultry production include the induction of bacterial resistance, environmental contamination and the accumulation of residues in poultry products. The use of these drugs must comply with strict withdrawal periods, doses and duration of treatment (Gouvea et al., 2015). It is utmost requirement to develop newer safer antimicrobials or to enhance the therapeutic effect of currently available agents with reduced dose, toxicity and increased efficiency against resistant bacterial infections.

Several dietary constituents and phytochemicals are now identified as important factors affecting drug disposition (Walter-Sack and Klotz, 1996; Evans, 2000). Combined use of herbs with drugs beneficially to improve pharmacokinetics of co-administered drug (Peng et al., 2006). Many natural compounds from medicinal plants have demonstrated capacity to enhance the bioavailability of co-administered drugs by inhibiting efflux pumps or oxidative metabolism, and perturbing the intestinal brush border membrane. These natural compounds include piperine, quercetin, genistein, naringin, sinomenine, glycyrrhizin and nitrile glycoside (Varma et al., 2003). Drug bioavailability after oral administration is dependent on an enterocyte P-glycoprotein (P-gp), a drug transporter protein from adenosine triphosphate-binding cassette (ABC) super family which can actively pump back drugs into the intestinal lumen, and also by enterocyte Cytochrome P450 (CYP450), drug metabolizing enzymes which metabolize the drug before it reaches the systemic circulation. Induction or inhibition of P-glycoprotein and enterocyte CYP450 can thus influence drug bioavailability (Cummins et al., 2002). Herbal bioenhancers are the agents of herbal origin or any phytomolecules, which are capable of enhancing bioavailability and/or bioefficacy of a specified drug at the low dose. Herbal bioenhancers can reduce the dose level and produce desired therapeutic effect, reduces the cost of treatment and toxicities in animals and tissue residues problems for human health significance (Kowalski et al., 2005; WHO, 2009).

Piperine, a nitrogenous pungent substance was discovered by Hans Christian Orsted in 1819 (Capasso et al., 2002). Piperine, a main active component in both
black pepper (Piper nigrum Linn.) and long pepper (Piper longum Linn.) has been validated as the world's first bioenhancer (Atal, 1979). Ability of piperine to inhibit drug metabolism was first recognized before 30 years ago, when its administration in rats, increased the oral bioavailability of the alkaloids sparteine and vasicine by factors of two and three, respectively (Atal et al., 1985).

Piperine combination treatment has increased plasma concentrations of several compounds such as theophylline, propranolol (Bano et al., 1991) and phenytoin (Pattanaik et al., 2006) in laboratory animals. It has also enhanced the bioavailability of antimicrobial agents like ciprofloxacin (Bhise and Pore, 2002), amoxicillin and cefotaxime (Hiwale et al., 2002), oxytetracycline (Singh et al., 2005), pefloxacin (Madhukar et al., 2008; Nduka et al., 2013), norfloxacin and ampicillin (Janakiraman and Manavalan, 2008) and gatifloxacin (Patel et al., 2011). Mechanism of action of piperine in increasing the drug bioavailability includes enhancement of blood supply through vasodilatation (Annamalai and Manavalan, 1989), inhibition of drug metabolism (Atal et al., 1985), inhibition of hepatic monooxygenase and UDP-glucouronyl transferase and intestinal glucuronidation (Singh et al., 1986), inhibition of CYP3A4 and p-glycoprotein (P-gp) (Bhardwaj et al., 2002).

Flavonoids, another group of phytochemicals are the most abundant polyphenols forming an integral component of our common diet. They are particularly abundant in vegetables, fruits and plant-derived beverages such as wine and tea. In addition, a variety of flavonoid-containing dietary supplements and herbal products are now available in the market because of their proposed health-promoting activities, such as antioxidant, anticarcinogenic, anti-inflammatory, antiproliferative, antiangiogenic, and antiestrogenic (or estrogenic) effects (Havsteen, 2002; Zhang et al., 2004). Flavonoids have attracted much attention in recent years because of their beneficial pharmacological activities and for their additional abilities to modulate both CYP3A4 and P-gp (Critchfield et al., 1994). These include in-vitro studies on the effect of flavonoids on intracellular accumulation of P-gp substrates using P-gp over expressing cells or a variety of in-vivo studies in P-gp knockout animals (Morris and Zhang, 2006).

Quercetin, a flavonoid group compound widely occurring in plants and foods including apples, berries, Brassica vegetables, capers, grapes, onions, shallots, tea, and tomatoes, as well as many seeds, nuts, flowers, barks, and leaves. It is also found in medicinal botanicals, including Ginkgo biloba, Hypericum perforatum (St. John’s
wort) and *Sambucus canadensis* (Williamson and Manach, 2005). Quercetin constitute a promising new class of natural modulators as it might affect the bioavailability of drugs that are metabolized by CYP 3A4 or effluxed by P-gp (Conseil *et al*., 1998).

Quercetin has many beneficial pharmacological effects like antioxidant (Meyers *et al*., 2008), antiallergic, antiasthmatic (Joskova *et al*., 2011), antihypertensive (Carlstrom *et al*., 2007), anti-inflammatory (Morikawa *et al*., 2003), antidiabetic (Adewole *et al*., 2006). Quercetin has increased the bioavailability of moxidectin (Dupuy, 2003), paclitaxel (Choi *et al*., 2004), diltiazem (Choi and Xiuguo, 2005), doxorubicin (Choi *et al*., 2011) and losartan (Swathilatha and Lakshmi, 2014).

Evaluation of pharmacokinetics in various animal species and birds reveals poor bioavailability of marbofloxacin following oral administration which limits its therapeutic effectiveness. Birds are having the presence of efflux pumps (P-gp) at the apical surface of enterocytes in the duodenum, jejunum and ileum (Haritova *et al*., 2010), that could interfere with the absorption of some antimicrobial agents such as fluoroquinolones, oxytetracycline, doxycycline and, to a lesser extent, macrolides when administered orally (Haritova, 2008).

The poultry industry suffers from wide spread bacterial infections and economical losses due to less frequent availability of doorstep veterinary services. Treatment of broiler and layer birds by farmers themselves by oral administration of antibacterial drug, leads to less efficacy, side effects and development of bacterial resistance.

The problem may be solved by enhancing the bioavailability of antimicrobial drugs with the use of herbal bio-enhancer agents. It would be rational and judicious to study the pharmacokinetics of newer antimicrobial drugs like marbofloxacin with bio-enhancers. The continued interest in marbofloxacin has led to several recent investigations describing its pharmacokinetics in various species of domestic animals like sheep, goats, cattle, buffalo, horses, dogs, pigs and birds. However, data are not available related to effect of piperine and quercetin on pharmacokinetics and safety profiles of marbofloxacin in broiler birds. Hence, present study was planned to generate valuable information about effect of piperine and quercetin administration on pharmacokinetics and safety profile of marbofloxacin; and on CYP3A37 and MDR1 mRNA expression levels in liver and duodenum of broiler chickens with following objectives:
1. To optimize and standardize the method for detection of marbofloxacin from plasma of broiler chickens by High Performance Liquid Chromatography (HPLC).
2. To study single and repeated dose pharmacokinetics of marbofloxacin (5 mg/kg) after intravenous and oral administration for 5 days in broiler chickens.
3. To study the effect of piperine pretreatment (10 mg/kg, p.o. for 3 days) on single and repeated dose pharmacokinetics of marbofloxacin (5 mg/kg, for 5 days) after oral administration in broiler chickens.
4. To study the effect of quercetin pretreatment (10 mg/kg, p.o. for 3 days) on single and repeated dose pharmacokinetics of marbofloxacin (5 mg/kg, for 5 days) after oral administration in broiler chickens.
5. To study the effect of piperine and quercetin combined pretreatment (10 mg/kg each, p.o. for 3 days) on single and repeated dose pharmacokinetics of marbofloxacin (5 mg/kg, for 5 days) after oral administration in broiler chickens.
6. To evaluate the effect of piperine, quercetin and combined pretreatment (10 mg/kg each, p.o. for 3 days) on safety profile of marbofloxacin (5 mg/kg, p.o., for 5) days in broiler chickens.
7. To evaluate the effect of piperine, quercetin and combined pretreatment (10 mg/kg each, p.o. for 3 days) on CYP3A37 and MDR1 mRNA expression levels in liver and duodenum of broiler chickens.