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## Effect of piperine and quercetin pretreatment on safety profile of marbofloxacin after repeated oral administration in broiler chickens

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### Abstract

The present study was carried out to investigate the effect of pretreatment of bioenhancers like piperine and quercetin on safety profile of marbofloxacin after repeated oral administration in broiler chickens. Hemato-biochemical and histopathological alterations after repeated dose oral administration of marbofloxacin (5 mg/kg, PO, 5 days) in normal, piperine, quercetin and both in combination (10 mg/kg, each, PO, for 3 days) pretreated broiler chickens were investigated. After repeated oral administration of marbofloxacin, mean hemoglobin, packed cell volume, total erythrocyte count and total leukocyte count in quercetin pretreated broiler chickens were  $9.52 \pm 0.33$  g/dl,  $28.33 \pm 1.00$  %,  $2.12 \pm 0.05 \times 10^6/\mu\text{l}$  and  $20.38 \pm 0.29 \times 10^3/\mu\text{l}$ , respectively which significantly ( $p < 0.05$ ) lower as compared respective values of  $12.48 \pm 0.47$  g/dl,  $35.72 \pm 1.39$  %,  $2.80 \pm 0.13 \times 10^6/\mu\text{l}$  and  $22.81 \pm 0.27 \times 10^3/\mu\text{l}$  of control group. However, these alterations were in the normal clinical range of broiler chickens. The differential leukocyte count, mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin were not significantly different as compared to control group. Biochemical parameters, viz., alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, total protein, creatinine, blood urea nitrogen and total bilirubin were also not significantly different from control group. No major histomorphological changes were observed in liver, intestine and kidney of broiler chickens. The present dosage regimen of piperine, quercetin and marbofloxacin was found to be safe and might be helpful to explore therapeutic efficacy of herbal drugs with antibiotics in broiler chickens.

### 1. Introduction

Various bacterial infections like colibacillosis, salmonellosis, pasteurellosis, campylobacteriosis, mycoplasmosis, tuberculosis, psittacosis, infectious coryza and coccidiosis are important threats to poultry industry (Hasan *et al.*, 2012). Therapeutic and prophylactic use of antibiotics has improved poultry production to achieve a significant growth (Apata, 2009). Marbofloxacin is a third generation, fluorinated quinolone compound, exclusively developed for use in animals (Brown, 1996). Marbofloxacin inhibits the topoisomerase II (DNA gyrase) and topoisomerase IV enzymes, which are responsible for supercoiling of bacterial DNA (Paradis *et al.*, 2001). Marbofloxacin has shown broad spectrum of antimicrobial activity against gram-negative, some gram-positive bacteria and *mycoplasma* spp., and used for respiratory, urinary tract and dermatological infections (Spreng *et al.*, 1995; Lefebvre

*et al.*, 1998). Marbofloxacin has shown low bioavailability (56.82 %) after oral administration in chickens (Anadon *et al.*, 2002). Single dose oral administration of marbofloxacin in various animal species and birds also revealed its low oral absorption, which limits its therapeutic effectiveness (Patel *et al.*, 2018).

Therapeutic effective concentration can be achieved with high dose of drug, however, it is associated with the risk of increased toxicity, tissue residues problems and development of antimicrobial resistance (Alhendi *et al.*, 2000). The use of fluoroquinolones must comply with strict withdrawal periods, doses and duration of treatment (Gouvea *et al.*, 2015).

The combination of bioenhancers like piperine, quercetin, genistein, naringin, niazeridine, lysergols, capmul, sinomenine and glycyrrhizin has shown improved bioavailability of various antibiotic drugs (Randhawa *et al.*, 2011). Piperine, an alkaloid present in black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) is recognized as the first bioenhancer drug (Atal, 1979). Piperine treatment has shown to increase the oral bioavailability of various fluoroquinolones like ciprofloxacin (Bhise and Pore, 2002), pefloxacin (Madhukar *et al.*, 2008), gatifloxacin (Patel *et al.*, 2011) and marbofloxacin (Chauhan *et al.*, 2020). Quercetin, a flavonoid compound present in various plants and foods including grapes, onions, berries, apples, capers, tea, shallots, and tomatoes having

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important pharmacological effects like antioxidant, anti-inflammatory, antihyperlipidemic, anticancerous and antiviral effects (Nijveldt *et al.*, 2001). Quercetin treatment has been reported to increase the bioavailability of moxidectin in sheep (Dupuy *et al.*, 2003). Piperine and quercetin both had shown to inhibit CYP3A37 (drug metabolizing enzyme) and MDR1 (P-glycoprotein/multi drug resistance gene 1) activity in liver and intestine of broiler chickens (Patel *et al.*, 2019).

Therapeutic effective dosage regimen of antibiotics can be determined by investigation of efficacy and safety of drugs. The safety profile of marbofloxacin after repeated administration in cat (Ishak *et al.*, 2008), sheep (Patel *et al.*, 2014) and rat (Chauhan *et al.*, 2017) was reported earlier. However, the safety profile of marbofloxacin after repeated oral administration in piperine and quercetin pretreated broiler chickens have not been evaluated so far. Hence, the present study was planned to investigate the effect of piperine and quercetin pretreatment on hemato-biochemical and histopathological changes after repeated oral administration of marbofloxacin in broiler chickens.

## 2. Materials and Methods

### 2.1 Experimental animals

The experiment was carried out on 30 broiler chickens of strain 'Cobb 400' which were procured from Venky's Pvt. Ltd., Anand, Gujarat. Day-old chicks were reared up to the age of three weeks as per standard CPCSEA guidelines for laboratory animal facility

(CPCSEA, 2003). Broiler starter and finisher feed of BIS (9862-1992) specification (Simran Feeds, Indore, India) and clean wholesome drinking water were provided *ad libitum* to chickens. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), College of Veterinary Science, Junagadh Agricultural University, Junagadh (Protocol No.: JAU/JVC/IAEC/SA/22/2017 dated: 26/05/2017).

### 2.2 Drugs and chemicals

Marbofloxacin tablet 50 mg (Marbomet<sup>®</sup>, Intas Pharmaceuticals Limited, Ahmedabad, Gujarat, India) was used for oral administration to broiler chickens. Ready to use analytical kits for estimation of serum enzymes like ALT, AST, LDH and AKP (Greiner Diagnostics, Bahlingen, Germany) and biochemical parameters, *viz.*, total protein, blood urea nitrogen, bilirubin total and creatinine (Diatek Health care Pvt. Ltd., Kolkata, West Bengal, India) were used. Piperine and quercetin for were purchased from Sigma Aldrich, Bangalore, India.

Various active principles from plant origin like piperine, quercetin, curcumin, naringin, rutin, genistein, sinomenin and carvacrol shown to have bioenhancing property. Such agents affect the activities of metabolizing enzymes as well as drug efflux transporters which lead to increase the bioavailability of drug upon simultaneous administration through oral route. Sources, chemical class and mechanism of action of some important bioenhancers are shown in Table 1.

**Table 1:** Sources, chemical class and mechanism of action of some important bioenhancers

Name of bio-enhancer	Plant source	Chemical class	Mechanism of action	References
Piperine	<i>Piper longum</i> and <i>Piper nigrum</i>	Alkaloid	Drug metabolizing enzyme (CYP3A37) inhibition and drug efflux transporter (MDR-1/P-gp) inhibition	(Atal, 1979; Patel <i>et al.</i> , 2019)
Quercetin	Grapes, onions, berries, apples, capers, tea, shallots and tomatoes	Flavonoid	Drug metabolizing enzyme (CYP3A37) inhibition and drug efflux transporter (MDR-1/P-gp) inhibition	(Nijveldt <i>et al.</i> , 2001; Patel <i>et al.</i> , 2019)
Curcumin	<i>Curcuma longa</i>	Flavonoid	Drug metabolizing enzyme (Uridine diphosphate glucuronyl transferase) inhibition	(Basu <i>et al.</i> , 2004)
Naringin	Grapes, apple, onion and tea	Flavonoid	Drug metabolizing enzyme (CYP3A4) inhibition and drug efflux transporter (P-gp) inhibition	(Choi and Shin, 2005)
Rutin	Apricots, cherries, grapes, grapefruit, plums, buckwheat and oranges.	Flavonoid	Inhibition of Breast Cancer Resistance Protein (BCRP) transporters at intestine	(Dogra <i>et al.</i> , 2020)
Genistein	<i>Pueraria lobata</i> (Kudzu) and Soybeans	Flavonoid	Drug efflux transporters (ABCB1, BCRP, MRP2) inhibition and drug metabolism (CYP3A4) inhibition	(Li and Choi, 2007)
Sinomenine	<i>Sinomenium acutum</i>	Alkaloid	Drug efflux transporters ABCB1/p-gp inhibitor	(Chan <i>et al.</i> , 2006)
Carvacrol	<i>Carum carvi</i> (Caraway)	Monoterpenoid phenol	Enhancement of intestine permeation of drug and inhibition of the P-glycoprotein activity	(Choudhary <i>et al.</i> , 2014)

### 2.3 Experimental design

At the age of 3 weeks, 30 broiler chickens were randomly allocated in to five groups (n=6); group I (control), group II (marbofloxacin 5 mg/kg, PO, 5 days), group III (pretreatment of piperine 10 mg/kg, PO, 3 days + marbofloxacin 5 mg/kg, PO, 5 days), group IV (pretreatment of quercetin 10 mg/kg, PO, 3 days + marbofloxacin 5 mg/kg, PO, 5 days) and group V (pretreatment of piperine and

quercetin 10 mg/kg, each, PO, for 3 days + marbofloxacin 5 mg/kg, PO, 5 days). Piperine, quercetin and marbofloxacin were administered with oral gavaging bent feeding needle (16 G x 4") as per above treatment schedule daily. Under local anesthesia (lignocaine gel 2.5 %), blood samples (approx. 2 ml) were collected at 0 day (before drug administration) and 6<sup>th</sup> day (after repeated oral administration of marbofloxacin) from jugular vein into sterile

K<sub>3</sub>EDTA and non-heparinized test tubes for hematological and serum biochemical analysis, respectively. Blood samples collected in non-heparinized test tubes were allowed to clot at room temperature. Serum from each sample was collected by centrifugation at 5000 rpm for 10 min at 4°C (Eppendorf 5430 R, Germany) and stored at -20°C for biochemical analysis.

#### 2.4 Hematological and biochemical parameters evaluation

Hemoglobin (Hb) estimation was carried out by Sahli's hemoglobinometer and expressed as gram per cent, PCV was estimated by microhematocrit method and expressed as per cent (Coles, 1986). Estimation of TEC, TLC and DLC was carried out as per standard method (Jain, 1986). Serum biochemical parameters were analyzed by using standard kits with automatic biochemistry analyzer (Model: Dia-chem 240 plus, Diatek, China).

#### 2.5 Histopathological evaluation

For histopathological examinations, liver, intestine, and kidney were collected in 10 % neutral buffered formalin. The formalin fixed tissues were subjected to paraffin wax embedding for tissue sectioning (5 micron) thickness with semi-automated rotary

microtome (Leica Biosystems, Germany) and were stained with haematoxylin and eosin stain (Luna, 1968). The stained slides were viewed under microscope (H & E stain x 100 and x 400) and histopathological lesions were recorded.

#### 2.6 Statistical analysis

The data obtained for hematological and biochemical parameters were presented as Mean  $\pm$  SE and differences among the various treatment groups were analyzed by one-way analysis of variance (ANOVA), followed by post hoc Tukey high significant difference test. Where  $p \leq 0.05$  was considered as statistically significant (Snedecor and Cochran, 1982).

### 3. Results

#### 3.1 Hematological and biochemical parameters

Following repeated dose oral administration of marbofloxacin (5 mg/kg, PO, for 5 days) in normal, piperine, quercetin alone and both in combination pretreated (10 mg/kg each, PO, for 3 days) broiler chickens, the hematological parameters are presented in Table 2 and Figure 1; and biochemical parameters are presented in Table 3 and Figure 2.

**Table 2:** Effect of piperine, quercetin alone and both in combination pretreatment (10 mg/kg each, PO, for 3 days) on hematological parameters (Mean  $\pm$  SE) following repeated dose oral administration of marbofloxacin (5 mg/kg, PO, for 5 days) in broiler chickens (n=6)

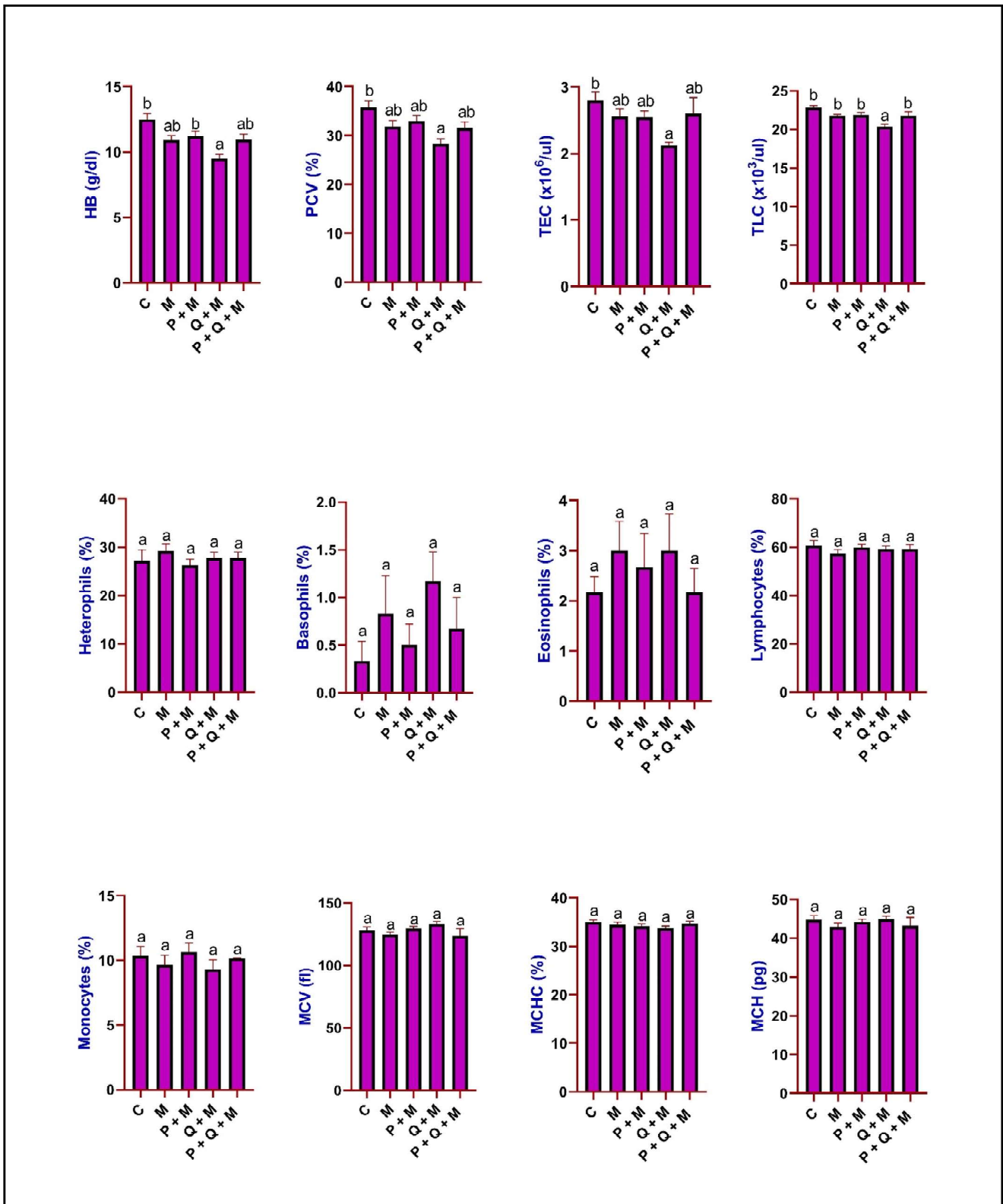
Hematological parameters	Control	Marbofloxacin	Piperine + Marbofloxacin	Quercetin + Marbofloxacin	Piperine + Quercetin + Marbofloxacin
HB (g/dl)	12.48 $\pm$ 0.47 <sup>b</sup>	10.93 $\pm$ 0.33 <sup>ab</sup>	11.23 $\pm$ 0.38 <sup>b</sup>	9.52 $\pm$ 0.33 <sup>a</sup>	10.95 $\pm$ 0.40 <sup>ab</sup>
PCV (%)	35.72 $\pm$ 1.39 <sup>b</sup>	31.88 $\pm$ 1.19 <sup>ab</sup>	32.97 $\pm$ 1.19 <sup>ab</sup>	28.33 $\pm$ 1.00 <sup>a</sup>	31.52 $\pm$ 1.22 <sup>ab</sup>
TEC ( $\times 10^6/\mu$ l)	2.80 $\pm$ 0.13 <sup>b</sup>	2.56 $\pm$ 0.11 <sup>ab</sup>	2.55 $\pm$ 0.09 <sup>ab</sup>	2.12 $\pm$ 0.05 <sup>a</sup>	2.60 $\pm$ 0.24 <sup>ab</sup>
TLC ( $\times 10^3/\mu$ l)	22.81 $\pm$ 0.27 <sup>b</sup>	21.82 $\pm$ 0.14 <sup>b</sup>	21.88 $\pm$ 0.31 <sup>b</sup>	20.38 $\pm$ 0.29 <sup>a</sup>	21.83 $\pm$ 0.46 <sup>b</sup>
Heterophils (%)	27.17 $\pm$ 2.26 <sup>a</sup>	29.17 $\pm$ 1.47 <sup>a</sup>	26.33 $\pm$ 1.12 <sup>a</sup>	27.67 $\pm$ 1.28 <sup>a</sup>	27.67 $\pm$ 1.33 <sup>a</sup>
Basophils (%)	0.33 $\pm$ 0.21 <sup>a</sup>	0.83 $\pm$ 0.40 <sup>a</sup>	0.50 $\pm$ 0.22 <sup>a</sup>	1.17 $\pm$ 0.31 <sup>a</sup>	0.67 $\pm$ 0.33 <sup>a</sup>
Eosinophils (%)	2.17 $\pm$ 0.31 <sup>a</sup>	3.00 $\pm$ 0.58 <sup>a</sup>	2.67 $\pm$ 0.67 <sup>a</sup>	3.00 $\pm$ 0.73 <sup>a</sup>	2.17 $\pm$ 0.48 <sup>a</sup>
Lymphocytes (%)	60.67 $\pm$ 2.11 <sup>a</sup>	57.33 $\pm$ 1.65 <sup>a</sup>	59.83 $\pm$ 1.35 <sup>a</sup>	59.33 $\pm$ 1.15 <sup>a</sup>	59.33 $\pm$ 1.71 <sup>a</sup>
Monocytes (%)	10.33 $\pm$ 0.76 <sup>a</sup>	9.67 $\pm$ 0.71 <sup>a</sup>	10.67 $\pm$ 0.67 <sup>a</sup>	9.33 $\pm$ 0.71 <sup>a</sup>	10.17 $\pm$ 0.01 <sup>a</sup>
MCV (fL)	128.13 $\pm$ 2.54 <sup>a</sup>	124.94 $\pm$ 1.74 <sup>a</sup>	129.38 $\pm$ 1.85 <sup>a</sup>	133.13 $\pm$ 2.23 <sup>a</sup>	123.90 $\pm$ 5.52 <sup>a</sup>
MCHC (%)	34.94 $\pm$ 0.52 <sup>a</sup>	34.36 $\pm$ 0.64 <sup>a</sup>	34.11 $\pm$ 0.53 <sup>a</sup>	33.72 $\pm$ 0.43 <sup>a</sup>	34.70 $\pm$ 0.50 <sup>a</sup>
MCH (pg)	44.74 $\pm$ 1.06 <sup>a</sup>	42.93 $\pm$ 0.95 <sup>a</sup>	44.09 $\pm$ 0.79 <sup>a</sup>	44.88 $\pm$ 0.78 <sup>a</sup>	43.14 $\pm$ 2.18 <sup>a</sup>

HB: hemoglobin, PCV: packed cell volume, TEC: total erythrocyte count, TLC: total leukocyte count, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCHC: mean corpuscular hemoglobin. Difference among the various treatment groups were analyzed by one way analysis of variance (ANOVA), followed by Tukey high significant difference test. Values with different superscripts in each row are significantly different ( $p < 0.05$ ).

**Table 3:** Effect of piperine, quercetin alone and both in combination pretreatment (10 mg/kg each, PO, for 3 days) on biochemical parameters (Mean  $\pm$  SE) following repeated dose oral administration of marbofloxacin (5 mg/kg, PO, for 5 days) in broiler chickens (n=6)

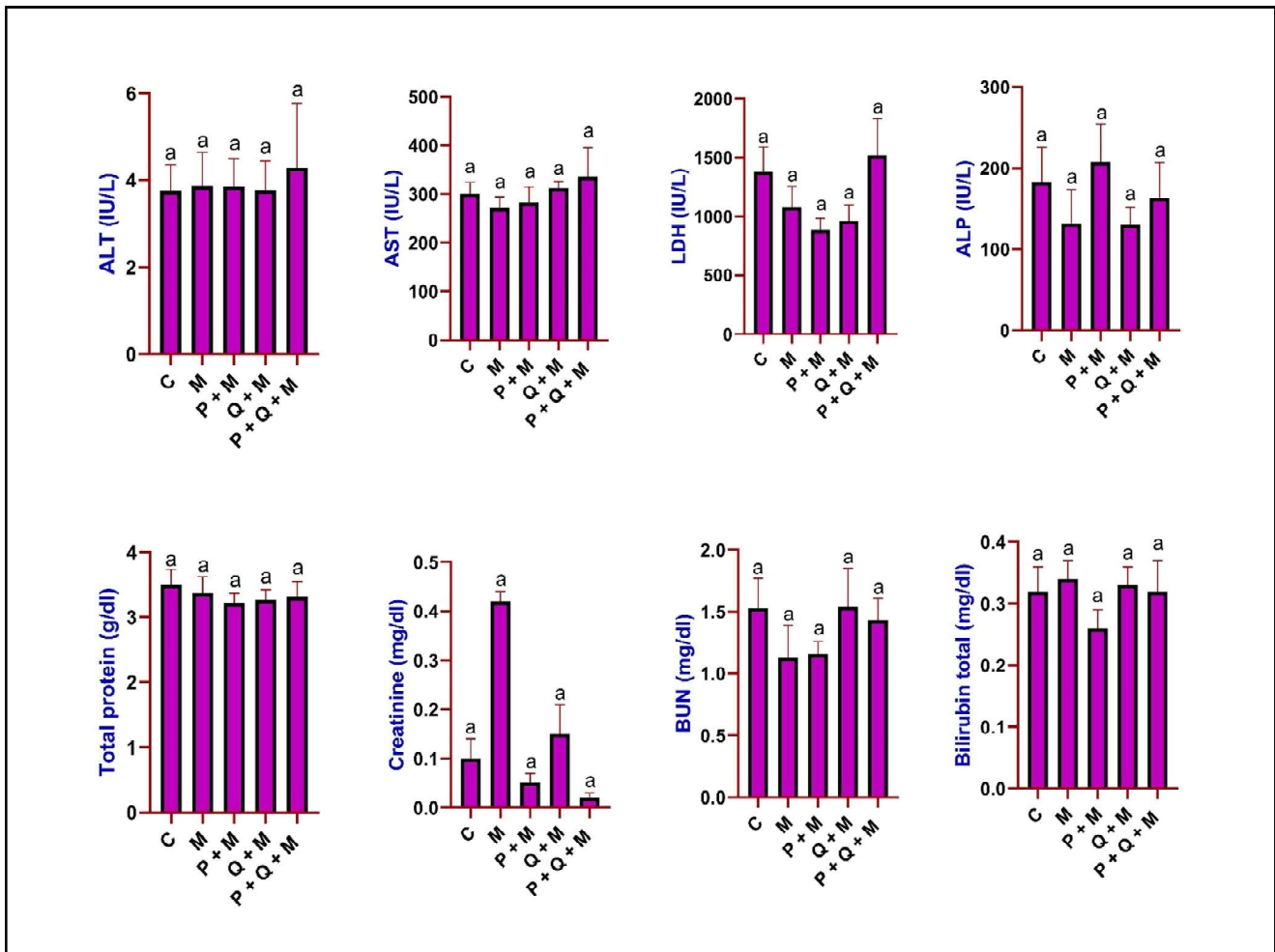
Biochemical parameters	Control	Marbofloxacin	Piperine + Marbofloxacin	Quercetin + Marbofloxacin	Piperine + Quercetin + Marbofloxacin
ALT (IU/L)	3.75 $\pm$ 0.61 <sup>a</sup>	3.87 $\pm$ 0.77 <sup>a</sup>	3.86 $\pm$ 0.64 <sup>a</sup>	3.77 $\pm$ 0.68 <sup>a</sup>	4.29 $\pm$ 1.48 <sup>a</sup>
AST (IU/L)	301.66 $\pm$ 22.44 <sup>a</sup>	271.94 $\pm$ 22.48 <sup>a</sup>	282.90 $\pm$ 31.91 <sup>a</sup>	312.48 $\pm$ 13.18 <sup>a</sup>	335.38 $\pm$ 60.33 <sup>a</sup>
LDH (IU/L)	1376.70 $\pm$ 212.40 <sup>a</sup>	1078.80 $\pm$ 174.80 <sup>a</sup>	884.4 $\pm$ 100.60 <sup>a</sup>	961.40 $\pm$ 138.20 <sup>a</sup>	1520.70 $\pm$ 306.30 <sup>a</sup>
ALP (IU/L)	183.00 $\pm$ 42.60 <sup>a</sup>	132.03 $\pm$ 41.38 <sup>a</sup>	207.52 $\pm$ 46.66 <sup>a</sup>	129.97 $\pm$ 21.70 <sup>a</sup>	163.55 $\pm$ 43.49 <sup>a</sup>
Total protein (g/dL)	3.50 $\pm$ 0.23 <sup>a</sup>	3.37 $\pm$ 0.25 <sup>a</sup>	3.22 $\pm$ 0.15 <sup>a</sup>	3.27 $\pm$ 0.15 <sup>a</sup>	3.31 $\pm$ 0.24 <sup>a</sup>
Creatinine (mg/dL)	0.10 $\pm$ 0.04 <sup>a</sup>	0.42 $\pm$ 0.28 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.15 $\pm$ 0.06 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>
BUN (mg/dL)	1.53 $\pm$ 0.24 <sup>a</sup>	1.13 $\pm$ 0.26 <sup>a</sup>	1.16 $\pm$ 0.10 <sup>a</sup>	1.54 $\pm$ 0.31 <sup>a</sup>	1.43 $\pm$ 0.18 <sup>a</sup>
Bilirubin total (mg/dL)	0.32 $\pm$ 0.04 <sup>a</sup>	0.34 $\pm$ 0.03 <sup>a</sup>	0.26 $\pm$ 0.03 <sup>a</sup>	0.33 $\pm$ 0.03 <sup>a</sup>	0.32 $\pm$ 0.05 <sup>a</sup>

ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, BUN: blood urea nitrogen. Difference among the various treatment groups were analyzed by one way analysis of variance (ANOVA), followed by Tukey high significant difference test. Values with different superscripts in each row are significantly different ( $p < 0.05$ ).



**Figure 1:** Effect of piperine, quercetin alone and both in combination pretreatment (10 mg/kg each, PO, for 3 days) on hematological parameters (Mean  $\pm$  SE) following repeated dose oral administration of marbofloxacin (5 mg/kg, PO, for 5 days) in broiler chickens (n=6)

HB: hemoglobin, PCV: packed cell volume, TEC: total erythrocyte count, TLC: total leukocyte count, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCHC: mean corpuscular hemoglobin.



**Figure 2:** Effect of piperine, quercetin alone and both in combination pretreatment (10 mg/kg each, PO, for 3 days) on biochemical parameters (Mean  $\pm$  SE) following repeated dose oral administration of marbofloxacin (5 mg/kg, PO, for 5 days) in broiler chickens (n=6)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, BUN: blood urea nitrogen.

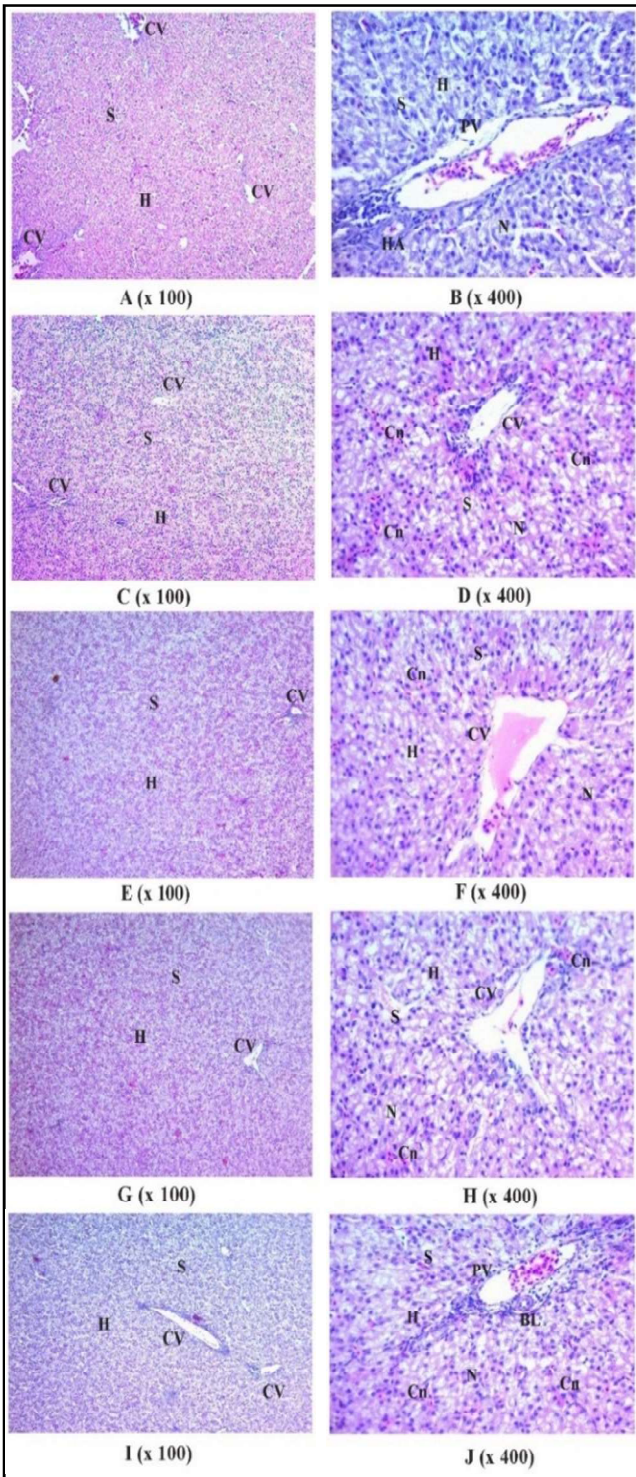
After repeated oral administration of marbofloxacin, the mean hemoglobin (HB) concentration in quercetin pretreated broiler chickens was  $9.52 \pm 0.33$  g/dl, which significantly ( $p < 0.05$ ) lower than respective values of  $12.48 \pm 0.47$ ,  $10.93 \pm 0.33$ ,  $11.23 \pm 0.38$  and  $10.95 \pm 0.40$  g/dl in control, marbofloxacin alone, piperine and both in combination pretreated broiler chickens, respectively. Similarly, mean packed cell volume (PCV) in quercetin pretreated broiler chickens was  $28.33 \pm 1.00$  %, which also significantly ( $p < 0.05$ ) lower than respective values of  $35.72 \pm 1.39$ ,  $31.88 \pm 1.19$ ,  $32.97 \pm 1.19$  and  $31.52 \pm 1.22$  % in control, marbofloxacin alone, piperine and both in combination pretreated broiler chickens, respectively. Total erythrocyte count (TEC) and total leukocyte count (TLC) were also significantly reduced in case of quercetin pretreated group as compared broiler chickens of all other groups. However, these alterations were in the normal clinical range of broiler chickens. Other hematological parameters like heterophils, basophils, eosinophils, lymphocytes, monocytes, MCV, MCHC and MCH values were not significantly ( $p > 0.05$ ) different among the various treatment groups. The biochemical parameters, viz., ALT,

AST, LDH, ALP, total protein, creatinine, BUN and total bilirubin were also not significantly differ among treatment groups as compared to control group.

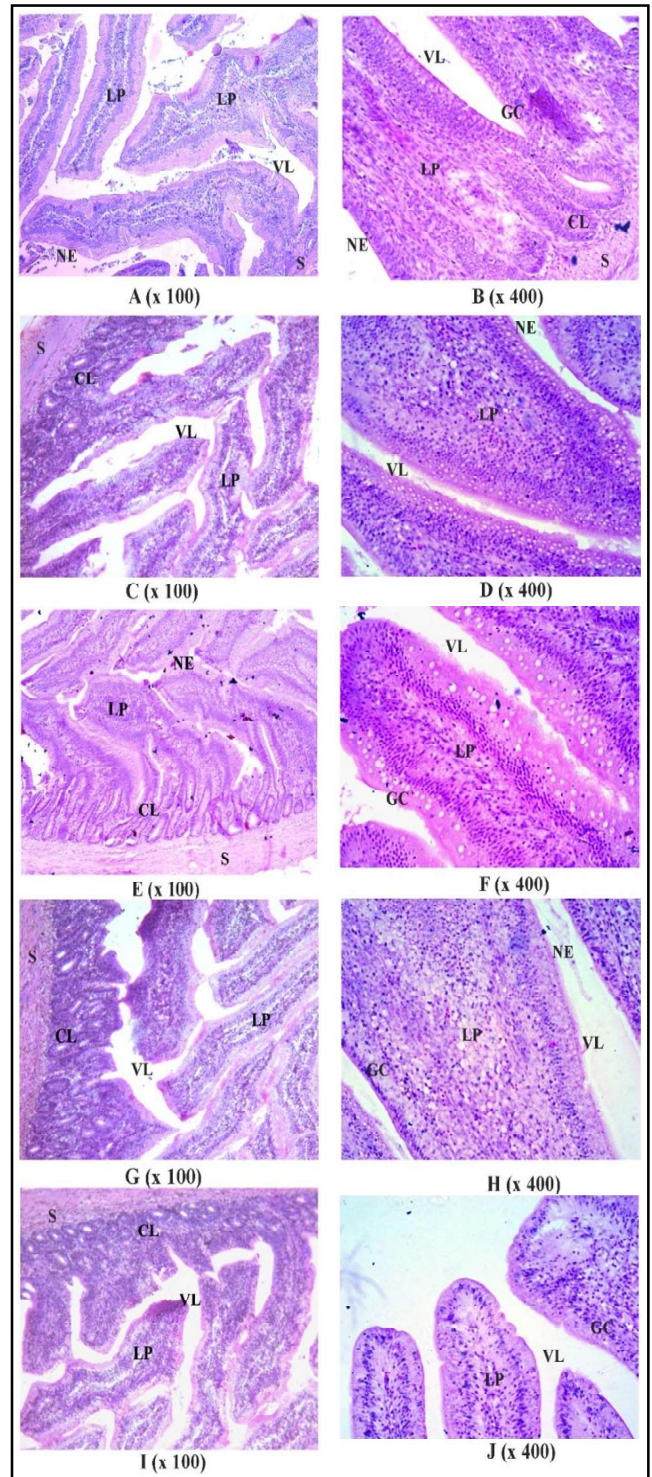
### 3.2 Histopathological changes in liver, intestine and kidney

Microscopic view and histopathological changes in liver, intestine and kidney of chickens under different treatments are depicted in Figure 3, Figure 4 and Figure 5, respectively. Upon histopathological examinations, after repeated oral administration of marbofloxacin, mild congestions of liver were observed in all treatment groups as compared control group. No other histopathological changes were observed in central vein, portal vein, bile duct, sinusoids and hepatocytes of liver in all groups. Microscopic examination of intestine revealed normal structure of villi, lamina propria, crypts of lieberkühn (intestinal glands) and goblet cells in all groups. In kidney, mild congestions of renal interstitium were observed in case of quercetin and combination pretreated broiler chickens, whereas other histological structures, viz., renal corpuscle, collecting tubules, bowman's capsule and urinary space were appeared normal in all treatment groups.



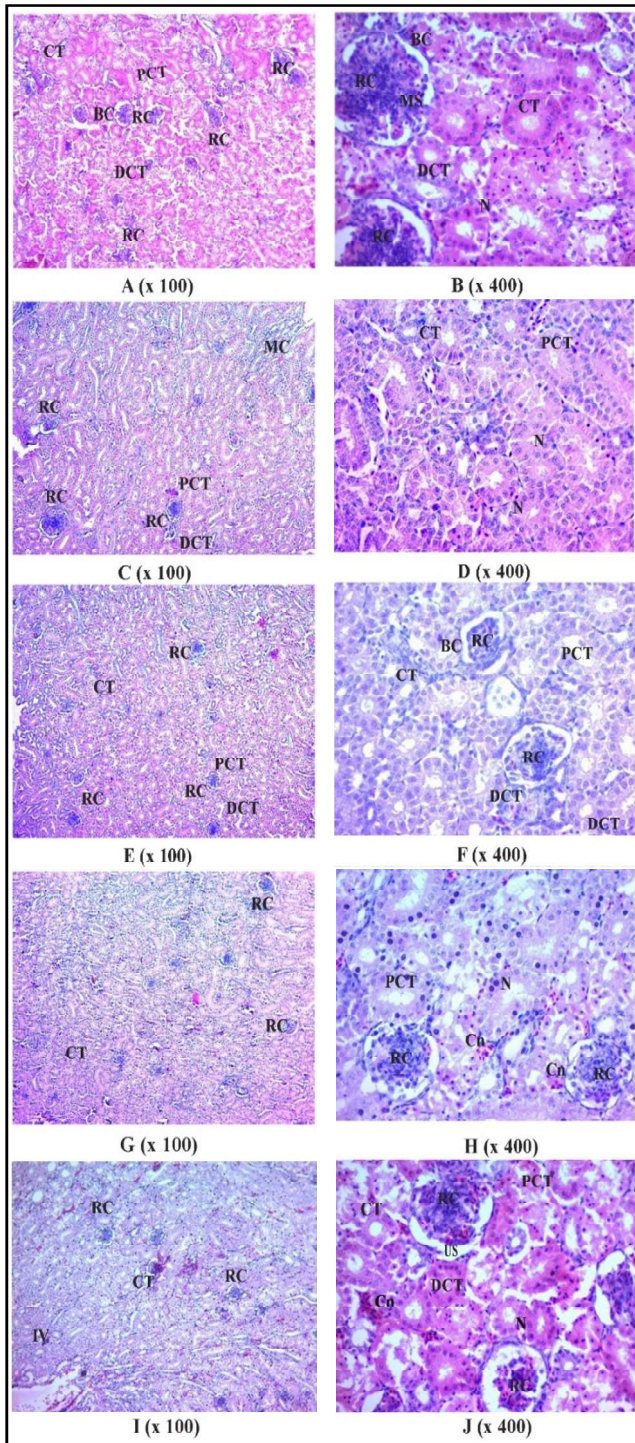


**Figure 3:** Microscopic view of liver following repeated dose oral administration of marbofloxacin showed normal hepatic lobules and architecture except mild congestions were observed in all treatment groups as compared to control group broiler chickens (H & E stain x 100, x 400). (A, B): Group I (CON), (C, D): Group II (MAR), (E, F): Group III (PIP + MAR), (G, H): Group IV (QUE + MAR) (I, J): Group V (PIP + QUE + MAR); CV: central vein, PV: Portal vein, HA: hepatic artery BL: bile duct, H: hepatocytes, S: sinusoids, N: nucleus, Cn: congestion.



**Figure 4:** Microscopic view of intestine following repeated dose oral administration of marbofloxacin in different treatment groups showed normal intestinal villi, epithelium, serosa and glands in broiler chickens (H & E stain x 100, x 400). (A, B): Group I (CON), (C, D): Group II (MAR), (E, F): Group III (PIP + MAR), (G, H): Group IV (QUE + MAR) (I, J): Group V (PIP + QUE + MAR); VL: villi, LP: lamina propria CL: crypts of Lieberkühn, S: serosa, GC: goblet cells, NE: normal epithelium.





**Figure 5:** Microscopic view of kidney following repeated dose oral administration of marbofloxacin in different treatment groups showed normal glomeruli and renal tubular epithelium in broiler chickens (H & E stain x 100, x 400). (A, B): Group I (CON), (C, D): Group II (MAR), (E, F): Group III (PIP + MAR), (G, H): Group IV (QUE + MAR) (I, J): Group V (PIP + QUE + MAR); RC: renal corpuscle, CT: collecting tubules, N: nucleus of podocytes, DCT: distal convoluted tubules, PCT: proximal convoluted tubules, MS: mesangial cells, BC: bowman's capsule, MC: medullary cone, IV: intralobular vein, US: urinary space, Cn: congestion.

#### 4. Discussion

In the present study, to the best of author's knowledge, effects of piperine and quercetin pretreatment on safety profile of marbofloxacin have been studied first time in broiler chickens. After repeated oral administration of marbofloxacin, the mean HB, PCV, TEC and TLC values were significantly ( $p < 0.05$ ) lower in quercetin pretreated broiler chickens as compared to other groups. However, these alterations were in the normal clinical range of broiler chickens (Hb: 7 - 13 g/dl; PCV: 22 - 35 %; RBC: 2.5 - 3.5  $\times 10^6/\mu\text{l}$  and WBC: 12 - 30  $\times 10^3/\mu\text{l}$ ) as reported by Bounous and Stedman (2000).

Quercetin is a well-known flavonoid compound possesses many important pharmacological activities including bioenhancing effects on co-administered drugs. Nevertheless, quercetin has quite adverse effects on hematopoietic system. Quercetin has iron chelating action and affects iron homeostasis in the body. Non-haem iron is ferric ( $\text{Fe}^{3+}$ ) which reduces to ferrous ( $\text{Fe}^{2+}$ ) form and influxed into the enterocyte by the action of apical iron transporter (divalent metal transporter-1/DMT1) (Gunshin *et al.*, 1997). Afterwards, ferrous iron is effluxed across the basolateral membrane *via* the iron exporter (ferroportin/FPN) and enters into systemic circulation (Abboud and Haile, 2000). After single dose of quercetin administration (50 mg/kg), significant down regulation of iron transporters, *viz.*, DMT1 and FPN mRNA levels were observed in duodenum of rats (Lesjak *et al.*, 2014). Authors opine that, due to these possible mechanisms, quercetin pretreatment for three days might have reduced HB, PCV, TEC and TLC count in broiler chickens. In agreement to our findings, after 28 days of repeated oral quercetin treatment (20 mg/kg) significant reductions ( $p < 0.05$ ) in HB (control:  $7.34 \pm 0.39$  g/dl; quercetin treated:  $5.56 \pm 0.88$  g/dl), TEC (control:  $4.18 \pm 0.32 \times 10^6/\mu\text{l}$ ; quercetin treated:  $2.32 \pm 0.32 \times 10^6/\mu\text{l}$ ) and TLC (control:  $7.90 \pm 0.60 \times 10^3/\mu\text{l}$ ; quercetin treated:  $6.50 \pm 0.89 \times 10^3/\mu\text{l}$ ) were observed in rabbits (Parabathina *et al.*, 2011).

In piperine pretreated broiler chickens, no hematological abnormalities were observed in the present study. Similarly, repeated marbofloxacin administration (5 mg/kg, intramuscular, for 5 days) along with piperine pretreatment (10 mg/kg, PO, for 5 days) reported being safe and well-tolerated without hematological abnormalities in rats (Chauhan *et al.*, 2017). Cardoso *et al.* (2012) also did not found significant alterations in HB, PCV, TEC and TLC values of broiler chickens fed with piperine (60 mg/kg) supplemented diet for 42 days.

The MCV is used to calculate the average size and volume of erythrocyte, the MCH to measure hemoglobin amount per erythrocyte and the MCHC to know the amount of haemoglobin relative to the size of the cell (Bounous and Stedman, 2000). The normal red blood cell indices of MCV, MCH and MCHC in present study suggests that repeated oral administration of marbofloxacin had no adverse effect on hemoglobin content and size of erythrocytes in broiler chickens. Furthermore, after 21 days of repeated oral administration of marbofloxacin (2 mg/kg), no significant changes were observed in HB, PCV, TEC, TLC and platelet count in Beagle dogs (Lei *et al.*, 2018). Marbofloxacin treatment (2.75 mg/kg, PO, daily for 14 days) had shown to improve the hemoglobin and PCV values in *Mycoplasma haemofelis* infected cats as compared to non-treated (control) infected cats (Ishak *et al.*,

2008). Similarly, after 28 days of oral exposure of other routinely used fluoroquinolones, *viz.*, enrofloxacin, ciprofloxacin and norfloxacin (0.25 mg/liter of drinking water) had shown no significant changes in hematological values like TLC, PCV, HB and in TEC in laying hens (Rehman *et al.*, 2019). Earlier researchers also did not observe hematological alterations in HB, PCV, TEC and TLC values after repeated oral administration of gatifloxacin (10 mg/kg, for 14 days) and gemifloxacin (10 mg/kg, for 5 days) in broiler chickens (Devada *et al.*, 2012; Gohel *et al.*, 2018).

During investigation of therapeutic efficacy and safety of target compound, it is essential to evaluate its systemic action on vital body organ performance indexes. In present study, the biochemical parameters, *viz.*, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total protein, creatinine, BUN and total bilirubin were not significantly different as compared to control group. Kuttappan *et al.* (2013) observed that increase in the AST and ALT serum levels indicate liver damage caused by the metabolism of target compound and other feed additives in broiler chickens. Creatinine is a biomarker of kidney function. The kidney plays an important role in excretion of waste products resulting from protein metabolism and muscle contraction. Increased level of serum creatinine indicates compromised kidney functions (Ileke *et al.*, 2014). Protein metabolism, stress and dehydration increase the concentration of blood urea nitrogen in the blood (Chernecky and Berger, 2008).

Normal range of AST, ALT, creatinine and BUN after repeated oral administration of marbofloxacin suggests minimal or no side effects of drug on liver and kidney functions of broiler chickens. In agreement to this, Mahmood (2013) also did not found significant changes in serum biochemical parameters following daily intramuscular administration marbofloxacin (2 mg/kg) for five days in sheep. Patel *et al.* (2014) also reported that after intravenous administration of marbofloxacin (2 mg/kg) no alterations were seen in biochemical parameters in sheep. The mean values of AST, ALT, ALP, LDH, BUN, total protein, albumin, creatinine and total bilirubin were also not significantly altered after repeated oral administration of levofloxacin (10 mg/kg) for 14 days in White Leg Horn layer birds (Patel *et al.*, 2009).

Upon histopathological examinations, after repeated oral administration of marbofloxacin, mild congestions of liver were observed in all treatment groups as compared control group. In agreement to our findings, after repeated oral administration of marbofloxacin (5 mg/kg for 5 days) alone and in piperine pretreated (10 mg/kg for 3 days) rats mild degree of congestion of hepatocytes was observed (Chauhan *et al.*, 2019). Microscopic examination of intestine revealed normal structure of villi, lamina propria, crypts of lieberkühn (intestinal glands) and goblet cells in all groups. In kidney, mild congestions of renal interstitium were observed in case of quercetin and combination pretreated broiler chickens. Similar to our findings, after 21 days of repeated oral administration of marbofloxacin (2 mg/kg), no major histopathological abnormalities were found in liver, heart, spleen, lungs and kidneys of Beagle dogs (Lei *et al.*, 2018).

## 5. Conclusion

The piperine alone and piperine and quercetin combined pretreatment had no significant effect on safety profile of marbofloxacin after repeated oral administration in broiler chickens. Quercetin a flavonoid compound had shown to produce reductions in HB, PCV, TEC and TLC values of broiler chickens. However, these hematological alterations were in the normal clinical range of broiler chickens. After repeated oral dose of marbofloxacin, major biochemical and histological alterations were not observed in broiler chickens. Overall, piperine and quercetin pretreatment along with repeated oral administration of marbofloxacin found to be safe and well tolerated in broiler chickens. Findings of present study pave way to design appropriate therapeutic dosage regimens of piperine and quercetin like bioenhancers with marbofloxacin for broiler chickens.

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## Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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