SUMMARY

The present study was undertaken to evaluate the single and repeated dose pharmacokinetics and safety profile of marbofloxacin after intravenous and oral administration (5 mg/kg, for 5 days) in broiler chickens. The effects of the piperine, quercetin as well as combined pretreatment (10 mg/kg each, p.o., for 3 days) on single and repeated dose pharmacokinetics and safety profile of marbofloxacin (5 mg/kg, for 5 days) after oral administration in broiler chickens were also studied. Additionally, effect of piperine, quercetin as well as combined pretreatment (10 mg/kg each, p.o., for 3 days) on CYP3A37 and MDR1 mRNA expression levels in liver and duodenum was also evaluated to support the pharmacokinetic data in broiler chickens. Drug concentration in plasma was determined using High Performance Liquid Chromatography (HPLC) and evaluation of mRNA expression was done with real time PCR analysis.

Following single dose intravenous administration of marbofloxacin, therapeutically effective concentration ≥ 0.10 ± 0.02 µg/mL was maintained up to 24 h in broiler chickens. The mean distribution half-life (t\(_{1/2α}\)) and elimination half-life were 0.16 ± 0.03 h and 5.55 ± 0.67 h, respectively. The average value for area under curve (AUC\(_{(0-∞)}\)) was 26.80 ± 2.08 µg.h/mL. The mean values of volume of distribution Vd\(_{(\text{area})}\) and Vd\(_{(ss)}\) were 1.49 ± 0.12 and 1.30 ± 0.09 L/kg, respectively. The mean value for total body clearance (Cl\(_B\)) and mean residence time (MRT) were 0.19 ± 0.02 L/h/kg and 6.78 ± 0.16 h, respectively. The mean rate constant for transfer of marbofloxacin from central to the tissue compartment (K\(_{12}\)) and tissue to the central compartment (K\(_{21}\)) were 3.05 ± 0.99 and 3.11 ± 0.80 h\(^{-1}\), respectively. The mean ratio of K\(_{12}\)/K\(_{21}\) was 0.91 ± 0.09.

Following repeated intravenous administration, the initial plasma concentration (C\(_p^0\)) marbofloxacin was 17.72 ± 5.28 µg/mL which was significantly (p < 0.01) higher than the respective value of 6.78 ± 0.28 µg/mL after single administration. The plasma concentrations of marbofloxacin of ≥ 0.10 ± 0.02 (after single dose) and ≥ 0.15 ± 0.03 µg/mL (after repeated dose) were maintained up to 24
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h after drug administration. The mean elimination half-life \((t_{1/2\beta})\) and \(V_d(\text{area})\) were \(5.12 \pm 0.25\) h and \(1.37 \pm 0.08\) L/kg, respectively. No significant difference in \((t_{1/2\beta})\) and \(V_d(\text{area})\) was observed after repeated i.v. administration in broiler chickens. The mean value of AUC of \(27.60 \pm 2.57\ \mu g.h/mL\) which was non-significantly higher compare to single dose administration. The total body clearance (ClB) and MRT were \(0.19 \pm 0.02\) L/h/kg and \(6.63 \pm 0.15\) h, respectively which where almost similar to that of single administration. The mean rate of transfer of marbofloxacin from central to the tissue compartment (K_{12}), tissue to the central compartment (K_{21}) and the mean ratio of K_{12}/K_{21} were \(1.87 \pm 0.20\) h\(^{-1}\), \(0.43 \pm 0.06\) h\(^{-1}\) and \(2.15 \pm 0.50\), respectively after single administration. The K_{21} rate constant was significantly \((p < 0.01)\) lower and mean ratio of K_{12}/K_{21} was significantly \((p < 0.01)\) higher after repeated i.v. administration compare to single.

Following single dose oral administration of marbofloxacin, the mean peak plasma concentration (C\(_{\text{max}}\)) in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were \(2.05 \pm 0.23\), \(1.96 \pm 0.20\), \(1.95 \pm 0.15\) and \(1.64 \pm 0.11\) µg/mL, respectively achieved at T\(_{\text{max}}\) \(0.83 \pm 0.11\), \(0.67 \pm 0.11\) h, \(0.83 \pm 0.25\) and \(1.50 \pm 0.22\) h, respectively. C\(_{\text{max}}\) values were non-significantly different among the groups, however T\(_{\text{max}}\) value of combination pretreated broiler chickens was significantly \((p < 0.05)\) higher than all three groups. However, in terminal phase at 24 h significantly \((p < 0.05)\) high concentrations were \(0.16 \pm 0.02\) and \(0.18 \pm 0.02\) µg/mL were observed in quercetin pretreated and both in combination pretreated broiler chickens, respectively compare to respective value of \(0.08 \pm 0.01\), \(0.09 \pm 0.02\) µg/mL in normal and piperine pretreated chickens.

Following single dose oral administration of marbofloxacin in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens mean absorption half-life \((t_{1/2\text{ka}})\) were \(0.22 \pm 0.04\), \(0.21 \pm 0.08\), \(0.15 \pm 0.06\) and \(0.44 \pm 0.06\) h, respectively whereas mean elimination half-life \((t_{1/2\beta})\) were \(4.62 \pm 0.42\), \(6.23 \pm 1.01\), \(5.69 \pm 0.39\) and \(7.71 \pm 0.59\) h, respectively observed in present study. Significantly \((p < 0.05)\) higher \(t_{1/2\beta}\) were found in case of combination pretreated broiler chickens as compare to other groups. Mean apparent volume of distribution \(V_d(\text{area})\) in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were \(1.32 \pm 0.10\), \(1.71 \pm 0.39\), \(1.65 \pm 0.26\) and \(2.30 \pm 0.27\) L/kg, respectively. \(V_d(\text{area})\) values were significantly \((p < 0.05)\) higher.
in all three groups as compared to normal chickens, however, the largest volume of distribution was observed in combination pretreated broiler chickens. The mean AUC values in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were 15.11 ± 2.19, 17.66 ± 1.94, 18.36 ± 2.12 and 18.60 ± 1.31 µg.h/mL, respectively, and area under the moment curve (AUMC) of 106.00 ± 7.21, 139.23 ± 21.97, 164.22 ± 22.01 and 200.27 ± 23.07 µg.h²/mL, respectively, observed in the present study. Mean AUMC values are significantly (p < 0.05) higher in combination pretreated broiler chickens as compared to other groups.

After single dose oral administration of marbofloxacin, the total body clearance (Cl) in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were 0.20 ± 0.02, 0.19 ± 0.02, 0.20 ± 0.03 and 0.21 ± 0.02 L/h/kg, respectively, and MRT were 7.03 ± 0.33, 7.67 ± 0.48, 8.88 ± 0.28 and 10.71 ± 0.70 h, respectively. MRT was significantly (p < 0.05) higher in the case of combination pretreated broiler chickens as compared to other groups. The mean values of bioavailabilities were 60.22 ± 8.07, 64.61 ± 7.78, 73.03 ± 12.35 and 75.39 ± 7.34 %, respectively. The bioavailability of the drug in piperine, quercetin and combination pretreatment group were non-significantly higher than in normal chickens.

Following repeated oral administration of marbofloxacin, the maximum plasma concentrations (C_max) in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were of 1.81 ± 0.25, 2.20 ± 1.08, 2.22 ± 0.17 and 1.91 ± 0.14 µg/mL, respectively. The mean absorption half-life (t_1/2ka) in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were 0.34 ± 0.07, 0.23 ± 0.06, 0.22 ± 0.03 and 0.51 ± 0.17 h, respectively which were non-significantly higher in each group after repeated oral administration compared to single administration. After repeated oral administration of marbofloxacin in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens mean elimination half-life (t_1/2β) were of 5.63 ± 0.60, 5.41 ± 0.35, 6.43 ± 1.66 and 7.11 ± 0.35 h, respectively, whereas mean Vd(area) were of 1.61 ± 0.21, 1.43 ± 0.14, 1.60 ± 0.23 and 2.08 ± 0.29 L/kg, respectively. Value of half-life (t_1/2β) and Vd(area) were non-significantly different after repeated administration compared to single administration. After repeated oral administration, the mean AUC in normal, piperine pretreated, quercetin pretreated and
both in combination pretreated broiler chickens were of 16.49 ± 1.88, 20.27 ± 2.07, 22.10 ± 4.48 and 23.20 ± 2.30 µg.h/mL, respectively. AUC value in combination pretreatment group was significantly (p < 0.05) higher after repeated administration as compare to single administration.

After repeated oral administration of marbofloxacin in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens, the mean (ClB) were 0.20 ± 0.02, 0.19 ± 0.02, 0.20 ± 0.02 and 0.20 ± 0.03 L/h/kg, respectively whereas the mean MRT values were 8.80 ± 0.81, 8.68 ± 0.71, 8.98 ± 1.42 and 10.62 ± 0.37 h, respectively. The mean ClB values are not altered after repeated administration, whereas MRT values were non-significantly increased after repeated administration in each group. The bioavailability following repeated oral administration of marbofloxacin in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens were 64.55 ± 7.95 %, 72.64 ± 6.02 %, 80.27 ± 12.35 and 89.60 ± 9.06 %, respectively. The bioavailability values in case of piperine and quercetin combination group was significantly (p < 0.05) higher after repeated administration compare to single administration.

The pharmacodynamic efficacy of marbofloxacin was determined by calculating the AUC/MIC90 and Cmax/MIC90 ratios following single dose intravenous and oral administration of marbofloxacin in normal, piperine pretreated, quercetin pretreated and both in combination pretreated broiler chickens. Following single dose intravenous administration of marbofloxacin (5 mg/kg) in broiler chickens would result in AUC/MIC ratio of 536, 268 and 134 h at MIC values of 0.05, 0.10 and 0.20 µg/mL respectively. Similarly, after oral administration of marbofloxacin, higher ratios of AUC/MIC have been observed at MIC levels of 0.05 and 0.10 µg/mL were: (302.20 and 151.10 h) in normal, (353.20 and 176.60 h) in piperine pretreated, (367.20 and 183.60 h) in quercetin pretreated, (372.00 and 186.60 h) in both in combination pretreated broiler chickens, respectively. After oral administration of marbofloxacin, higher ratios of Cmax/MIC have been observed at MIC levels of 0.05, 0.10 and 0.20 µg/mL were: (39.20, 19.60 and 9.80) in normal, (41.00, 20.50 and 10.25) in piperine pretreated, (39.00, 19.50 and 9.75) in quercetin pretreated, (32.80, 16.40 and 8.20) in both in combination pretreated broiler chickens, respectively. Based on PK-PD indices in could be indicated that after intravenous administration
and oral administration of marbofloxacin (5 mg/kg) would be highly efficacious against bacteria with MIC value of ≤ 0.2 μg/mL in broiler chickens.

In gene expression study, CYP3A37 mRNA expression level in liver was non-significantly down regulated by 3.79 fold in piperine pretreated chickens, whereas in quercetin pretreated and combination pretreated chickens significant down regulation by 13.21 (p < 0.05) and 24.35 fold (p < 0.01), respectively were observed as compare to control group. Similarly, CYP3A37 mRNA expression levels in duodenum were significantly down regulated by 5.93 (p < 0.05), 7.21 (p < 0.05) and 17.59 fold (p < 0.01) in piperine, quercetin and combination pretreated broiler chickens, respectively compared to control. MDR1 mRNA expression level in liver following piperine, quercetin and both in combination treatment were significantly (p < 0.05) down regulated by 7.08, 5.47 and 7.65 fold, respectively compare to control boiler chickens. Similarly, MDR1 mRNA expression levels in duodenum was non-significantly down regulated by 8.42 fold in piperine pretreated chickens, whereas significantly (p < 0.05) down regulated by 9.59 and 21.59 fold in quercetin pretreated and combination pretreated chickens, respectively compared to control.

In safety study, following multiple intravenous administrations of marbofloxacin in broiler chickens, at 6th day post treatment, significant (p < 0.05) reductions were observed in total erythrocyte count and lymphocyte count and increased heterophil count in broiler chickens. After multiple oral administrations, significant (p < 0.05) reductions were observed in hemoglobin, packed cell volume and total erythrocyte count in quercetin pretreated chickens and MCH values were significantly increased in normal chickens, piperine pretreated and quercetin pretreated broiler chickens. However, the alterations in hematological parameters were in the normal clinical range for broiler chickens. Following multiple oral administrations of marbofloxacin, significantly (p < 0.05) increased mean values of aspartate aminotransferase (AST) in normal and quercetin pretreated chickens and total protein in quercetin and in combination pretreated chickens were observed. Significantly decreased mean values of lactate dehydrogenase in piperine pretreated chickens was observed at 6th day post treatment compare to 0 day (before treatment) in present study. However, the alterations in biochemical parameters were in the normal clinical range for broiler chickens. No significant alterations were found for alanine aminotransferase, alkaline phosphatase, total protein, albumin, globulin,
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creatinine, blood urea nitrogen and bilirubin (total) in all groups compared to that of control. After repeated intravenous and oral administrations of marbofloxacin no any gross and histopathological lesions were observed in broiler chickens.

CONCLUSIONS

Following conclusions can be drawn from the present study:

1. Following single dose oral administration of marbofloxacin, the maximum plasma drug concentration ($C_{\text{max}}$) value were similar in all groups; however, $T_{\text{max}}$ values were significantly reduced ($p < 0.05$) in piperine pretreated chickens compare to normal chickens. The plasma concentration at 24 h (last observed concentration) in quercetin pretreated and combination pretreated groups were significantly ($p < 0.05$) higher as compare to normal and piperine pretreated broiler chickens. Thus, the piperine pretreatment has enhanced the marbofloxacin absorption rate after oral administration and combination pretreatment has delayed the excretion of drug from body.

2. Following single dose oral administration of marbofloxacin, significantly ($p < 0.05$) higher elimination half-life ($t_{1/2\beta}$), volume of distribution ($V_{\text{darea}}$) and the mean residence time (MRT) were observed in combination pretreated broiler chickens as compare to normal chickens.

3. After repeated oral administration of marbofloxacin, the area under curve (AUC) and area under first moment curve (AUMC) and bioavailability were significantly ($p < 0.05$) higher compare to single dose in combination pretreated broiler chickens.

4. Piperine and quercetin pretreatment has no any significant effect on body clearance rate of marbofloxacin after repeated oral administration.

5. The $C_{\text{max}}$/MIC$_{90}$ ratio after single oral administration was similar in all groups of broiler chickens, whereas AUC/MIC$_{90}$ ratio were higher in piperine, quercetin and combination pretreatment group compared to normal chickens. That indicates efficacy of drug may be increased after piperine, quercetin and combined pretreatment.

6. CYP3A37 and MDR1 mRNA expression levels in liver and duodenum were significantly down regulated after piperine, quercetin and both in combination pretreatment compare to normal chickens. However, after piperine and quercetin combination pretreatment, significantly ($p < 0.01$) higher down regulation of MDR1 mRNA level were observed in duodenum compare to liver.
7. Following multiple intravenous administration of marbofloxacin, significant reductions in TEC and lymphocyte count and increase in heterophil count were observed in broiler chickens. Following multiple oral administration, significant reduction in Hb level, PCV and TEC and increased MCH values were observed in quercetin pretreated broiler chickens. However, these alterations were in the normal range for broiler chickens.

8. Following multiple oral administration of marbofloxacin, significantly increased AST level (normal and quercetin pretreated), total protein (combination pretreated) and decreased LDH level (piperine pretreated) were observed in broiler chickens. However, these alterations were in the normal range for broiler chickens.

9. After repeated intravenous and oral administrations of marbofloxacin no any gross and histopathological lesions were observed in organs like liver, kidney, intestine, lung, heart and proventriculus.

10. Piperine and quercetin combined pretreatment has improved the pharmacokinetic profile of marbofloxacin (5 mg/kg) after single and repeated oral administration by inhibition of drug efflux protein (P-gp)/MDR1 and drug metabolizing enzyme (CYP3A37).