



## Effect of Berberine on Some Histopathological, Enrofloxacin Residue and Anti-Diarrheagic *Escherichia coli* in Broiler Chicken

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**Abstract** | Background Antibiotic residues in poultry have the potential to have a number of harmful effects on health, including the emergence of multidrug-resistant microbial strains, allergic and an alteration of the normal flora in the intestines. The study aimed to evaluate the residue levels of enrofloxacin in chicken meat after administering berberine (BBR). *Escherichia coli* (*E. coli*) was isolated from chickens suffering from diarrhea, and its isolation was confirmed by culturing it on MacConkey agar medium. In this study, a total of one hundred chicken chicks were reared, and on the tenth day of the experiment, they were divided into five equal groups: NCG: This group represented the control and was given normal drinking water. T1: The chickens in this group were orally inoculated with *E. coli* at a dose of 0.5 ml containing 6x10<sup>8</sup> CFU/ml of *E. coli*. T2: The chickens in this group were orally inoculated with *E. coli* at a dose of 0.5 ml containing 6x10<sup>8</sup> CFU/ml of *E. coli* and were treated with enrofloxacin at a dose of 12.5 mg/kg orally through drinking water. T3 group: The chickens in this group were orally inoculated with *E. coli* at a dose of 0.5 ml containing 6x10<sup>8</sup> CFU/ml of *E. coli* and were treated with 250 mg/kg BBR through drinking water. T4 group: The chickens in this group were orally inoculated with *E. coli* at a dose of 0.5 ml containing 6x10<sup>8</sup> CFU/ml of *E. coli* and were treated with both combination enrofloxacin and BBR. Conclusion: study showed that berberine increased the residue levels of enrofloxacin in chicken meat, specifically in the thigh and liver. The docking study confirmed inhibitory activity of Cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (P-gp) an enzyme responsible for the metabolism of antibiotics. Additionally, berberine demonstrated antibacterial effects against *E. coli*, as well as anti-inflammatory and antioxidant properties. Furthermore, histopathological examination of the intestinal broiler received BBR showed improvements and restore tissue near to normal.

**Keywords** | Enrofloxacin, Berberine, *E. coli*, Antibiotic residues, CYP450, Antioxidant, Drug docking

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## INTRODUCTION

Poultry chickens are commercially bred for egg and meat production because they grow quickly and are

fairly inexpensive (Atsbeha and Hailu, 2021). In fact, the demand for meat is rising day by day, as various drugs have been used to promote growth and control infection. When antibiotics are introduced to poultry, some of the

## MATERIALS AND METHODS

## MATERIALS

Enrofloxacin having a purity of 99.9% and Berberine 90% were purchased from Adooq (USA) and TCI (USA), respectively. Phosphoric acid was obtained Valsad (India). HPLC grade Acetonitrile was purchased from fisher scientific (UK).

DIAGNOSIS OF *E. COLI*

*E. coli* was isolated from suffering from diarrhea after confirming the isolation by culturing it on macConkey agar medium. Pink colonies appeared, indicating lactose fermentation in MacConkey medium [Figure 1](#).



**Figure 1:** Pink colonies of *E. coli* grown on macConkey agar medium.

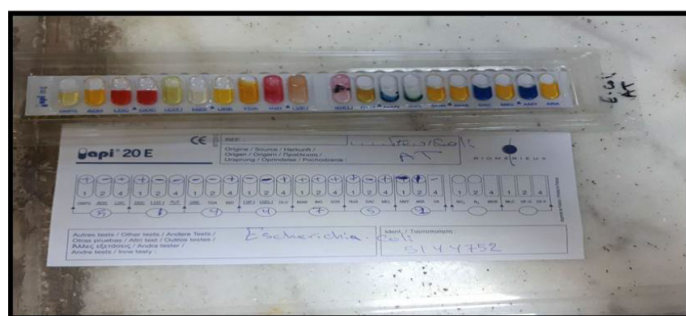


**Figure 2:** Metallic green luster of *E. coli* grown on Eosin Methylene Blue (EMB) agar, and gave.

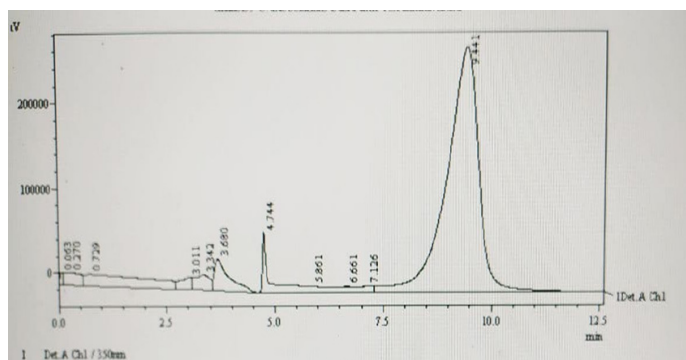
It was also detected on a selective medium, which is Eosin Methylene Blue (EMB) agar, and gave metallic green luster [Figure 2](#).

antibiotic and its derivatives are released into the meat and eggs, and after a few weeks, the level gradually tapers off due to metabolic regression (Yousif and Jwher, 2021). The antibiotic withdrawal period is the time that passes between the final doses of antibiotic given to the chicken and the time that the level of antibiotic residue in the meat passes (Jabber *et al.*, 2022). Prior to withdrawal periods, chicken meat or eggs are not fit for human consumption. Berberine powder is isoquinoline alkaloid has yellow color and bitter taste, commonly isolated from different plant families (Ibrahim *et al.*, 2023). It has been utilized since the 50 years ago as an active medication for the treatment of human and animal gastro-enteritis and secretory type diarrhea (Luo *et al.*, 2013). Additionally, it serves well to avoid and treat diarrhea in animals, including poultry. Moreover, studies have confirmed that berberine support the P-glycoprotein (P-gp) and co-administered with P-gp substrate result in mediate drug-drug interactions (Murakami *et al.*, 2023). Berberine is a substrate of P-gp chicken and down-regulation of P-gp expression in chicken tissues, resulting enhancing the absorption of P-gp substrates as well as berberine boost the bioavailability other substrate specially orally co-administered with other P-gp substrates like enrofloxacin that resulting toxicity and antibiotics accumulation (Zhang *et al.*, 2019). In recent years, berberine as a broad-spectrum antimicrobial activity has attracted more and more attention (Hao *et al.*, 2022). It is known that berberine has wide medical effects in the treatment of many diseases related to humans and animals, such as gastroenteritis, chronic diarrhea, malaria, polycystic ovaries, Alzheimers disease, obesity, diabetes and lowering blood pressure (Imenshahidi and Hosseinzadeh, 2019; Alnuqaydan *et al.*, 2022). Several studies reported the inhibition effect of berberine on biofilm formation by altering the quorum-sensing system in a drug-resistant *Escherichia coli* confirmed by Sun *et al.* (2019). Enrofloxacin is fluoroquinolone from second generation, restricted developed for veterinary use only and has excellent potency versus aerobic bacteria from the Gram-negative type (Dai *et al.*, 2023). Enrofloxacin is until now still essential part in the veterinarian's armamentarium for the treating of colibacillosis in numerous cities of the world (Joosten *et al.*, 2019; Fairbrother and Nadeau, 2019). The study aimed to use the berberine compound in poultry *in vivo*, as well as the activity of berberine on inhibition of P-gp using drug docking technique and its influence on the ability *Escherichia coli* to induce pathogenesis in broiler from side and accumulation of enrofloxacin alone or in combination with berberine in thigh and liver to get suitable data about drug-drug interaction of berberine with antibiotic as well as regulate dose of enrofloxacin to reduce accumulation in broiler meat as well as reduce toxicity and enrofloxacin resistance in human consumption.

After confirming the presence of *E. coli* bacteria, a series of decimal dilutions of the bacteria was conducted for the purpose of counting the bacteria (Al-Ahbabi *et al.*, 2016). The fifth dilution was taken and a sensitivity test was performed on Mueller-Hinton Agar medium using the drilling method and injecting a ratio of (0.5) into the hole and after incubating in the incubator at 37 degrees Celsius for 24 hours (Figure 3). The test result was read by reading the zoom with a result of 16 mm (Salman *et al.*, 2022; Othman *et al.*, 2023).



**Figure 3:** Api 20 E strip shows a specimen diagnosed as *E. coli*.

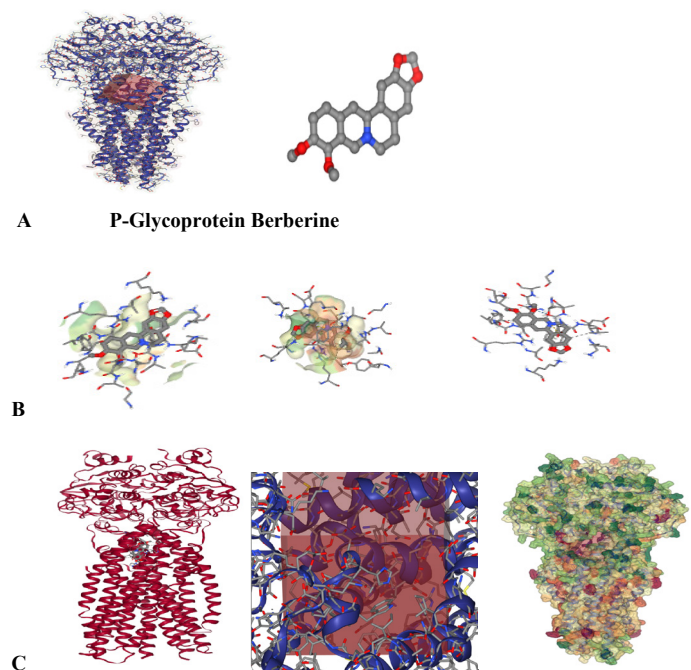


**Figure 4:** Retention time of enrofloxacin in liver using HPLC.

**EXPERIMENTAL OF DESIGN**

This study was conducted in the animal house of the College of Veterinary Medicine, Babylon Province. The study was carried out in a semi-closed hall divided into four sections and its dimensions were 3 x 2 m<sup>2</sup> divided by wooden partitions, wire mesh and independent doors. Wood sawdust with a height of 8-10 cm. It is equipped with all breeding requirements such as feeders, manholes and gas incubators. The poultry field was air-conditioned, the ambient temperature stabilized at approximately 25 to 30°C, and the relative humidity was maintained between 44 and 60%. The light cycle was identical to that of a commercial farm (15 hours light/9 hours dark). No clinical signs of disease were noted before beginning the experiment. In this current study, one hundred chicken chicks were reared, and on the tenth day of the experiment, they were divided into five equal groups, as the following division included: The first group represented the control

and left the festive river water (NCG), The second group (T1) was inoculated orally with *E. coli* at dose 0.5 ml involving 6x10<sup>8</sup> CFU/ml of *E. coli* (Al-Taii and Yousif, 2019; Mohammad and Al-Mahmood, 2022). The third group (T2), was orally inoculated with *E. coli* at dose 0.5 ml containing 6x10<sup>8</sup> CFU/ml of *E. coli* and given enrofloxacin 12.5 mg / kg orally with river water. The fourth group(T3) was inoculated inoculated with *E. coli* at dose 0.5 ml has 6x10<sup>8</sup> CFU/ml of *E. coli* and treated by berberine extract with drinking water. The last five group (T4) was inoculated with *E. coli* at dose 0.5 ml with 6x10<sup>8</sup> CFU/ml of *E. coli* and treated by berberine extract and enrofloxacin 12.5 mg/ kg with drinking water.



**Figure 5:** AutoDock Vina was used to obtain the berberine molecular docking solution against the p-glycoprotein target berberine (A) in the ligand binding domain of (B) Ligand interaction diagram of the berberine docking pose (dark green conventional hydrogen bond; light green carbon hydrogen bond; dark pink-pi-pi stacked interaction; light pink pi-alkyl interaction; yellow pi-sulfur interaction); (C) berberine in the active sites defined by the hydrophobic surface and surrounding residues which are labelled and represented as thin grey lines). For clarity, hydrogen atoms are not displayed. (D) illustrates the dynamic character of interactions.

The current experiment lasted for five days. During this period, clinical symptoms were recorded on the chicks, including anorexia and diarrhea as well as macroscopically showed congestion of liver, kidney and slightly hemorrhage in small intestine with mortality rate was 5%. On the fifth day, the chicks were sacrificed. Blood samples were collected from heart puncture and serum was isolated to determine immunological parameters. Sections were taken from the

intestines for the purpose of histological examination, and a swab was taken from the intestines to find out the effect of the treatment on the formation of bacterial colonies, The specimen 20 from liver were collected from different chicken received enrofloxacin and mixed enrofloxacin with berberine then samples were placed at -20 °C for HPLC analysis.

### HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ASSAY

Enrofloxacin residues were determined quantitatively by High Performance Liquid Chromatography (HPLC). Enrofloxacin standard. The stock solutions of enrofloxacin was performed at a concentration of 100 µg powdered dissolving in 10 ml working standard solutions of acetonitrile and phosphoric acid to get concentration of 10 µg/mL of antibiotic to get Standard solutions and kept at 4 °C (Aslam *et al.*, 2016; Mahmood *et al.*, 2019).

### APPARATUS UTILIZE IN THE STUDY

The analyses were done using shimadzu (Japan) high performance liquid chromatographic system. A separation was perfects using C18 column (4.6 mm × 250 mm, 5µm) for stationary phase. The temperature of column was set at 25 °C and the injection volume was 20 µL; the mobile part consist of phosphoric acid buffer (0.01 M, pH 3) and acetonitrile and (75:25% v/v) were used with a flow rate of 1.0 mL/min and wave length was 278 nm (Figure 4). The concentration of antibiotic was calculated from Samples preparation for HPLC (Aresta *et al.*, 2019).

$$\text{Concentration} = \frac{\text{AUC standard concentration}}{\text{AUS}}$$

AUC= area under the curve of sample; AUS= area under the curve of standard.

### SAMPLES PREPARATION FOR HPLC

The liver and muscle chicken cold samples were crushed by grinder separately and 5g of each liver and muscle was accurately transferred into a 10 mL glass tube. Then, 5 mL of 25 mM phosphoric acid: acetonitrile (25:75 v/v) solution was transfer to the glass tube and shaken for 5 minutes. The tubes were centrifuged for 8 min at 7000 rpm at room temperature. The supernatant of samples was filtered using a 0.45 µm milipore filter and transferred into second glass tubes to make more suitable for HPLC inspection (Moghadam *et al.*, 2018).

### STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to assess the data for the presence of normal distribution preconditions. The SPSS 22.0 program (USA) was used for statistical comparisons of parameters between groups. One-way Anova (Posthoc Duncan) was utilized (Nunes *et al.*, 2015).

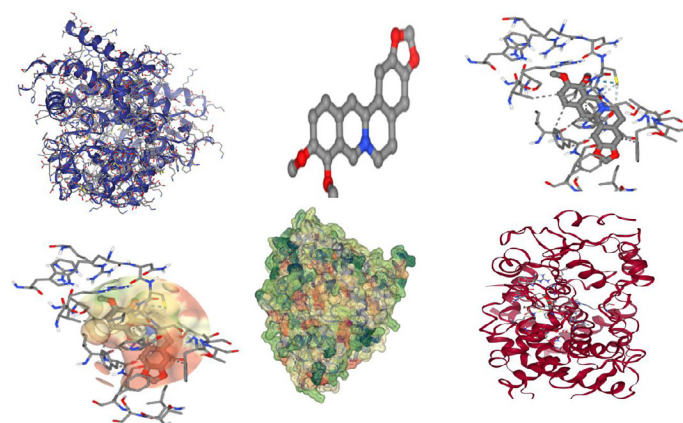
### HISTOLOGICAL ANALYSIS

Broiler of each group were sacrificed at end of study by decapitation, the small intestine, were isolated and fixed in 10% formalin, dehydrated by ethyl alcohol in ascending grades, cleared in xylol, embedded in paraffin at 56°C and rotator microtome for cutting with size 5µ. The staining was by hematoxylin and eosin (Luna *et al.*, 1972).

## RESULTS AND DISCUSSION

### DRUG DOCKING

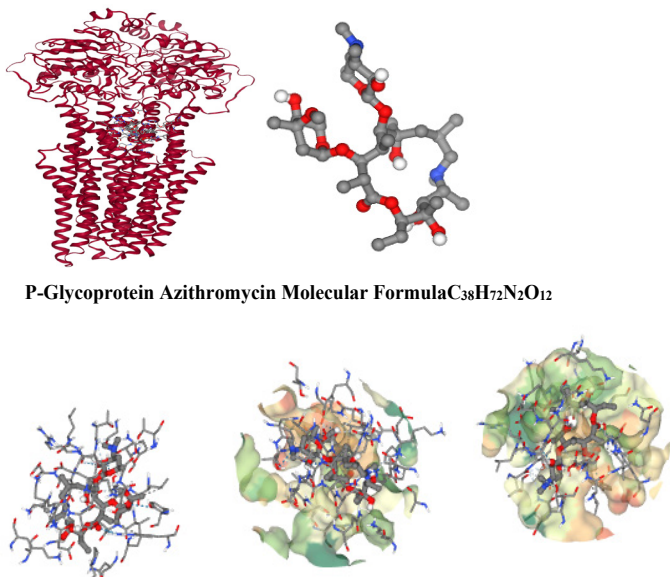
In the present study azithromycin and berberine have been studied for their potential inhibitory effects on P-glycoprotein (P-gp), which is a protein involved in drug transport and resistance (Figure 7). However, it's important to note that the research on this topic is still limited, and the precise mechanisms and clinical implications are not fully understood molecules Based on information on the Figure 6.



**Figure 6:** Receptor protein CYP3A4 that berberine and its analogues bind to, the top compounds were chosen, and their ADMET graphs were drawn. Top-hitting chemicals docked with a high binding energy score.

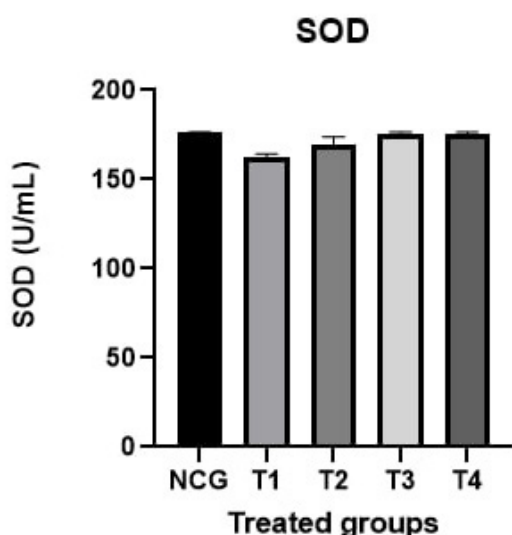
### ANTIOXIDANT AND ANTI-INFLAMMATORY

The present study showed that serum collected from infected poultry with *E. coli* and received berberine extract alone or in combination with enrofloxacin 12.5 mg / kg orally with drinking water clear reduction in symptoms of bacteria in broiler Chickens of treated in both enrofloxacin and extract or in combination while the un treated chicks showed sever, dehydration, depression, lowered appetite, cough, and labored breathing and diarrhea. On the other aspect clear reduction in pro-inflammatory cytokines in infected poultry and treated with enrofloxacin when compared with positive control group , as well as excellent reduction was noted in broiler chicken received berberine extract alone or in combination with enrofloxacin to recorded mean value (22.5±1.04, 21.5±0.64 and 21.7±1.65) of serum IL6 for negative control, extract and drugscombination while serum TNFa aclear reduced in this group to recorded mean value (30.5 ±n2.02, 35±0.61 and 32±1.73).



P-Glycoprotein Azithromycin Molecular Formula  $C_{38}H_{72}N_2O_{12}$

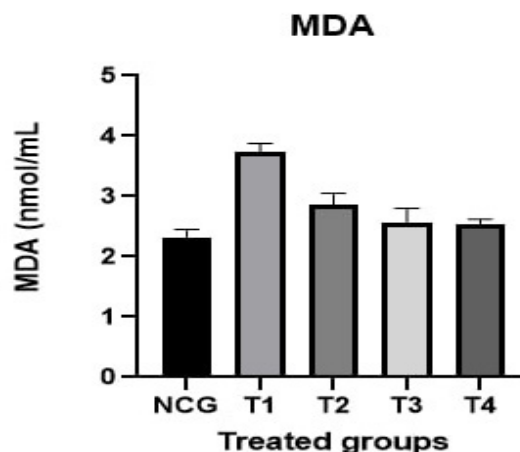
**Figure 7:** A representative 3D structure of the complex docked target protein molecule (surface p-glycoprotein) with azithromycin is shown in the molecular modeling and docking investigations of both chosen medicines with this protein.



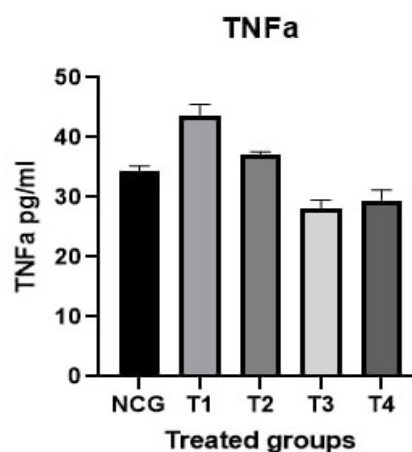
**Figure 8:** SOD value result from influence of that berberine and enrofloxacin against *E. coli* infection induced in broiler chicks. NCG: Negative control group T1: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli*. T2: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* treated by enrofloxacin. T3 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with BBR. T4 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with both combination enrofloxacin and BBR.

In addition to anti-inflammatory of berberine also noted via a significant reduction of IL6 as compared with (T1) group Figure 10. Serum super oxide dismutase (SOD) broiler chicken showed highly reduced in infected group to  $(159.2 \pm 3.95)$  as compared with other treated group with extract  $(173 \pm 1.9)$  and  $(175 \pm 1.19)$  while poultry infected with *E. coli* and treated with enrofloxacin showed

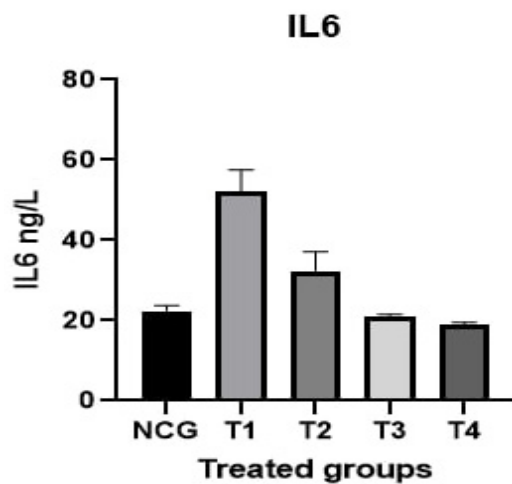
improvement of SOD but still not a significant as compared with control group Figure 8. Actually, the berberine extract and or enrofloxacin treated the infected *E. coli* in broiler chicks neutralize the oxidative stress in among groups to recorded mean value for malondialdehyde (MDA)  $(2.4 \pm 0.14, 2.9 \pm 0.13, 2.67 \pm 0.19)$  and  $(2.72 \pm 0.20)$  respectively for control and infected chicken treated with enrofloxacin, extract and their combination Figure 9.



**Figure 9:** MDA value result from influence of that berberine and enrofloxacin against *E. coli* infection induced in poultry chicks. NCG: Negative control group T1: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli*. T2: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* treated by enrofloxacin. T3 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with BBR. T4 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with both combination enrofloxacin and BBR.



**Figure 10:** SOD value result from influence of that berberine and enrofloxacin against *E. coli* infection induced in poultry chicks. NCG: Negative control group T1: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli*. T2: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* treated by enrofloxacin. T3 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with BBR. T4 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with both combination enrofloxacin and BBR.



**Figure 11:** SOD value result from influence of that berberine and enrofloxacin against *E. coli* infection induced in poultry chicks. NCG: Negative control group T1: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli*. T2: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* treated by enrofloxacin. T3 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with BBR. T4 group: Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* and were treated with both combination enrofloxacin and BBR.

### ENEROFLOXACIN LIVER AND MUSCLE RESIDUAL

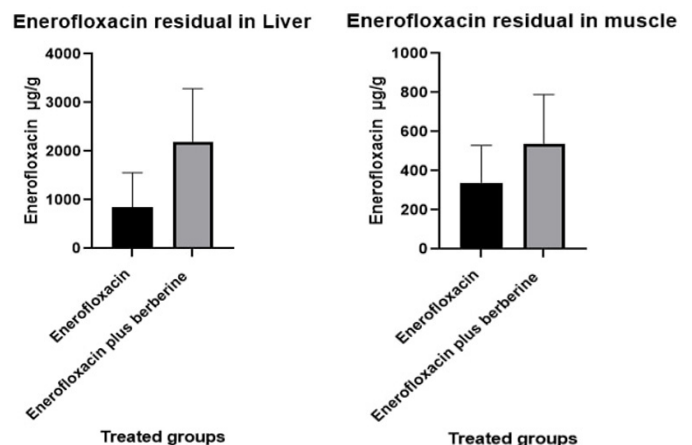
The present data in Figure 11 showed that berberine administrated to chicken lead to a significant increase accumulation of enrofloxacin in liver and muscle (tissue residual) as compared with chicken received enrofloxacin alone. Our results demonstrate that berberine could inhibit P-gp expression and activity, that result in change the bioavailability of oral enrofloxacin when it is administered to the broiler's different organs in chicken. The results of the present study could guide the responsible use of berberine and additives in the poultry industry.

### HISTOPATHOLOGICAL STUDY

Inoculated with  $6 \times 10^8$  CFU/ml of *E. coli* appear several clinical signs such as fever, emaciation, irregular of feathering with severe diarrhea, thus the macroscopically broiler in T1 group specially noted there is some congestion was seen on liver and small intestine. The current study on histopathology of intestine showed clear restore the tissue to normal state and concord with many studies was done recently. Inoculated broiler with *E. coli* and then treated with berberine showed hyperplasia of the intestinal associated lymphoid tissues with increase in goblet cells number with normal intestinal villi (Figure 13), thus BBR restore the damage effect result from bacteria and enrofloxacin toxicity Figure 12.

The present study noted that berberine may inhibit P-gp and CP3A4 activity by evaluate drug docking activity of on T137(A) OG1Y247(A) CE2D177(A) OD1 and

C442(A) SGF302(A) CBI118(A) CG2 which could have implications for drug interactions and the efficacy of certain medications on this enzyme to alter or increase metabolism of drugs. However, it's worth mentioning that the evidence regarding the extent and clinical significance of P-gp inhibition by both azithromycin and berberine is still limited and conflicting. Moreover, the inhibitory effects of these compounds may affect according to concentration, cell type, and experimental conditions used in different studies. Our findings proven that berberine elevates the bioavailability of enrofloxacin and that interactions between berberine-enrofloxacin important to reduce dose of P-gp substrates especially narrow therapeutic windows. However, berberine influence on withdrawal period of many drugs that considered P-gp substrates in human and animal (Li *et al.*, 2022). The recent study confirmed by (Mohammad and Al-Mahmood, 2022) showed that chicks oral received of  $6 \times 10^8$  CFU/ml will cause the clear pathological lesions in the internal organ specially kidney and intestine, with clear mononuclear penetration of inflammatory cells in hepatic tissue from side and reduce body weight with emaciation of chicks with reduce weight of liver and kidney.



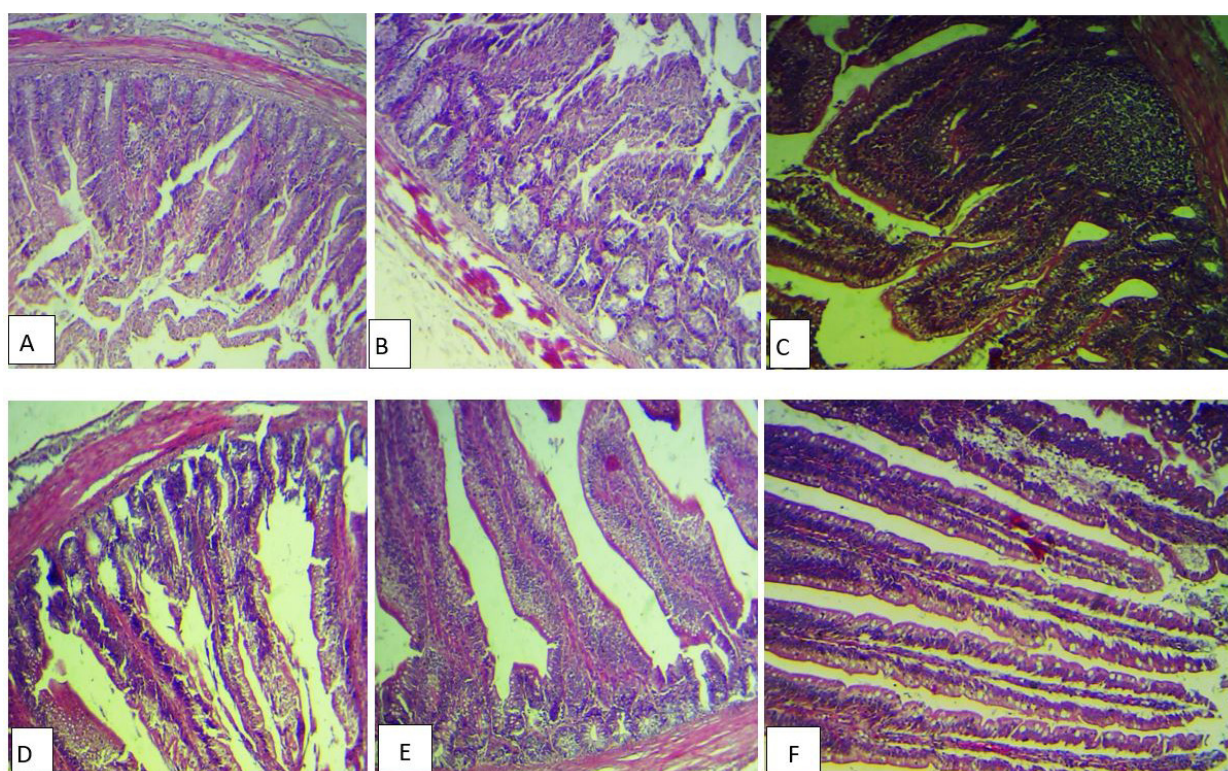
**Figure 12:** Refer to influence of that berberine on enrofloxacin concentration in liver and thigh muscle of broiler.

The present study was agreement with recent study reported by Aziza *et al.* (2019) that noted dietary berberine significantly alleviate the LPS- affect by reduce in the mRNA expression of inducible nitrite synthase, TNF- $\alpha$ , IL-1 $\beta$ , cyclooxygenase-2, nuclear factor-kappa B (NF- $\kappa$ B) in the liver with enhance antioxidant enzyme activity and suppression of inflammatory markers.

Tabatabaei *et al.* (2015) reported that poultry chicks infected with *E. coli* significantly serum levels of TNF- $\alpha$  and IL6 NF- $\kappa$ B as well as some liver enzymes including lactate-dehydrogenase (LDH), creating-kinase (CK), alanine-transferase (ALT) and aspartate-transferase (AST) with clear reduction of antioxidant SOD as

compared with healthy control group. Berberine (BE) has been appear to have numerous pharmacological activities, including enhance immunity, antibacterial activity, as well as potent antioxidant and anti-inflammatory effects. Dietary supplementation with Forsythia suspense extract (FSE) and berberine (BE) at dose 100 mg/kg, or both can ameliorate the growth performance may be via boost immunity, decrease reactive oxygen species, and intestinal colonization promoting by normal microbiota of broilers (Tabatabaei *et al.*, 2015) (Figure 1). Dehau *et al.* (2023) study confirmed that supplementation of berberine at a high concentration augment chicken gut morphology toward decreased inflammation and boost villus length

and lowering crypt depth and infiltration in the bowels tissue of poultry by CD3+ T-lymphocyte. Berberine also affected the diversity of the microbiota of gut from the jejunum to the colon, both at a compositional and active level, with huge effects showed in the large intestine. The ileal proinflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) were considerably higher in broiler chicks with necrotic enteritis brought on by *Clostridium perfringens* so treatment with berberine at 100mg/kg lower the number of *C. perfringens* in the cecum, growth performance was restored to normal state, improved inflammatory cytokines, intestinal morphology, and protein expression of cell tight junction (Yuan *et al.*, 2021).



**Figure 13:** (A) Histopathological section of intestine broiler inoculated with *E. coli* alone showing congestion of the serosa blood vessels ( ), sloughing and fusion of intestinal villi ( ), with inflammatory cells infiltration (H&E stains ,40x). B, Histopathological section of intestine for *E. coli* group showing atrophy of intestinal villi ( ), necrosis in some glands ( ), with intermuscularis edema (H&E stains, 40x). C, Histopathological section of intestine inoculated with *E. coli* and treated with berberine group showing hyperplasia of the intestinal associated lymphoid tissues with increase in goblet cells number (H&E stains, 40x). D, Histopathological section of intestine for *E. coli* treated with berberine group showing normal intestinal villi with increase in the numbers of the goblet cells (H&E stains, 40x). E, Histopathological section of intestine for *E. coli* treated with enerofloxacin group showing necrosis of the intestinal villi with inflammatory cells infiltration (H&E stains, 40x). F, Histopathological section of intestine for *E. coli* treated with berberine and enerofloxacin showing normal intestinal villi with increase in the numbers of the goblet cells (H&E stains, 40x).

**Table 1:** Using the estimated binding affinities, docking scores of berberine against a subset of P-gp substrates and inhibitors were generated.

No.	Protein	Ligand	Binding energy (kcal/mol)	Inhibition constant	Receptor	No. of H bond
1	P-GP	Berberine	-8.7	33 $\mu$ M	T137(A)OG1, Y247(A)CE2, D177(A)OD1	8
1	CYP3A4	Berberine	-9.9, -9.5	21 $\mu$ M	C442(A)SG, F302(A)CB, I118(A)CG2	6
1	P-Gp	Azithromycin	-6.9	34 $\mu$ M	T173(A)OG1, R148(A)NH1, N887(A)CB, Y920(A)OH	3

The present study showed that berberine administrated to chicken lead to a significant increase accumulation of enrofloxacin in liver and muscle (tissue residual) as compared with chicken received enrofloxacin alone. The current study was agreement with Li *et al.* (2022) noted that berberine is a substrate of poultry P-gp and down-regulates P-gp in tissues of chicken, thereby enhancement the absorption of P-gp substrates. Enrofloxacin is one of P-gp substrate in chicken and pig intestines. Therefore, one of the most important lines of evaluation of P-gp is the pharmacokinetic interactions with feed or feed additives and their potential impact on the clinical efficacy of antibiotics (Bhutto *et al.*, 2018). This study confirmed that a substrate of P-gp (beberine) lead to down-regulate (expression) and lower activity of P-gp in birds, thus elevating the bioavailability of P-gp substrates in intestine. The introducer P-gp and P-gp inhibitors, and now many technologies depend on synthesis of drug loaded by delivery systems oral taken that can easily penetrate and inhibit P-gp to accelerate the enteral absorption and improve bioavailability of substrates. Since many drugs are reduced efficacy by P-gp efflux system (Jain *et al.*, 2022). The drugs that cause CYP450 enzymes inducer accelerate drug metabolism, while the inhibition of CYP450 enzymes, which causes a delay in drug metabolism (Zhang *et al.*, 2022). The main enzymes for metabolism of enrofloxacin in microsomal liver chicken CYP3A4 and CYP2A6. Additionally, this study supported that this tilmicosin lead to inhibition was greatly associated with CYP3A4 in animal and *in vitro* (Zhang *et al.*, 2022). Recent study confirmed that enrofloxacin and ciprofloxacin are tendency to accumulate in liver rather than other organ as skin, muscle and kidney (Xu *et al.*, 2023). Here, we investigated the effects of berberine on the activity of enrofloxacin as well as interactions between berberine and enrofloxacin in liver and muscle residual. Our results demonstrate that berberine could inhibit P-gp expression and activity, that result in change the bioavailability of oral enrofloxacin when it is administered to the broiler's different organs in chicken. The results of the present study could guide the responsible use of berberine and additives in the poultry industry. Histopathological section of intestine infected broiler and received enrofloxacin appear necrosis of the intestinal villi with inflammatory cells infiltration while berberine ameliorate defect of infection due to antioxidant and antibacterial activity from side and modulate adverse effect of enrofloxacin recording normal intestinal villi with increase in the numbers of the goblet cells. The present data was agreement with Yuan *et al.* (2021) noted that berberine significantly lowered the number of *Clostridium perfringens* in the cecum, improved tissue intestinal, lowered inflammatory cytokines, and potentiate immune function of broilers. Our study also agree with Dehau *et al.* (2023) that noted that berberine feed

additive at a high dose improves chicken gut histology and minimize inflammation, with inhibited the growth of many butyrate-producing strains while not influence on Enterobacteriaceae.

## CONCLUSION AND RECOMMENDATIONS

Berberine has increase enrofloxacin residual in chicken meat of thigh and liver by inhibit activity of CYP450 that responsible of metabolism antibiotic as well as has antibacterial effect on colibacillosis, anti-inflammatory and antioxidant from side and improvement of histopathological of intestinal tissue.

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## NOVELTY STATEMENT

The special goal and novelty of this investigation is to seek out the berberine antibacterial effects against *E. coli*, furthermore the anti-inflammatory and antioxidant properties, along with its approachability and accessibility.

## AUTHOR'S CONTRIBUTION

Conceived and planned to experiments design of study, contributed to broiler management, drugs and samples preparation. Contributed to the sample analysis and accomplishment of the results.

## ETHICAL APPROVAL

The Ethics Commission of Veterinary Medicine has authorized this study under reference number 533FD2. Additionally, Al-Qasim Green University's institutional animal ethics council approved the procedure.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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