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# Evaluation of therapeutic effects of an herbal mixture (*Echinacea purpurea* and *Glycyrrhiza glabra*) for treatment of clinical coccidiosis in broilers

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## Abstract

**Background:** Avian coccidiosis is thought to be one of the most expensive infectious diseases in the poultry industry.

**Objectives:** Safe and alternative anti-coccidial drugs are herbal extracts because they do not result in tissue residue and drug resistance. The objective of the present study was to evaluate the anti-coccidial effect of the herbal mixture, a complex of two plants (*Echinacea purpurea*, *Glycyrrhiza glabra*) in broiler chickens in comparison with toltrazuril.

**Methods:** One hundred twenty broiler chickens were used in this experiment and divided into 4 equally numbered groups. All the groups, except Group D, were experimentally infected with mixed *Eimeria* spp. (*E. Tenella*, *E. maxima*, *E. necatrix* and *E. brunetti*) on day 14. Group A treated with an herbal mixture [*Glycyrrhiza glabra* Extract 5% (standardised to 5.4% glycyrrhizic acid) and *Echinacea purpurea* Extract 2% (standardised to 4% total phenolic content based on chlorogenic acid); Coxinin-EC®; Shamim Teb Sepid Giti]. Group B treated with toltrazuril. Group C was experimentally infected with mixed *Eimeria* spp. but they did not have any treatment, this group was our positive control. Performance indices, faecal oocyst excretion, and intestinal lesion score were determined during the experiment.

**Results:** Positive control group had the poorest results and more mortality than other groups. Group D was not infected and was healthy all the experiment period. Treatment with herbal complex significantly reduced the negative performance and pathogenic effects associated with *Eimeria* spp. at a level that was comparable with toltrazuril.

**Conclusions:** In summary, the anti-coccidial activity of the studied herbal complex suggests its use as an alternative anti-coccidial agent to chemotherapeutic drugs for controlling coccidiosis in poultry.

Amir Ebrahim Tavanaee Tamannaei, Soheil Sadr and Ali Charbgoo contributed equally to this work.

KEYWORDS
chicken, coccidiosis control, herbal medicine

**Highlights**

- Coccidiosis is an important infectious disease that causes serious financial loss to the poultry industry.
- Chemical anti-coccidial drugs and vaccines are the main control strategies to combat the disease. However, these tools have some constraints.
- Herbal remedies are suitable alternatives to chemical compounds for control of losses associated with coccidiosis in poultry.
- An herbal mixture (*Echinacea purpurea*, *Glycyrrhiza glabra*) has promising effects for controlling of coccidiosis in broiler chickens.

## 1 | INTRODUCTION

Avian coccidiosis is a remarkable enteric disease in poultries with the ability to inflict a large economic impact on farm profitability (Kostadinović et al., 2019). It is known for its extraordinary effects and huge damage to poultry industries among the parasitic diseases around the world (Blake et al., 2020). The economic loss to the poultry enterprise due to coccidiosis is considerable, which is specifically because of prophylactic or healing in-feed medications and additionally due to the impact of the ailment on birds' health (Kostadinović & Lević, 2018; Puvača et al., 2019). The global cost of the pathogen to the poultry industry is estimated to be over US$2.4 billion per annum (Shirley & Chapman, 2005). There are seven species of avian coccidian. *Eimeria* species multiply inside the bird's intestinal tract, inflicting considerable tissue damage. The harm caused to tissue can interfere with nutrient absorption, feeding and digestion (Chapman, 2009). This can lead to blood loss, poor skin pigmentation, dehydration and escalated vulnerability to other diseases including necrotic enteritis (Shirley & Millard, 1986). Control of coccidiosis is substantially established by using chemotherapeutic agents. During the fight against coccidiosis, to avert and control the disease, the poultry industry relied heavily on anti-coccidial drugs. The intensive use of anti-coccidial drugs has led to the development of resistance (Abbas et al., 2011; Noack et al., 2019). So far, chemotherapeutic drugs and anti-coccidial feed additives have managed coccidiosis but have been complicated by the emergence of drug resistance and their toxic effects on animal health (Peek & Landman, 2011). Furthermore, drug residues in poultry meat and other products are a potential constraint to the consumer (Sundar et al., 2017). Also, some achievement is done with vaccines for controlling avian coccidiosis, especially in broiler breeders (Tewari & Maharana, 2011). But, proscribing issue for using vaccines in opposition to coccidia is that the inclusion of numerous species of *Eimeria* in one vaccine can purpose similarly despair in weight benefit and feed conversion (Alfaro et al., 2007). Therefore, alternative tactics are being required for more effective and safer control of coccidiosis in chickens in both developed and underdeveloped countries (Crespy & Williamson, 2004). Among alternative methods, herbal compounds are the most potential candidates for the control of avian coccidiosis (Nidaullah, 2010). Based on the directions provided by world health organisations (WHO), FDA and EMEA for veterinary medicine in any possible case, synthetic drugs should be replaced with plant-based materials to lower the amount of synthetic drugs and their metabolites (residues) in the animal products. Due to their influential properties and complex bioactivity, medicinal plant supplements and their essential oils can be considered the best alternatives to synthetic drugs (Arafat & Abbas, 2018). During the last decade the consumption of medicinal plant supplements and their other products has expanded according to their antioxidation (Elmahallawy et al., 2021), hypocholesterolemic (Yong et al., 2020) and antibacterial activity (Li et al., 2018). Moreover, a correlation between some of the components and diverse stimulatory effects on the digestive system and digestive enzyme production has been detected (Vinus et al., 2018). Medicinal plant components also have been proved to exhibit anti-parasitic (Gohel et al., 2019), antiviral (Alagbe et al., 2018), antioxygenic, anti-mycotic (Abed et al., 2021) and insecticidal effects.

In this study, a comparative model was designed to assess the effectiveness of herbal medicine based on two plant extracts (*Glycyrrhiza glabra*, and *Echinacea purpurea*) for the treatment of coccidiosis in broilers. The goal of the current study was to evaluate the effect of a commercial multi-plant extract compound, in experimental coccidiosis in broiler chickens.

## 2 | MATERIALS AND METHODS

### 2.1 | Birds, housing

In total, 120 one-day-old Ross 308 broiler chicks were purchased from a local hatchery. The chicks were kept on a slotted floor in a specific place for raising birds. The birds were fostered under standard

environmental conditions, following the breeding standards of the Ross breed. The temperature was adjusted on 33°C on the first days of chick arrival, which was held until the end of the first week. Then the temperature was gradually reduced to 21°C on day 22 and held at this level by the end of the period. During the experiment water, and food were provided as much as desired and there were no anti-coccidial or coccidiostats drugs in the food. Gohar Daneh Shargh Corporation provided a standard commercial diet for feeding the birds. At the end of the second week, the birds were transferred from the litter to the cages, and birds were grouped randomly. The birds were divided into 4 groups of 30 and 3 replicates of 10 within each group. Group A takes an herbal mixture [*Glycyrrhiza glabra* Extract 5%) standardised to 5.4% glycyrrhizic acid) and *Echinacea purpurea* Extract 2% (standardised to 4% total phenolic content based on chlorogenic acid); Coxinin-EC®; Shamim Teb Sepid Giti] at a dose of 1 cc in 1 L of water for 4 days. The second group (Group B) receives toltrazuril 2.5% (Behroodatrak Co.) with the dose of 1 cc/L for 4 days. In the third group (Group C), birds become infected with *Eimeria* oocysts but do not receive treatment; this is positive control group. The fourth group (Group D) is the one that faces no challenge and needs no treatment. All procedures in this study including birds and their care were approved by institutional Ethics Committee.

## 2.2 | Weighing birds, measuring feed consumption and calculating feed conversion ratio

The birds were weighed collectively until the fourteenth day. But at the end of the second week, the birds were grouped, and the weighing procedure was performed for groups, and then the average weight of each group was calculated. The weighing of the birds continued until day 42 of breeding (end of the experiment).

By feeding oocysts and starting the challenge in birds, the amount of daily feed consumed in each replicate of the groups was recorded separately.

To calculate the feed conversion ratio by calculating the amount of feed consumed and also the increase in bird weight during the week, the feed conversion ratio was calculated.

## 2.3 | Challenge birds using *Eimeria* oocysts

A mixture of sporulated *Eimeria* oocysts was purchased from a local supplier. From the prepared mixture, a direct slide sample was taken and under the microscope, the diversity of *Eimeria* sporulated oocyst species was observed. Species of this mixture included 50% of *Eimeria tenella*, 25% of *Eimeria maxima* and the remaining 25% included other species such as *Eimeria acervulina*, *Eimeria mitis* and *Eimeria necatrix*. To challenge the birds, about 250,000 mixture oocysts of *Eimeria* spp. were fed to each bird through an oral gavage on day 14 of rearing. The estimated challenge dose was based on the infective inoculums that cause obvious lesions and negligible mortality in the birds.

## 2.4 | Registration of clinical symptoms and investigation of possible casualties

During the experimental period, birds in all groups underwent daily care for clinical signs, especially those related to coccidiosis, and the symptoms were recorded. Symptoms include decreased daily feed intake, lethargy and somnolence of the birds, and symptoms of dysentery. In addition, in cases where casualties were observed in the groups, the carcass was necropsied as soon as possible and the cause of death and injury were recorded.

## 2.5 | Treatment using drugs

Treatment was started by observing symptoms in birds that included lethargy, reduced feed intake, dysentery and death. In the present experiment, on the fourth day after feeding oocysts, specific symptoms of the disease were observed in Groups A, B and C, then treatment was started. The medicines – to the extent mentioned in the grouping section of the birds – were provided to the birds of each group on a daily basis. The length of the treatment period is based on the manufacturer's recommendation.

## 2.6 | Calculation of output per gram (OPG) in different groups

In order to reach this goal, according to the standard method and by using the McMaster counting slide, the number of oocysts per gram of faeces was counted (Haug et al., 2006). On this matter, 3 g of faeces from birds of each group were mixed with 42 ccs of water and shaken vigorously to obtain a uniform mixture. Then 15 cc of this mixture is centrifuged at a speed of 2000 rpm for 10 min and in the next step, the supernatant is discarded while water and saturated salt are added to the formed sediment to bring the volume to 15 cc. In the next step, we pour some part of it on the cells of the McMaster slide, and by placing the slide on a flat surface for 5 min, the oocysts have a chance to float. The oocysts are counted with a 10× magnification of the compound microscope and the average obtained from the two cells of the McMaster slide is expressed as OPG in that sample.

During the trial period, birds from each group were sampled and faecal oocysts were counted on four different days: the first time on the fifth day after the challenge; the second time on the seventh day after the challenge; the third time on the ninth day after the challenge; the fourth time is the twelfth day after the challenge. The increasing and decreasing trend in the number of excreted oocysts in the faeces of birds were recorded.

## 2.7 | Lesion scoring

The descriptive method of Johnson and Reid (1970) was used on this matter. In this method, based on the lesions observed in different parts

**TABLE 1** Mean ± SEM weight of birds in four groups of study during the 42 days

| Group | First day | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|---|
| Herbal mixture (Group A) | 48 ± 0.9 | 171 ± 6 | 408 ± 16 | 645 ± 50[a] | 867 ± 42[a] | 1430 ± 46[a] | 1910 ± 124[a] |
| Toltrazuril (Group B) | 48 ± 0.9 | 171 ± 6 | 408 ± 16 | 650 ± 50[a] | 865 ± 34[a] | 1310 ± 77[a] | 1833 ± 82[a] |
| Challenged and not treated (Group C) | 48 ± 0.9 | 171 ± 6 | 408 ± 16 | 550 ± 33[a] | 751 ± 42[a] | 1259 ± 44[a] | 1655 ± 118[a] |
| No challenge No treatment (Group D) | 48 ± 0.9 | 171 ± 6 | 408 ± 16 | 670 ± 50[a] | 955 ± 82[a] | 1431 ± 103[a] | 1881 ± 151[a] |

*Note*: Means denoted by different superscript letters show significant differences between groups in each column ($p < 0.05$).

of the intestine, a score of 0 (healthy) to +4 (the most severe lesions) is considered. It should be noted that for scoring, four areas of the intestine, namely the beginning of the intestine including the duodenum, the middle part of the intestine including the jejunum and ileum, the end of the intestine including the colon and finally, the cecum were examined. At the end of treatment, two birds from each replicate were slaughtered humanely and lesions of different parts of the intestine were recorded, and then the average lesions in the carcasses were obtained.

### 2.8 | Statistical analysis

The data on body weight, FCR and OPG parameters were analysed by ANOVA coupled with post hoc tests (SPSS® Software version 16). The discrepancy was considered statistically significant at the $p$ value $< 0.05$ for all the analysed data.

## 3 | RESULT

### 3.1 | Weekly weight gains of birds

The average weight of birds in different groups during the breeding period (42 days) is given in Table 1.

Accordingly, the most noticeable weight gains were seen in the groups that did not have challenges by *Eimeria* oocysts, Group D, and consistently had the most remarkable average weight during the period. The least weight gain had a place with the Group C which was not treated during the challenge. The weight contrast between the other treated groups is not noticeable. The important thing to mention about coccidiosis is that this disease will lead to a decrease in weight gain in meat herds, which causes significant economic damage. In the present experiment, Group D gained weight more than the other groups, so the control and prevention of coccidiosis can reduce the damage caused by this disease.

### 3.2 | Feed conversion ratio

The results of the feed conversion ratio (weekly) in different groups from the beginning of the period to the end of the experiment period are given in Table 2. As can be seen in Table 2, the best results were seen during the rearing period – except on day 42 – in Group D. The best feed conversion ratio on day 42, was observed in the group that received an herbal mixture. It is vital about the adverse effect of coccidiosis on feed conversion ratio, which is very clear in the table above on day 21 and prompted a huge contrast in the feed conversion ratio of Group D with other study groups. Yet, with the use of medication in Groups A and B, the condition of birds has also improved and does not show a distinction from the unchallenged group.

### 3.3 | Registration of clinical signs and possible casualties

The birds were perfectly healthy on the day of feeding the oocysts and had no particular clinical problems. Stool consistency was also normal. Oocysts were fed on day 14 in Groups A, B and C. Two days after the challenge, diarrhoea was seen in the birds. Yet, the birds were clinically and appetizingly normal. On the fourth day after the challenge, chocolate-coloured dysentery and diarrhoea were seen in various groups and lethargy and anorexia were seen in birds. The decrease in food consumption was also clear in the groups. Four days after the challenge and after observing signs, the treatment began in Groups A and B. Three days after taking the medication, the stool status got back to normal, and the overall state of the birds was normal.

In the group receiving herbal mixture (Group A), only one death was observed before treatment. In Group B, three deaths were observed, one related to before starting treatment and the other two related to one day after starting treatment. In Group C, two deaths occurred on the second and third days of treatment. No casualties were observed in Group D. Necropsies were performed on all carcasses and specific coccidiosis lesions were observed in all carcasses. The most obvious finding was related to the cecum, where a severe lesion of +4 was observed.

### 3.4 | Results of scoring lesions

The best time to score lesions in coccidiosis is days 5–7 after infection. Based on lesions observed in different parts of the intestine. The average scores in each part are given in Table 3.

The results showed that in the Group D, no lesion was observed in the intestines. The score of Group D was zero or equivalent to a healthy

**TABLE 2** Weekly FCR values of birds of four groups during the study

| Group | Day 7 | Day 14 | Day 21 | Day 28 | Day 35 | Day 42 |
|---|---|---|---|---|---|---|
| Herbal mixture (Group A) | 2.71 | 2.62 | $3.1 \pm 0.1^{a}$ | $2.81 \pm 0.05^{a}$ | $1.67 \pm 0.03^{a}$ | $1.75 \pm 0.13^{a}$ |
| Toltrazuril (Group B) | 2.71 | 2.62 | $3.5 \pm 0.05^{b}$ | $3.48 \pm 0.38^{b}$ | $2.31 \pm 0.11^{b}$ | $1.98 \pm 0.09^{a}$ |
| Challenged and not treated (Group C) | 2.71 | 2.62 | $5.4 \pm 0.13^{c}$ | $5.43 \pm 0.22^{c}$ | $2.75 \pm 0.13^{c}$ | $2.42 \pm 0.19^{b}$ |
| No challenge No treatment (Group D) | 2.71 | 2.62 | $2.3 \pm 0.15^{d}$ | $2.39 \pm 0.2^{d}$ | $1.4 \pm 0.1^{d}$ | $1.8 \pm 0.15^{a}$ |

*Note*: Means denoted by different superscript letters show significant differences between groups in each column ($p < 0.05$).

**TABLE 3** Lesion scoring of the intestine in different groups

| Group | Proximal part of intestine | Middle part of intestine | caecum | Distal end of intestine | Average lesion scores in each group |
|---|---|---|---|---|---|
| Herbal mixture (Group A) | 1 | 0 | 2.66 | 0 | 0.915 |
| Toltrazuril (Group B) | 0.66 | 0 | 2 | 0 | 0.66 |
| Challenged and not treated (Group C) | 1.66 | 0.66 | 3 | 0 | 1.33 |
| No challenge No treatment (Group D) | 0 | 0 | 0 | 0 | 0 |

**TABLE 4** Mean ± SEM OPG values of different groups at 5, 7, 9 and 12 days post-challenge

| Group | Day 5 | Day 7 | Day 9 | Day 12 |
|---|---|---|---|---|
| Herbal mixture (Group A) | $72{,}000 \pm 1000^{a}$ | $180{,}000 \pm 3000^{a}$ | $84{,}000 \pm 2000^{a}$ | $70{,}000 \pm 2000^{a}$ |
| Toltrazuril (Group B) | $45{,}000 \pm 2000^{b}$ | $210{,}000 \pm 3000^{b}$ | $89{,}000 \pm 2000^{b}$ | $50{,}000 \pm 1000^{b}$ |
| Challenged and not treated (Group C) | $32{,}000 \pm 500^{c}$ | $150{,}000 \pm 3000^{c}$ | $132{,}500 \pm 3000^{c}$ | $32{,}700 \pm 700^{c}$ |
| No challenge No treatment (Group D) | $0^{d}$ | $0^{d}$ | $0^{d}$ | $0^{d}$ |

*Note*: Means denoted by different superscript letters show significant differences between groups in each column ($p < 0.05$).

gut. Most injuries were observed in Group C, (a score of 1.33), which is quite expected. It is notable that the intestinal lesions score of Group A, compared to Group B, decreased and showed acceptable performance of an herbal mixture.

### 3.5 | Counting the number of oocysts per gram of faeces (OPG)

The results of counting excreted oocysts from birds in each group in four sampling stages (days 5, 7, 9 and 12 after the challenge) in different groups are presented in Table 4. The results of counting the number of oocysts per gram of faeces show that the herbal mixture has established a good performance and has reduced the number of oocysts per gram of faeces. In the case of Toltrazuril, the results of drug use have been satisfactory and the number of oocysts excreted from birds has decreased with drug use. The point to consider in the results is that with the use of drugs (herbal mixture and toltrazuril), a sharp decrease in faecal oocysts is observed on day 9 after the challenge. But in the untreated group, a sharp decrease in excreted oocysts was observed on day 12 after the challenge.

## 4 | DISCUSSION

Broiler herds infected with coccidiosis experience an important reduction in weight gain that will lead to huge economic losses. Coccidiosis is an infectious disease that damages intestinal epithelial cells and results in dreadful haematochezia (Chand et al., 2016; Kadykalo et al., 2018). Prolonged use of anti-coccidial chemicals promotes drug resistance and results in tissue residues in chickens; therefore, a healthy anti-coccidial treatment based on herbs is essential (Nahed et al., 2022; Naidoo et al., 2008). Based on the observation of this study, birds in the non-challenged Group D had a difference in weight gain in comparison to birds of other groups, hence the importance of control and prevention in management and reduction of the damages of the coccidiosis can be concluded (Scheurer et al., 2013). Our experiment was held in highly hygienic conditions. Environmental factors such as density and stress status of the herds are essential in detecting performance responses to plant extracts used as treatment. Continuing failures of vaccines, broad emergence of anti-coccidial drug resistance, and residual toxic effects of drugs are the reasons behind the efforts on finding alternative methods to control coccidiosis. On this matter, various botanicals have been proved to be

beneficial for their use as anti-coccidial agents or immunomodulatory.

Common clinical signs were observed during the present study such as anorexia, paleness, ruffled feathers, depression and huddling together that complied with the observation provided in the studies by Dubey (2019) and Tanweer et al. (2014). Based on the results of this experiment, Group D ingests more feed in comparison to other groups. The result of the current experiment is backed by Hashmi et al. (1994) and Tipu et al. (2002) who mentioned that coccidiosis infection leads to a reduction in feed intake (Hayat et al., 1991). Notable weight loss and reduced FCR are the results of all *Eimeria* isolates infection (Logan et al., 1993). The FCR reduction happens because of the process in which the organism damages the absorptive mucosal surface and contests for micronutrients. The outcome is metabolic disturbance and therefore unfavourably impacts nutrient utilisation (Ali et al., 2019). All seven *Eimeria* species evolving in the chick's digestive tract in a specific site can induce a wide range of symptoms from mild subclinical enteric infection to subacute high mortality. The clinical conclusion of coccidial infection can be affected by *Eimeria* species, strains, stress, environmental factors, host genetics, simultaneous infections, infective dose and flock size (Nahed et al., 2022; Taylor et al., 2022). It is believed that weight gain is the more sensitive variable to coccidiosis and anti-coccidial treatments (Gerhold et al., 2016). When infective sporozoites arrive at the cecum mucosa by penetrating villus epithelial cells, this results in widespread damage to the cecum epithelia, a bloody stool and a huge number of oocyst excretion (Dutta et al., 1990; Kawazoe & Di Fabio, 1994). In this study, infected broilers were very depressed, revealing ruffled feathers and a lesser feed intake, which may have been due to altered gut homeostasis that led to poor feed intake and metabolism and thus reduced weight gains (Abbas et al., 2013). Interestingly, FCR and body weight at day 42 in Group A were better than in other groups that would be linked to growth-promoting properties of the herbal complex. The lower OPG recorded in the infected group given the herbal extracts (Group A) was comparable with that obtained in the group being administered the toltrazuril (Group B) and was probably the effect of the phenolic compounds in the herbal extracts. Phenols can interact with cytoplasmic membranes and change their cation permeability, leading to impairment of crucial processes in the coccidia cells and, finally, their death (Arczewska-Wlosek & Swiatkiewicz, 2012; Sikkema et al., 1995). Our findings showed a significant reduction in oocysts output in the herbal-treated group (Group A) in comparison with the toltrazuril-treated group (Group B) and the result of the current experiment is backed by Qaid et al. (2021). Birds in Groups A and B had higher OPG levels compared with Group C. This would be linked to the over-multiplication of the uninhibited *Eimeria* species (Pop et al., 2019). Birds in groups A and B had better growth performance than group C, despite the higher OPG levels. This may be linked to the effect of diminished injury to intestinal epithelial cells or stimulation of enterocyte renewal, which can provide the substrate for *Eimeria* species multiplication (Pop et al., 2019). Based on the investigation of Arczewska-Wlosek and Swiatkiewicz (2012), the usage of an herbal extract mixture comprising *Echinacea purpurea*, *Thymus vulgaris*, *Allium sativum*, *Origanum vulgare* and *Salvia officinalisi* to some extents reduces the negative effects of *Eimeria* experimental infections (*E. acervulina*, *E. tenella*, *E. maxima* and *E. necatrix*) in broiler chickens. In a recent study by Abbas et al. (2010) and Conway et al. (2002), broiler chickens, which experimentally infected with *Eimeria*, showed more cecal lesion score and lower weight gain and higher oocyst counts as compared to the non-medicated group, which exactly support our findings (Abbas et al., 2010; Conway et al., 2002). All species of *Echinacea* are herbaceous, perennial plants of the family *Asteraceae*. *Echinacea* is well-thought-out one of the top 10 species of herbs known for their disease-resistance activities (Miller & Yu, 2004). *Echinacea* consists of nine spp., three of which (*Echinacea purpurea*, *Echinacea pallida*, and *Echinacea angustifolia*) have been used medicinally all over the world. *Echinacea* is used for the treatment of upper respiratory tract infections in humans, helping to decrease the duration of the cold symptoms (Caruso & Gwaltney, 2005). It also has many other pharmacological potentials, including wound-healing and anti-inflammatory actions (Speroni et al., 2002). Based on the findings of another study, the extracted juice of *Echinacea purpurea* enhanced humoral and cellular immunity in mice by increasing the number of peripheral lymphocytes and monocytes. Moreover, it can also boost the immune system by increasing total immunoglobulins (IgM, IgG) levels (Mishima et al., 2004). Positive effects of this herbal mixture including *Echinacea purpurea* in the treatment of coccidiosis in broilers are also recorded in this study. The *Echinacea purpurea*'s anti-coccidial effect has been referred to as its immunomodulating properties, broadly reported (Arczewska-Włosek et al., 2018; Fabia et al., 2021). *E. purpurea* extract triggers the innate immune response by activating macrophages (Goel et al., 2002), macrophage-derived cytokine creation and activation of natural killer cells and polymorphonuclear leukocytes, therefore, acting as an immunostimulant (Currier & Miller, 2001; Srinivasu et al., 2020). *Glycyrrhiza glabra* extract has bioactive components, such as glycyrrhizin and flavonoids, which have medicinal and pharmacological activities (Pop et al., 2019). The Glycyrrhiza polysaccharide is known to have a solid immune action and extensive involvement in some processes of the immune system. Significantly, the usage of licorice in broiler diets developed the immune organs such as the bursa or, spleen, and as a result of that immune efficacy, livability and health status improve. These herb extracts have been found to show antioxidant and immunogenic potentials, which might increase the feed efficiency, blood biochemical indices of the poultry birds, growth performance and carcass traits and act as a potential solution for solving respiratory, immune and digestive problems in poultries (Alagawany et al., 2019; Hussain et al., 2017).

It is concluded from the present study that the herbal drug containing *Glycyrrhiza glabra and Echinacea purpurea* show improved performance and anti-coccidiosis effects in the broilers challenged by *Eimeria* spp. More research is recommended to ascertain the effectiveness of this drug for controlling coccidiosis in layers, breeders, and turkeys. Furthermore, extraction of active ingredients in these herbs for development of newer drugs may lead to better anti-coccidial efficacy.

## AUTHOR CONTRIBUTION

Conceptualisation: Ali Ghafouri. Methodology: Ali Ghafouri, Abolfazl Ghaniei and Soheil Sadr. Formal analysis and investigation: Abolfazl Ghaniei and Soheil Sadr. Writing – original draft preparation: Soheil Sadr, Amir Ebrahim Tavanaee Tamannaei, Ali Charbgoo and Shakila Ghiassi. Writing – review and editing: Abolfazl Ghaniei and Soheil Sadr. Resources: Morteza Abuali. Supervision: Abolfazl Ghaniei.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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## PEER REVIEW

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