Assessment of Anticoccidial Efficacy of Novel Triazine Compound and Sulfaclozine against Experimentally Induced Caecal Coccidiosis in Broiler Chickens

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INTRODUCTION

Chickens considered as a good source of high biological value animal protein for human consumption. Rising of public awareness of the poultry industry will likely continue to make proper prevention and treatment of several diseases, of which avian coccidiosis. Coccidiosis is still major problems worldwide; caused by a single-cell protozoan parasite belonging to the genus *Eimeria*. It has a significant economic impact on poultry by reducing performance and decreasing productivity, with high rate of mortality and morbidity (Abbas et al. 2017). Conventional control of this problem based mainly on managerial skills...
and the use of anticoccidial drugs (Tewari & Maharana 2011).

Synthetic anticoccidial drugs remain the mainstream agents used in control of chicken coccidiosis globally. Moreover, anticoccidial compounds should be highly effective against all developmental stages of *Eimeria* species, do not disturb the host immune response as well as have no residues in the tissues. In this respect, Toltrazuril, a triazinetrione derivative is licensed drug for not only as prophylactic agent but also as curative drugs against all developmental stages of *Eimeria* spp. (Shivaramaiah et al. 2014). Toltrazuril interferes with the division of the nucleus and with the activity of the mitochondria, which is responsible for the respiratory metabolism of Coccidia. In the macrogamete, toltrazuril damages the so-called wall-forming bodies. In all intracellular developmental stages, severe vacuolization occurs due to inflation of the endoplasmic reticulum (Harfoush et al. 2010; Ramadan et al. 1997).

On the other hand, Sulfaclozine sodium is an efficacious sulphonamide derivative with antibacterial and anticoccidial effect (Li & Bu 2014). As other sulphonamides, it has a good coccidiostatic effects on control and treatment of chicken coccidiosis in experimental and natural infection (McDougald & Fitz-Coy 2008). Sulfaclozine is a competitive antagonist of para-aminobenzoic acid (PABA), a precursor of folic acid, in protozoa and bacteria. Folic acid is a coenzyme necessary for the synthesis of nucleic acid; hence sensitive species do not multiply in the presence of sulfaclozine (Harfoush et al. 2010). Furthermore, feeding of sulphonamides may prevent clinical signs and reduce oocyst production thereby allowing development of protective immunity.

As the world’s poultry industry continues to grow, so does concerns about the control of coccidiosis, which remains one of the most commonly reported disease of chickens (Xie et al. 2001). Therefore, the present study was conducted to investigate the anticoccidial efficacy of toltrazuril, sulfaclozine sodium and their simultaneous use against experimentally induced *E. tenella* in broiler chickens.

**MATERIALS AND METHODS**

**DRUGS**

Toltrazuril oral solution (Kimzuril 2.5%®), each 100 g containing 2.5 g toltrazuril. It is recommended at level of 1 mL kimzuril/liter of drinking water for three successive days. Sulfaclozine sodium monohydrate water soluble powder (sulfaclozimed 30%®), each 100 g contains 30 g sulfaclozine sodium monohydrate. It is recommended at level of 2 g sulfaclozimed/liter of drinking water for three successive days. The two drugs were purchased from Arab company for medical products, Egypt.

**EXPERIMENTAL BIRDS**

A total of 75 one-day old Erbo plus strain chicks from Taraq Deiab Farm, Kotor, Egypt with an average body weight of 45 to 50 g were used in these trials. The birds were kept in individual well-isolated floor pens with wood shawing litter, plastics waters, and feeders. All tested birds were fed a standard commercial diet and were formulated without any anticoccidial medication (Abd El-Salam Hegazy Company, Cairo, Egypt). Strict sanitation practices were maintained in the experimental house before and during the course of experiment. Temperature was adjusted according to the age (at the first week of age it was 32 °C and decreased 2 °C per week till reached 26 °C at one month of age and was fixed at this degree till the end of experiment. Continuous light was also provided throughout the experimental period (Harrison & Harrison 1986). Furthermore, Birds were vaccinated against infectious bronchitis, Newcastle disease and infectious bursal disease according to the standard schedule.

**PREPARATION OF Eimeria INFECTION**

*Eimeria tenella* oocysts were collected from ceci of naturally infected chickens. The infected chickens were selected from a commercial broiler farm obtained from Alpha Laboratory, Gharbia governorate, Egypt. The protocol of isolation and preparation of *E. tenella* oocyst was performed according to Lovelu et al. (2016). The cecal contents were homogenized with water and sieved in a beaker through a fine wire mesh. The filtrate was let to sediment, the supernatant was discarded and the pellet was re-suspended in potassium dichromate 2.5% in the presence of suitable humidity and temperature in a group of petri dishes. The thickness of fluid was not higher than 5 mm to facilitate the oxygen diffusion. Forced aeration was achieved (2-3 time daily) by removing the cover and shaking the suspension for few minutes. The plates were examined microscopically to assign the degree of sporulation. After sporulation, the sporulated oocysts were removed from the fecal debris by a series of centrifugation using NaCl (concentration flotation technique). The suspension was centrifuged at a moderate speed (1500 rpm) for 5-10 min to allow the oocysts to suspend at the top of supernatant and sediment the solids. The floated oocysts were collected by Pasteur pipette. Sporulated oocysts count (oocysts per gram of feces, OPG) was estimated by the use of the Mc Master technique (Lloyd & Soulsby 1978). The coccidial species were microscopically identified following its sporulation in 2.5% solution by using morphological parameters (Eckert et al. 1995).

**EXPERIMENTAL DESIGN**

The chickens were weighed and randomly divided at 15th day of age into five equal groups (each of 15) as the following:
Group\textsubscript{1}: non-infected non treated (control negative). Group\textsubscript{2}: infected non treated (control positive). Group\textsubscript{3}: infected treated with toltrazuril (1 mL/L). Group\textsubscript{4}: infected treated with sulfaclozine sodium (2 g/L). Group\textsubscript{5}: infected treated toltrazuril with (1 mL/L) + sulfaclozine sodium (2 g/L).

Except Group\textsubscript{1}, all groups were infected with $5 \times 10^6$ oocysts of 
\textit{E. tenella} (field isolate) orally by direct administration into the crop using rubber syringe on day 15\textsuperscript{th} of the experiment (Harfoush et al. 2010). Moreover, the application of drug treatment was started at day 20\textsuperscript{th} of age and continued for three successive days.

### ASSESSMENT CRITERIA

#### CLINICAL SIGNS AND GROWTH PERFORMANCE MEASUREMENTS

Throughout the experiment, the morbidity and the numbers of dead birds were recorded daily representing the mortality rate, beside any obvious clinical signs were also recorded. Furthermore, birds were individually weighted weekly, beginning from 15\textsuperscript{th} day of age. The weight gain was calculated by subtracting the initial weight of birds from the final weight of the birds. Feed conversion ratio (FCR) was calculated using the following formula: 

$$ FCR = \frac{Feed \ consumption \ in \ a \ given \ period}{gain \ produced \ in \ the \ same \ period} \quad (El-Ghoneimy \ & El-Shahawy 2017). $$

#### NUMBER OF OOCYSTS PER GRAM (OPG) OF FAECES

The reduction of oocyst output was taken as a criterion for the efficacy of tested drugs. The faecal samples were collected on day 0 (pretreatment) at 20 days old and consequently at day 1 till 13 days post coccidian treatment (from 21 till 33 days old). The oocysts counting were done through Mc Master Techniques as described by Chand et al. (2016). Additionally, the percentage efficacy for tested drugs was determined (Moskey & Harwood 1941).

#### LESION SCORE

Five birds were sacrificed/group at 8\textsuperscript{th} and 22\textsuperscript{nd} days post infection for demonstration of macroscopical caecal lesion score. The lesions were scored on a scale of 0 to +4 according to the severity of the lesions (Johnson & Reid 1970). A score (0) denoted no lesions whereas (+4) denoted severe lesion.

#### HEMATOLOGICAL AND BIOCHEMICAL ANALYSIS

Two blood samples were collected from each group (five birds/group) at 23\textsuperscript{th} and 37\textsuperscript{th} days of age. For hematological parameters, about 1 mL of blood was withdrawn from the wing vein and immediately transferred into sterile test tube containing 1 mg EDTA as an anticoagulant. Complete blood picture (hemoglobin concentration, packed cell volume, red blood cell count, white cells count, and differential leucocyte count) were calculated (Reagan et al. 2019). For biochemical analysis on the other hand, blood was collected without anticoagulant for serum biochemistry determination. Serum was separated after centrifugation at 3000 rpm for 15 min and stored at -20 °C until tested. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Reitman & Frankel 1957), alkaline phosphates (Kind & King 1954), total serum protein (Henry 1964), albumin (Doumas et al. 1971), creatinine (Henry 1974) and uric acid (Barham & Trinder 1972) were measured.

#### HISTOPATHOLOGICAL EXAMINATION

Tissue specimens from cecum, liver, and kidney were taken from chickens in all groups (five birds/group) at the day 23\textsuperscript{th} and 30\textsuperscript{th} of age. Specimens fixed in 10% neutral formalin before dehydration in ascending grading of ethyl alcohol, followed by clearing in xylene and processed in paraffin wax cubes. Specimens cubes were sectioned at 5 μm thickness, then stained with haematoxylin and eosin (H&E) according to the method described by Pop et al. (2015). The count of the parasitic stages within the intestinal tissues was performed blindly and expressed as the average number/mm$^2$ in about 8 HPF. Regarding to histological scoring of both hepatic and renal lesions, eight points score was assessed according 4 parameters (2 points to each) including vascular lesions as congestion and hemorrhage, degenerative changes, necrosis, and inflammation. Each parameter was assessed as 0 no obvious lesions, 1 mild changes and 2 advanced diffuse changes.

#### STATISTICAL ANALYSIS

All the data were expressed as means ± standard deviation (SD). The statistical significance evaluated by one-way analysis of variance (ANOVA) using the statistical software program (SPSS, ver.16.00, USA). Values were considered statistically significant at (p ≤ 0.05).

### RESULTS

#### CLINICAL SIGNS AND MORTALITY RATE

In this study, the birds in the infected non treated group (G\textsubscript{3}) showed ruffled feathers, huddling, depression, loss of appetite, anorexia, and intensive bloody diarrhea, which began on the 6\textsuperscript{th} day post-infection. These signs severed by 7\textsuperscript{th} day post-infection and subsided gradually with the observation of few discolored droppings and varying degrees of depression until the end of the experiment. This signs declined after treatment with toltrazuril (G\textsubscript{4}), and sulfaclozine sodium (G\textsubscript{5}) each alone or in combination (G\textsubscript{4}) and chickens were more apparently healthy during this period. Moreover, the mortality % in G\textsubscript{3} was 33.33% and it
was reduced to 13.33% in G₅, with no mortality observed in G₆, G₇ (Table 2).

GROWTH PERFORMANCE
Infected non treated group (G₁) showed a significant (p ≤ 0.05) decrease in the body weight gain with poor feed conversion ratio. Moreover, there was a significant improvement in these growth performance parameters in G₂, G₄ and G₅ as compared with G₁ (Table 1).

OCOCYST OUTPUT AND LESION SCORE
The infected non treated control group (G₁) showed the highest oocyst counting which reached its maximum count on the 8th day post infection. This highest oocyst counting was significantly decreased after treatment with toltrazuril (G₂) and sulfadiazine sodium (G₃) each alone or in combination (G₄) (Table 3). Based on percentage of reduction in the OPG in faeces, it can be concluded that the combination between Toltrazuril and Sulfadiazine sodium (G₄) was superior followed by Toltrazuril alone (G₂) and Sulfadiazine sodium alone (G₃), respectively (Table 3). On the other hand, the caeca of the infected non treated group (G₁) showed the highest lesion scores which ranged from +3 to +4. The lesion scores were improved by all drugs treatments and ranged from +1 to +2 in G₄ and from 0 to +1 in G₂, G₃.

HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS
Infected non treated group (G₁) showed a significant decrease in RBCs count, HB concentration and PCV% at 23rd and 37th days of age compared with control group (G₀). This decreased number was significantly increased after treatment with toltrazuril (G₂) and sulfadiazine sodium (G₃) each alone or in combination (G₄). There was a marked increase of the total leucocytic count, lymphocytes, heterophils, monocytes, and eosinophils in infected non treated group (G₁) and treated groups (G₂, G₃, G₄) then changed by time towards the normal value till the end of the experiment. With regarding G₄, there was a significant increase in total leucocytic count and eosinophils at 37 days of age when compared with G₁ (Tables 4 & 5).

Infected non treated group (G₁) showed a significant increase in serum AST, ALT, ALP, uric acid, and creatinine, with a significant decrease in total protein and albumin compared with G₁ at 23rd and 37th days of age. Moreover, infected treated groups (G₂, G₃, G₄) showed a significant decrease in AST, ALT, uric acid, and creatinine and a significant increase in total protein and albumin compared with G₁ (Tables 6 & 7).

HISTOPATHOLOGICAL FINDINGS
Quantitative scoring of the histopathological findings as illustrated in Figures 1 to 3. The cecal, hepatic, and renal scores markedly elevated in G₁. While the different tissue scores decreased in treated groups (G₂, G₃, & G₄). The parasitic stages count in cecal tissue significantly reduced in all treated groups. G₁ showed a significant decrease of coccidial stages in comparison with G₀ and G₄ (p ≤ 0.05). However, the hepatic and renal scores in G₅ did not show any significance in compared with every single treatment.

On the 23rd, the histological examination of cecal sections of G₁ showed normal mucosal folds with normal intestinal crypts. G₁ showed necrotic enteritis associated with partial or complete sloughing of the mucosa as most of intestinal gland showed presence of coccidial schizonts. Some of the parasitic stages were extended to the muscle layer associated with extensive necrosis, inflammation, and marked inflammatory cells infiltrated such as heterophils, eosinophils, and mononuclear cells. G₄ showed a marked decrease in the necrotic lesion accompanied by decreasing the parasitic stages within lining mucosa and hyperplastic regenerative changes seen within the mucosal lining. Similarly, G₄ showed to decrease the parasitic stages and necrotic enteritis but with a lesser degree than G₁. G₅ showed a marked decrease in parasitic stages, mild degenerative and hyperplastic changes within the mucosa. The liver of G₁ showed normal hepatic tissues. G₁ showed multifocal periportal inflammation consisted of mononuclear cell infiltration mixed with eosinophils. G₁ showed a mild to moderate degree of portal inflammation. G₁ showed to a marked degree of hepatic vacuolation and degeneration. The liver of G₄ showed periportal inflammatory cell infiltration. The kidney of G₁ showed normal renal glomeruli and tubules. G₁ demonstrated coagulative necrosis of the renal tubules. G₄ showed mild degenerative change of renal tubules. G₅ showed degenerative changes within the renal tubules such as vacuolation, myelin membrane on the renal tubules epithelial lining and hyaline cast. G₅ showed similar lesions of G₁.

On the 37th, the cecal mucosa was improved than the previous sacrifice, unless G₁ showed the still presence of parasitic stages seen within the lamina propria and muscle layer. Meanwhile, G₁, G₃, and G₅ showed a marked decrease of coccidial stages which mostly noticed as a dead vacuolated cyst. The liver of G₅ showed still perportal inflammation consisted mainly of mononuclear cells and an abundant number of eosinophils. Also, the kidney showed degenerative changes within the renal tubules with marked regenerative tubular basophilia. The liver and kidney of G₁ were showed mild lesion while G₁ and G₅ showed moderate degenerative changes of the renal tubular epithelium.
TABLE 1. Body weight gain (g) and feed conversion ratio (FCR) (mean±SD) in control and treated groups

| Groups | Body wt. gain (At day 22) | Feed conversion ratio (FCR) (At day 22) | Body wt. gain (At day 29) | Feed conversion ratio (FCR) (At day 29) | Body wt. gain (At day 36) | Feed conversion ratio (FCR) (At day 36) |
|--------|---------------------------|------------------------------------------|---------------------------|------------------------------------------|---------------------------|------------------------------------------|
| G₁     | 444.7±12.15a              | 1.38±0.02c                               | 591.05±25.97a             | 1.35±0.03b                               | 714.09±25.14a             | 1.49±0.06c                               |
| G₂     | 264.79±11.15c             | 1.89±0.05a                               | 524.13±11.24b             | 1.43±0.01a                               | 527.27±30.59b             | 1.7±0.01a                                |
| G₃     | 352.6±13.76b              | 1.68±0.03b                               | 585.59±14.15a             | 1.31±0.02b                               | 683.46±20.19a             | 1.59±0.05b                               |
| G₄     | 344.67±16.35b             | 1.71±0.06b                               | 584.33±16.35b             | 1.30±0.03b                               | 674.23±17.73b             | 1.6±0.02b                                |
| G₅     | 338.93±19.73b             | 1.77±0.02b                               | 587.56±16.14b             | 1.32±0.01b                               | 690.19±16.16b             | 1.57±0.03b                               |

Means within rows with different superscripts differ at P ≤ 0.05. G₁: Non-infected non-treated. G₂: Infected non-treated. G₃: Infected, treated by toltrazuril. G₄: Infected, treated by sulfaclozine sodium. G₅: Infected, treated by toltrazuril + sulfaclozine sodium.

TABLE 2. Lesion score and mortality % in control and treated groups

| Group | Lesion score | NO. of deaths | Mortality % |
|-------|--------------|---------------|-------------|
|       | At 23rd day  | At 37th day   |             |
| G₁    | 0            | 0             | 00.00       |
| G₂    | +4           | +3            | 33.33       |
| G₃    | +1           | 0             | 00.00       |
| G₄    | +2           | +1            | 13.33       |
| G₅    | +1           | 0             | 00.00       |

G₁: Non-infected non-treated. G₂: Infected non-treated. G₃: Infected, treated by toltrazuril. G₄: Infected, treated by sulfaclozine sodium. G₅: Infected, treated by toltrazuril + sulfaclozine sodium.

TABLE 3. Oocysts count (×10⁵)/gram of feces (OPG) (mean± SD) and % of OPG reduction (OPGR%) in infected non treated and treated groups (N=5)

| Age | Group | Infected groups oocyst count (×10⁵)/gm feces |
|-----|-------|---------------------------------------------|
|     | G₆    | G₇                                          | G₈                                          |
|      | Pretreatment oocyst count (20 day)         |                                              |                                              |
| 21 day | OPG   | 400.00±31.24a                              | 108.60±17.21b                              | 156.00±8.63b                              |
|       | OPGR% | -                                           | 71.26                                       | 60.20                                      |
| 22 day | OPG   | 600.00±24.36a                              | 185.20±20.32b                              | 265.00±41.23b                             |
|       | OPGR% | -                                           | 51.005                                      | 32.39                                      |
| 23 day | OPG   | 436.20±22.14a                              | 165.10±5.90b                               | 192.00±37.21b                             |
|       | OPGR% | -                                           | 56.32                                       | 51.02                                      |

Means within rows with different superscripts differ at P ≤ 0.05.
Means within rows with different superscripts differ at $P \leq 0.05$. $G_1$: Non-infected non-treated. $G_2$: Infected non-treated. $G_3$: Infected, treated by toltrazuril. $G_4$: Infected, treated by sulfaclozine sodium. $G_5$: Infected, treated by toltrazuril + sulfaclozine sodium

### TABLE 4. Hematological parameters (mean ± SD) in control and treated groups at day 23 of age

| Parameters      | $G_1$    | $G_2$    | $G_3$    | $G_4$    | $G_5$    |
|-----------------|----------|----------|----------|----------|----------|
| RBCs ($\times 10^6/\mu$L) | 2.72±0.09$^a$ | 2.41±0.01$^b$ | 2.25±0.01$^b$ | 2.3±0.03$^b$ | 1.44±0.13$^c$ |
| Hb (g/dl)       | 9.88±0.14$^a$ | 7.95±0.03$^b$ | 6.8±0.01$^d$ | 7.5±0.09$^e$ | 5.18±0.11$^c$ |
| PCV (%)         | 33.46±0.38$^a$ | 27.17±0.09$^b$ | 24.65±0.04$^e$ | 26.06±0.25$^c$ | 23.25±0.45$^e$ |
| WBCs ($\times 10^3/\mu$L) | 14.24±0.82$^c$ | 16.88±0.24$^b$ | 17.88±1.02$^b$ | 17.55±0.21$^b$ | 19.78±0.41$^a$ |
| Lymphocyte (%)  | 58.24±0.49$^b$ | 60.5±0.16$^b$ | 59.53±0.29$^b$ | 60.92±0.82$^b$ | 65.2±1.59$^c$ |
| Heterophil (%)  | 33.62±0.45$^e$ | 35.1±1.52$^b,c$ | 36.77±1.73$^{a,b}$ | 35.9±0.39$^{a,c}$ | 39.73±0.94$^c$ |
| Eosinophil (%)  | 3.53±0.23$^{a,d}$ | 4.8±0.59$^{a,d}$ | 6.1±0.58$^{a,b}$ | 5.3±0.52$^{a,c}$ | 7.2±0.25$^a$ |
| Monocyte (%)    | 3.4±0.29$^{a,d}$ | 4.35±0.38$^{a,d}$ | 5.4±0.59$^{a,b}$ | 4.62±0.64$^{a,c}$ | 6.6±0.29$^a$ |

Means within rows with different superscripts differ at $P \leq 0.05$. $G_1$: Non-infected non-treated. $G_2$: Infected non-treated. $G_3$: Infected, treated by toltrazuril. $G_4$: Infected, treated by sulfaclozine sodium. $G_5$: Infected, treated by toltrazuril + sulfaclozine sodium
### Table 5. Hematological parameters (mean ± SD) in control and treated groups at day 37 of age

| Parameters               | G₁   | G₂   | G₃   | G₄   | G₅   |
|--------------------------|------|------|------|------|------|
| RBCs (×106/μL)           | 2.85±0.04 a | 1.88±0.12 b | 2.83±0.01 a | 2.81±0.02 a | 2.84±0.03 a |
| Hb (g/dl)                | 9.75±0.09 a | 6.06±0.07 c | 8.32±0.04 c | 7.7±0.17 d | 8.94±0.03 b |
| PCV (%)                  | 33.63±0.15 a | 25.07±0.31 d | 28.57±0.06 b | 25.99±0.3 c | 28.84±0.09 b |
| WBCs (×103/μL)           | 14.32±0.22 a | 17.25±0.62 a | 15.14±0.98 a | 15.88±0.35 b | 15.12±0.73 b |
| Lymphocyte (%)           | 58.47±0.98 a | 60.15±1.95 a | 59±1.4 a | 59.1±0.92 a | 58.93±1.17 a |
| Heterophil (%)           | 34.17±0.65 a | 35.21±0.97 a | 34.71±1.53 a | 34.95±0.65 a | 34.6±1.88 a |
| Eosinophil (%)           | 3.54±0.59 a | 5.67±0.3 a | 3.57±0.47 a | 3.6±0.49 b | 3.57±0.49 b |
| Monocyte (%)             | 3.2±0.23 a | 4±0.38 a | 3.33±0.6 a | 3.43±0.35 a | 3.33±0.6 a |

Means within rows with different superscripts differ at P ≤ 0.05. G₁: Non-infected non-treated. G₂: Infected non-treated. G₃: Infected, treated by toltrazuril. G₄: Infected, treated by sulfaclozine sodium. G₅: Infected, treated by toltrazuril and sulfaclozine sodium

### Table 6. Biochemical parameters (mean ± SD) in control and treated groups at day 23 of age

| Parameters      | G₁   | G₂   | G₃   | G₄   | G₅   |
|-----------------|------|------|------|------|------|
| AST (U/L)       | 43±0.00 e | 92.1±0.36 a | 74.1±0.34 b | 76.7±0.35 c | 80.0±0.08 b |
| ALT (U/L)       | 40±0.69 d | 88.9±0.68 a | 69.0±0.74 b | 70.8±0.8 c | 73.0±0.39 b |
| ALP (U/dl)      | 43.76±0.04 e | 93.2±0.01 a | 76.1±0.04 d | 77.6±0.04 c | 79.5±0.01 b |
| TP (g/dl)       | 3.5±0.03 a | 1±0.06 a | 1.9±0.06 b,c | 1.78±0.01 c,d | 1.69±0.06 a |
| Albumin (g/dl)  | 1.93±0.03 a | 0.49±0.01 a | 0.72±0.01 b | 0.68±0.01 b | 0.61±0.02 c |
| Creatinine (mg/dl) | 0.4±0.003 | 1.69±0.04 a | 0.8±0.12 b,c | 0.88±0.01 b,c | 0.95±0.09 b |
| Uric acid (mg/dl) | 6.9±0.051 | 9.9±0.03 a | 8.3±0.04 b,d | 8.5±0.01 b,c | 8.7±0.06 b |

Means within rows with different superscripts differ at P ≤ 0.05. G₁: Non-infected non-treated. G₂: Infected non-treated. G₃: Infected, treated by toltrazuril. G₄: Infected, treated by sulfaclozine sodium. G₅: Infected, treated by toltrazuril and sulfaclozine sodium

### Table 7. Biochemical parameters (mean ± SD) in control and treated groups at day 37 of age

| Parameters      | G₁   | G₂   | G₃   | G₄   | G₅   |
|-----------------|------|------|------|------|------|
| AST (U/L)       | 44±0.03 a | 88.6±0.04 b | 70±0.02 c | 73±0.15 c | 75.1±0.08 b |
| ALT (U/L)       | 41±0.28 a | 85.1±0.49 a | 67.3±0.59 a | 67.8±0.54 a | 71.2±0.24 b |
| ALP (U/dl)      | 44.27±0.02 a | 87.9±0.03 a | 68.6±0.01 c | 73.2±0.05 c | 75.0±0.01 b |
| TP (g/dl)       | 3.21±0.05 b | 1.31±0.05 a | 2.5±0.06 b | 1.9±0.03 c | 1.8±0.01 c |
| Albumin (g/dl)  | 2.03±0.01 b | 0.56±0.01 c | 0.78±0.01 b | 0.75±0.02 b | 0.64±0.01 c |
| Creatinine (mg/dl) | 0.5±0.1 a | 1.33±0.01 b | 0.7±0.01 c | 0.82±0.01 b | 0.89±0.01 b |
| Uric acid (mg/dl) | 7±0.07 c | 9±0.07 a | 8±0.04 b c | 8.3±0.04 b | 8.4±0.05 b |

Means within rows with different superscripts differ at P ≤ 0.05. G₁: Non-infected non-treated. G₂: Infected non-treated. G₃: Infected, treated by toltrazuril. G₄: Infected, treated by sulfaclozine sodium. G₅: Infected, treated by toltrazuril and sulfaclozine sodium
FIGURE 1. A) represents the histological count of coccidial stages, B) represents hepatic score, C) renal score. Data expressed as Means with different superscripts differ at $P \leq 0.05$. G$_1$: Infected non-treated. G$_2$: Infected, treated by toltrazuril. G$_3$: Infected, treated by sulfadiazine sodium. G$_4$: Infected, treated by toltrazuril and sulfadiazine sodium.

FIGURE 2. Histopathological findings of the cecal, hepatic, and renal tissues of the different groups sacrificed on the 23rd day. The first lane represents the cecum, liver and kidney of G1, 2nd lane represents the cecum, liver and kidney of G2, 3rd lane represents the cecum, liver and kidney of G3, 4th lane represents the cecum, liver and kidney of G4, 5th lane represents the cecum, liver and kidney of G5. Arrows indicate parasitic stages; arrowheads reveal periportal inflammatory cells infiltration and curved-arrow shows necrotic and degenerative changes within the renal tubules, bar= 100 $\mu$m.
DISCUSSION

Toltrazuril and sulfadiazine sodium blocked the production of coccidia stages in the cecum, which in turn reduced the number of oocysts in feces. Toltrazuril is effective against all intracellular developmental stages including those of schizogony and gametogenic (Mehlhorn et al. 1988). Toltrazuril enhances natural immunity (Greif 2000). Due to antibacterial activity, the sulfonamide drug prevents secondary bacterial infections, which often occur after coccidiosis (Yegani & Korver 2008). Sulfadiazine sodium may reduce the deleterious effects of coccidiosis in broilers (Ashraf 2011). It interferes with the synthesis of folic acid, which required for deoxyribonucleic acid synthesis (Haritova et al. 2013). Therefore, toltrazuril when used alone, or in combination with sulfadiazine sodium, has a potent anticoccidial effect and improved body weight gain. In addition, the slight clinical manifestation of illness all over the experimental period, decreased lesion score and oocyst output was observed (Ashraf 2011; Lovelu et al. 2016).

Infection with *E. tenella* induced a significant decrease in RBCs, Hb, and PCV; this may be due to the profuse cecal hemorrhages caused by *E. tenella* infection, which leads to anemia. It also induced a significant increase in total leucocytic count and differential leucocytic count as (lymphocytosis, heterophilias, monocytosis, and eosinophilias). These hematological findings were in agreement with Adamu et al. (2013) and Akhtar et al. (2015). Increase in leucocytic count expressed as lymphocytosis, heterophilias, monocytosis, and eosinophilias confirmed by Bremner (2018) who stated that heterophils are the major phagocytic cells in the population of leucocytes, mast cells could play a role in primary inflammatory cells, eosinophils which mainly increased during parasitic infestation, monocytes as 2nd line of defense increased in case of protozoal infection and lymphocytes which consider as a wandering cells by migration during the inflammatory immune response. Moreover, hematological parameters markedly improved in toltrazuril and toltrazuril plus sulfadiazine sodium.
treated groups than sulfaclozine sodium only treated group. Similar improvement in hematological parameters was also recorded by Harfoush et al. (2010) and Youssef et al. (2015).

A significant increase in serum ALT, AST, ALP, uric acid, and creatinine and a significant decrease in total protein and albumin in infected non treated group may be due to the impaired liver function and injury of liver and kidney parenchyma due to harmful effect of *Eimeria* parasite. These findings are following those obtained by Harfoush et al. (2010) who found that infection with *E. tenella* showed unexpected increase in ALT and AST. This noticed that although *Eimeria* infection might not have a direct effect on the liver. On the other hand, more support could be obtained from the fact that *Eimeria* caused diarrhea and favors secondary bacterial infection like clostridia and their toxins may affect the enzymes of the liver. The liver is considered the precursor of most serum protein. Generally, hypoproteinemia occurs in chronic renal, hepatic diseases, malnutrition, and malabsorption of nutrients. In the present study, the pattern of total protein and albumin indicates hypoproteinemia which may be attributed to AL-Saeed et al. (2017) and El-Maksoud et al. (2014).

Moreover, Mondal et al. (2011) and Patra et al. (2010) reported that, liver function test of the infected broiler chickens with *Eimeria* spp. showed a significant increase in the serum ALT, AST. They suggested that significant damage of cell lining of the cecal wall along with their inflammation, severe blood loss causing tissue loss from the body may attribute to increased AST activity, and the fall in total plasma protein in the coccidia infected birds might be due to acute stress that leads to cortisol secretion and catabolism of protein. The noticeably increased serum activities of ALP in our study might be associated with the metabolic alteration and damage of the bone marrow as compensation for the blood losses; the bone marrow might force to produce excessive blood cellular components. Alkaline phosphatase found mainly in the bone, liver, and intestinal wall, with high levels found in young animals with high osteoblastic activity (Kerr 2008). The changes in the biochemical parameters were significantly improved in toltrazuril treated group than sulfaclozine sodium, toltrazuril plus sulfaclozine sodium groups.

Histopathological findings in G4 were in agreement with Bould et al. (2009) who reported that this severe destruction in cecal mucosal layer, penetrating villus epithelial cells resulting in extensive desquamation of the cecal epithelium and hemorrhagic feces due to the initial adherence and invasion of *E. tenella* to the intestinal epithelium of the host cells which must occur across this mucus interface. At the end of the experiment, we observed the capacity of cecal mucosa to repair itself. This might be explained by developing the chronic stage when the host can modulate its defensive mechanisms through enhancing local mucosa immune responses, and this assumption could be proved by Khalafalla (2009) who cleared that in the absence of reinfection, the life cycle of the parasite in an infected host is self-limiting as a result of building up of immunity during propagation of infective agents through the course of the disease and/or a result of repeated infection. G1, G2, and G3 showed absence of any developmental stages of *E. tenella* accompanied by mild degenerative and hyperplastic changes with the mucosa, these finding agree with Ashraf (2011). The histopathological examination of liver and kidney samples collected from G1 showed multifocal periporal inflammation consisted of mononuclear cells infiltration mixed with eosinophils and coagulative necrosis of the renal tubules, these finding agree with finding reported by Saber (1995). The hepatic and kidney samples from G3, G4, and G5 showed mild to moderate periporal inflammatory cells infiltration and degenerative changes of renal tubules epithelium. There was an improvement in livers and kidneys of G4, G5, and G6 in comparison with G2.

**CONCLUSION**

Through these trials, the use of toltrazuril alone and in combination with sulfaclozine was able to control experimental caecal coccidiosis infection in chickens. The combined use of both drugs can be proven to better manage the infection adequately due to the improved effect of both drugs. But this result needs to be further investigated in the other species of coccidia that infect chickens.

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