

Clinicopathological Studies on the Effect of *Artemisia cina* (Sheih Baladi) on Coccidiosis in Chickens

Fatma M.A. Youssef^{*}, Hala A. Abd El-Hamid^{*} and Effat A. El Sheshtawy^{**}

^{*}Department of Clinical Pathology, Animal Health Research Institute and ^{**}Department of Poultry, Animal Health Research Institute, Ministry of Agriculture, Cairo, Egypt.

THIS EXPERIMENT was conducted to determine the effect of *Artemisia cina* on coccidiosis in poultry. A total of one hundred and fifty broiler chicks were divided to five equal groups, first group kept as control, second group was orally infected with 1×10^4 of *Eimeria tenella* oocysts. Third group was infected with the same dose of *Eimeria tenella* and treated with Artemisia water extract. Fourth group was infected with the same dose of *Eimeria tenella* and treated by Toltrazuril and fifth group treated by Artemisia and non-infected. The birds of all groups were kept under observation for 3 weeks post infection. Group II showed increased mortality rate (16.7%), with very high oocyste shedding 12×10^4 at the 6th day post infection, increased total leucocytic count mainly heterophil, monocyte & eosinophil, and reduction of body weight as well as anemia. Liver function test showed increased in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) accompanied with hypoproteinemia and hypoalbuminemia. While, kidney function test showed increase in the level of uric acid and creatinine. Artemisia water extract treatment relatively minimize the infection, lowered mortality rate and oocyste shedding after and returned the liver and kidney function activities to normal level gradually as nearly as Toltrazuril. Also, Artemisia water extract induces significant increase in body weight of broiler as well as increased lymphocytic count, gamma and beta globulin, these findings indicating that Artemisia has a good effect on the immunity of the bird as compared with the group of chicks treated with Toltrazuril. It could be concluded that Artemisia were effective for controlling coccidia and have positive effect on body weight and survival rates nearly as Toltrazuril.

Keywords: Artemisia, Toltrazuril, *Eimeria tenella*, Poultry.

Avian coccidiosis is an enteric parasitic disease caused by multiple species of the protozoan parasite of the genus *Eimeria* and is considered one of the commonest and economically most important diseases of poultry world-wide, causing production losses, high morbidity (due to acute, bloody enteritis) and mortality rates (Shirley *et al.*, 2005 and Usman *et al.*, 2011). Infection with *Eimeria tenella* is followed by cecal lesions (petechial, thickening, ecchymosis, accumulation of

blood and caseous necrotic material) and bloody diarrhea. Coccidiosis is responsible for 6–10% of all broiler mortalities, and the global economic losses occur as a result of reduction in growth rate and feed conversion efficiency (Usman *et al.*, 2011). It is estimated that the annual loss worldwide is more than 3 billion US\$ (Dalloul & Lillehoj, 2006 and Usman *et al.*, 2011). Consequently, there is increasing interest in the development of alternative strategies of disease prevention (Dalloul and Lillehoj, 2006). As part of this effort, there are studies on the effect of natural products on *Eimeria* infections and on protective immunity against these infections (Dalloul *et al.*, 2006, Hassan *et al.*, 2008 and Lee *et al.*, 2008).

Toltrazuril is one of the most effective anticoccidial drugs for controlling coccidiosis in chicken by increase immune index, body weight gain and survival rates (Salwa *et al.*, 1998). *Artemisia annua* is a plant whose dried leaves have been used in traditional Chinese medicine for over 2 millennia (Drăgan *et al.*, 2010). The effect of *Artemisia* plants on poultry coccidiosis was reported from USA for the first time (Allen *et al.*, 1997 and Abbas, 2012). Extensive studies have shown that artemisinin, an extract from *A. annua*, exhibits multiple studies on its anticoccidial in chickens infected with several species of *Eimeria* (Allen *et al.*, 1997, 1998, Youn & Noh, 2001, Arab *et al.*, 2006, Naidoo *et al.*, 2008 and Abbas, 2012). Moreover, it is also known to be very good modulators of the immune system in animals (Chew, 1995). Aside from antioxidants, and compared to traditional forages, *Artemisia* species also have high concentrations of essential oils which are useful in the maintenance of a favorable micro flora balance, suppression of rumen protozoa, increases nitrogen uptake and reduces methane production (Greathead, 2003). In the last few years were become established more restriction regarding using in poultry feeding of additives, growth promoters, antibiotics and Coccidiostatic. European Union forbidden antimicrobial growth promoters since 2006, and regarding the coccidiostats starting with 2012 is not clear if they will be still in use (Titilincu *et al.*, 2008).

This work highlights the effect of *Artemisia* on coccidiosis in chickens with studies to hematological and biochemical changes.

Material and Methods

Chicks

One hundred and fifty broiler chicks (Cobb) at one day old were used. Chicks were assigned to five groups consisting of 30 chicks for each group. The birds were kept floor reared in separate pens at a density of 8 birds/m², and offered a commercial balanced ration ad libitum (free from anticoccidial drug). At the 20th day, chickens were infected with 1X10⁴ *E. tenella* oocytes. The group treatment with water extract of *Artemisia* started in the same day with infection (at the 20th day) and continued till the end of the study (41 days). The birds of all groups were kept under observation for 3 weeks post infection for clinical signs and mortality. All birds were vaccinated against Newcastle and Gumboro

diseases with Hitcher, LaSota at the 4th, 18th and the 28th days-old and Gumboro vaccines at the 14th and the 24th day-old.

Experimental groups

There were five experimental groups and each was having 30 chicks. The groups include: Group (I): The birds of this group were kept without any infection or medications as a control. Group (II): Birds were infected with coccidian (1 X104) /chick. Group (III): Birds were infected with coccidian (1 X104) /chick and treated with watery Artemisia extract at dose of (7.5ml/liter) in drinking water (Drăgan, et al., 2010) till the end of experiment. Group (IV): Birds were infected with coccidian (1 X104)/ chick and treated with Toltrazuril at dose of 1ml/liter for 48 hours at (20-21, 28 - 29 day old). Group (V): Birds were kept without any infection and treated with watery Artemisia extract at dose of (7.5ml/liter) in drinking water till the end of experiment.

Parasite and dose

The strain of *Eimeria tenella* used was obtained from the field strain from commercial poultry shops in Ismailia. The oocysts were preserved in 2.5% potassium dichromate solution to induce sporulation and kept in a refrigerator (2–5°C) until use. Each bird was challenged with 10,000 oocysts/chicken of *E. tenella* at the 20th day of age.

Preparation of watery Artemisia cina extract

A 500 grams of crumbled Artemisia from a local retailer in Ismailia, Egypt were added to 1 liter of boiled distilled water in a sterile flask, then the flask left for 30 minutes at 20 °C for making Artemisia tea 5/10 (w/v). After this time period, the content of the flask was heated at 95°C for 1 minute, and filtered through sterile cheesecloth to another sterile flask and then filtered through a filter paper into another sterile flask. The content of the flask was cooled to a room temperature and used in the experiment (Youn and Noh, 2001).

Clinical observation and weight measurements

During periods of the study, the birds were examined daily for morbidity and mortality any clinical signs and changes in body weight were checked. Body weights were individually measured for 2 weeks post-infection.

Fecal sampling and oocyst counting

Fecal materials were collected from 5 to 10 days post infection. The fecal samples were analyzed for the presence of coccidial oocysts using a standard fecal flotation technique (Lee *et al.*, 2011). Briefly, 1 g from each sample was suspended with 5 mL saline and pelleted by centrifugation at 1500×g for 5 min. The resulting pellet was re-suspended in saturated sodium chloride (aqueous) and passed through a sieve with a mesh size of 1 mm to remove coarse fecal debris. The resulting filtrate was used in a standard gravity vial fecal flotation using 22 mm×22 mm cover slips. After flotation, the cover slip was mounted on a slide and examined in its entirety for the presence of coccidial oocysts. Total numbers of oocysts were calculated using the following formula:

Total number of oocysts = oocyst count \times dilution factor \times (fecal sample volume/counting chamber volume)/number of birds per cage. Lesions core scores as evaluated at the 7th day (10 chicks) post-challenge using a score of 0-4 (Johnson and Reid, 1970).

Blood sampling

Two blood samples, were taken from wing vein from 10 birds of each group at the 7th, the 14th and the 21st days post infection, one sample was taken with anticoagulant for hematological examination and other for serum separation and biochemical analysis.

Hematological analysis

Complete blood picture (hemoglobin concentration, packed cell volume, red blood cell count and white cells count, as well as differential leukocytic count) were investigated (Jain, 2000).

Serum biochemical analysis

The collected sera were assayed for serum biochemistry. The level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to Reitman and Frankel (1957), creatinine (Young *et al.*, 1975) and uric acid (Caraway, 1963), total serum proteins (Henry, 1964), total albumin (Doumas, 1975) were determined by using Auto analyzer Hitachi 912. Serum protein electrophoresis: Fractionation of serum protein by polyacrylamide gel electrophoresis was done after Laemmli (1970).

Statistical analysis

The data were reported as the mean \pm S.E. Statistical significance was determined using analysis of variance according to (Snedecor and Cochran, 1982). Means were compared by Least Significance Difference (LSD) test at $P < 0.5$ significance level according to (Steel and Torrie, 1980).

Results

Clinical signs: Bloody diarrhea of almost all treated and infected experimental groups was observed from the 4th to the 6th day after challenge with *E. tenella*. In the groups treated with watery extract of Artemisia (0.75%) and Toltrazuril, the extent of bloody diarrhea was milder than that observed in other groups and as compared with the control negative groups (Table 1). The survival rates of chickens infected and treated with *A. cina* and anticoccidial Toltrazuril were (100%) in both groups as compared with that of infected non medicated group (83.3%). The lesion scores increased in infected non-treated groups in comparison to treated groups during the experimental period (Table 2). The mean values of body weight gain in various groups at different weeks after the treatment are shown in Table 3. At the end of experiment, the body weight gain in the group treated with 0.75% water extract of *A. cina* and toltrazuril (1420g and 1360g) respectively were significantly higher than that of infected group (920g). Excreted oocysts in the groups treated with ration supplemented with

Egypt. J. Vet. Sci. Vol. 45-46 (2014 - 2015)

0.75% water extract of *A. cina* (GIII) and Toltrazuril (GIV) (38.000, and 65.000) respectively were lower than that of the infected group (199.000/g of feces) (Table 4). Results of hematological changes were illustrated in Tables 5-7. The biochemical tests were tabulated in Tables 8 & 9.

TABLE 1. Bloody diarrhea of chickens challenged with *Eimeriatenella* and treated with *A. cina* or toltrazuril.

Groups	Blood in feces (days afterinfection)				
	3	4	5	6	7
GI	-	-	-	-	-
GII	-	+	+++	+	-
GIII	-	+	+	-	-
GIV	-	+	+	-	-
GV	-	-	-	-	-

TABLE 2. Mortality rate, lesion score and survival rate in treated and untreated groups.

Groups	No	0-5 days post challenge		6-15 days post challenge		16-21 days post challenge		Total Mortality	Mortality %	Survival rate
		Dead bird	lesion score	Dead bird	lesion score	Dead bird	lesion score			
GI	30	0	0	0	0	0	0	0	0	100
GII	30	2	2.7	1	2.5	2	2.8	5	16.7	83.3
GIII	30	-	0.8	-	0.5	-	0	-	-	100
GIV	30	-	1	-	0.5	-	0	0	0	100
GV	30	0	0	0	0	0	0	0	0	100

TABLE 3. Mean body weight in treated and untreated groups at the end of the experiment

Groups	Average body weight
GI	1350± 8.6 ^b
GII	920±2.1 ^a
GIII	1420±5.6 ^c
GIV	1360± 8.6 ^b
GV	1440±3.4 ^c
LSD	28

TABLE 4. Oocyste excretions from chickens treated with *Artemisia* and challenged with *Eimeriatenella*(x1000).

Groups	Days						Total
	5	6	7	8	9	10	
GI	0	0	0	0	0	0	0
GII	0	120	58	22	7	2	199
GIII	0	9	22	6	1	0	38
GIV	0	22	30	8	4	1	65
GV	0	0	0	0	0	0	0

Discussion

Coccidiosis remains one of the most economically important diseases in poultry industry. Control of coccidiosis has been focused on prophylaxis with anticoccidial drugs in food. Their extensive use has inevitably led to the development of drug resistance, and as a consequence, alternative control strategies against avian coccidiosis were studied. The new approaches include the use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the chicken immune system (Allen & Fetter, 2002, Dalloul & Lillehoj, 2005, Titilincu *et al.*, 2008 and Abbas, 2012).

It has been reported that the water extract of *A. cina* were suppressive towards the development of coccidiosis in chicks (Allen *et al.*, 1998 and Abbas, 2012). Bloody diarrhea of all treated and infected groups were seen during 4-6 days after infection with *E. tenella*. But the extent of bloody diarrhea in the groups treated with water extract of *A. cina* was milder than that of other groups. Also, *A. cina* water extract causes an increase in body weight gain, improves lesion scores and decrease oocyst output were noticed in the infected and treated groups. Oocyst counts were carried out on 5, 6, 7, 8, 9, 10 days after the infection. The counts were zero in control group and non-infected treated *A. cina* group (GV). Large number of oocysts was produced in all the infected groups. However the oocyst count of birds treated with Toltrazuril and water extract of *A. cina* and were significantly lower than the infected non treated group. Kostadinovic *et al.* (2012) reported that bloody diarrhea, the oocyst output and mortality rate were lower in all the treated groups as compared to the infected un-medicated control group. Comparing the medicated groups, the birds treated with 3 mg/kg *Artemisia absinthium* extract showed better results in terms of oocyst count per gram of feces. Titilincu *et al.* (2008) and Brisibe *et al.* (2008) reported that the growth of the broilers were enhanced following the addition of *A. annua* leaves in the starter and finisher diets because of its high protein content and presence of essential minerals such as sodium, potassium, zinc and manganese, amino acids and vitamins. *A. cina* also has an inhibitory effect directly on the oocyst sporulation and damaging effect of sporulated oocysts (Arab *et al.*, 2006, Drăgan, *et al.*, 2010 and Kostadinovic *et al.*, 2012). Mehlhorn *et al.* (1988) reported that toltrazuril is effective against all species of *Eimeria* infecting chickens. Also, reported that toltrazuril is active against all intracellular developmental stages including those of schizogony and gametogony. Greif (2000) who reported that despite high efficacy of toltrazuril, it does not interfere with the development of natural immunity but can even enhance it. Mathis *et al.* (2004) demonstrated that toltrazuril was an effective aid to certain anticoccidial program as well as effective as a stand-alone anticoccidial.

It was clear that infection with *E. tenella* causes macrocytic hypochromic anemia at 1st week. Fatma *et al.* (2008) stated that the infection with *E. tenella* caused severe anemia due to severe hemorrhage which is caused by the second generation schizonts development.

TABLE 5. Hematological picture in broiler chicks experimentally infected with Coccidia and treated groups (n=10)

Time	Parameter	Group					P≤ 0.05
		GI	GII	GIII	GIV	GV	
1W	RBCs ($\times 10^6/\mu\text{l}$)	2.5 ± 0.06 ^a	1.35 ± 0.1 ^b	1.55 ± 0.07 ^b	1.54 ± 0.19 ^b	2.3 ± 0.06 ^a	0.06
	Hb (g/dl)	6.5 ± 0.3 ^a	4.21 ± 0.2 ^b	4.3 ± 0.16 ^b	4.5 ± 0.23 ^b	6.2 ± 0.3 ^a	1.0
	PCV (%)	36.4 ± 0.4 ^a	31.8 ± 0.9 ^b	31.3 ± 0.35 ^b	31.6 ± 0.3 ^b	35.9 ± 0.4 ^a	2.0
	MCV (fl)	14.4 ± 0.5 ^a	23.6 ± 0.4 ^b	20.2 ± 0.5 ^b	20.5 ± 0.42 ^b	15.6 ± 0.5 ^a	2.1
	MCH (pg)	26.0 ± 0.21 ^a	31.2 ± 0.31 ^b	27.7 ± 0.33 ^b	29.2 ± 0.5 ^b	26.95 ± 0.21 ^a	1.2
	MCHC (g/dl)	17.9 ± 0.7 ^a	13.2 ± 0.05 ^b	13.8 ± 0.06 ^b	14.2 ± 0.43 ^b	17.3 ± 0.7 ^a	2.2
	WBCs ($\times 10^3/\mu\text{l}$)	1.76 ± 0.03 ^a	2.53 ± 0.36 ^b	2.56 ± 0.2 ^b	2.50 ± 0.2 ^b	1.80 ± 0.03 ^a	0.5
	H ($\times 10^3/\mu\text{l}$)	0.91 ± 0.01 ^a	1.30 ± 0.3 ^b	1.36 ± 0.1 ^b	1.23 ± 0.2 ^b	0.90 ± 0.01 ^a	0.3
	L ($\times 10^3/\mu\text{l}$)	0.75 ± 0.02 ^a	0.50 ± 0.02 ^b	0.55 ± 0.1 ^b	0.56 ± 0.3 ^b	0.80 ± 0.02 ^a	0.1
	M ($\times 10^3/\mu\text{l}$)	0.04 ± 0.01 ^a	0.18 ± 0.01 ^c	0.19 ± 0.01 ^c	0.14 ± 0.01 ^c	0.10 ± 0.01 ^b	0.07
E ($\times 10^3/\mu\text{l}$)	0.05 ± 0.01 ^a	0.55 ± 0.03 ^b	0.56 ± 0.03 ^b	0.57 ± 0.02 ^b	0.05 ± 0.01 ^a	0.15	
2W	RBCs ($\times 10^6/\mu\text{l}$)	2.28 ± 0.1a	1.49 ± 0.11b	2.24 ± 0.1a	2.10 ± 0.1a	2.26 ± 0.1a	0.3
	Hb (g/dl)	14.3 ± 0.5a	4.13 ± 0.2b	13.6 ± 0.31a	14.13 ± 0.4a	14.6 ± 0.5a	4.0
	PCV (%)	28.5 ± 0.4a	9.9 ± 0.6b	28.4 ± 0.5a	27.6 ± 0.4a	28.6 ± 0.2a	6.0
	MCV (fl)	12.5 ± 0.4a	6.64 ± 0.7b	12.6 ± 0.26a	13.1 ± 0.5a	12.7 ± 0.4a	4.2
	MCH (pg)	62.7 ± 0.5a	27.7 ± 0.4b	60.7 ± 0.47a	67.3 ± 0.27a	64.6 ± 0.3a	7.8
	MCHC (g/dl)	50.2 ± 1.9a.	41.7 ± 0.9b	47.8 ± 4.11a	51.1 ± 2.09a	51.0 ± 0.6a	5.1
	WBCs ($\times 10^3/\mu\text{l}$)	1.19 ± 0.09a	1.22 ± 0.08a	1.86 ± 0.23a	1.26 ± 0.2a	1.98 ± 0.23a	0.5
	H ($\times 10^3/\mu\text{l}$)	0.37 ± 0.3a	0.36 ± 0.3a	0.28 ± 0.3a	0.30 ± 0.4a	0.30 ± 0.3a	0.1
	L ($\times 10^3/\mu\text{l}$)	0.74 ± 0.2b	0.76 ± 0.2a	1.50 ± 0.4b	0.87 ± 0.3a	1.60 ± 0.4b	0.2
	M ($\times 10^3/\mu\text{l}$)	0.05 ± 0.01a	0.08 ± 0.01a	0.05 ± 0.03a	0.06 ± 0.02a	0.05 ± 0.03a	0.1
E ($\times 10^3/\mu\text{l}$)	0.03 ± 0.01b	0.02 ± 0.01a	0.03 ± 0.03b	0.03 ± 0.02b	0.03 ± 0.03b	0.05	
3W	RBCs ($\times 10^6/\mu\text{l}$)	3.2 ± 0.1a	1.34 ± 0.1b	3.3 ± 0.12a	3.38 ± 0.18a	3.4 ± 0.14a	1.2
	Hb (g/dl)	9.8 ± 0.8a	4.18 ± 0.37b	9.9 ± 1.09a	9.8 ± 0.9a	9.7 ± 0.9a	2.5
	PCV (%)	26.5 ± 1.4a	10.11 ± 0.8b	27.0 ± 2.35a	26.7 ± 1.7a	26.9 ± 2.35a	4.8
	MCV (fl)	82.8 ± 0.9a	75.4 ± 1.6b	81.8 ± 1.8a	78.99 ± 1.3a	79.1 ± 1.8a	3.0
	MCH (pg)	30.6 ± 0.5a	31.2 ± 0.46a	30.0 ± 0.7a	28.99 ± 0.58a	28.5 ± 0.7a	3.6
	MCHC (g/dl)	36.98 ± 3.4a	41.3 ± 3.87ab	36.7 ± 3.6a	36.7 ± 3.6a	36.1 ± 3.6ab	4.9
	WBCs ($\times 10^3/\mu\text{l}$)	1.76 ± 0.20a	2.34 ± 0.14b	1.99 ± 0.27a	1.86 ± 0.29a	1.89 ± 0.27a	0.3
	H ($\times 10^3/\mu\text{l}$)	0.74 ± 0.2a	1.29 ± 0.3b	0.79 ± 0.5a	0.78 ± 0.4a	0.75 ± 0.5a	0.4
	L ($\times 10^3/\mu\text{l}$)	0.92 ± 0.1a	0.68 ± 0.3b	1.08 ± 0.4a	0.96 ± 0.3a	1.15 ± 0.4a	0.2
	M ($\times 10^3/\mu\text{l}$)	0.05 ± 0.01a	0.14 ± 0.01b	0.06 ± 0.02a	0.05 ± 0.03a	0.06 ± 0.02a	0.03
E ($\times 10^3/\mu\text{l}$)	0.05 ± 0.01a	0.23 ± 0.05b	0.06 ± 0.03a	0.07 ± 0.02a	0.06 ± 0.03a	0.04	

RBCs = total erythrocytic count Hb=hemoglobin. PCV = packed cell volume. MCV= mean corpuscular volume MCH Pg. = mean corpuscular hemoglobin MCHC = mean corpuscular hemoglobin concentration. WBCs= Total leucocytic count. H= Heterophils. L= Lymphocytes M=Monocytes. E= Eosinophils.

TABLE 6. Some biochemical tests on broiler chicks experimentally infected with coccidia and infected treated groups.

Groups	AST μ l			ALT μ /l			Uric acid mg/dl			Creatinine mg/dl		
	1w	2w	3w	1w	2w	3w	1w	2w	3w	1w	2w	3w
G I	47.1 \pm 3.3 ^a	85.9 \pm 3.4 ^a	66.5 \pm 0.6 ^a	12.96 \pm 0.2 ^a	15.16 \pm 0.3 ^a	14.9 \pm 0.3 ^a	4.9 \pm 0.9 ^a	6.7 \pm 0.5 ^a	5.8 \pm 0.8 ^a	0.48 \pm 0.06 ^a	0.44 \pm 0.05 ^a	0.48 \pm 0.03 ^a
G II	123.6 \pm 4.5 ^b	142.1 \pm 0.9 ^b	127.2 \pm 1.9 ^b	24.3 \pm 0.34 ^b	19.32 \pm 0.3 ^b	21.2 \pm 0.8 ^b	9.4 \pm 1.0 ^b	10.6 \pm 0.5 ^b	9.8 \pm 1.2 ^b	1.0 \pm 0.1 ^b	1.12 \pm 0.13 ^b	0.72 \pm 0.1 ^b
G-III	121.6 \pm 1.6 ^b	91.2 \pm 1.13 ^a	69.8 \pm 2.4 ^a	20.88 \pm 0.2 ^b	16.1 \pm 0.44 ^a	15.8 \pm 0.3 ^a	8.4 \pm 0.8 ^b	6.1 \pm 0.8 ^a	6.1 \pm 0.6 ^a	0.62 \pm 0.11 ^a	0.48 \pm 0.05 ^a	0.55 \pm 0.07 ^a
G IV	120.9 \pm 0.8 ^b	90.3 \pm 1.6 ^a	71.1 \pm 2.98 ^a	21.9 \pm 0.15 ^b	14.16 \pm 0.3 ^a	15.9 \pm 0.2 ^b	8.8 \pm 0.9 ^b	6.6 \pm 0.8 ^a	6.4 \pm 0.6 ^a	0.68 \pm 0.1 ^a	0.54 \pm 0.05 ^a	0.54 \pm 0.04 ^a
G V	46.5 \pm 2.1 ^a	79.9 \pm 3.4 ^a	64.5 \pm 0.3 ^a	13.16 \pm 0.2 ^a	14.16 \pm 0.1 ^a	14.1 \pm 0.1 ^a	4.5 \pm 0.3 ^a	6.2 \pm 0.4 ^a	5.1 \pm 0.2 ^a	0.5 \pm 0.06 ^a	0.48 \pm 0.05 ^a	0.51 \pm 0.03 ^a
$P \leq 0.05$	12.46	5.98	7.4	5.2	2.2	2.3	2.7	1.9	2.5	0.23	0.23	0.2

There was a marked increase of the total leucocytic count, heterophil, monocyte, and eosinophil accompanied with lymphocytopenia during the first weeks in infected non treated and treated groups (II, III and IV) and that may be attributed to stress condition of infection then changed by time towards the normal value till the end of experiment (Fatma *et al.*, 2008). With regarding to the treated groups by *A. cina* there was significant increase in lymphocytic count in the second and third weeks this may be due to the immunostimulant effect of *Artemisia* (Allen & Fetter, 2002, Dalloul & Lillehoj, 2005 and Zhongjuan, 2011) stated that the immunostimulant effect of *Artemisia* on chicken by increase the expression of IL-6 and TNF- α in macrophages and promote T cellular immunity.

Serum ALT and AST activities, uric acid and creatinine were significantly increased in infected groups of chickens and this may be due to the impaired liver function and injury of liver and kidney parenchyma due to the harmful effect of *Eimeria* parasite. These findings agreed with Sena *et al.* (1997), Salwa *et al.* (1998) and Fatma *et al.* (2008). The infected and treated groups showed an increase in liver and renal function tests at the first week then returned gradually to the normal levels in the second and third weeks. The observed decrease in total serum proteins on first week post-inoculation with *E. tenella* in chickens, which may be due to cease of feeding, sloughing of mucosal epithelial cells of caecum and bloody diarrhea (Ruff & Augustine, 1982 and Allen *et al.*, 1998).

Hypoproteinemia, hypoalbuminemia and increased gamma and alpha globulins were observed at the 1st week post infection in all infected and treated groups. Ruff and Augustine (1982) reported that in *E. tenella*-infected chickens, the albumin was decreased, but the alpha, beta, and gamma globulins were increased and had returned to normal by the day 10 post inoculation. On the other hand, there were significant increase in gamma and alpha globulins during the 2nd and the 3rd weeks in treated groups by *A. cina* this may be due to their immunostimulant effects (Allen & Fetter, 2002, Dalloul & Lillehoj, 2005 and Titilincu *et al.*, 2008).

Conclusions

Through this study, we noticed that *A. cina* prevent *Coccidia* similar to Toltrazuril. Coccidiostatic effect was observed with *A. cina* extract at dose of (7.5ml/liter) in drinking water, significantly reduced bloody diarrhea, lesion score and oocytes shedding as compared to the infected groups. *A. cina* could be a potential source of protection agents against coccidiosis in chickens.

TABLE 7. Proteins profile (g/dl)in Serum of broiler chicks experimentally infected with Coccidia and infected treated groups

Time	Parameter	Group					P ≤ 0.05
		GI	GII	GIII	GIV	GV	
W1	Total proteins	3.1 ± 0.03 ^a	2.68 ± 0.1 ^b	2.69 ± 0.01 ^b	2.71 ± 0.02 ^b	3.0 ± 0.03 ^a	0.3
	Albumin	2.1 ± 0.04 ^a	1.38 ± 0.02 ^b	1.35 ± 0.05 ^b	1.42 ± 0.05 ^b	1.9 ± 0.04 ^a	0.2
	Globulin	1.0 ± 0.09 ^a	1.3 ± 0.11 ^a	1.34 ± 0.06 ^a	1.29 ± 0.04 ^a	1.24 ± 0.1 ^a	0.5
	α- globulin	0.3 ± 0.02 ^a	0.4 ± 0.022 ^b	0.4 ± 0.03 ^b	0.42 ± 0.13 ^b	0.40 ± 0.03 ^b	0.05
	βglobulin	0.2 ± 0.04 ^a	0.3 ± 0.03 ^b	0.3 ± 0.04 ^b	0.32 ± 0.12 ^b	0.30 ± 0.04 ^b	0.07
	γ-globulin	0.4 ± 0.03 ^a	0.6 ± 0.02 ^b	0.64 ± 0.12 ^b	0.55 ± 0.04 ^b	0.54 ± 0.12 ^b	0.1
W2	Total proteins	2.82 ± 0.01 ^a	1.79 ± 0.1 ^b	3.26 ± 0.02 ^a	3.22 ± 0.02 ^a	3.10 ± 0.1 ^a	0.4
	Albumin	1.48 ± 0.1 ^a	1.2 ± 0.03 ^b	1.41 ± 0.1 ^a	1.44 ± 0.05 ^a	1.4 ± 0.1 ^a	0.18
	Globulin	1.34 ± 0.1 ^b	1.2 ± 0.1 ^b	1.85 ± 0.1 ^a	1.48 ± 0.05 ^b	1.7 ± 0.1 ^a	0.3
	α- globulin	0.24 ± 0.01 ^a	0.20 ± 0.1 ^a	0.35 ± 0.11 ^b	0.28 ± 0.22 ^a	0.30 ± 0.11 ^b	0.2
	βglobulin	0.2 ± 0.01 ^a	0.2 ± 0.21 ^a	0.20 ± 0.04 ^a	0.2 ± 0.11 ^a	0.20 ± 0.04 ^a	0.15
	γ-globulin	0.9 ± 0.01 ^a	0.8 ± 0.02 ^a	1.30 ± 0.05 ^b	1.0 ± 0.45 ^a	1.20 ± 0.05 ^b	0.1
W3	Total proteins	2.78 ± 0.02 ^a	2.89 ± 0.03 ^a	3.12 ± 0.01 ^a	2.67 ± 0.01 ^a	3.25 ± 0.01 ^a	0.3
	Albumin	1.54 ± 0.1 ^a	1.60 ± 0.02 ^a	1.56 ± 0.02 ^a	1.42 ± 0.04 ^a	1.50 ± 0.02 ^a	0.28
	Globulin	1.24 ± 0.04 ^a	1.29 ± 0.1 ^a	1.57 ± 0.1 ^b	1.25 ± 0.1 ^a	1.75 ± 0.1 ^b	0.12
	α- globulin	0.24 ± 0.02 ^a	0.22 ± 0.1 ^a	0.36 ± 0.13 ^b	0.23 ± 0.11 ^a	0.34 ± 0.13 ^b	0.1
	βglobulin	0.2 ± 0.12 ^a	0.22 ± 0.11 ^a	0.21 ± 0.2 ^a	0.2 ± 0.23 ^a	0.21 ± 0.2 ^a	0.14
	γ-globulin	0.8 ± 0.012 ^b	0.82 ± 0.11 ^a	1.00 ± 0.03 ^b	0.8 ± 0.13 ^a	1.20 ± 0.03 ^b	0.15

References

- Abbas, T.E. (2012)** Phytogetic feed additives as a coccidiostat in poultry bull. *Environ. Pharmacol. Life Sci.*, **1** (7), 22 – 24.
- Allen, P.C., Lyndon, J. and Danforth, H.D. (1997)** Effect of component of Artemisia annua on coccidian infection in chickens. *Poult. Sci.*, **76**, 1156-1163.
- Allen, P.C., Danforth, H.D., Augustine, P.C. (1998)** Dietary modulation of avian coccidiosis. *Int. J. Parasitol.*, **28**, 1131-1140.
- Allen, P.C. and Fetter, R.H. (2002)** Recent advances in biology and immunology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin. Microbiol. Rev.*, **15**, 58-62.
- Arab, H.A., Rahbari, S., Rassouli, A., Moslemi, M.H. and Khosravirad, F. (2006)** Determination of Artemisinin in Artemisia sieberiana and anticoccidial effects of the plant extract in broiler chickens. *Trop. Anim. Health. Prod.*, **38**, 497-503.
- Brisibe, E.A., Umoren, U.E., Owai, P.U. and Brisibe, F. (2008)** Dietary inclusion of dried Artemisia annua leaves for management of coccidiosis and growth enhancement in chickens. *Afr. J. Biotechnology.*, **7**, 4083-4092.
- Caraway, W.T. (1963)** Standard Methods of Clinical Chemistry. Edited by Seligsol, D. Academic Press, *New York and London*, 4.
- Chew, B.P. (1995)** Antioxidant vitamins affect food animal immunity and health. *J. Nutr.*, **125**, 1804 - 1818.
- Dalloul, R.A. and Lillehoj, H.S. (2005)** Recent advances in immunomodulation and vaccination strategies against coccidiosis. *Avian Dis.*, **49**, 1–8.
- Dalloul, R.A. and Lillehoj, H.S. (2006)** Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Rev. Vaccines*, **5**, 143-163.
- Dalloul, R.A., Lillehoj, H.S., Lee, J.S., Lee, S.H. and Chung, K.S. (2006)** Immunopotentiating effect of a Fomitella fraxinea-derived lectin on chicken immunity and resistance to coccidiosis. *Poult. Sci.*, **85**, 446-451.
- Doumas, B.T. (1975)** "Standard Method for Clinical Chemistry", vol. 7, Academic Press, New York. *Clinical Chemistry*, **21**, 10 Acta, 31-78.
- Drăgan, L., Titilincu, A., Dan, I., Dunca, I., Drăgan, M. and Mircean, V. (2010)** Effects of Artemisia annua and Pimpinella anisum on Eimeria tenella (Phylum Apicomplexa) low infection in chickens. *Sci. Parasitol.*, **11**(2), 77-82.
- Fatma M. Yousseff, Dalia H. Mansour, Ramadan, M.T. and Amina A. Dessouki (2008)** Comparative clinicopathological studies on the effect of some herbal plants as anticoccidial agents in broiler chickens. 8th scientific conference of the Egyptian Veterinary poultry association, 197-208.

- Giorio, J.D. (1974)** Clinical chemistry- principle sand techniques Henry *et al.*, Harper and Row. Hagerstown. p. 543.
- Greathead, H. (2003)** Plants and plant extracts for improving animal productivity. *Proc. Nutr. Soc.*, **62**, 279 - 290.
- Greif, G. (2000) Immunity to coccidiosis after treatment with toltrazuril. *Parasitol. Res.*, **86**, 787-790.
- Hassan, S.M., El-Gayar, A.K., Cadwell, D.J., Bailey, C.A. and Cartwright, A.L. (2008)** Guar meal ameliorates Eimeriatenellainfection in broiler chicks. *Vet. Parasitol.*, **157**, 133-138.
- Henry, R.J. (1964)** "Clinical Chemistry, Principles and Techniques", Harper and Row Publishers, New York, p.181.
- Jain, N.C. (2000)** "Schally's Veterinary Hematology", 8th ed., Lea and Fibiger, Philadelphia, U.S.A.
- Johnson, J. and Reid, W.M. (1970)** Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. *Exp. Parasitol.*, **28**, 30-36.
- Klayman, D.L. (1985)** Qinghaosu (Artemisinin): an antimalarial form china. *Science*, **228**, 1049-1055.
- Kostadinovic, L.,Jovanka, L., Galonja-Coghill, T. and Ruzicic, L. (2012)** Anticoccidian effects of the Artemisia absinthium L. extracts in broiler chickens. *Archive Zootechnica*, **15**, 69-77.
- Laemmli, U.K. (1970)** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680-685.
- Lee, S.H., Lillehoj, H.S.,Lillehoj, E.P., Cho, S.M., Park, D.W., Hong ,Y.H., Chun, H.K. and Park, H.J. (2008)** Immunomodulatory properties of dietary plum on coccidiosis. *Comp. Immunol. Microbiol. Infect. Dis.*, **31**, 389-402.
- Lee H.A., Hong, S., Chung, Y. and Kim, O. (2011)** Sensitive and specific identification by polymerase chain reaction of Eimeriatenella and Eimeria maxima, important protozoan pathogens in laboratory avian facilities. *Lab. Anim. Res.*, **27**, 255–258.
- Mathis, G.F., Froyman, R. and Kennedy, T. (2004)** Coccidiosis control by administering toltrazuril in the drinking water for a 2-day period. *Vet. Parasitol.*, **121**,1-9.
- Mehlhorn, H.,Schmahl, G. and Haberkorn, A. (1988)** Toltrazuril effective against a broad spectrum of protozoan parasites. *Parasitol. Res.*, **75**, 6-10.
- Naidoo, V., McGaw, L.J., Bisschop, S.P., Duncan, N. and Eloff, J.N. (2008)** The value of plant extracts withantioxidant activity in attenuating coccidiosisinbroiler chickens. *Vet. Parasitol.*, **153**, 214-219.

- Reitman, S. and Frankel, S. (1957)** A colorimetric method for determine-ation of serum glutamic oxaloacetate and glutamim pyruvic transaminases. *J. Clin. Path.*, **28**, 56-62.
- Ruff, M.D. and Augustine, P.C. (1982)** Effects of coccidiosis on the electrophoretic pattern of serum proteins in chickens. *J. Parasitol.*, **68**,107-111.
- Salwa F. Awadallah, Effat A. ElShishtawy and Sohair Y. Mohamed (1998)** Effectivity of some anticoccdial on immunity and performance index in broiler chickens. Egypt. *J. V. Sci.*, **32**, 53-66.
- Sena, D.S., Suryanarayana, C. and Rao, D.S.T. (1997)** Hematological, biochemical and therapeutic studies on coccidiosis in rabbits. *Indian Vet. Med. J.*, **42**, 225-231.
- Shirley, M.W., Smith, A.L. and Tomley, F.M. (2005)** The biology of avian Eimeria with an emphasis on their control by vaccination. *Adv. Parasitol.*, **60**, 285-330.
- Snedecor, G.W. and Cochran, W.G. (1982)** "Statistical Method", 7th ed., Iowa State, U.S.A.
- Steal R.G. and Torrie, J.H. (1980)** "*Principle and Procedures of Static. A Biochemical Approach*", 2nd ed., McGavous Hill Booh Company, New York, U.S.A.
- Titilincu, A., Santha, B. and Cozma, V. (2008)** Effects of polioel 3 on sporulation and infectivity of Eimeria oocysts... *Lucr. Stiint. Med. Vet.Timisoara*, **41**, 372-378.
- Usman, J.G., Usman, N.G., Ayi, V.K. and Hanna, T.M. (2011)** Anticoccdial Resistance in Poultry: A Review. *New York Science Journal*, **4**, 102-109.
- Youn, H.J.and Noh, J.W. (2001)** Screening of the anticoccdial effects of herb extracts against Eimeriatenella. *Vet. Parasitol.*, **96**, 257-263.
- Young, D.,Pestaner, L. and Giberman, V. (1975)** *Clinical Chemistry*, **21**,10-15.
- Zherng, W. and Wang, S.Y. (2001)** Antioxidant activity and phenolic compound in selected herbs, *J. Agric. Chem.*, **94**, 5165-5170.
- Zhongjuan, L., Chaoling, Y., Jinghui, W., Xin, Y., Hongliang, W. and Zhongliang, Z. (2011)** Effect of Artemisia argyi polysaccharide on immunity activity of macrophages and T cells in mouse. 4th International Conference on: Biomedical Engineering and Informatics (BMEI), 1416-1419.

(Received 16/ 7/2014;
accepted 27/ 9/2015)

دراسات باثولوجيه اكلينيكية على تأثير الشبح البلدي (الارتيميا) على الكوكسيديا في الدجاج

فاطمة محمد احمد يوسف* ، هاله عبد المنعم عبد الحميد* و عفت عاصم الشيشتاوي**
 *قسم الباثولوجيا الاكلينيكية و**قسم الدواجن - معهد بحوث صحة الحيوان -
 وزارة الزراعة - القاهرة - مصر.

أجريت هذه التجربة لتحديد أثر الشبح البلدي على الكوكسيديا في الدواجن. إجمالي مائة وخمسون فرخ تسمين قسمت إلى خمسة مجموعات متساوية، المجموعة الأولى كضابطة والمجموعة الثانية اعطيت ايميريا تينبلا بجرعة 1×10^4 عن طريق الفم والمجموعة الثالثة وكان مصابة بنفس جرعة تينبلا ايميريا وعولجت بمستخلص الشبح المائي والمجموعة الرابعة كان مصاباً بنفس جرعة ايميريالتينبلا وعولجت بالتولترازوريل و المجموعة الخامسة تمت معاملتها بمستخلص الشبح المائي وغير مصابة. وأقيمت الطيور من جميع الفئات تحت الملاحظة لمدة 3 أسابيع بعد الإصابة. المجموعة الثانية أظهرت زيادة في معدل الوفيات (16,7 %) ، مع زيادة عالية جدا في معدل تبويض الكوكسيديا (12×10) في اليوم السادس بعد العدوى، ووجد زيادة عدد كرات الدم البيضاء وهيتروفيل والمونوسايت والايزينوفيل وانخفض في وزن الجسم مع فقر الدم. وزيادة في انزيمات الكبد مصحوبة بنقص نسبة البروتين والألبومين بينما اسفرت وظائف الكلى عن وجود زيادة في نسبة حمض اليوريك والكرياتين. وقد اسفر استخدام مستخلص الشبح الى تقليل العدوى بخفض معدل الوفيات ومعدل التبويض لطفيل الكوكسيديا مع عودة أنشطة وظائف الكبد والكلى الى المستوى الطبيعي تدريجيا مثل اثر العلاج بالتولترازوريل. ايضا كان تأثير مستخلص الشبح واضحا في زيادة وزن جسم الطائر مع زيادة عدد الليمفوسايت والجاما والبيتا جلوبيولين. وهذه النتائج تشير الى ان مستخلص الشبح له تأثير جيد على مناعة الطائر بالمقارنة بالعلاج بالتولترازوريل. ومن ذلك يمكن الاستنتاج بأن الشبح كانت له الفاعلية للسيطرة على الكوكسيديا وله تأثير ايجابي على معدلات الوزن والبقاء على قيد الحياة تقريبا مثل تأثير التولترازوريل.