Impact of *Artemisia Annua* L. Supplementation On Growth Performance And Control Of Coccidiosis In Rabbits

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Abstract: In this study, growth performance and anticoccidial protective effects of *Artemisia annua l.* powder or extract as dietary supplement were studied. Ninety growing new Zealand White rabbits (932 ±32.0g) were randomly distributed in nine experimental groups which established: (T_1) control negative; (T_2) control positive; (T_3) infected and fed 0.5ml of *Artemisia annua* extract;(T_4) infected and fed 1.0 ml extract; (T_5) Infected and supplemented 5 g *Artemisia annua* powder /kg diet;(T_6) infected and supplemented 10g powder/kg diet;(T_7) infected and treated with (Salphaqenioxaline Sodium 30%;(T_8) infected and fed 2.5 ml extract and (T_9) infected and fed 5 ml extract. Artemisinin analysis was performed using HPLC–MS system (Dionex Ultimate 3000 Bremen, Germany). The effects of *A. annua* infection were assessed by clinical signs, mortality, fecal oocyst shedding, lesion score, body weight gain, feed conversion (FCR), relative growth rate (RGR) and Performance Index (PI %). Caecal content pH, volatile fatty acids (TVFA) and total bacterial counts (TBC) were determined. *Artemisia annua l.* extract has a good anticoccidial effect and improved the bacterial count showed the lowest value. Rabbits treated with *A. annua* significantly reduced fecal oocytes (90.76%). The best values of FCR; RGR and PI % of rabbits as affected by *Artemisia annua l* powder or extract doses were significantly ($p<0.05$) achieved by T_8; T_9 groups (3.51; 86.28; 85.54 and 3.77; 61.87; 65.93%, respectively). Also, the best values of caecal TVFA and TBC were significantly ($p<0.05$) recorded by group fed 5ml of *Artemisia annua* extract (T_9) (4.85 meq/100ml juice and 4.73 log cfu/g) compared with other tested groups. The use of *Artemisia annua* extract in 0.5; 1.0 and 2.5 ml (T_3; T_4 and T_8) was better in controlling coccidiosis of rabbits than other experimental groups. ***Conclusively,*** that *Artemisinin* is a promising natural drug for prevention and control of coccidiosis in rabbits. *Artemisia annua* powder at 5g/kg diet achieved a good growth performance and *coccidiosis* prevention, Meanwhile, *Artemisia annua* extract at 2.5ml oral /3days per week recorded the best results of performance and prevention of coccidiosis in growing rabbits, but further studies must be done with adjusted infective doses.

[M.S. Abousekken; M.F. Azazy; A.O.El-Khtam and Walaa K.S. Zaglool. **Impact of *Artemisia Annua* L. Supplementation On Growth Performance And Control Of Coccidiosis In Rabbits.** *J Am Sci* 2015;11(5):159-169]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 19

Key words: *Artemisia annua* extract, Rabbit coccidiosis, Growth performance, lesion score, Anticoccidial effect.

1. Introduction

Artemisia annua is a plant whose dried leaves have been used in management of cocidiosis and growth enhancement in poultry **(Brisibe *et al.*, 2008).** Some studies have shown that artemisinin, an extract from *A. annua,* exhibits high efficacy against several stages of *Plasmodium* **(Golenser *et al.*, 2006).** Also, multiple studies have demonstrated the anticoccidial effects of *A. annua* in experiments performed with chickens infected with several species of *Eimeria* **(Youn and Noh, 2001; Arab *et al.*, 2006 and Naidoo *et al.*, 2008).**

Artemisia annua l. has a high content of flavonoid compounds which are responsible for its high antioxidant activity. There are potential uses of the *Artemisia annua* plant extracts for humans and livestock based on the synergistic effects of flavonoids, artemisinin precursors, etc., including antimalarial effects reported for the *A. annua* traditional tea **(Mueller *et al.*, 2004; Blanke *et al.*, 2008).** *Artemisia annua* and artemisinin uses for the livestock industry based on current reports of its anti-protozoal, anti-bacterial and antioxidant activities of the plant, its extracts, and its essential oil**. (Albert *et al.*, 2010).**

Coccidiosis in the poultry and rabbits industry is caused by any of the species of the genus *Eimeria,* individually or in combination. Outbreaks usually result in enormous economic losses as a result of the associated morbidity and mortality **(Oluyemi & Roberts, 2000 and Alen & Fetterer, 2002).** Also, Coccidiosis causes reduction in poultry feed conversion ratio and body weight gain, as well as a temporary reduction in egg production (**Min *et al.*, 2004 and Dalloul and Lillehoj, 2005)**. Moreover, consumers request poultry products that are free from residual antiparasitic drugs **(Harper and Makatouni, 2002).** The development of alternative control

strategies against avian coccidiosis were studied (**Drăgan *et al*, 2010**). The new approaches include the use of natural products, probiotics, live vaccines, improved farm management practices, and modulation of the rabbit's immune system **(Dalloul and Lillehoj, 2005).** Coccidiosis is mainly controlled by the hygienic measures and the use of chemotherapeutic agents or chemical anticoccidial agents **(Soulsby and Helminths, 1982)**. Increasing of interest in the development of alternative strategies of disease prevention **(Dalloul and Lillehoj, 2006)** led to developed and introduced a lot of anticoccidial drugs and natural products in the poultry industry all over the world. **(Shahbazfar *et al.,* 2011; El-Khtam *et al.*, 2014).**

. Several Studies has been conducted on the effect of natural products on *Eimeria* infections; and protective immunity against these infections **(Giannenas *et al.*,2003; Perazzo *et al.,* 2003; Saini *et al.*,2003; Dalloul *et al.,* 2006; Marques *et al.,* 2006and Rondón *et al.*,2006)** and to find new substitutes to chemical anticoccidials (**El-Khtam *et al.,* 2014**). Aromatic plants, their extracts and essential oils have a variety of functional bioactive compounds. *Therefore,* The objective of this study was to demonstrate growth performance; meat quality and anticoccidial effect as affected by dietary *Artemisia annua* in ether extract or powder form (as a natural feed additive) fed to growing rabbits suffered from coccidiosis.

2. Materials and Methods

The present study was carried out at Rabbit Research Unit, Poultry Research Station, belonging to Environmental Studies and Research Institute, University of Sadat City, Minufiya province, Egypt, during autumn 2014. A total number of Ninety growing new Zealand White (NZW) rabbits at 5 weeks of age (with an average weight 932 ±32.0g) were kept under the same managerial and hygienic conditions. Rabbits were randomly divided into 9 equal groups, 10 for each in 5 replicates. Rabbits were fed two control diets (control negative and isolated control positive) and 7 experimental diets.

2.1. Preparation of powder and ethanolic extract from *Artemisia annua*.

The aerial parts of *Artemisia annua* were collected at flowering stage from the Egyptian western desert and University of Sadat City farm, Sadat City, Minufiya province of Egypt.

In this study, the fresh aerial parts of *Artemisia annua* were harvested, then spread out and dried under shade at room temperature for one week, so they can be crispy for easy milling. The leaves were then ground into a powder which was incorporated to the experimental diets.

About of 100 grams of grinded air dried aerial parts of plant was exhaustively extracted with 750 ml of ethanol in a Soxhlet apparatus at 60 °C then filtered through 0.45 µm membranes prior to use. The collected ethanolic extract was evaporated via rotary evaporator at 40-50 0C under reduced pressure yielding 19 g. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -20°C until use.

2.2. Chemicals and reagents

All solvents and reagents were of the highest purity needed for each application. HPLC–MS grade Methanol, water and formic acid (99.8%) were purchased from Sigma Aldrish Fluka (6 of October city, Cairo). Phytochemical screening of the powdered aerial parts was carried out according to procedures described in **Trease and Evans (1989).**

2.3. Pharmacope ial standards

Methods for the determination of pharmacopoeia standards such as the moisture content, total ash values, water soluble ash values, acid insoluble ash values and extractive values of *A. annua* (aerial parts), were carried out as described by **World Health Organisation's (2006)** publication on quality of medicinal plants.

2. 4. Preparation of Extracts for HPLC- MS Analysis

An aliquot of 25 Grams of grinded air dried aerial parts of Artemisia annua was exhaustively extracted with 750 ml of methanol in a Soxhlet apparatus at 60 °C then filtered through 0.45µm membrane prior to use. The collected methanolic extracts evaporated via rotavapour at 40-50 ◦C under reduced pressure yielded 5.06 gm of the crude methanolic extract. Artemisinin extracts of 100 mg was accurately transferred into a 10 ml volumetric flask and dissolved in methanol. Final volume was made up with methanol to give 10mg/ml solution of *A. annua*. Aliquots of 10µl was injected into the LC-DAD/MS analysis system. Analysis were performed using a Dionex Ultimate 300 (Bremen, Germany) composed of a quaternary pump with an on line degasser, a thermostatted column compartment, a photodiode array detector (DAD), an auto sampler, and Chromelon software. The HPLC–MS system consisted of electro spray ionization (ESI) interfaced Bruker Daltonik Esquire- LC Amazon SL Ion Trap Mass spectrometer (Bremen, Germany) and Dionex Ultimate 300 (Germany) composed of a quaternary pump with an on line degasser, a thermostatted column compartment, a photodiode array detector (DAD), an auto sampler, and Hystar software.

2.5. Experimental design.

The Experimental groups were arranged as the following:

Group 1: (T_1) No infected – non treated (control negative).
Group 2: (T_2) Infected - non treated (isolated control positive).
Group 3: (T_3) Infected and oral by fed with 0.5ml of *Artemisia annua* extract **(100mg/l)** 3days/ weekly.
Group 4: (T_4) Infected and oral by fed with1.0 ml of *Artemisia annua* extract **(100mg/l)** /3days / weekly.
Group 5: (T_5) Infected and supplemented with 5 g of *Artemisia annua* powder /kg diet. **Group 6:** (T_6) Infected and supplemented with 10 g of *Artemisia annua* powder /kg diet
Group 7: (T_7) Infected and treated with Salphaqenioxaline Sodium 30%.
Group 8: (T_8) Infected and oral by fed with 2.5 ml of *Artemisia annua* extract **(200mg/l)** /3days/ weekly.
Group 9: (T_9) Infected and oral by fed with 5 ml of *Artemisia annua* extract **(200mg/l)** /3days/ weekly. All rabbit groups except group 1 were naturally infected with mixed ***Eimeria spp.***. Group 7 treated with Salphaqenioxaline Sodium 30%, Water soluble powder) at 1 g / liter drinking water at the same day of starting till the end of experiment (15 weeks of age). All rabbits were kept under the same managerial conditions. Feed and water were offered *ad libitum* throughout the experimental period (5 to 13 weeks of age). Live body weight (BW), feed intake (FI) and number of dead rabbits were recorded weekly. Daily weight gain (BWG), feed conversion ratio (FCR), performance index (PI) and mortality rate were calculated.

Table 1. Formulation and chemical composition of the basal diet used in the trial*

Ingredients	Clover hay (CH),	Wheat bran,	Barley,	Soybean meal (44%CP),	Yellow corn,	Limestone,	Nacl,	Vit & Min. premix**	Total
%	28.00	28.00	20.00	12.00	10.00	1.00	0.50	0.50	100.00

Chemical composition of the basal diet (DM basis).									
DM (%)	OM%	CP %	CF %	EE %	NFE %	Ash %	DE $_{kcal/kg}$***	ADF %	ADL %
91.20	88.77	17.18	12.42	3.11	56.06	11.23	2543	22.43	2.81

* Calculated according to **NRC (1977).**
** Each one kg of vitamin & mineral mixture contains: Vit.A 4000000 IU; Vit D_3 50000IU; Vit E 16.7g.; Vit K_3,0.67g.; Vit.B_1 67g; VitB_2 2.00g; Vit. B_6 0.67g; Vit B_{12} 3.33mg; Cholin chloride 400g.; Biotin 0.07g;Niacin 16.7g.; pantothenic acid 6.7g; Folic acid 1.7g;; Copper 1.7g; Iron 25.00g; Manganese 10.00g; Iodine 0.25g; Selenium 33.3g; Zinc 23.3g and Magnesium 133.3g.
*** DE *(Mcal/kg)* = 4.36 – 0.049 x NDF, NDF% = 28.924 + 0.657 (CF%) according to **Cheeke (1987).**
DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

2.6. Dosage and solution preparation.

Table 2 cleared the methods of solution preparation according to **Erhirhie *et al.* (2014)** and Dry alcohol extract dissolution and volume selection according to **Karl-Heinz, *et al.* (2001) and Nebendahl (2000).**

Table 2.The *A. annua L.* dosage preparations for solution extract concentrations and powdered leaves fed to experimental rabbits.

Artemisia annua extract (mg/L dist. Water)		*Artemisia annua* Powder (g/kg diet)
0.5ml from100mg/L	1.5ml from100mg/L	5.0g/kg diet
2.5 ml from 200mg/L	5.0ml from 200mg/L	10.0g/kg diet

2.7. Caecal activity.

At the end of experimental period (10 weeks), six rabbits(15 weeks old) per group were taken randomly, fasted for 12 hrs, weighed and slaughtered in a specialised slaughterhouse the caeca were tied at both ends, separated by sterile instruments from the rest of the gastrointestinal tract, placed in tightly closed plastic bags and put in pre-warmed thermos. After sampling, the material was transported as soon as possible (about 1 h) to the laboratories. In the laboratory, caecal content was collected; Values of pH were immediately measured in ceaecal content using pH meter (Model HI 8424) and submitted to chemical analysis **(AOAC, 2005).** In diluted caecal content, volatile fatty acids VFA's concentration was determined by steam distillation as mentioned by **Eadie *et al.*(1967)** and according to methods described by **Bovera *et al.* (2012).**

Total anaerobic bacterial counts (as a log of colony forming unit cfu/g) were estimated and

recorded according to the microbiological method described by **Collin *et al*. (1995)** and **Awad (2003).**

2.8. Ethical Considerations.

Experiments with rabbits were done according to the ethical standards formulated in the Declaration of Helsinki, and measures taken to protect rabbits from pain or discomfort. It has been approved by institutional ethical review board according to Ethical Committee of the University of Sadat City, Egypt, 2014.

2.8. Experimental drug.

Salphaqenioxaline (Salphaqenioxaline Sodium 30%, Water soluble powder, Pharma swede Pharmaceuticals company Ltd., Egypt) a commercially available anticoccidial drug for the routine treatment of avian coccidiosis (due to Eimeria species) was used to compare the anticoccidial effects of the tested plant powder.

2.9. Clinical and Parasitological examination (Oocyts count).

The clinical signs as growth depression, ruffled hair, diarrhea, loss of appetite were recorded during experimental period. Fresh feces were collected from the litter spread on the battery tare from each group for oocysts count biweekly when only few or no oocysts could be detected in feces. The mean number of oocysts per gram faeces for each group was counted using the Mc Master counting technique according to the method described by **Long and Joyner (1976)**. The daily number of dead rabbits as a result of coccidian infection were recorded and calculated as a percentage of mortality.

2.10. Post mortem examination

It was carried out at 0 day, 2th, 4th, 6th and 8weeks. Three rabbits from each group were randomly picked up for P.M. examination as well as all rabbits that died during the experiment. Lesion scores were determined by macroscopic examination of the intestine and ceci of each rabbit according to **Elbahy *et al* (2006).** Lesion score was 0 when no evident lesions were detected while a score 3 referred to the severely affected rabbits.

Table 3. Lesion Score according to Elbahy *et al*. (2006).

Grade	Remarks
Grade 0 (-)	• No evident lesions.
Grade 1 (+)	• Light redness of the intestinal wall. • Mild thickening of the intestinal wall. • 1-3 focal lesions in 3 cm. of the intestinal wall.
Grade 2(++)	• Moderate redness of the intestinal wall. • Moderate thickening of the intestinal wall. • 3-6 focal lesions in 3 cm. of the intestinal wall. • Ballooning in the caecum.
Grade 3 (+++)	• Severe congestion of the intestinal wall. • Increase the thickening of the intestinal wall. • Ballooning in the caecum and presence of "bloody cecal core".

2.11. Statistical analysis.

The statistical analysis of data obtained were performed by using analysis of variance (one way analysis) as described in SAS 9.1 program **(SAS® institute, 2003).** Significant differences between treatment means were distinguished by using Duncan's Multiple Range Test **(Duncan, 1955).** All statements of significance were based on P≤0.05. The statistical model used in the experiment was: $Y_{ij} = M + T_i + E_{ij}$ where: Y_{ij} = The individual observation.; M = The overall mean.; T_i = The effect of dietary treatment (i=1, 2,,.,.,.,.,4.,) and E_{ij} = The experimental error.

3. Results and Discussion

Artemisia annua l. has a high content of flavonoid compounds which are responsible for its high antioxidant activity. There are potential uses of the *Artemisia annua* plant extracts for humans and livestock based on the synergistic effects of flavonoids, artemisinin precursors, etc.,

3.1- **HPLC-DAD and HPLC-MS analysis of *Artemisia annua L*. extract.**

HPLC-positive mode ESI-MS base peak chromatogram (BPC) of the crude ethanolic extract of *Artemisia annua l.* showing artemisinin peak eluted at 35.2 min as the main major active ingredient is represented in Figure 1 (A). Meanwhile, HPLC-DAD chromatogram of the crude ethanolic extract of Artemisia annua monitored at 214, 217, 290 and 280 nm and UV spectra (DAD) recorded between 195 and 500 nm showing very low absorbance of artemisinin peak in the ultraviolet (UV) regions compared with the LC-positive mode ESI-MSn base peak chromatogram (BPC) profile which is presented in Figure 1 (B).

The developed analytical system HPLC/DAD/MSn in our study made it possible to

separate (Rt =35.2) and quantify artemisinin in both plant materials and pure samples. Identification of artemisinin was based on retention time, injection of different dilutions of artemisinin standard and confirmed by comparing the mass spectral fragmentation pattern of both pure standard and artemisinin peak in the ethanolic extract of *Artemisia annua* with each other's and to those reported in literature.

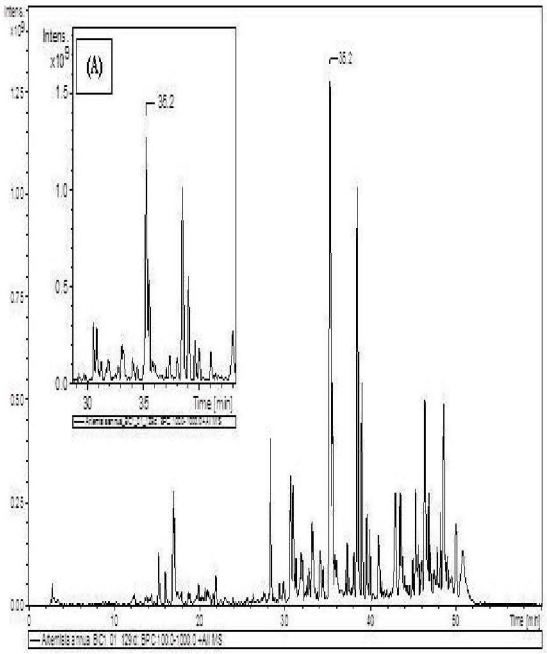

Fig. 1(A): Typical HPLC-positive mode ESI-MSn base peak chromatogram (BPC) profile of the methanolic extract of *Artemisia annua* showing artemisinin peak eluted at 35.2 min as the major active ingredient.

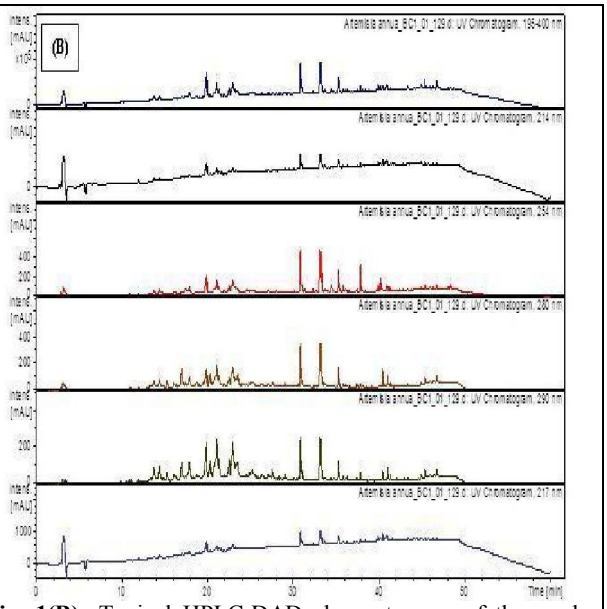

Fig. 1(B): Typical HPLC-DAD chromatogram of the crude methanolic extract of *Artemisia annua* monitored at 214, 217, 290 and 280 nm and UV spectra (DAD) recorded between 195 and 500 nm showing very low absorbance of artemisinin peak in the ultraviolet (UV) regions compared with the LC-positive mode ESI-MSn base peak chromatogram (BPC) profile

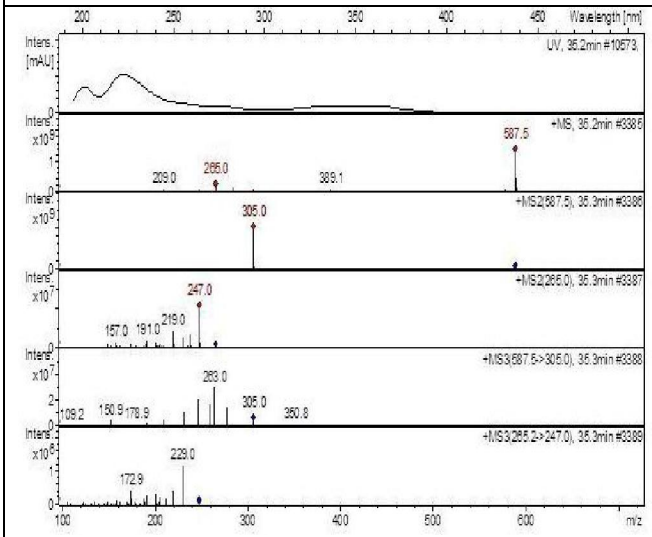

Fig. 2: The HPLC/DAD/MS3 mass spectra and fragmentation pattern of artemisinin peak present in the methanolic extract of *A.annua* as well as that of artemisinin standard.

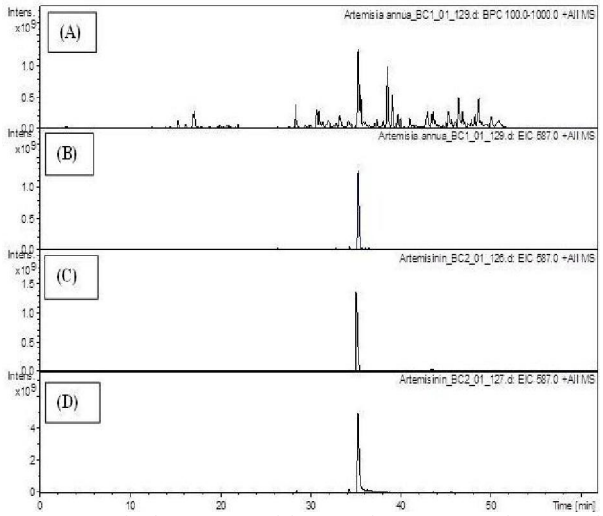

Fig 3: Typical HPLC-positive mode ESI-MSn base peak chromatogram (BPC) profile of the methanolic extract of *Artemisia annua* showing artemisin in peak as the major active ingredient.

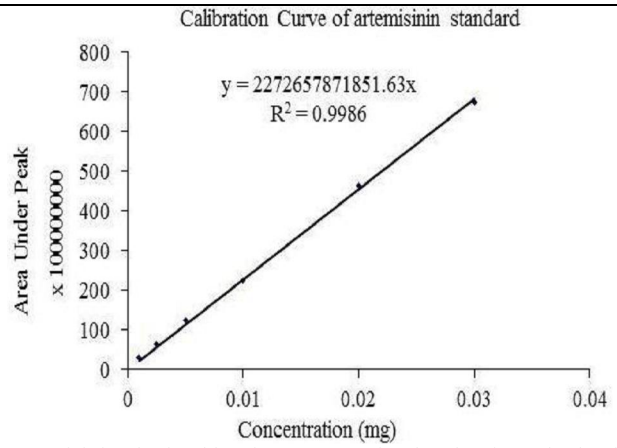

Fig.4: Calibration Curve of Artemisinin obtained by six concentration levels of standard, with three injections per level.

3.2. Rabbits Performance.

3.2.1. Growth Performance.

Results presented in Table 4 cleared that final body weight significantly (p<0.05) improved with T_3 and T_9 groups while, insignificant difference was observed between T_2 and T_8 groups. There were significant (p<0.05) differences in feed consumption and weight gain between all experimental rabbit groups,Meanwhile, the best gain was obtained by group (T_9)(5 ml) (1.44 Kg) compared with other experimental groups. Results of feed intake indicated that there were insignificant difference among experimental rabbits fed diets containing 5 and 10g *Artemisia annua* powder /Kg diet or groups fed 2.5; 5 ml *Artemisia annua* extract and T_7 being received chemical drug group (CD).The reduction of feed intake values were recorded with T_2 group (control +). These results were in agreement with those reported by **Li and Peggins (1998)** who showed that decrease in feed and water consumptions in rats after administration of the first or second doses of intramuscular artemether, arteether, and dihydroartemisinin. Better results were registered for weight gain (1.44 kg/rabbit) with group T_9 (5ml) and feed conversion (3.51 kg feed/ kg gain), with group T_8 (2.5ml).

Among feed conversion ratio (FCR); relative growth rate (RGR) and performance index (PI %) of rabbits as affected by *Artemisia annua l* powder or extract doses, results showed that the best values significantly (p<0.05) achieved by T_8; T_9 groups (3.51; 86.28; 85.54 and 3.77; 61.87; 65.93%, respectively). The mode of action of artemisinin has been attributed both to its potential to induce a state of oxidative stress through the free radical cascade generated by the endoperoxide function **(Levander *et al.*, 1989 and Patricia *et al.*, 1998)**, and to the ability of the free radical to alkylate protein **(Yang *et al.*, 1994).**

Two separate modes of action can be attributed to the efficacy of plant extracts for treating parasitic infections: their immunomodulatory properties and their antiparasitic effects **(Anthony *et al.*, 2005).** In our case *Artemisia annua l* extract was a good anticoccidial effect and improved both of weight gain and feed conversion.

3.2.2. Caecum activity.

Data illustrated in Table 5 show caecum morphology (weight and length); pH, total bacterial counts (TBC) and total volatile fatty acids (TVFA) of caecal contents for growing rabbits naturally infected with mixed *Eimeria spp.* and supplemented with dietary *Artemisia annua* either in ether extract or powder form. Results of caecum weight as percent of live body weight cleared that groups fed **T_6** (10g *Artemisia annua* powder /Kg diet); **T_7** (Salphaqenioxaline Sodium 30 %,); **T_8**: (2.5 ml *Artemisia annua* extract) and **T_9** (5ml of *Artemisia annua* extract) did not significantly (p<0.05) differ from **T_1**(Control +). This means that the infected rabbits which treated by either 10g *Artemisia annua* powder **(T_6)** or 2.5 ml *Artemisia annua* extract**(T_8)** or 5ml of *Artemisia annua* extract **(T_9)** and Salphaqenioxaline Sodium 30% **(T_7)** has significantly (p<0.05) normal caecum weight compared with T_1(control -) group. The results of total bacterial counts (TBC) of caecal content showed that the lowest values were significantly (p<0.05) recorded by groups fed 5ml of *Artemisia annua* extract(T_9) (4.73 log cfu/g) compared with control and other tested groups. These results are in agreement with the results of **Kim *et al.* (2011)**, who found that supplementation of 0.05% mannan-oligosaccharide (MOS) in broiler diets significantly decreased the populations of *Clostridium perfringens* and *Escherichia coli*, while lactobacilli and total bacteria increased as compared with control group. The digestion of carbohydrate in rabbits is primarily in the

large intestine (caecum) due to intestinal microflora, therefore, we can hypothesize that the additives used in the trial are able to modify the microbial bacterial population or its activity in the caecum **(Bovera *et al.*, 2006 and 2012)**.

The same trend was observed among the total volatile fatty acids which showed that the best value of total volatile fatty acid (TVFA) contents significantly (p<0.05) were recorded by group fed 5ml of *Artemisia annua* extract (T_9) (4.85 meq/100ml juice), Meanwhile, control- group (T_1) was the worst one (2.24 meq/100ml juice).This means that 5ml of *Artemisia annua* extract may play a role as antimicrobial substrate under coccidian infection stress. The same results were obtained by **Awad (2003); Abousekken *et al.* (2007 and 2008)**. In this connection, **Radwan and Kahlil (2002)** reported that rabbits with neutral pH value had the highest bacterial number. **De Blas and Wiseman(1998)** detected that the presence of microbial populations in rabbit caecum practicing cecotrophy permit obtaining additional energy and nutrients. These results are in agreement with findings by **Mateos *et al.* (2010)** who indicated that supplementation of rabbit feeds with certain oligosaccharides and increases volatile fatty acids in the caecam of weanling rabbits, decreasing the caecal ammonia concentration

3.3. Veterinary Studies.

3.3.1. Clinical signs

The symptoms of coccidiosis in rabbits were depression, wasting, decreased activity, diarrhea, ascitis, polyuria, distension of the abdomen and some deaths in some groups. The symptoms were mild in non-infected non treated group (T_1) and treated or supplemented groups.

The symptoms were severing in infected non treated group (T_s) with mortality rate (30%). Symptoms of intestinal coccidiosis started from 5th dpi with maximum strength at 7th and 9th d.p.i. and then regression and disappearance of diarrhea till the end of the experiment (Table 6).

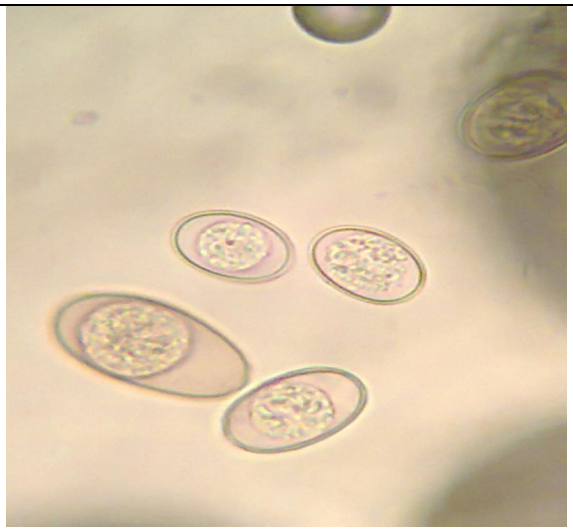

Fig. 5 A Sporulated Oocytes

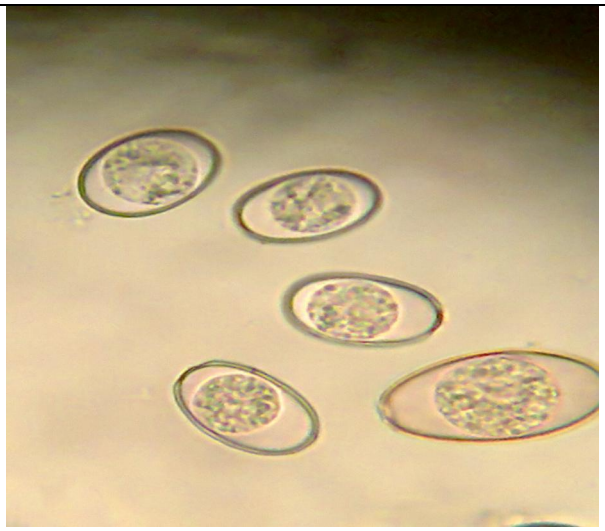

Fig. 5 B Non-sporulated Oocytes

Fig. 5(A, B) Oocyte in different Developmental Stages and different size Sporulated and non-sporulated.

3.3.2. Lesion score

Positive control group (T_1) showed no lesions till the end of experiment. Negative control group (T_2) showed an increasing congestion of intestinal mucosa, ballooning of caeci, increasing the thickness of intestinal mucosa and liver was enlarged and studded with white nodules (Grade 3) from ^{5th} day post-infection till the end of the experiment(Table 6). Groups (T_3, T_4, T_7) showed slight congestion of all intestinal mucosa and the caeci were nearly normal till the end of the experiment (Grade 1). Groups (T_5, T_6, T_8, and T_9) showed congestion and hemorrhagic patches of all intestinal mucosa and the caeci were ballooned (Grade 2). The oocyst count was decreased in groups (T_3, T_4, and T_7) than other groups. In the control positive group, the peak oocyst count was 1500 oocyst /gram, while in the negative control group, oocyst count per gram was zero till the end of experiment. Oocyst count in groups (T_3, T_4, T_6 and T_7) reached zero oocyst /gram after 4 weeks of treatment. Groups supplemented with 0.5, 1,2 and 5 ml Artemisia annua extract and group treated with sulphaquinoxalin(T_7) showed high reduction in total oocysts. This study attempts to evaluate the effect of *Artemisia annua* on, performance, coccidiosis and weight gain of growing rabbits. Supplemented groups with *Artemisia annua* and those treated with sulphaquinoxalin showed slight clinical manifestation

of illness all over the experimental period, decreased lesion score, and oocyst output. These results are in agreements with previously reported work of **Allen *et al.* (1997)**. Therefore, the use of *Artemisia annua* extract in 0.5; 1.0 and 2.5 ml (T_3; T_4 and T_8) is better in controlling coccidiosis of rabbits than other experimental groups.(Table 6)

***Conclusively**, from these results it could be stated that* artemisinin is a promising natural drug for prevention and control of coccidiosis in rabbits and its side effects are not too much serious especially at therapeutic doses. *Artemisia annua* powder at 5g/kg diet achieved a good growth performance and *coccidiosis* prevention, Meanwhile, *Artemisia annua* extract at 2.5 ml oral /3days per week recorded the best results of performance and prevention of coccidiosis in growing rabbits, but further analysis must be done to standardize their extraction and composition properly with a higher infective doses.

Acknowledgements

We are thankful to prof. Dr. Naser El-Bahy- professor of Parasitology, faculty of Veterinary medicine, University of Sadat City, for the revision of this manuscript.

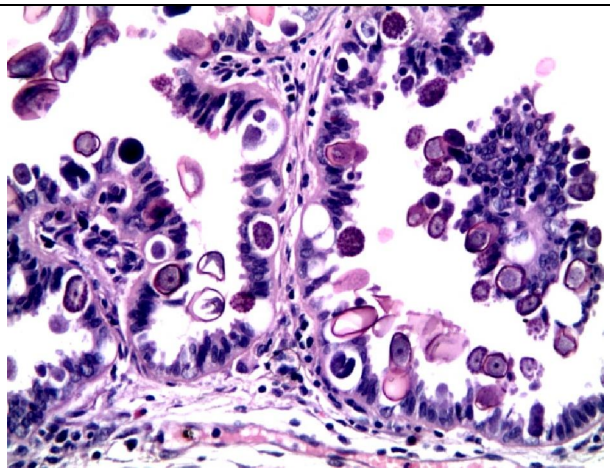

Fig. 6 A

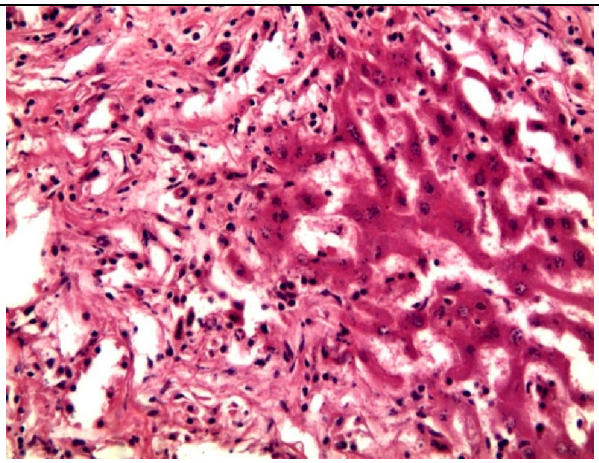

Fig. 6 B

Fig. 6 A: Rabbit liver: Eimeria Steadii infestation; finger like projections having the developmental stages of the parasite (arrows) in the lumen of the major bile ducks (black stars). H & E stain, X20.

Fig. 6 B: Rabbit liver: Eimeria Steadii infestation; coagulative necrosis in hepatocytes (arrow); fibrosis in necrotic area in hepatic parenchyma (white stars). H & E stain, X20.

Table 4: Growth performance of NZW rabbits naturally infected with mixed *Eimeria spp.* and supplemented with dietary *Artemisia annua* in Ether extract or powder form. (Mean ± **SE).**

Diets Item	T_1** Control (-)	T_2 Control (+)	T_3 0.5ml	T_4 1.0 ml	T_5 5 g	T_6 10 g	T_7 CD	T_8 2.5 ml	T_9 5 ml	Sig.
Initial wt. (kg)	0.92 ±0.04	0.96±0.04	0.99 ±0.04	0.94±0.05	0.93±0.03	1.04±0.02	1.02±0.05	0.89±0.14	0.96±0.05	NS
Final weight (kg)	1.80 ±0.11 c	2.02 ±0.14 abc	2.22 ±0.10 a	1.89 ±0.10 bc	1.99 ±0.17 bc	1.81 ±0.12 c	1.86 ±0.12 bc	2.16 ±0.14 abc	2.39 ±0.11 a	*
Total BWG(kg)	0.85 ±0.13 bc	1.11 ±0.16 abc	1.24 ±0.12 abc	0.96 ±0.12 bc	1.05 ±0.20 abc	0.78 ±0.14 c	0.80 ±0.14 c	1.30 ±0.16 ab	1.44 ±0.13 a	*
Feed intake (kg)	4.73 bc ±0.31	3.84 c ±0.39	5.06 ±0.28 ab	5.9 a ±0.28	5.4 ±0.48 ab	4.43 ±0.34 bc	4.86 ±0.34 bc	4.53 ±0.39 bc	5.24 ±028 ab	*
Feed conversion (FCR)	4.72 abc ±0.87	3.97 ±1.06 bc	4.38 ±0.61 abc	6.32 ±0.61 ab	5.18 ±1.06 abc	4.17 ±0.87 abc	6.77 ±0.75 a	3.51 ±0.87 c	3.77 ±0.67 bc	*
Relative Growth Rate (RGR)	61.27 ab ±7.32	75.59 ±9.45 ab	76.07 ±6.68 ab	67.96 ± 6.68 ab	71.43 ±11.57 ab	53.24 ±8.18 b	54.35 ±8.18 b	86.28 ±9.45 a	85.54 ±7.32 a	*
Performance Index (PI%)***	41.99 abc ±9.17	57.47 ±11.23 abc	55.69 ±4.48 abc	30.44 ±6.48 c	38.40 ±11.23 abc	48.28 ±9. 17 abc	34.24 bc ±7.94	61.87 ab ±9.17	65.93 ±7.10 a	*

*a,b,c …..etc.: Means in the same row at each item with different letters, differ significantly at $p<0.05$, NS=not significant

**T_1: Control (-);T_2: Control (+);T_3: 0.5ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly;T_4: 1.0 ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly; T_5; 5 g of *Artemisia annua* powder /Kg diet; T_6: 10 g of *Artemisia annua* powder /Kg diet; T_7: Salphaqenioxaline Sodium 30%, Water soluble powder) at 1 g / liter drinking water.; T_8: 2.5 ml of *Artemisia annua* extract (200mg/l) /rabbit/3days weekly and T_9: 5ml of *Artemisia annua* extract(200mg/l) /rabbit/3days weekly.; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

*** Calculated according to **North (1981)**

Table 5: Caecum activity of NZW growing rabbits naturally infected with mixed *Eimeria spp.* and supplemented with dietary *Artemisia annua* in Ether extract or powder form (Means ± SE).

Item	T_1 Control(+)	T_2 Control (-)	Ether extract and powder of *Artemisia annua* levels,							Sig.
			T_3	T_4	T_5	T_6	T_7	T_8	T_9	
Caecum weight,%	9.17 ±0.68 ^{ab}	7.03 ±0.39 ^{c}	8.74 ±0.43 ^{abc}	8.47±1.13 abc	8.25 ±0.52 ^{bc}	9.46 ±0.67 ^{ab}	9.11 ±0.76 ^{ab}	10.50 ±0.03 ^{a}	9.26 ±0.19 ^{ab}	*
Caecum length, cm	9.67±0.60	10.00±0.29	10.07±0.43	10.25±0.22	10.27±0.62	11.17±0.44	10.58±0.22	9.77±0.54	9.68±0.54	NS
Caecum pH	6.30 ^{c} ±0.24	6.95 ^{bc} ±0.28	6.74 ^{c} ±0.41	6.93 ±0.33 ^{bc}	8.07 ±0.28 ^{ab}	6.71 ±0.22 ^{c}	8.26 ±0.41 ^{a}	8.07 ±0.28 ^{ab}	8.46 ±0.81 ^{a}	*
Total bacterial counts (TBC) (Log cfu§/g) of caecal contents	6.49 ^{bc} ±0.15	7.11 ^{abc} ±0.07	7.70 ^{a} ±0.53	6.95 ±0.63 ^{abc}	7.61 ±0.26 ^{ab}	6.48 ±0.12 ^{bc}	5.02 ±0.33 ^{d}	6.40 ±0.23 ^{c}	4.73 ±0.26 ^{d}	*
TVFA (meq/100ml cecal juice)	2.24 ^{d} ±0.11	3.40 ^{c} ±0.14	3.80 ^{bc} ±0.39	3.60 ±0.42 ^{bc}	3.41 ±0.29 ^{c}	4.51 ±0.51 ^{ab}	3.60 ±0.39 ^{bc}	3.08 ±0.05 ^{cd}	4.85 ±0.20 ^{a}	*

* a, b,c…. Means in the same row with different super scripts are significantly different ($p<0.05$).
NS: not significant; § Colony Forming Unit.
T_1: Control (-);T_2: Control (+);T_3: 0.5ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly;**T_4**: 1.0 ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly; **T_5**; 5 g of *Artemisia annua* powder /Kg diet; **T_6**: 10 g of *Artemisia annua* powder /Kg diet; **T_7**: Salphaqenioxaline Sodium 30%, Water soluble powder) at 1 g / liter drinking water.; **T_8**: 2.5 ml of *Artemisia annua* extract (200mg/l) /rabbit/3days weekly and **T_9**: 5ml of *Artemisia annua* extract(200mg/l**)** /rabbit/3days weekly.

Table 6. The oocysts count (x10^3 / g feces), mortality (%) and lesion score of NZW growing rabbits naturally infected with mixed *Eimeria spp.*, and supplemented with dietary *Artemisia annua* in Ether extract or powder form. (Mean ± S.E.).

Week	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
0 WK	0.30 ±0.057 ^{d}	1.33 ±0.06 ^{d}	0.5 ±0.12 ^{d}	2.07 ±1.3 ^{cd}	2.7 ±1.3 ^{cd}	15.13 ±6.96 ^{b}	7.87 ±7.07 ^{bcd}	13.0 ±0.58 ^{bc}	45.0 ±2.89 ^{a}
2 WKs	0.57 ±0.15 ^{b}	1.50±0.05^{b}	8.47 ±4.04 ^{b}	25.03 ±10.47 ^{a}	12.97 ±10.46 ^{ab}	5.17 ^{b} ±1.36	2.47 ±2.07 ^{b}	6.23 ±0.15 ^{b}	12.5 ±0.29 ^{ab}
4 WKs	0.2 ±0.01 ^{c}	1.47 ±0.09 ^{d}	5.08 ±0.02 ^{ab}	5.5 ±0.76 ^{a}	1.4 ±0.6 ^{d}	2.7 ^{c} ±0.12	2.77 ±0.15 ^{c}	4.3 ±0.12 ^{b}	5.28 ±0.15 ^{a}
6 WKs	0.15 ±0.07 ^{b}	1.01 ±0.01 ^{a}	0.0 ±0.0 ^{c}	0. 0 ±0.01 ^{c}	0.1 ±0.01 ^{b}	0.0 ^{c} ±0.00	0.00 ±0.00 ^{c}	0.00 ±0.00 ^{c}	0.10 ±0.03 ^{b}
8 WKs	0.47 ±0.24 ^{c}	1.3 ±0.06 ^{a}	0.0 ±0.00 ^{d}	0.0 ±0.02 ^{d}	0.17 ±0.02 ^{d}	0.60 ^{c} ±0.06	0.00 ^{d} ±0.00	0.00 ±0.00 ^{d}	1.00 ±0.03 ^{b}
Mortality (%)	10.00	30.0	0.00	0.00	20.00	20.0	10.00	00.00	10.00
Lesion score	-	+++	-	-	-	++	-	-	++

a,b, and e: Means in the same row at each item with different letters, differ significantly at $p<0.05$, NS=not significant
** T_1: Control (-);T_2: Control (+);T_3: 0.5ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly;T_4: 1.0 ml of *Artemisia annua* extract(100mg/l) /rabbit/3days weekly; T_5; 5 g of *Artemisia annua* powder /Kg diet; T_6: 10 g of *Artemisia annua* powder /Kg diet; T_7: Salphaqenioxaline Sodium 30%, Water soluble powder) at 1 g / liter drinking water.; T_8: 2.5 ml of *Artemisia annua* extract (200mg/l) /rabbit/3days weekly and T_9: 5ml of *Artemisia annua* extract(200mg/l) /rabbit/3days weekly.

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4/20/2015