



## Biochemical role of zinc oxide and propolis nanoparticles in protection rabbits against coccidiosis

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### ABSTRACT

Coccidiosis is a protozoan infection of animals which causes growth retardation and high mortality in rabbits. There are two forms of coccidiosis were recognized in rabbits; hepatic and intestinal coccidiosis. The hepatic coccidiosis is due to *Eimeria stiedae* cause severe damage for liver which leads to death. Nanotechnology is the most important technology in our life where the use of nanotechnology in medicine offers some exciting possibilities. So, this study uses zinc oxide nanoparticles as chemical therapy and propolis nanoparticles as natural therapy. Twenty male rabbits aged 1 - 2 months and weighting 1250 – 1400 gm was used. Animals were divided into 4 groups (5 animals per each): control negative group; uninfected male rabbits and saved healthy, control positive group; infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and not treated. Zinc oxide nanoparticles treated group; infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and treated orally with 10 mg ZnO-NPs/Kg body weight daily for 5 consecutive days. Propolis nanoparticles treated group; infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and treated orally with 100 mg propolis-NPs/Kg body weight daily for 7 consecutive days. Serum ALT, CRP, zinc, cholinesterase, plasma GR, SOD and CAT were measured in addition to; histopathological examination of liver was done. The results indicated that the serum ALT and CRP were increased but serum cholinesterase, zinc, plasma GR, SOD and CAT were decreased in infected group, with treatment all parameters were improved, also examination of histological sections of livers from all treated groups did not detect any protozoal stage in their parenchyma nor bile ducts. Also, no or minimal papillary projection of epithelium of bile duct with decrease of peribiliary fibrosis could be seen in comparison control positive group. In conclusion, zinc oxide nanoparticles and propolis nanoparticles play a protective role against *Eimeria* infestation in rabbits.

**Keywords:** *Coccidiosis, Nanotechnology, Propolis-NPs, ZnO-NPs.*

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### 1. INTRODUCTION

Coccidiosis is a protozoan infection of animals which causes growth retardation and high mortality in rabbits (El-Akabawy et al., 2004). There are two forms of coccidiosis were recognized in rabbits; hepatic and

intestinal coccidiosis. The hepatic coccidiosis is due to *Eimeria stiedae* cause severe damage for liver which led to death (Oncel et al., 2011). The characteristic symptoms associated with hepatic coccidiosis in

rabbits include loss of appetite, anorexia, hair loss, diarrhea, yellowish mucous membranes, fatigue, abdomen swelling and significant loss in body weight (Al-Mathal, 2010), as well as increase in serum AST, ALT, GGT and L-MDA but decrease erythrocyte CAT and SOD activities which have been reported in the rabbits with hepatic coccidiosis (Cam et al., 2008).

Nanotechnology is the most important technology in our life where the use of nanotechnology in medicine offers some exciting possibilities. Now, Researchers try to use more effective drugs against coccidia as nanoparticles because nano medicine has recently emerged as a better option for the treatment of various diseases and researches into nano technological approaches to infectious diseases increases quickly (Allahverdiyev et al., 2011). There is more expectation that nano particles will be able to be used in the treatment of various diseases in the future (Angeli et al., 2008). In this study, we used zinc oxide nano particles due to zinc supplementation have been successfully used as a therapeutic and preventive agent for many conditions (Prasad, 2004). ZnO-NPs have effective antibacterial activity and antimicrobial efficacy against various bacterial and fungal pathogens (Gunalan et al., 2012). Zinc oxide nanoparticles have anticoccidial properties (Dkhil et al., 2015). Dietary supplementation with low dose of zinc oxide nanoparticles improved growth performance and intestinal morphology, reducing diarrhea and intestinal inflammatory in weaning piglets (Long et al., 2017). And we used propolis nanoparticles because propolis has many biological and pharmacological properties (Bufalo et al., 2009). Propolis (blue glue) is a natural nontoxic resinous sticky substance with very complex chemical composition produced by mixing secretions of honey bees secretions hypopharyngeal glands with the digested

product of resins collected from plant particularly from flowers of plants, leaf buds and the bark of trees (Coutinho, 2012). Propolis extracts from Serbia has antioxidant activity (Potkonjak et al., 2012). Propolis-NPs have antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* (Rangasamy et al., 2012). Propolis-NPs have antibacterial and antifungal activity against *Staphylococcus aureus* and *Candida albicans* where it is used efficiently in controlling of bacterial and fungal diseases (Afrouzan et al., 2012). Propolis-NPs was effective in the treatment of diabetes due to the reduction of blood sugar level and the regeneration of damaged  $\beta$ -cells observed in streptozotocin-induced diabetic rats (Nyun-Ki et al., 2010). So, this study investigated the potential protective role of zinc oxide nanoparticles and propolis nanoparticles against *Eimeria* infestation in rabbits.

## 2. Materials and methods

### 2.1. Experimental animals:

A total number of 20 animals male rabbits divided into 4 groups each group contain 5 rabbits weighting 1250 – 1400 gm and aged 1 - 2 months old were used for the experimental investigation of this study. Rabbits were housed separately in clean wire floored cages in the lab. They were kept 12 days after setting up the groups before beginning the experiment for adaptation with the environment. The rabbits were fed on diet in the form of pellets obtained from El – Baraka Company in Cairo, Egypt. This diet composed of 58 % Carbohydrate, 20 % Protein, 4.0 % Lipid, 3.0 % Cellulose, 3.0 % (Minerals, Calcium, Phosphorous) 12 % Moisture.

### 2.2. Drugs:-

*Propolis-NPs*: Natural propolis was obtained from Imtenan Pharma, Cairo, Egypt. Then, it was converted into propolis-NPs by grinding

in RTZ-400 for 3 hours by dry ball milling mechanism till reach to size 100nm. In Egyptian Petroleum Research Institute in Naser city, Cairo, Egypt.

*ZnO-NPs:* Zinc oxide was obtained from Biostc Company, Cairo, Egypt. Then, it was converted into ZnO-NPs by grinding in RTZ-400 for 3 hours by dry ball milling mechanism till reach to size 100nm. In Egyptian Petroleum Research Institute in Naser city, Cairo, Egypt.

### 2.3. Dose of sporulated oocysts:

The dose of sporulated oocyst was calculated according to (Long et al., 1976). Where each rabbit was given 1 ml under the tongue (Coudert et al., 2000) containing  $4 \times 10^4$  sporulated oocyst of *Eimeria* spp. using McMaster Technique (Fisher and Kelley, 1977).

### 2.4. Animal grouping:

Rabbits were divided into 4 groups:-

*Group 1:* (Control negative group) contained 5 uninfected male rabbits and saved healthy.

*Group 2:* (Control positive group) contained 5 infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and not treated.

*Group 3:* (ZnO-NPs treated group) contained 5 infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and treated orally with 10 mg nano ZnO-NPs /Kg body weight daily for 5 consecutive days (Dkhil et al., 2015).

*Group 4:* (Propolis-NPs treated group) contained 5 infected male rabbits by 40.000 sporulated oocyst of *Eimeria* spp. and treated orally with 100 mg propolis nanoparticles / Kg body weight daily for 7 consecutive days (Rangasamy et al., 2012).

### 2.5. Biochemical investigations:

Blood samples were collected after overnight fasting from all animals from the marginal ear

vein of all rabbits for making serum alanine aminotransferase (ALT) determination according to the method described by (Henry, 1974) using the kit supplied by BIOSTC company, Egypt. Serum CRP was determined according to the method described by (Macleod and Avery, 1941) using the kit supplied by BIOSTC company, Egypt. Serum zinc was determined according to the method described by (Johnsen and Eliasson., 1987) using the kit supplied by Salucea, BioSTC company, Egypt. Serum cholinesterase (CHE) was determined according to the method described by (Szasz, 1968) using the kit supplied by BEN, BIOSTC company, Egypt. Plasma glutathione reductase (GR) was determined according to the method described by (Goldberg and Spooner., 1983) using the kit supplied by Biodiagnostic, company, Egypt. Plasma SOD activity was determined according to the method described by (Nishikimi et al., 1972). Plasma catalase (CAT) was determined according to the method described by (Aebi, 1984) using the kit supplied by Biodiagnostic, company, Egypt. We measured all parameters before treatment and one week, two weeks, three weeks post treatment.

### 2.6. Histopathological specimens:

At the end of experiment, all rabbits were decapitated. Livers of all rabbits were thoroughly examined for the gross lesions and liver tissues specimens were collected in 10 % neutral buffer formalin. After fixation of liver specimens in 10 % neutral buffer formalin, dehydration in ascending grades of alcohol, clearance in xylene were done. Finally, liver specimens were embedded in paraffin. Paraffin sections of 5  $\mu$ m were cut and stained for H & E as outlined by (Bancroft and Marilyne, 2008).

## 3. RESULTS

Infection with *Eimeria* spp. to normal rabbits exhibited a significant increase in serum ALT

activity and serum CRP concentration but exhibited a significant decrease in serum zinc concentration, serum cholinesterase activity, plasma GR, SOD and CAT activities before treatment, one week, two weeks and three weeks post treatment compared to control negative group but with administration of ZnO-NPs and propolis-NPs to infected rabbits after 2 weeks of infection exhibited a significant decrease in serum ALT activity and serum CRP concentration but exhibited a significant increase in serum zinc concentration, serum cholinesterase activity, plasma GR, SOD and CAT activities.

**Histopathological results:**

**Liver of control negative group:**

Histological sections showed normal feature of liver structure; normal central vein, and hepatocytes were arranged in cords and separated by blood sinusoid (Fig.1).

**Liver of control positive group:**

Sections through the lesions of liver from this group showed the different protozoal stages including microgametocytes, macrogametocytes and oocysts that were

separated from hepatic parenchyma by thick connective tissue capsule (Figs.2). Hyperplasia of biliary columnar epithelium with papillary projections in addition to, peribiliary fibrosis with infiltration of mononuclear cells was also common features in the bile ducts (Fig.3). Within the hepatic parenchyma, congestion of central vein and sinusoids (Fig.4) and focal mononuclear cells infiltration (Fig.5) could be observed.

**Liver of treated groups:**

Examination of histological sections of livers from treated groups did not detect any protozoal stage in their parenchyma or bile ducts. Congestion of the central vein, blood sinusoid and hepatic vein was the common feature for livers from ZnO-NPs (6) and propolis-NPs (8) treated groups. In ZnO-NPs treated group, no papillary projection of biliary duct epithelium with obvious decrease of peribiliary fibrosis were identified (Fig.7) in comparison to control positive group. In propolis-NPs treated group, liver showed hyperplasia of epithelium of bile duct with fibrous coat (Fig.9).

Table 1: Serum ALT activity (U/L) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with ZnO-NPs, propolis-NPs (mean±SE).

Groups		Period				Mean
		Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (Healthy)	(-ve)	34.40±1.36 <sup>dA</sup>	34.57±0.45 <sup>cA</sup>	33.77±0.61 <sup>dA</sup>	32.93±1.34 <sup>dA</sup>	33.92±0.48 <sup>e</sup>
Control (Infected, non treated)	(+ve)	93.40±4.83 <sup>aC</sup>	106.97±4.24 <sup>aA</sup>	103.70±2.70 <sup>aB</sup>	92.63±1.42 <sup>aC</sup>	99.18±2.42 <sup>a</sup>
Exp. infected and ZnO-NPs treated		78.07±4.88 <sup>bA</sup>	30.57±1.87 <sup>dB</sup>	30.20±0.64 <sup>eB</sup>	31.17±1.32 <sup>deB</sup>	42.50±6.30 <sup>c</sup>
Exp. infected and propolis-NPs treated		74.23±0.81 <sup>cA</sup>	40.77±0.73 <sup>bC</sup>	41.77±0.66 <sup>bC</sup>	44.37±0.47 <sup>bB</sup>	50.28±4.20 <sup>b</sup>

a, b & c: There is a significant difference (P<0.05) between any two means, within the same column have the different superscript letter

.A, B & C: There is a significant difference ( $P<0.05$ ) between any two means, within the same row have the different superscript letter.

Table 2: Serum CRP concentration (mg/L) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean $\pm$ SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	23.00 $\pm$ 0.58 <sup>e</sup> B	22.67 $\pm$ 0.88 <sup>d</sup> B	23.33 $\pm$ 0.88 <sup>e</sup> B	28.00 $\pm$ 0.58 <sup>d</sup> A	24.25 $\pm$ 0.73 <sup>e</sup>
Control (+ve) (Infected, non treated)	192.33 $\pm$ 1.45 bD	200.67 $\pm$ 1.45 aC	204.33 $\pm$ 2.73 aB	217.67 $\pm$ 4.33 aA	203.75 $\pm$ 3.00 <sup>a</sup>
Exp. infected and ZnO-NPs treated	195.00 $\pm$ 5.03 bA	22.67 $\pm$ 0.88 <sup>d</sup> B	23.67 $\pm$ 0.88 <sup>e</sup> B	25.00 $\pm$ 1.00 <sup>e</sup> B	66.58 $\pm$ 22.38 <sup>d</sup>
Exp. infected and propolis-NPs treated	200.00 $\pm$ 3.46 aA	51.33 $\pm$ 1.76 <sup>b</sup> C	56.67 $\pm$ 1.86 <sup>b</sup> B	58.67 $\pm$ 1.76 <sup>b</sup> B	91.67 $\pm$ 18.90 <sup>b</sup>

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A, B & C: There is a significant difference ( $P<0.05$ ) between any two means, within the same row have the different superscript letter.

Table 3: Serum zinc concentration ( $\mu$ g/dl) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean $\pm$ SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	84.73 $\pm$ 2.22 <sup>aB</sup>	86.63 $\pm$ 1.72 <sup>dA</sup>	86.60 $\pm$ 2.25 <sup>cA</sup>	88.17 $\pm$ 2.31 <sup>cA</sup>	86.53 $\pm$ 0.98 <sup>bc</sup>
Control (+ve) (Infected, non treated)	76.93 $\pm$ 1.00 <sup>bA</sup>	74.67 $\pm$ 0.64 <sup>eBC</sup>	75.60 $\pm$ 0.35 <sup>eAB</sup>	73.37 $\pm$ 0.43 <sup>cC</sup>	75.14 $\pm$ 0.48 <sup>e</sup>
Exp. infected and ZnO-NPs treated	76.00 $\pm$ 1.45 <sup>bc</sup>	100.27 $\pm$ 0.20 <sup>aA</sup>	99.33 $\pm$ 0.54 <sup>aAB</sup>	98.43 $\pm$ 0.83 <sup>aB</sup>	93.51 $\pm$ 3.08 <sup>a</sup>
Exp. infected and propolis-NPs treated	76.93 $\pm$ 1.17 <sup>bd</sup>	86.70 $\pm$ 1.97 <sup>dA</sup>	82.90 $\pm$ 0.98 <sup>dB</sup>	80.40 $\pm$ 0.53 <sup>dC</sup>	81.73 $\pm$ 1.20 <sup>d</sup>

a, b & c: There is a significant difference ( $P<0.05$ ) between any two means, within the same column have the different superscript letter. A, B & C: There is a significant difference ( $P<0.05$ ) between any two means, within the same row have the different superscript letter.

Table 4: Serum cholinesterase activity (U/L) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean±SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	6593.67±199.81 <sup>aB</sup>	7097.33±127.46 <sup>aA</sup>	7081.00±217.56 <sup>aA</sup>	7160.33±144.31 <sup>aA</sup>	6983.08±101.6 <sup>2<sup>a</sup></sup>
Control (+ve) (Infected, non treated)	4246.33±33.86 <sup>d</sup> <sub>A</sub>	3975.33±72.00 <sup>e</sup> <sub>C</sub>	3748.00±47.72 <sup>e</sup> <sub>D</sub>	4014.33±59.65 <sup>e</sup> <sub>B</sub>	3996.00±58.24 <sub>e</sub>
Exp. infected and ZnO-NPs treated	4512.33±87.21 <sup>c</sup> <sub>C</sub>	6282.67±31.42 <sup>c</sup> <sub>B</sub>	6387.00±85.29 <sup>c</sup> <sub>B</sub>	6658.33±38.08 <sup>c</sup> <sub>A</sub>	5960.08±256.9 <sub>3<sup>c</sup></sub>
Exp. infected and propolis-NPs treated	4670.00±102.21 <sup>bD</sup>	5367.00±64.38 <sup>d</sup> <sub>C</sub>	5666.67±140.36 <sup>dB</sup>	5898.33±74.37 <sup>d</sup> <sub>A</sub>	5400.5±145.62 <sub>d</sub>

a, b & c: There is a significant difference (P<0.05) between any two means, within the same column have the different superscript letter. A, B & C: There is a significant difference (P<0.05) between any two means, within the same row have the different superscript letter.

Table 5: Plasma glutathione reductase activity (U/L) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean±SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	48.65±0.23 <sup>aA</sup>	46.82±2.46 <sup>aB</sup>	46.37±3.05 <sup>aB</sup>	49.48±0.32 <sup>aA</sup>	47.83±0.92 <sup>a</sup>
Control (+ve) (Infected, non treated)	29.03±0.16 <sup>bA</sup>	28.77±0.20 <sup>eA</sup>	28.66±0.23 <sup>dA</sup>	28.55±0.23 <sup>eA</sup>	28.75±0.10 <sup>e</sup>
Exp. infected and ZnO-NPs treated	28.75±0.17 <sup>bB</sup>	45.31±0.27 <sup>bA</sup>	45.40±0.10 <sup>aA</sup>	45.41±0.23 <sup>bA</sup>	41.22±2.17 <sup>b</sup>

Exp. infected and propolis-NPs treated	28.85±0.05 <sup>bB</sup>	40.89±0.19 <sup>dA</sup>	40.70±0.20 <sup>cA</sup>	40.69±0.24 <sup>dA</sup>	37.78±1.56 <sup>d</sup>
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Table 6: Plasma SOD activity (U/ml) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean±SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	44.03±0.94 <sup>aA</sup>	44.77±2.09 <sup>aA</sup>	44.13±0.83 <sup>aA</sup>	42.60±1.22 <sup>aB</sup>	43.88±0.63 <sup>a</sup>
Control (+ve) (Infected, non treated)	28.33±0.98 <sup>bA</sup>	26.93±0.80 <sup>fB</sup>	27.67±0.84 <sup>eAB</sup>	22.47±1.10 <sup>cC</sup>	26.35±0.80 <sup>e</sup>
Exp. infected and ZnO-NPs treated	29.37±0.74 <sup>bD</sup>	39.60±0.36 <sup>cC</sup>	42.27±0.55 <sup>bA</sup>	41.13±0.83 <sup>bB</sup>	38.09±1.57 <sup>b</sup>
Exp. infected and propolis-NPs treated	29.23±1.29 <sup>bD</sup>	30.53±0.61 <sup>cC</sup>	33.70±0.55 <sup>dA</sup>	31.80±1.71 <sup>dB</sup>	31.32±0.70 <sup>d</sup>

a, b & c: There is a significant difference ( $P<0.05$ ) between any two means, within the same column have the different superscript letter.

A, B & C: There is a significant difference ( $P<0.05$ ) between any two means, within the same row have the different superscript letter.

Table 7: Plasma catalase activity (U/L) of experimentally infected male rabbits with *Eimeria* spp. before and after treatment with with ZnO-NPs, propolis-NPs (mean±SE).

Groups	Period				Mean
	Before treatment	One week post treatment	Two weeks post treatment	Three weeks post treatment	
Control (-ve) (Healthy)	1229.67±41.80 <sup>a</sup> B	1279.00±17.35 <sup>a</sup> A	1206.67±13.42 <sup>a</sup> C	1271.67±24.69 <sup>a</sup> A	1246.75±14.49 a
Control (+ve) (Infected, non treated)	649.00±14.18 <sup>cA</sup>	628.67±14.66 <sup>fB</sup>	635.00±13.11 <sup>fA</sup> B	623.67±14.68 <sup>fB</sup>	634.08±6.69 <sup>f</sup>
Exp. infected	614.00±10.02 <sup>dB</sup>	1191.33±7.86 <sup>bA</sup>	1176.00±17.62 <sup>b</sup> A	1183.33±4.26 <sup>bA</sup>	1041.17±74.53 b

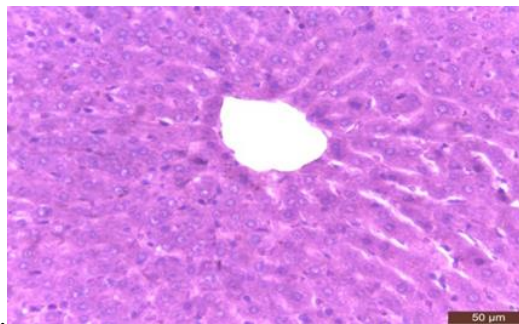
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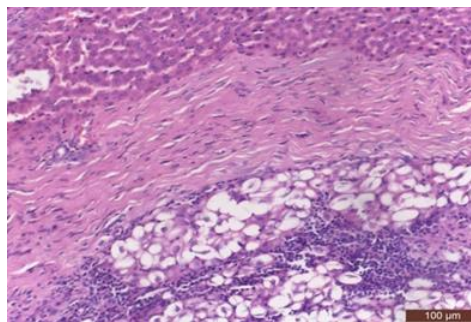
720.33±16.7 <sup>bC</sup>	913.00±11.36 <sup>eA</sup>	904.00±15.52 <sup>eA</sup>	845.33±6.57 <sup>eB</sup>	845.67±23.85 <sup>e</sup>
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a, b & c: There is a significant difference ( $P<0.05$ ) between any two means, within the same column have the different superscript letter.

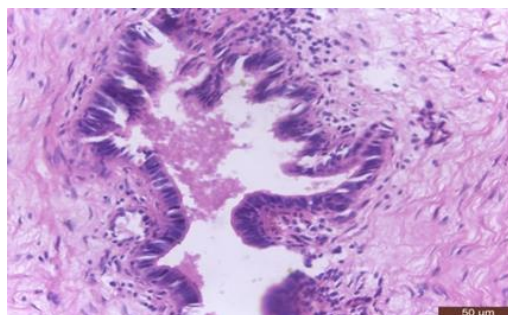
A, B & C: There is a significant difference ( $P<0.05$ ) between any two means, within the same row have the different superscript letter.



**Fig.1.** Liver from control negative group showing normal feature of histological liver structure; normal central vein, hepatocytes arranged in cords and separated blood sinusoid (H&E stain, scale bar = 50  $\mu$ m).

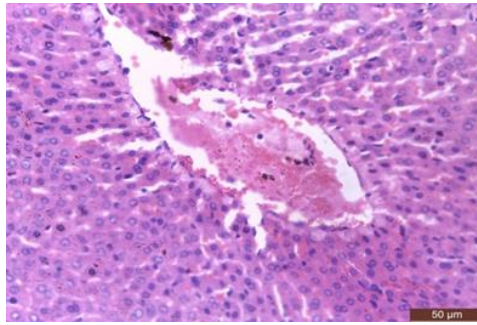


**Fig.2.** Liver from control positive group showing different protozoal stages. Notice thick C.T capsule surrounding the cyst (H&E stain, scale bar = 100  $\mu$ m).

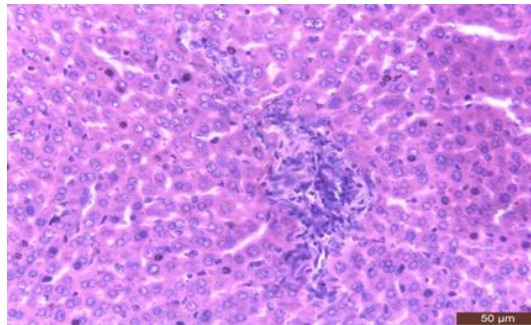




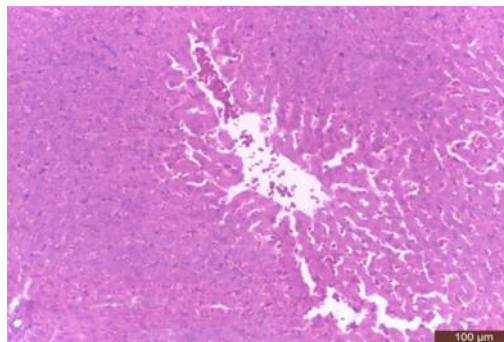
**Fig.3.** Liver from control positive group showing hyperplasia of biliary columnar epithelial cells (H&E stain, scale bar =50  $\mu$ m).



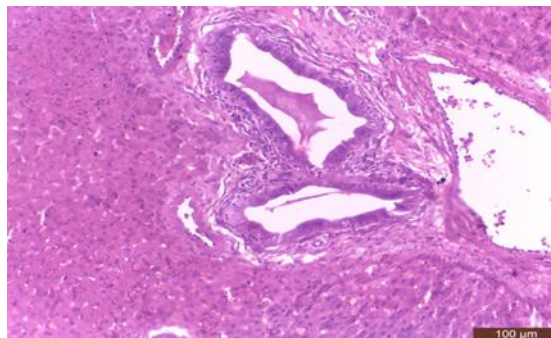
**Fig.4.** Liver from control positive group showing dilation and congestion of central vein with rupture of lining endothelial cells (H&E stain, scale bar =50  $\mu$ m).



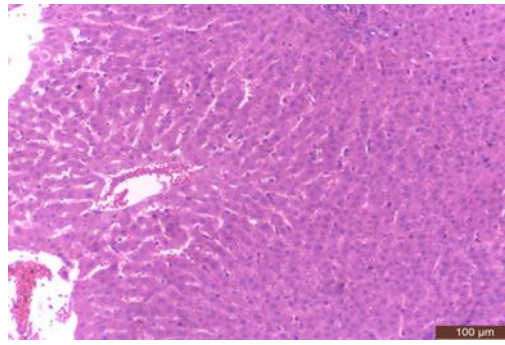
**Fig.5.** Liver from control positive group showing focal infiltration of mononuclear cells within hepatic parenchyma (H&E stain, scale bar =50  $\mu$ m).



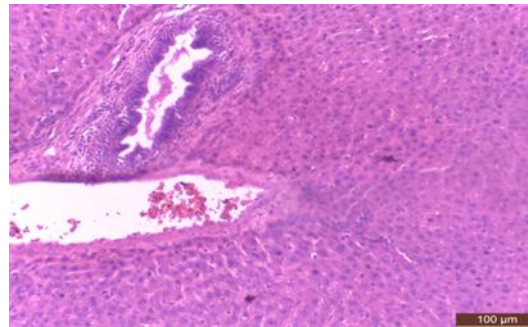
**Fig.6.** Liver from ZnO-NPs treated group showing congestion of central vein and blood sinusoid (H&E stain, scale bar =100  $\mu$ m)



**Fig.7.** Liver from ZnO-NPs treated group showing no papillary projection of epithelium of bile duct in comparison to control positive group. Also, notice decrease of peribiliary fibrosis (H&E stain, scale bar =100  $\mu$ m).



**Fig.8.** Liver from propolis-NPs treated group showing severe congestion of central vein and blood sinusoid (H&E stain, scale bar =100 μm).



**Fig.9.** Liver from propolis-NPs treated group showing hyperplasia of epithelium of bile duct with fibrous coat (H&E stain, scale bar =100 μm).

#### 4. DISCUSSION

Our results showed that ALT activity of infected rabbits with *Eimeria* spp. exhibited a significant increase, these results agreed with Cam et al. (2008) who reported that *Eimeria stiedae* in rabbits causes increasing of serum ALT activity compared to healthy control group. Administration of ZnO-NPs to infected rabbits after 2 weeks of infection exhibited a significant decrease in serum ALT activity if compared to ZnO-NPs infected group before treatment, these results agreed with Ahmadi et al.(2014) who reported that the dietary ZnO-NPs in broiler chicken showed decreasing serum alanine transferase (ALT). Administration of propolis-NPs to infected rabbits after 2 weeks of infection exhibited a significant decrease in ALT activity if compared to propolis-NPs infected group before treatment, these results agreed with Ambardekar et al. (2012) who reported that propolis by fabrication of liposomes has a

platform nano-formulation for multi-component natural medicine where formulation was able to suppress serum ALT activity in hepatotoxicity induced experimental animals and promote tissue healing.

In this study, cholinesterase activity exhibited a significant decrease; these results agreed with Jin et al. (2016) who recorded that cholinesterase were significantly different from the non-infected control. Administration of ZnO-NPs to infected rabbits after 2 weeks of infection exhibited a significant increase in cholinesterase if compared to ZnO-NPs infected group before treatment these results agreed with Bashandy et al.( 2017) who reported that thioacetamide (TAA) treatment led to a significant increase in liver enzymes, these parameters were reduced after treatment with ZnO-NPs. Administration of propolis-NPs to infected rabbits after 2 weeks of infection exhibited a significant increase in

cholinesterase if compared to propolis-NPs infected group before treatment these results agreed with Ambardekar et al.(2012) who reported that propolis by fabrication of liposomes has a platform nano-formulation has hepatoprotective properties.

The obtained data showed that serum CRP concentration of infected rabbits with *Eimeria* spp. exhibited a significant increase, these results agreed with Ragab et al .(2015) who reported that coccidiosis causes inflammation in tissues so levels of inflammatory mediators such as CRP showed increasing. Also, Pepys and Hirschfield. (2003) who declared that CRP is a sensitive systemic marker of inflammation and tissue damage. Administration of ZnO-NPs to infected rabbits after 2 weeks of infection exhibited a significant decrease in CRP concentration if compared to ZnO-NPs infected group before treatment these results agreed with Bashandy et al .(2017) who reported that thioacetamide (TAA) treatment led to a significant increase in plasma inflammatory markers but ZnO-NPs decreased it . Administration of propolis -NPs to infected rabbits after 2 weeks of infection exhibited a significant decrease in CRP concentration if compared to propolis -NPs infected group before treatment these results agreed with Nyun-Ki et al.(2010) who showed that the propolis -NPs was effective in the treatment of diabetes due to the reduction of blood sugar level and the regeneration of damaged  $\beta$ -cells observed in streptozotocin-induced diabetic rats so, nano propolis can decrease the inflammation of cells .

Our results showed that serum zinc concentration of infected rabbits with *Eimeria* spp. exhibited a significant decrease, these results agreed with Southern and Baker (1983) who reported that Liver Zn concentration was decreased by coccidiosis. Administration of ZnO-NPs to infected

rabbits after 2 weeks of infection exhibited a significant increase in zinc concentration if compared to ZnO-NPs infected group before treatment these results agreed with Hassan et al. (2017) who reported that diets of rabbits supplemented with zinc nanoparticles showed that the value of hepatic zinc increased. Administration of propolis-NPs to infected rabbits after 2 weeks of infection exhibited a significant increase in zinc concentration if compared to propolis-NPs infected group before treatment these results agreed with Lotfy( 2006) who reported that propolis contains minerals like zinc (Zn).

The obtained data showed that plasma GR, SOD and CAT activities of infected rabbits with *Eimeria* spp. exhibited a significant decrease, these results agreed with Kizil and Yuce (2009) who showed that coccidiosis in dogs revealed decreasing in GSH concentration and CAT activity. And Ghanem et al. (2009) who reported that there is a significant decrease in erythrocytes SOD, CAT and GSH concentration in *Eimeria* – infected kids. Administration of ZnO-NPs to infected rabbits after 2 weeks of infection exhibited a significant increase in plasma GR, SOD and CAT activities if compared to ZnO-NPs infected group before treatment these results agreed with (Atef et al., 2016) who declared that aflatoxicosis increased the concentration of NO and MDA, while decreased the level of GSH and activities of SOD, CAT and GPx. These changes were improved by administration of ZnO-NPs to aflatoxicated animal through the immune strengthening effect and protection of lipid and protein from oxidative damage. Administration of propolis -NPs to infected rabbits after 2 weeks of infection exhibited a significant increase in plasma GR, SOD and CAT activities if compared to propolis -NPs infected group before treatment these results agreed with Hong-zhuan et al .(2008) who declared that propolis-NPs showed

antioxidation activity where propolis nanometer could scavenge excessive free radicals in body better than common propolis and reduce the generation of superoxide lipids, which is a new anti-oxidation product. In conclusion, some nanoparticles such as ZnO-NPs and propolis-NPs act as hepatoprotective and antioxidative agents against *Eimeria* infestation in rabbits.

#### *Histopathological of liver*

The liver of infected male rabbit with coccidiosis showing different stages of the protozoal stages that were agreed with those reported by Wang et al. (1991). Extensive hyperplasia of biliary columnar epithelial cells was identified that was agreed with Rosmini and Simoni (1979). Within the hepatic parenchyma, congestion of central vein and sinusoids like Al-Mathal (2008) and focal mononuclear cells infiltration like Singla et al. (2000) were recorded. The liver of infected ZnO-NPs treated group showed no papillary projection of epithelium of bile duct with obvious decrease of peribiliary fibrosis were identified in comparison to control positive group. Also, there are no microgametocytes, macrogametocytes or oocytes in liver so ZnO-NPs can decrease damaging of liver. These results were agreed with those reported by Bashandy et al. (2017) who reported the protective effect of ZnO-NPs.

The liver of propolis-NPs treated group, showed sever congestion of central vein and blood sinusoid without microgametocytes, macrogametocytes and oocytes in liver and liver showed hyperplasia of epithelium of bile duct with fibrous coat so propolis-NPs can decrease damaging of liver. These results were supported by finding of Nyun-Ki et al. (2010) and Hasan et al. (2016) who reported that the effective of propolis-NPs in the treatment of diabetes and regeneration of

damaged cells and the treatment of rat mammary gland tumors respectively.

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