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Therapeutic effect of *Moringa oleifera* and *Thymus vulgaris* oils against hepatic coccidiosis in experimentally infected rabbits

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Abstract. The present study was conducted to detect the therapeutic effect of *Moringa oleifera* and *Thymus vulgaris* oils on hepatic coccidiosis in experimentally infected rabbits. Also, immunomodulatory effect of the two oils was detected. Twenty-four Newzealand rabbits were used in this study and divided into 4 groups; healthy rabbits, experimentally infected rabbits with *Eimeria stiedae* oocysts, and two infected treated groups (one with moringa (200 mg/kg) and the other with thyme (500 mg/kg) oils). The results showed highly significant reduction in oocysts shedding ($P<0.001$ and $P<0.05$) in the two infected and treated rabbits than the infected non-treated rabbits in almost all days post infection (PI). Thyme oil was more potent and stopped oocysts shedding earlier at the day 34 PI compared to moringa oil at the day 41 PI. Microscopically, there was a damage in the oocysts shed by treated rabbits. Macroscopically, the livers of thyme oil treated rabbits showed more enhancement with protection percentage 75% than those treated with moringa oil in which protection percentage was 55%. The highest titer of antibodies was detected in moringa oil treated rabbits. It was concluded that both moringa and thyme oils had an anti-coccidial effect with thyme oil superiority. So, thyme oil could be useful as an alternative product for the control of rabbit coccidiosis.

INTRODUCTION

Rabbit meat is a good source of high animal protein with low fat content (Nistor *et al.*, 2013). From 2018, Egypt is considered the third largest producer of rabbit meat in the world following China and North Korea, where, Egyptian rabbit meat production was estimated by 62,143 tons (FAO, 2020). Coccidian parasites could affect this high commercial value of rabbits with direct and indirect losses as a result of acute illness, weight loss and high mortality and morbidity (El-Shahawi *et al.*, 2012). Coccidiosis is a worldwide challenging disease of wild and domestic rabbits that affects different rabbit types (Chowdhury & Fraser, 2008; Yin *et al.*, 2016). In Egypt, Rabbit coccidiosis natural infection among rabbit herds reached 70% (El-Shahawi *et al.*, 2012). Its prevalence rate was found to be 26.87% in Iran (Tehrani *et al.*, 2013), 11.5% in Kenya (Okumu *et al.*, 2014) and 1.01% in India (Chacko *et al.*, 2017). Two forms of coccidiosis were recognized in rabbits; intestinal and hepatic coccidiosis. Hepatic coccidiosis is a serious and lethal disease in rabbits caused by *Eimeria stiedae* (*E. stiedae*) (Sivajothi *et al.*, 2016). Infection of the epithelial cells of the bile ducts occurs by ingestion of *E. stiedae* sporulated oocysts causing severe liver damage in rabbits (Oliveira *et al.*, 2011; Abu El Ezz *et al.*, 2012). Clinical symptoms of the disease are dullness, diarrhea or constipation, reduced food consumption, ascites, liver enlargement, icterus, a distended abdomen and nally death (Karaer, 2001). Young rabbits are more susceptible; however, infected adults can be carriers of the disease and act as a source of infection (Sivajothi *et al.*, 2016).

Traditional control strategies of the disease had counted mainly on chemoprophylaxis, which is expensive (Dalloul & Lillehoj, 2005). Furthermore, the continuous use of anticoccidial drugs led to the emergence of drug resistance (El Banna *et al.*, 2016). In addition to concerns about drug resistance, there are also food safety and public health concerns about drug residues in animal products and so, this stimulates the researchers to find safer alternatives (Kheirabadi *et al.*, 2014). Plant products could provide an alternative choice for coccidial control to which resistance has not yet developed (Abbas *et al.*, 2012), reducing the farmer input costs and protect animal health (Abu El Ezz, 2005). Plant oils can be used as a replacement to current antiparasitic drugs (Anthony *et al.*, 2005).

Previous studies had shown that moringa (*Moringa oleifera; M. Oleifera*) has many bioactive compounds including vitamins, essential amino acids, polyphenols, avonoids and phenolic acids (Leone *et al.*, 2015). Many *in vitro* and *in vivo* studies have widely confirmed numerous pharmacological properties of moringa (Konmy *et al.*, 2016). It exhibited curative properties such as; immune-boosting (Miyachi *et al.*, 2004), antioxidant (Singh *et al.*, 2009), anti-inammatory, anti-diarrheal (Kesharwani *et al.*, 2014) and antiparasitic (Hegazi *et al.*, 2018; Kandil *et al.*, 2018; Aboelsoued *et al.*, 2019).

Thyme *(Thymus vulgaris*, *T. vulgaris*), belonging to the Lamiacea family, is an aromatic native herb of the Mediterranean region. This plant possesses various beneficial effects such as: antibacterial (Dorman and Deans, 2000) anticoccidial (Jamroz *et al.*, 2003), anthelmintic (Rasooli *et al.*, 2006) and antifungal properties (Shen *et al.*, 2016) as it contains many compounds with therapeutic potentials like flavonoids, thymol, eugenol, carvacrol, saponins and phenols (Amarowicz *et al.*, 2008).

The emergence of parasites that are resistant to current chemotherapies highlights the importance of plants as novel antiparasitic agents. So, the present study was designed to investigate the therapeutic effect of *M. oleifera* and *T. vulgaris* oils on hepatic coccidiosis in experimentally infected rabbits. Also, to detect immunomodulatory effect of the two oils.

MATERIAL AND METHODS

Ethical approval

Rabbits were housed in good conditions in the animal house of the National Research Centre (NRC), Egypt, in accordance with the ethical standards. The study protocol was approved by the Medical Research Ethics Committee of NRC, Egypt, No: 18-118.

Parasite

Collection of *E. stiedae* sporulated oocysts: The oocysts of *E. stiedae* were collected from gall bladders and necrotic hepatic lesions of naturally infected rabbits. The livers and gall bladders were removed, minced and digested in 0.25% trypsin in normal saline. Then, the digested materials were sieved and washed several times by centrifugation at 2000 rpm for 10 minutes/each. Oocysts were counted as described by Ryley *et al.* (1976) and identified according to Levine (1985). The oocysts were allowed to sporulate by incubation for 3 days in 2.5% potassium dichromate solution at 26°C. Then, the sporulated oocysts were kept at 4°C until use in experimental infection and antigen preparation.

Plant oils

T. vulgaris oil was purchased from El Huawag Company, Egypt. *M. oleifera* seed oil was obtained from Moringa Production Unit, NRC, Egypt. Oils were emulsified with water and presented to rabbits by gastric tubes, 1 h before meals, for 5 consecutive days starting from the 16th day post infection (PI) in a dose of 500 mg/kg body weight for thyme oil (Abdel-Aziem *et al.*, 2014) and 200 mg/kg body weight for moringa oil (Khalifa *et al.*, 2016).

Experimental infection

In this study, twenty-four New Zealand rabbits (5 weeks old and about 1.5 kg body weight) were used. They were reared in

metal wire floored cages and fecal samples from all animals were examined daily for 2 weeks to ensure that animals were coccidian free. Rabbits were divided into 4 groups each one contained 6 rabbits. The first group was healthy rabbits, the 2nd group was experimentally infected with 50000 sporulated oocysts (Abu El Ezz *et al.*, 2012), the 3rd group was infected and treated with moringa oil and the last group was infected and treated with thyme oil. All rabbits were examined for 7 weeks. At the end of observation period, all rabbits were sacrificed.

Fecal analysis and oocysts count

Fresh fecal samples were collected daily from experimentally infected rabbits into sterile containers from the 14th day PI till the end of the experiment to determine the number of *E. stiedae* oocysts per gram using the McMaster counting chamber (Long *et al.*, 1976).

Liver lesion scores

Liver focal lesions were scored for severity of hepatic coccidiosis and the percentage of protection against lesions were performed according to Abdel Megeed & Abu El Ezz (2005).

Serological Studies

Collection of rabbit Sera: Blood samples were collected twice a week from each rabbit in each group from zero day till the 7th week PI., placed in plain centrifuge tubes and sera were separated and stored at -20°C for further work.

Preparation of Antigen: *E. stiedae* oocyst antigen was prepared as described by Mousa *et al.* (1996) and the protein content of antigen was determined according to Lowry *et al.* (1951). The antigen was aliquoted and stored at -20°C until use.

Enzyme Linked Immunosorbent Assay (ELISA): The immunomodulatory effect of moringa and thyme oils was evaluated through the detection of antibodies level by ELISA according to Santiago *et al.* (1986). The optimum antigen concentrations, sera and conjugate dilutions were determined by checkerboard titration. The absorbance was read by spectrophotometer at 405 nm and the cut off value of optical density (OD) was calculated according to Hillyer *et al.* (1992).

Statistical Analysis

Data of oocysts count and IgG antibody response were analyzed for the means and standard deviations. Significance of the results was evaluated using Analysis of variance (ANOVA) and Duncan using Statistical Package for Social Science (SPSS) computer program (2015).

RESULTS

Oocysts count

The first observation of *E. stiedae* oocysts in feces was observed in the 16th day PI (prepatent period was 16 days). Overall, there was a gradual reduction in the number of oocysts in the infected non-treated rabbits from the day 25 PI and continued till no oocysts were found by the day 46 PI. While, treatment of rabbits with moringa and thyme oils with the doses: 200 mg/kg and 500 mg/kg, respectively, stated to reduce oocysts shedding at 21st and 22nd day PI, respectively. There was a highly significant reduction in oocyst shedding ($P<0.001$ and $P<0.05$) in the infected moringa and thyme oils treated rabbits than the infected non-treated ones in almost all days PI. The effect of moringa and thyme oils on oocyst shedding was statistically the same in almost days although, thyme oil was more potent and stopped oocysts shedding earlier at the day 34 compared with moringa oil at the day 41 (Table 1). During the examination of feces of the moringa and thyme oils treated rabbits, deformed oocysts were observed after the peak of oocysts count at the 24th day PI (9th day post treatment) for moringa oil and at the 25th day PI (10th day post treatment) for thyme oil treated rabbits, respectively (Fig. 1).

Table 1. The number of oocysts in infected treated and non-treated rabbits at different days post infection

Rabbit Groups / Days Post Infection	Infected, Non-Treated	Infected, Treated with Moringa Oil	Infected, Treated with Thyme Oil	F- Value
Day 14	0±0	0±0	0±0	–
Day 15	0±0	0±0	0±0	–
Day 16	22050±1450	23200±900	22400±1400	0.64^{NS}
Day 17	$25150±350^{b}$	$25850±150^{a}$	$25900±300^{a}$	6.73^{*}
Day 18	25650±50	25750±50	25700±200	0.5^{NS}
Day 19	$33500±1500^{a}$	$26750±1150^{b}$	$25750±250^{b}$	44.0^{***}
Day 20	$49000±1000^{a}$	$26250±250^{b}$	$25600±400^{b}$	1307.42^{***}
Day 21	$84750±5250^{a}$	$25800±300^{b}$	$26250±250^{b}$	373.3^{***}
Day 22	$132500±12500^{a}$	$22000±2000^{b}$	$21500±1500^{b}$	226.45^{***}
Day 23	$298500±31500^{a}$	$21000±1000^{b}$	$19000±2000^{b}$	233.34^{***}
Day 24	$291900±26100^{a}$	$16000±4000^{b}$	$13500±1500^{b}$	329.47^{***}
Day 25	$130000±4500^{a}$	$13000±3000^{b}$	$7000±1000^{b}$	1430.8^{***}
Day 26	$126900±4600^{a}$	$12000±2000^{b}$	$4500±500^{c}$	1667.1^{***}
Day 27	$124000±4000^{a}$	$8000±2000^{b}$	$3000±0^{b}$	2109.2^{***}
Day 28	$112750±2750^{a}$	$5500±500^{b}$	$1900±100^{c}$	4564.4^{***}
Day 29	$105600±8400^{a}$	$4500±500^{b}$	$1350±150^{b}$	446.8^{***}
Day 30	$101400±10900^{a}$	$2850±550^{b}$	$550±50^{b}$	250.4^{***}
Day 31	$95850±9650^{a}$	$2350±50^{b}$	$300±0^{b}$	287.9^{***}
Day 32	$90950±5950^{a}$	$1300±300^{b}$	$100±0^{b}$	688.6^{***}
Day 33	$79000±13500^{a}$	$1150±150^{b}$	$50±0^{b}$	101.2^{***}
Day 34	$76100±15900^{a}$	$900±100^{b}$	$0±0^{b}$	67.9^{***}
Day 35	$73000±17000^{a}$	$600±0^{b}$	$0±0^{b}$	54.87^{***}
Day 36	$70150±15050^{a}$	$400±100^{b}$	$0±0^{b}$	64.81^{***}
Day 37	$65250±15050^{a}$	$150±50^{b}$	$0±0^{b}$	56.3^{***}
Day 38	$49650±19150^{a}$	$100±0^{b}$	$0±0^{b}$	20.13^{**}
Day 39	$42800±17500^{a}$	$50±0^{b}$	$0±0^{b}$	17.9^{**}
Day 40	$37500±17500^{a}$	$33±14^{b}$	$0±0^{b}$	13.76^{**}
Day 41	$25350±9650^{a}$	$0±0^{b}$	$0±0^{b}$	20.7^{**}
Day 42	$16500±7500^{a}$	$0±0^{b}$	$0±0^{b}$	14.52^{**}
Day 43	$12000±3000^{a}$	$0±0^{b}$	$0±0^{b}$	48^{***}
Day 44	5500±4500	0±0	0±0	4.48^{NS}
Day 45	$667±289^{a}$	$0±0^{b}$	$0±0^{b}$	16^{**}
Day 46	0±0	0±0	0±0	–

Data are expressed as Mean ± SD. Means that are followed by different letters indicated significance.
N.S. Non-significant, *Significant differences at $P<0.05$, $^{**}P<0.01$, $^{***}P < 0.001$.

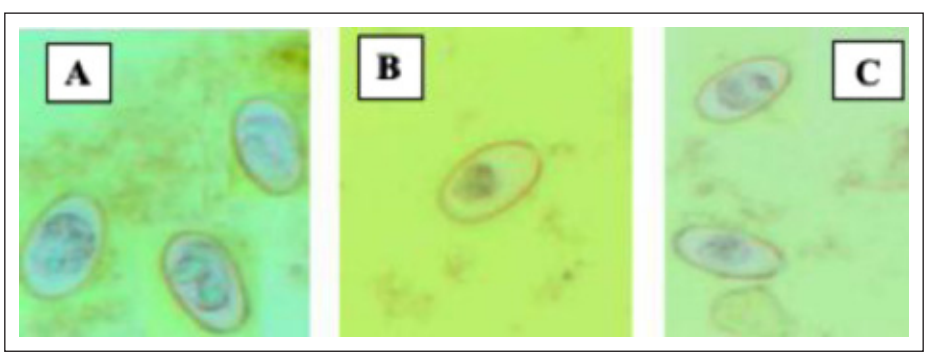

Figure 1. Sporulated oocysts of *Eimeria stiedae* isolated from infected non-treated rabbits (X400) (A). Deformed *Eimeria stiedae* oocysts observed in moringa (B) and thyme (C) oils treated rabbit feces (X400).

Macroscopic Lesions

Examined livers of healthy non-infected rabbits showed normal structure of liver tissue (Fig. 2A). Infected non-treated rabbit livers were extensively enlarged and pale in color. Many scattered yellowish-white nodules of variable sizes containing creamy fluid packed with oocysts were observed on liver surface tissue and the gall bladder was enlarged and greatly distended with creamy yellowish fluid (Fig. 2B). Livers of infected and moringa and thyme oils treated rabbits showed enhancement than livers of infected non-treated rabbits, whereas, thyme oil treated rabbits showed more enhancement in liver's morphology (Fig. 2C) than moringa oil treated rabbits (Fig. 2D).

Lesion Score

Lesion score was used as a parameter of infection severity (Fig. 2). It was found that mean focal lesions score in liver was 4 in the infected non-treated rabbits, 1.8 in the infected moringa oil treated rabbits and 1 in the infected thyme oil treated rabbits. Non-infected rabbits exhibited no lesions (Table 2). Rabbits infected and treated with moringa and thyme oils showed high level of protection (55 and 75%, respectively) against lesion score compared to the protection percentage of infected non treated rabbits (0%) (Table 2).

Detection the level of antibodies in treated and non-treated rabbits:

As shown in Figure 3, using *E. stiedae* oocyst antigen in ELISA, IgG antibody response in experimentally infected non treated rabbits started from 7th day PI and reached its maximum level at third week, then declined slightly till the fourth week before take plateau shape till the end of

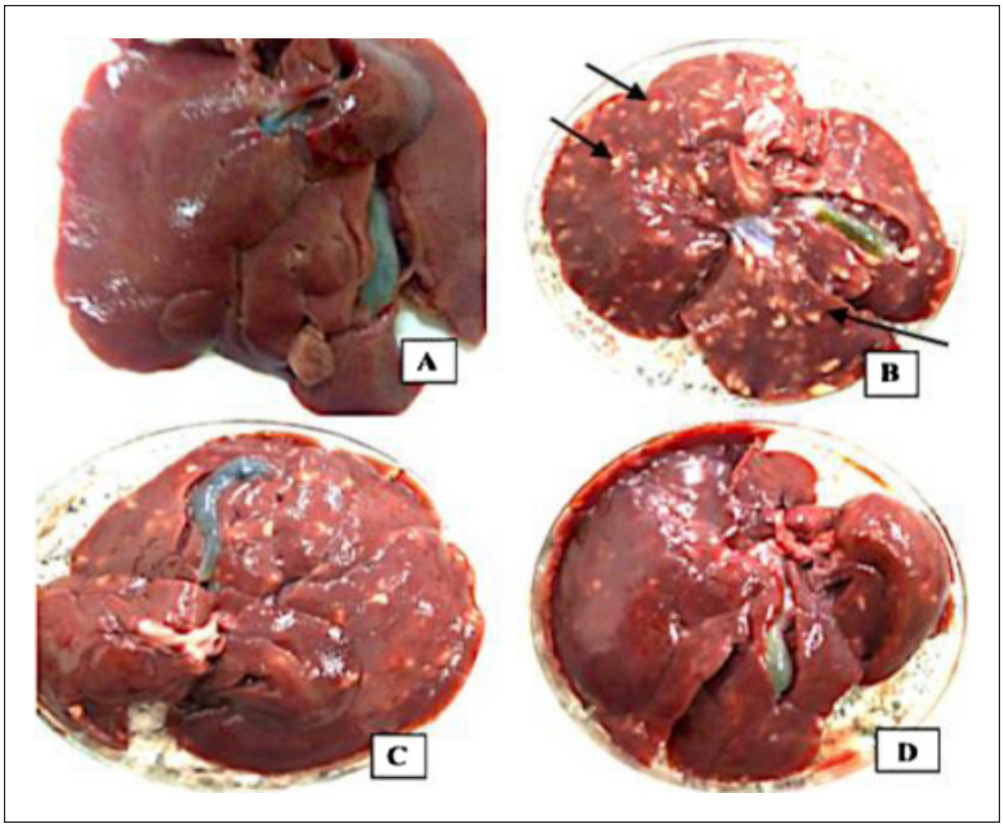

Figure 2. Examined livers of healthy rabbits (A), Irregular yellowish-white nodules of variable sizes (some showed by arrows) on the surface tissue of liver of domestic rabbits infected with hepatic coccidiosis (B). Livers of moringa and thyme oils treated rabbits (C and D, respectively) showing enhancement than livers of infected non-treated rabbits.

Table 2. Liver lesion score of healthy and infected rabbits

Group \ Parameter	Mean lesion score	Percentage of protection
Non-infected rabbits	0	100%
Infected non treated rabbits	4	0%
Infected moringa oil treated rabbits	1.8	55%
Infected thyme oil treated rabbits	1	75%

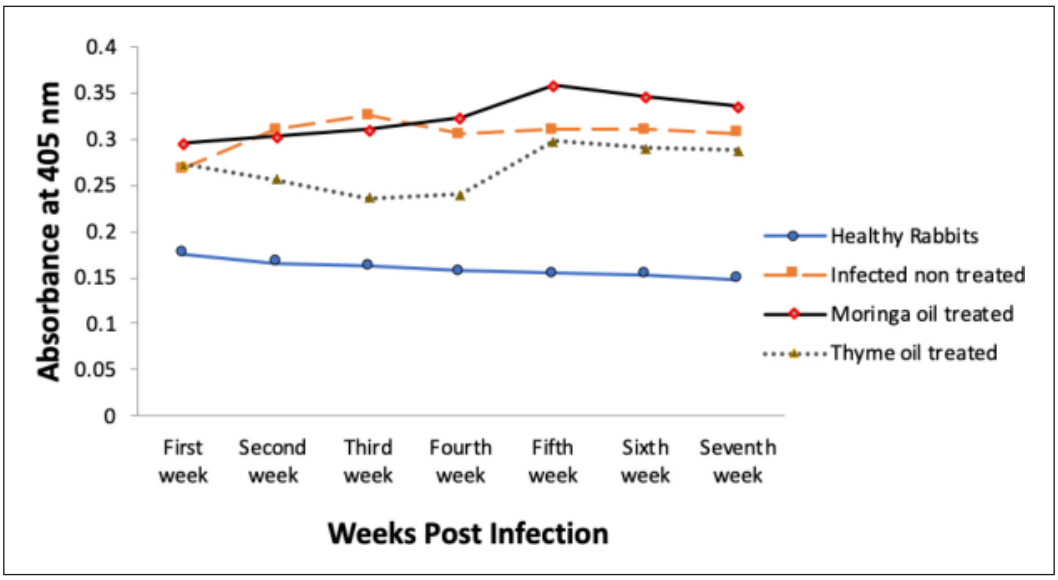

Figure 3. IgG antibody response in experimentally infected, treated and non-treated rabbits.

experiment. In the two treated groups the level of antibodies increased after treatment with moringa and thyme oils at third week PI (first week post treatment) till reached their maximum level at fifth week PI. Then declined slightly till the end of the experiment.

Statistically, starting from the 3rd week PI, which is considered the first week post treatment, till the end of the 4th week, thyme oil showed a significant decrease ($P<0.05$) than moringa oil treated and the infected non treated ones which were similar statistically. At the 5th week PI, IgG antibodies response increased significantly ($P<0.05$) in moringa oil treated rabbits than the other two infected groups. At the same week, IgG antibodies response in thyme oil treated rabbits didn't differ significantly from those of the infected non treated ones. At the 6th and 7th weeks PI, IgG antibodies response showed a significant increase ($P<0.05$) in moringa oil treated rabbits and a significant decrease ($P<0.05$) in thyme oil treated rabbits compared with the infected non treated ones.

DISCUSSION

Rabbit hepatic coccidiosis is one of the most important diseases affecting rabbit production industry. In the current study, parasitological examination of experimentally infected rabbit feces revealed that the prepatent period of *E. stiedae* infection was 16 days. Similar results were observed by Abdel Megeed & Abu El Ezz (2005) and Abu El Ezz *et al.* (2012). There was a gradual

reduction in the number of oocysts in the infected non-treated rabbits from the day 25 PI and continued till no oocysts were found by the day 46 PI. Comparable results were detected by Abu El Ezz *et al.* (2012) in experimentally infected rabbits with *E. stiedae*. In the current experiment, after treatment of infected rabbits with moringa (200 mg/kg) and thyme (500 mg/kg) oils, the number of shedding oocysts was decreased starting from the 21st and 22nd days PI, respectively, then completely disappeared at 34th day for thyme which the most potent followed by moringa, where, the disappearance occurred at 41st day PI. Moringa and thyme oils not only affect the number of shedding oocysts but also the deformed oocysts were observed during microscopic examination. This effect of moringa and thyme oils might be due to the potent therapeutic compounds found in these plants. Studies had shown that *M. oleifera* contained many bioactive compounds including vitamins, essential amino acids, polyphenols, flavonoids and phenolic acids (Leone *et al.*, 2015). Mature moringa seeds exhibited curative effects as they contain cysteine, benzyl isothiocynate (Katre *et al.*, 2008). In addition, the low density of plant oil and its rapid diffusion through cell membranes could enhance the targeting of active components within the oil against parasites (Anthony *et al.*, 2005). Also, Ola-Fadunsin & Ademola (2013) reported that *Moringa Oleifera* were used to treatment broiler chickens naturally infected with *Eimeria* species. El Banna *et al.* (2016) confirmed the anticoccidial activity of *M. Oleifera*. Furthermore, thyme contains many compounds that had therapeutic potentials like flavonoids, thymol, eugenol, carvacrol, saponins and phenols (Amarowicz *et al.*, 2008). Thyme extract containing thymol caused destruction of oocysts of *E. tenella* (Abbas *et al.*, 2012). *T. vulgaris* was able to destroy parasites, including oocysts and sporozoites (Muthamilselvan *et al.*, 2016). Essential oils derived from *T. vulgaris* and other plants showed inhibition of *Eimeria* species at different developmental stages (Muthamilselvan *et al.*, 2016). Also, Evans *et al.* (2001) reported a reduction of coccidia oocyst excretion in chicks fed the diets mixed with clove, thyme, lemon and peppermint essential oil mixture. In this study, the diference between the anticoccidial effect of the two oils might be due to the differences in active components of each oil which might act in a different way.

In the current study, livers of infected non-treated rabbits were extensively enlarged and pale in color with crowded yellowish-white nodules packed with oocysts of variable sizes containing creamy fluid. The gall bladders were enlarged and greatly distended with creamy yellowish fluid. These results matched with those observed by Cam *et al.* (2008), Abu-Akkada *et al.* (2010) and Abu El Ezz *et al.* (2012). These characteristic lesions of rabbit coccidiosis might be due to fibrosis and intense biliary hyperplasia. The livers of infected rabbits treated with thyme oil showed more enhancement in morphology than moringa oil treated livers and this might be due to that the thyme extract has an inhibitory effect on lipid peroxidation, which could decrease the strength of inflammatory response (Bozin *et al.*, 2006). In addition, this result might be due to the ability of thyme oil to eliminate infection in less time than the moringa oil, which helped to keep the liver in a better condition. Also, lesion scores recorded from livers of experimentally infected rabbits that were treated with moringa and thyme oils could be considered as a strong indication of the potent effect of these oils with the superiority of thyme oil.

In the present study, IgG antibody response in experimentally infected non-treated rabbits started from 7th day PI and reached its maximum level at third week, then declined slightly before taking plateau shape and still in high level till the end of the experiment. These results were comparable to those obtained by Constantinoiu *et al.* (2007) and Abu El Ezz *et al.* (2012). IgG antibody response in infected rabbits treated with moringa oil increased after the third week PI (first week post treatment) and reached its maximum level at the fifth week and still in high level till the end of the

experiment. Similar immunomodulatory effect of *M. oleifera* was reported by Miyachi *et al.* (2004) and Nfambi *et al.* (2015). The current result was not consistent with El Shanawany *et al.* (2019) who reported that *M. oleifera* leaves aqueous extract caused reduction in the level of IgG in *Fasciola gigantica* infected sheep. This difference in immune response might be due to that they used moringa leaves extract while, in current study, oil extract was used in addition to difference in used host and parasite. In the current study, rabbits treated with thyme oil showed an elevation in IgG antibody response from the third week PI (first week post treatment) and reached its peak at the fifth week PI then declined till the end of experiment and still in level less than IgG in moringa treated rabbits. This may be due to the rapid disappearance of the infection, resulting in a decrease in the level of antibodies or inability of the thyme to memorize the immune system of infected treated rabbits. In a previous study, the supplementation of thyme extract in drinking water did not improve the immune status in broiler chickens (Abdulkarimi, 2011) and this confirmed our results. In a previous study, the success of volatile plant oils as an alternative treatment for parasites depended on their anti-parasitic effect and also their improvement of host immune system (Anthony *et al.*, 2005). In the current study, although moringa oil proved immunomodulatory effect for rabbit immune system, thyme oil proved more anticoccidial effect and eliminated the infection in time less than moringa oil. Therefore, thyme oil needs further research to clarify this phenomenon in order to be widely used in the treatment of coccidiosis.

CONCLUSION

This study proved that both *M. oleifera* and *T. vulgaris* oils had an anti-coccidial effect with *T. vulgaris* oil superiority. So, *T. vulgaris* oil could be useful as an alternative product for the control of rabbit coccidiosis.

Authors' contributions

All authors prepared the research plan and designed experiments. NMTA contributed to isolation of oocysts, experimental infection, counting of oocysts, examination of livers and lesion score determination. DA shared in isolation of oocysts, experimental infection, counting of oocysts, antigen preparation, ELISA, data interpretation and statistical analysis. SEH contributed to dose preparation, antigen preparation, ELISA and data interpretation. KNA shared in isolation of oocysts, experimental infection, antigen preparation, ELISA and laboratory work analysis. TME contributed to sample collection. All authors shared in manuscript preparation and approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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