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ORIGINAL ARTICLE

Immunomodulatory effects of *Moringa oleifera* leaves aqueous extract in sheep naturally co-infected with *Fasciola gigantica* and *Clostridium novyi*

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Abstract The current study was designed to evaluate the in vivo fasciolicidal activity of *Moringa* (*M.*) *oleifera* leaf aqueous extract oral administration as well as its antibacterial activity against *Clostridium* (*C.*) *novyi in* sheep naturally co-infected with fascioliasis and *C. novyi*. Sheep naturally infected with fascioliasis were divided into 3 groups, heavily infected treated group, lightly infected treated group and mixed infection control (non-treated) group. Treatment groups were orally administered *M. oleifera* leaves aqueous extract at a dose of 150 mg/kg every 48 h for 21 days. Animal body weights, fecal egg count, serum levels of anti-*Fasciola* IgG, cytokines (IL-2, IL-17, IL-10), and bacterial count of *C. novyi* were evaluated. The results showed that treatment with *M. oleifera* improved the body weight gain and decreased fecal egg count in lightly and heavily infected groups compared to the nontreated group with 100% reduction in egg count in lightly infected sheep. Furthermore, the treatment with *M. oleifera* significantly reduced the serum levels of IgG, IL-2, and IL-17. Interestingly, elevated levels of IL-10 were recorded in both heavily and lightly infected sheep. The treatment with *Moringa* extract significantly decreased the fecal bacterial count of *C. novyi* in both heavily and lightly infected groups. In conclusion, this study highlights the potential beneficial effects of *M. oleifera* leaf against *Fasciola* (*F.*) *gigantica* and *C. novyi*.

Keywords *Moringa oleifera* · Anthelmintic · Antimicrobial · Liver flukes · Cytokines · Th17 · Immunoglobulins

Introduction

Fascioliasis, a parasitic infection caused by *Fasciola* (*F.*) *hepatica* and *F. gigantica*, *is* considered an important disease of ruminants causing significant weight loss, increased rates of liver condemnation and animal mortalities (Mas-Coma et al. 2014; Nyindo and Lukambagire 2015). Moreover, *Fasciola* species have zoonotic importance, affecting approximately 50 million people worldwide, and are listed as a foodborne infection by the World Health Organization (2015).*Clostridium* (*C.*) species are normal bacterial inhabitants found in the gastrointestinal tract of ruminants. Migration of *Fasciola* species to the liver and the subsequent necrosis and damage to the hepatic parenchyma facilitate the transmission and anaerobic medium required for systemic clostridial infection (Benavides et al. 2015; Cullen and Stalker 2015; Redford et al. 2017). Infection with *Clostridium novyi* type B causes "black disease" in sheep. It is associated with necrotic hepatitis due to migration of immature liver fluke that release toxins destroying the animal liver and causing sudden death (Uzal et al. 2016). The synergistic interaction between the two infections alters the infection duration, transmission risks, and clinical symptoms (Cox 2001; Vaumourin et al. 2015). Infection with *F. gigantica* is able to elicit Th2 immune response and downregulated Th1 and Th17- type

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inflammatory immune responses, leading to increased secretion of IFN-γ, Il-2, IL-6, IL-12, IL-17, and IL-1β cytokines in order to support the parasite's existence within the infected host (Pleasance et al. 2011; Zhang et al. 2017). The down regulation of protective Th1 responses results in an increase in the host's susceptibility to many bacterial infections (Cerf-Bensussan and Gaboriau-Routhiau 2010).

The use of anthelmintics and antibiotics in food animals is fundamental to animal health and to the agricultural economy as well. Drugs used in food-animals and drug residues in food products do increase the health risk in persons who consume products from treated animals (Hao et al. 2014). Moreover, during the last decade, there has been an increasing report in antibiotic resistance to different clostridial infections in animals and humans (Gholamiandehkordi et al. 2009; Bannam et al. 2011; Zidaric et al. 2012). Similarly, multiple reports confirmed anthelmintic resistance of *Fasciola* species in sheep, cattle and humans (Ortiz et al. 2013; Brockwell et al. 2014; Kelley et al. 2016). Medicinal plants have been widely utilized, either as a single drug or in combination with synthetic drugs to aid in control/treatment of various bacterial and parasitic infections in animals and human. These plants provide valuable natural products and drugs for development of medicines against various disorders and diseases (Pan et al. 2013; Bahmani et al. 2014).

Moringa oleifera, commonly known as Drumstick, have many active components such as alkaloids, tannins, flavonoids, saponins and triterpenoids (Vats and Gupta 2017), which showed potent anthelmintic activity and displayed a potential antibacterial activity against Gram negative and Gram-positive bacteria (El-Kholy et al. 2018). Aqueous extract of *M. oleifera* has been shown a potent immunomodulatory effect that can modulate the activation of B cells and stimulate production of IgM, IgA and IgG level after administration (Ojeka et al. 2018), and T cells where it will result for induction of IL 10 production (Fard et al. 2015; Tan et al. 2015) and inhibit production of TNFα, IL-6, IL-8 (Kooltheat et al. 2014) and IL-2 (Sashidhara et al. 2009). Studies on humans and animals revealed that *M. oleifera* is considered safe for consumption (Stohs and Hartman 2015). The ovicidal activity for *Moringa* extracts on *Fasciola* eggs in vitro was previously reported by Hegazi et al. (2018).

When these findings taking together we should warrant further consideration of *M. olifiera* as an immunomodulatory for immune disorders induced by fascioliasis. Therefore, we hypothesized that the oral administration of *M. oleifera* aqueous extract can provide protection against *F. gigantica* and *C. novyi* by regulating the immune responses. To test this hypothesis, The present study was designed to evaluate the oral administration effect of *M. olifiera* aqueous extract on the body weight, fecal egg count, IgG, cytokines (IL-2, IL-17, IL-10), and the bacterial load of *C. novyi* in sheep naturally co-infected with *F. gigantica* and *C. novyi*.

Materials and methods

Ethical approval

All experimental procedures were performed in accordance with the institutional guidelines of the National Research Centre's Animal Research Committee under protocol number 16/219.

Animals and ethical standard

A number of 30 reared sheep, aged 6–12 months, were examined in this study. The animals were determined for infection with *F. gigantica* using egg detection in fecal samples by sedimentation technique according to the method of Happich and Boray (1969). The examined animals were raised in the River Nile's basin and fed regularly on clover grasses and a concentrate mix. The infected animals were classified based on the severity of fascioliasis according to Attallah et al. (2013). The animals were further classified to a lightly infection treated group, a heavily infection treated group, a mixed lightly/heavily infection control non treated group and healthy, non-infected, sheep as a negative control.

Preparation of M. oleifera leaf aqueous extract and animal treatment

Moringa leaves powder was a courtesy from Dr. Aboelfetoh Abdalla's research group at the National Research Centre. 150 g of dried *M. oleifera* powder was soaked in a 1 liter distilled water and incubated at 4° C on a shaking incubator for 24 h then filtered using a filter paper into a clean conical flask. The *M. oleifera* extract was orally administrated to the lightly and heavily infection treated groups every 48 h for 21 days at a dose of 150 mg/kg body weight (Almanzor et al. 2014).

Body weight, fecal and blood sampling

All sheep were weighed on day 0 and re-weighed on day 21. Fecal samples were collected aseptically using rectal digital palpation from each animal on days 0, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21. The degree of body condition was scored 0–5 according to Russel et al. (1969) at the zero days of treatment and 21 day post treatment (dpt). A portion of each fecal sample was saved for *Fasciola* egg count per gram using the sedimentation technique. The remaining

portion of the egg sample was saved for bacteriological examination to estimate *C. novyi* colony forming unit per gram (CFU/g). Blood samples were obtained via Jugular venipuncture at different treatment intervals. Serum was separated and stored at − 20 °C until further analyses.

F. gigantica egg count and morphology

Briefly, 1 g of fecal sample was added to 45 ml of distilled water in a graduated cylinder and thoroughly mixed using a glass rod. The solution was filtrated using a sieve and allowed to sediment. *F. gigantica* egg count was then calculated and reported as eggs per gram (Happich and Boray 1969).

Detection of *F. gigantica* specific antibodies

Antigen preparation

Adult *F. gigantica* flukes were obtained from infected livers of water buffaloes freshly slaughtered at El Monib local abattoir, Giza Governorate and washed several times with distilled water. The crude worm antigen was prepared according to the method previously described (Abdel-Rahman et al. 2016). Adult flukes were homogenized in 0.2 M Tris–Hcl buffer, pH 7.4, with a glass homogenizer at 4 °C. The homogenate was then centrifuged at 14,000 round per minute (rpm) for 30 min at 4 °C, then the supernatant was collected and assayed for protein content using Lowery method (Lowry et al. 1951). The supernatant was aliquoted and stored at − 20 °C.

F. gigantica specific IgG assay

The assay was utilized to evaluate the total IgG antibody response of sheep after treatment using *M. oleifera* leaf aqueous extract at different time points in treated and control groups. The assay was performed according to the method previously described (Oldham 1983), and the optimal dilution of serum and concentration of the antigen were determined using checkerboard titration. The cutoff point of optical density values was determined as described by Almazán et al. (2001).

Quantification of cytokines in sheep sera

Sandwich enzyme-linked immunosorbent assay (ELISA) was used to measure serum cytokine levels. Cytokine concentrations for sheep IL-10, IL-2 and IL-17 determined with commercially available reagents and ELISA kits purchased from Bioneovan Co., China and following the manufacturer's instructions.

Determination of *C. novyi* levels (bacterial count CFU/g)

One gram from each fecal sample was homogenized in 9 ml of Phosphate Buffer Saline (1 × PBS). 10 fold serial dilutions of the homogenates in 1 × PBS was cultured in duplicates on neomycin blood agar using 100 µl of the diluted specimen for each plate. The inoculated plates were incubated at 37 °C and 5% CO2 for 24–48 h then the bacterial colonies were counted using colony counter according to the predefined criteria as described before (Black 1996). The colonies of *C. novyi* were confirmed microscopically and biochemically.

Statistical analysis

Data are expressed as mean ± standard deviation of the mean (SD). Statistical analyses were performed using two-way ANOVA followed by a post hoc test when appropriate. The result were considered significant at the level $p < 0.05$. Statistics were computed using GraphPad Prism software (version 6; GraphPad Software, Inc, La Jolla, CA, USA).

Results

The effects of *M. oleifera* aqueous extract oral administration on the body weight of sheep naturally infected with *F. gigantica*

The heavy infected treatment group showed a significant weight loss compared to the light and mixed infected groups before initiation of the treatment with *M. oleifera* aqueous extract (Day 0). Oral treatment with *M. oleifera* resulted in a significant weight gain at day 21 in the lightly infection treated group compared to heavily infected treated and mixed infection control groups (Fig. 1). The treatment with *M. olifera* improved the body weight of heavily infected sheep by 3.9 ± 0.1 and 4.5 ± 0.5 in treated lightly infected ones.

The effects of *M. oleifera* aqueous extract oral administration on the fecal egg count and morphology

The lightly and heavily infected treated groups showed a significant lower EPG across treatment time compared to the mixed infection control groups. Significant differences were noted between the lightly and heavly infected groups at all-time points. The treatment with *M. oleifera* aqueous extract resulted in a significant decrease in EPG in the lightly infected treated group with complete resolution of

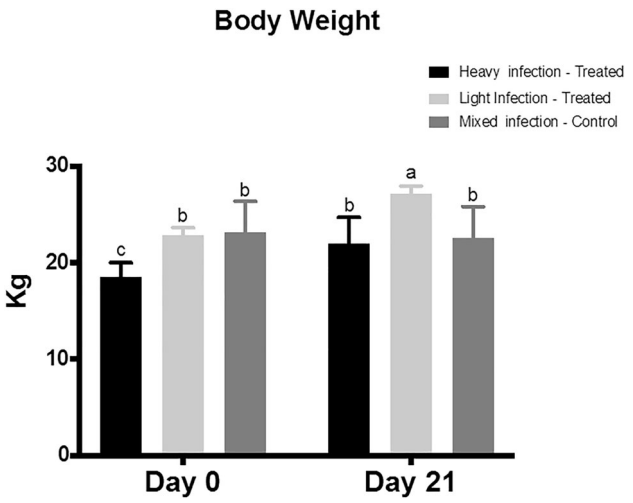

Fig. 1 The effect of *Moringa* (*M.*) *oleifera* aqueous extract oral adminstration on the body weight of sheep naturally infected with *Fasciola* (*F.*) *gigantica*. The Sheep (6–12 months age) were classified based on the severity of fascioliasis into three groups; lightly infected treated group, heavily infected treated group, and mixed infected control group. The lightly and heavily infected groups were treated with *M. oleifera* aqueous extract for 21 days. The body weight of sheep in the three groups was recorded at 0 and 21 days. Two-way ANOVA was performed followed by multiple comparison tests. Data represented in mean ± standard deviation (SD). Different letters denote significance ($p < 0.05$)

the infection (Fig. 2a). However, in heavily infected group the mean egg count was significantly ($p \leq 0.001$) decreased with 99.2% reduction after 21 dpt. Furthermore, the treatment with *M. oleifera* aqueous extract resulted in a degeneration of *Fasciola* eggs and accumulated dark embryo after the first dose of treatment at the end of experiment the egg showed empty without cells or embryo (Fig. 2b) This observation illustrates that treatment using *Moringa* extract have an obivious effect on egg viability and development.

The effects of *M. oleifera* aqueous extract oral administration on the fecal bacterial load of *C. novyi*

The *C. novyi* was numerous short, thick, straight; round-ended, gram positive rod occurs singly or in short chains under a microscope. On blood agar, the pure culture was small, irregular, whitish pale colored, finely granular and surrounded by a typical zone of β-hemolysis. In cooked meat medium, organism produced turbidity and gas with pinkish discoloration of meat particles. Biochemical characterization showed that all isolates fermented dextrose, lactose, sucrose, maltose and produced acid and gas but did not ferment mannitol. They were negative catalase, oxidase, Indole, Voges–Proskauer, and methyl red. The bacterial load of *C. novyi* was reduced significantly during the treatment with *M. oleifera* extract in both lightly infected and heavily infected groups compared to untreated control group till reach 2×10^4 after 21 day post treatment (dpt) (Fig. 3).

The effects of *M. oleifera* aqueous extract oral administration on serum IgG expression

The levels of IgG showed a decrease in the lightly infected group compared to the heavily and mixed infection groups. Significant differences were noted between the lightly and heavily infected groups at most of the time points. The

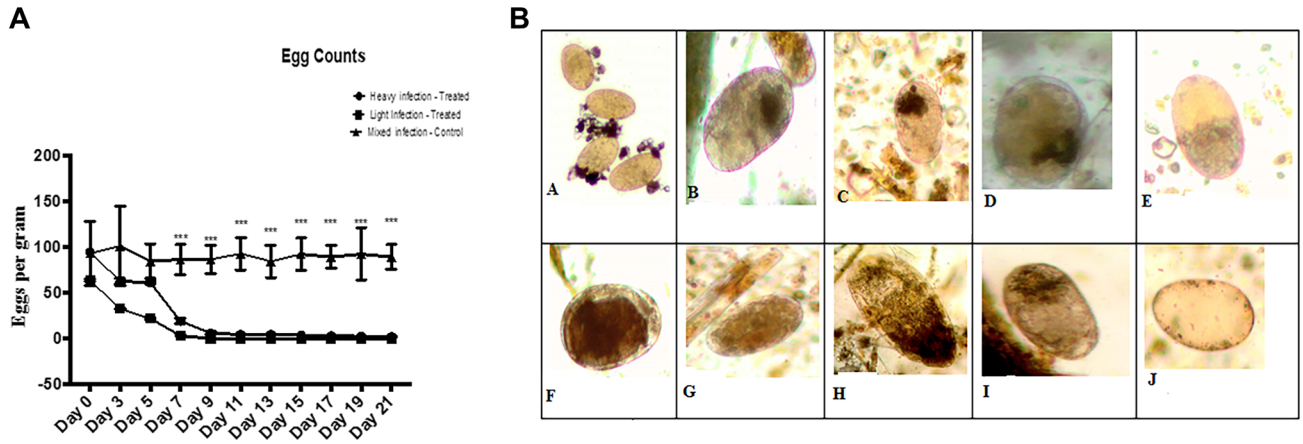

Fig. 2 **a** The effect of *M. oleifera* aqueous extract oral adminstrationon the fecal egg count of sheep naturally infected with *F. gigantica*. Eggs per gram (EPG) were counted using fecal sedimentation technique in the three groups; lightly infected treated group, heavily infected treated group, and mixed infected control group. Data represented in mean ± standard deviation (SD). *Denotes significance ($p < 0.05$) between the three groups. Two-way ANOVA followed by multiple comparisons test was applied. **b** Photomicrograph to show the ovicidal activity of *M. oleifera* aqueous extracts treatment on sheep. *A* Normal *F. gigantica* egg. *B*, *C*, *D*, *E*, *F*, *G*, *H*, *I* showed degenerated *Fasciola* egg and accumulated dark embryo at 3, 5, 7, 9, 11, 13, 15, 17 dpt respectively. *J* Showed empty *F. gigantica* egg at 21 dpt (×100)

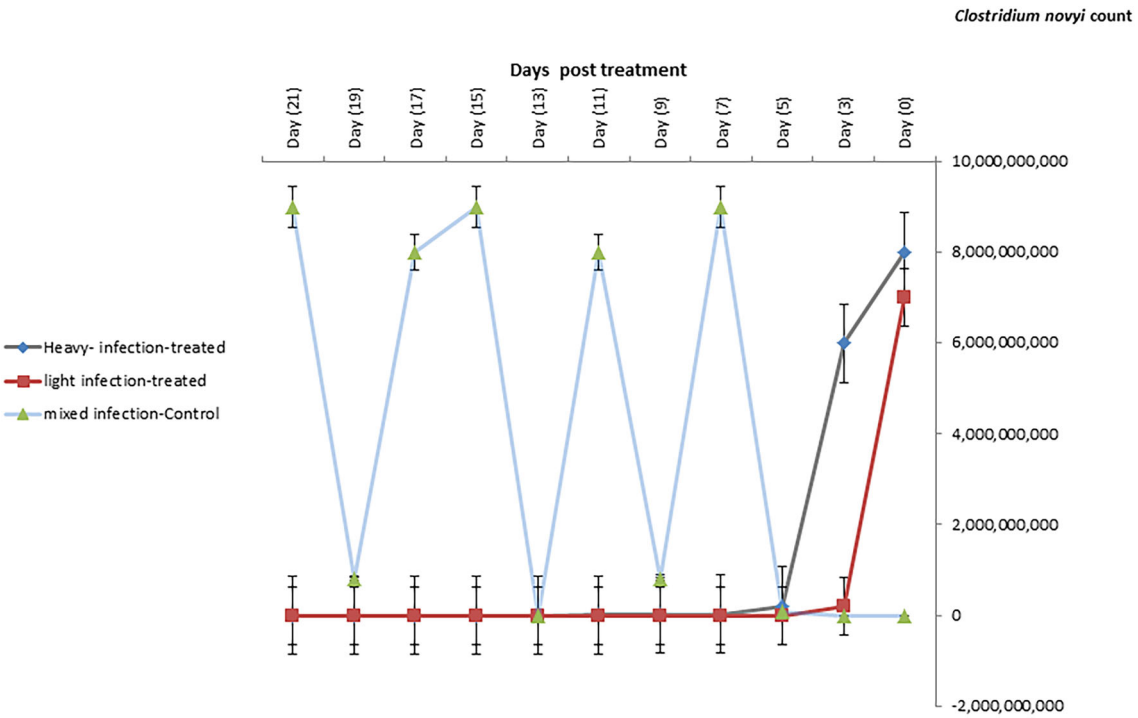

Fig. 3 The bacterial counts of *C. novyi* in heavily, lightly, and mixed infected sheep with *F. gigantica* and treated with *M. oleifera* leaves. Data represented in mean ± standard deviation (SD). *Denotes significance ($p < 0.05$) between the three groups. Two-way ANOVA followed by multiple comparisons test was applied

treatment with *M. oleifera* aqueous extract resulted in a gradual significant decrease in IgG in the lightly infected treated group with complete resolution of the infection (Fig. 4).

The effects of *M. oleifera* aqueous extract oral administration on serum levels of IL-2, IL-17, and IL-10

The treatment with *M. oleifera* aqueous extract resulted in a significant decrease in serum level of both IL-17 and IL-2 and increase in the level of IL-10 regulatory cytokine in the lightly infected treated group compared to the heavily infected and mixed infection groups (Fig. 5a–c). The temporal patterns of IL-2 and IL-17 showed an increase after treatment with *M. oleifera* aqueous extract then started to decrease at day 5 and continuous decrease until day 21 (Fig. 5a–c).

Discussion

The current study was designed to investigate the in vivo fasciolicidal and antibacterial effect of *M. oleifera* leaf aqueous extract oral administration *in* naturally infected sheep. The study showed a significant body weight gain in the infected sheep treated with *M. oleifera*. This result is

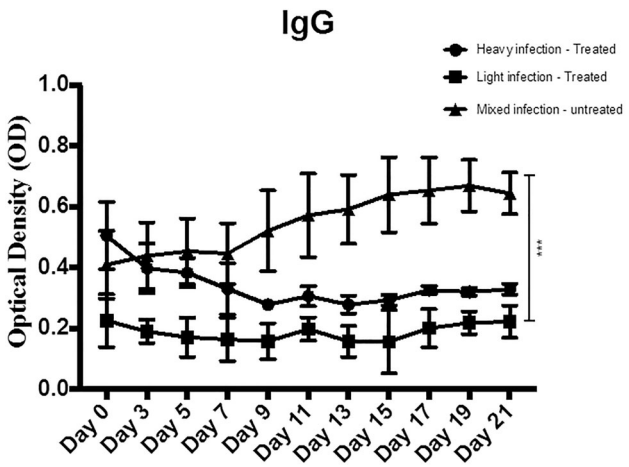

Fig. 4 The effect of *M. oleifera* aqueous extract oral adminstration on the serum IgG expression in sheep naturally infected with *F. gigantica*. IgG levels were quantified using specific IgG assay in the three groups; lightly infected treated group, heavily infected treated group, and mixed infected control group. Data represented in mean ± standard deviation (SD). *Denotes significance ($p < 0.05$) between the three groups. Two-way ANOVA followed by multiple comparisons test was applied

anticipated as a reduction in parasites load would result in improved growth rates and body condition scores. Moreover, it is obvious that *M. oleifera* contained a considerable amount of protein, minerals and antioxidant that may have improved digestion and absorption resulting in increased

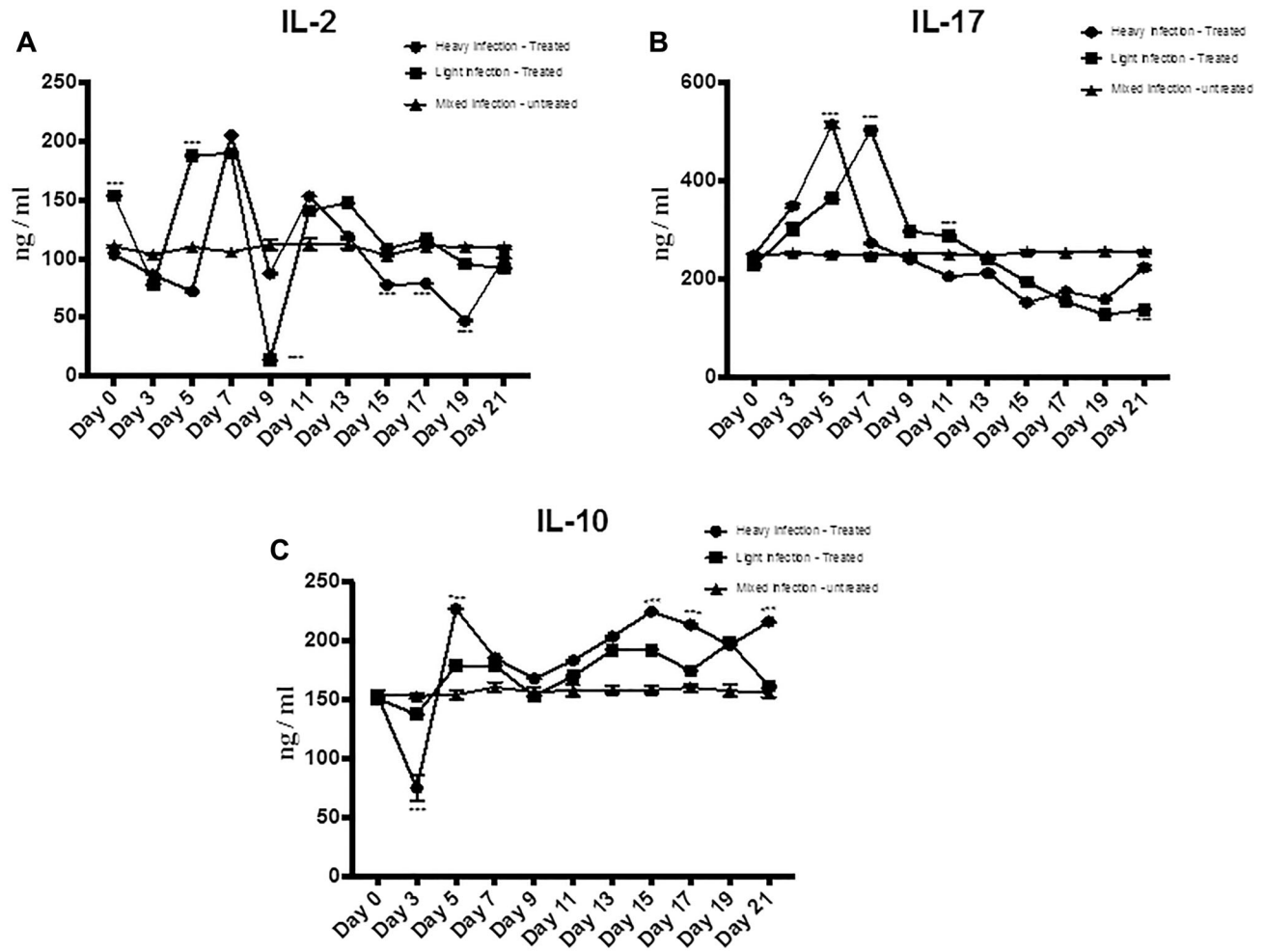

Fig. 5 The effect of *M. oleifera* aqueous extract oral administration on the serum levels of IL-2, IL-17, and IL-10 in sheep naturally infected with *F. gigantica*. The serum levels of **a** IL-2, **b** IL-17, and **c** IL-10 were quantified using ELISA in the three groups; lightly infected treated group, heavily infected treated group, and mixed infected control group. Data represented in mean ± standard deviation (SD), *Denotes significance ($p < 0.05$) between the three groups. Two-way ANOVA followed by multiple comparisons test was applied

body weight and host-resistance to the helminthes. These results are in agreement with Moyo et al. (2013) who reported an improved body condition score and a decrease in coccidian oocysts and helminthes load in goats after dietary supplementation with *M. oleifera.*

The fecal egg count of the infected sheep treated with *M. oleifera* significantly decreased over the 21 days course of treatment in heavily infection with completely disappearance of eggs in lightly infection. Furthermore, the treatment resulted in egg morphological changes; as *Fasciola* eggs obtained from fecal samples showed marked degeneration and accumulated dark embryo. These results came in agreement with our previous research (Hegazi et al. 2018) which revealed the in vitro ovicidal activity of alcoholic and aqueous extract of *M. oleifera* leaf on *Fasciola* eggs. One explanations for this finding is that *M. oleifera* may induce toxic effect on *F. gigantica* worms, resulting in alternation in worm fecundity and distortion of their eggs similar to the effect reported on *Haemonchus contortus* (Cabardo Jr and Portugaliza 2017). This toxic effect may be related to the presence of saponins, tannins, and flavonoids *in M. oleifera* leaves (Fatima et al. 2014). Furthermore, *M. oleifera* treatment proved an immunomodulatory effect in rats (Jayanthi et al. 2015) leading to increasing the immune response which positively correlated with the reduction of worm number then reduction in egg shedding. However, the mechanism by which *M. oleifera* might function as fasciolicidal agent needs further investigation.

In the present study, *M. oleifera* leaf aqueous extract oral administration resulted in a significant decrease in IgG levels in highly infected sheep, from day 3 and continued until the end of the experiment. However, mean IgG titers remained significantly higher than the assay control

negative sera obtained from healthy, non-infected sheep. Meanwhile, lightly infected treated sheep showed significant decreases in serum IgG level to levels equivalent to those of the assay negative control sera. This finding indicated that *M. oleifera* oral administration may lower the severity of the infection. Previous research reports concluded that *M. oleifera* is an immune boosting agent that can stimulate IgG, IgM and IgA production to fight invading organisms until neutralization (Abd-Elhakim et al. 2018). The resulted antibody may interact with critical sites in the surface membrane of the parasite. It is also possible that the anti fasciolicidal activity of *M. oleifera* aqueous extract may be related to tannins which can cause paralysis of the *Fasciola* adult worm (Hoste et al. 2006; Molan et al. 2000). Similarly, the presence of saponins is known to destabilize the cell membrane and cuticle collagen of the parasite (D'addabbo et al. 2011).

Cytokines are key regulators of immune inflammatory responses (Pleasance et al. 2011; Zhang et al. 2005). The beneficial effects of *M. oleifera* extract on the regulation of Th1/Th2/Th17 balance were evaluated (Sudha et al. 2010). Current results proved that both inflammatory Th1 cytokine; IL- 2 and Th17 cytokine; IL-17 in heavily and lightly infected sheep were suppressed by *M. oleifera* extract treatment with the lowest level at 21 dpt. This result running parallel with the previous reports which proved that *M. oleifera* downregulated the expression and production of IL-2 cytokine (Sashidhara et al. 2009), which inhibited the inflammatory reaction due to infection. On the other hand, the presented results demonstrated a significant gradual increase in anti-inflammatory cytokine IL 10 to reach to its highest level at the 21 dpt in both high and lightly infected sheep treated with *M. oleifera* extract. The present result comes in accord with the previous study of Fard et al. (2015) who proved that *M. oleifera* hydroethanolic bioactive leaves extract significantly modulate the production of IL- 10 from macrophage. However, the exact effect of *M. oleifera* on Th1/Th2 during fascioliasis remains obscured and needs further study.

The current results showed that the bacterial count of *C. novyi* at zero days of treatment was 7×10^9–9×10^9 and after treatment with *Moringa* extract the count were reduced in both heavly and lightly naturally infected sheep reached 2×10^4 after 21 dpt. M. *oleifera* was a potential source of the antimicrobial molecule against pathogenic *Clostridium spp*. The mechanism of reducing *C. novyi* count was due to direct contact between the *M. oleifera* and bacteria (El-Kholy et al. 2018). Moreover, *M. oleifera* downregulated the levels of IL-2 and IL-17 and up-regulated IL-10 expression resulting in a protective immune mechanism against *C. novyi*.

More studies are required to fully understand the immunological implications of using *M. oliefera* as target drug to control fascioliasis inflammation-based disorder. Further studies on understanding the effect of *M. oliefera* on Nf-kB transcription factor may shed light on immune modulatory mechanism of *M. oliefera.*

In conclusion, this study provides evidence that *M. oleifera* may have potential therapeutic effects against *F. gigantica*. However, further studies are warranted to investigate the implicated molecular pathways and cell–cell interaction during the treatment with *M. oleifera* in naturally infected animals.

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Author contributions EHA and AGH designed the study. HGK and EAF clinically diagnosed the infection. EEE, HGK participated in preparation of *M. oleifera* leaf aqueous extract, and animal's treatment. EEE, HGK and EAF weighed animals and collected fecal, and blood samples. SEH identified and counted *F. gigantica* eggs in feces with determination of its morphology. EEE and SEH carried out ELISA for detection of *F. gigantica* specific antibodies. EEE conducted in cytokines quantification in sheep sera. EAF isolated, identified, characterized and counted *C. novyi*. AGH, EHA and EEE were conducting in data analyses and interpretation and in manuscript preparation. EEE was write manuscript. All authors have reviewed and approved the final manuscript.

Compliance with ethical standard

Conflict of interest The authors declare that they have no conflicts or competing interests.

References

Abd-Elhakim YM, El Bohi KM, Hassan SK, El Sayed S, Abd-Elmotal SM (2018) Palliative effects of *Moringa olifera* ethanolic extract on hemato-immunologic impacts of melamine in rats. Food Chem Toxicol 114:1–10. https://doi.org/10.1016/j.fct.2018.02.020

Abdel-Rahman EH, Mohamed AH, Abdel-Rahman AAH, El Shanawany EE (2016) The role of Ser-(Arg-Ser-Arg-Ser-GlucNAc)$_{19}$-GlucNAc *Fasciola gigantica* glycoprotein in the diagnosis of prepatent fasciolosis in rabbits. JOPD 40:11–21. https://doi.org/10.1007/s12639-014-0461-3

Almanzor D, Clemente R, Fornillos M, Gomez F, Ladiao B, Calzada BD, Tamodtamod R (2014) In vivo trials of *Moringa oleifera* Lam. extracts as antischistosomal treatment on *Schistosoma japonicum* infected mice. IJIMS 2:49–56

Almazán C, Avila G, Quiroz H, Ibarra F, Ochoa P (2001) Effect of parasite burden on the detection of *Fasciola hepatica* antigens in sera and feces of experimentally infected sheep. Vet Parasitol 97:101–112. https://doi.org/10.1016/S0304-4017(01)00376-4

Attallah AM, Bughdadi FA, El-Shazly AM, Ismail H (2013) Immunodetection of *Fasciola gigantica* circulating antigen in sera of infected individuals for laboratory diagnosis of human fascioliasis. Clin Vaccine Immunol 20:1569–1577. https://doi.org/10.1128/cvi.00305-13

Bahmani M, Rafieian-Kopaei M, Hassanzadazar H, Saki K, Karamati SA, Delfan B (2014) A review on most important herbal and

synthetic antihelmintic drugs. Asian Pac J Trop Med 7:S29–S33. https://doi.org/10.1016/s1995-7645(14)60200-5

Bannam TL, Yan XX, Harrison PF, Seemann T, Keyburn AL, Stubenrauch C, Weeramantri LH, Cheung JK, McClane BA, Boyce JD, Moore RJ, Rood JI (2011) Necrotic enteritis-derived *Clostridium perfringens* strain with three closely related independently conjugative toxin and antibiotic resistance plasmids. MBio 2:e00190–e00211. https://doi.org/10.1128/mBio.00190-11

Benavides J, González L, Dagleish M, Pérez V (2015) Diagnostic pathology in microbial diseases of sheep or goats. Vet Microbiol 181:15–26. https://doi.org/10.1016/j.vetmic.2015.07.012

Black JG (1996) Microbiology: principles and applications, 3rd edn. Prentice Hall, Upper Saddle River

Brockwell YM, Elliott TP, Anderson GR, Stanton R, Spithill TW, Sangster NC (2014) Confirmation of *Fasciola hepatica* resistant to triclabendazole in naturally infected Australian beef and dairy cattle. Int J Parasitol Drugs Drug Resist 4:48–54. https://doi.org/10.1016/j.ijpddr.2013.11.005

Cabardo DE Jr, Portugaliza HP (2017) Anthelmintic activity of *Moringa oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae. IJVSM 5:30–34. https://doi.org/10.1016/j.ijvsm.2017.02.001

Cerf-Bensussan N, Gaboriau-Routhiau V (2010) The immune system and the gut microbiota: friends or foes? Nat Rev Immunol 10:735. https://doi.org/10.1038/nri2850

Cox F (2001) Concomitant infections, parasites and immune responses. Parasitol 122:S23–S38. https://doi.org/10.1017/S0031182000001698X

Cullen JM, Stalker MJ (2015) Liver and biliary system. Jubb Kennedy Palmer's Pathol Domest Anim 2:258–352

D'addabbo T, Carbonara T, Leonetti P, Radicci V, Tava A, Avato P (2011) Control of plant parasitic nematodes with active saponins and biomass from *Medicago sativa*. Phytochem Rev 10:503–519. https://doi.org/10.1007/s11101-010-9180-2

El-Kholy K, Barakat SA, Morsy W, Abdel-Maboud K, Seif-Elnaser M, Ghazal MN (2018) Effect of aqueous extract of *Moringa oleifera* leaves on some production performance and microbial ecology of the gastrointestinal tract in growing rabbits. Pak J Nutr 17:1–7. https://doi.org/10.3923/pjn.2018.1.7

Fard MT, Arulselvan P, Karthivashan G, Adam SK, Fakurazi S (2015) Bioactive extract from *Moringa oleifera* inhibits the pro-inflammatory mediators in lipopolysaccharide stimulated macrophages. Pharmacogn Mag 11:S556. https://doi.org/10.4103/0973-1296.172961

Fatima T, Sajid MS, Jawad-ul-Hassan M, Siddique RM, Iqbal Z (2014) Phytomedicinal value of *Moringa oleifera* with special reference to antiparasitics Pak. J Agric Sci 51:251–262

Gholamiandehkordi A, Eeckhaut V, Lanckriet A, Timbermont L, Bjerrum L, Ducatelle R, Haesebrouck F, Van Immerseel F (2009) Antimicrobial resistance in *Clostridium perfringens* isolates from broilers in Belgium. Vet Res Commun 33:1031. https://doi.org/10.1007/s11259-009-9306-4

Hao H, Cheng G, Iqbal Z, Ai X, Hussain HI, Huang L, Dai M, Wang Y, Liu Z, Yuan Z (2014) Benefits and risks of antimicrobial use in food-producing animals. Front Microbiol 5:288. https://doi.org/10.3389/fmicb.2014.00288

Happich F, Boray J (1969) Quantitative diagnosis of chronic fasciolosis: 1. Comparative studies on quantitative faecal examinations for chronic *Fasciola hepatica* infection in sheep. Aust Vet J 45:326–328. https://doi.org/10.1111/j.1751-0813.1969.tb05009.x

Hegazi AG, Megeed KNA, Hassan SE, Abdelaziz M, Toaleb NI, El Shanawany EE, Aboelsoued D (2018) Comparative ovicidal activity of *Moringa oleifera* leaf extracts on *Fasciola gigantica* eggs. Vet World 11:215. https://doi.org/10.14202/vetworld.2018.215-220

Hoste H, Jackson F, Athanasiadou S, Thamsborg SM, Hoskin SO (2006) The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol 22:253–261. https://doi.org/10.1016/j.pt.2006.04.004

Jayanthi M, Garg SK, Yadav P, Bhatia A, Goel A (2015) Some newer marker phytoconstituents in methanolic extract of *Moringa oleifera* leaves and evaluation of its immunomodulatory and splenocytes proliferation potential in rats. Indian J Pharmacol 47:518. https://doi.org/10.4103/0253-7613.165199

Kelley JM, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW (2016) Current threat of triclabendazole resistance in *Fasciola hepatica*. Trends Parasitol 32:458–469. https://doi.org/10.1016/j.pt.2016.03.002

Kooltheat N, Sranujit R, Chumark P, Potup P, Laytragoon-Lewin N, Usuwanthim K (2014) An ethyl acetate fraction of *Moringa oleifera* Lam inhibits human macrophage cytokine production induced by cigarette smoke. Nutrients 6:697–710. https://doi.org/10.3390/nu6020697

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Mas-Coma S, Valero MA, Bargues MD (2014) Fascioliasis. Adv Exp Med Biol 766:77–114. https://doi.org/10.1007/978-1-4939-0915-5_4

Molan A, Alexander R, Brookes I, McNabb W (2000) Effects of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes in vitro. In: Proceedings of the New Zealand society of animal production, pp 21–25

Moyo B, Masika PJ, Muchenje V (2013) Effects of supplementing cross-bred Xhosa lop eared goats with *Moringa oleifera* Lam. on helminth load and corresponding body condition score, packed cell volume. Afr J Agric 8:5327–5335. https://doi.org/10.1007/s11250-011-9970-6

Nyindo M, Lukambagire A-H (2015) Fascioliasis: an ongoing zoonotic trematode infection. BioMed Res Int. https://doi.org/10.1155/2015/786195

Ojeka S, Obia O, Dapper D (2018) Effect of acute administration of aqueous leaf extract of *Moringa oleifera* on immunoglobulin levels in wistar rats. EJMP 14:1–7. https://doi.org/10.9734/ejmp/2016/24880

Oldham G (1983) Antibodies to *Fasciola hepatica* antigens during experimental infections in cattle measured by ELISA. Vet Parasitol 13:151–158. https://doi.org/10.1016/0304-4017(83)90075-4

Ortiz P, Scarcella S, Cerna C, Rosales C, Cabrera M, Guzmán M, Lamenza P, Solana H (2013) Resistance of *Fasciola hepatica* against Triclabendazole in cattle in Cajamarca (Peru): a clinical trial and an in vivo efficacy test in sheep. Vet Parasitol 195:118–121. https://doi.org/10.1016/j.vetpar.2013.01.001

Pan SY, Zhou SF, Gao SH, Yu ZL, Zhang SF, Tang MK (2013) New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evid Complement Altern Med 2013:627375. https://doi.org/10.1155/2013/627375

Pleasance J, Wiedosari E, Raadsma H, Meeusen E, Piedrafita D (2011) Resistance to liver fluke infection in the natural sheep host is correlated with a type-1 cytokine response. Parasite Immunol 33:495–505. https://doi.org/10.1111/j.1365-3024.2011.01305.x

Redford T, Cubberley JC, Hengeveld P, Zabek E, Britton AP (2017) Myocardial necrosis associated with *Clostridium novyi* infection in a bighorn sheep (*Ovis canadensis*). J Wildl Dis 53:695–698. https://doi.org/10.7589/2016-09-221

Russel A, Doney J, Gunn R (1969) Subjective assessment of body fat in live sheep. J Agric Sci 72:451–454. https://doi.org/10.1017/S0021859600024874

Sashidhara KV, Rosaiah JN, Tyagi E, Shukla R, Raghubir R, Rajendran SM (2009) Rare dipeptide and urea derivatives from roots of *Moringa oleifera* as potential anti-inflammatory and antinociceptive agents. Eur J Med Chem 44:432–436. https://doi.org/10.1016/j.ejmech.2007.12.018

Stohs SJ, Hartman MJ (2015) Review of the safety and efficacy of *Moringa oleifera*. Phytother Res 29:796–804. https://doi.org/10.1002/ptr.5325

Sudha P, Asdaq S, Dhamingi SS, Chandrakala GK (2010) Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals Indian. J Physiol Pharmacol 54:133–140

Tan WS, Arulselvan P, Karthivashan G, Fakurazi S (2015) *Moringa oleifera* flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF-κB pathway. Mediat Inflamm 2015:1–11. https://doi.org/10.1155/2015/720171

Uzal FA, Songer JG, Prescott JF, Popoff MR (2016) Clostridial diseases of animals. Wiley Online Library, Hoboken. ISBN 978-1-118-72840-6

Vats S, Gupta T (2017) Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. Physiol Mol Biol Plants 23:239–248. https://doi.org/10.1007/s12298-016-0407-6

Vaumourin E, Vourc'h G, Gasqui P, Vayssier-Taussat M (2015) The importance of multiparasitism: examining the consequences of co-infections for human and animal health. Parasit Vectors 8:545. https://doi.org/10.1186/s13071-015-1167-9

WHO (2015) WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007–2015. World Health Organization, Geneva

Zhang FK, Guo AJ, Hou JL, Sun MM, Sheng ZA, Zhang XX, Huang WY, Elsheikha HM, Zhu XQ (2017) Serum levels of cytokines in water buffaloes experimentally infected with *Fasciola gigantica*. Vet Parasitol 244:97–101. https://doi.org/10.1016/j.vetpar.2017.07.028

Zhang W, Moreau E, Hope J, Howard C, Huang W, Chauvin A (2005) *Fasciola hepatica* and *Fasciola gigantica*: comparison of cellular response to experimental infection in sheep. Exp Parasitol 111:154–159

Zidaric V, Pardon B, dos Vultos T, Deprez P, Brouwer MSM, Roberts AP, Henriques AO (2012) Different antibiotic resistance and sporulation properties within multiclonal *Clostridium* difficile PCR ribotypes 078, 126, and 033 in a single calf farm. Appl Environ Microbiol 78:8515–8522. https://doi.org/10.1128/AEM.02185-12