

The Aqueous Leaf Extract of *Moringa Oleifera* had Immunomodulatory Effects on Sheep that had Been Infected by *Fasciola Gigantica*, *Clostridium Novyi* Naturally and Impact to *Fasciola gigantica* Non-Embryonated

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ABSTRACT

M., which received a (C), was the study's main objective. The whole thing was eaten. a couple of quotes from (C. dispersed in a liquid. has antibacterial properties (E. elegans). It's necessary to remove the body. C. Eczema on the face is comparable to this. Sheep and novyi both had organic infections. novyi. Following different combinations of treatments, a heavy dose of treatment, and a light dose, fasciolitis manifested in all three groups of infected sheep. The treatment groups received an oral 150 mg/kg aqueous M. oleifera leaf extract every 48 hours for 21 days. Body mass, fecal egg counts, blood anti-Fasciola IgG levels, cytokines (IL-2, IL-17, and IL-10), and C. the elegans' bacterial inhabitants jokingly strike up a conversation with everyone there. Sheep with light and heavy infections gained more weight after receiving M. oleifera treatment and had lower fecal egg counts than control groups; sheep with light infection had a 100% reduction in egg count. The serum concentrations of IL-2, IL-17, and IgG all significantly decreased after M. oleifera treatment. There were significantly higher levels of IL-10 in sheep with both mild and severe infections. C's feces contained noticeably fewer bacteria after taking moringa extract. The two novyi groups had varying degrees of infection severity. Fabricio (F. (as well as OleiferaMdot). C. noteworthy and novyi. M. oleifera aqueous extracts showed a concentration-dependent ovicidal effect on F. gigantica non-embryonated.

Key words: *Moringa oleifera*, Anthelmintic, Antimicrobial, Cytokines, Public Health.

INTRODUCTION

Caused by the parasite *Fasciola* (F. either hepatitis type. In (MasComa) is a ruminant-specific illness called gigantica. Nyindo, Lukambagire, and Nyindo made their debuts in 2015. Because they are significant zoonotic agents that affect about 50 million people worldwide, the *Fasciola* species are categorized by the World Health Organization¹ as a food borne infection. *C. clostridium*). In the digestive tracts of ruminants, species are frequently found. The movement of *Fasciola* species to the liver, followed by the hepatic parenchyma's subsequent necrosis and damage, causes infection transmission and creates the anaerobic environment required for systemic clostridial infection.² The business is Ddot. Movies like *Stalker*, *Cullen*, and other projects came to fruition in 2015. Sheep exposed to *Clostridium novyi* type B developed black disease. It resembles necrotic hepatitis because young liver flukes that migrate there release toxins that damage the animal's liver and result in sudden death³ similar to *Vaumourin et al.* According to *Cox*⁴ the synergistic interaction of the two infections is said to have an effect on the length of the infection, the risk of transmission, and the clinical symptoms. The F virus is found in that area. To keep the parasite alive inside the infected host, *Massivea* has the ability to secrete cytokines like IFN-c, IL-2, IL-6, IL-12, IL-17, and IL-1b as well as a Th2 immune response. *Massivea* can also reduce Th1 and Th17 immune responses that are

inflammator. The protective Th1 responses of the host are downregulated, increasing susceptibility to various bacterial infections, according to research by *Cerf-Bensussan* and *Gaboriau-Routhiau* published in 2010. Animal health and the agricultural industry both greatly benefit from the use of antibiotics and helminthics on animals raised for human consumption.

People who eat food products made from treated animals or the drugs used on them for food run the risk of developing drug-related health problems due to the presence of drug residues in these products.⁵ Based on *Gholamiandehkordi*, various clostridial infections have increased in frequency in both humans and animals over the past ten years. One of the best writers of the year 2010 is *Bannam*. It was released in 2011 by *Zidaric* and colleagues. Numerous *Ortiz's* coworkers are present.

Medicinal plants have been used alone or in conjunction with synthetic drugs to control and treat a variety of bacterial and parasitic infections in both humans and animals related to Pan. I claim that to be accurate. The beneficial natural compounds found in these plants are being studied for a range of conditions and diseases as well as for the creation of cutting-edge therapeutic modalities. Between 2013 and 2014, *Bahmani* and others were involved. *Moringa oleifera*, also known as the drumstick plant, contains a number of powerful chemicals, including triterpenoids, alkaloids, tannins, flavonoids, and saponins.⁶ Strongly effective as anthelmintics, these

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substances may also have antibacterial properties that can kill both Gram-positive and Gram-negative bacteria. Ojeka is also brought up. I do so courteously. The *M. oleifera* aqueous extract has a potent immunomodulatory effect after consumption that can decrease B cell activation and raise IgM, IgA, and IgG levels. Fard and his friends produced it. As a result, IL 10 will be created (2018). Tan and the rest of his crew. 2015. concerning Koltheat and associates. *M. oleifera* is safe to eat, according to research on both humans and animals (Stoys and Hartman 2015). Hegazi and his coworkers are included. On *Fasciola* eggs, occidiostatic effects of *Moringa* extracts were first identified. (2018). Given all these findings, *M. oleifera* should be viewed more as an immunomodulatory with respect to immune disorders caused by fascioliasis. We conclude that administration of an aqueous *M. oleifera* extract may offer protection from *F. as well as the C key*. by regulating immune reactions. The current study investigated the impact of oral administration of the *M. oleifera* aqueous extract on fecal egg count, IgG levels, and body weight. *M. oleifera* is commonly known as Drumstick and contains many active compounds such as alkaloids, flavonoids, saponins, tannins, and triterpenoids. Extracts from this plant have several pharmacological effects such as anthelmintic, anti-inflammatory, antimicrobial and antioxidant.⁷

METHOD

The study included 30 raised sheep, with lambs ranging in age from 6 to 12 months. Animal research was useful in identifying *F. The Happich and Boray*⁸ sedimentation technique was used by Gianta to find eggs in fecal samples. The study animals were raised in the River Nile basin and fed mostly clover grasses and a concentrate mixture. Atallah is one of the others, too. It was brought up by one. Animal infections with fascioliasis were given severity ratings. Then, the animals were divided into four groups: the control group, which was made up of the remaining sheep; the control group, which was made up of sheep that had been mildly, moderately, or severely infected; and the negative control group, which was made up of healthy, uninfected sheep.

Generously donated moringa leaf powder. On days 0 and 21, each sheep was weighed again. On days 0 through 15, we used rectal digital palpation to take an aseptic sample of each animal's feces. Russell, along with his associates, make this claim. The number can range from 0 to 5 depending on how the body is functioning first and second days of therapy.⁸ For each fecal sample, the number of *Fasciola* eggs per gram was calculated using the sedimentation method. On the last egg sample, a bacteriological test was run to establish *C. New colonies*, gram for gram. Jugular venipuncture marks were used to identify the various treatment checkpoints and make blood sampling easier. Before the next round of testing, the serum was split into two portions and chilled to -20 C.

A glass rod was used to thoroughly mix the sample in the graduated cylinder with 1 g of the fecal sample and 45 ml of distilled water. After passing through a sieve filter, the cure was allowed to settle. *F. Following that*, the egg density of the *gigantica* was determined using the number of eggs per gram as a proxy⁸ a knowledgeable person. A nearby butcher shop in El Monib, Giza Governorate, carefully removed the *gigantica* flukes from the water buffalo livers of recently deceased animals. AbdelRahman was developed and officially acknowledged as the first worm antigen. 2016). The adult flukes were homogenized at 4 C in a solution containing a buffer with a pH of 7 M. and a concentration of 0 M Tris-Hcl. The homogenate was then subjected to a 30-minute, 4 C centrifugation procedure at 14,000 rpm. Lowry and his friends follow. Lowry and other individuals a plan by Lowery the supernatant using a pour.

The long-term responses of sheep in the treated and control groups were compared using an aqueous leaf extract from *M. oleifera* in the

study. The assay was carried out in accordance with the aforementioned steps, and checkerboard titration was used to establish the ideal serum dilution and antigen concentration⁹ coworkers in Almaza. Speak formally. The optical density cutoff value appears immediately after the description. (2001). An ELISA sandwich test was used to gauge serum cytokine levels. Co. Bioneovan. a toolkit for ELISA testing preparation. Using straightforward techniques and tools, the cytokine concentrations of IL-10, IL-2, and IL-17 in sheep were measured by adhering to the creator's guidelines and sourcing Chinese producers.

For each 1 g of the weight of the fecal sample, 19 PBS, or phosphate buffer saline, were present. Each plate received 100 μ l of the homogenates sample, which had been serially diluted ten times in 1-9 PBS. The inoculated plates were then counted for the number of bacterial colonies using a colony counter and the previously mentioned predefined criteria after being incubated for 24-48 hours at 37°C and 5 percent CO₂. The *C. colonies* biochemical and microscopic data were used to support the *novyi*. 150 g of dried *M. oleifera* powder were incubated in 1 liter of distilled water for 24 hours at 4 C to produce a clean conical flask. The *M. oleifera* extract was given to the light and heavy infection treatment groups over the course of 21 days in equal doses for a total of 48 hours each.¹⁰ The supernatant was stored at -20 C following aliquoting. Two experiments were involved in the present study; one was for non-embryonated and the other was for developed (morula stage) *F. gigantica* eggs.

RESULT

On Day 0, the first day of the *M. oleifera* aqueous extract treatment, the heavy infected treatment group displayed a significant weight loss in comparison to the light and mixed infected groups. The lightly infected group gained weight noticeably more quickly than the heavily infected group and the mixed infection control groups after receiving oral *M. oleifera* treatment for 21 days (Figure 1). Following *M. oleifera* treatment, sheep with severe infections gained 3 to 9 points more body weight, whereas sheep with mild infections gained 4 to 5 points more.

Compared to the mixed infection control groups, the EPGs in the groups treated for light and heavy infection decreased significantly over the course of the treatment. The groups that consistently stood out as distinct from one another were those that were both severely and moderately infected. Following administration of *M. oleifera* aqueous extract, the group that had only mild infections noticed a sizable decline in EPG and total eradication of the infection (Figure 2a). Despite this, after 21 days post-transplant, the mean egg count had decreased significantly ($p < 0.001$), with a 99.2 percent reduction in the severely infected group. Furthermore, after the first dose of treatment with the *M. oleifera* aqueous extract, *Fasciola* eggs started to degenerate and accumulate dark embryo, and by the end of the experiment, the egg was empty and had no cells or embryo (Figure 2b) The findings of this study show that the viability and growth of eggs are significantly impacted by treatment with a moringa extract.

The *C. When examined under a microscope, novyi* was discovered to be a large number of short, thick, straight, gram-positive rods with round ends that can be found both individually and in short chains. The pure culture was small, irregular, whitish pale in color, finely granular, and encircled by a typical zone of β -hemolysis on blood agar. a turbidity and gas production by an organism in the medium containing the cooked meat, along with a pinkish discoloration of the meat particles. All isolates fermented dextrose, lactose, sucrose, and maltose, producing gas and acid but not mannitol, according to biochemical characterization of the samples. These included Voges-Proskauer, Indole, Oxase, Methyl Red, and in the negative. How many bacteria are present in *C. When given M. oleifera* extract, *novyi* was significantly reduced in both the lightly and heavily infected groups

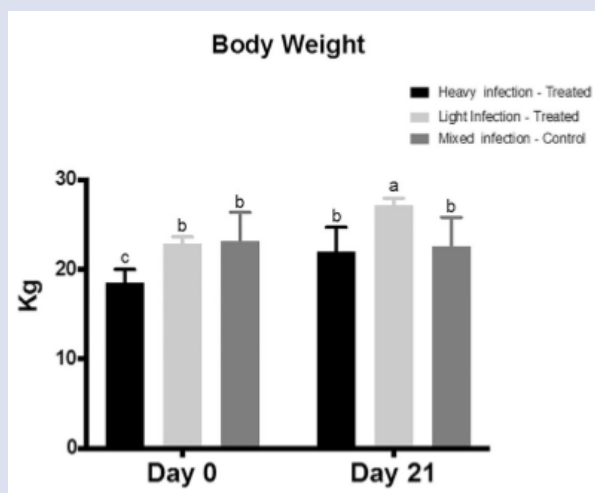


Figure 1: Due to the moringa tree (*M. sheep* that had been infected naturally with *Fasciola* (*F. oleifera*) were given an oral dose of the aqueous extract) *gigantica*.

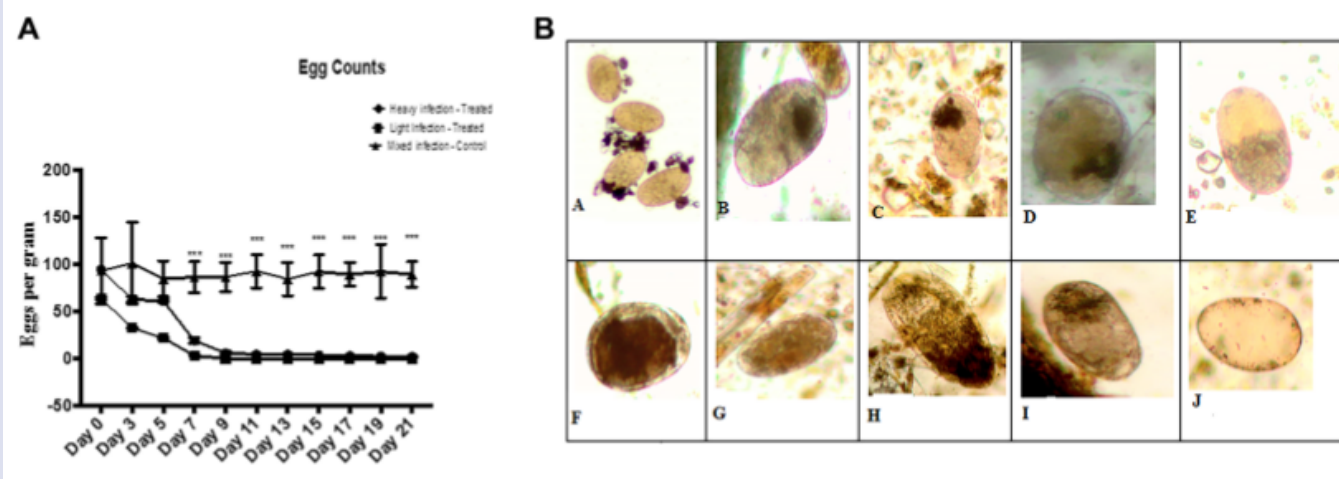


Figure 2: Effects of *M. oleifera* aqueous extract oral administration on naturally *F. ovine* pneumonia-infected sheep. *gigantica*. Eggs per gram (EPG) was calculated in each of the three groups using the fecal sedimentation method.

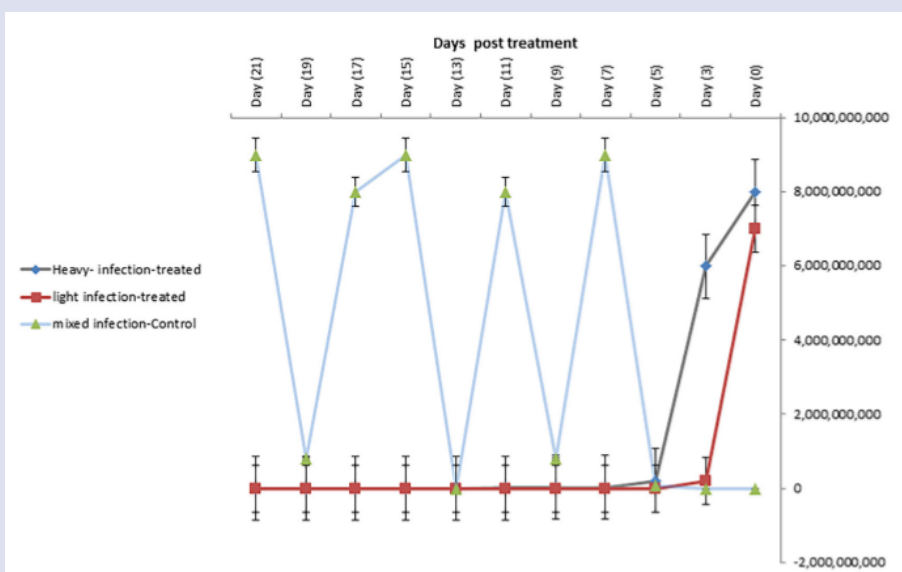


Figure 3: The number of bacteria discovered in C. Both sheep with a light and heavy *F* infection had *novyi*. *Massivea* was treated with *M. oleifera* leaf extracts.

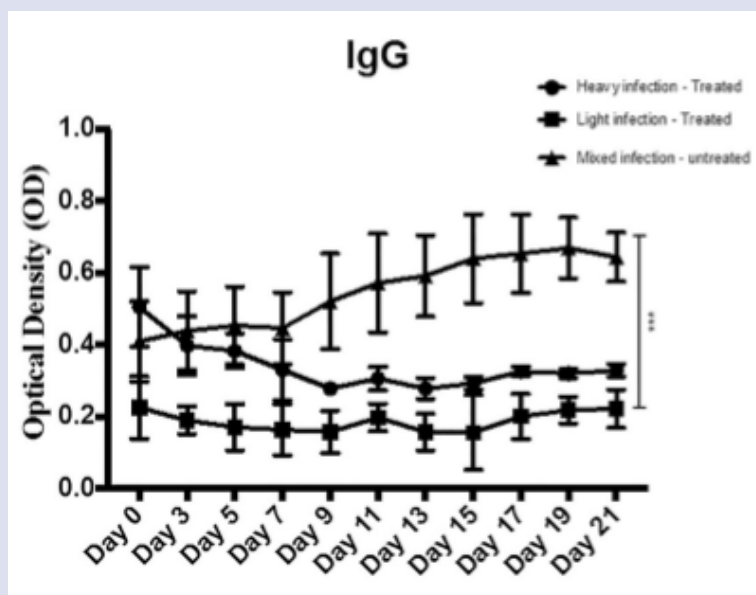


Figure 4: The impact of oral administration of *M. oleifera*'s aqueous extract on sheep that were naturally infected with F's serum IgG expression. *gigantica*. IgG levels were measured in each of the three groups using a particular IgG assay.

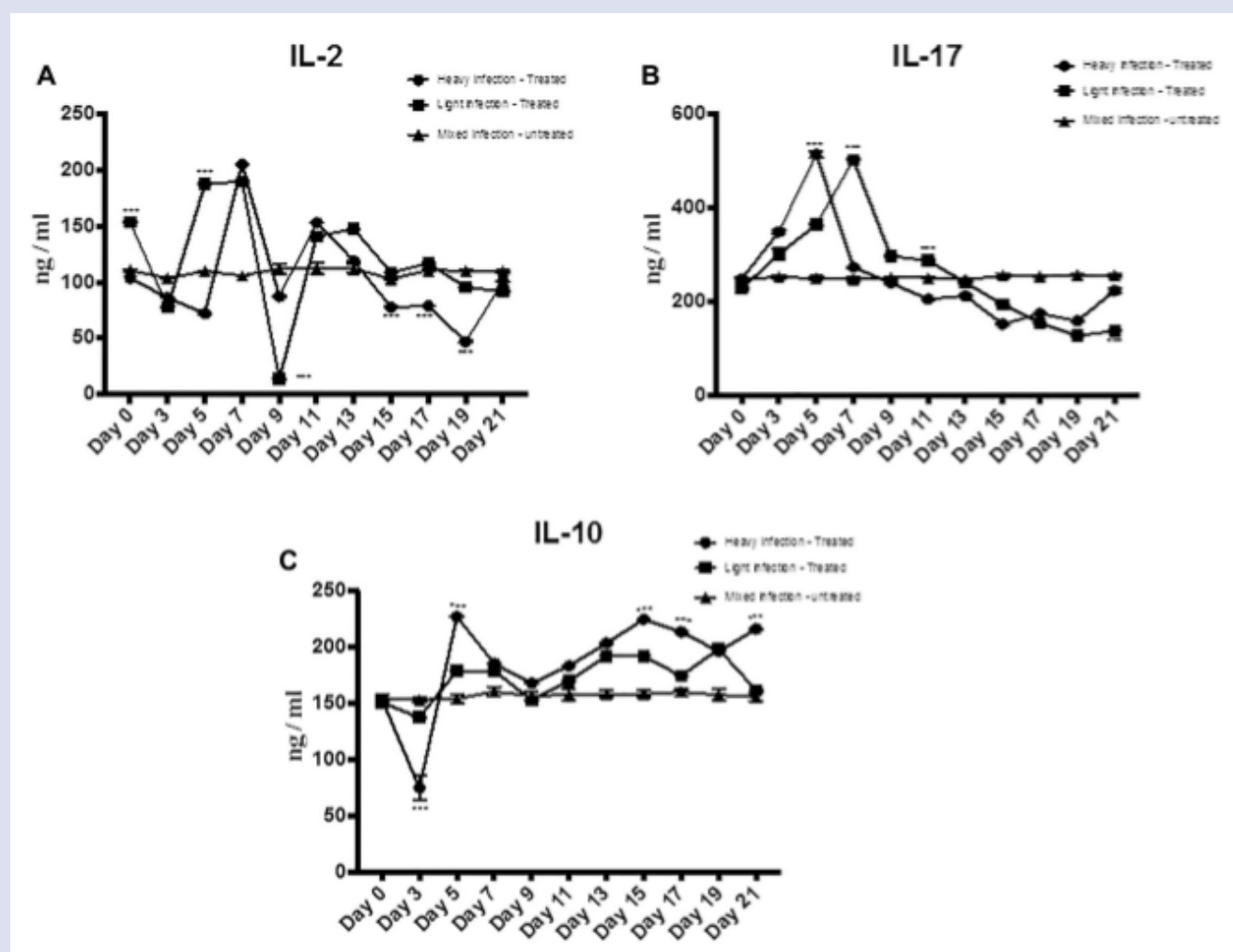


Figure 5: The impact of oral administration of *M. oleifera* aqueous extract on the IL-2, IL-17, and IL-10 serum levels in sheep naturally infected with *F. gigantica*.

<i>Fasciola</i> egg	<i>Moringa</i> extract	LC ₅₀		
		Conc. (mg/ml)	Lower limit	Upper limit
Non-embryonated eggs	Methanol	19.98	12.42	26.42
	Ethanol	24.39	10.7	36.93
	Water	2.6	0.03	12.31
Developed eggs	Methanol	15.85	13.21	18.45
	Ethanol	12.38	4.15	16.36
	Water	60.69	44.12	76.46

Table 1: LC50 of different Moringa extracts on *Fasciola* non-embryonated and developed eggs.

when compared to the untreated control group, reaching 2 9 104 after 21 days (dpt) (Figure 3).

In comparison to the groups with heavy and mixed infections, the IgG levels in the group with light infection were lower. At most time points, there were noticeable differences between the groups that had been mildly and severely infected. In the *M. oleifera* aqueous extract-treated group, where the animals had only been mildly infected, the infection completely vanished and IgG levels gradually dropped significantly (Figure 4). When compared to the heavily infected and mixed infection groups, the lightly infected treated group showed a significant decrease in serum levels of both IL-17 and IL-2 and an increase in the level of the regulatory cytokine IL-10 (Figure 5a-c). Following treatment with the *M. oleifera* aqueous extract, the temporal patterns of IL-2 and IL-17 showed an increase, a decline that began at day 5 and persisted through day 21 (Figure 5a-c).

DISCUSSION

The objective of the current study was to investigate the potential antibacterial or fasciolicidal effects of orally administering an aqueous extract of *M. oleifera* leaves to sheep that already had fascioli that were naturally infected. Both the sheep given *M. oleifera* treatment and the sheep that contracted the illness during the study gained a lot of weight. Lower parasite loads would encourage quicker growth and better body condition scores, so this outcome was anticipated. The amount of protein, minerals, and antioxidants in *M. oleifera* is surprisingly high. The host may have benefited from these nutrients by gaining weight and strengthening their helminths defenses because they improved digestion and absorption. Results as described by Moyo and associates bolstering these. (2013) found that administering *M. oleifera* to the goats increased their body condition score and decreased the number of helminths and coccidian oocysts in their bodies. Infected sheep receiving *M. oleifera* treatment experienced a significant decline in fecal egg count in cases of heavy infection and a complete disappearance in cases of light infection over the course of the 21-day treatment period. In order to show how the treatment changed the eggs' morphology, *Fabioloia* eggs isolated from feces exhibit severe degeneration and an accumulation of dark embryos.

Although worm eggs look like *Haemonchus contortus*, they are sterile. Like Portugaliza¹¹ but better. Fatima along with a friend.¹² I can confirm that without a doubt. It's possible that the tannins, saponins, and flavonoids in *M. oleifera* leaves are what's causing this toxic reaction. Jayanthi et al. claim as much. rats were given *M. oleifera* treatment also displayed immunomodulatory effects¹³ which increased protection, reduced worm populations, and resulted in egg loss. Any possible *M. oleifera* fasciolicidal effects must be thoroughly assessed. In the current study, the severely infected sheep had significantly lower IgG levels on day 3 after receiving an oral dose of *M. oleifera* leaf aqueous extract, and this effect persisted throughout the experiment. Even when sera from unharmed, healthy sheep were used as a negative assay control, the mean IgG titers were consistently higher. After treatment, serum

IgG levels in sheep with minor infections significantly decreased, and this decrease was comparable to that observed in control sera with assay-negative results. A verbal M. This discovery was the outcome. The severity of the infection might be lessened with treatment. Invaders can be repelled until they are defeated by immune-stimulating *M. oleifera*.¹⁴ The resulting antibody may interact with crucial membrane sites on the parasite's surface. Tannins in *M. oleifera*'s aqueous extract have been shown to paralyze adult *Fasciola* worms, indicating that it may possess anti-fasciolocidal properties¹⁵ was a collaborative project between Molan and other authors¹⁶ D'addabbo and others". claim in their investigation. The parasite cell membrane and the collagen in the cuticle are both weakened by saponins, which has a similar effect.

21 days after treatment, the cytokine levels were lowest in the sheep who had both a heavy and light infection. Reduced Th1 and Th17 cytokines, which promote inflammation, have been observed following treatment with *M. oleifera* extract. Historical studies¹⁷ *M. oleifera*, IL-2 expression and production decreased (2013, 2009), which reduced the inflammation brought on by the infection. When *M. oleifera* extract was given to sheep with high and low infection levels, the anti-inflammatory cytokine IL 10 gradually increased and peaked 21 days later in both groups of sheep. Fard is another option. The most recent discovery supports this included in its conclusions. first-level research. (2015) presented convincing evidence that the hydroethanolic bioactive leaf extract of *M. oleifera* significantly reduced macrophage IL-10 production. How *M. oleifera* controls Th1/Th2 during fascioliasis is still a mystery to us. According to recent studies, *C. Novyi* was 7 9 109-9 9 109 at the start of the treatment in both severely and mildly naturally infected sheep, but by the end of the 21-day moringa extract regimen, it had decreased to 2 9 104. The substance that has been responsible for eliminating harmful *Clostridium* species. *M. oleifera* might be used to produce something. The process for lowering *C. They are here with El-Kholy. I beg of you, please. As a direct result of the bacteria and M. oleifera* interacting, the *novyi* count was generated. 2018). Additionally, *M. oleifera* increased the expression and level of IL-10, triggering an immune response against *C. novyi*. To fully understand the effects of *M. oleifera* targeting on the immune system, more study will be required.

Anti-inflammatory drugs are used to treat fascioliasis, an inflammatory condition. By focusing on how it affects the Nf-kB transcription factor, we might be able to comprehend how *M. oleifera* modulates the immune system. Hegazi and associates presented the study's findings and came to the following conclusions: g, 2008). *In vitro* ovicidal activity for the leaf alcohol and aqueous extract of *M. oleifera* has been demonstrated (*M. oleifera*, 2018). This finding supports a hypothesis that *M. oleifera* may be detrimental to *F. massive* worms. Based on LC50 values, water extract presented the highest ovicidal activity since it registered the lowest values of 2.6 mg/ml on immature non-embryonated eggs suggesting that non-embryonated eggs were more susceptible to aqueous extract than developed eggs. Poné et al. found that LC50 value of the ethanol extract of *C. manni* was relatively low, indicating that this extract is more active on non-embryonated eggs than aqueous extracts.

CONCLUSION

Based on the findings of this study, *M. oleifera* may be useful in the treatment of *F. gigantica*. It is important to investigate the proposed molecular pathways and cell-cell interactions that occur when *M. oleifera* is administered to animals that are already naturally infected. Non-embryonated eggs were more susceptible to aqueous extract than developed eggs.

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