HEPATOPROTECTIVE ROLE OF NANOURCUMIN AND PUMPKIN SEED OIL IN TRICHINELLA SPIRALIS INFECTION: PATHOGENESIS AND MODULATION OF MATRIX METALLOPROTEINASIS (MMP9)

Running title: Hepatoprotection of Nanocurcumin against T. spiralis infection.

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ABSTRACT

Background: Trichinella spiralis is a unique zoonotic parasite have two phases during its life cycle, enteral and parenteral phase. To the best of our knowledge, few literatures have been demonstrated the effect of T. spiralis infection on hepatic tissue. Aim: The present study aimed to investigate the impact of Nanocurcumin and Pumpkin oil, as natural compounds, against the hepatic pathogenesis and MMP-9 production during T. spiralis infection compared to Albendazole. Methods: One hundred and twenty mice were divided into four groups: the control group, the infected untreated group, the Nanocurcumin treated group, the Pumpkin treated group, and the Albendazole treated group. Histology and Immunohistochemical techniques were applied. Results: The infected untreated group showed acute liver inflammation with coagulative necrosis during the enteral and parenteral phase of infection. The treated groups showed more or less normal hepatic tissue, however necrobiotic changes were observed in the Pumpkin treated group. The immunohistochemical staining showed significant reduction in the expression of MMP-9 in the treated groups. Conclusion: Our results suggests that Nanocurcumin could effectively against hepatic inflammation associated with T. spiralis infection through reduction MMP-9 activity. However, the present results suggested that Nanocurcumin exhibited high efficacy compared to the Pumpkin seed oil. Additional research and clinical studies are necessary to validate these findings and determine the practical applications of these treatment strategies.

Key words: Curcumin chitosan nanoparticles, pumpkin seed oil, trichinellosis, immunohistochemistry of MMP9 mediator

INTRODUCTION

Trichinella spiralis is a zoonotic parasite widely spreading around the world which infects human and animals (Dupouy-Camet and Murrell 2007). The infection of human with Trichinella species can be occurred by oral ingestion of the infected larvae in skeletal muscles, or adult worms that lived in the small intestine, or newborn larvae in the blood and lymphatic vessels (Farid et al., 2019; El-Moghazy and Shalaby
2005) *Trichinella spiralis* life cycle is divided into three stages: intestinal, migratory and muscular stage (Despommier 2009). Ex-cystation of consumed larvae that penetrate the epithelial lining of the stomach happens as a result of stomach acidity and adult worms form in the upper region of the small intestine. In two to three weeks, the newborn larvae (NBL) generated by fertilised females move through the blood and lymphatic systems to attack various organs before encapsulating in skeletal muscles (Saad et al., 2016a).

Liver is the primary organ for biochemical reactions in the human and animal body. Previous literatures showed acute liver inflammation and a series of biochemical changes in response to infection with *T. spiralis* in rats (Farid et al., 2019). *T. spiralis* secretes many proteins at various phases of parasite growth in an attempt to cope with the host immune response and evade or inhibit host defense systems. These proteins are regarded to be crucial for invasion of the host by the parasite and its maintenance there (Yang et al., 2014).

**Matrix metalloproteinasis** (MMPs) are endogenous regulators involved in tissue regeneration and inflammation. Previous research studied the role of these proteins in parasitic infections such as Malaria, Neurocysticercous and Angiostrongylus (Bruschi and Pinto 2013; Chiu and Lai 2014). It had an essential role in granuloma formation during infection through encouragement infiltration of inflammatory cells and degeneration the extracellular matrix proteins (Shyu Chen et al., 2019).

The commonly used drugs for trichinellosis are Benzimidazole (McKellar and Scott 1990). Several literatures have been demonstrated the resistance of anthelmintic drugs in nematode species which affect animals or humans (Kaplan 2004; De Clercq et al., 1997; Flohr et al., 2007). Unfortunately, the drug do not effectively kill the muscle encysted larvae or the NBL of *T. spiralis* (Saad et al., 2016b). They have strong resistance and poor action when it comes to encapsulating larvae due to their weak water solubility, low bioavailability and potential side effects from long-term use, they have a limited impact resistance to drugs (García et al., 2014).

Curcumin has medical benefits including anti-inflammatory, cancer fighting, antioxidant, anti-atherosclerotic, antibacterial, antiviral and wound healing properties (Hussein et al., 2021), as well as anti-parasitic properties (Gressler et al., 2015; Hussein et al., 2017). Moreover, Curcumin demonstrated several cardiovascular protective benefits and benefited animal models of liver damage, inflammation, and cirrhosis (Lee et al., 2017). Lipophilic therapeutic small drug molecules face limitations in clinical use due to factors like low hydrophilicity, dissolving rate, physicochemical instability, fast metabolization, limited bioactive absorption, poor pharmacokinetics/bioavailability, and low penetration/targeting effectiveness. These limitations hinder their effectiveness in treating disease conditions (Burgos-Morón et al., 2010; Yang et al., 2008). So, employing nanotechnology should disperse or target targeted tissues with the least amount of inadvertent harm to neighboring healthy cells and tissue. Recently, the nanoparticles were frequently utilized as delivery systems for medications or vaccinations to increase their therapeutic efficacy (Jiang et al., 2013). A naturally occurring polymer created by deacetylating chitin is chitosan. Due to its biocompatibility, nontoxicity, mucoadhesion, minimal immunogenicity and environmental safety, it is employed in medicinal applications (Priotti et al., 2017).

Pumpkin (*Cucurbita pepo*) seeds are popular worldwide, especially in Egypt, which has a good nutritional value and can be eaten raw or roasted and making bread and cakes (Abou-Zeid et al., 2018). Pumpkin seed oil mostly contains the important fatty acids linoleic, stearic, oleic and palmitic acids. It has a very moderate composition of fatty acids.
acids (Gohari Farhoosh, and Haddad 2011). However, it is a significant tropical vine with leaves and seeds that has strong traditional nutritional and therapeutic properties (Hussein and Shukur 2020). So when used on people and animals it has been found to have anthelmintic characteristics (Marie-Magdeleine et al., 2009; Guarrera 1999).

The aim of the study: evaluate the anti-inflammatory and the hepatic regeneration of Nanocurcumin and Pumpkin seed oil (Cucurbita pepo) through regulation the pathogenesis in the liver and expression of MMP-9. For this purpose, experimental infection with T. spiralis as inflammatory parasitic disease in mice model was used.

MATERIALS AND METHODS

Mice
One hundred and twenty mice aged 8-12 weeks and weighing 25-30 g were acclimated for two weeks in the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

Parasite strains
The strains of T. spiralis were raised in BALB/c mice at the Animal House in the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt under suitable and pathogen-free conditions. After 30 to 90 days post infection (dpi), the infected mice were slaughtered, skinned, and the skeletal muscles were isolated for obtaining larvae. The muscles were minced and digested using artificial digestive fluid containing 7.5 g of pepsin + 10 ml of conc. HCl+ 1L of distilled water. The Larvae were filtered, precipitated and counted.

Preparation of Nanocurcumin and Pumpkin seed oil
The synthesis and characterization of NanoCurcumin were done according to (Yadav et al., 2012) Briefly, 0.5 ml of Tween 80 was added to 20 ml of a 0.15% solution of chitosan in dilute acetic acid with constant stirring for 1 hour. Then, 250 μl of Curcumin solution was added in aliquots of 20 μl with stirring for a further 1 hour. After that, 0.5 ml of 20% sodium sulfate solution was added drop by drop with stirring for more 30 minutes. To crosslink the nanoparticles, 0.1 ml of glutaraldehyde was added to the solution and stirring for more 30 minutes. Finally, 1 ml of 10% sodium metabisulfite was added to the solution with continuous stirring. The solution was suspension and yellow slurry in color. The encapsulation efficiency was 84.9% and measured using a UV-Vis spectrophotometer (Cary series UV-Vis-NIR, Australia) at 420 nm. The physical properties and morphology of the nanoparticles were determined using a JEOL transmission electron microscope (JEM 2000EX200, Tokyo, Japan) at the Electron Microscope Unit in Assiut University, Egypt (Abdel Hakeem et al., 2022). The particles were spherical shape and the average size was 100 ±20 nm.

Pumpkin seed oil was purchased from IMTENAN brand for Natural Oils & Herbs in Egypt. The Cucurbita Pepita oil was extracted from the seeds of the Cucurbits Gourd family through a cold-pressed method, ensuring that the oil retains its natural benefits. The oil is dark brown green to dark red, with an intense nutty scent. It is a 100% pure, natural product, certified by ISO, COA, and MSDS.

Experimental design
A total of 300 T. spiralis larvae/mouse were given orally to each mouse. Four groups of infected mice were formed:

Group I (10 mice) is a control group, did not receive any infection or treatment.
Group II (30 mice) is an infected untreated group.
Group III (30 mice) is infected and treated with NanoCurcumin (oral administration with 15 ml/kg BW of NanoCurcumin three times/ week (Yadav et al., 2012) and divided into 3 subgroups:
  Subgroup IIIa: the diseased mice received treatment from 2nd day till 7th dpi.
  Subgroup IIIb: the diseased mice received treatment from 7th till 25th dpi.
  Subgroup IIIc: the diseased mice received treatment from 7th till 54th dpi.

Group IV (30 mice) is infected and treated with Pumpkin seed oil (oral administration with 1.5 ml/kg BW three times/ week) and divided into 3 subgroups:
Subgroup IVa: the diseased mice received treatment from 2\textsuperscript{nd} day till 7\textsuperscript{th} dpi.
Subgroup IVb: the diseased mice received treatment from 7\textsuperscript{th} till 25\textsuperscript{th} dpi.
Subgroup IVc: the diseased mice received treatment from 7\textsuperscript{th} till 54\textsuperscript{th} dpi.

Group V (20 mice): is infected and treated with Albendazole (orally with 50 mg/kg BW) and divided into 2 subgroups:
Subgroup Va: the infected mice received treatment from 2\textsuperscript{nd} till 7\textsuperscript{th} dpi for three successive days according to (Gönnert and Andrews 1977) against the intestinal phase.
Subgroup Vb: the infected mice received treatment from 31\textsuperscript{st} till 54\textsuperscript{th} dpi for three successive days against the muscular phase.

**Histopathology**
Following sacrifice, the liver tissues from the different groups were promptly isolated for 48 h. They were submerged in a formalin-alcohol solution. The fixed tissues were washed twice in 70% ethanol and dehydrated in a series of graded alcohols. The dehydrated tissues are then cleared with a solvent such as xylene to remove the alcohol and make the tissue transparent. Then, they were impregnated and embedded in paraffin wax. The embedded tissue was then cut into thin sections 3-5 \( \mu \)m thickness using a microtome (a Reichert Leica Microtome) (RM 2125, Germany), placed on glass slides with hematoxylin and eosin staining. The stained tissue sections were then mounted with a coverslip using a mounting medium to protect the tissue and enhance visualization (Hafez et al., 2020).

**Immunohistochemical staining of MMP-9**
Formalin fixed paraffin-embedded liver tissue sections were cut at 3\( \mu \)m thick for immunohistochemical staining (IHC). The IHC were done using automated Dako instrument at the South Egypt Cancer Institute, Assiut University, Assiut, Egypt. Antigen retrieval was done using Citric buffer, low PH 6.1 (50x). Peroxidase Blocking Reagent (Code SM801) was applied for 5-10 min at room temperature. Rabbit monoclonal antibody MMP-9 (Catalog No. BSB 2538; Bio SB clone EP127, USA) was used, whereas the secondary antibody was applied horseradish peroxidase for 20 min at room temperature. DAB solution was applied to the slides for 5 – 10 min. Sections were stained with iron hematoxylin and mounted with Dibutyl Phthalate Polystyrene Xylene. The scored of immunostaining was assessed using Image J software (Java image processing program inspired by NIH) on a five random images per mouse with a fixed high-power field at \( \times 400 \) under a light microscope (OPTICA, Italy). The data was determined a percentage of antibody-stained area and were expressed as mean for each group (Alnasser et al., 2023).

**Statistical analysis**
Quantitative variables were expressed as mean \( \pm \) standard deviation and analyzed using SPSS software (version 20). The two-tailed unpaired data was used to assess differences in MMP-9 level. Differences were considered significant at \( P \leq 0.05 \).

**RESULTS**

**Synthesis of nanoparticles**
The cross-linked of curcumin encapsulated in chitosan nanoparticles were done according to Yadav et al. (2012). The solution was suspension and yellow slurry in color. The particles were spherical shape and average size was 100 \( \pm \)20 nm. The encapsulation efficiency was 84.9 % and loading capacity was 17.7 % (Fig1.).

**Histopathological results**
The histopathological results indicated a significant change in the parenchymal cells of the liver in the infected non-treated group in the enteral phase after 7\textsuperscript{th} dpi. The observed changes including, severe leukocytic reaction, Kupffer cells activation in the sinusoids and multi-focal areas of coagulative necrosis (Figs. 2 A and B). Diffuse necrotic changes (Fig. 2 C) and severe vacuolar and fatty degeneration was observed (Fig. 2 D). The treated groups showed different levels of improvement in the hepatic cells (Figs. 3, 4 and 5).

In the migratory and muscular phases of infection, the infected non-treated group showed significant changes in the parenchymal cells of the liver (Fig. 6). In 25\textsuperscript{th} dpi (migratory phase) the infected non-treated group showed multiple foci of inflammatory cell in the hepatic parenchyma (Figs. 6 A and B). Coagulative necrosis of the hepatocytes was observed (Figs. 6 B and C). The nucleus of hepatocytes was prominently enlarged (Fig. 6 C). Infiltration of
inflammatory cells mainly of eosinophil type was observed (Fig. 6 D). However, in the Nanocurcumin and Pumpkin oil treated groups, the hepatic cells showed more or less normal (Figs. 7 and 8). In the muscular phase, the infected non-treated group showed acute histopathological changes in the liver (Fig. 9). However, the treated groups showed mostly normal hepatocytes (Figs. 10, 11 and 12), although a few hepatocytes showed necrobiotic changes in the Pumpkin treated group (Fig. 11).

**Down regulation in the expression of MMP-9 in the liver tissue**

The immunohistochemical analysis of MMP-9 in the liver of the different groups was shown in (Fig. 13. A). Brown granular cytoplasmic expression of the MMP-9 in the hepatocytes and sinusoidal reaction was observed in the infected non-treated group (Fig. 13 A). The treated groups showed a significant reduction in the expression of MMP-9 compared to the infected nontreated group (Figs. 13 B, C, and D). Moreover, Nanocurcumin treated group showed the most significant reduction in the expression of MMP-9 compared to the other treated groups (Fig. 13 B).

**Fig. (1) shows the TEM images of the prepared Curcumin chitosan nanoparticles.**

**Fig. 2: Photomicrograph of liver of the infected non treated group in intestinal phase showing severe pathological reaction.**

A) Low power showing congestion and thrombosis of blood vessels, inflammatory foci distributed between hepatocytes and perivascular inflammatory cell reaction.

B) Magnification showing congestion of the CV, focal area of coagulative necrosis (black arrow), swollen and congestion of sinusoids with Kupffer cell reaction.

C) Prominent enlarged of nucleus of hepatocyte (head arrow), presence of Diffuse necrotic hemorrhagic area.

D) Magnification showing severe perivascular inflammatory cell reaction mainly from eosinophil type, severe vacuolar degeneration of the hepatocyte (head arrow).
Fig. (3): Photomicrograph of liver of the curcumin chitosan treated group in intestinal phase showing: A) Lower power show more or less normal hepatic tissue with inflammatory cell infiltration mainly of eosinophil type (arrow), B, C, D) Magnification showing inflammatory cells infiltration mainly of eosinophil and monocytes.

Fig. (4): Photomicrograph of liver of the pumpkin oil treated group in intestinal phase showing: A) Lower power show more or less normal hepatic tissue. B) Magnification showing congestion of blood vessels and perivascular inflammatory cell infiltration mainly of eosinophil and monocytes and mitotic figure appear inside the nucleus of hepatocyte. C, D) congestion of central vein and portal vein with perivascular cell infiltration of inflammatory cells and edema, some of hepatocyte show vacuolation.

Fig. (5): Photomicrograph of liver of the albendazole treated group in intestinal phase showing: A) Low power showing more or less normal hepatic parenchyma. B) Normal central vein within normal hepatocyte arrangement. C) Mild Swollen of sinusoids with Kupffer cell activation (black arrow), mitotic picture in nucleus of hepatocyte and mild vacuolation of hepatocyte. D) Mitotic figure of nucleus of hepatocyte (black arrow), inflammatory cell infiltration mainly eosinophil infiltration (square).
Fig. (6): Photomicrograph of liver of the infected non treated group in migratory phase showing severe pathological reaction. **A)** Low power showing congestion of blood vessels and perivascular inflammatory cell infiltration. **B)** Magnification showing focal area of coagulative necrosis, swollen of sinusoids with Kupffer cell reaction and focal inflammatory cell infiltration mainly of eosinophil type. **C)** Thrombosis of CV within Perivascular inflammatory cell reaction. **D)** Fatty changes of hepatocyte, inflammatory cell infiltration mainly of eosinophil type.

Fig. (7): Photomicrograph of liver of curcumin nano chitosan treated group treated group in migratory phase showing. **A)** Lower power show more or less normal hepatic tissue. **B)** Magnification showing some blood vessels congested with Kupffer cell activation **C, D)** few cases showed thrombosis of blood vessels with more or less normal hepatocyte.

Fig. (8): Photomicrograph of liver of the pumpkin oil treated group in migratory phase showing. **A)** Lower power show more or less normal hepatic tissue with inflammatory cell activation in portal area. **B)** Magnification showing normal blood vessels with mild swollen of sinusoids with mild Kupffer cell activation. **C)** Some of hepatocytes show vacuolar degeneration (arrow) with Kupffer cell reaction. **D)** Hyperplasia of portal duct
Fig. (9): Photomicrograph of liver of the infected non treated group in muscular phase showing severe pathological reaction. A) Low power showing severe inflammatory cell reaction between hepatocytes and some vacuolation of the hepatocyte. B) Magnification showing, fatty degeneration of some of hepatocyte, focal area of coagulative necrosis, swollen of sinusoids with Kupffer cell reaction. C) Prominent enlarged of nucleus of hepatocyte, congestion of the portal vein with inflammatory cell reaction D) Inflammatory cells infiltration in portal area with hyperplasia of bile duct.

Fig. (10): Photomicrograph of liver of the curcumin chitosan treated group in muscular phase showing. A) Low power show more or less normal liver tissue. B) magnification showing more or less normal hepatic parenchyma, minute area of vacuolar degeneration. C, D) Minutes of coagulative necrosis

Fig. (11): Photomicrograph of liver of the pumpkin oil treated group in muscular phase showing. A) Low power show more or less normal liver tissue. B, C, D) magnification showing more or less normal hepatic parenchyma,
**DISCUSSION**

Parasite larvae spreading to different tissues and organs in the body induce hyper-allergic reactions due to the release of structural antigens. This triggers significant systemic pathologic alterations, including localized inflammation, necrosis, tissue damage and the release of altered self-proteins, occurring alongside the demise of the larvae (Zanc 2001a).
Hepatic problems in trichinellosis, though less studied than other organ involvement, have significant effects on protein synthesis and liver function observed during or after the intestinal phase (Neghina and Neghina 2011).

Our results showed severe vacuolar degeneration and fatty degeneration in most of parenchymal cells during enteral phase in the liver of the infected non-treated group. This is a reversible damage, so we can say that *T. spiralis* causes acute hepatitis, this agreed with (Kocięcka 2000) and (Neghina et al., 2011). This could be attributed role of liver in toxic and allergic mechanisms (Zanc 2001b). In the present study, multifocal areas of coagulative necrosis were observed in the liver of the infected non-treated group, suggesting the deficiency in IL-10 production which has a critical role in hepatoprotection (Bliss et al., 2003). In the migratory phase, mild congestion of the blood vessels in perivascular area was observed with multiple areas of inflammatory cell foci mainly of eosinophil type within the hepatic parenchyma. This may be due to migration of parasite (antigen) in blood to citation in default sites. This is agreed with (Neghina and Neghina 2011) who reported that the lesions may develop as a result of direct or indirect larval damage (e.g., through eosinophils or immunologic reactions). Moreover, focal areas of coagulative necrosis were noticed in the pericentral, periportal and midzonal areas in the muscular phase. These results were agreed with (Farid et al., 2019) who reported that rats infected with *T. spiralis* had acute hepatitis, and their serum paraoxonase activity dropped during the enteric phase. Eosinophilia is a sign of the host immune system's reaction to parasitic diseases which have the ability to fend against parasite infection, including *T. spiralis* (Klion and Nutman 2004).

In an attempt for in development an alternative source of anthelmintics drugs and a growing interest with medicinal plants as antiparasitic agents with consideration drug resistance, we conducted this study to evaluate the role of Nanocurcumin and Pumpkin seed oil through regulation the pathological changes and the expression of MMP-9, as inflammatory marker, in the hepatic tissue during *T. spiralis* infection. The obtained data presented some information on the anti-inflammatory degree of Pumpkin seed oil in comparison to Nanocurcumin which occasionally accompany parasitic infection with *T. spiralis* in mice.

Our findings revealed more or less normal hepatic cells with some congestion in blood vessels and minute collection of inflammatory cells in the Nanocurcumin and Pumpkin treated groups. The hepatoprotective effects of medicinal plants are mediated by their phytochemical’s contents including flavonoids, alkaloids, tannin, saponin etc, which have antioxidant and free radical-scavenging properties (Adeneye and Benebo 2008).

The nematocidal activity of Pumpkin extract was previously studied by (Grzybek et al., 2016) who reported high antiparasitic effect of Pumpkin ethanolic extract in the high doses. Asgary et al. (2010) mentioned that Pumpkin powder treatments showed a substantial effect in lowering the score of liver inflammation in diabetic rats. In addition, a considerable reduction in the oxidative stress was observed in the liver of albino rats-induced alcohol and treatment with Pumpkin oil (Abou Seif 2014). Besides, (El gendy et al., 2020) demonstrated that certain substances may aid larval migration and movement causing pathological changes in vascular organs. In the current study, we evaluated the influence of *T. spiralis* infection on the expression of MMP9 as inflammatory mediator which highly involved in the pathogenesis and degenerative disorders.

MMP-9 is an inflammatory marker which involved in many physiological and pathological processes. To the best of our knowledge, no information on MMP-9...
expression is available in the liver tissue of *T. spiralis*-infected mice. Moreover it is reported that MMP-9 is a good marker of inflammation in patients infected with *T. britovi* compared with MMP-2. Interestingly, we found a significant upregulation of MMP-9 expression in the liver of mice infected with *T. spiralis* in the late phase of infection, which was harmonized with the results of (Bruschi et al., 2016). Our results are partially consistent with data obtained in other helminth infections, such as, in *Toxocara canis* infection where highly activity of MMP-9 in the lung, muscle, liver, and brain tissues of infected mice (Lai et al., 2005). Curcumin and Pumpkin are abundant in nutrients and antioxidants; however there is no evidence of any research into their immunomodulatory effects on MMP-9 as inflammatory mediators. To our knowledge, only a few research have reported on the effectiveness of Nanocurcumin or Pumpkin seed oil extract on MMP-9 expression and levels (Tsai et al., 2015). In this study, we showed a statistically significant reduction in the expression of MMP-9 in the infected mice treated with Nanocurcumin and Pumpkin oil, suggesting the role of them as anti-inflammatory agents. Given the overall results, the anti-inflammatory degree of the Pumpkin seed oil was less than Nanocurcumin in the liver tissue. This could be explained the potential role of Nanocurcumin in scavenge free radicals and minimize oxidative stress due to its secondary metabolites and antioxidant properties.

The anti-inflammatory action was observed on the reduction in the expression of MMP-9. However, significant effect on the pathogenesis of the liver tissue was observed in the Nanocurcumin and Pumpkin oil treated groups compared to the infected non-treated group. This can be suggested that because higher creation of immune system cells may suggest improved immune system activity, Nanocurcumin and Pumpkin have a stimulatory effect on immunological responses. Because higher creation of immune system cells may suggest improved immune system activity, Nanocurcumin and Pumpkin have a stimulatory effect on immunological responses (Sainis et al., 1997).

**CONCLUSION**

According to the current findings, *T. spiralis* infection caused a significant uptick in MMP-9 synthesis in mice with the infected livers for the first time. Furthermore, the function of Nanocurcumin and Pumpkin seed oil as anti-inflammatory agents was correlate with downregulation of MMP-9 expression in the liver tissue. Consequently, acute histopathological changes were observed which gave information on the role of these chemicals in the pathophysiology of the liver via larval migration and invasion. However, assessment of MMPs in the different tissues during different phases of *T. spiralis* infection should be studied.

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دور النانو كركم وزيت اليقطين في حالة الإصابة بعدوى التراينكليا سبيراليس

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