Egyptian Journal of Zoology (EJZ), Vol. 78: 1-14 (December, 2022) p-ISSN: 1110-6344
Publisher: The Zoological Society of A. R. Egypt e-ISSN: 2682-3160
DOI: 10.21608/ejz.2022.113530.1068

RESEARCH ARTICLE

INHIBITION OF GRANULOMA INDUCED BY GRANULOCYTES ISOLATED FROM PATIENTS CO-INFECTED WITH *SCHISTOSOMA MANSONI* AND HEPATITIS C VIRUS: ROLE OF THE *MORINGA OLEIFERA* AQUEOUS EXTRACT

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Article History:
Received: 23 March 2022
Revised: 5 April 2022
Accepted: 5 April 2022

Published Online:
5 May 2022

Keywords:
Granulomatosis
IL-17
Moringa oleifera
Praziquantel
Soluble egg antigen

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ABSTRACT

The current study reported the potential activity of the aqueous extract of *Moringa oleifera* leaves in reducing granulomatosis induced by *Schistosoma* soluble egg antigen (SEA). Twenty-five healthy individuals and fifty *Schistosoma mansoni*/hepatitis C virus (HCV) co-infected patients were enrolled in the current study. The granulocytes were isolated and used to induce *in vitro* granuloma using SEA-conjugated polyacrylamide beads in the presence of interleukin (IL)-17 (125 pg/mL). After 3 days, the granuloma was treated with the aqueous extract of *Moringa* leaves (0.1 mg/mL). Praziquantel (PZQ, 0.1 mg/mL) was used as a reference drug. On the 7^{th} and 14^{th} days, the granuloma index (G.I) was determined and tumor necrosis factor (TNF)-α, H_2O_2, and nitric oxide (NO) levels were measured in the culture supernatant. The microscopic examination showed a significant reduction (P<0.05) in the granuloma size in response to PZQ or *Moringa* extract after 7 and 14 days, respectively, in the healthy subjects; the reduction induced by *Moringa* was significant comparing with PZQ after 14 days. A significant decrease was also recorded in the granulocytes' mediators as H_2O_2 (in both healthy subjects and co-infected patients) and NO (in healthy subjects only) in response to *Moringa* extract comparing with PZQ after 14 days. However, TNF-α showed a significant increase (only after 14 days) in the granuloma treated with PZQ or *Moringa* extract compared with the untreated granuloma of healthy individuals. In conclusion, the *Moringa* extract showed a higher protective activity than PZQ in reducing granuloma, possibly due to affecting the production of granulocytes' mediators.

INTRODUCTION

Schistosomiasis (bilharzia) is an endemic disease in 74 countries across the world caused by parasitic flatworms of the genus *Schistosoma* that currently infects more than 200 million people worldwide[1]. Schistosomiasis is a major health problem in the developing world; 20 million persons are described as having severe morbidity, with 24000-200000 deaths annually[1]. Egypt has the highest prevalence of hepatitis C virus (HCV) country-wide, ranging from

6-40% among regions and demographic groups[2,3] with frequently reported co infection with schistosomiasis[4].

Schistosoma mansoni is the most studied and geographically widespread species, and is the most common agent of schistosomiasis disease[5]. Infection with schistosomes results in the production of parasitic eggs, which provoke macrophages and other immune cells to gather around the egg and encapsulate it within an immune structure known as a granuloma resulting in granulomatous lesions[5,6]. Chronic granulomas have a complex cellular composition of myeloid cells, eosinophils, lymphocytes, and fibroblasts that cause tissue pathology. In hepatic tissue, they may be considered as the main cause of pathogenesis and morbidity[6]. At the onset of granuloma formation, neutrophils are recruited into the granuloma core resulting in the neutrophil-mediated inflammatory response, which ultimately leads to tissue damage[7]. The T cell-mediated response, especially $CD4^+$ T cell response, has been reported to participate in neutrophil induction in schistosomiasis[8].

There is no vaccine available against any species of *Schistosoma*, although valuable progress has been made in the control of schistosomiasis in recent decades, which focuses on periodic and preventive chemotherapy using praziquantel (PZQ)[9]. The effectiveness of PZQ against *Schistosoma* sp. is well recognized, but evidence is accumulating that it can cause allergic and hypersensitivity reactions and cannot prevent re-infection[10,11]. Preventive chemotherapy alone is not enough to break the transmission cycle and chronic infections that lead to organ damage, growth retardation, anemia, and cognitive impairment[9]. Therefore, it has become necessary to search for alternative medicines and bioactive natural products for the treatment of schistosomiasis.

Moringa is a genus of medicinal plants, commonly referred to as the miracle tree due to the beneficial uses of its various parts[12] and has been used to treat wounds and various diseases. In addition, *Moringa oleifera* leaves have anti-inflammatory, antioxidant, antidiabetic, and anticancer properties[12,13]. Most of its biological activity is due to the high content of flavonoids, glucosinolates, and glucosides. The main anti-inflammatory mechanism reported for *Moringa oleifera* was the inhibition of the nuclear factor (NF)-κB pathway[13]. The hepatoprotective properties of *Moringa* seed extract were discovered from the anti-fibrotic study conducted by Hamza[14] which indicated that *Moringa* also possesses anti-inflammatory properties against CCl_4-induced liver fibrosis. Therefore, the current study investigates the potential effect of *Moringa oleifera* aqueous extract on *in vitro* granulomatosis induced by *Schistosoma* soluble egg antigen (SEA).

PATIENTS AND METHODS

Patients

Fifteen schistosomiasis/hepatitis C patients (28 males and 22 females, mean age ± standard deviation: 43.8±9.8 and 45.5±8.2, respectively) and 25 healthy subjects (12 males and 13 females, mean age ± standard deviation: 29±5.4 and 38.6±13.6, respectively) were enrolled in the current study. Participants were enrolled from Al Kasr El Einy University Hospital, Cairo University, from October 2012 to June 2015. Using anticoagulant ethylene diamine tetra acetic acid (EDTA) tubes, approximately 15 mL of blood was drawn by venipuncture from all participants. All participants underwent full clinical examinations. All patients were diagnosed with chronic schistosomiasis/hepatitis C infection by histological examination (liver biopsy), presence of anti-HCV in the serum, and detection of *S. mansoni* ova in the stool and seropositivity for antischistosomal antibodies (indirect hemagglutination; Femouz laboratories, Cedex, France), and have elevated serum alanine transaminase and aspartate transaminase activities. Healthy subjects had no past or current history of schistosomiasis or any other viral infection. The study was approved by the research ethics committee

of Cairo University, Egypt (24-3-2012). All participants were given and signed informed consent.

Preparation of praziquantel (PZQ)

PZQ is a white crystalline powder, stable under normal storage conditions, almost insoluble in water, but soluble in some organic solvents. One tablet of biltricide from the commercial pharmacy (600 mg PZQ) dissolved in 6 mL of Dulbecco's modified eagle medium (DMEM) and centrifuged at 200 x*g* for 10 minutes. The supernatant became an aqueous extract of 100 mg PZQ/mL. PZQ was used immediately or stored at 4ºC until used according to the method of Allan *et al*.[15].

Preparation of *Moringa oleifera* aqueous extract

Dried powdered of *Moringa oleifera* leaves was purchased from the local markets in Cairo, Egypt. The method for preparing aqueous extract of *Moringa oleifera* leaves was previously described[16]. Briefly, *Moringa* leaves powder (1.0 g) was added to 10 mL of boiling water, brewed for 5 minutes, cooled to room temperature, and filtered. The supernatant was filtered through filter paper and the resulting filtrate was sterilized by passing through a 0.22 µm cellulose nitrate membrane filter to give an aqueous extract of 100 mg of *Moringa* tea/mL. *Moringa* tea was used immediately or stored at 4ºC until used.

Antigens

S. mansoni SEA was purchased as a lyophilized endotoxin-free preparation from Theodor Bilharz Research Institute, Giza, Egypt.

Isolation of granulocytes from whole blood and *in vitro* granuloma formation

Separation of human granulocytes from blood was performed by Ficoll–Paque density gradient[17]. Granulocytes were suspended in DMEM supplemented with 10% fetal bovine serum (FBS) (HyClone Ltd, Cramlington UK) and were counted using a hemocytometer. The viability of the isolated granulocytes was >98% as measured with trypan blue dye (ADWIC, Qalyubiyya, Egypt) using the exclusion technique. Isolation of granulocytes from whole blood was performed and granuloma induction *in vitro* were measured and classified according to the method of Nady *et al*.[17].

Formation of granuloma *in vitro*

The ability of SEA to stimulate granulocytes in the presence of various cytokines was evaluated using the *in vitro* granuloma technique as previously described[18,19]. Polyacrylamide beads (200 mg, 130±40 µm in diameter, Bio-Rad, Bio-Gel P-4, Richmond, CA, USA) were activated by hydration in sterile distilled water for 24 hours at 4ºC. The hydrated beads were washed several times and then autoclaved with sterile distilled water. The beads (10 mL) were added to pH 7.4 fixing solution [5 mL of 1.0 mol sterile phosphate-buffered saline (PBS), 10 mL of 25% glutaraldehyde, and 25 mL sterile distilled water) and incubated overnight at 37ºC. The activated beads were after that washed several times until no glutaraldehyde was detected in the supernatant. To combine the beads with SEA, 10 mL of activated beads were incubated with 50 mg SEA and then stirred on a rotating mixer overnight at 4ºC. By using a sterile PBS the unbound antigen is removed by successive washing. Antigen-conjugated beads were stored at 4°C in sterile PBS until use.

Optimization of granuloma formation using granulocytes

Bead dilution and granulocyte counting were optimized by using different beads dilutions and different granulocytes concentrations. The optimal dilution of beads was 1:200 and the optimal concentration of granulocytes was 1×10^6 cells/well.

Induction of granuloma

Granuloma was induced *in vitro* as previously described[19]. In a volume of 100 µL, 200 SEA–conjugated poly-acrylamide beads were added to the bottom of 24-well tissue culture plate (Corning

costar, 3524, Sigma Aldrich, St. Louis, MO, USA) in the presence of 1×10^6 granulocytes in 2 mL of DMEM media supplemented with 10% FBS. IL-17 (125 pg/mL) was added to the culture to induce the optimum size of granuloma within 3-7 days. The culture was maintained at 37ºC in 5% CO_2 incubator for 14 days. Supernatants were collected on the 7th and 14th days and stored at –70ºC for further analysis.

Optimization of PZQ and *Moringa* extract

Different concentrations of PZQ and *Moringa* (0.1 mg/mL, 1.0 mg/mL, 5 mg/mL, and 10 mg/mL) were added to granuloma and incubated in 37°C and 5% CO_2 in 24-well flat-bottomed tissue culture plates. The optimal concentration of PZQ and *Moringa* was 0.1 mg/mL as shown in Figure "1".

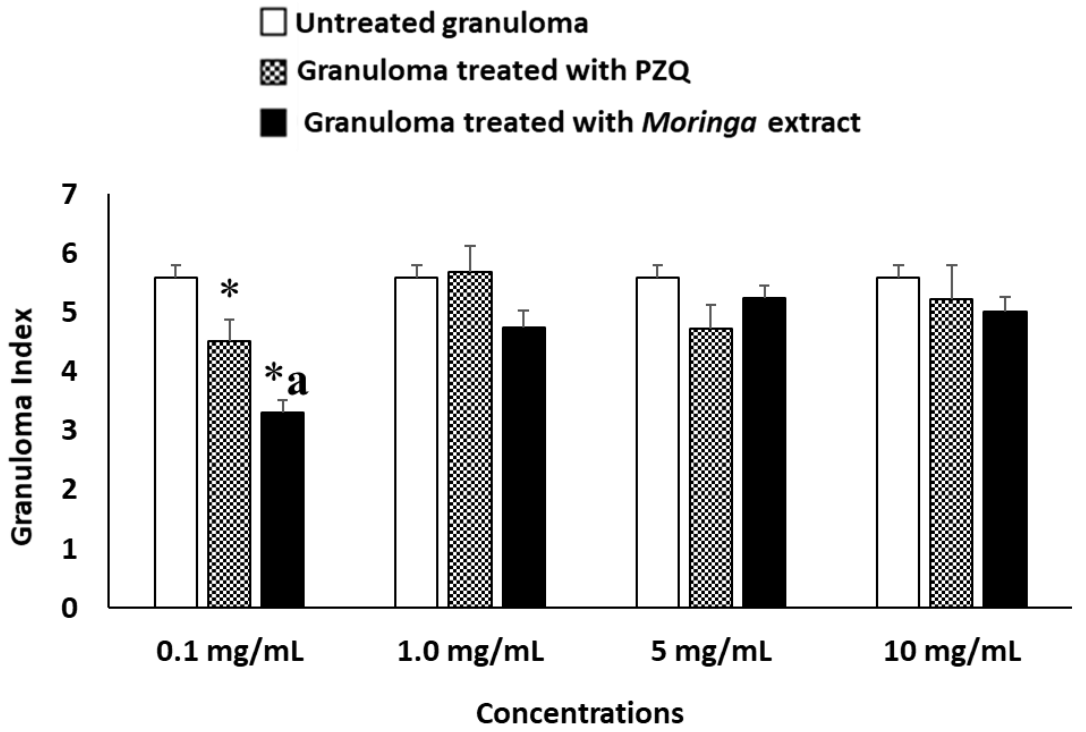

Figure (1): Optimization of praziquantel (PZQ) and *Moringa* extract concentrations used in the treatment of granuloma generated *in vitro* from granulocytes of healthy individuals (not infected with *Schistosoma* and/or HCV) (after 14 days). Data is represented as mean ± standard deviation of 3 different experiments. *****: Significant at $P<0.05$ as compared with their corresponding value of granuloma index of untreated granuloma around soluble egg antigen (SEA)-conjugated polyacrylamide beads in the presence of interleukin 17. **a**: Significant at $P<0.05$ as compared with their corresponding value of granuloma index produced by granuloma treated with PZQ.

Determination of granuloma size

Granuloma reactivity was assessed *in vitro* by measuring the cellular activity around the beads after examining the culture[18] using an inverted tissue culture microscope (Olympus, CKX41, London, UK). The granuloma was photographed in the culture using a digital camera (Premiere MA88-300, Microscopes America, Inc., Cumming, GA, USA) attached to the inverted phase-contrast microscope. The reactivity of cells was determined by morphological observations based on the number of cells attached to the beads. A numerical score corresponding to the following classification was assigned to bead-cell interaction[17]. The *in vitro* classification of bead granuloma was as follows: (1) No adhering cells to the beads, (2) less than 5 adhering cells, (3) greater than 5 adhering cells bind to the beads,

(4) greater than 5 adhering cells accompanied by peripheral mononuclear cell migration and blast formation, (5) adherent cell layer attached to the beads accompanied by peripheral mononuclear cell migration, and (6) several cell layers surrounding the beads accompanied by mononuclear cell migration as shown in Figure "2".

The total score was calculated for 100 beads and the mean was expressed as the granuloma index (G.I) of each experimental group, culture reactivity of all SEA-conjugated beads was compared with the reactivity of SEA unconjugated beads (negative control) according to the study of Nady *et al*.[17].

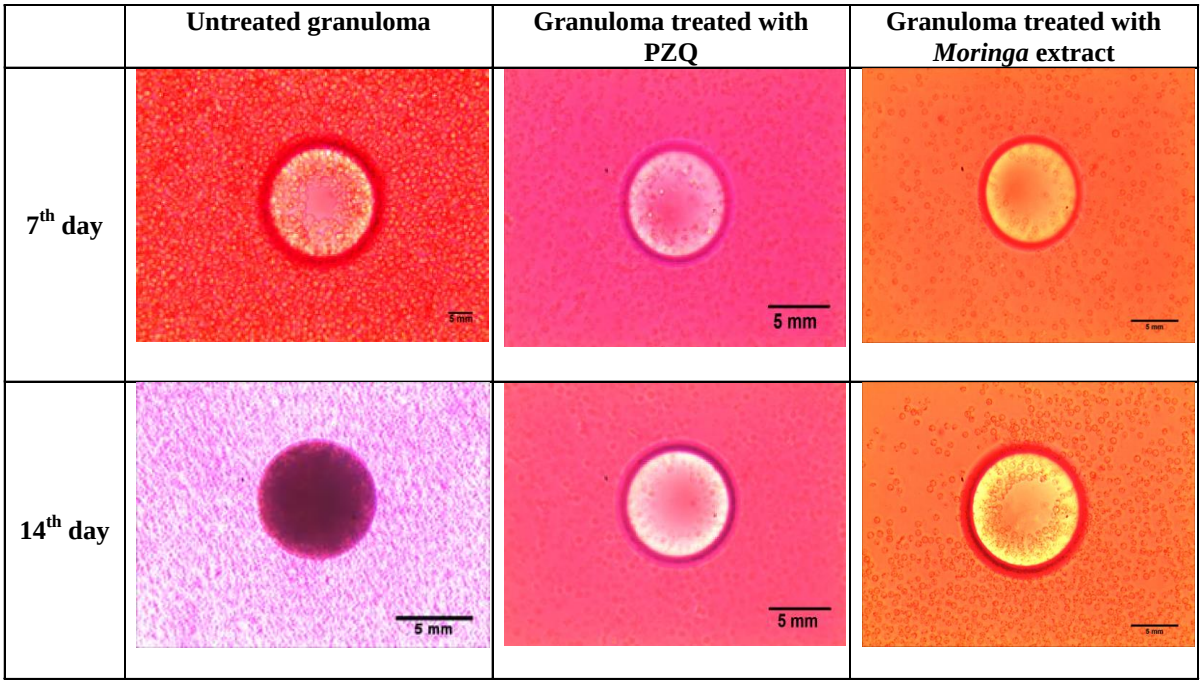

Figure (2): Selected photomicrographs of untreated granuloma, granuloma treated with PZQ, and granuloma treated with *Moringa* extract (0.1 mg/mL) after 7 and 14 days (magnification: 20×, scale bar: 5 mm). PZQ: praziquantel.

Measurements of granulocytes mediators

Tumor necrosis factor (TNF)-α was measured using human TNF-α enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Boster, Pleasanton, CA, USA). H_2O_2 was measured using H_2O_2 colorimetric methods according to the manufacturer's instructions (Bio-diagnostic, Giza, Egypt). Nitric oxide (NO) was measured as previously described[20]. NO in the supernatant was assessed by reaction of Griess, NO has the potential to produce a chromophore with Griess reagent. The reading of color changes was measured using a microtiter plate reader (BioTek, Winooski, VT, USA) at a dual wavelength of 450 and 640 nm. The standard curve was used to measure the nitrite concentration.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 3 software (GraphPad Company San Diego, CA. USA). Analysis of variance (ANOVA) and Bonferroni post-hoc test were used to analyze the effect of different treatments on granuloma as comparing to non-treated granuloma at each time point (after 7 or 14 days) and to compare the granulocytes functions of *Schistosoma*/HCV co-infected individuals with those of non-infected individuals. The data obtained were represented as mean ± standard deviation. Results with a *P* value of < 0.05 were considered significant.

RESULTS

Optimization of PZQ and *Moringa* extract concentrations after 14 days of granuloma treatment

Different concentrations of PZQ and *Moringa* extract (0.1 mg/mL, 1.0 mg/mL, 5 mg/mL, and 10 mg/mL) were used in the treatment of granuloma induced *in vitro* from granulocytes of healthy individuals (not infected with *Schistosoma* and/or HCV). The optimum concentration of PZQ, as well as *Moringa* extract, was 0.1 mg/mL; this dose reduced the number of cells attracted around the beads conjugated with SEA after 14 days as shown in Figure "1".

Effect of PZQ and *Moringa* extract, compared with PZQ, on morphology of the obtained granuloma from healthy subjects

Figure "2" showed the granuloma reactivity as determined by the morphological observations based on the number of cells attached to polyacrylamide beads conjugated with SEA in granuloma of healthy individuals treated with PZQ (0.1 mg/mL) and *Moringa* (0.1 mg/mL) compared with the untreated granuloma after 7 and 14 days of treatment. The untreated granuloma showed multiple cell layers attached to the bead accompanied by peripheral cell migration. After seven days of treatment with PZQ and *Moringa* extract, granuloma showed more than 5 cell layers attached to the beads. After 14 days of treatment, PZQ showed less than 5 cell layers attached to the beads, while *Moringa* extract totally inhibited the granuloma formation.

Effect of *Moringa* extract, compared with PZQ, on granuloma index (G.I)

PZQ (0.1 mg/mL, as a reference drug) caused a reduction in G.I of granuloma induced by *S. mansoni* SEA and granulocytes isolated from healthy subjects (–11.3% and –9.4%) or *Schistosoma*/HCV co-infected patients (–13.7% and –6.8%) in the presence of IL-17 after 7 and 14 days, respectively, compared with the corresponding values of untreated granuloma (Figure 3). PZQ inhibited significantly (P<0.05) G.I in either healthy subjects or co-infected patients after 7 days only.

Moringa extract (0.1 mg/mL) significantly inhibited (P<0.05) granuloma formation after 14 days only in healthy subjects, but not in co-infected patients. Moreover, the results recorded a reduction in G.I of granuloma induced from granulocytes of healthy subjects, but not of *Schistosoma*/HCV co-infected patients, in the presence of *Moringa* extract on the 14th days compared with the corresponding value in the presence of PZQ (Figure 3).

Effect of *Moringa* extract, compared with PZQ, on mediators produced by stimulated granulocytes

Both PZQ and *Moringa* extract elevated significantly (P<0.05, 114.1% and 100.7%, respectively) TNF-α production from granulocytes of healthy subjects that were stimulated with SEA+IL-17 at day 14 only (Figure 4). PZQ decreased significantly (P<0.05) the H_2O_2 production from granulocytes of healthy subjects (at day 14 only) and *Schistosoma*/HCV co-infected patients (at day 7 only) that were stimulated with SEA+IL-17 (Figure 5). On the contrary, a significant elevation (P<0.05) was observed in the H_2O_2 production from stimulated granulocytes of *Schistosoma*/HCV co-infected patients on the 7th and 14th days (156.5% and 146.5%, respectively) as compared with their corresponding values of granulocytes isolated from healthy subjects. *Moringa* extract decreased significantly (P<0.05) the H_2O_2 production from granulocytes of healthy subjects (at day 14 only) and *Schistosoma*/HCV co-infected patients (at both 7 and 14 days) that were stimulated with SEA+IL-17 (Figure 5). In comparison with PZQ treatment, *Moringa* extract induced a significant decrease (P<0.05) in the H_2O_2 production by stimulated granulocytes of the healthy subjects and co-infected patients at day 14 only (Figure 5).

PZQ didn't show any significant change in the NO production from granulocytes of

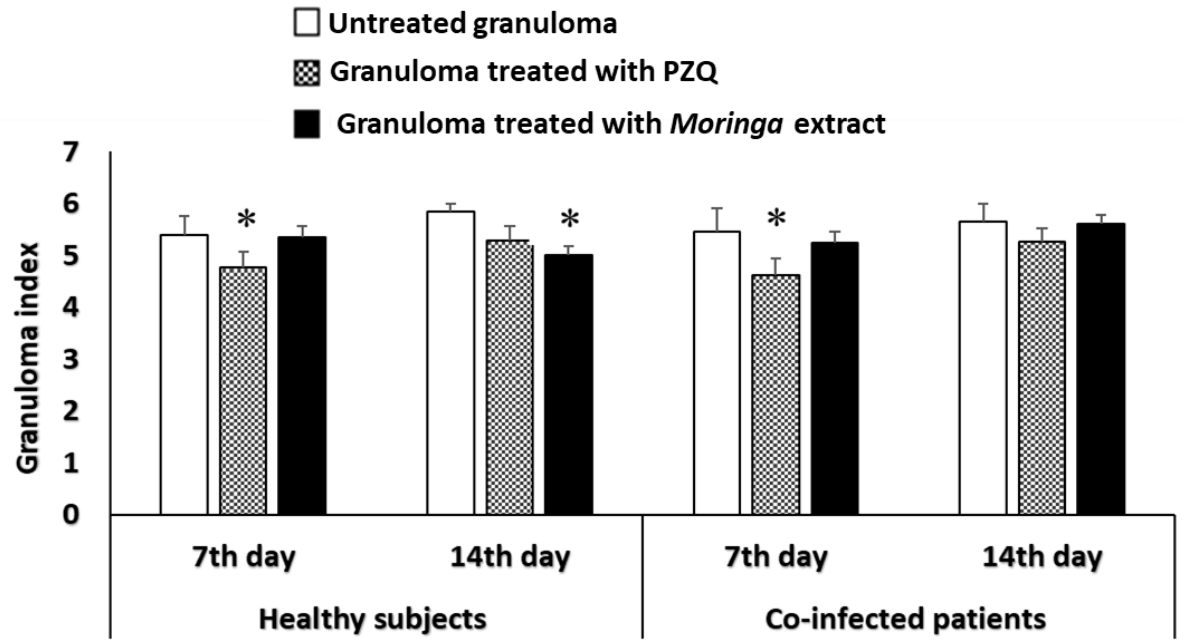

Figure (3): Effect of *Moringa* extract on granuloma index of granuloma formed by granulocytes stimulated with *S. mansoni* soluble egg antigen (SEA)-conjugated polyacrylamide beads in the presence of interleukin 17. Data is represented as mean ± standard deviation of 3 different experiments. *****: Significant at $P<0.05$ as compared with their corresponding value of granuloma index of untreated granuloma.

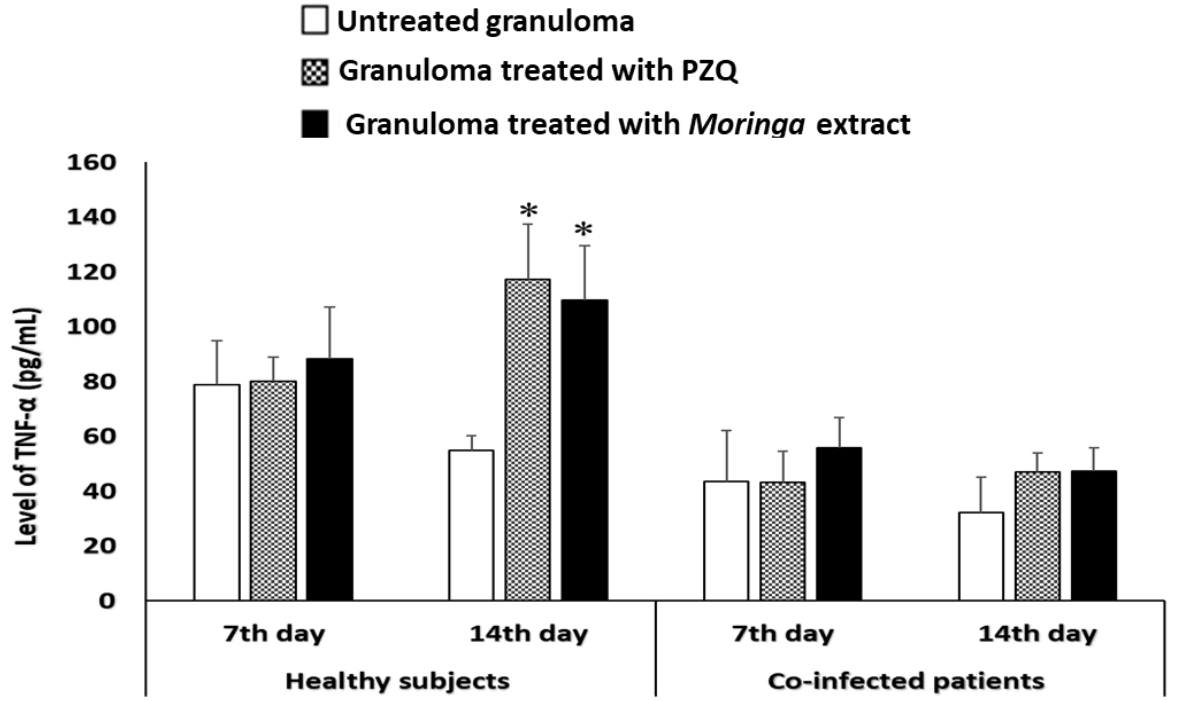

Figure (4): Effect of *Moringa* extract on tumor necrosis factor (TNF)-α produced by granulocytes stimulated with *S. mansoni* soluble egg antigen (SEA)-conjugated polyacrylamide beads in the presence of interleukin 17. Data is represented as mean ± standard deviation of 3 different experiments. *****: Significant at $P<0.05$ as compared with their corresponding value of granuloma index of untreated granuloma.

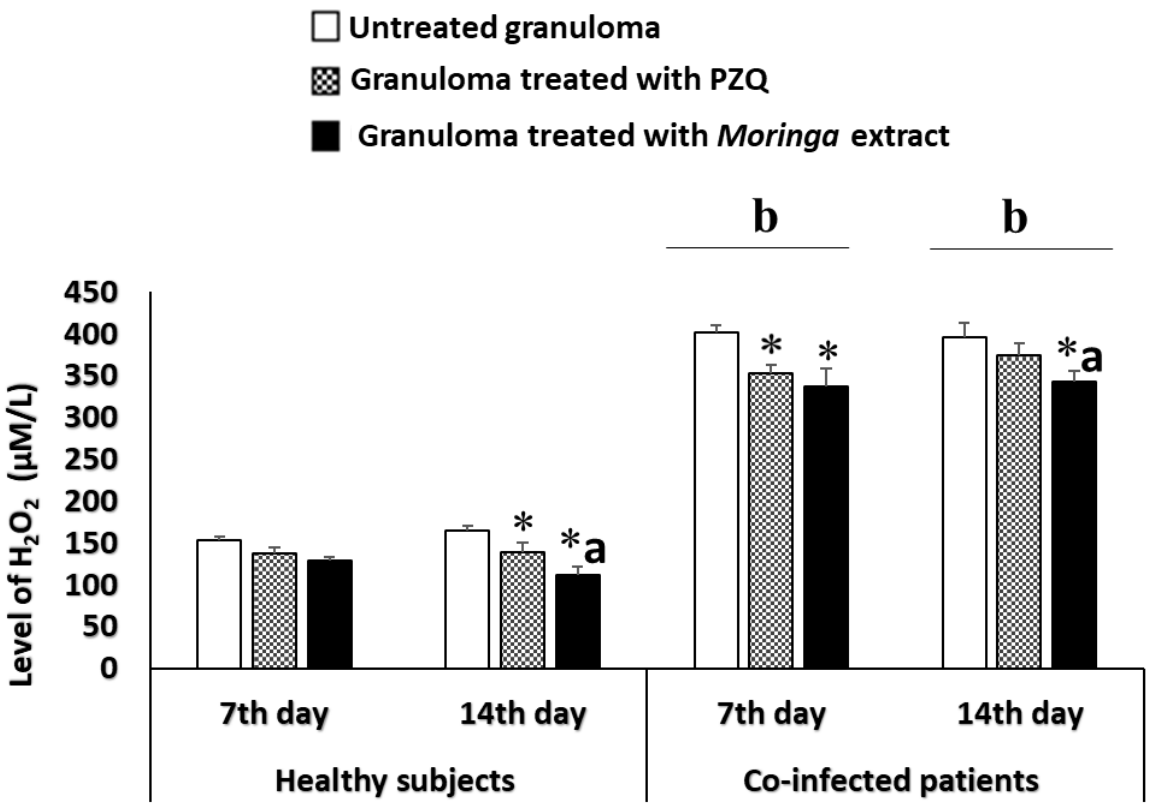

Figure (5): Effect of *Moringa* extract on H_2O_2 produced by granulocytes stimulated with *S. mansoni* soluble egg antigen (SEA)-conjugated polyacrylamide beads in the presence of interleukin 17. Data is represented as mean ± standard deviation of 3 different experiments. *****: Significant at $P<0.05$ as compared with their corresponding value of granuloma index of untreated granuloma. **a**: Significant at $P<0.05$ as compared with their corresponding value of granuloma index produced by granuloma treated with praziquantel (PZQ). **b**: Significant at $P<0.05$ as compared to their corresponding value of H_2O_2 level produced by granulocytes isolated from healthy subjects.

healthy subjects or *Schistosoma*/HCV co-infected patients (at days 7 and 14) that were stimulated with SEA+IL-17 (Figure 6). In addition, a non-significant change ($P\geq0.05$) was observed in the NO production from stimulated granulocytes of *Schistosoma*/ HCV co-infected patients on days 7^{th} and 14^{th} as compared with their corresponding values of granulocytes isolated from healthy subjects. *Moringa* extract decreased significantly ($P<0.05$) the NO production from only granulocytes of healthy subjects at days 7 and 14 that were stimulated with SEA+IL-17 (Figure 6). In comparison with PZQ treatment, *Moringa* extract induced a significant decrease ($P<0.05$) in the NO production from stimulated granulocytes of the healthy subjects at day 14 only (Figure 6).

DISCUSSION

This study revealed the attenuating effects of *Moringa* on granulocytes functions of healthy and *Schistosoma*/HCV co-infected patients stimulated with *S. mansoni* SEA-conjugated polyacrylamide beads in the presence of IL-17. The *Moringa* extract inhibited significantly the formation of granuloma from granulocytes of healthy individuals after 14 days more than the inhibition observed with PZQ treatment. Additionally, *Moringa* statistically affects the activity of granulocytes in granuloma as follows: (a) it increased significantly the level of TNF-α after 14 days from granulocytes of healthy individuals as PZQ, (b) it decreased significantly the NO level more than PZQ after 14 days from granulocytes of healthy individuals, and

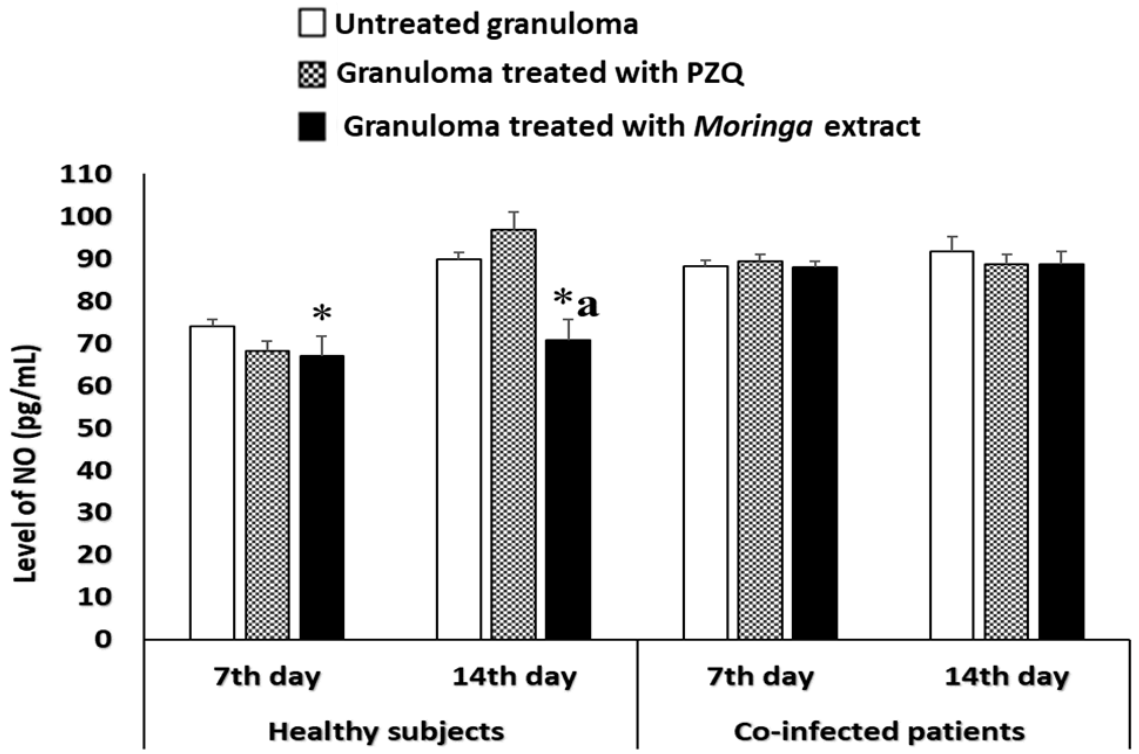

Figure (6): Effect of *Moringa* extract on nitric oxide (NO) produced by granulocytes stimulated with *S. mansoni* soluble egg antigen (SEA)-conjugated polyacrylamide beads in the presence of interleukin 17. Data is represented as mean ± standard deviation of 3 different experiments. *****: Significant at $P<0.05$ as compared with their corresponding value of granuloma index of untreated granuloma. **a**: Significant at $P<0.05$ as compared with their corresponding value of granuloma index produced by granuloma treated with praziquantel (PZQ).

(c) it decreased significantly the H_2O_2 levels more than PZQ after 14 days from granulocytes of healthy individuals and *Schistosoma/*HCV co-infected patients.

Granuloma cells mainly include neutrophils, eosinophils, monocytes, lymphocytes, and epithelioid cells[21]. SEA attracted the granulocytes and upon contact with the egg they gain chemical activity that exceeds the activity of the egg resulting in subsequent recruitment of other cells[5]. These cells aggregate then clump together to encapsulate SEA resulting in the granuloma formation. The present study showed that treatment of granuloma with PZQ induced a significant inhibition in G.I after 7 days, but not after 14 days, when using granulocytes either from healthy subjects or *Schistosoma*/HCV co-infected patients. However, treatment of granuloma generated in healthy subject with *Moringa* leaves extract recorded a significant decrease in G.I only after 14th days and more than that observed with PZQ treatment. The whole pharmacological mechanisms of *Moringa* leaves have not yet precisely documented despite their increasing use. The decrease in G.I by *Moringa* may be explained on the basis that *Moringa* leaves extract act as a good source of natural antioxidants because they contain different types of antioxidant compounds such as ascorbic acid, flavonoids, phenols, and carotenoids[22]. Such compounds, as flavonoids, may also have anti-inflammatory activity[23]. However, in the current study *Moringa* extract enhanced the TNF-α (a proinflammatory cytokine) production of granulocytes isolated from healthy individuals, as PZQ, after 14 days.

The result of the present study showed a significant increase in the level of TNF-α

produced by granulocytes of healthy subjects (after 14 days), but not of that of *Schistosoma*/HCV co-infected patients, after *Moringa* or PZQ treatment in the presence of SEA and IL-17. Therefore, this increase in TNF-α production should be not only due to SEA, which is known to stimulate the expression of TNF-α [24,25], but also due to PZQ and the compounds found in *Moringa* extract. The obtained results are in contrast with that found by a study of Muangnoi *et al*.[26] who reported that phenolic rich fraction of *Moringa* inhibited cytokine production by human macrophages in an *in vitro* model of macrophage production of TNF-α, IL-6, and IL-8. Another study also reported that an ethyl acetate fraction of *Moringa oleifera* depressed the expression of an important gene in NF-κB signaling inflammatory reaction in human macrophages stimulated by cigarette smoke[27]. On the other hand, Gupta *et al.*[28] reported that *Moringa* leaves extract increased the count of leucocytes and neutrophils in healthy and immuno-suppressed mice. In addition, Takaki *et al*.[29] reported that TNF-α mediated granulomatous hyperplasia rather than granuloma initiation.

Several studies have shown that H_2O_2 causes tissue damage when its level is increased and is not regulated by antioxidants[30-33]. In the current study, granuloma induced by *S. mansoni* SEA-conjugated polyacrylamide beads in the presence of IL-17 and treated with aqueous extract of *Moringa* leaves caused a significant inhibition in H_2O_2 level production by granulocytes isolated from healthy subjects after 14 days and from *Schistosoma/*HCV co-infected patients after 7 and 14 days. Compared with PZQ treatment, *Moringa* led to a significant decrease in the level of H_2O_2 production by granulocytes isolated from both *Schistosoma*/HCV co-infected patients and healthy subjects after 14 days. The decrease in the level of H_2O_2 released from granulocytes after treatment with *Moringa* was in agreement with the results of Madeo *et al*.[34], who reported that *Moringa* leaf extract reduces the harmful effects of H_2O_2 by scavenging hydroxyl radical ($OH^{\bullet}$). Hydroxyl radical was a highly reactive species that readily oxidizes all cellular macromolecules including proteins, sugars, lipids, and deoxyribonucleic acid. It has been shown that naturally occurring antioxidants inhibit the generation of reactive oxygen species, scavenge free radicals and initiate the alteration of the redox potential within cells[35]. There was sufficient evidence revealing that polyphenolic antioxidants prevent radical-mediated cell damage and indirectly suppress apoptosis[36].

In the present study, treatment of granuloma with *Moringa* extract resulted in a significant decrease in the level of NO production by granulocytes of healthy subjects only on the 7^{th} and 14^{th} day as compared with untreated granuloma. This inhibition in NO production induced by *Moringa* was significant as compared with PZQ after 14 days, but not after 7 days. Waterman *et al*.[37] observed that *Moringa* extract isolated from leaves reduced significantly the gene expression and production of inflammatory markers including NO from macrophages. The reduction in NO production by granulocytes shown in response to *Moringa* treatment in the present study might be due to the antioxidant activity of the bioactive compounds of *Moringa* leaves, such as flavonoids and phenolic acids. Also, as previously reported, *Moringa* can scavenge free radicals, attenuating expression of inducible nitric oxide synthase, leading to reduction of NO production[26,27,37], which support our findings.

In the current study, the effect of *Moringa* compared with PZQ treatment was evaluated as inhibitory agents in granulocytes activity. The observed decrease in the level of H_2O_2 and NO by both agents indicated a decrease in oxidative stress. In conclusion, treatment of granuloma using *Moringa* showed a more protective effect than treatment observed with PZQ treatment. An increase of TNF-α

and a decrease in H_2O_2, and NO of granuloma after *Moringa* treatment may explain the mechanism of the inhibition of granuloma formation.

ACKNOWLEDGEMENTS

The authors would like to thank the Egyptian Science and Technology Development Fund (STDF) for supporting this study through grant No. 1814 awarded to Dr. Soad Nady under the framework of the Egypt/US cooperation program. The authors express their gratitude to Dr. Gamal Esmat, Professor of Hepatology (Department of Endemic Medicine and Hepatology, Faculty of Medicine, Cairo University) for providing blood samples from Schistosoma/HCV co-infected patients and healthy subjects through the STDF grant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

SNI planned the study and designed the experiment. AAE carried out the experiments and performed the statistical analysis. RMH summarized, discussed, and interpreted the results, as well as drafted the manuscript.

REFERENCES

[1] Schistosomiasis (Fact Sheet 18 May 2021). WHO (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis).

[2] El Gaafary, M. M.; Rekacewicz, C.; Abdel-Rahman, A. G. *et al*. (2005). Surveillance of acute hepatitis C in Cairo, Egypt. J Med Virol, 76(4): 520-525.

[3] Lehman, E. M. and Wilson, M. L. (2009). Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J Viral Hepat, 16(9): 650-658.

[4] El-kady, I. M.; El-Masry, S. A.; Badra, G. *et al*. (2004). Different cytokine patterns in patients coinfected with hepatitis C virus and *Schistosoma mansoni*. Egypt J Immunol, 11 (1): 23-29.

[5] Takaki, K. K.; Rinaldi, G.; Berriman, M. *et al*. (2021). *Schistosoma mansoni* eggs modulate the timing of granuloma formation to promote transmission. Cell Host Microbe, 29: 58-67.

[6] Pagán, A. J. and Ramakrishnan, L. (2018). The formation and function of granulomas. Annu Rev Immunol, 36: 639-665.

[7] Chuah, C.; Jones, M. K.; Burke, M. L. *et al*. (2013). Spatial and temporal transcriptomics of *Schistosoma japonicum*-induced hepatic granuloma formation reveals novel roles for neutrophils. J Leukoc Biol, 94(2): 353-365.

[8] Seki, T.; Kumagai, T.; Kwansa-Bentum, B. *et al*. (2012). Interleukin-4 (IL-4) and IL-13 suppress excessive neutrophil infiltration and hepatocyte damage during acute murine *Schistosomiasis japonica*. Infect Immun, 80: 159-168.

[9] Llanwarne, F. and Helmby, H. (2021). Granuloma formation and tissue pathology in *Schistosoma japonicum* versus *Schistosoma mansoni* infections. Parasite Immunol, 43(2): e12778 (DOI: 10.1111/pim.12778).

[10] de Lima Aires, A.; Ximenes, E. C. P. A.; Barbosa, V. X. *et al*. (2014). β-Lapachone: a naphthoquinone with promising antischistosomal properties in mice. Phytomedicine, 21(3): 261-267.

[11] Goupil, L. S. and McKerrow, J. H. (2014). Introduction: drug discovery and development for neglected diseases. Chem Rev, 114(22): 11131-11137.

[12] Obembe, O. O. and Raji, Y. (2018). Effects of aqueous extract of *Moringa oleifera* seed on cadmium-induced reproductive toxicity in male Wistar rats. Afr Health Sci, 18(3): 653-663.

[13] Abd Rani, N. Z.; Husain, K. and Kumolosasi, E. (2018). *Moringa* genus: a review of phytochemistry and pharmacology. Front Pharmacol, 9: 108 (DOI: 10.3389/fphar.2018. 00108).

[14] Hamza, A. A. (2010). Ameliorative effects of *Moringa oleifera* Lam seed extract on liver fibrosis in rats. Food Chem Toxicol, 48: 345-355.

[15] Allan, L. A.; Kutima, H. L.; Muya, S. *et al*. (2014). The efficacy of a herbal drug, schitozim over praziquantel in the management of *Schistosoma mansoni* infection in BALB/c mice. J Biol Agric Health Care, 4: 77-87.

[16] Jaiswal, D.; Rai, P. K.; Kumar, A. *et al*. (2009). Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. J Ethnopharmacol, 123(3): 392-396.

[17] Nady, S.; Shata, M. T. M.; Mohey, M. A. *et al*. (2017). Protective role of IL-22 against *Schistosoma mansoni* soluble egg antigen-induced granuloma *in vitro*. Parasite Immunol, 39: e12392 (DOI: 10.1111/pim. 12392).

[18] Doughty, B. L.; Ottesen, E. A.; Nash, T. E. *et al*. (1984). Delayed hypersensitivity granuloma formation around *Schistosoma mansoni* eggs in vitro. III. Granuloma formation and modulation in human Schistosomiasis mansoni. J Immunol, 133(2): 993-997.

[19] Doughty, B. L.; Goes, A. M.; Parra, J. C. *et al*. (1987). Granulomatous hypersensitivity to *Schistosoma mansoni* egg antigens in human schistosomiasis. I. Granuloma formation and modulation around polyacrylamide antigen-conjugated beads. Mem Inst Oswaldo Cruz, 82 (Suppl 4): 47-54.

[20] Green, L. C.; Wagner, D. A.; Glogowski, J. *et al*. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem, 126: 131-138.

[21] Burke, M. L.; McManus, D. P.; Ramm, G. A. *et al*. (2010). Temporal expression of chemokines dictates the hepatic inflammatory infiltrate in a murine model of schistosomiasis. PLoS Negl Trop Dis, 4(2): e598 (DOI: 10.1371/journal.pntd.0000598).

[22] Sivasankari, B.; Anandharaj, M. and Gunasekaran, P. (2014). An ethnobotanical study of indigenous knowledge on medicinal plants used by the village peoples of Thoppampatti, Dindigul district, Tamilnadu, India. J Ethnopharmacol, 153(2): 408-423.

[23] Bhujbal, S. S.; Chitlange, S. S.; Suralkar, A. *et al*. (2008). Anti-inflammatory activity of an isolated flavonoid fraction from *Celosia argentea* Linn. J Med Plant Res, 2(3): 052-054.

[24] Nascimento, M.; Huang, S. C.; Smith, A. *et al*. (2014). Ly6Chi monocyte recruitment is responsible for Th2 associated host-protective macrophage accumulation in liver inflammation due to schistosomiasis. PLoS Pathog, 10(8): e1004282 (DOI: 0.1371/journal.ppat.1004282).

[25] Chiu, B.-C.; Freeman, C. M.; Stolberg, V. R. *et al*. (2004). The innate pulmonary granuloma: characterization and demonstration of dendritic cell recruitment and function. Am J Pathol, 164(3): 1021-1030.

[26] Muangnoi, C.; Chingsuwanrote, P.; Praengamthanachoti, P. *et al*. (2012). *Moringa oleifera* pod inhibits inflammatory mediator production by lipopolysaccharide-stimulated RAW 264.7 murine macrophage cell lines. Inflammation, 35(2): 445-455.

[27] Kooltheat, N.; Sranujit, R. P.; Chumark, P. *et al*. (2014). An ethyl acetate fraction of *Moringa oleifera* Lam. inhibits human macrophage cytokine production induced by cigarette smoke. Nutrients, 6(2): 697-710.

[28] Gupta, A.; Gautam, M. K.; Singh, R. K. *et al*. (2010). Immunomodulatory effect of *Moringa oleifera* Lam. extract on cyclophosphamide induced toxicity in mice. Indian J Exp Biol, 48(11): 1157-1160.

[29] Takaki, K. K.; Roca, F. J.; Schramm, G. *et al*. (2021). Tumor necrosis factor and *Schistosoma mansoni* egg antigen omega-1 shape distinct aspects of the early egg-induced granulomatous response. PLoS Negl Trop Dis, 15(1): e0008814 (DOI: 10.1371/journal.pntd. 0008814).

[30] Halliwell, B. (1999). Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic Res, 31(4): 261-272.

[31] Long, L. H.; Lan, A. N.; Hsuan, F. T. *et al*. (1999). Generation of hydrogen peroxide by "antioxidant" beverages and the effect of milk addition. Is cocoa the best beverage? Free Radic Res, 31(1): 67-71.

[32] Halliwell, B.; Clement, M. V. and Long, L. H. (2000). Hydrogen peroxide in the human body. FEBS lett, 486: 10-13.

[33] Halliwell, B.; Clement, M. V. and Ramalingam, J. *et al*. (2000). Hydrogen peroxide. Ubiquitous in cell culture and *in vivo*? IUBMB life, 50(4-5): 251-257.

[34] Madeo, F.; Fröhlich, E.; Ligr, M. *et al*. (1999). Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol, 145(4): 757-767.

[35] Finkel, T. and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. Nature, 408(6809): 239-247.

[36] Sroka, Z. and Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem Toxicol, 41(6): 753-758.

[37] Waterman, C.; Cheng, D. M.; Rojas-Silva, P. *et al*. (2014). Stable, water extractable isothio-cyanates from *Moringa oleifera* leaves attenuate inflammation *in vitro*. Phytochemistry, 103: 114-122.

How to cite this article:
Ibrahim, S. N.; El-Shorbagy, A. A. and Hussein, R. M. (2022). Inhibition of granuloma induced by granulocytes isolated from patients co-infected with *Schistosoma mansoni* and hepatitis C virus: role of the *Moringa oleifera* aqueous extract. Egyptian Journal of Zoology, 78: 1-14 (DOI: 10.21608/ejz.2022.113530.1068).

تثبيط الورم الحبيبي المُستحث بواسطة خلايا الدم البيضاء المُحَبَّبَة المعزولة من مرضى مصابين بكل من دودة البلهارسيا "*Schistosoma mansoni*" وفيروس التهاب الكبد الوبائي "C": دورالمستخلص المائي لنبات"*Moringa oleifera*"

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تشير الدراسة الحالية إلى النشاط المحتمل للمستخلص المائي لأوراق نبات المورينجا "*Moringa oleifera*" في تخفيف الورم الحُبيبي المتكون في المختبر والناجم عن المستضد القابل للذوبان لبيض البلهارسيا. شارك فى الدراسة الحالية "25" فردًا سليمًا و "50" مريضًا مصابًا بالبلهارسيا وفيروس الالتهاب الكبد الوبائي سي. تم عزل واستخدام خلايا الدم البيضاء المُحَبَّبَة للحث على الورم الحُبيبي في المختبر باستخدام حبات بولي أكريلاميد المشبعة بالمستضد القابل للذوبان لبيض البلهارسيا في وجود إنترلوكين-17 (125 بيكوجم/مل). وبعد ثلاثة أيام، تمت معاملة الورم الحُبيبي بمستخلص المورينجا (0.1 مجم/مل). وتم استخدام عقار "Praziquantel" (0.1 مجم/مل) كعلاج مرجعي. في اليومين السابع والرابع عشر، تم تحديد مؤشر الورم الحبيبي وقياس مستويات عامل نخر الورم-ألفا وفوق أكسيد الهيدروجين وأكسيد النيتريك في وسط استزراع الورم الحبيبي. وقد أظهر الفحص المجهري انخفاضًا ملحوظًا إحصائيًا في حجم الورم الحُبيبي استجابةً لعقار "Praziquantel" أو مستخلص المورينجا بعد 7 و 14 يوم، على التوالي، في الأشخاص الأصحاء. وكان الانخفاض الناجم عن المورينجا ملحوظة إحصائيًا مقارنة بـعقار "Praziquantel" بعد 14 يومًا. وقد تم تسجيل انخفاض ملحوظًا إحصائيًا في وسطاء الخلايا المحببة مثل فوق أكسيد الهيدروجين (في كل من الأشخاص الأصحاء والمرضى المصابين بالعدوى المشتركة) وأكسيد النيتريك (في الأشخاص الأصحاء فقط) استجابةً للمورينجا مقارنة بـعقار "Praziquantel" بعد 14 يومًا. ومع ذلك، أظهر عامل نخر الورم-ألفا زيادة ملحوظًا إحصائيًا (فقط بعد 14 يومًا) في الورم الحبيبي المعالج بـعقار "Praziquantel" أو مستخلص المورينجا مقارنة بالقيم المقابلة للورم الحبيبي غير المعالج في الأفراد الأصحاء. والخلاصة، أظهر مستخلص المورينجا نشاطًا وقائيًا أعلى من عقار "Praziquantel" في الحد من الورم الحبيبي، ربما بسبب التأثير على إنتاج وسطاء الخلايا الحبيبية.