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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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 7th and 14th days, the granuloma index (G.I) was determined and tumor necrosis factor (TNF)-α, H₂O₂, 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 H₂O₂ (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\textsuperscript{[2,3]} with frequently reported co-infection with schistosomiasis\textsuperscript{[4]}. Schistosoma mansoni is the most studied and geographically widespread species, and is the most common agent of schistosomiasis disease\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[7]}. The T cell-mediated response, especially CD4\textsuperscript{+} T cell response, has been reported to participate in neutrophil induction in schistosomiasis\textsuperscript{[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)\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[12]} and has been used to treat wounds and various diseases. In addition, Moringa oleifera leaves have anti-inflammatory, antioxidant, antidiabetic, and anticancer properties\textsuperscript{[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)-\textgreek{kappa}B pathway\textsuperscript{[13]}. The hepatoprotective properties of Moringa seed extract were discovered from the anti-fibrotic study conducted by Hamza\textsuperscript{[14]} which indicated that Moringa also possesses anti-inflammatory properties against CCl\textsubscript{4}-induced liver fibrosis. Therefore, the current study investigates the potential effect of Moringa oleifera aqueous extract on \textit{in vitro} granulomatosis induced by \textit{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 tetraacetic 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 sero-positivity 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, Qalyubiyaa, 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 4ºC 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 polycrylamide beads were added to the bottom of 24-well tissue culture plate (Corning
Moringa oleifera inhibits granulocytes-induced granuloma

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% CO₂ 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% CO₂ 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 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. 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,
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].

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<tr>
<th>Untreated granuloma</th>
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**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$_2$O$_2$ was measured using H$_2$O$_2$ 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, Winnoski, 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 H2O2 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 H2O2 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 H2O2 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 H2O2 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.
**Moringa oleifera** inhibits granulocytes-induced granuloma

Figure (5): Effect of *Moringa* extract on H$_2$O$_2$ 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$_2$O$_2$ 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 ($P>0.05$) was observed in the NO production from stimulated granulocytes of *Schistosoma/HCV* co-infected patients on days 7th and 14th 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_{2}O_{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\textsuperscript{[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\textsuperscript{[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 14\textsuperscript{th} 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\textsuperscript{[22]}. Such compounds, as flavonoids, may also have anti-inflammatory activity\textsuperscript{[23]}. However, in the current study Moringa extract enhanced the TNF-\alpha (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-\alpha
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 H₂O₂ 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 H₂O₂ 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 H₂O₂ production by granulocytes isolated from both Schistosoma/HCV co-infected patients and healthy subjects after 14 days. The decrease in the level of H₂O₂ 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 H₂O₂ by scavenging hydroxyl radical (OH·). 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 7th and 14th 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.[35] 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 H₂O₂ 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 H₂O₂, and NO of granuloma after Moringa treatment may explain the mechanism of the inhibition of granuloma formation.

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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
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ٍدٌ/ٍو). وحٌ اسخخذاً عقاس

Praziquantel

( "0.1

ٍدٌ/ٍو) معلاج ٍشخعٍ. فٍ اىُىٍُِ اىسابع

واىشابع عشش، حٌ ححذَذ ٍؤشش اىىسً اىحبُب

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وقُاط ٍسخىَاث عاٍو ّخش اىىسً

- أىفا

و

فىق أمسُذ اىهُذسوخُِ

وأمسُذ

اىُْخشَل فٍ

وسؾ اسخضساع اىىسً اىحبُبٍ

. وٍع رىل، أظهش

عاٍو ّخش اىىسً

- أىفا

صَادة

ٍيحىظـًا إحظائُـًا

فٍ حدٌ اىىسً

اىحُبُبٍ اسخدابتً

ى

عقاس "Praziquantel"

أو

ٍسخخيض اىَىسَْدا بعذ

11

َىٍ

ا. وقذ حٌ حسدُو

اّخفاع

ٍيحىظت إحظائُـًا

فٍ وسطاء اىخلاَا اىحببت ٍثو

فىق أمسُذ اىهُذسوخُِ

(فٍ مو ٍِ الأشخاص الأطحاء

واىَشػً اىَظابُِ باىعذوي اىَشخشمت) وأمسُذ اىُْخشَل (فٍ الأشخاص الأطحاء فقؾ) اسخدابتً ىيَىسَْدا ٍقاسّت ب

ـعقاس "Praziquantel"بعذ

11

َىٍـًا

فٍ

اىىسً اىحبُبٍ، سبَا بسبب اىخأثُش عيً إّخاج وسطاء اىخلاَا اىحبُبُت.

وكان الانخفضات الناجم عن المورينجا ملحوظة إحصائياً مقارنة بعقار "Praziquantel" بعد 14 يوما. وقد تم تسجيل

انخفض ملحوظة إحصائياً في وسطاء الخلايا المحبة مثل فوقي أكسي الهيدروجين (في كل من الأشخاص الأصحاء والمرضى المصابين بالدواء المشترك) وأكسي النتريك (في الأشخاص الأصحاء فقط) استجابةً للمورينجا مقارنة بعقار "Praziquantel" بعد 14 يوما، ومع ذلك، أظهر عامل نخر الورم-ألفا زيادة ملحوظة إحصائياً (فقط بعد 14 يوما) في الورم الحبيبي المعالج بعقار "Praziquantel" أو مستخلص المورينجا مقارنة بالقيم المقابلة للورم الحبيبي غير المعالج في الأفراد الأصحاء، والخاصة، أظهر مستخلص المورينجا نشاطاً وقائياً أعلى من عقار "Praziquantel" في الحد من الورم الحبيبي، ربما بسبب التأثير على إنتاج وسطاء الخلايا الحبيبية.