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In vitro anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol

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ABSTRACT

Objective: To study *in vitro* anti-hydatic and immunomodulatory effects of ginger and [6]-gingerol as an alternative therapy for Cystic echinococcosis. **Methods:** Effect of a commonly used herbal product and ginger (*Zingiber officinale*) towards protoscoleces (PSC) and cyst wall *in vitro* was studied. The effect of [6]-gingerol, and the pungent constituent of ginger, was also evaluated on PSC culture. Furthermore, the activity of both extracts in association with interferon-gamma (IFN- γ) on PSC co-cultured with mononuclear cells of hydatic patients was evaluated. The nitric oxide (NO) production was measured in each co-culture. **Results:** Ginger exhibited a concentration- and time-dependent cytotoxic effect against PSC and cyst wall. Interestingly, ginger was more effective than the [6]-gingerol. Moreover, additional parasitic effect between extracts and IFN- γ are also observed in co-cultures. Furthermore, both extracts attenuated the NO production elicited by this infection or by the IFN- γ . **Conclusions:** Ginger has an important anti-hydatic effect *in vitro*. This effect is amplified in the presence of IFN- γ . Moreover, this herbal product may protect against host's cell death by reducing the high levels of NO. Ginger may act, at least, through the [6]-gingerol. All our data suggest the promising use of ginger in the treatment of *Echinococcus granulosus* infection.

1. Introduction

Cystic echinococcosis is a zoonotic disease in humans and sheep. This infection is caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*). Man is being infected after ingestion of disseminated eggs in water, plants or on over the definitive hosts (dogs). Each egg, containing an oncosphere, gives a unilocular cyst especially in the liver and lungs. Surrounded by a thin wall: cyst

wall (CW), the cyst is filled with fluid containing brood capsules and protoscoleces (PSC).

The development of hydatic cyst for a long period induces many complications like organ malfunction and can lead to death[1]. The preferred therapy is cyst removal by surgery. However, the protoscoleces dissemination can occur during surgery leading to a high frequency of relapse. Chemotherapy with benzimidazole carbamate derivatives, amphotericin B and praziquantel is also used. This is the only option in many inoperable cases like cyst in brain or multiple cysts and in the immune-depressed host. Nevertheless, these drugs are not efficient in some cases and leads to liver toxicity and other side effects[2]. All these data prompted us to find new drugs for therapy.

The natural products are attracting attention as new therapeutic drugs against a number of diseases. Among their effects we found antiparasitic and antioxidant activities[3]. One of commonly used herbal product in traditional medicine all over the world is ginger (*Zingiber officinale*)[4,5]. There are few studies about the direct effect

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of this rhizome or other natural products against the helminthes. Some of these studies are related to *Echinococcus granulosus* and *Echinococcus multilocularis* using ginseng derivatives or other herbs or natural products[6]. However, as far as we know, there are no studies about the effect of ginger on *Echinococcus granulosus* metacestode in culture or coculture with mononuclear cells (PBMC) of hydatid patients. In this sense, we assessed here the effect of crude aqueous extract of dried ginger on *Echinococcus granulosus* CW and PSC in culture. Furthermore, we evaluated the activity of this extract in association with IFN- γ on PSC viability and nitric oxide (NO) production in coculture and culture performed with PBMC of hydatid patients and healthy donors. The effects of ginger were also compared with those of [6]-gingerol, one of the active components of ginger.

2. Materials and methods

2.1. Ginger and [6]-gingerol

The dried rhizome of *Zingiber officinale* Roscoe (family Zingiberaceae), was procured from a local market in Algiers, Algeria and then has been identified and authenticated by a botanist. The bioactive compound [6]-gingerol was obtained from Calbiochem (San Diego, CA).

2.2. Preparation of ginger crude aqueous extract and [6]-gingerol

The crude aqueous extract was prepared by dissolving 1mg of the dried extract of ginger in 1ml of RPMI-1640 medium supplemented with 5% of heat-inactivated FCS (fetal calf serum, sigma) and antibiotics. Tubes were then mixed and centrifuged. The aqueous extract was then filtered through 0.22 μm (Millipore) and stored at -20 $^{\circ}\text{C}$ until use.

The [6]-gingerol was diluted from the stock solution (dissolved in DMSO) with the culture medium (RPMI 1640) at the maximum final concentration of DMSO at 0.1%.

2.3. Patients

The peripheral blood samples were obtained from twenty Algerian patients carrying hydatid cyst in lungs ($n=20$) at Department of Surgery, Mustapha Bacha Hospital, Algiers, Algeria. The blood samples were obtained before (no more than 1 week) and after (24-72) h surgery. All patients having other infection or inflammatory chronic diseases were excluded from this study. Blood samples from healthy donors ($n=10$, from the same region of Algeria) were included in this study as healthy controls. All samples were used

immediately after collection for PBMC preparation and culture.

All participants (patients and healthy donors) gave informed consent for this study, which was in accordance with the guidelines of the ethic committee of the Algerian agency of research and development in health (ATRSS). All procedures were performed in accordance with Helsinki declaration of 2008.

2.4. Preparation of PSC and CW

The hydatid cysts ($n=5$) were obtained after surgery in lungs and livers at Mustapha Bacha hospital, Algiers, Algeria. Cysts were washed several times with sterile PBS, pH 7.4 supplemented with antibiotics.

CW was prepared as previously described[7]. Briefly, cyst wall, obtained after hydatid fluid removal, was washed several times with sterile PBS. This membrane was then cut into small pieces (1 cm \times 1 cm) and washed again. CW was finally maintained in RPMI-1640 medium supplemented with 5 % of heat-inactivated FCS and antibiotics until culture.

PSC were prepared as previously described[8,9]. Briefly, free PSC were obtained by centrifugation of hydatid fluid followed by washing in sterile PBS. The viability of PSC was determined prior the cultures by microscopic examination using 0.1% eosin as a vital stain. While viable PSC still unstained, dead PSC become stained. The appropriate percentage of viability was 95% or more. PSC were then maintained in RPMI-1640 medium supplemented with 5% of heat-inactivated FCS until culture and coculture.

2.5. CW and PSC culture and treatment

Two pieces of CW and 2×10^3 viable PSC were placed in each well of 24-well plastic trays. They were cultured in RPMI1640 medium supplemented with antibiotics and 10% heat-inactivated FCS and treated with different concentrations of ginger (100 $\mu\text{g}/\text{mL}$ for CW and 1, 10 and 100 $\mu\text{g}/\text{mL}$ for PSC). PSC cultures were also treated with [6]-gingerol (100 $\mu\text{g}/\text{mL}$). The untreated cultures were used as a negative control. PSC treated with 0.1% DMSO vehicle without [6]-gingerol were also considered as a control. Cultures were then incubated at 37 $^{\circ}\text{C}$ in a humid atmosphere and 5% CO_2 for 72 h for CW and 24 h and 48 h for PSC.

Cultures were observed under microscope before and after incubation. Percentages of viable proto scoleces were determined by microscopic examination using 0.1% eosin as a vital stain.

2.6. PBMC culture and coculture with PSC

PBMC were prepared from blood of hydatid patients and healthy donors as previously described[8]. Briefly, PBMC isolated by density gradient were washed three times with sterile PBS. Cell viability

was checked using 0.2% trypan blue dye. The appropriate percent of viable cells was always > 98%. Viable of 10^6 cells/mL were then resuspended in RPMI-1640 medium supplemented with antibiotics and 10% of FCS.

Cells were immediately cocultured with PSC and stimulated with 100 $\mu\text{g/mL}$ of ginger or [6]-gingerol alone or with IFN- γ (100 U/mL). The unstimulated cocultures were used as negative control. The cocultures treated with 0.1% DMSO vehicle without [6]-gingerol were also considered as a control. Cells were also stimulated with ginger extract (1, 10 and 100 $\mu\text{g/mL}$) and [6]-gingerol (100 $\mu\text{g/mL}$).

The effects of ginger and [6]-gingerol on nitrites production was demonstrated using L-NMMA (0.5 mM), (Sigma) and human recombinant IFN- γ (100 U/mL)[8] (HuIFN- γ is a gift from Dr. J. Wietzerbin, INSERM U365- 'Interférons et cytokines'- Institut Curie, INSERM U932- 'Immunité et cancer'- Institut Curie, Paris, France).

Cocultures and cultures were then incubated at 37 °C in humid atmosphere and 5% CO₂. After 20 h, percentages of viable proto scoleces were determined in coculture as described previously in section 2.4. Moreover, nitrites production was evaluated in coculture and culture supernatants.

2.7. Nitrites measurement

Nitric oxide production was evaluated by measuring nitrites concentration (NO₂⁻) in each supernatant by Griess method as previously described[10]. Briefly, 100 μL of supernatant were incubated for 5 min with 0.05% naphthylamine dihydrochloride and 0.5% sulfanilamide prepared in 5% HCl. The OD was measured by spectrophotometer at 543 nm. The data were expressed as μM of nitrites per 10^6 cells.

2.8. Statistical analysis

The results obtained were expressed as mean \pm SD. Data analysis was performed using the Minitab 16. The comparison between different groups was performed using Student's *t*-test. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Cytotoxic effect of ginger on cyst wall

The effect of ginger on CW was examined *in vitro*. In this sense, morphological change in CW was evaluated by microscopic examination in the absence and presence of crude aqueous extract of dried ginger (100 $\mu\text{g/mL}$) for 72 h.

Our results showed that ginger effect correlated with exposure

times. In fact, between 12 to 24 h, the germinal layer (GL) becomes darker and small areas of degeneration are observed after 36 h of exposure. After 48 h, GL loses completely its integrity followed by the laminated layer (LL). CW loses its integrity after 60 to 72 h (Figure 1).

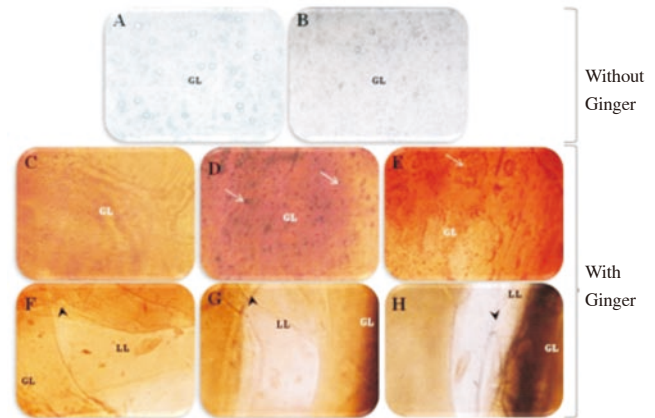


Figure 1. Progressive morphological alterations of CW after treatment with 100 $\mu\text{g/mL}$ of ginger for 72 h showing by light microscopy.

A: CW culture at $t=0$. B: CW culture after 72 h without ginger. C, D, E, F, G and H: CW culture with ginger (100 $\mu\text{g/mL}$) after 12 h, 24 h, 36 h, 48 h, 60 h and 72 h, respectively. Discontinued arrow: Altered germinal layer (GL); Arrow: degeneration of GL. Arrowhead: loss of layers in the laminated layer (LL). (Ob \times 05).

3.2. Cytotoxic effect of ginger and [6]-gingerol on protoscoleces

In the PSC culture, our results show that ginger exhibited a dose-dependent cytotoxic effect on PSC (Figure 2).

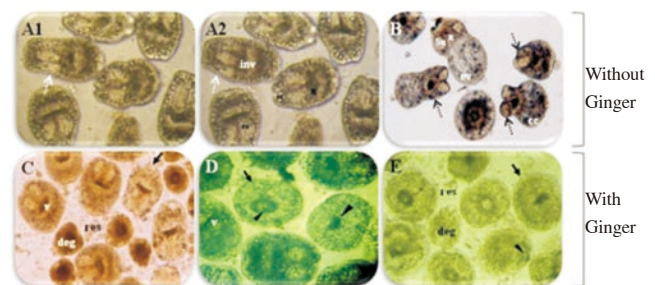


Figure 2. Protoscolicidal effect of ginger showing by light microscopy.

PSC were cultured and treated with ginger for 24 h, at 37 °C, in 5% CO₂. A: PSC culture at $t=0$ (97% of viability). B: PSC culture after 24 h. C, D and E: PSC culture after 24 h with Ginger at 1, 10 and 100 $\mu\text{g/mL}$, respectively. cc: calcareous corpuscles; deg: degeneration; ev: evaginated PSC; H: hooks; inv: invaginated PSC; R: rostellum; res: PSC residues; su: suckers; v: viable PSC. Arrow: Altered tegument; Arrowhead: loss of hooks. Black discontinued arrow: evagination and invagination of the head; White discontinued arrow: tegument shift. (\times 108).

Remarkably, ginger induced a strong morphological change with loss of the membrane integrity, the order of hooks, and the homogeneity of the calcareous corpuscles. Moreover, contractile movements of membrane and suckers disappear gradually followed by complete PSC degeneration and death. In contrast, no appreciable change was detected in the PSC viability of untreated culture (Figure 3).

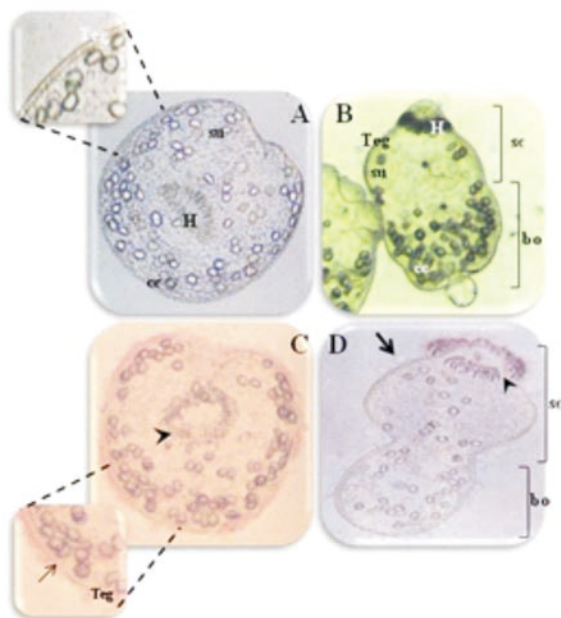


Figure 3. Morphological alterations of protoscoleces after treatment with ginger showing by light microscopy.

A and B: viable PSC, C and D: dead PSC. A and C: invaginated PSC. B and D: evaginated PSC. bo: body; cc: calcareous corpuscles; H: hooks; R: rostellum; sc: scolex; su: suckers. Teg: tegument. Arrow: Altered tegument. Arrowhead: loss of hooks. ($\times 269$).

To confirm these observations, we also evaluated the PSC viability using 0.1% eosin as a vital stain (Figure 3). The figure 4a shows the percentage of viable PSC after ginger exposure for 24 h and 48 h of culture. We notice that scolicidal effect of ginger is strongly related to concentration and exposure times. Interestingly, concentrations of 1 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ of ginger kill respectively 51.80% and 89.72% ($P < 0.0001$) of parasite after 24 h of culture (Figure 4). Ginger exhibited also a time-dependent protoscolocidal effect after 24h and 48h with respective maximum reduction of 89.72% and 100% ($P < 0.0001$) in PSC viability for 100 $\mu\text{g}/\text{mL}$ (Figure 4A).

In the presence of 100 $\mu\text{g}/\text{mL}$ [6]-gingerol, time-dependently reduced cell viability was also observed (Figure 4B). Interestingly, [6]-gingerol was less effective than ginger ($P < 0.0001$). For note, PSC treated with vehicle alone remained viable throughout the incubation period (24 and 48 h with respectively $P = 0.897$ and $P = 0.371$).

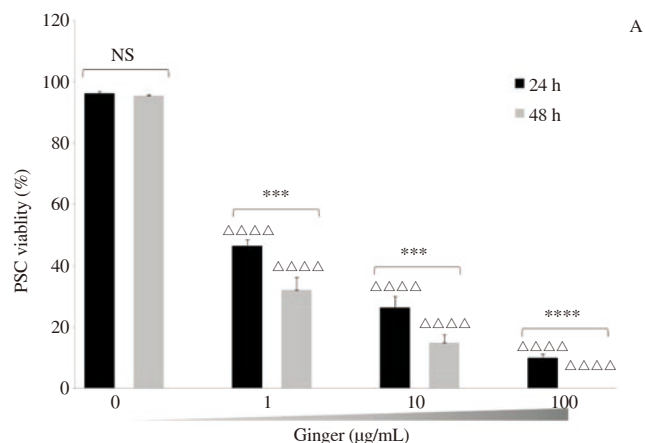


Figure 4. Killing of *E. granulosus* protoscoleces after exposure to ginger (A) and [6]-gingerol (B) for 24 h and 48 h of culture.

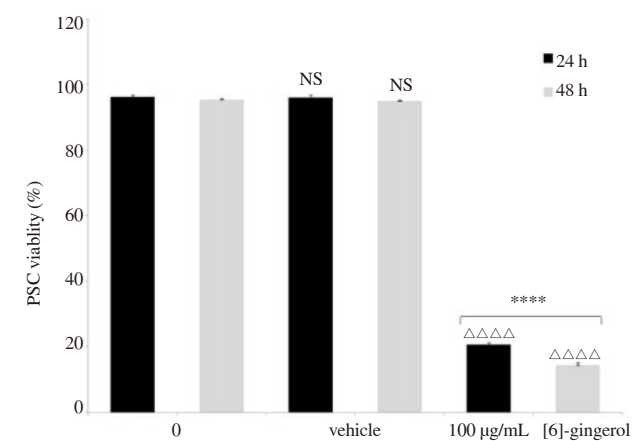


Figure 4. Killing of *E. granulosus* protoscoleces after exposure to ginger (A) and [6]-gingerol (B) for 24 h and 48 h of culture.

Each histogram represents the mean percentage of viable protoscoleces from five different experiments. Differences between different groups are indicated ($\Delta\Delta P < 0.01$, $\Delta\Delta\Delta P < 0.001$, $\Delta\Delta\Delta\Delta P < 0.0001$; NS: no significance). Triangles (Δ) and NS above single bars indicate differences with the negative control (untreated cocultures) for 24 h and 48 h. Asterisks above horizontal brackets indicate difference between 24 h and 48 h.

3.3. Scolicidal effect of ginger and [6]-gingerol in mononuclear cells– protoscoleces cocultures

In the second part of our study, we investigate the scolicidal effect of ginger and [6]-gingerol on PSC cocultured with mononuclear cells (PBMC) in presence or absence of $\text{IFN-}\gamma$. For note, we have previously highlighted the role of this cytokine in host defense and parasite killing[8].

Our results show that 100 $\mu\text{g}/\text{mL}$ of ginger and [6]-gingerol reduces protoscoleces viability *in vitro* from $61.94\% \pm 2.72\%$ to respectively $39.82\% \pm 0.57\%$ ($P < 0.0001$) and $46.63\% \pm 1.67\%$ ($P < 0.0001$) (for PBMC from patients before surgery). Interestingly, synergistic scolicidal effects between these two extracts and $\text{IFN-}\gamma$ are also observed in PSC-PBMC co-cultures. In fact, incubation with extracts and $\text{IFN-}\gamma$ induced the most damage to the protoscoleces as indicated by viability loss in comparison with co-cultures treated with $\text{IFN-}\gamma$ or extracts alone (Figure 5A).

The same results are reported for co-cultures performed with PBMC from patients before and after surgery and healthy donors.

However, incubation of PBMC from healthy donors with ginger or [6]-gingerol induced less damage as manifested by the high levels of PSC viability.

3.4. Inhibitory effect of ginger and [6]-gingerol on NO production by mononuclear cells

In our previous study, we have reported that the scolocidal effect of IFN- γ is associated with an increase in NO production[8]. On the basis of these results, we investigated here if ginger and [6]-gingerol act by the same mechanism. During our experiment, loss of viability in the presence of ginger and [6]-gingerol is associated with a decrease in nitrites levels. This effect contrasts with the increase observed in the presence of IFN- γ [8]. Interestingly, ginger and [6]-gingerol also attenuate the increase in NO production elicited by IFN- γ (Figure 5B).

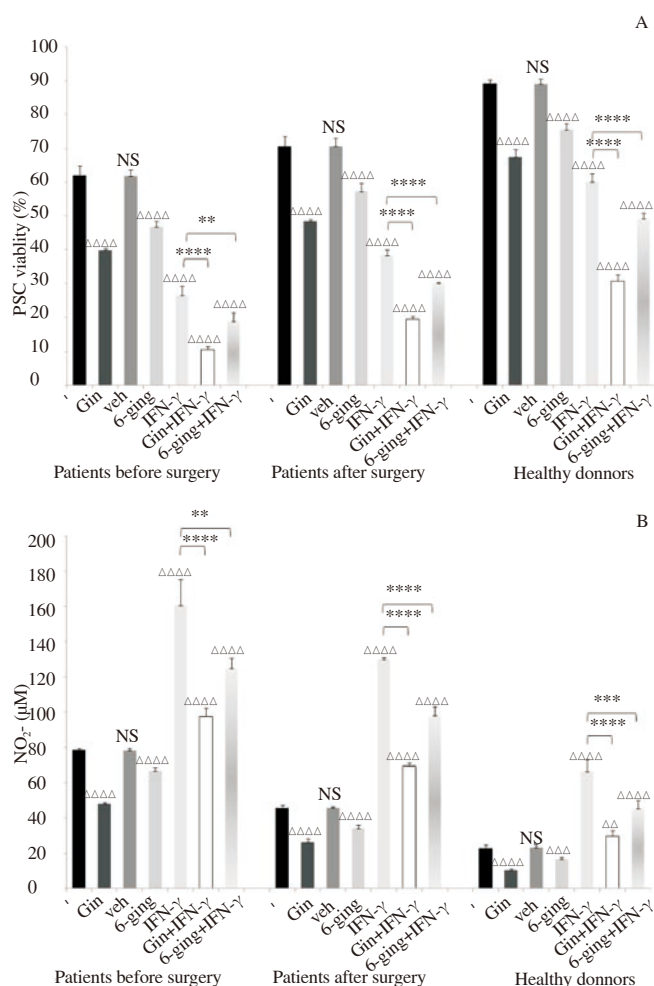


Figure 5. Killing of *E. granulosus* protoscoleces. (A) in coculture with mononuclear cells (PBMC) treated with ginger or [6]-gingerol (100 µg/mL) alone or with human recombinant IFN- γ (100 U/mL) for 20 h. Correlation with nitrites production (B). Each histogram represents the mean percentage of viable protoscoleces from different experiments ($n=20$ and $n=10$ for patients and controls respectively). Differences between different groups are indicated ($\Delta\Delta P<0.01$, $\Delta\Delta\Delta P<0.001$, $\Delta\Delta\Delta\Delta P<0.0001$; NS: no significance). Triangles (Δ) and NS above single bars indicate differences with the negative control (untreated cocultures). Asterisks above horizontal brackets indicate difference between IFN- γ alone or with ginger or [6]-gingerol. Gin: ginger, veh: vehicle, 6-ging: [6]-gingerol.

The same results are also observed for culture with a dose-dependent inhibitory effect of ginger on nitrites production. However, the inhibition observed using 1 µg/mL of ginger is statistically not significant for all cultures (Figure 6).

To confirm the effects of ginger on NO production, L-NMMA (NO Synthase inhibitor) and IFN- γ are used in PBMC culture. While L-NMMA decreases nitrites production, IFN- γ has an opposite effect. Remarkably, ginger and [6]-gingerol decrease the levels on nitrites when used alone or with IFN- γ (Figure 6).

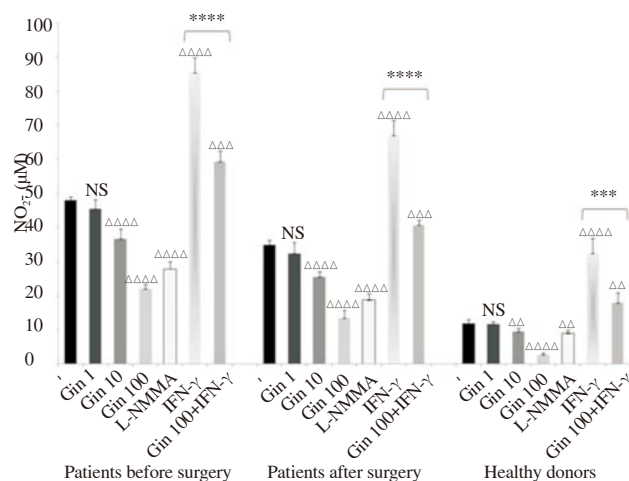


Figure 6. Nitric oxide production in PBMC cultures treated with ginger (1, 10 and 100 µg/mL) for 20 h. Human recombinant IFN- γ (100 U/mL) and L-NMMA (0.5 mM) were also used to confirm the effect of ginger on NO production. Each histogram represents the mean nitrite levels from different experiments ($n=20$ and $n=10$ for patients and controls respectively). Differences between different groups are indicated ($\Delta\Delta P<0.01$, $\Delta\Delta\Delta P<0.001$, $\Delta\Delta\Delta\Delta P<0.0001$; NS: no significance). Triangles (Δ) and NS above single bars indicate differences with the negative control (untreated cultures). Asterisks above horizontal brackets indicate difference between IFN- γ alone or with ginger. Gin 1, 10 and 100 (concentrations of ginger in µg/mL).

4. Discussion

Nowadays, several studies have been done to find new natural compounds that treat parasitic diseases. However, hydatidosis is among the most neglected parasitic infections, and development of new therapy and drugs received very little attention. In this study, the role of ginger (*Zingiber officinale*) against *Echinococcus granulosus* larvae has been investigated. Interestingly, we found that ginger exhibited dose- and time-dependent larvicidal effect against *Echinococcus granulosus* metacestode and protoscoleces.

Our results are in the same line with previous studies showing that ginger has an anthelmintic activity against some parasitic species

both *in vitro* and *in vivo*. In their report, Iqbal et al. reported that methanol extracts of ginger killed all the *Haemonchus contortus* worms within two hours post exposure[11]. These findings agree with other works revealing that ginger extract affects the viability of *Schistosoma mansoni* adult pairs[12] and *Ascaridia galli*[13], both *in vitro* and *in vivo* in mice.

Our present results are also in harmony with previous *in vivo* studies conducted by Datta and Sukul showing a significant anthelmintic efficacy of ginger alcoholic extract in dogs, naturally infected with *Dirofilaria immitis*[14]. Later, Iqbal et al. demonstrated that crude powder and crude aqueous extract of dried ginger exhibit anthelmintic activity in sheep naturally infected with different species of gastrointestinal nematodes including: *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Oesophagostomum columbianum*, *Strongyloides papillosus* and *Trichuris ovis*[15]. More recently, Mostafa et al. and Aly and Mantawy reported that the crude aqueous extract of ginger exhibited antischistosomal activity in mice as showed by the reduction in worm and egg burden in the hepatic tissue and faeces of infected mice[16,17].

Our data show that *in vitro* treatment with ginger extract induces significant alterations in cyst wall and PSC leading to parasite death. *In vivo*, such damage to the cyst wall destroys the defense system of the parasite. Thus, the PSC could easily be attacked by the protective host immune responses. We have found that ginger induces a loss of spontaneous movement of PSC and damage to the hooks in a time- and dose-dependent manner. These effects cause loss of PSC ability to adhere to the host tissues. Moreover, the observed tegumental alterations could be a committed mechanism by ginger to destroy the parasite. Our observations are more or less similar to the alterations observed after treatment with genistein as observed by Naguleswaran et al.[6]. The alterations in the surface architecture of *E. granulosus* metacystode were used by several investigators as a deleterious indicator for the evaluation of anti-hydatic activities of different drugs. Moreover, the reduction observed in PSC viability was considered by several authors as a strong evidence of the efficiency of anti-hydatic drugs[6,18,19].

Interestingly, ginger acts synergistically with IFN- γ to kill PSC. However, the immunomodulatory effect of ginger on host immune response has not been evaluated during *E. granulosus* infection. Based on our previous work showing a significant induction of nitric oxide (NO) production after *in vitro* treatment with IFN- γ [8], we wished to determine the effect of ginger on NO production. This present work demonstrates, for the first time, that ginger reduces NO production by host cells in response to *E. granulosus* infection *in vitro*.

It is known that pro-inflammatory cytokines (likes IFN- γ and TNF- α) simulate iNOS expression and activity in macrophages[20,21]. Thus, when the production of these cytokines is inhibited, the generation of NO is likely to be reduced. Several studies have shown that ginger inhibited the expression of genes encoding pro-inflammatory cytokines by various cells[22,23]. These findings are consistent with *in vivo* study showing that ginger extract inhibit the production of NO, TNF- α and IL-12 in mice infected with *Shistosoma mansoni*[17].

Although our previous studies have demonstrated that IFN- γ and iNOS pathways are involved in protective immunity against *E. granulosus*[8,9,24]. Our present study indicates that IFN- γ kills the parasite even although NO production was reduced by ginger. Thus, our data suggest the commitment of another mechanism by which IFN- γ kills the protoscoleces *in vitro*.

In summary, our study indicates for the first time that ginger possesses cestocidal activity against *E. granulosus*. However; further studies are required to clarify the mechanisms involved in this effect. Sanderson et al. and Iqbal et al. reported that ginger kills *Shistosoma mansoni* and gastrointestinal nematode *in vivo* through its binding to parasite beta-tubulin and inhibition of glucose uptake[12,15]. Another study attributed the significant reduction in tissue egg after ginger treatment to the disturbance in worm fecundity[25]. Nevertheless, the bioactivity of ginger may be due to its constituents. In fact, phytochemical reports show that ginger contains over 400 different compounds. The major constituents are zingerone, paradol, gingerols and shogaols. According to several literature reviews, [6]-gingerol is the most abundant active constituent of whole ginger. It has various pharmacological and microbiological properties, including anti-parasitic, anti-tumorigenic, anti-inflammatory and anti-oxidant activities. Interestingly, in many study, researchers used the [6]-gingerol as a marker substance of ginger[4,26,27].

These data prompted us to investigate the effect of [6]-gingerol on *E. granulosus* PSC and NO production. In this study, we have found that [6]-gingerol significantly kills PSC *in vitro* both in culture and coculture. Moreover, [6]-gingerol reduces NO production by host mononuclear cells. These results confirm our hypothesis about the implication of [6]-gingerol in the cytotoxic and regulatory effects of ginger respectively on PSC and NO production. Various studies reported that [6]-gingerol possess a variety of pharmacological effects like anthelmintic and anti-inflammatory activities[4,26,27]. Our results are in the line with those reported by Goto et al. indicating a significant anti-nematodal activity of [6]-gingerol against *Anisakis* larvae *in vitro*[28]. In the same way, Adewunmi et al. demonstrated that gingerol completely abolished the infectivity of *Shistosoma mansoni* miracidia and cercariae in *Biomphalaria glabrata* snail

and mice, respectively[29]. [6]-gingerol was also found to kill *Angiostrongylus cantonensis* larvae or to reduce their spontaneous movements[25]. Our findings are in agreement with a recent *in vivo* study in mice assigning a significant anthelmintic activity to [6]-gingerol against *Schistosoma mansoni*[17].

According to Lee *et al.*, [6]-gingerol reduces iNOS and TNF-expression by blocking NF- κ B and PKC signaling pathway in lipopolysaccharide-stimulated mouse macrophages[30]. It is well known that PKC activates NF- κ B[31] which in turn regulates at the transcriptional level the iNOS expression[32]. Results reported by another team suggested that [6]-gingerol inhibits NO production and iNOS expression in activated J774.1 mouse macrophages[33]. In the same way, Tripathi *et al.* also demonstrated that [6]-gingerol inhibits the production of TNF- α , IL-1 β , and IL-12 in murine peritoneal macrophages[34].

It is well known that inflammatory mediators (like NO) play a crucial role in chronic inflammation, oxidative stress, and fibrosis that affect tissue architecture and impair organ function[35,36]. Thus, we can conclude here, that ginger extract and [6]-gingerol could minimize the deleterious effects of this parasite on the vital functions of infected organs[1] through its immunomodulatory effect on the iNOS pathway. This active component of ginger may have a direct effect on the vitality and viability of PSC and NO production. However, ginger had a higher efficacy than that of [6]-gingerol. Another component of ginger such as the [6]-shogaol may act in synergy with [6]-gingerol[17,25]. Further studies to explore the effect of these components on *E. granulosus* metacestode appear to be useful.

This study is the first to determine the larvicidal activity of ginger and [6]-gingerol on *E. granulosus* metacestode and protoscolexes. Interestingly, we found that ginger and its main component have also an immunomodulatory effect that did not adversely affect their larvicidal activity. Further investigations for the mechanisms underlying ginger effects are necessary. Above results might contribute to the use of ginger as new alternative therapy. However, *in vivo* studies on an animal model should be done before using ginger or ginger derivatives for treatment of human hydatidosis.

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Conflict of interest statement

We declare that we have no conflict of interest.

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