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Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf. fruit extract

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SHORT COMMUNICATION

Chemical composition, protoscolicidal effects and acute toxicity of *Pistacia atlantica* Desf. fruit extract

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ABSTRACT

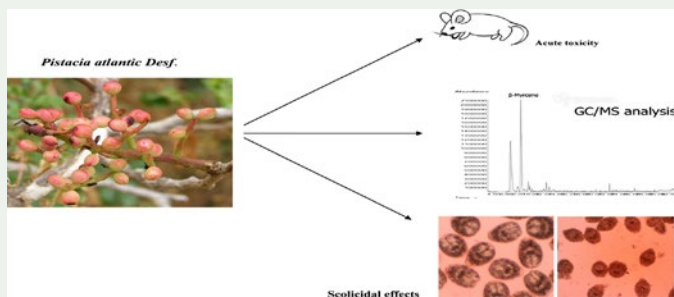
The present study was designed to evaluate the chemical composition and scolicidal effects of *Pistacia atlantica* Desf. extract against protoscoleces of hydatid cysts and its acute toxicity in mice model. Various concentrations of the methanolic extract (5–50 mg/mL) were used for 10–60 min. Viability of protoscoleces was confirmed using eosin exclusion test (0.1%). Acute toxicity was also determined in mice model. The main components were β -myrcene (41.4%), α -pinene (32.48%) and limonene (4.66%). Findings demonstrated that *P. atlantica* extract at the concentrations of 25 and 50 mg/mL after 20 and 10 min of exposure killed 100% protoscoleces. The LD₅₀ of the intraperitoneal injection of the *P. atlantica* methanolic extract was 2.43 g/kg and the maximum non-fatal dose was 1.66 g/kg. Obtained results showed the potential of *P. atlantica* extract as a natural source with no significant toxicity for the production of new scolicidal agent to use in hydatid cyst surgery.


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1. Introduction

Hydatid cyst or cystic echinococcosis (CE), a widely zoonotic disease caused by the infection with metacestodes (larval stage) of the tapeworm *Echinococcus granulosus*, remains a major public health concern on several continents (WHO 1996). For many years, surgery has been considered the only treatment available for CE because of the potential radical removal of the parasite (Brunetti et al. 2010). Nowadays, to reduce the risk of intraoperative spillage of the cyst contents (protoscoleces) and subsequent recurrence of CE and secondary infection during surgery, the use of effective scolicidal agents are necessary. Current protoscolicidal agents are associated with serious side effects such as sclerosing cholangitis (biliary tract fibrosis), liver necrosis and methaemoglobinaemia (Mahmoudvand, Saedi Dezaki, et al. 2014). Therefore, the development of new scolicidal agents especially from natural resources with low side effects and more efficacies is urgent for surgeons. *Pistacia atlantica* Desf. commonly grows in the Mediterranean and Middle East countries for the last 3000 years. The different parts of *P. atlantica* have been used widely as traditional medicine for the treatment and prevention of different disease conditions such as gastrointestinal, respiratory, skin, renal and infectious diseases (Bozorgi et al. 2013). Moreover, previous studies have proven anti-inflammatory, antioxidant, antitumor, antiasthmatic and antimicrobial properties of this plant (Bozorgi et al. 2013). The present study was designed to evaluate the chemical composition and scolicidal effects of *P. atlantica* fruit extract against protoscoleces of hydatid cysts and also its acute toxicity in mice model.

2. Results and discussion

Table S1 indicates the results obtained by GC/MS analysis of *P. atlantica* extract. Twenty-six compounds were identified, representing 98.7% of the total extract. The main components of the fruit extract were β -myrcene (41.4%), α -pinene (32.48%) and limonene (4.66%). Obtained results demonstrated that *P. atlantica* extract at the concentration of 50 mg/mL after 10 min of exposure killed 100% protoscoleces. Similarly, the mean of mortality rate of protoscoleces after 20 min of exposure to the concentration of 25 mg/mL was 100%. *P. atlantica* extract at the concentration of 10 mg/mL killed 12.6, 38.3, 75 and 100% of the protoscoleces and at the concentration of 5 mg/mL also killed 8.3, 22.3, 57.3, and 77.6% of the protoscoleces after 10, 20, 30 and 60 min of incubation, respectively. The mortality rate of protoscoleces in the negative and positive controls was 4.3% after 30 min and 100% after 5 min exposure, respectively. Results also showed that *P. atlantica* extract at all concentrations had significant ($p < 0.05$) scolicidal effects compared with the control group. The LD₅₀ of the intraperitoneal injection of the *P. atlantica* methanolic extract was 2.43 g/kg and the maximum non-fatal dose was 1.66 g/kg.

Nowadays, an ideal scolicidal agent described by its potency at lower concentrations, high efficacy in a shorter time of exposure, lower toxicity, higher availability and ability for rapid preparation (WHO 1996). We found that *P. atlantica* extract at the concentrations of 50 and 25 mg/mL after 10 and 20 min of exposure killed 100% protoscoleces. At present, the scolicidal activity of several chemical and natural products including hypertonic saline, silver nitrate, mannitol, cetrimide, ethyl alcohol (95%), H₂O₂ and 10% povidone iodine, chlorhexidine gluconate, selenium nanoparticles, honey and some plant extracts have been demonstrated (Mahmoudvand, Fasihi Harandi, et al. 2014). Our findings revealed that *P. atlantica* extract had potent scolicidal activity which is comparable with the existing scolicidal agents

such as 20% hypertonic saline (15 min), 20% silver nitrate (20 min), 0.5–1% cetrimide (10 min), H₂O₂ 3% (15 min) and 95% ethyl alcohol (15 min). We found that the main components of *P. atlantica* extract are the monoterpene hydrocarbons such as β -myrcene (41.4%), α -pinene (32.48%) and limonene. However, some factors such as geographical origin of the variety and harvest season could affect on the chemical composition and functional activity of plants (Yesil Celiktas et al. 2007). Individual activities of these compounds have been previously demonstrated (Cowan 1999). In addition, in various studies, potent antimicrobial activities of these compounds such as α -pinene, limonene, terpinene and linalool have been reported (Sokovic & van Griensven 2006; Rodrigues et al. 2015). Thus, the present phytoconstituents in this plant could be responsible for their scolicidal effects though their exact mode of action is poorly understood. Considering antimicrobial mechanism of some terpenoid compounds such as monoterpenes, some studies suggested that the antimicrobial activity is related to the ability of terpenes to affect not only permeability but also other functions of cell membranes, these compounds might cross the cell membranes, thus penetrating into the interior of the cell and interacting with critical intracellular sites (Cristani et al. 2007). In regard to toxicity effects of *P. atlantica*, the LD₅₀ of the intraperitoneal injection of the *P. atlantica* methanolic extract was 2.43 g/kg and the maximum non-fatal dose was 1.66 g/kg. According to a toxicity classification, the methanolic extract of *P. atlantica* had no significant toxicity against male NMRI mice (Loomis 1968).

3. Conclusion

Our results demonstrated that *P. atlantica* fruit extract might be a natural source for the production of new scolicidal agents to reduce the risk of protoscolices spillage during hydatid cyst surgery. However, further studies will be needed to confirm these results by checking the extract in a clinical setting as a new scolicidal agent.

Disclosure statement

No potential conflict of interest was reported by the authors.

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