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Short Communication

Efficacy of *Pistacia khinjuk* Fruits on Viability of Hydatid Cyst Protoscoleces and Its Acute Toxicity in Mice Model

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Abstract

Background: This investigation aimed to evaluate the *in vitro* scolicidal effects of *Pistacia khinjuk* methanolic extract against protoscoleces of hydatid cysts and its acute toxicity in mice NMRI model.

Methods: Protoscoleces were aseptically extracted from sheep livers having hydatid cysts. Various concentrations of the essential oil (12.5- 100 mg/mL) were used for 10 to 60 min. Viability of protoscoleces was confirmed using eosin exclusion test (0.1% eosin staining). Twenty-four male NMRI mice were used to assess the acute toxicity of *P. khinjuk*.

Results: *P. khinjuk* extract at the concentrations of 100 mg/mL after 10 min of exposure killed 100% of protoscoleces. Similarly, the mean of mortality rate of protoscoleces after 20 min of exposure to the concentration of 50 mg/mL was 100%. The LD_{50} of the intraperitoneal injection of the *P. khinjuk* methanolic extract was 2.8 g/kg and the maximum non-fatal dose was 1.7 g/kg.

Conclusion: The findings demonstrated effective scolicidal effects of *P. khinjuk* extract with no considerable toxicity that might be a natural source for the producing of new scolicidal agent.

Introduction

Human cystic echinococcosis (hydatid cyst), triggered by the cyst-like tapeworm of *Echinococcus granulosus*, is one of the most important community health problem on several continents which is recurring in some countries (1, 2). During last decades, surgery was the merely method for treatment of hydatid cyst (3). However,

through surgery to diminish the threat of intraoperative release of the cyst substances (protoscoleces) and consequently reappearance of CE and secondary infection, witnessed in closely 10% of the postoperative patients, the consumption of effective scolicidal drugs are compulsory (4, 5). Right now, there are numerous scolicidal agents comprising hypertonic saline, silver-nitrate, cetrimide, and ethanol which have been applied for elimination of the cyst substances. However, these scolicidal agents are accompanying with a number of side effects for instance sclerosing colangitis (6, 7). Therefore, vast efforts have been performed to gain novel scolicidal agents specifically from natural resources with little side effects and more abilities for hydatid cyst surgery.

Now, herbal medicines and purified natural crops deliver a rich source for new antimicrobial agent development. One of these interesting plants is *Pistacia khinjuk* Stocks from family of Anacardiaceae, which generally cultivates in the Mediterranean and Middle East countries from last centuries (8, 9). In traditional remedy, the various parts of the plant for example resin, leaf, bark, fruit and aerial parts have been broadly applied for management and prevention of many illness conditions including stomach discomfort, nausea, vomiting, and motion sickness. Moreover, in modern medicine, previous studies have demonstrated *P. khinjuk* as having anti-inflammatory, antioxidant, antitumor, antiasthmatic and antimicrobial effects (10). To the best of our knowledge, no study has been conducted on the scolicidal activity of this plant.

Therefore, the present study was designed to investigate the in vitro scolicidal effects of *P. khinjuk* extract against protoscoleces of hydatid cysts and its acute toxicity in mice model.

Materials and Methods

Collection of plant materials

P. khinjuk fruits were collected from rural regions of Kerman Province, south east of Iran, in September 2013. They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Science, Iran (KF 1135).

Preparation of extract

Air-dried plant materials (100 g) were separately extracted by percolation method with 80 % methanol successively for 72 h. in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20°C, until use (11).

Scolicidal effect on protoscoleces

Scolicidal effects of different concentrations of the *P. khinjuk* extract (12.5 – 100 mg/mL) against hydatid cysts protoscoleces were assessed as stated by the technique designated elsewhere (13). The protoscoleces of hydatid cysts were acquired from the liver of the naturally infected sheep and goats slaughtered at Kerman abattoir, southeastern Iran. Initially, using a 50 mL syringe hydatid liquid was extracted and aseptically moved into a flask and was left for 30 min for protoscoleces to settle down. After collection of protoscoleces, they washed two times with PBS (pH 7.2) solution and the number of protoscoleces/mL was adjusted as 2×10^3 protoscoleces in 0.9% NaCl solution with as a minimum 90% viability proportion (12).

Five hundred µL of the protoscoleces (2×10^3/mL) solution was located in experiment tubes. Then 0.5 mL of different concentrations of the extract (dissolved in normal saline) was added to every one-examination tube. The substances of the tubes were quietly mixed and then were kept warm at 37 °C for 10, 20, 30, and 60 min. In the last part of each

incubation time, the superior phase was cautiously removed so as not to disturb the protoscoleces. Finally, 50 µL of 0.1% eosin stain (Sigma-Aldrich, St Louis, MO, USA) was added to the residual settled protoscoleces and mixed slightly. The upper quota of the solution was cast-off after 10 min of incubation. The lasting pellet of protoscoleces was then smeared on a glass slide, covered with a cover glass and examined under a light microscope. The proportions of dead protoscoleces were calculated by counting 300 protoscoleces. In the present investigation, normal saline and hypertonic saline 20% were also applied as negative and positive control group, respectively.

Viability test

The viability of hydatid cysts protoscoleces was determined using eosin omission examination (14). To do this, after contact to the 0.1% eosin solution (1 g of eosin powder in 1000 mL distilled water), live protoscoleces stayed neutral and exhibited characteristic muscular and flame cell activity; in contrast, dead protoscoleces immersed eosin and colored red.

Acute toxicity

Animals

Twenty-four male NMRI mice (6–8 weeks old) were obtained from the Animal Breeding Stock Facility of Razi Institute of Iran (Karaj, Iran). Animals were housed in a colony room with a 12:12 h light/ dark cycle at 21 ± 2°C and were handled according to standard protocols for the use of laboratory animals.

Ethical statement

The experimental procedures carried out in this study complied with the guidelines of the Kerman University of Medical Science (Kerman, Iran) for the care and use of laboratory animals (Permit No. 92/279).

Toxicity effects

To determine the acute toxicity, various doses of *P. khinjuk* extract (0.5-4 g/kg) were injected as intraperitoneally into groups of six mice. Normal saline as solvent of the extract was used as negative control group. The number of deaths was counted at 48 h after treatment. LD_{50} values were determined by the probit test SPSS software (15).

Statistical analysis

All the tests were performed in triplicate. Data analysis was carried out by using SPSS (version 17.0) (SPSS Inc., Chicago, IL, USA). Differences between test and control groups were analyzed by *t*-test. In addition, $P<0.05$ was considered statistically significant.

Results

Scolicidal effects

Table 1 indicated scolicidal effects of *P. khinjuk* methanolic extract at the various concentrations following different exposure times. *P. khinjuk* extract at the concentrations of 100 mg/mL after 10 min of contact killed 100% protoscoleces. In the same way, the mean of mortality level of protoscoleces after 20 min of exposure to the concentration of 50 mg/mL was 100%. Furthermore, lesser concentrations of *P. khinjuk* extract triggered a postponed protoscolicidal effects. These outcomes also confirmed that *P. khinjuk* extract at all of concentrations had significant ($P<0.05$) scolicidal effects compared with the negative control group.

Acute toxicity

The LD_{50} of the intraperitoneal injection of the *P. khinjuk* methanolic extract was 2.8 g/kg and the maximum non-fatal dose was 1.7 g/kg. No death was observed until the dose of 2 g/kg.

Table 1: Scolicidal effects of *P. khinjuk* extract against protoscoleces of hydatid cyst at various concentrations following various exposure times

Concentration (mg/mL)	Mean of mortality rate (%)	Exposure time (min)
	100	10
	100	20
100	100	30
	100	60
50	46.6	10
	100	20
	100	30
	100	60
25	18.6	10
	48.3	20
	85	30
	100	60
12.5	6.3	10
	22.3	20
	61.6	30
	89.6	60
Normal saline + Tween 20	1.3	10
	2.6	20
	4.3	30
	9.1	60
20% Hypertonic saline	100	10
	100	20
	100	30
	100	60

Discussion

This study investigated the scolicidal effects of *P. khinjuk* methanolic extract on an *in vitro* model and its acute toxicity in mice model. *P. khinjuk* extract at the concentrations of 100 and 50 mg/mL after 10 and 20 min of exposure killed 100% protoscoleces. Equally, the mean of mortality level of protoscoleces after 20 min of exposure to the concentration of 5 mg/mL was 100%.

Plant extracts and their pure components because of possessing low toxicity, low cost, high effectiveness, and high accessibility are responsible for infinite prospects for new drug discoveries due the unparalleled accessibility of chemical variety (15).

Previously a proper scolicidal agent was described by its effectiveness at lesser concentrations, high ability in a shorter period of contact, constancy in the existence of cystic liquid, scolicidal capability inside a cyst, lower harmfulness, higher obtainability, and facility for quick preparation (1). At the moment, the scolicidal effects of numerous chemical agents such as hypertonic saline, silver nitrate and mannitol, cetrimide, SeNPs, and a number of plant extracts including *Pistacia vera*, *Zataria multiflora*, *Nigella sativa*, *Berberis vulgaris*, *Myrtus comminus*, and *Pistacia atlantic*a have been demonstrated (16-20). Conversely, they are concomitant with a number of adverse effects and their ability is debatable. Our results verified that *P. khinjuk* extract had strong scolicidal activity which is as good as the standing

scolicidal agents such as 20% hypertonic saline (15 min), 20% silver nitrate (20 min), 0.5–1% cetrimide (10 minutes), H_2O_2 3% (15 minutes), and 95% ethyl alcohol (15 min). Accordingly, results of this study supported the knowledge that *P. khinjuk* extract may possibly be a natural resource for the creation of a new scolicidal agent for usage in hydatid cyst surgery.

Concerning phytochemical screening of *P. khinjuk* extract, Bozorgi et al., have described the attendance of terpenoids, flavonoids, and tannins in this plant (9). To this point, specific biological properties of these components have been validated (21). Hence, the phytoconstituents in *P. khinjuk* extract may perhaps be answerable for their scolicidal properties nevertheless, their precise manner of action is not clear. On the other hand, some terpenoids components for example monoterpens can drawn-out into pathogen and harm cell wall constructions (22, 23). Other investigators proposed that the antimicrobial activity of terpenoids components is correlated to capability of terpenes to affect further functions of cell membranes; for instance, they piercing into the cell and interrelating with vital intracellular locates (23, 24).

On the subject of toxicity, the LD_{50} of the intraperitoneal inoculation of the *P. khinjuk* methanolic extract was 2.8 g/kg and the maximum non-fatal dose was 1.7 g/kg. Based on a toxicity classification, the methanolic extract of *P. khinjuk* had no considerable harmfulness against male NMRI mice (25).

Conclusion

The findings demonstrated potent scolicidal effects of *P. khinjuk* extract with no significant toxicity that might be a natural source for the producing of new scolicidal agent.

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The author declares that there is no conflict of interests in this study.

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