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Original Article

Effect of *Taxus baccata L.* Extract *on* Hydatid Cyst Protoscolices *In vitro*

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Abstract

Hydatidosis is the most important global parasitic infectious disease both in humans and animals, which can lodge at different organs of the host, such as liver, lung (even heart), and brain which may lead to death. Surgery is the main method for the treatment of hydatidosis. In surgical therapy of hydatidosis, the use of sporicidal agents is very important since these agents inactivate live protoscolices and prevent recurrence of infection. Presently, numerous scolicidal chemical agents have been administrated to inactivate the hydatid cyst contents. Recently, there has been a high tendency among researchers to evaluate and present herbal plants as alternative option due to inexpensiveness, availability, low side effects, and toxicity. This study aimed to evaluate the scolicidal effect of hydro alcoholic *Taxus baccata L.* extract *in vitro* for the first time. The scolicidal activities of the extract were tested in concentrations of 50, 100, and 150 mg/ml following 10, 30, and 60 min of incubation, and the experiments were performed in triplicate. Viability of protoscolices was confirmed by 0.1% eosin vital staining. The data were analyzed in SAS software (version 9.4). The results showed that the hydroalcoholic extract of *Taxus baccata L.* at the concentration of 150 mg/ml led to killing 66.6% of protoscolices at 60 min. according to the results of this investigation, it is recommended to use this plant as a scolicidal plant. The findings of the present study showed that *Taxus baccata L.* had potent scolicidal effects. However, further studies are required to evaluate the efficacy of *Taxus baccata L. in vivo.*

Keywords: Hydatid cyst, In vitro, Scolicidal, *Taxus baccata L*

Effet de l'extrait de *Taxus baccata* L. sur les protoscolices de Kystes Hydatiques *In vitro*

Résumé: L'hydatidose est la maladie infectieuse parasitaire mondiale la plus importante chez les humains et les animaux, qui peut se loger dans différents organes de l'hôte, tels que le foie, les poumons (même le cœur) et le cerveau, entraînant parfois la mort. La chirurgie est la principale méthode de traitement de l'hydatidose. Dans la thérapie chirurgicale de l'hydatidose, l'utilisation d'agents sporicides est très importante car ces agents inactivent les protoscolices vivants et préviennent la récurrence de l'infection. Actuellement, de nombreux agents chimiques scolicides ont été administrés pour inactiver le contenu du kyste hydatique. Récemment, les chercheurs portent une attention particulière aux plantes médicinales comme traitement alternatif en raison de leur faible coût, de leur disponibilité, de leurs faibles effets secondaires et toxicité. Cette étude visait à évaluer pour la première fois l'effet scolicide de l'extrait hydro-alcoolique du *Taxus baccata L.* in vitro. L' activité scolicide de l'extrait a été testée à des concentrations de 50, 100 et 150 mg/ml après 10, 30 et 60 minutes d'incubation, et les expériences ont été réalisées en triple. La viabilité des protoscolices a été confirmée par une coloration vitale à l'éosine à 0,1%. Les données ont été analysées par le logiciel SAS (version 9.4). Les résultats ont montré que l'extrait hydroalcoolique du *Taxus baccata L.* dans une concentration de 150 mg/ml a éliminé 66,6% des protoscolices à 60 min. Selon les résultats de cette étude, l'utilisation de cette plante comme un agent

scolicide est recommandée. Ces résultats montrent que l'extrait de *Taxus baccata L.* possède un puissant effet scolicide. Cependant, d'autres études sont nécessaires pour évaluer l'efficacité de *Taxus baccata L. in vivo*.
Mots-clés: Kyste hydatique, In vitro, Scolicide, *Taxus baccata L*

Introduction

Hydatidosis is an important zoonotic disease across the world occurring due to the infection of the larval stages of some species belonging to the genus *Echinococcus* (Otero-Abad and Torgerson, 2013). Larval stages (hydatid cysts) develop in different organs of the host, such as liver, lung (even heart), brain, bone, spleen, and kidneys which may lead to death (Moro and Schantz, 2009). This disease affects humans as well as domestic livestock, including cattle, sheep, camels, pigs, horses, and others (Fasihi Harandi et al., 2012).

Although surgery remains one of the most important therapy, chemotherapy is the preferred treatment where surgeons are not available (Moazeni et al., 2015). Therefore the development of new scolicidal agents with low side effects and more efficacies is an urgent need for surgeons (Adas et al., 2009). Chemotherapy with benzimidazoles is accompanied with some limitations, such as low solubility and poor absorption. In addition, long-term therapies are usually required; however, some side effects and improper results possibly occur (Elissondo et al., 2008).

Nonetheless, benzimidazoles are extensively used for the treatment of hydatid disease (Daniel-Mwuambete et al., 2003). Furthermore, scolicidal agents used through hydatid cyst surgery are vital for surgical success owing to a reduction in the risk of spillage of viable protoscolices. A perfect scolicidal agent is defined as being effective in low concentrations in shorter exposure time, as well as stable after dilution with the cyst fluid. Moreover, it should be the eliminator of cyst protoscolices, as well as non-toxic, more efficient, less harmful for tissue host, low cost, and available (Anthony et al., 2005). Many efforts have been recently performed on herbal medicine extracts against protoscolices of hydatid cysts throughout the world.

English yew (*Taxus baccata L.*) is one of the limited coniferous species in the Caspian forests of Iran that is remained from tertiary third. Afratakhteh yew reserve is one of the yew sites in Caspian forests; accordingly, there are dense stands of ancient yew trees (the age of some trees is greater than 1000 years) that occasionally are forming pure yew stand (Esmailzadeh et al., 2007). The leaves of *T. baccata L.* have been used in traditional medicine as an abortifacient, antimalarial, antirheumatic, asthma, bronchitis, antifungal, and significant activity against some gram-negative bacteria but no activity against tested gram-positive bacteria (Erdemoglu and Sener, 2001).

During recent years, the use of medicinal plants has been attracted the attention of researchers in Iran. This *in vitro* study aimed to evaluate the scolicidal effect of hydroalcoholic *Taxus baccata L.* extract for the first time.

Material and Methods

Protoscolices preparation. Hydatid cysts were collected from livers and lungs of sheep, which were infected with hydatid cyst in an industrial slaughterhouse, Tabriz, Iran. They were then transferred to the parasitology laboratory of Veterinary Medicine Faculty. Subsequently, the surface of the cysts was disinfected by 70% alcohol, and 25 ml of the cyst fluid were aspirated by sterile syringe, transferred into glass cylinders, and left to set for 30 min. The protoscolices were settled down at the bottom of the cylinders. The supernatant was removed, and the yielded protoscolices were washed three times with phosphate-buffered saline and tested by 0.1% eosin for the assessment of the viability of protoscolices. The

samples of protoscolices which were over 90% viability were selected for testing.

Plant collection. In this experimental study, *Taxus baccata L.* was collected from Gorgan region (Golestan province, Iran), and species were identified and authenticated (with herbarium number1560) in the botany section of Gorgan Agricultural Research Center, Gorgan, Iran. The whole dried *Taxus baccata L.* was powdered (10 g) and dissolved in distilled water (100 ml) for overnight at room temperature, and the yielded suspension was used. Concentrations and dosages of *Taxus baccata L.* were expressed as the crude amount of the dried plant used in preparing the stock solution.

Gas-chromatography/mass spectrometry (GC-MS) procedure. Chromatography was performed using Agilent 19091S-433(USA), and column dimensions (0.25 mm in diameter, 30 meters in length, and 0.25 microns in film thickness) was used for preparation. Furthermore, the aqueous extract was added in a flask, and it was equally added to hexane. Following that, it was placed on the shaker for 1 h until it was homogeneously mixed. In the next stage, it was put in a separator for 15 min to be doubled. The hexane phase was isolated using gas-chromatography/mass spectrometry (GC/MS) for injection.

Preparation of hydroalcoholic extract of the Taxus baccata L.. The hydroethanolic extract of the plant was prepared by maceration of 370 g of dried powder of plant's gum resin in absolute ethanol (70%) for two weeks at room temperature. The materials after filtration through filter paper (Whatmann Ltd.) were concentrated under low pressure at 40ºC and lyophilized in order to obtain dry powder extract.

Evaluation of the scolicidal effect of the Taxus baccata L. extract in vitro. To evaluate the scolicidal effect of the *Taxus baccata L.* extract, concentrations of 50, 100, and 150 mg/ml were disposed in distilled sterile water and added to the microtubes to which a drop of protoscolex rich sediment was added. The contents of the tubes were gently mixed, and the tubes were incubated at 37ºC for 10, 30, and 60 min. At the end of each incubation time, the upper phase was carefully removed so as not to disturb the protoscolices. One milliliter of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. Following that, the viability of protoscolices was confirmed by 0.1% eosin staining. The protoscolices, which did not absorb the dye with the movement of the flame cells, were potentially recorded as viable five min after exposure to the eosin stain; otherwise, they were considered dead protoscolices.

The upper portion of the solution was discarded after 15 min of incubation. The remaining pellet of protoscolices was then smeared on a manually scaled glass slide, covered with a cover glass (24×50 mm), and examined under a light microscope. The percentages of the dead protoscolices were determined by counting a minimum of 500 protoscolices. The experiments were performed in triplicate, normal saline was considered a negative control group, and mebendazole (5g/ 100 ml) was used as a positive control group.

Statistical Analysis. The data were analyzed in SAS software (version 9.4).

Results

The results showed that *Taxus baccata L.* extract at the concentration of 150 mg/ml killed 66.6% of the protoscolices after 60 min of application. Moreover, the scolicidal effect of *Taxus baccata L.* extract at the concentration of 50 mg/ml was lower than that in other concentrations (25%). Figures 1 and 2 illustrate the live and dead protoscolices after exposure to *Taxus baccata L.* The mortality rate of hydatid cyst protoscolices after exposure to different concentrations of the hydroalcoholic extract of *Taxus baccata L.* following various exposure times are presented in Table 1 and Figure 3. The results of GC/MS *Taxus baccata L.* extract are also presented in Figure 4. The GC-MS

analysis showed that major compounds in *Taxusbaccata L.* included Octane (13.36%), 4-methoxycarbonyl-3,5-diphenyl-1 (8.30%), and 9,12,15-octadecatrienoic acid (10.75%).

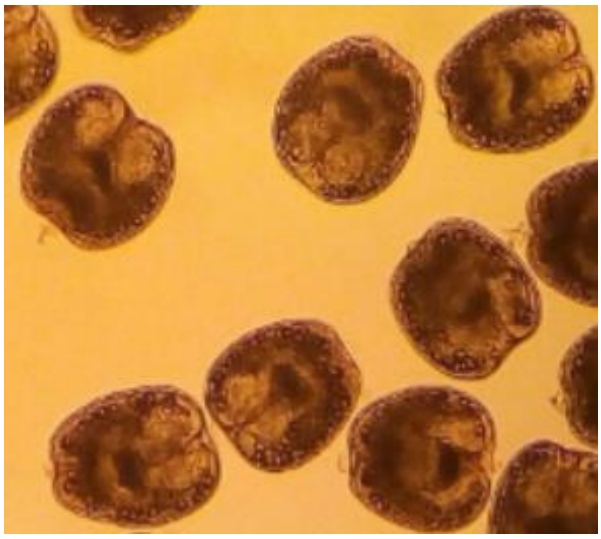

Figure 1. Live protoscolices

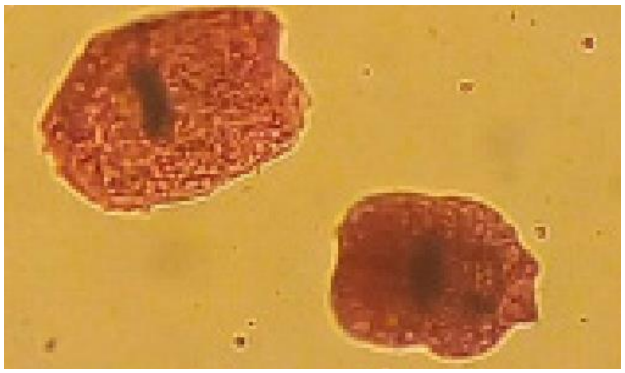

Figure2. Dead protoscolices

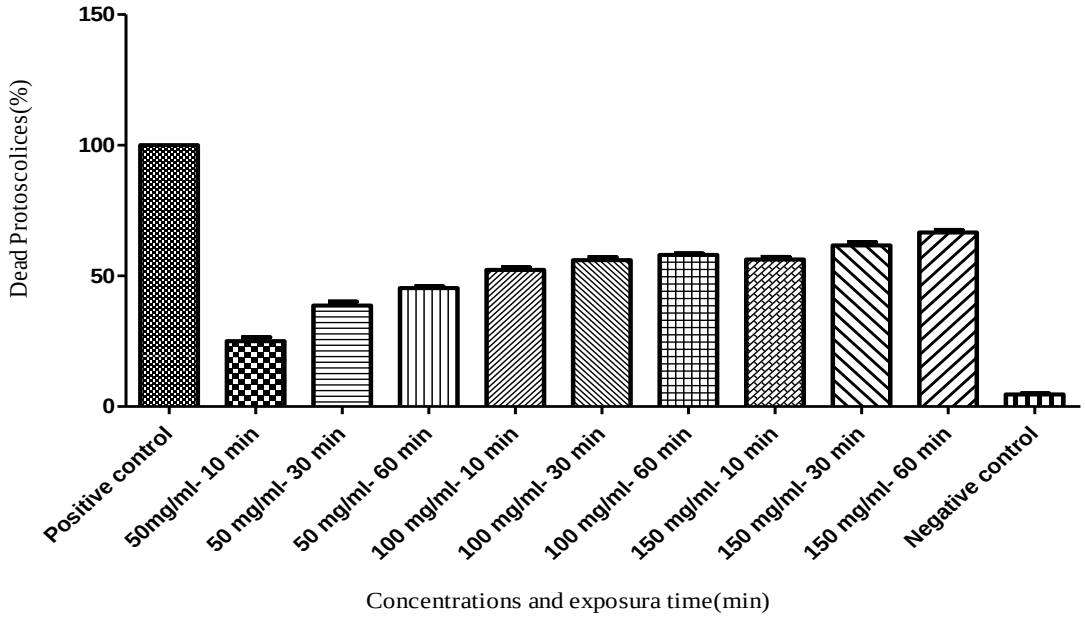

Figure 3. Scolicidal effects of different concentrations of *Taxus baccata L.* and various exposure times

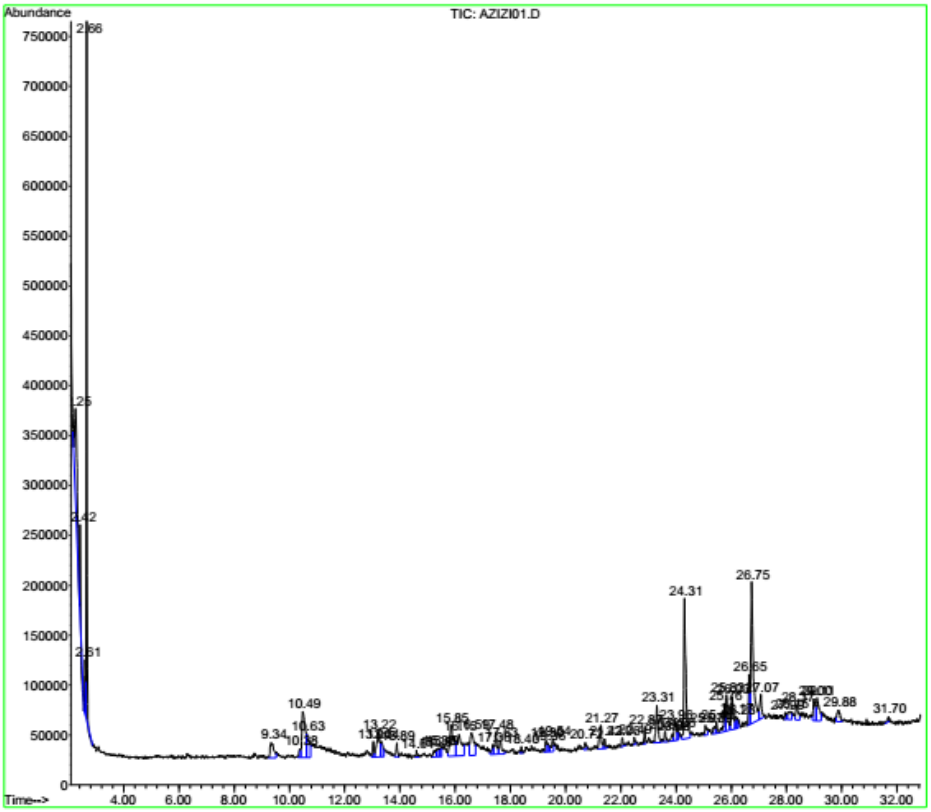

Figure 4. Gas-chromatography/mass spectrometry analysis results of *Taxus baccata L.* extract

Table 1. Scolicidal effect of *Taxus baccata L.* extract at different concentrations following various exposure times

Agents	Repeat	Time		
		10 min	30 min	60 min
Positive control	1	100	100	100
	2	100	100	100
	3	100	100	100
	(Mean±SD)	(100±0.00)	(100±0.00)	(100±0.00)
50 mg/ml	1	28	36	46
	2	24	41	44
	3	23	39	46
	(Mean±SD)	(25±2.64)	(38.6±2.51)	(45.3±1.15)
100 mg/ml	1	52	56	59
	2	54	58	57
	3	51	54	58
	(Mean±SD)	(52.3±1.52)	(56±2.00)	(58±1.00)
150 mg/ml	1	58	60	67
	2	55	64	65
	3	56	61	68
	(Mean±SD)	(56.3±1.52)	(61.6±2.08)	(66.6±1.52)
Negative control	1	5	6	4
	2	4	7	5
	3	5	5	4
	(Mean±SD)	(4.6±0.57)	(6.0±1.00)	(4.3±0.57)

Discussion

The use of medicinal plants has long been in existence and are widely registered in records kept in ancient China, India, Egypt, and Iran. Moreover, they have been used as traditional treatments for human diseases for years ago. These ancient native actions were discovered by a series of "trial and error" which then could not be supported by demonstrating scientific theories. The consumption of chemical drugs is restricted due to side effects, low efficacy, parasite resistance, high toxicity, extend of treatment, and high cost. The search for better drugs should be continued since antiparasitic vaccines may not become accessible imminently. Natural products may present a limitless origin of chemical variety to identify new drug modules. New drugs against parasites should be safe, non-toxic, low cost, and available antiparasitic agents (Rouhani et al., 2001).

Herbal therapy can be used as alternative therapies depending on their conditions and efficacy. Moreover, these remedies do not have complications of chemical therapy and are acceptable in terms of sustainability and compatibility with the environment (Elissondo et al., 2008). Therefore, several studies have been carried out to investigate the use of medicinal plants for the treatment of bacterial, viral, and parasitic diseases (Valadbeigi and Shaddel, 2014; Valadbeigi and Shaddel, 2015; Valadbeigi and Shaddel, 2016).

In the same line, multiple studies have been performed on herbal medicine against protozoan infections in Iran. English yew (*Taxus baccata L.*) is one of the limited coniferous species in the Caspian forests of Iran that is remained from tertiary third. Afratakhteh yew reserve is one of the yew sites in Caspian forests (Esmailzadeh et al., 2007). The GC-MS investigation showed that the major portions in *Taxus baccata L.* were octane (13.36%), 4-methoxycarbonyl-3,5-diphenyl-1 (8.30 %), and 9,12,15-Octadecatrienoic acid (10.75%) as main ingredients isolated from this plant.

The present study investigated the effect of hydroalcoholic extract of *Taxus baccata L.* on the protoscolices of hydatid cyst. According to the results, *Taxus baccata L.* extract at the concentration of 150 mg/ml killed 66.6% of the protoscolices after 60 min of application. In addition, scolicidal effect of *Taxus baccata L.* extract at the concentration of 50 mg/ml was lower than that in other concentrations (25%).

Sadjjadi et al. (2008) used *Allium Sativum* extracts, and the results showed that chloroformic extract of the plant with 200 mg/mL concentration had the highest protoscolicidal activity (99.58±1.63). In the same vein, Moazeni et al. (2014) indicated a high scolicidal effect of methanolic extract of *Zataria multiflora* on hydatid cyst. The concentrations of 10 and 25 mg/mL killed 100% of protoscolices after 3 and 1 min, respectively. In another study, Kavoosi and Purfard (2013) revealed that all protoscolices were killed after 10 min of exposure at concentrations of more than 17µg/mL of essential oil from *Z. multiflora*.

According to a study conducted by Mahmoudvand et al. (2014), the essential oil of *Nigella sativa* at the concentration of 10 mg/mL after 10 min of exposure eliminated 100% of the protoscolices. Similalry, Rouhani et al. (2013) in a study on the scolicidal effect of *barberry* with different concentrations (0.5, 1, 2, and 4 mg/ml diluted form) at different exposure times (5, 15, and 30 min) observed that 4 mg/mL dilution with the mean of scolicidal activity had 100% efficacy after 5 min. *In vitro* scolicidal effect of Satureja khuzistanica essential oil indicated that 81% and 100% of the protoscolices were killed at the concentrations of 5 and 10 mg/mL, respectively.

Moreover, the results of a study exhibited both dose-dependent and time-dependent scolicidal effect of this plant (Moazeni et al., 2012). It seems that the variations in the results of different studies are due to the diversities in plants, concentration, and time exposure.

Generally, the findings of this study indicated a scolicidal activity of hydroalcoholic extract of *Taxus baccata L.* and its potential as a natural component for the synthesis of a new scolicidal agent in hydatid cyst surgery.

Authors' Contribution

Study concept and design: R. N. and M. H.
Acquisition of data: R. N.
Analysis and interpretation of data: M. H.
Drafting of the manuscript: R. N.
Critical revision of the manuscript for important Intellectual content: A. A.
Statistical analysis: R. N.
Administrative, technical, and material support: D. A.

Ethics

Since this study was an in vitro one, there was no need for ethical committee approval.

Conflict of Interest

The authors declare that they have no conflict of interest.

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