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The effect of *Taxus baccata L.* extract on hydatid cyst protoscolices *In vitro*

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ABSTRACT

Hydatidosis is most important global parasitic infectious diseases, both in humans and animals, which can be lodge at different organs of host such as liver, lung even in heart and brain which may lead to death. The mainly methods for treatment of hydatidosis include surgery. In surgical therapy of hydatidosis, use of scolicidai agents is very important, because these agents inactivate live protoscolices and prevent recurrence of infection. Presently, numerous scolicidal chemical agents have been administrated for inactivation of the hydatid cyst contents. Recently, there is a high tendency among researchers to evaluate and present herbal plants as alternative option due to being inexpensive, easy available, low side effects and toxicity. This study was undertaken for the first time to evaluate the scolicidal effect of hydro alcoholic *Taxus baccata L.* extract *in vitro*. 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. Data were analyzed by SAS software version 9.4. The results showed that the hydroalcoholic extract of *Taxus baccata L.* at the concentration of 150 mg/ml leads to killing 66.6% of protoscolices at 60 minutes, then this investigation is recommended to use this plant as a scolicidal plant. The findings of present study proven that *Taxus baccata L.* have potent scolicidal effects. However, further studies are required to evaluate the efficacy of *Taxus baccata L. in vivo.*

Keywords: Hydatid cyst, scolicidal, *Taxus baccata L*, *In vitro*.

INTRODUCTION

Hydatidosis is an important zoonotic disease all over the world occurring due to the infection of the larval stages of some species belonging to the genus *Echinococcus* (Otero and Torgerson, 2013). Larval stages (hydatid cysts) develop in the different organs of host such as liver, lung even in heart, brain, bone, spleen and kidneys which may lead to death (Moro and Schantz, 2009). The

disease affects humans as well as domestic livestock including cattle, sheep, camels, pigs, horses and others (Fasihi Harandi et al., 2013).

Although, surgery remains one of the most important therapy; chemotherapy is the preferred therapy 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 have some limitations such as low solubility and poor absorption, in addition, long-term therapies are required usually, and however some side effects and improper results possibly occur (Elissondo et al., 2008). However, benzimidazoles are extensively used for therapy of hydatid disease (Daniel et al., 2003). Scolicidal agents used through hydatid cyst surgery are vital for surgical success owing to reduction in the risk of spillage of viable protoscolices. A perfect scolicidal agent is defined as being effective in low concentrations in a shorter exposure time, stable after by dilution with the cyst fluid, non-toxic, eliminator of cyst protoscolices, more efficient, less harmful for tissue host, low cost and easy available (Anthony et al., 2005).Till now, many efforts have been 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 Caspian forests of Iran that is remained from tertiary third. Afratakhteh yew reserve is one of the yew sites in Caspian forests so there are dense stands of ancient yew trees (the age of some trees is greater than 1000 year) that occasionally are forming pure yew stand (Esmailzadeh et al., 2007). The leaves of *T. baccata L.* have been used in traditional medicine as 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 have been attracted the attention of researchers, in the Iran. This study was undertaken for the first time to evaluate the scolicidal effect of hydro alcoholic *Taxus baccata L.* extract *In vitro* study.

MATERIAL AND METHODS

Protoscolices preparation. Hydatid cysts from livers and lungs of sheep which were infected to hydatid cyst were collected from Tabriz industrial slaughterhouse and transferred to the parasitology laboratory of Veterinary Medicine Faculty. Then the surface of cysts were disinfected by 70% Alcohol and 25 ml of cyst fluid were aspirated by sterile syringe and transferred to into

glass cylinders and left to set for 30 min. The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the yielded protoscolices were washed three times with PBS and tested by 0.1% eosin for assessment of 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 spices was identified and authenticated (with herbarium number1560) in the botany section of Gorgan Agricultural Research Center. The whole dried *Taxus baccata L.* was powdered (10 g) and dissolved in distillated water (100 ml) for overnight at room temperature and the yielded suspension was used. Concentrations and dosages of *Taxus baccata L.* were expressed as crude amount of the dried plant used in preparing the stock solution.

Gas-chromatography/mass spectrometry (GC-MS) procedure. Chromatography was performed by using (Agilent 19091S-433, USA) and column dimensions 0.25 mm in diameter, 30 meters in length, 0.25 microns in film thickness was used for prep. Add the aqueous extract in a flask and add it equally to hexane, then place it on the shaker for 1 hour until it is homogeneously mixed. Then put it in a separator, wait 15 minutes to double. The hexane phase is isolated to GC/MS for injection.

Preparation of hydroalcoholic extract of the *Taxus baccata L.* The Hydro ethanolic extract of the plant was prepared by maceration of 370 g of dried powder of plant's gum resin in absolute ethanol 70% two a week at room temperature. The materials after filtration through filter paper (Whatmann Ltd.), the filtrates were concentrated under low pressure at 40 C and lyophilised in order to obtain dry powder extract.

Evaluation of the scolicidal effect of the *Taxus baccata L.* extract *in vitro*. For evaluation of 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. The tubes were incubated at 37 °C 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. Viability of protoscolices was confirmed by 0.1% eosin staining. Five min after exposure times to the eosin stain the protoscolices which did not absorbed dye with the movement of the flame cells were potentially recorded as viable, otherwise, they were considered as 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 dead protoscolices were determined by counting a minimum of 500 protoscolices. The experiments were performed in triplicate. Normal saline was considered as negative control group and mebendazole (5g/ 100 ml) were used as positive control group.

Statistical Analysis. Data were analyzed by SAS software version 9.4.

RESULTS

The results were showed that *Taxus baccata L.* extract at the concentration of 150 mg/ml killed 66.6% of protoscolices after 60 minutes of application. Scolicidal effect of *Taxus baccata L.* extract at the concentration of 50 mg/ml was lower than that in others concentration (25%). Image of live and dead protoscolices after exposure to *Taxus baccata L.* showed in Figures 1 and 2. The mortality rate of hydatid cyst protoscolices after exposure to different concentrations of the hydro alcoholic extract of *Taxus baccata L.* following various exposure times are presented in Tables 1 and Figure 3. The results of Gas-chromatography/mass spectrometry (GC/MS) *Taxus baccata L.* extract showed in Figure 4. Gas-chromatography/mass spectrometry (GC-MS) investigation showed that major portion in *Taxus baccata L.* is Octane (13.36%), 4-methoxycarbonyl-3,5-diphenyl-1 (8.30 %) and 9,12,15-Octadecatrienoic acid (10.75%) as main ingredients have been isolated from this plant.

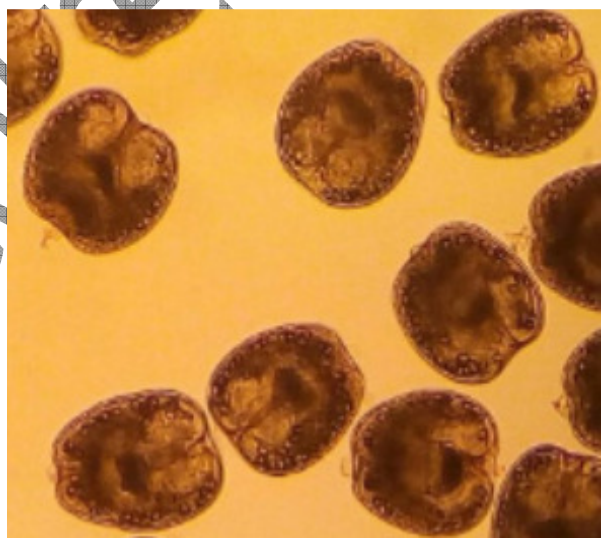

Figure 1. Image of live protoscolices

Figure2. Image of dead protoscolices

Table 1. Scolicidal effect of the *Taxus baccata L.* extract at the 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)

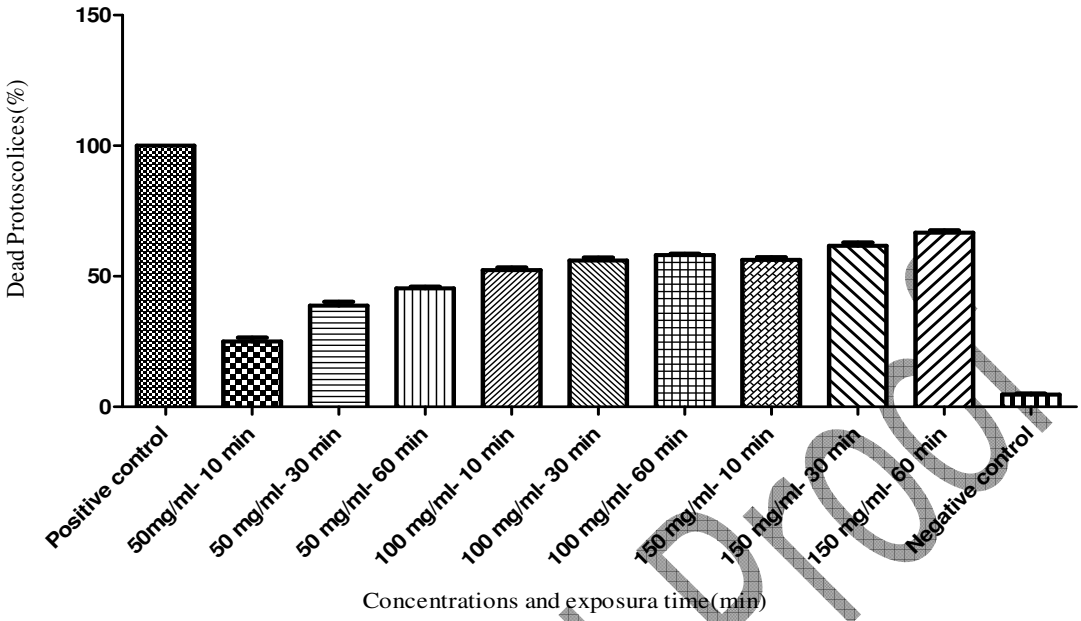

Figure 3. Scolicidal effects of different concentrations of *Taxus baccata L.* and various exposure times

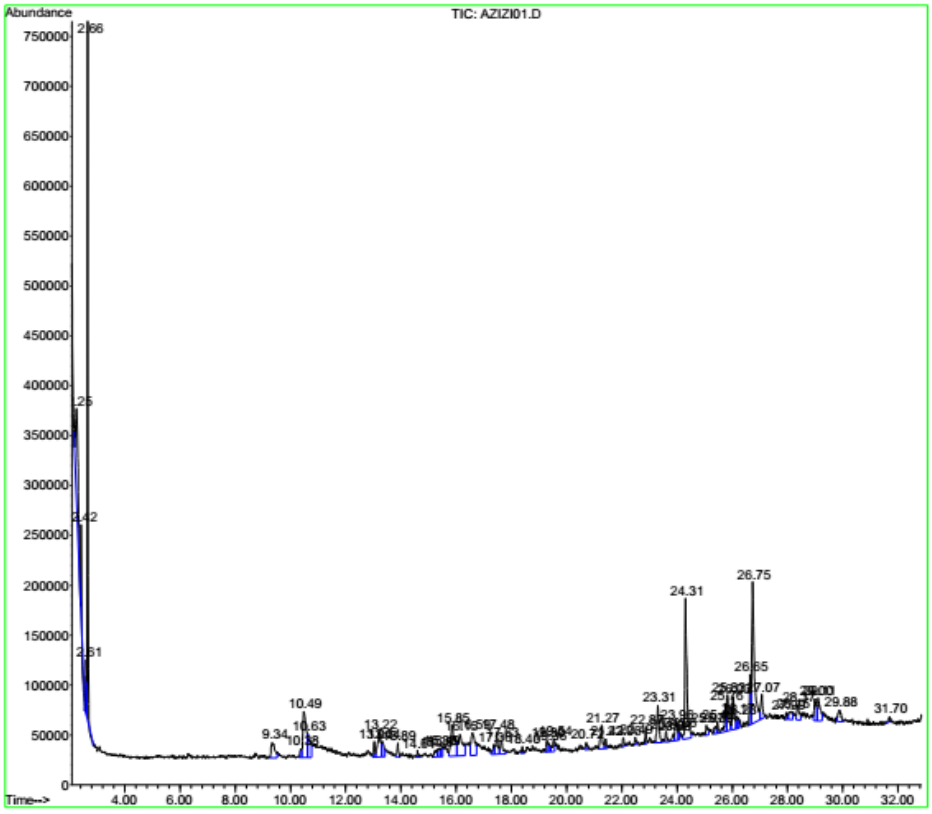

Figure 4. Gas-chromatography/mass spectrometry (GC-MS) analysis results of *Taxus baccata L.* extract

DISCUSSION

The use of medicinal plants has being long in existence and are widely registered in records kept in ancient China, India, Egypt and Iran and 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 demonstrate scientific theories. The use 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 because antiparasitic vaccines may not become accessible in the near future. 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 (Rohanni et al., 2001).

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

Many studies have been performed on herbal medicine against protozoan infections from Iran. English yew (*Taxus baccata L.*) is one of the limited coniferous species in 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). Gas-chromatography/mass spectrometry (GC-MS) investigation showed that major portion in *Taxus baccata L.* is Octane (13.36%), 4-methoxycarbonyl-3,5-diphenyl-1 (8.30 %) and 9,12,15-Octadecatrienoic acid (10.75%) as main ingredients have been isolated from this plant.

In the present study the effect of hydro alcoholic extract of *Taxus baccata L.* on the protoscolices of hydatid cyst investigated. According to the results, *Taxus baccata L.* extract at the concentration of 150 mg/ml killed 66.6% of protoscolices after 60 minutes of application. Scolicidal effect of *Taxus baccata L.* extract at the concentration of 50 mg/ml was lower than that in others concentration (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) (Sadjjadi et al., 2008). Moazeni *et al.* (2014) indicated high scolicidal effect of methanolic extract of *Zataria multiflora* on hydatid cyst. The concentrations of 10 mg/mL and 25 mg/mL killed 100%

of protoscolices after 3 min and 1 min, respectively (Moazeni et al., 2014). In another study, Kavoosi *et al*.(2013) showed that all protoscolices were killed after 10 min of exposure time at concentrations more than 17µg/mL of essential oil from *Z. multiflora* (Kavoosi et al., 2013).

Mahmoudvand *et al.* (2014) indicated that the essential oil of *Nigella sativa* at the concentration of 10 mg/mL after 10 min of exposure time eliminated 100% of protoscolices (Mahmoudvand et al., 2014).

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) and at different exposure times (5, 15, and 30 min) observed that in 4 mg/mL dilution with the mean of scolicidal activity had 100% efficacy after 5 min (Rouhani et al. 2013). *In vitro* scolicidal effect of *Satureja khuzistanica* essential oil indicated that at the concentration of 5 and 10 mg/mL, 81 and 100%, of protoscolices were killed, respectively. Result of the study exhibited both dose-dependent and time-dependent scolicidal effect of this plant (Moazeni et al., 2012).

It seems that the differences in result of different studies are due to the differences in plants, concentration and times exposure.

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

Ethics

This work has been performed in vitro condition; so ethical committee approval was not needed.

Conflict of interests

None of authors had conflict of interests.

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