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INTRODUCTION TO ECHINOCOCCOSIS AND A REVIEW OF TREATMENT PANELS

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INTRODUCTION

Echinococcosis is a neglected silent cyclozoonotic parasitic disease, caused by metacestodal stages of organisms of genus *Echinococcus,* belonging to family *Taeniidae.* These parasites affect wide spectrum of animal species including livestock and wildlife, and also possess zoonotic implications (Singh et al. 2012; Sadjjadi et al. 2013; Ma et al, 2015; Rialch et al. 2018). At present, there is a huge debate over taxonomy of genus *Echinococcus* at the species level. There are considerable variations shown by this genus at the level of species in terms of morphology, developmental phases, modes of transmission, antigenicity and host specificity (Thompson and McManus 2001; Lymbery and Thompson 2012; Thompson 2008). Genus *Echinococcus* contains at least 10 valid species, each having different strains and genotypes namely *E. granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarhtus, E. canadensis, E. equines, E. felidis, E. shiquicus* and *E. ortleppi* (Nakao et al. 2013).

Echinococcus species require two different hosts, the intermediate and the definitive. The definitive hosts are the carnivores, especially the dogs that carry this parasite in their small intestines. Both wild and domesticated ruminants, camels and human beings serve as intermediate hosts of different *Echinococcus* species (Eckert et al. 2001; Jenkins et al. 2005; Elham et al. 2014). The size of adult worm varies from 2-11 mm, having 2-7 proglottid segments. Each of the proglottid has single genital opening and mature proglottid is called as penultimate segment. Scolex has two rows of rosttelar hooks.

After entering the small intestine of the definitive host, the scolex with its suckers and rostellar hooklets, becomes exvaginated and develops into adult worm. After fertilization, eggs are fully developed in the uterus and gravid proglottids release eggs that are passed into the environment along with dog feces (Figure 1). Ingestion of contaminated water or vegetation by the intermediate host leads to release of oncosphere from embyronated eggs that penetrate the intestinal wall, spreading to various other tissues of the body through circulation. Cyst formation primarily occurs in the lungs and liver and such infected tissue when eaten by the canids leads to release of protoscoleces, which become mature worm resulting in the completion of the life-cycle of parasite (Thompson and McManus 2002; Brehm 2010; Pourseif et al. 2017; Conceição et al. 2017;).

Geographical distribution

Echinococcosis is included in the World Health Organization (WHO) list of neglected tropical diseases (NTDs) (Butt and Khan, 2020). This disease is widespread in its distribution and persists in a variety of environmental conditions, varying from temperate to circumpolar, tropical and sub-tropical regions. The parasite has ability to survive very well in climatic conditions varying from arid to subpolar oceanic environment. Eurasia, Australia, Africa and South America have very high prevalence of the disease and around 50 milllion people are infected with the disease worldwide (Eckert et al. 2001; Hammad et al. 2018). This ailment encompasses wide geographical area, extending from Eastern parts of Asia to the Northern America and from upper northern hemisphere to the southern countries of African continent (Schneider et al. 2010; Sadjjadi et al. 2006) (Figure 2).

Pathology of echinococcosis

Echinococcosis can appear in four different forms namely Cystic Echinococcosis (CE), Alveolar Echinococcosis (AE), Poly-cystic Echinococcosis (PE) and Uni-cystic Echinococcosis (UE), caused by *Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus vogeli and Echinococcus oligarthrus*, respectively (Table 1). All of these species share the common definitive hosts which are the canids, except *E. oligarthrus* which have members of the family Felidae as the definitive host. Definitive hosts harbor the adult stage of worms in their small intestine and shed the embryonated eggs of the parasite in the environment with the feces. *Echinococcus granulosus* and *E. multilocularis* enjoy wide range of intermediate hosts, including bovines which harbor the larval stages of the parasite in their visceral organs after ingestion of the embryonated eggs (Pleydell et al. 2008; Santa et al. 2018).

For all of the species of *Echinococcus*, human and monkeys serve as aberrant hosts and the disease may take its course from asymptomatic to severe clinical infection, which may lead to death (Eckert and Thompson 2017). Disease pattern of Echinococcosis is usually asymptomatic in livestock and diagnosis is generally made on necropsy findings in the abattoir and it has serious implications in terms of condemnation of the carcasses. In human Cystic Echinococcosis (CE), clinical

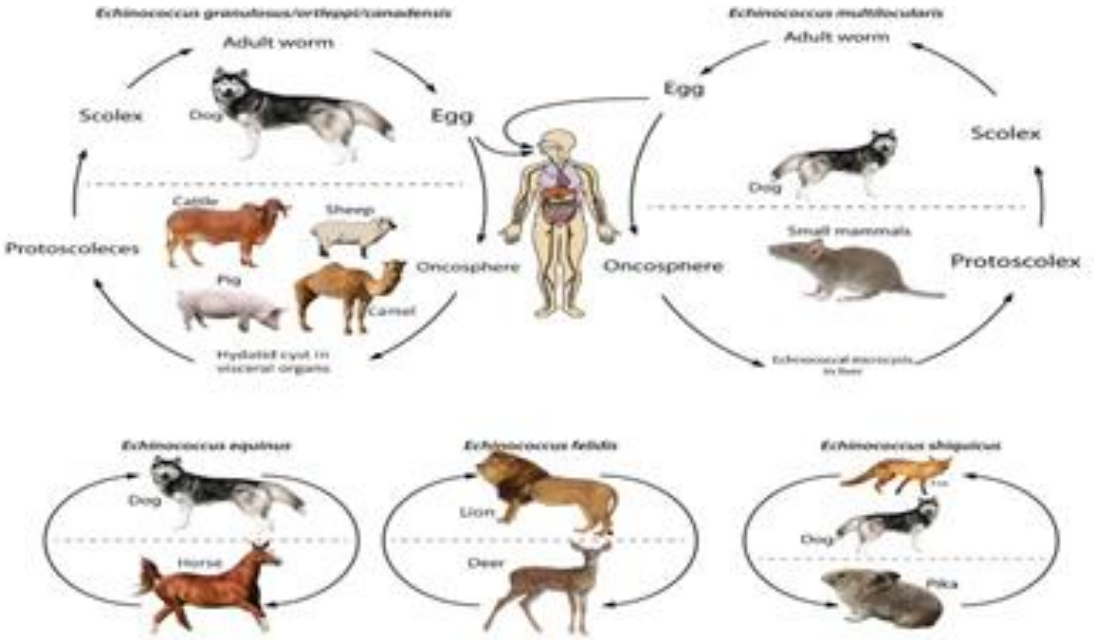

Fig. 1: Overview of life cycle of *Echinococcus* species.

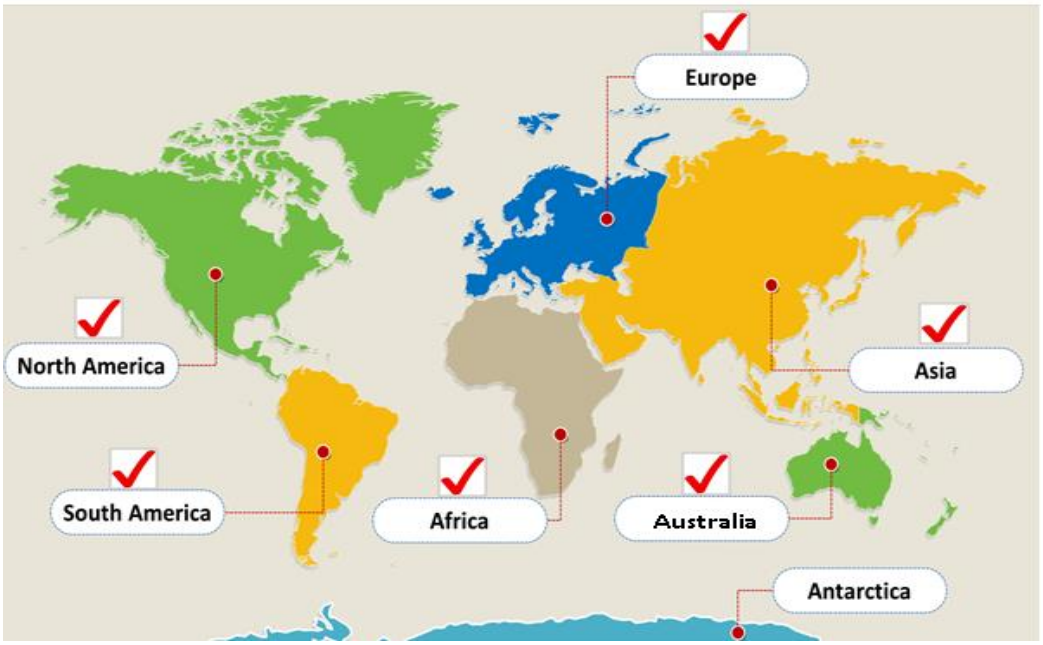

Fig. 2: Cosmopolitan distribution of *Echinococcus* species.

Table 1: Forms of Echinococcosis*

Forms of Echinococcosis	Causative agent	Typical definitive host	Typical intermediate host	Aberrant host
Cystic Echinococcosis	*E. granulosus*	Dog, wolf, jackal and other canids	Sheep, goat, cattle, pig, horse	Human, monkey, and many other mammals
Alveolar Echinococcosis	*E. multilocularis*	Fox, wolf, raccon dog, domestic dog, cat	Small herbivorous mammals and rodents	Human, monkey, dog, pig, horse
Polycystic Echinococcosis	*E. vogeli*	Bush dog, domestic dog	Paca, agouti	Human, monkey
Unicystic Echinococcosis	*E. oligarthrus*	Wild cats	Agouti, Paca, Opossums	Human

*Table derived from Thompson et al. (2017).

signs include asthenia, weight loss, epigastric pain, hepatomegaly and cholestatic jaundice (Brunetti et al. 2010). It may become a fatal disease when the cysts rupture and their fluid contents and protoscoleces are drained into peritoneal cavity, leading to anaphylactic shock (Yang et al. 2015).

Economic thrashes and public health significance

Echinococcosis is a neglected tropical disease of public health concern and has serious economic setbacks. Out of total ten known species of *Echinococcus*, *E. granulosus* and *E. multolocularis* pose significant threats to human

and animal health in addition to substantial economic losses (Torgerson and Macpherson 2011). This infection leads to economic and social thrashed in terms of treatment cost, production loss and mortality in the infected animals and aberrant human host infections. Cosmopolitan distribution of this ailment has led to losses of US dollar (USD) 3.0 billion annually (WHO Echinococcosis Fact Sheet 2017).

According to Ehsan et al. (2017), Echinococcosis has caused annual economic losses of USD 212.35 million in India, USD 232.3 million in Iran and USD 7.708 million in Turkey. It has been estimated that in Chile Cystic Echinococcosis leads to economic losses amounting to USD 14.35 million per year (Rojas et al. 2017). Followed by *Fasciola hepatica*, Cystic Echinococcosis is the most important cause of condemnation of livestock viscera.

Echinococcosis also causes substantial economic losses in Pakistan. It has been estimated that the malady causes losses accounting to USD 276.20 per 100 infected goats and sheep and USD 165.72 per 100 infected large ruminants and camels. Losses occur in terms of poor quantity and quality milk, poor quality wool and meat, retarded growth, reduced fertility and condemnation of carcasses (Latif et al. 2010).

The burden of human Echinococcosis is expressed in terms of disability adjusted life years (DALYs). For Alveolar Echinococcosis, overall global burden of disease was approximated to be 18,200 cases per annum that account up to about 666,000 DALYs (37 DALYs per case) (Torgerson et al. 2010). About 91% of cases and 95% of the DALYs were reported from China and 1600 cases per annum from Europe, Russia and central Asian countries, resulting in 21 DALYs per case. Survival analyses investigations of French and Swiss Alveolar Echinococcosis patients have indicated that modern treatment techniques like resection of liver lesions and extended therapy with benzimidazoles can lead to survival of Alveolar Echinococcosis patients comparable to those of healthy human populations (Torgerson et al. 2008; Piarroux et al. 2011). The regions where treatment choices are available, DALYs burden is moderate because of the better prognosis. For example, in Switzerland, there is a total burden of 3.7 DALYs per case which is 10 times less than that of global estimates (Torgerson et al. 2008). This accounts for one of the most important factors for the dominance of the global burden in China, where the greater part of cases were estimated to come from resource poor communities like the Tibetan plateau. In the central Asian countries, the situation seems to be quite comparable (Torgerson 2017). The most recent global estimate for the Cystic Echinococcosis burden is 188,000 cases per annum, resulting in 0.98 DALYs per case (Torgerson et al. 2015), which is quiet lower than Alveolar Echinococcosis burden, and is due to the lower death rate of Cystic Echinococcosis patients as compared to Alveolar Echinococcosis ones.

Treatment

Treatment of Cystic Echinococcosis cases involves destruction of the metacestode which can be achieved by a number of ways like sterilization of the parasite contents, aspiration of cystic fluid or by surgical removal of the entire cyst (Sayek and Onat 2001; Buttenschoen et al. 2003; Junghanss et al. 2008; Dziri et al. 2009). Surgical procedures are often complicated due to spillage of the cystic fluid into the body cavity, leading to immunological reactions (Brunetti et al. 2010).

To overcome such situations, use of anthelmintics and herbal medications is among other resorts without any risk of side-effects. Some of the medicinal based (chemical, herbal and allopatheic) options to treat Cystic Echinococcosis cases are summarized below.

Treatment with anthelmintics

Mebendazole (MBZ)

In humans, mebendazole (MBZ), an anthelmintic, is only effective against small cysts having size <5 cm (Kern 1983). Long term treatment with MBZ is not well-tolerated by all the patients equally because investigations have shown that 5-40% of patients exhibited adverse effects related to MBZ like hair loss, gastro-intestinal tract (GIT) distress, anaphylactic reaction, headache, hematotoxic effects and altered level of transaminases. Moreover, MBZ can lead to congenital anomalies in the fetus when administered during the first trimester of pregnancy (De Silva et al. 1999). When an epoxy group is introduced to MBZ, two isoforms (M-C1 and M-C2) are obtained, with M-C2 having higher parasiticidal effect than M-C1, as well as simple MBZ (Xu et al. 2019).

Albendazole (ABZ) and their combinations

Initially, MBZ was considered as the first line of treatment of Echinococcosis. But with time, this drug was proved to be less efficacious than albendazole (10 mg/kg/day, maximum 800 mg in 2 doses orally) due to its low solubility in water and poor bioavailability (Davis et al. 1989). Also, the results of a study conducted in murine models have shown that the therapeutic activity of albendazole increases when co-administered with *Zataria multiflora* (herbal product) (Moazeni et al. 2019). In humans, absorption of ABZ shows wide fluctuations and co-administration of ABZ with a fatty meal can enhance its absorption (Anand et al. 2012).

The synergistic effect of ABZ has been reported in recent studies when it is administered in combination with other drugs. The combined treatment of ABZ with metformin appears to be a good option, having admirable results than the therapy done by using ABZ alone (Gorgas et al. 2017). The co-administration of metformin with ABZ has proved effective *in vivo* and *in vitro* (Loos et al. 2015; Loos et al. 2017). Similarly, the combination of ABZ with atovaquone killed protoscoleces of *Echinococcus* within 24 hours, while atovaquone alone took a long time for showing the therapeutic effects against protoscoleces (Kouguchi et al. 2021). The combined treatment of ABZ plus thymol also showed higher efficacy as compared to

that when these drugs were used singly against *E. multilocularis* (Albani et al. 2015).

Recent studies have also shown that there is increased sensitivity of *Echinococcus* protoscoleces to ABZ when given in combination with sodium arsenite. A 100% protoscolicidal effect was seen when 80 µM albendazole and 20 µM sodium arsenite were given in combination (Xing et al. 2019). The nano-crystal formulation of ABZ demonstrated inhibition of cyst 3.7 times higher than that of the albendazole commercial oral product (Albenda) (Hu et al. 2019). When ABZ was loaded into mesoporous material (SBA-15 and SBA-16), the highest drug loading, as well as dissolution, rate was showed by SBA-15 carriers. It enhanced ABZ bioavailability and could be formulated as capsules (Adrover et al. 2020). Lv et al. (2013) conducted an experiment on protoscoleces by using albendazole liposome (L-ABZ), Huaier aqueous extract, and their combination. The results revealed that there was maximum treatment efficacy when L-ABZ and Huaier aqueous extract were given in combination *in vitro,* as well as *in vivo.*

According to Spincher et al. (2008), 2-methoxyestradiol destroyed metacestodes of *E. multilocularis* alone or in combination with ABZ but neither of the drugs had a true killing effect. Both of these drugs showed good results when they were given in combination *in vitro* and also resulted in the reduction of parasite load after 6 weeks *in vivo*. Albandazole 100 µg/ml and povidone iodine (1/10) killed all protoscoleces *in vitro* after 15 minutes (Polat et al. 2009). Tibetan medicine *Elsholtzia eriostachya* in combination with ABZ damaged germinal membrane cells of metacestodes and exhibited good efficacy against secondary *Echinococcus multilocularis* infection in rats (Ji-hai et al. 2020).

The efficacy of ABZ is reported to be higher in the case of the pulmonary or hepatic cysts as compared to that of other anthelmintics. Patients having effective immune status show better response to ABZ than the other patients with CD4+ less than 500 cells/mm^3 (Magdalena et al. 2015). Another study on HIV patients indicated that the continuous treatment with ABZ for more than six months was ineffective and CD4+ count might have been the important factor for poor response to the treatment (Dumitru et al. 2015).

Albendazole is considered to be a safe drug and is well tolerated by the patients; therefore, it is preferred to use ABZ continuously rather than discontinuous therapy. Side effects of ABZ include GIT disturbances, liver toxicity, hair loss, jaundice (less common), cough, itching, and vertigo (Karabulut et al. 2014). In pregnant women, ABZ can lead to teratogenic and embryotoxic effects (Horton, 1989). Age is an important factor that influences outcome of the treatment because drug effectiveness is better in young patients as compared to that in old ones. The site of the cyst also affects the response of infected animal to the treatment; susceptibility of the bone cyst to ABZ is much less as compared to that of alveolar or hepatic cysts (Hemphill et al. 2010; Dumitru et al. 2015).

In the last few years, the development of resistance against ABZ has been widely discussed which considerably restricted cystic echinococcosis treatment. Both miRNA and genetic profile of helminths influence their response to albendazole sulfoxide (Mortezaei et al. 2019). Now-a-days, researchers are making efforts to assemble new ABZ preparations with enhanced organ and tissue penetration to improve the therapeutic effects of the drug (Panwar et al. 2010).

Praziquantel (PZQ) and its combinations

Antiparasitic efficacy of **praziquantel** (PZQ) for cystic echinococcosis is less than that of ABZ. However, it has shown promising results in diffused and incurable cases when it was administered in combination with ABZ (Jamshidi et al. 2008; Bygott et al. 2009). It has also been proved that PZQ becomes more effective when co-administered with ABZ. In combination, the PZQ is given once a week with a dose rate of 40 mg/kg with 800 mg/day albendazole, both before PAIR (puncture, aspiration, injection and reaspiration)/ surgical procedure and in inoperable cases (Jamshidi et al. 2008; Jiang et al. 2017). Jelowdar et al. (2017) evaluated the effect of ABZ and PZQ loaded solid lipid nanoparticles on Cystic Echinococcosis and found that their efficacy was more than that of the pure ABZ and PZQ.

Other anthelmintics

Richter et al. (2013) investigated the efficacy of triclabendazole and clorsulon on *E. multilocularis* larvae and found that maximum damage was caused by triclabendazole at a concentration of 20 µg/ml within 12 days and at 25 µg/ml within 13 days; triclabendazole sulfoxide showed the highest efficacy at concentrations of 20 and 25 µg/ml within 20 and 14 days, respectively. On the other hand, clorsulon showed no effects on vesicles at 5, 10, and 15 µg/ml concentrations in the *in vitro* culture.

According to Hokelek et al. (2002), ivermectin, a nematocidal drug, was effective against scoleces when it was injected into cysts and cyst size was significantly decreased. Ahmadpour et al. (2019) observed that nano lipid carrier combined with ivermectin brought about 100% death of cestodes after 1 and 2 hrs of administration at 800 µg/ml and 400 µg/ml concentration, respectively and was also responsible for the augmented expression of mRNA caspase 3 that showed its strong apoptotic activity against the parasite.

Insecticides

Lufenuron

Lufenuron (benzylphenylurea) is an inhibitor of insect growth and is commonly used to control fleas on companion animals. When it was used against *E. granulosus* hydatid cyst in mice, a 20-30% reduction in cyst size was observed which indicates that it can be used to augment the scolicidal activity of ABZ (Breijo et al. 2011).

Fluralaner

The drug fluralaner is used to control canine ectoparasites like mites, fleas and ticks. L-glutamate gated channels and GABA-gated chloride channels are potently inhibited by this drug. Studies have revealed that the activation of caspase 3, which is an indicator of apoptosis, was prominent after treatment with fluralaner. Treatment with this drug also causes damage to metacestode layers and protoscoleces, which shows that fluralaner can be effective for the treatment of Echinococcosis (Zahran et al. 2020).

Antiprotozoals

Nitazoxanide

Nitazoxanide kills all vesicles *in vitro* culture. It causes disintegration of the vesicles at a high dose of 10 µg/ml after 7 days and its effects are rapid than those of ABZ. Nitazoxanide, itraconazole and methiazole possess parasitostatic effects, while combination of ABZ with nitazoxanide shows parasitocidal effects *in vitro* (Reuter et al. 2006).

Buparvaquone

Buparvaquone is an anti-theilerial drug, with cytotoxic effects (Xing et al. 2019). This drug damages mitochondria of *in vitro* cultured *E. multilocularis* metacestodes and might be an effective choice for the treatment of patients with Cystic and Alveolar Echinococcosis (Rufener et al. 2018).

Anti-malarial drugs

Mefloquine is another drug with antiparasitic efficacy and is used in the treatment of malaria caused by *Plasmodium* species and has proven to have efficacy against metacestodes of *E. multilocularis* (Stadelmann et al. 2011; Küster et al. 2011; Küster et al. 2015; Rufener et al. 2018; Lundström-Stadelmann et al. 2020). Similarly, atovaquone (ATV) is another antimalarial drug and enzymatic analysis has shown its inhibitory action on mitochondrial complex III. Culture experiments have revealed the activity of ATV under aerobic condition, but not under anaerobic condition, against protoscoleces. The combination of ATV with atpenin completely destroys protoscoleces in culture, whereas *in vivo* experiments also demonstrated a significant reduction in cyst formation in mouse liver after oral drug administration (Enkai et al. 2020). In a recent study, Enkai et al. (2021) treated protoscoleces of *E. multilocularis* with atovaquone, praziquantel, rotenone, artemisinin and pyrvinium pamoate in different media culture at a concentration of 50 µM, and observed that the co-administration of artemisinin or praziquantel with ATV completely eliminated the protoscoleces. Li et al. (2020) used pyronaridine, an anti-plasmodium drug, against Cystic Echinococcosis and suggested that intraperitoneal injection with pyronaridine at the dose rate of 57 mg/kg, once a day for 3 days induced 100% cyst inhibition. When it was given orally with the same dose rate q.d. for 30 days, there was a significant reduction in number of parasites in experimentally infected mice as compared with the ABZ treated group.

The use of dihydroartemisinin, another anti-malarial drug, resulted in the morphological changes and loss of viability of protoscoleces. It induced apoptosis by endoplasmic stress-caspase3 pathway *in vitro* (Ma et al. 2020). Artesunate is responsible for damaging the DNA of metacestodes and protoscoleces of *E. granulosus* and this damage is provoked by oxidative stress (Wen et al. 2020). Li et al. (2020) reported that the combined effect of veliparib and artesunate at a higher dose (325 µM) on cysts of *E. granulosus* showed reasonably high scolicidal activity *in vitro* and *in vivo*.

Plant Extracts

Eskandarian (2012) evaluated the efficacy of hydroalcoholic and chloroformic extracts of squash seeds, hazelnuts, and garlic and observed that among these plant extracts the chloroformic extract of garlic had the highest potency (98%) against protoscoleces at a concentration of 50 mg/ml after 20 min of exposure. For *Allium sativum* (garlic), ultrasonic flower extract showed a potent scolicidal activity at concentration of 100 mg/ml at different time intervals (Rahimi-Esboei et al. 2016). In another study, Barzin et al. (2019) found that garlic chloroformic extract possessed a high protoscolicidal effect. The effectiveness of garlic extract was also compared with silver nitrate and sodium chloride at one and two minutes of exposure and it was found that the anti-protoscoleces effect of garlic was higher than that of other chemicals, whereas no difference was noticed between the garlic and sodium chloride effects when the exposure time was increased to 5 minutes.

According to No et al. (2016), ethanolic extract of *Zingiber officinale* (ginger) exhibited a strong activity against hydatid disease after 20 and 10 minutes at concentrations of 30 mg/ml and 50 mg/ml, respectively. Significant scolicidal effect of ginger and eucalyptus (Blue gum) was reported by Faizei et al. (2015); the methanolic extract of ginger and eucalyptus at the concentration of 100 mg/ml killed 100% protoscolices after 40 min of exposure. Ginger extract exhibits high scolicidal activity *in vitro;* the efficacy was 100% at a concentration of 200 mg/ml after half an hour of exposure (Houshmand et al. 2019).

El-Bahy et al. (2019) conducted a study on protoscoleces of camel hydatid cysts by using *Nigella sativa* (black cumin) and *Punica granatum* (pomegranate) extracts. The highest scolicidal activity of *Nigella sativa* was noted at concentrations of 100 mg/ml and 10 mg/ml after 30 and 60 minutes exposure, respectively, while *P. granatum* showed its highest efficacy at a concentration of 100 mg/ml after 120 minutes of exposure. Essential oil of *Curcuma zadoaria* (white turmeric) showed remarkably high scolicidal activity against protoscoleces at 300 and 150 µl/ml concentration and could eliminate parasites

after exposure of 5 and 10 minutes, respectively *in vitro* (Mahmoudvand et al. 2020). Similarly, essential oil of *Cinnamomum zeylanicum* (true cinnamon) and cinnamaldehyde exhibited dose and time-dependent efficacy against protoscoleces, with the maximum efficacy was found at 50 µg/ml of cinnamaldehyde. The viability of protoscoleces decreased after 4 days of treatment and reached 0% on the 8th day (Fabbri et al. 2020).

The methanolic extract of *Myrtus communis* (myrtle) and *Tripleurospermum disciforme* (mayweed) have been found to exhibit scolicidal effects (Barzin et al. 2019). Both of these plant extracts can be given during cyst surgery, as they also inhibit secondary bacterial infections. The maximum scolicidal effects could be achieved at concentrations of 100 mg/ml and 50 mg/ml of *Myrtus communis*. The type and nature of active ingredients present in these extracts is not yet clear (Amiri et al. 2019). Moreover, the combination of pomegranate extract and albendazole possesses anti-inflammatory and anti-hydating effects which help in preventing relapse (Labsi et al. 2019).

Yuan et al. (2019) have reported that anacardic acid, a product obtained from Brazilian cashew nut shell liquid, has higher efficacy than albendazole and dihydroartemisinin against *E. multilocularis* and *E. granulosus* sensu stricto metacestodes *in vivo* and *in vitro*. Ampelopsin, which is extracted from *Ampelopsis grossedentata* (moyeam), has been used for the treatment of different types of diseases like cancer. It also indicated profound efficacy against protoscoleces of *E. granulosus* and metacestodes of *E. multilocularis in vitro* (Xin et al. 2019). *Ferula gummosa* (galbanum) and *Pelargonium roseum* (geranium) oils have activity against scolexes with no side effects (Tabari et al. 2019).

Moudgil et al. (2020) compared the protoscolicidal effect of methanolic extract of three herbs *Ferula asafetida* (dried latex), *Trachyspermum ammi* (fruits), and *Hippophae salicifolia* (leaves) for 20, 40 and 60 minutes interval at different concentrations with ABZ and the extracts of two herbs (*F. asafetida* and *T. ammi*) showed comparable or better results than those of *H. salicifolia* extract. In a recent study, Ranjbar et al. (2020) investigated the scolicidal activity of extract of *Mentha aquatic* (water mint), *M. spicata* (spear mint), *M. longifolia* (Asian mint) and *M. piperita* (peppermint). The results revealed that the activity of methanol extract of *Mentha aquatic* was the highest (99.54%) half an hour after application.

Pestalotiopsis sp. (plant pathogen), which is an endophytic fungus sp. from the Neem plant, showed 97% scolicidal activity due to its bioactive compound responsible for damaging protoscoleces of *E. granulosus* (Verma et al. 2013). Another study performed by Verma et al. (2014) revealed changes in ultrastructures of protoscoleces following their expoxure to an endophytic extract of *Eupencillium* and *Chaetomium* fungi.

Metabolite of *Piper longum* (Pippali) disrupted the ultrastructure of hydatid cysts and its effectiveness was highest in 50 mg/ml within one hour of exposure to the extract (Cheraghipour et al. 2020). Trans retinoic acid was used against protoscoleces at different concentrations including 1.67 and 0.167 µM, and 16.7 nM/L and it was observed that the death of protoscoleces was dose-dependent and occurred within a few minutes to seven days of exposure. The ultrastructural changes observed were disorganization of rosteller, changes in hook shape, and loss of hook (Yones et al. 2014). When protoscoleces were exposed to essential oils of *Pinus nigra* subsp. *pallasiana* (lamb), 100% deaths of protoscoleces were recorded after 60 min of exposure at a concentration of 50 mg/ml (Kozan et al. 2019). Moazeni et al. (2019) carried out a study on live protoscoleces of sheep liver and reported that essential oil (1%) of *Eucalyptus globulus* (blue gum), povidone-iodine (10%), and silver nitrate (0.5%) showed 100% protoscolicidal activity.

Vakili et al. (2019) exposed *E. granulosus* protoscoleces to three concentrations of extract of *Artemisia sieberi* (white wormwood) and observed that the mortality at different time intervals (2, 5 and 10 minutes) with concentration of 75 mg/ml was 80.0, 78.0, and 86.4%, respectively. *Ziziphora tenuior* (kohi purchink) extract killed protoscoleces in 20 minutes at a concentration of 10 mg/ml. By increasing the concentration of the extract, time of exposure was decreased to achieve the desired effects (Shahnazi et al. 2016).

According to Larki et al. (2017), gallic acid at 35 mg/ml concentration resulted in 92.08 and 100% mortality of protoscoleces after 1 and 3 minutes of exposure, respectively. Berberine, which is an active compound obtained from the root extract of *Berberis vulgaris* (barberry), showed 100% inhibition of protoscoleces at 2 mg/ml concentration after 10 minutes exposure (Mahmoudvand et al. 2014). Scolicidal effect of aqueous extract of *B. vulgaris* was observed at a low concentration of 4 mg/ml after 5 minutes (Rouhani et al. 2013). Similarly, carvacrol, which is the main chemical constituent obtained from *Satureja khuzistanica* (Marzeh Khuzestani), has shown 100% scolicidal effect at 10 mg/ml concentration after 10 minutes exposure *in vitro* (Moazeni et al. 2012). When protoscoleces and cysts of *Echinococcus* were treated with carvacrol, maximum scolicidal activity was observed at a concentration of 10 µg/ml and reduction in cyst weight was also observed at a concentration of 40 mg/kg 20 days after treatment in mice (Fabbri et al. 2016).

Thymoquinone is a principal active scolicidal agent found in *Nigella sativa* extract and found to have potent activity against protoscoleces at 1 mg/ml concentration after 10 minutes exposure *in vitro* (Mahmoudvand et al. 2014). Similarly, thymol, gamma-terpinene and p-cymene constitute 50.07, 23.92, and 22.9% of ajowan *(Trachyspermum ammi*) essential oil, and showed the highest scolicidal activity at a concentration of 10 mg/ml after 10 minutes exposure (Moazeni et al. 2012). An experiment conducted by Bahrami et al. (2016) showed that essential oil of *Lepidium sativum* (gardencress) possessed a high level of activity against scoleces.

According to Haleem et al. (2019), trials on experimental animals revealed that plants like *B. wallichiana* (zeresk),

B. vulgaris (barberry), and *E. heliscopia* (umbrella milkweed) are effective against protoscoleces of *E. granulosus* and could be used as a treatment. Similarly, Norouzi et al. (2021) indicated that hydroalcoholic extract of *Taxus baccata* L. (common yew) possessed scolicidal properties and killed 66.6% protoscoleces after 60 minutes exposure at a concentration of 150 mg/ml *in vitro*. Yazdi et al. (2020) found solicidal activity of *Zataria multiflora* (shirazi Thyme) essential oil nanoemulsion and emulsion at different concentrations (1, 2, 5, 10, 15 and 20 µl/ml). Emulsion at a concentration of 20 µl/ml for 15 minutes and nanoemulsion at the same concentration for 10 minutes resulted in 100% mortality of protoscoleces. Protoscoleces were killed by 100% when *Thymus capitatus* (zaatar) essential oil was used at concentrations of 2 and 3 mg/ml after 5- and 1-min exposure, respectively (Hizem et al. 2020).

Methanolic extract of *Rhus coriaria* (sumac) has 98.89 and 100% efficacy against scoleces at a concentration of 30 mg/ml after 10 and 20 minutes, respectively (Moazeni et al. 2012). Youssefi et al. (2020) studied the effect of important phytoconstituents including isofuranodiene, a-bisabolol and farnisol on scoleces and concluded that isofuranodiene possessed the highest activity against protoscoleces, followed by a-bisabolol and farnisol. Osthole, a coumarin derivative from medicinal plant, eliminated protoscoleces by 100% with 120 µM concentration within 3 days *in vitro*. When three infected groups of mice were treated with osthole (100 mg/kg), ABZ (100 mg/kg), and honey/PBS (100 mg/kg) daily for 6 weeks, there was a significant reduction in metacestodes in osthole and ABZ treated groups compared to the control group (Yuan et al. 2016).

Gholami et al. (2013) assessed the effect of methanolic extract of *Sambucus ebulus* (danewort). They used four concentrations of extract 1%, 10%, 50%, and 100% with 5, 10, 30, and 60 minutes of exposure and found highest scolicidal activity *in vitro* after 60 minutes of exposure. The extract of *Satureja khuzestanica* (Marzeh Khuzestani) and *Olea europaea* (olive) leaves showed scolicidal effects on protoscoleces of hydatid cysts, however, the extract of *S. khuzestanica* showed better effect than that of the *O. europaea* (Zibaei et al. 2012). Protoscoleces of *E. granulosus* can also be killed by using propolis (resinous material) at a concentration of 1 µg/ml for 3 minutes *in vitro*; it has no side effects when used intraperitonially (Kismet et al. 2006).

In another study, Jasim et al. (2020) evaluated the bioactivity of leaf extract of *Lepidium sativum* (gardencress) on scoleces of sheep origin and showed that it caused 100% mortality at a concentration of 100 mg/ml after 15 minutes of exposure. Methanolic extract of *Sideritis perfoliata* (cyprus) also showed scolicidal activity (57.9, 71.8, and 79.1%) on protoscoleces of *E. granulosus* at a concentration of 0.4 mg/ml after 10, 20, and 30 minutes, respectively which indicates that scolicidal activity is increased by increasing the exposure time (Çelik et al. 2021). Derakhshan et al. (2017) evaluated the scolicidal activity of *Bunium persicum* (boiss). The rate of dead protoscoleces was 100% at 15 mg/ml concentration after 10-60 minutes exposure *in vitro*. *Tordylium persicum* and fruit of *Citrullus colocynthis* (bitter apple) possessed scolicidal activity in a dose-dependent manner (Sharifi-Rad et al. 2016; Hussein et al. 2019). Similarly, the methanolic extract of *Hymenocarter longiflorus* (Lamiaceae) showed larvicidal effects against *E. granulosus* (Taran et al. 2013).

Anti-fungal drugs

Amphotericin B, an antifungal agent, has been proved to have effective activity *in vitro* against *E. multilocularis* at 2.7 µM concentration (Reuter et al. 2003a; Reuter et al. 2003b; Reuter et al. 2010). As salvage therapy in human patients, amphotericin B has activity against Alveolar Echinococcosis (Reuter et al. 2010) but it is not commonly used due to its nephrotoxic effects (Reuter et al. 2003a; Reuter et al. 2003b).

Anti-cancerous drugs

BI2536, originally designed to inhibit the human ortholog of EmPlk1, can effectively inactivate germinative cells of *E multilocularis* larvae *in vitro* by inactivation of *E. multilocularis* EmPlk1 protein and can be considered a promising compound in the treatment of Alveolar Echinococcosis cases. Direct inhibition of EmPlk1 provokes mitotic arrest and killing of germinative cells (Schubert et al. 2014).

Hemer et al. (2012) revealed the scolicidal effect of anti-cancerous imatinib and it was found to be highly active in killing stem cells, protoscoleces, and metacestode vesicle of *Echinococcus* at a concentration of 25 µM *in vitro*. Stadelmann et al. (2014) suggested that bortezomib, another anti-cancerous drug, had anti-metacestode activity *in vitro* due to inhibition of proteasome of cestodes. Tamoxifen, a non-steroidal antioestrogen, represents a significant advance in treatment of female breast cancer; it may also be used in human *Echinococcus* treatment. The reduction in cyst weight of *E. granulosus* was observed after 3 or 6 months of treatment with 20 mg/kg concentration in mice (Nicolao et al. 2014). Similarly, pyrvinium pamoate completely destroyed the protoscoleces after 5 days under aerobic and 7 days under anaerobic conditions (Enkai et al. 2020).

According to Xin et al. (2020), lonidamine and 6-aminonicotinamide showed remarkable effects against both adult and larval stage of *E. granulosus* and *E. multilocularis in vitro*, and combined drug treatment revealed significantly higher efficacy than the single drug treatment. *E multilocularis* MPK2 activity can be inhibited by ML3403 and SB202190, which are inhibitors of p38MAPKs, cause EmMPK2 dephosphorylation and effectively damage vesicles of parasite at concentrations that do not harm mammalian cells in tissue culture (Gelmedin *et al.* 2008). In another study, the anti-echinococcosis effect of SB202190 was evaluated by using different concentrations (10, 20, 40, and 80 µM) and results indicated dose-dependent death of scoleces *in vitro* (Lv et al. 2013). Egp38, a 368 amino acid MAPK

protein was identified in *E. granulosus* and when protoscoleces were treated with p38- MAPK inhibitor ML3403 *in vitro*, it suppressed Egp38 activity, leading to protoscoleces mortality within 5 days, hence, MAPK Epg38 is believed as a target site for anti-cystic echinococcosis drugs (Lü et al. 2016).

Nano Particles

Mahmoudvand et al. (2014) evaluated the scolicidal efficacy of selenium nanoparticles (SeNPs) prepared from *Bacillus* species Msh-1 and revealed that SeNPs showed scolicidal activity at different concentrations, particularly at 250 and 500 µg/ml, after 20 and 10 minutes exposure, respectively. According to Rahimi et al. (2015), different silver nanoparticle have been used in different concentrations (0.025, 0.05, 0.1 and 0.15 mg/ml) and concentration of 0.15 mg/ml after 2 hours exposure has shown 90% activity against protoscoleces. Another study on the same drug found that the drug was less effective against protoscoleces and eliminated only 71.6% protoscoleces in 1 hour at a concentration of 4 mg/ml, whereas hypertonic saline (20%) eliminated 100% protoscoleces in 10 minutes (Lashkarizadeh et al. 2015).
Nassaf et al. (2019) reported that ABZ-loaded silver nanoparticles showed the highest activity when compared with silver nanoparticles and ABZ alone. There was a marked decrease in the weight of the cyst and size of granuloma with albendazole-loaded silver nanoparticles. In another study conducted by Norouzi et al. (2020), the scolicidal activity of silver (Ag), silicon (Si), copper (Cu), iron (Fe), and zinc (Zn) nanoparticles was compared. Results revealed that Ag-NPs had the strongest protoscolicidal effect (80%) at 1 mg/ml after 1 hour of treatment. Furthermore, Si-NPs at concentration of 1 mg/ml, Cu-NPs at concentration of 0.5mg/ml, Fe-NPs at concentration of 1 mg/ml and Zn-NPs at concentration of 1 mg/ml exhibited 52.33, 41, 28 and 15.67% scolicidal effects, respectively, after 1 hour exposure *in vitro*.
Cerium dioxide and *Holothuria leucospilota* are also found to show effective scolicidal activity when used separately or in combination. Studies have shown that *H. leucospilota* extract showed 70% scolocidal effect at a concentration of 20 mg/ml after 1 hour of exposure, followed by the combination (63%) at a concentration of 15 mg/ml after the same time (Aryamand et al. 2019). Ibrahim et al. (2020) evaluated zirconium dioxide nanoparticles at 1000, 2000, and 4000 µg/ml concentration. After 1 hour, it damaged 49.6, 52.7, and 53.1% of scoleces. Napooni et al. (2019) found that gold nanoparticles possessed the strongest scolicidal effect at a concentration of 4000 µg/ml after 1 hour of treatment. According to Barabadi et al. (2017), gold nanoparticles, formed by a green process through utilizing *Penicillium aculeatum,* showed potent scolicidal activity. Different concentrations (0.05, 0.10, 0.20 and 0.30 mg/ml) were used for 10, 30, 60 and 120 minutes and variation between scolicidal effects was significant statistically at different concentrations and different time intervals. Gold nanoparticles effectively killed protoscoleces at a concentration of 1 mg/ml after 60 minutes (Malekifard, 2017).
The highest scolicidal activity of copper nanoparticles is reported at a concentration of 750 mg/ml *in vitro* and copper nanoparticles killed 73.3% protoscoleces after 60 minutes of treatment. The maximum mortality is reported when copper nanoparticles are given along with ABZ at a concentration of 750 mg/ml and 200 mg/ml, respectively for 10 minutes exposure time (Ezzatkhah et al. 2021). Silver-copper nanoparticles (core-shell) have a dramatic adverse effect on the vitality of protoscoleces compared with ABZ. Even the lowest concentration of 50 mg/ml showed a significant effect. The killing rate was 100% at the concentration of 500 mg/ml of nanoparticles (Aljanabi et al. 2021).
In a recent study, Navvabi et al. (2019) indicated that the scolicidal effect was 84% when gonad extract of sea urchin was used in combination with titanium dioxide at 15 µg/ml concentration for 60 minutes exposure time *in vitro*. When gonad extract and titanium dioxide nanoparticles were given orally in infected mice for three months, significant reductions in size, volume and weight of hydatid cysts were observed. Napooni et al. (2019) suggested that nano-particles of chitosan having curcumin (Ch-Cu NPs) could be regarded as an anti-protoscolex drug that showed good efficacy against *E granulosus*.

Chemical solutions

Hypertonic saline was evaluated by Adas et al. (2009) for its scolicidal activity. A comparison of effectiveness of hypertonic saline with ABZ sulfone and ABZ sulfoxide combinations was also made. The results showed that hypertonic saline possessed similar efficacy as shown by ABZ combinations used in the comparative study. Hosseini et al. (2006) also conducted a comparative study to evaluate the activity of hypertonic glucose with silver nitrate (0.5%), cetrimide (0.5%), and hypertonic saline (20%) against protoscolices of *Echinococcus*. It was observed that 50% hypertonic glucose had more scolicidal activity than 0.5% silver nitrate but fewer efficacies were found when compared with cetrimide (0.5%).
Honey, which has antibacterial activity as well, was used by Kilicoglu et al. (2006) in order to determine its effects on protoscoleces. Different concentrations (1, 5, 10, 25, and 50%) of honey were used for the treatment of protoscolices *in vitro*. All protoscoleces were killed at 10% or higher concentrations, indicating anthelmintic potential of honey.
Topcu et al. (2009) studied the effect of chlorhexidine gluconate on hydatid cyst during surgery of a patient suffering from Cystic Echinococcosis. It was found that all protoscoleces were killed with 0.04% chlorhexidine-gluconate solution after 5 minutes of intra-cystic injection. In another study, commercial chitosan was found to show high level of deacetylation and was proven to have anti-scoleces activity *in vitro*. It was also noted that chitosan from fungus could also be used as effective as commercial chitosan for the treatment of hydatidosis (Rahimi-Esboei et al. 2013).

Zeghir-Bouteldja et al. (2009) studied the effect of peroxynitrite, nitrite, and nitrate on the viability of protoscoleces. Peroxynitrite and nitrite damaged the germinal layers after 24hr and 3hr when used at the concentrations of 320 and 80 µM, respectively. Kuster et al. (2013) suggested that dicationic diguanidino
drugs having thiophene core groups were effective against metacestodes of *E. multilocularis*.
Auranofin (MMV688978) is a thioredoxin-glutathione reductase inhibitor, and shows anti-protoscoleces activity against metacestodes of *E. multilocularis*, and *E. granulosus* (Bonilla et al. 2008; Ross et al. 2012). Good efficacy was reported after 48 hrs of administration *in-vitro* (Saiz et al. 2014; Salinas et al. 2017).
In another study, Aydin et al. (2012) exposed Echinococcocus protoscoleces to taurolidine in petri plates. All protoscoleces were killed in 90 minutes. Moreover, administration of taurolidine *in vivo* also showed significant results. Ekçi et al. (2010) reported that when mice were treated with polyvinylprolidone iodine (1%) and taurolidine (2%) for 2 and 5 minutes, polyvinylprolidone seemed to have strong activity as compared with taurolidine. According to Abdulkareem et al. (2020), ozonated saline solution has the potential to kill scoleces *in vitro* (100%) and can be used during hydatid cyst surgery *in vivo* without any fear of toxicity because ozone is converted into oxygen afterwards.

Other options

The venom of *Androctonus crassicauda* (scorpion) was used against protoscoleces and the efficacy was found to be 100% at a concentration of 100 µg/ml after 240 minutes of exposure (Al-Malki et al. 2020). Likewise, octenidine dihydrochloride (used for skin, mucous membrane and wound antisepsis) has been found to have strong efficacy against scoleces at a concentration of 0.1% *in vitro* after 15 minutes (Ciftci et al. 2007).
Treatment with interleukin IL-17A at various concentrations including 100, 125 and 150 pg/mL *in vivo* stopped metacestode growth by 72.3, 93.8, and 96.9%, respectively (Labsi et al. 2018). Metformin, which is an antihyperglycemic and antiproliferative agent, induced dose-dependent killing of stem cells and protoscoleces *in vitro*. When it was given orally at a concentration of 50 mg/kg per day for 8 weeks, it caused a significant reduction in parasite weight and suppression of Em-TOR in Alveolar Echinococcosis models (Loos et al. 2020). Shi et al. (2016) treated protoscoleces with chenodeoxycholic acid at different concentrations (500, 1000, 2000, and 3000

Compound	Disease	Experiment Setting	Dosage	Treatment Duration	Efficiency Assessment	References
Albendazole + Sodium Arsenate	CE	In vitro	80µM +20µM	48 hr	100%	Xing et al., 2019
Albendazole + povidone Iodine	CE	In vitro	100 µg/ml + 1/10	15 min	100%	Polat et al., 2009
Praziquantel + Albendazole	CE	In vivo	40mg/kg + 800mg/day	Once a week	Significant	Jamshidi et al., 2008
Triclabendazole	CE	In vitro	25 µg/ml	13 days	Significant	Richter et al., 2013.
Ivermectin Nano-lipid carrier	CE	In vitro	800 µg/ml	1 hr	100%	Ahmadpour et al., 2019
Nitazoxanide	CE	In vitro	10 µg/ml	7 days	Significant	Reuter et al., 2006
Artemisinin or Praziquantel + Atovaquone	CE	In vitro	50 µM	7 days	Significant	Enkai et al., 2021
Pyronaridine	CE	In vivo	57 mg/kg (IP)	3 days	100%	Li et al., 2020
Imatinib	CE	In vitro	25 µM	7 days	Significant	Hemer et al., 2012
Tamoxifen	CE	In vivo	20 mg/kg	3 months	Significant	Nicolao et al., 2014
Pyrvinium pamoate	CE	In vitro	50 µM	5 days (Aerobic)	100%	Enkai et al., 2021
SB202190	CE	In vitro	80 µM	4 days	95%	Lv et al., 2013
Selenium nanoparticles	CE	In vitro	500 µg/ml	10 min	Significant	Mahmoudvand et al., 2014
Silver nanoparticle	CE	In vitro	0.15mg/ml	2 hr	90%	Rahimi et al., 2015
Hypertonic Saline	CE	In vitro	20%	10 min	100%	Lashkarizadeh et al., 2015
Gold nanoparticles	CE	In vitro	4000 µg/ml	1 hr	Significant	Napooni et al., 2019
Copper nanoparticles + Albendazole	CE	In vitro	750mg/ml + 200mg/ml	10 min	Significant	Ezzatkhah et al., 2021
Gonad extract of Sea Urchin + Titanium dioxide	CE	In vitro	15 µg/ml	60 min	84%	Navvabi et al., 2019
Garlic chloroformic extract	CE	In vitro	50 mg/ml	20 min	98%	Eskandarian 2012
Piper longum extract	CE	In vitro	50 mg/ml	1 hr	Significant	Cheraghipour et al., 2020
Eucalyptus extract	CE	In vitro	100 mg/ml	40 min	100%	Faizei et al., 2015
Carvacrol	CE	In vitro	10 mg/ml	10 min	Significant	Moazeni et al., 2012
Thymoquinone	CE	In vitro	1 mg/ml	10 min	Significant	Mahmoudvand et al., 2014
Curcuma zadoaria essential oil	CE	In vitro	300 mg/ml	5 min	Significant	Mahmoudvand et al., 2020
Thymus capitatus essential oil	CE	In vitro	3 mg/ml	1 min	100%	Hizem et al., 2020
Androctonus crassicauda (Scorpion)	CE	In vitro	100 µg/ml	240 min	100%	Al-Malki et al., 2020
Metformin	AE	In vitro	50 mg/kg	8 weeks	Significant	Loos et al. 2020
FBG	CE	In vitro In vivo	20%	8 hr	98%	Rostami et al., 2015

µmol/L) and recorded mortality of protoscoleces and changes in morphology like loss of hooks, microtriches destruction, stroma region contraction, blebs, and lipid droplets formation. Fluoride containing bioactive glass (FBG) is a novel scolicidal agent used to prevent post-surgical infections particularly of hydatidosis. FBG damaged 98% protoscoleces with 20% fluoride after 8 hours of treatment. By increasing the concentration of bioactive glass and fluoride ratio, scolicidal activity was also increased (Rostami et al. 2015). A recent study (Wang et al. 2021) demonstrated the effect of galactosidases on metacestodes of *E. multilocularis in vitro* and revealed that the metacestode vesicle of the parasite was unable to infect mice after glycan digestion induced by galactosidases.

Knocking down of EgRad54 gene and treatment combined with harmine and harmine derivatives, DNA damage of *E. granulosus* was enhanced through down-regulation of Topo2a and Rad54 and up-regulation of H2A and ATM which eventually inhibited growth of *E. granulosus* (Gong et al. 2021). Signaling inhibitors, including CI-1033, U0126 and BIBW2992, showed activity against protoscoleces and metacestodes of *E. multilocularis in vitro* by changing the ultrastructure of metacestode. CI-1033 and BIBW2992 caused apoptosis of metacestodes and germinal cells. U0126 and BIBW2992 exhibited efficacy against *E. multilocularis* (Cheng et al. 2020). Among the seven mTOR inhibitors, the tacrolimus (TAC) was found to be more effective than other inhibitors against hydatid cyst *in vitro* and also there was an effective reduction in weight and number of the parasitic cyst at a concentration of 4 mg/kg/day *in vivo* (Muhedier et al. 2020). Chen et al. (2020) studied the effect of hMASP-2 DNA-nanolipoplexes against Cystic Echinococcosis and reported that they induced degradation of germinal layer cells and up-regulated T-cell immunity in mice, therefore, could be used as an alternate for Cystic Echinococcosis treatment.

Conclusion

A huge research work on treatment protocols for Cystic Echinococcosis has been done *in vitro*. As the location of the cyst, its size and type determine the response to treatment, there is a dire need of conducting investigations on standardization and drug therapy duration *in vivo*. Studies have explored that some drugs have abilities to kill the parasite *in vivo* and more research is necessary to discover new drugs/chemical agents which have high anti-*Echinococcus* activity *in vivo* for the treatment of clinically ill patients.

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