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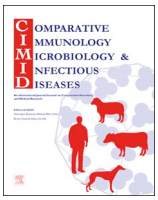

# *Zataria Multiflora bois* as an auspicious therapeutic approach against *Echinococcus granulosus*: Current status and future perspectives

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ABSTRACT

Hydatidosis caused by *Echinococcus granulosus* is a major zoonotic diseases. In addition to imposing heavy economic losses, the disease is a public health problem worldwide. The larval stage of the parasite (hydatid cyst) is formed in a wide range of domestic, wild and human beings as intermediate hosts. On the other, its recurrence has been reported anywhere as a reemerging disease. Although the cysts have some evading mechanisms, both human TH1 and TH2 cells subsets are stimulated. Because of increasing resistance and adverse effects of medications such as abnormalities of liver and other organs functions and abdominal pain, seeking alternative therapeutic approaches to be inexpensive, easy available, with low side effects and toxicity seems essential. However, the lack of information on the social and economic welfares of herbal medicines for the industrial scale application is a limitation. *Zataria Multiflora bois* (*ZMB*) has exhibited huge advantages and tremendous protoscolicidal effects as demonstrated by numerous studies and its combination therapies with anti-parasitic drugs have exerted desirable outcomes *in vitro* and *in vivo*. Noticeably, the compound confers negligible side effects or toxicity even at high concentrations. ZMB has exhibited promising inhibitory effects against hydatid cyst, particularly when combined with chemical drugs and in formulations of nanoemulsions. Its immunomodulatory effects include increase of nitric oxide production (NO) and protection of hepatic cells (Kupffer cells, fat-storing cells, and endothelial cells), enhancement of macrophages and T cells and increase of cytokines production. This study aimed at assessment of ZMB traits for application against hydatid cyst protoscolices.

## 1. Introduction

Hydatid cyst is typically a rural and occupational disease, since particular human activities, such as dogs feeding with the viscera of slaughtered livestock is associated with the increase of contamination. Contamination with the adult worm eggs from the carnivores leads to the infection initiation in the intermediate hosts (donkeys, hogs, sheep, deer, etc.) [1]. In the life cycle of this parasite there are two host mammals, the first host, Canideae family, where the adult worm lives in the small intestine of this animal. The adult worm being less than 7 mm lays eggs into the terminal segment forms to the environment contaminating there broadly [2]. Each egg is spherical with a size of 30–40 μm and contains an embryo with six hooks (known as oncospheres). Parasite uptaking may also initiated following consumption of food or water contaminated with infectious eggs through direct contact or environmental contamination [3]. After eating eggs by the host, the hydatid cysts are formed in the internal organs, especially the liver and the lung. Structurally, hydatid cyst contains liquid and consists of a cyst with a non-cellular outer layer and an interior germinal layer which produces capsules, in which protoscolices grow. Each of the protoscolices in the cyst has the ability to become an adult parasite. While adult worms in carnivores (definitive hosts) exert very low pathogenicity, hydatid cysts in intermediate hosts cause huge complications and economic and health problems [4]. The disease is commonly observed in 65% of people without clinical signs and not distinguishable from any other diseases with similar signs. Clinical symptoms depend on the involved organ (lung, liver, brain, bone, etc.), the number and size of cysts, their location, the distribution of cysts into adjacent organs, and the induction of immunological reactions such as asthma and anaphylaxis [5].

Human echinococcosis is caused by ingestion of parasite eggs, which are dispersed from the final host stool in the environment. At the

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initial stage of echinococcosis, small, encapsulated cysts are formed without clinical signs and 5 days after ingestion, a small cyst sizing 60 to 70 microns containing a cellular (granular) layer and a non-cellular outer layer is formed. There is evidence that liver cysts have a slower growth rate compared to pulmonary cysts, although the growth rate of cysts in various organs and even persons and in different regions is different. The growth rate of cysts varies from 1 to 5 cm in diameter per year. These cysts are slowly growing and host granulomatous reaction occurs, in which fibrous tissue and connective tissue form around the cysts. The size of the cysts varies, mostly reaching 1–15 cm, but cysts with a size of 20 cm may also be formed [6]. The time required for the formation of protoscolices in the cyst in humans is not known, but is occurred possibly more than 10 months after the infection. Protoscolices are usually formed in cysts regardless of limited size. Accordingly, there is no relation between fertility and the size of the cysts [7]. In some cysts, protoscolices are not produced and thus they are sterilized. Hydatidosis therapy is accomplished based on three approaches including surgical procedure, puncture, aspiration, injection, re-aspiration (PAIR) technique, and chemotherapy. The surgery is the most reliable and effective strategy (preferred from other approaches) performed in 90% of cases to completely eliminate cyst in patients, but there may be side effects and risks such as death or recurrence of the cyst after surgery. In the PAIR technique, the contents of the hydatid cyst are aspirated first, then a protoscolicidal substance is injected into the cyst for 30 min and eventually aspirated [8]. This technique also increases the possibility of contamination with protoscolices during process. Eventually, chemical drugs have some drawbacks such as side effects, high costs and resistance to anti-parasites.

At the beginning of the infection there are no symptoms. Small cysts and those without growth, calcium and encapsulated cysts do not cause specific pathological effects, and the patient suffers from illness for years without clinical signs. Sonographic studies have demonstrated that the average cyst size is less than 4 cm in the infected patients without clinical symptoms, while in patients with clinical symptoms reaches up to 10 cm [9]. Echinococcosis symptoms are variable and mostly affect the limb. The slow growth of cysts is often well tolerated as long as their size does not impair function of the organs. If the cysts be ruptured following the pressure, the symptoms of the infection will appear. In addition, the recurrence of post-surgical disease may occur after the protoscolices release and dissemination [10].

Following oral uptake of eggs by humans, an oncosphere larva is released from the egg and penetrates the intestinal lamina propria, distributing into the blood and lymph vessels through which it is transported to liver, lungs and other organs, where oncosphere larvae can develop into metacestodes (also known as hydatid cysts). The growth of cysts is associated with a host immune reaction and the thus development of a fibrous layer, called the pericyst. Some cysts may have tumor-like, infiltrative behavior, leading to tissue destruction and eventually organ failure [11]. Firstly, a cell-mediated immune response involving macrophages, neutrophils and eosinophils is established. During the first week, antibodies are detectable with early significant enhancement of IgE, IgG2 and IgG4 levels [12]. Allergic reactions following IgE response mostly include itching, urticaria and anaphylactic shock. both TH1 and TH2 responses are exerted which cause increase of cytokines, especially interferon-γ (IFN-γ) and also TH2 cytokines, such as IL-4, IL-5 and IL-6. However, TH1 has a higher role in the eradication of cysts following chemotherapies. For some cysts, macrophages, T-lymphocytes and myofibroblasts increase. It is worth considering that cysts employ several mechanisms to evade the immune system. The applications of some herbal medicines have exerted proliferator effects on immune responses.

Early diagnosis of cystic and alveolar echinococcosis has a significant impact on the management and treatment of the disease. Often the early stages of infection are unchecked, so using inexpensive and quite simple diagnostic approaches is necessary to find a population at high risk. The definitive diagnosis of most cases of cystic and alveolar echinococcosis in humans is carried out using physical imaging techniques such as radiology, ultrasonography, CT scan and magnetic resonance imaging (MAV), although this equipment is not readily available in less developed communities [13]. Diagnosis using immunological approaches is useful not only in the initial diagnosis, but also for subsequent post-surgical or drug therapies [14]. The determination of the common antigens of *E. granulosus* in the serum, though a selective approach, is less sensitive in contrast to antibodies. ELISA, indirect hemoblotting antibody assays, Latex agglutination test, and immune-blotting tests are commonly used diagnostic approaches based on safety tests being used extensively [15].

Hydatid cyst fluid materials are the common source of antigenic agents in immune diagnosis. In addition, lipoprotein B antigens and antigens 5 are important hydatid cyst fluid compounds that are widely used in immunological tests to detect echinococcosis cysts. The application of both types of antigens is limited to its scientific applications and, in general, none are available for general applications. In addition, there are problems with their low sensitivity and specificity and standardization for consumption. Cross-reactivity with other parasitic cysts is a drawback associated with their use, limiting the serological tests for low sensitivity and specificity [16]. However, serum antibody activity is used to determine the infection at the herd level. This test may be useful in screening for hydatidosis, especially when cysts are smaller. Antibodies effective against hydatid cyst fluid antigen can be detected after 4 weeks of exposure to the parasite. The application of antigen B in an enriched hydatid cyst fluid has more than 90% sensitivity. However, this technique is also associated with problems caused by cross-reactivity with *Tinea hydatigena, Tinea ovis,* and *Fasciola hepatica*. In countries where the disease is endemic, in order to overcome this cross-reactivity, the identification of novel antigens that are prominent will be of great importance. It should also be noted that 10–20% of patients with liver cysts and 40% of patients with pulmonary cysts do not produce specific antibodies to be detectable in the serum, resulting in false negative reactions in their serological tests. Also, patients with cysts in the brain, bones, eyes, or those with calcite cysts often develop antibody responses either at low levels in the serum or do not respond. At least two tests are necessary to obtain the desired outcomes of these patients in their common tests [17]. The choice of a serological diagnostic test usually depends on the sensitivity and specificity of the test. The first problem with most common tests is the high percentage of false negative results (over 25%). Moreover, false-positive reactions are observed in areas with co-contamination of both *E. granulosus* and *E. multilucolaris*. Therefore, rapid, efficient and highly sensitive tests should be developed [18]. This study aimed at assessment of ZMB traits for application against hydatid cyst protoscolices.

## 2. Methods

For this systematic review, the words “Hydatidosis”, “hydatid cyst”, “*Zataria Multiflora Bois*”, “therapy” and “zoonosis” were searched in valid searching engines such as PubMed, Medline, SCOPUS, Emabase and also Persian publications. Inclusion criteria included every study which had evaluated the effect of ZMB in vitro and in vivo. Studies in which chemotherapy had been investigated were excluded. Furthermore, we did not use meta-analysis because of variety in the samples and methods in studies. The duplicate records and those deemed not relevant after perusal of title and abstract were removed. Eventually, full texts were screened and eligible publications were included and reviewed.

## 3. Results

A total of 258 studies were found by searching the keywords. After study of abstract of them and further evaluation, 29 studies met the criteria. The results of reviewed studies have been included in this systematic review.

The plant grows mostly in tropical areas such as Iran, Afghanistan and Pakistan. The Lamiaceae family is one of the largest plant families with an approximately global distribution, with 200–250 genera and about 5000 species [19]. Some plants belonging to this family have anti-microbial activities. Thyme name is used for a group of plants belonging to the mint family. ZMB locally known as the Shirazi thyme is highly adapted to the humans and animals and contains various properties [2,20]. This plant is safe or non-toxic for the embryo digestive system in pregnant Balb /C mice. It also stimulates innate and acquired immune system in laboratory animals. Based on the compilation and study of the methanolic extract of ZMB, it is very effective in eliminating hydatid cyst protoscolices over short periods of time and in appropriate concentrations. This extract at concentration of 10 mg /ml eliminated 68.9%, 93.7% and 100% of hydatid cysts after 2.1 and 3 and 20 min, respectively *in vitro*. Also, the concentration of 25 mg /ml could eliminate 100% of protoscolices after 1 min [21]. The effect of its methanolic extract (at a concentration of 8 g /L for 30 days) and commercial efficacy of ZMB (40 cc /L for 30 days and 4 g /L in drinking water for 8 months) has been confirmed in prevention of hydatid cyst formation in experimental mice.

Shokri et al. Studies demonstrated that ZMB can significantly stimulate the function of the body's innate immunity, and significantly increase phagocytosis and TNF-α secretion on days 4 and 7 after intraperitoneal injection of essential oil in comparison with the control group [22]. It was also observed that ZMB extract plays an important role in inhibiting muscarinic receptors of smooth muscle affected by propranolol. This is due to the stimulating effect of the β-adrenergic receptor that acts as a histamine receptor antagonist. Previous in vitro studies have revealed the scolicidal effects of methanolic extract of ZMB [23]. In addition, *in vivo* studies during recent years have revealed the therapeutic effects of methanolic extracts of ZMB on hydatid cyst [24]. Noticeably, in order to increase the therapeutic effects of albendazole on hydatid cyst, simultaneous administration of this drug and other herbal medicines and chemical agents with scolicidal properties have revealed significant efficiency enhancement in comparison to its single application. It has been also observed that combination therapy using albendazole and ZMB exerts higher scolicidal effects against cysts. In addition to anti-oxidant and immuno-stimulatory effects, ZMB has protective effects to liver cells [25–31].

## 4. Discussion

Based on the opinion of the European Medicines Agency (ECHA), thymol, as the main ingredient in the ZMB essential oil, has not shown any chronic or teratogenic side effects (harmful to the fetus) in previous studies and can be considered as safe alternative compound. As mentioned earlier, carvacrol is another essential ingredient found in the ZMB essential oil. Carvacrol's eliminatory effect on the *E. granulosus* larval stages has recently been demonstrated *in vivo* and *in vitro* studies [32]. Recently, the essential oil (EO) of *Thymus capitatus in vitro* exhibited that of seven fractions carvacrol was the major compound exhibiting low toxicity to mammalian cells and high toxicity against *E. multilocularis* metacestodes and germ line based on Alamar Blue, as well as transmission electron microscopy [33]. On the other hand, the mean lethal dose (LD50) of carvacrol after oral administration in mice was 919 mg /kg [34], hence carvacrol is considered to be safe or non-toxic as a healthy food supplement. It is also worth mentioning that the nanoemulsion of ZMB essential oil, especially when used with Albendazole, was revealed a significant effect on the treatment of hydatidosis. Nanoemulsions can be more easily applied to the target organ due to the small size of their particles. High stability, more water solubility and higher ability to delivery to biological membranes are other advantages of nanoemulsions [35,36]. On the other hand, previous studies have revealed that the total essential oil and its compounds may have a higher antimicrobial effects than any of its major or minor compounds, and may have synergistic effects with other chemical compounds. It is necessary in the context of the harmlessness of concomitant administration of albendazole with nanoemulsion of the ZMB essential oil on the Liver cells and biliary system. More intraocular studies are needed in animals. Therefore, ZMB has demonstrated incredible effects against hydatid cyst protoscolices. Its immunomodulatory effects include increase of nitric oxide production (NO) and protect of hepatic cells (Kupffer cells, fat-storing cells, and endothelial cells), enhancement of macrophages, T cells and increase of cytokines production. However, more studies are needed to completely clarify ZMB immunomodulatory effects against hydatid cysts. Several suggestions regarding application of ZMB for parasitic infections in future studies are offered such as A) Evaluation of the effect of long-term consumption of ZMB on changes in liver enzymes and some blood factors in laboratory animals (pharmacokinetics); B) Whole blood assay (WBA) following long-term consumption of ZMB singly and combined with chemical drugs; C) The effect of long-term consumption of nanoemulsion of ZMB essential oil on changes in liver enzymes and some blood factors in laboratory animals; D) The effect of long-term application of nanoemulsion on essential oil, along with Albendazole, on changes in liver enzymes and some blood factors in laboratory animals; F) Evaluation of the effect of nanoemulsion of the high values essential oils (20–50 cc/l) with albendazole various doses (50–150 mg/kg) for the treatment of hydatidosis in laboratory animals; G) Examination of ZMB and its formulations in clinical trials; H) Combination therapy of ZMB with other antiparasitic drugs; I) Application of nanoemulsions and nanocarriers for efficient and specific cyst therapy; J) Vaccine designation for hydatid cyst antigenic structures

## 5. Conclusion

Chemotherapy of hydatid cyst has had adverse events in terms of toxicity and infection recurrence. Hence, application of alternative compounds formulations for its eradication seems essential exerting promising insight for future studies. ZMB has exhibited promising inhibitory effects against hydatid cyst, particularly when combined with chemical drugs and in formulations of nanoemulsions. Additionally, ZMB immunomodulatory effects include increase of NO and protect of hepatic cells (Kupffer cells, fat-storing cells, and endothelial cells), enhancement of macrophages, T cells and increase of cytokines production. Owing to the both Th1 and Th2 responses against hydatid cyst protoscolices, their stimulation with ZMB is a promising insight. In addition, lack of ZMB cell toxicity on host body organs even at high concentrations and hepato-protective properties is worth considering, highlighting its safety as an efficient herbal medicine. However, more investigations are necessary to achieve efficient therapeutic approaches applying ZMB formulations.

## Declaration of Competing Interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial in the subject matter or materials discussed in this manuscript.

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