



Therapeutic Effect of Hydatid Cyst Liquid on Melanoma Tumor Growth in Mouse Model

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Authors' contributions

This work was carried out in collaboration between all authors. Author HYD contributed in designing the study, performed the experimental work and wrote the first draft of the manuscript. Author SMS designed the study, supervised the research project and wrote the manuscript. Author FM was cancer consultant of the project. Author MY contributed in writing the manuscript. Author SAS helped with data analysis. Author RJ contributed in doing experimental works. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Hydatid cyst is the larval stage of *Echinococcus granulosus* which develops in various human and livestock animals tissues. This hydatid cyst composed of several layers. Previous investigations have shown that the prevalence of hydatid cyst in cancer patients is significantly lower than in healthy individuals. In animal models preventive effect of the hydatid cyst antigens in reduction of tumor growth has been shown. Due to the effect of live protoscolex and

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hydatid cyst antigens on reducing the growth of cancer cells in culture medium, in this study therapeutic effect of hydatid cyst liquid on the growth of melanoma tumor in mice (C57 black) has been investigated.

Methods: In this Experimental study, the population studies were C57 black mice. Six groups of mice were injected with 10^6 melanoma cells in their chest site subcutaneously. Then the mice were injected with hydatid cyst fluid either with or without albendazole. From the second week, the tumor size was measured using a calipers and tumor area was calculated and the mean tumor area compared with those of control groups using One-way ANOVA test.

Results: The mean tumor area, in groups which injected with hydatid cyst fluid (case groups), was less than the mean tumor area in the control groups and this difference was statistically significant.

Conclusion: This study showed that hydatid cyst fluid may have therapeutic effect on melanoma cancer growth.

Keywords: Melanoma; hydatid cyst fluid; C57 black mouse.

1. INTRODUCTION

Hydatid cyst is the larval stage of *Echinococcus granulosus* that is formed in various human and animal tissues. This parasite has different genotypes [1]. Spherical hydatid cyst outwardly consisted of laminated and germinal layers which are filled with a liquid. The cyst germinal wall produce larval stage called protoscolex. Hydatid cyst fluid, is clear liquid with a specific weight of 1007 to 1015, which contains albumin, protein, phosphate salts, calcium sulfate, succinate and some sugar substance [2]. Anti-cancer properties of different parasites particularly hydatid cysts have shown in various studies [3-10].

In recent years, several researchers have reported a negative association between some parasitic infections and cancer. Statistical results also showed a negative relationship between cancer and the prevalence of parasitic infections [10]. In a retrospective study with a large population the prevalence of hydatid cysts in patients with cancer was significantly lower than that of control group [11]. It has been shown that protoscolex of hydatid cysts in cell culture medium, increased cell death and decreased living Fibrosarcoma cells [6]. It was also reported that immunization with various antigens of hydatid cyst especially cyst fluid resulted in reducing of the growth of living Hela cells in culture medium and increasing of the dead Hela cells [3]. Also it has been shown that immunization with the cyst fluid lead to decrease colon cancer growth in animal models [12]. In another study it was shown that size of melanoma tumor in the C57 / Black mice that received live protoscolex was significantly smaller than that of control group [9]. Also the melanoma tumor size of mice that had been immunized with cyst fluid and excretory and secretory antigens were smaller than that of

control mice [13]. In another study it has shown that patient's serum with hydatid cyst has toxic effect on lung cancer cells in vitro [14]. Finally it has been shown that certain peptides of *E. granulosus* have anti-cancer properties [15].

The protective effect of hydatid cyst fluid in cancer inhibition has been shown in animal models in the previous investigations [6,13], so in this study therapeutic effect of hydatid cyst fluid on melanoma tumor growth in animal models has been studied.

2. METHODS

In this Experimental study, the populations study were C57 black mice. To prepare the antigens, hydatid cyst fluids were aspirated from liver and lung of livestock and then examined for the presence of protoscolex. The hydatid cyst fluid was centrifuged for 2 minutes (2000 rpm) and then the supernatant was stored at -20°C as hydatid cyst fluid antigen. Melanoma cancer cells were purchased from Pasteur Institute of Iran and were cultured in CO₂ incubator. The cancer cells in culture flasks were harvested and washed. Each group of mice was injected with a million of cells subcutaneously in chest area using insulin syringe.

In the first experiment mice that received cancer cells were divided into 3 groups as following: The first group received hydatid cyst fluid and alum as adjuvant intraperitonally and albendazole orally. The second group treated like the first group except they did not receive albendazole. The third group left intact as control.

In the second experiment mice that received cancer cells were divided into 3 groups as following: The first group received hydatid cyst fluid and alum as adjuvant subcutaneously in

tumor margin and albendazole orally. The second group treated like the first group except they did not receive albendazole. The third group left intact as control.

Albendazole was used with dose of 1200 mg daily for 14 days and then tumor diameters were measured every day with calipers. Tumor area was calculated and the mean tumor area in different group compared with control group using One-way ANOVA test.

3. RESULTS

The results of the tumor area measurement in the first and second tests are shown in Figs. 1 and 2 respectively. In the first experiment, all mice that received cyst fluid intraperitoneally with albendazole died and the mean tumor area in the group that received cyst fluid intraperitoneally without albendazole was smaller than that of control group and the difference was statistically significant ($p < 0.05$).

The results of the first experiment have been presented in Table 1. In the second experiment, the means tumor area in the group received cyst fluid via tumor margin with and without albendazole was also smaller than that of control group and the difference was statistically significant ($p < 0.05$). The results of the second experiment have been presented in Table 2.

4. DISCUSSION

The results of this study showed that hydatid cyst fluid injection (peritoneal or tumor margin) into C57 black mice with melanoma tumor resulted in decreasing tumor size. Tumor size in mice that received cyst fluid into the Peritoneum and also tumor margins was significantly smaller than that of control group. This anticancer effect of the hydatid cyst fluid may be due to the presence of common antigens between cyst fluid and melanoma cells. Presence of common antigens between this parasites and some cancer has been shown in previous investigations [10].

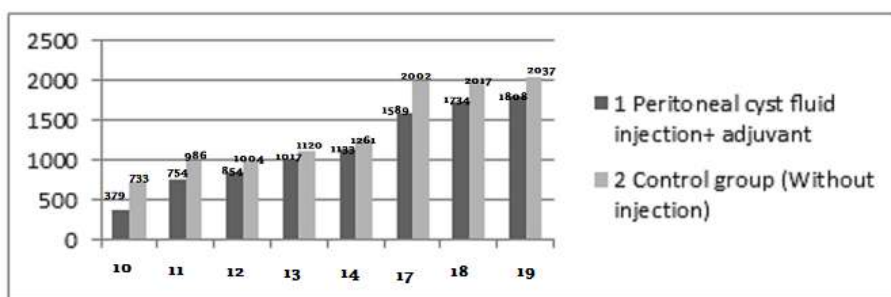


Fig. 1. The results of the tumor area measurement in the mice that received 10^6 melanoma cells and then injected with hydatid cyst fluid and alum as adjuvant interperitoneally (case group) in comparison with control group which received melanoma cells without any treatment 10-19 day following cancer cell injection

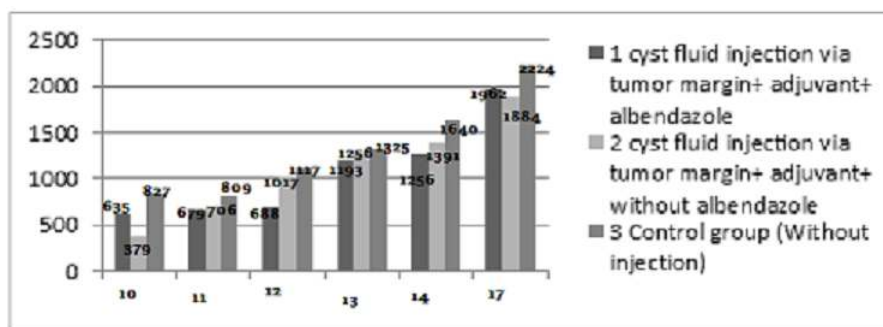


Fig. 2. The results of the tumor area measurement in the mice that received 10^6 melanoma cells and then injected with hydatid cyst fluid and alum as adjuvant in tumor margin with or without albendazole (case group) in comparison with control group which received melanoma cells without any treatment 10-17 day following cancer cell injection

Table 1. The results of the mean tumor area (mm²) of mice that received cyst fluid interperitounly (with or without albendazole) in comparison with control group which received melanoma cells without any treatment 10-19 days after melanoma cell injection

Groups	10 days after melanoma cell injection	11 days after melanoma cell injection	12 days after melanoma cell injection	13 days after melanoma cell injection	14 days after melanoma cell injection	17 days after melanoma cell injection	18 days after melanoma cell injection	19 days after melanoma cell injection
1 Peritoneal cyst fluid injection+ adjuvant+ albendazole	-	-	-	-	-	-	-	-
2 Peritoneal cyst fluid injection+ adjuvant+ without albendazole	379.94	754.86	854.86	1017.36	1133.54	1589.62	1734	1808.64
3 Control group (Without injection)	733.34	986.418	1004.58	1120.78	1261.20	2002.53	2017.18	2037.07

Table 2. The results of the mean tumor area (mm²) of mice that received cyst fluid via tumor margin (with and without albendazole) in comparison with control group which received melanoma cells without any treatment 10-17 days after melanoma cell injection

Groups	10 days after melanoma cell injection	11 days after melanoma cell injection	12 days after melanoma cell injection	13 days after melanoma cell injection	14 days after melanoma cell injection	17 days after melanoma cell injection
1 Cyst fluid injection via tumor margin+ adjuvant+ albendazole	635.06	679.02	688.44	1193.98	1256	1962.5
2 Cyst fluid injection via tumor margin+ adjuvant+ without albendazole	379.94	706.5	1017.36	1256	1391	1884.78
3 Control group (Without injection)	827.23	809.64	1117.20	1325.70	1640.96	2224.84

In animal models it has been shown that some parasites or parasitic compounds can inhibit cancer growth [8,9,16,17]. For example *Toxocara canis* and *Toxoplasma gondii* antigens reduce tumor size of fibrosarcoma in animal model [8]. Also it has been shown that *Trypanosoma cruzi* can decrease tumor size in mice [18]. Anti-cancer effect of hydatid cyst antigens has been shown in different investigations [3-6,8,19-21]. Yousofi et al. [6] investigated the effect of protoscolex of hydatid cyst on proliferation and death of fibrosarcoma and fibroblast cells. The results of this study showed that hydatid cyst protoscolex prevented proliferation of WEHI-164 and induced cell death in fibroblast cells. Also it has been shown that hydatid cyst antigens have inhibitory effects on cancer cells growth in culture medium [4]. Moreover effect of hydatid cyst antigens in inhibiting of tumor growth in mice was shown [12]. The results of our investigation are in agreement with the above studies and confirm that hydatid cyst fluid has anti-cancer effect.

Immunotherapy, like chemotherapy is performed systemically to prevent spread of malignancy cells. It only combats tumor cells with no lethal effect on normal cells so it is more specific than chemotherapy and radiotherapy.

Injection of monoclonal antibodies and cytokines, transfer of efficient immune cells, stimulate the immune system with materials like BCG as nonspecific vaccination, and specific vaccination with substances such as certain antigens of virus and tumor cells, are common examples of immunotherapy in cancer patients. BCG injection in tumor area resulted in macrophages activation. Also in several studies effect of BCG injection was showed on decrease of tumor growth [22].

Protective effect of Streptococcal and Gonococcal toxins injection, on tumor growth has been shown. Also it has been shown that suspension injection of *Bacillus prodigiosus* and *Serasia marcescens* alone or in combination with streptococci resulted in rapid and complete disappearance of the Lymphosarcoma grafted in dogs [23]. Also it has been shown that fractions obtained from *Mycobacterium bovis* strain have strong anti-tumor activity [24].

Moreover the antitumor activity of synthetic oligonucleotides with sequences of cDNA that encode the protein of *Mycobacterium bovis* strain BCG has been reported [25].

In agreement with the above works, in the present study effect of hydatid cyst fluid in reduction of melanoma cancer growth has been shown. So probably this liquid can be a curative material for cancer treatment. Regulation of the immune system by hydatid cyst [26,27] may be considered as a mechanism for anti-cancer effect of this parasite. In this context it has been shown that cytokine secretion and dendritic cell differentiation is modulated by the hydatid cyst fluid of *Echinococcus granulosus* [28].

5. CONCLUSION

According to the results of this work injection of hydatid cyst fluid to mice with established melanoma cancer resulted in significant reduction of tumor size in comparison with mice that had melanoma cancer without hydatid cyst fluid injection. However more research is recommended to find effective mechanisms of anti-tumor activity of hydatid fluid cyst.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and also national laws were followed. All experiments performed in this work were approved by the Isfahan University of Medical Sciences ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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