RESEARCH ARTICLE

Editorial Process: Submission:17/12/2025 Acceptance:18/08/2026 Published:26/08/2026

Evaluation of the Biological Activity of *Echinococcus granulosus* Hydatid Cyst Antigens on HRT-18 Colorectal Cancer Cells: Cytotoxicity and DNA Damage

Mustafa Najim Abdalla[1], Nagham Y. Albayati[1], Maeda Hussain Mohammad[2]*

Abstract

Introduction: Cystic echinococcosis (CE) is a zoonotic infection caused by the larvae of Echinococcus granulosus. Studies have indicated an indirect relationship between infection with parasites and many types of cancer, especially digestive tumors. **Aim:** The following study aims to evaluate the anticancer activity of two antigens extracted from hydatid cyst antigens (extracted from cyst fluid and protoscoleces) of Echinococcus granulosus on the growth and proliferation of colorectal cancer cells compared to normal cells, and study their mechanism of action by DNA damage and protein expression of p53 and caspase-3. **Methods:** In this study, the MTT assay was used to study the anticancer effect by exposing each antigen to a range of concentrations (100, 50, 25, 12.5, 6.2, 3.1, or 0.0 µg/ml). Then, the IC_{50} for each antigen on both the HRT-18 colorectal cancer cell line and the normal NHF cell line (normal fibroblasts derived from adipose tissue) was used to evaluate the mechanism of cell death by DNA damage and immunofluorescence staining for p53 and caspase-3 protein expression. **Results:** The results showed a significant decrease in the viability of colorectal cancer cells with increasing concentrations for all antigens used, with a higher effect on protoscoleces antigens (69.49%) and a slight effect on the normal cell line (21.13%). While the cyst fluid antigen showed a higher cytotoxicity (72.65%), it also showed an effect on the normal cell line (52.11%). Also, the IC_{50} for protoscoleces and cyst fluid antigens on colorectal cancer cells were (29.93 and 24.09) µg/ml, respectively, compared with IC_{50} for normal cells (119.4 and 25.95) µg/ml for protoscoleces and cyst fluid antigen, respectively. Therefore, treatment with the protoscoleces antigen gave the best cytotoxicity in the MTT test with more safety on the normal cell line. Also, the results showed the occurrence of programmed cell death induced by DNA damage and expression of both p53 and caspase-3 proteins in cultured cells after treatment. **Conclusion:** Antigens possess toxicity against cancer cells due to their ability to induce apoptosis and damage DNA; they can be considered promising sources for the development of natural antitumor agents, especially protoscoleces antigens.

Keywords: apoptosis, antigen, cytotoxicity, immunofluorescence staining, parasite

Asian Pac J Cancer Prev, **27 (8)**, 2949-2957

Introduction

Cancer is the second leading cause of death worldwide, with an estimated 14.1 million new cases and 8.2 million deaths annually [1], therefore its remains a significant and well-known health threat, despite the extensive research being conducted worldwide. Globally colorectal cancer is the third most common type of cancer, it's a leading cause of cancer-related deaths. Approximately 60% of col colorectal on cancer patients are diagnosed at stage II or III, and surgery is often the only treatment option for these patients [2]. Current treatments for cancer include radiation therapy, chemotherapy, and, eventually, surgery. Radiation therapy and chemotherapy destroy healthy cells, causing many side effects. Drug resistance and disease recurrence also lead to a poor prognosis [3, 4]. Humans have been searching for less invasive and more effective ways to treat cancer [5]. Infectious carcinogens in humans include Helicobacter pylori, human papillomavirus (HPV), and hepatitis B and C viruses [6]. However, a hypothesis has been proposed some parasitic infections cause that induce innate immune responses that exhibit antitumor activity. Previous studies indicate that patients with echinococcosis have a significantly lower incidence of cancer than healthy individuals [7]. In recent years, a different approach has emerged regarding the antitumor effects of derivatives and parasitic substances produced by certain protozoa and parasitic worms [8]. These parasitic substances exert numerous cancer-like effects, but the mechanisms involved remain incompletely understood. However, some studies suggest beneficial effects of these parasites, such

*[1]Department of Biology, College of Education for Pure Sciences, University of Diyala, Diyala, Iraq. [2]Experimental Therapy Department, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq. *For Correspondence: maeda.hm@uomustansiriyah.edu.iq*

as inducing apoptosis in the host, influencing the immune system by It activates the immune system, limits cancer spread and angiogenesis, acts on proliferative signals, and regulates and stimulates inflammatory responses in the host body [9, 10]. Echinococcosis is a zoonotic disease with specific genotypes or strains caused by parasites of the genus Echinococcaceae. The close relationship between humans and animals provides opportunities for the global transmission of this zoonotic disease [11]. The larval stage of Echinococcus granulosus, a tapeworm belonging to the family Tapeworms, causes echinococcosis. This disease is classified as one of the most serious tropical parasitic diseases affecting livestock and has significant economic and health consequences in both developed and developing countries [12] The hydatid cyst is a stage in the life cycle of the Echinococcus granulosus parasite; it is a larval stage found within the intermediate host. It is a cystic structure usually filled with a clear fluid (cystic fluid).and other components. Approximately 5 days after the intermediate host ingests the egg, the hydatid cyst begins to form and develop into a small cyst (60–70 µm in diameter) consisting of an inner cellular layer (germ layer), an outer non-cellular layer, and an outer layer of host origin formed as a result of the interaction between the parasite and the host. This inner cyst gradually expands and stimulates a granulation reaction in the host, followed by a fibrous reaction and the formation of a connective tissue layer (cystic envelope). The size of cysts in humans or animal intermediate hosts varies considerably, typically ranging from 1 to 15 cm, although much larger cysts (over 20 cm in diameter) can be found [13]. The hydatid cyst fluid of Echinococcus granulosus consists of a mixture of glycoproteins, glycolipids, carbohydrates, cyclophilins, and ferritin [14]. HCF contains various antigens such as antigen B, antigen A, and the 78 kDa fraction [15]. According to research, HCF can these compounds inhibit the growth of certain cancers in both in vitro cell cultures and animal models [16]. Some antigenic similarities have been established between the Echinococcus granulosus parasite and certain tumors; for example, some worm parasites express myosin-type glycan O antigens for certain cancers [17]. These similarities are attributed to the stimulation of cross-reactivity that inhibits cancer growth [18]. Hydrocarbon compounds, such as mucin, facilitate the adhesion of cancer cells to surrounding tissues, thereby preventing cell death and lysis by natural killer cells and cytotoxic lymphocytes. These hydatid cysts provide mucin-containing antigens, making them a useful agent for stimulating the immune system against mucin-secreting cancer cells [19]. Cell death occurs as a result of nuclear chromatin condensation, changes in membrane phosphatidyl structure, and DNA cleavage by enzymes. Ultimately, cell division by apoptotic components leads to Cell destruction [20].

In this study, the efficacy of two hydatid cyst antigens (extracted from cyste fluid, and Protoscoles of the Echinococcus granulosus) on the colorectal cancer was evaluated using cytological methods, specifically cytotoxicity assays by MTT assay and then study their cell death by DNA damage and proteins expression for P53 and Cas3 by immunofluorescence staining compared with normal cell line used in this study.

Materials and Methods

This study was carried out and the protocols were approved by ethics committee of scientific committee of Department of Biology, College of Education for Pure Sciences, University of Diyala, Diyala provenance, Iraq with Reference number (CEPEc/01) at 19/2/2026. Also, animal samples were handled in accordance with biosafety standards. Appropriate precautions were taken to ensure the safety of personnel and to maintain the integrity of the biological material throughout the study.

Hydatid Fluid Antigen Preparation (Cystic fluid antigen)

Samples of livers infected with hydatid cysts were collected from local butcher shops then transported and characterized to the scientific laboratories in the College of Education for Pure Sciences building at Diyala University, they washed with saline and sterilized with 70% alcohol. The cysts were then separated and isolated from the infected liver tissue. Cystic fluid antigen was prepared according to the method of Hadige et al. [21]. 100 mL of cystic fluid was washed with 5 mM acetate solution (pH 5), and centrifuged at 10,000 rpm for 30 minutes. The precipitate was then dissolved in 0.2 M phosphate buffer saline PBS (pH 8). Finally, 2.31 g of ammonium sulfate was added for 1 hour to remove any remaining host antibodies, followed by centrifugation for 30 minutes at 3,000 g. then filter by millipore (0.22) microns for use it in the next experiments. The antigen of the hydatid fluid used as concentration 0.625 mg/ml .

Preparation of Protoscoles Antigen

Using the method of Sapolard et al. [22], the protoscoles were fixed for 2 hours in PBS containing 4% paraformaldehyde. A soluble extract of the protoscoles, it was prepared by homogenization in a phosphate solution of a concentration of 0.4 M (pH 7.4). The extract was then centrifuged at 10,000 rpm at 4°C for 30 minutes. The precipitate was collected and used as a soluble antigen for the protoscoles as concentration 2.44 mg/ml.

Cell culture

Cell lines were cultured in MEM medium (US Biological, USA) supplemented with 10% (v/v) commercially available fetal bovine serum (FBS) (Capricorn-Scientific, Germany), 100 IU of penicillin antibiotics, and 100 µg/ml of streptomycin (Capricorn-Scientific, Germany). The cells were incubated in a humidified environment at 37°C. Subsequently, the proliferating cells were used in the required proportions for laboratory experiments using antigens as a treatment material [23, 24].

MTT procedure

Cells were seeded at a density of 10,000 cells per well of a 96-well microplate and incubated at 37°C for 24h until a homogeneous monolayer was formed. Cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For

each antigen, the cells were exposed to a range of concentrations (100, 50, 25, 12.5, 6.2, and 3.1 µg) for 72 h exposure time. Then after that, cells were stained by 28 µL of MTT stain solution (as concentration 2 mg/mL) for each well, the incubation continued for three hours, then, 100 µL of dimethyl sulfoxide (DMSO) was added to each well, and incubation continued for 15 minutes. Absorbance was measured at a wavelength of 492 nm using a microplate reader [25]. Cytotoxicity was calculated using the following equation:

Cytotoxicity % = (OD Control – OD sample)/OD Control × 100

OD control is the average optical density of the wells not treated with the antigens used, and OD Sample is the optical density of the wells treated with the antigens used for the study [26].

DNA Damage

Apoptosis study and morphological alteration:

Apoptosis estimation (propidium iodide/Acridine orange assay), the apoptotic attentions in cell lines (infected with antigens and control) were measured using (AO/PI). 5000 cells/well were seeded in plate, next infected with (cyst fluid antigen) for 24h in a 37 °C incubator. For traditional dual staining, the tested wells received exactly 50µl of the AO/PI stain mixture (at room temperature) for 30 seconds. After then, the stain was removed. The images were taken using a Leica fluorescent microscope [27]. Fluorescent intensity measured by fluorescent microscopy and via using image j software.

Indirect immunofluorescence staining

Indirect immunofluorescence was performed to detect protein expression in cultured cells and FFPE tissue sections using an unconjugated primary antibody followed by a fluorophore-conjugated secondary antibody. All steps involving fluorescent reagents were carried out in reduced light conditions to protect fluorophores from photobleaching.

Preparation of Cultured Cells, Cells were seeded onto sterile coverslips or chambered slides and allowed to attach overnight at 37 °C. confluency of 70–80% was used to ensure optimal morphology and antibody penetration. The cells were then stabilized by incubating them with a 4% paraformaldehyde solution for 15 minutes at room temperature. After fixation, the samples were washed three times with PBS solution. To permeabilize cellular membranes, slides were incubated in 0.1–0.5% Triton X-100 prepared in PBS for 3–15 minutes at room temperature, then washed in PBS three additional times.

Blocking, to reduce nonspecific binding, slides were incubated with 10% FBS, and then blocking was performed for 30–60 minutes at room temperature in a humidified chamber. Excess blocking solution was gently removed without allowing samples to dry.

Primary Antibody Incubation, Primary antibodies were diluted to a final concentration of 5 µg/mL in PBS containing 1.5% FBS. Then 500 µL was used to completely cover each coverslip. Slides were incubated for 1 hour at room temperature in a sealed humidified chamber to prevent evaporation. The slides were washed three times with PBS solution for 5 minutes per wash with gentle agitation.

Secondary Antibody Incubation, Fluorophore-conjugated secondary antibodies were diluted to 5 µg/mL in PBS containing 1.5% FBS. Slides were incubated with the secondary antibody for 1 hour at room temperature, protected from light, in a humidified chamber. Following incubation, The slides were washed three times with PBS for 5 minutes to remove unbound antibodies.

Mounting and Imaging, Coverslips were mounted using and allowed to polymerize as required. Slides were examined using a fluorescence microscope equipped with appropriate filter sets. Images were acquired at multiple magnifications and exported in TIFF format for analysis.

Statistical analysis

The obtained data were statically analyzed using Tukey's ANOVA multiple comparisons test with GraphPad Prism 8. The values were presented as the mean ± SD of triplicate measurements [28].

Results

Cytotoxicity

The cytotoxicity of Hydatid Cyst Antigens extracted from cyst fluid, and Protoscoles antigen was determined in colorectal cancer (HRT-18) and normal (NHF) cell line using the MTT assay. Microscopic images (Figure 1) showed distinct morphological changes in antigen-treated cells compared to the control group. Morphological analysis of the cell showed changes in cell morphology such as cell shrinkage, membrane swelling, or the formation of bodies programmed for cell death, leading to cell death. Also, cell density analysis showed decreased cell density compared to control wells indicate cytotoxic effects in treated antigens at concentrations of (100, 50, 25, 12.5, 6.2, and 3.1 µg/ml) which showed a concentration depended effect led to toxicity on both antigens used.

The results of MTT assay showed that the cytotoxicity increased gradually with increasing antigen concentration, where the highest toxicity rate was recorded at a concentration of 100 µg/ml and the lowest rate at a concentration of 3.12 µg/ml, Statistical analyses indicate that both are significant differences ($p \leq 0.01$) between the different concentrations, indicating that the effect of the antigen depends directly on its concentration. Although the highest concentrations in cyst fluid antigen (72.65%) compared with 69.49% in protoscoles antigen, the best antigens was Protoscoles because it's showed slight effect on normal cell line (21.13%) compared with cyst fluid antigen that showed a higher cytotoxicity (72.65%) but with their effect on normal cell line (52.11%) as showed in Figure 2.

Also, the IC_{50} for Protoscoles and cyst fluid antigens on colorectal cancer cells were (29.93, and 24.09) µg/ml respectively compared with IC50 for normal cells (119.4, and 25.95) µg/ml for Protoscoles, and cyst fluid antigen respectively (Figure 3). Therefore, treatment with the Protoscoles antigen gave the best cytotoxicity in the MTT

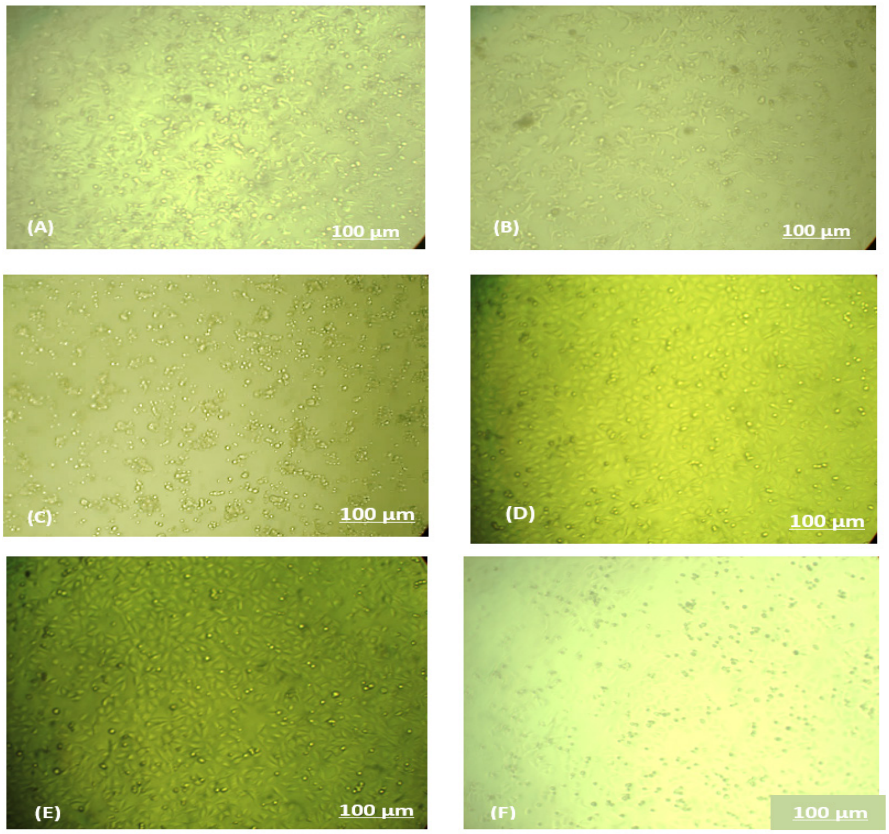

Figure 1. Morphological Changes after Exposed to Antigens, (A): Un treated cells of colorectal cancer cell line HRT-18, (B): Cells treated with 100 µg/ml Protoscoles antigen for HRT-18, (C): Cells treated with100 µg/ml cyst fluid antigen for HRT-18, (D): Un treated cells for normal cell line NHF, (E): Cells treated with 100 µg/ml Protoscoles antigen for NHF, (F): Cells treated with 100 µg/ml cyst fluid antigen for NHF.

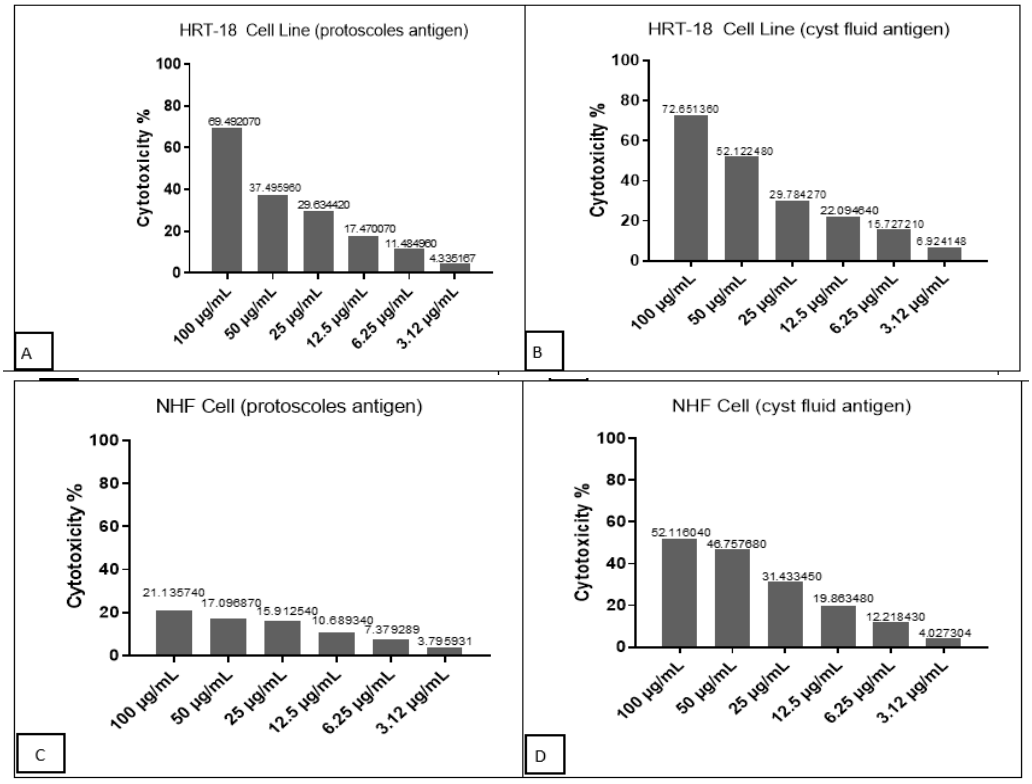

Figure 2. Inhibition Curve, which Shows the Effect of Protoscoles and Cyst Fluid Antigen on Cell Viability. The x-axis is the concentration of antigen (A): protoscoles antigen for colorectal cancer HRT-18, (B): cyst fluid antigen for colorectal cancer HRT-18, (C): protoscoles antigen for normal cell line NHF, (D): cyst fluid antigen for normal cell line NHF. The y-axis represents the percentage of cell death (cytotoxicity).

test with more safety on normal cell line.

DNA Damage

Apoptosis study and morphological

According to the results above mechanism of action were studied for IC50 concentration, a DNA damage assay and morphological changes accompanying apoptosis were conducted using antigens prepared from antigens. The results showed that antigens with high concentrations caused significant morphological changes compared to the untreated group. Figure (4 A & B) shows the morphological changes in HRT-18 cancer cell line after treatment with antigens extracts, which included nucleus condensation, fragmentation, and the appearance of signs of programmed cell death.

Also, the results of fluorescence test showed a clear significant difference between the group treated and the control group, as the fluorescence intensity of live cells (viable) decreased significantly compared to the control group by $p < 0.001$, while the results of dead cells showed a significant increase by $p < 0.001$. The group treated showed a higher fluorescence intensity for dead cells compared to live cells, while the control group showed higher fluorescence intensity for live cells compared to dead cells. There is a significant difference in the fluorescence intensity between the two groups, especially in dead cells, which indicates that the treatment had an effect (Figure 4 C).

Detection of P53 and Cas3 Proteins Expression by indirect immunofluorescence stainin

Figure 5 showed that both P53 and Cas3 protein levels of HRT-18 colorectal cancer cells was increased significantly at $p<0.05$ after exposed to antigen with higher protein levels at P53, compared with untreated cells (as negative control) which recorded no protein levels.

Discussion

In the present study, the effect of antigens from two hydatid cyst antigens (extracted from cyst fluid, and Protoscoles) of the Echinococcus granulosus on HRT-18 colorectal cancer cells was studied to determine their ability to inhibit cell growth and showed their ability to induce apoptosis using cellular methods including cytotoxicity and apoptosis testing with both DNA damage and protein expression of both P53 and Cas3.

Previous in vitro and animal studies have shown that some parasites have anticancer effects. These studies demonstrated that the heads of the parasites, present in the fluid of the cyst, induce cell death in myofibroblastic tumor cells such as (WEHI 164) [29]. Another study indicated that the fluid in the hydatid cyst contains a complex mixture of antigens, meaning there are more than one type that stimulate and activate the immune system [30]. Therefore, one of the characteristics of the hydatid cyst is its ability to stimulate both cellular and humoral immune systems, and it is not limited to a single type [31], another study showed that various molecules present in the hydatid cyst have an inhibitory effect on HeLa and Vero cell lines [32]. One study reported that AgB antigen extracted from the parasite's hydatid cyst fluid has inhibitory activity against melanoma cells. Other studies have highlighted the importance of the interaction between hydatid cyst antigens and cancer cell secretions [33]. Furthermore, the antitumor activity of human HCF

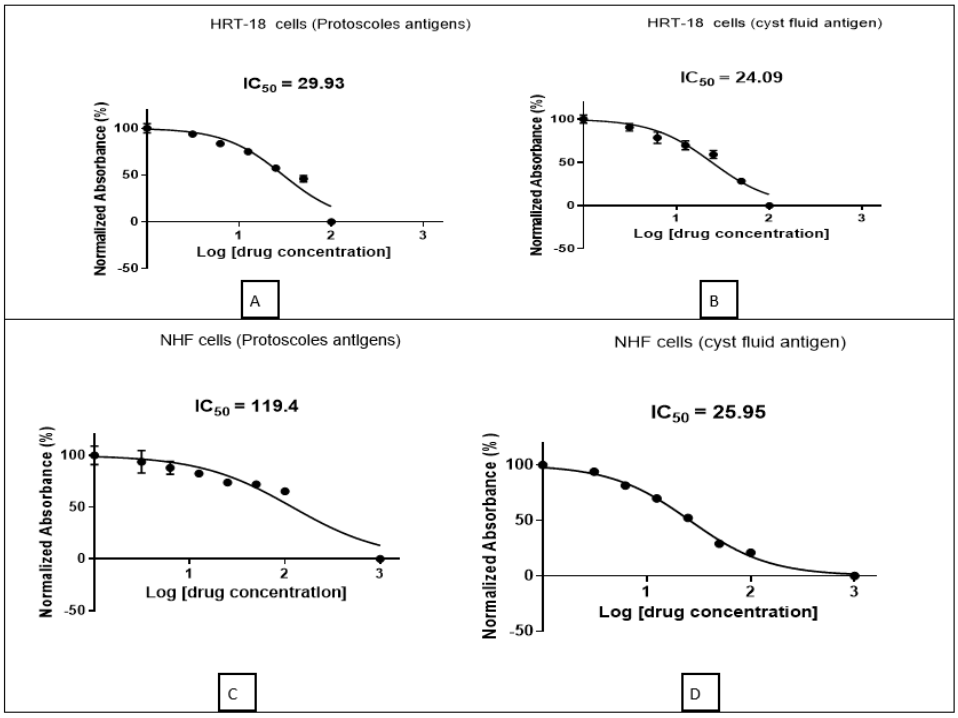

Figure 3. Dose-Response Curve by IC_{50} of Antigens Treated (A): Protoscoles antigens for colorectal cancer HRT-18, (B) cyst fluid antigen for colorectal cancer HRT-18. (C): Protoscoles antigens for normal cell NHF, (D): cyst fluid antigen for normal cell NHF. The x-axis is the concentration of the antigens used in the study (in micrograms), while the y-axis represents the percentage of cell inhibition by the antigens.

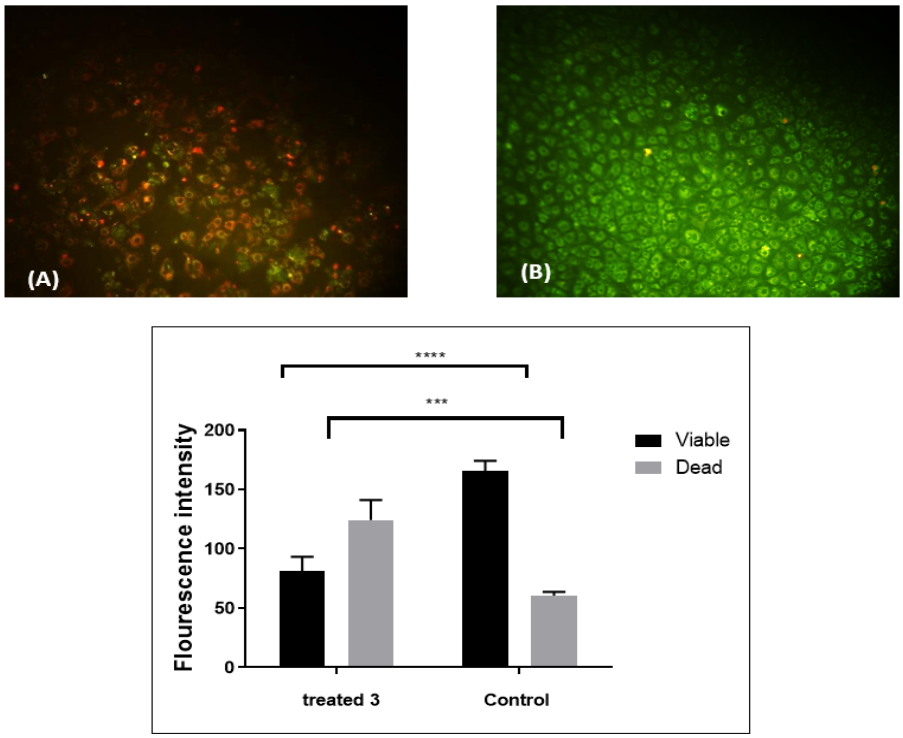

Figure 4. Apoptosis Study and Morphological Changes after Treatment of the HRT-18 Cancer Cell Line. (A): Treated, (B): Un treated as control, (C): fluorescence intensity of live and dead cells in the treated antigens compared with control groups.

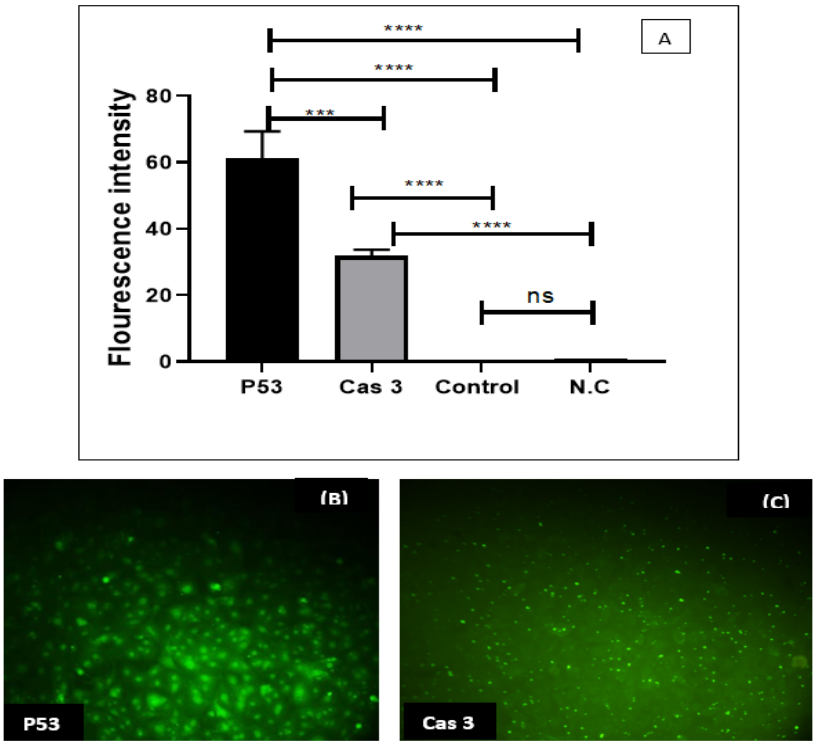

Figure 5. Shows the Proteins Expression Levels of the Immunofluorescence for P53 and Cas3 Antibodies of HRT-18 Colorectal Cancer Cells after Induced by Antigens, (A): fluorescence intensity, (B): P53, (C): Cas 3.

(hydroxycystic fluid antigen) was investigated using a source of hydatid cyst antigen isolated from human cysts and tested in a mouse model of colon cancer [18]. Karaday et al. also demonstrated that hydatid disease has protective effects against lung cancer [34]. In another study, two cell types normal and cancerous HeK293 and B16F10, were used. HeK293 cells, being normal cells, showed lower sensitivity to the AgB antigen present in the fluid of the cyst, while B16F10 cells, being a cancerous cell line, showed higher sensitivity compared to normal cells [29].

In a study by Mohammadi et al., human melanoma cells A375 were treated with an HCF extract from fertile and non-fertile hydatid cysts. Cell viability was assessed using the MTT assay, and they were sensitive to hydatid cyst fluid antigen [35, 36]. In a study, it was shown that hydatid cyst induces apoptosis by increasing levels of the Bcl-2 family [36]. The cytotoxic properties of Echinococcus granulosus have been previously established. In another study, the rate of apoptosis in lymphocytes treated with fertile hydatid fluid containing protoscoles was significantly higher than in untreated cells [37]. This supports the concept that Echinococcus granulosus hydatid fluid antigens induce apoptosis in lymphocytes [38]. Nuno et al. demonstrated that the excretory/excretory secretions of Echinococcus multilocularis (E. multilocularis) induce apoptosis in dendritic cells in vitro [39]. Regarding the role of hydatid cyst antigen in inducing apoptosis, studies have shown that apoptosis increases after exposure to AgB antigen, a component of hydatid cyst fluid. One hypothesis suggests that proteins that induce apoptosis accumulate on the mitochondrial surface, leading to the formation of Channels located in the mitochondrial membrane, causing the contents of the mitochondria to leak into the cytoplasm [40]. Programmed cell death is a key mechanism used by the host body for immune defense against parasites. Echinococcus granulosus has the characteristic ability to modulate the host's immune response through multiple mechanisms. Some of these mechanisms include influencing dendritic cell maturation and stimulating a non-protective type II (Th2) helper T cell response via the B antigen (AgB) [41].

In conclusion, the antigens from processed Protoscoles antigens components have best cytotoxicity compared to normal cells, and their toxicity is directly proportional to antigen concentration. The results also confirm that antigens induce programmed cell death (apoptosis) by induced DNA damage, also expressed each P53 and Cas3 protein in cultured cells after treatment in HRT-18 colorectal cancer cells. Therefore, Protoscoles antigens may a good natural therapeutic agent for inducing apoptosis in cancer cells.

Author Contribution Statement

Mustafa Najm Abdullah; Nagham Y. Albayati; and Maeda Hussain Mohammad performed the experimental tests. Mustafa Najm Abdullah performed the statistical analysis. Mustafa Najm Abdullah; Nagham Y. Albayati; and Maeda Hussain Mohammad; wrote the article.

Acknowledgements

This study is part of the first author's doctoral thesis. The protocols were approved by the Ethics Committee of the Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq (Reference number: CEPEc/01, dated 19/2/2026). Animal samples were handled in accordance with biosafety standards, and appropriate precautions were taken to ensure the safety of personnel and the integrity of the biological material.

Ethical issue and approval

All experimental procedures was carried out and the protocols were approved by ethics committee of scientific committee of Department of Biology, College of Education for Pure Sciences, University of Diyala, Diyala provenance, Iraq with Reference number (CEPEc/01) at 19/2/2026. Also, animal samples were handled in accordance with biosafety standards. Appropriate precautions were taken to ensure the safety of personnel and to maintain the integrity of the biological material throughout the study.

Conflict of interest

The authors declare no potential conflict of interest.

Consent for publication

All authors have given consent for publication.

Approval

This work approved by the scientific committee of University of Diyala.

References

1. Dalamaga M, Christodoulatos GS, Mantzoros CS. The role of extracellular and intracellular nicotinamide phosphoribosyltransferase in cancer: Diagnostic and therapeutic perspectives and challenges. Metabolism. 2018;82:72-87. https://doi.org/10.1016/j.metabol.2018.01.001.
2. Rabeneck L, Horton S, Zauber A, Earle C, Gelband H, Jha P, et al. Cancer: Disease control priorities. In: Disease control priorities. 3rd ed. Washington (DC): World Bank; 2015. P. 101–119.
3. Manoochehri H, Jalali A, Tanzadehpanah H, Taherkhani A, Saidijam M. Identification of key gene targets for sensitizing colorectal cancer to chemoradiation: An integrative network analysis on multiple transcriptomics data. J Gastrointest Cancer. 2021;53. https://doi.org/10.1007/s12029-021-00690-2.
4. Mozaffari Nejad AS, Fotouhi F, Mehrbod P, Keshavarz M, Alikhani MY, Ghaemi A. Oncolytic effects of Hitchner B1strain of Newcastle disease virus against cervical cancer cell proliferation is mediated by the increased expression of cytochrome c, autophagy and apoptotic pathways. Microb Pathog. 2020;147:104438. https://doi.org/10.1016/j.micpath.2020.104438.
5. Daneshpour S, Kefayat AH, Mofid MR, Rostami Rad S, Yousofi Darani H. Effect of hydatid cyst fluid antigens on induction of apoptosis on breast cancer cells. Adv Biomed Res. 2019;8:27. https://doi.org/10.4103/abr.abr_220_18.
6. Barati N, Nikpoor A, Mosaffa F, Razazan A, Badiee A, Motavallihaghi S, et al. AE36 HER2/neu-derived peptide linked to positively charged liposomes with CpG-ODN as an effective therapeutic and prophylactic vaccine for breast cancer. J Drug Deliv Sci Technol. 2021;67:102904. https://doi.org/10.1016/j.jddst.2021.102904.
7. Akgül H, Tez M, Unal AE, Keşkek M, Sayek I, Ozçelik T. Echinococcus against cancer: Why not? Cancer. 2003;98(9):1999-2000. https://doi.org/10.1002/cncr.11752.
8. Sardarian K, Maghsood AH, Farimani M, Hajiloii M, Saidijam M, Farahpour M, et al. Detection of Toxoplasma gondii B1 gene in placenta does not prove congenital toxoplasmosis. Hum Antibodies. 2019;27(1):31-5. https://doi.org/10.3233/hab-180346.
9. Daneshpour S, Rostamirad S, Kefayat A, Mofid M, Safavi

A, Darani HY. Identifying the most effective hydatid cyst fluid fraction for anticancer vaccination of 4t1 breast tumor-bearing mice. Int J Prev Med. 2019;10:143. https://doi.org/10.4103/ijpvm.IJPVM_508_18.
10. Shafaati M, Saidijam M, Soleimani M, Hazrati F, Mirzaei R, Amirheidari B, et al. A brief review on DNA vaccines in the era of COVID-19. Future Virol. 2021. https://doi.org/10.2217/fvl-2021-0170.
11. Nyirongo M. Detection and molecular epidemiology of echinococcosis spp in the wild and domestic carnivores in the wildlife-livestock interface areas of the South Luangwa national park in Mfuwe of Mambwe district of Zambia [PhD thesis]. Lusaka: University of Zambia; 2025.
12. Pal M, Alemu H, Megersa L, Garedo D, Bodena E. Cystic echincoccoosis: A comprehensive review on life cycle, epidemiology, pathogenesis, clinical spectrum, diagnosis, public health and economic implications, treatment, and control. Int J Clin Exp Med Res. 2022;6. https://doi.org/10.26855/ijcemr.2022.04.005.
13. Cai D, Zhang X. Ultrasound in the diagnosis of echinococcosis. In: Li J, Wang W, Mehlhorn H, editors. Echinococcus: Control and elimination of echinococcosis with a focus on China and Europe. Cham: Springer Nature Switzerland; 2024. p. 173-205. https://doi.org/10.1007/978-3-031-54479-8_11
14. Aziz A, Zhang W, Li J, Loukas A, McManus DP, Mulvenna J. Proteomic characterisation of Echinococcus granulosus hydatid cyst fluid from sheep, cattle and humans. J Proteomics. 2011;74(9):1560-72. https://doi.org/10.1016/j.jprot.2011.02.021.
15. Shahbazi AE, Saidijam M, Maghsood AH, Matini M, Haghi MM, Fallah M. Genotyping of fresh and paraffinized human hydatid cysts using nad1 and cox1 genes in Hamadan Province, West of Iran. Iran J Parasitol. 2020;15(2):259-65.
16. Banihashemi SM, Soleymani E, Abdizadeh R, Motavalli Haghi M, Khalili B. Intestinal protozoan infections in cancer patients undergoing chemotherapy in Shahrekord the Central Southwest of Iran in 2018. Int J Epidemiol Res. 2020;7(4):144-52. https://doi.org/10.34172/ijer.2020.26.
17. Osinaga E. Expression of cancer-associated simple mucin-type O-glycosylated antigens in parasites. IUBMB Life. 2007;59(4-5):269-73. https://doi.org/10.1080/15216540601188553.
18. Berriel E, Russo S, Monin L, Festari MF, Berois N, Fernández G, et al. Antitumor activity of human hydatid cyst fluid in a murine model of colon cancer. Sci World J. 2013;2013:230176. https://doi.org/10.1155/2013/230176.
19. Sun S, Zheng XJ, Huo CX, Song C, Li Q, Ye XS. Synthesis and evaluation of glycoconjugates comprising n-acyl-modified thomsen-friedenreich antigens as anticancer vaccines. ChemMedChem. 2016;11(10):1090-6. https://doi.org/10.1002/cmdc.201600094.
20. Sborgi L, Barrera-Vilarmau S, Obregón P, de Alba E. Characterization of a novel interaction between bcl-2 members diva and harakiri. PLoS One. 2010;5(12):e15575. https://doi.org/10.1371/journal.pone.0015575.
21. Hadighi R, Mirhadi F, Rokni MB. Evaluation of a dot-elisa for the serodiagnosis of human hydatid disease. Pak J Med Sci. 2003;19:268-71.
22. Saboulard D, Lahmar S, Petavy AF, Bosquet G. The Echinococcus granulosus antigen ega31: Localization during development and immunogenic properties. Parasite Immunol. 2003;25(10):489-501. https://doi.org/10.1111/j.1365-3024.2003.00663.x.
23. Alsaraf KM, Mohamed MH, Al-shammari AM, Abbas IS. Broad-spectrum cytotoxic effect of Calendula officinalis L l against breast cancer cells. Indian J Forensic Med Toxicol. 2020;14(1):803–9. https://doi.org/10.37506/v14/i1/2020/ijfmt/193002.
24. Al-Attar HM, Mohammad MH, Alwan AH. Laser ablation of asphalt and coal in different solvents an in vitro study. Lasers Med Sci. 2023;38(1):135. https://doi.org/10.1007/s10103-023-03796-0.
25. Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications. Hoboken (NJ): John Wiley & Sons; 2015.
26. Al-Zeiny SSM, Mohammad MH, Almzaien AK, Al-Shammari AM, Ahmed AA, Shaker HK. Cytotoxic effect of Phoenix dactylifera (Iraqi date) leaves and fruits extracts against breast cancers cell lines. Asian Pac J Cancer Prev. 2024;25(7):2391-6. https://doi.org/10.31557/apjcp.2024.25.7.2391.
27. Al-Shammari AM, Salman MI. Antimetastatic and antitumor activities of oncolytic ndv amha1 in a 3d culture model of breast cancer. Front Mol Biosci. 2024;11:1331369. https://doi.org/10.3389/fmolb.2024.1331369.
28. Hamad MA, Almzaien AK, Jameel FR, Mohammad MH, Al-Shammari AM. The oncolytic effect of Newcastle disease virus attenuated AMHA1 strain against digestive system tumors. Vet World. 2024;17(11):2688-93. https://doi.org/10.14202/vetworld.2024.2688-2693.
29. Yousofi Darani H, Soozangar N, Khorami S, Taji F, Yousofi M, Shirzad H. Hydatid cyst protoscolices induce cell death in WEHI-164 fibrosarcoma cells and inhibit the proliferation of baby hamster kidney fibroblasts in vitro. J Parasitol Res. 2012;2012:304183. https://doi.org/10.1155/2012/304183.
30. Chookami MB, Sharafi SM, Sefiddashti RR, Jafari R, Bahadoran M, Pestechian N, et al. Effect of two hydatid cyst antigens on the growth of melanoma cancer in C57/black mice. J Parasit Dis. 2016;40(4):1170-3. https://doi.org/10.1007/s12639-015-0643-7.
31. Riganò R, Buttari B, De Falco E, Profumo E, Ortona E, Margutti P, et al. Echinococcus granulosus-specific T-cell lines derived from patients at various clinical stages of cystic echinococcosis. Parasite Immunol. 2004;26(1):45-52. https://doi.org/10.1111/j.0141-9838.2004.00682.x.
32. Aref N, Shirzad H, Yousefi M, Yousofi Darani H. Effect of different hydatid cyst molecules on HeLa and Vero cell lines growth in vitro. J Immunodefic Disor. 2013;02. https://doi.org/10.4172/2324-853X.1000105.
33. Daneshpour S, Bahadoran M, Hejazi SH, Eskandarian AA, Mahmoudzadeh M, Darani HY. Common antigens between hydatid cyst and cancers. Adv Biomed Res. 2016;5:9. https://doi.org/10.4103/2277-9175.175242.
34. Karadayi S, Arslan S, Sumer Z, Turan M, Sumer H, Karadayi K. Does hydatid disease have protective effects against lung cancer? Mol Biol Rep. 2013;40(8):4701-4. https://doi.org/10.1007/s11033-013-2565-8.
35. Mohammadi M, Spotin A, Mahami-Oskouei M, Shanehbandi D, Ahmadpour E, Casulli A, et al. MicroRNA-365 promotes apoptosis in human melanoma cell a375 treated with hydatid cyst fluid of Echinococcus granulosus sensu stricto. Microb Pathog. 2021;153:104804. https://doi.org/10.1016/j.micpath.2021.104804.
36. Janssen D, De Rycke PH, Osuna A. Dose-dependent effects of hydatid fluid toxins from Echinococcus granulosus on mouse peritoneal macrophages. Folia Parasitol. 1993;40(2):109-13.
37. Mokhtari Amirmajdi M, Sankian M, Eftekharzadeh Mashhadi I, Varasteh A, Vahedi F, Sadrizadeh A, et al. Apoptosis of human lymphocytes after exposure to hydatid fluid. Iran J Parasitol. 2011;6(2):9-16.
38. Janssen D, Osuna A, Lazuen J, de Rycke PH. Comparative cytotoxicity of secondary hydatid cysts, protoscoleces, and in vitro developed microcysts of Echinococcus granulosus.

J Helminthol. 1992;66(2):124-31. https://doi.org/10.1017/s0022149x00012700.
39. Nono JK, Pletinckx K, Lutz MB, Brehm K. Excretory/secretory-products of Echinococcus multilocularis larvae induce apoptosis and tolerogenic properties in dendritic cells in vitro. PLoS Negl Trop Dis. 2012;6(2):e1516. https://doi.org/10.1371/journal.pntd.0001516.
40. Moradi M, Najafi R, Amini R, Solgi R, Tanzadehpanah H, Esfahani AM, et al. Remarkable apoptotic pathway of Hemiscorpius lepturus scorpion venom on ct26 cell line. Cell Biol Toxicol. 2019;35(4):373-85. https://doi.org/10.1007/s10565-018-09455-3.
41. Riganò R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, et al. Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a TH2 cell response. Infect Immun. 2007;75(4):1667-78. https://doi.org/10.1128/iai.01156-06.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.