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Original Article

Investigation of Excretory–Secretory (ES) Antigens from Zoonotic Helminths, *Fasciola* spp. and *Paramphistomum* spp., on Colorectal Cancer Cell Line Proliferation

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Abstract

Introduction: Colorectal cancer (CRC) is one of the most diagnosed cancers worldwide and remains a major cause of cancer-related mortality. Recent studies suggest that helminth-derived molecules may influence tumor progression. This study evaluated the antiproliferative effects of excretory–secretory (ES) antigens derived from *Fasciola* spp. and *Paramphistomum* spp. on the human colorectal cancer cell line HCT116. **Methods:** ES antigens were collected from adult parasites obtained from infected bovines. The antiproliferative activity was assessed using an MTT assay at concentrations of 10 µg/mL and 20 µg/mL after 24 and 48 hours. **Results:** MTT results demonstrated reduced cell viability, particularly at the concentration of 20 µg/mL. **Conclusion:** These findings suggest that helminth-derived ES antigens possess potential antiproliferative properties.

Keywords: *Excretory–Secretory Antigens; Fasciola Spp.; HCT116 Cell Line; Paramphistomum Spp.*

Introduction

Colorectal cancer (CRC) is a major global health concern and represents one of the leading causes of cancer-related mortality worldwide. CRC develops through genetic and epigenetic alterations leading to uncontrolled cell proliferation (Dekker *et al*., 2019). Despite advances in treatment, limitations such as toxicity and resistance persist.

Conventional chemotherapy targets rapidly dividing cells but often causes significant toxicity in healthy tissues. As a result, researchers are increasingly exploring alternative bioactive compounds from natural sources as potential anticancer agents (Lu *et al*., 2018).

Helminth parasites secrete a diverse range of excretory–secretory (ES) molecules during infection. These molecules include enzymes, proteases, glycoproteins, and immunomodulatory proteins that enable parasites to survive within the host environment (Hewitson *et al*., 2009). ES products are known to interact with host immune pathways and may modulate inflammatory responses, cellular signaling, and metabolic activity. Similar immunomodulatory effects of helminth-secreted proteins have also been reported in several host–parasite interaction studies (Hewitson *et al*., 2009; Smallwood *et al*., 2017); Zhang *et al*. (2026) demonstrate that *S. japonicum* SEA contributes to the

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activation of CRC-related signaling pathways, so offering empirical evidence for the influence of schistosomiasis in endemic regions on CRC.

Recent evidence indicates that helminth-derived molecules may influence cancer progression through immune modulation and signaling pathway interference. Recent evidence indicates that helminth-derived molecules may influence cancer progression through immune modulation and signaling pathway interference (Maizels *et al*., 2018). Helminths and their products can promote or inhibit colorectal cancer progression depending on parasite and host factors (Sánchez-Barrera *et al*., 2025). The Fasciola spp. and Paramphistomum spp. are trematode parasites commonly infecting ruminants, and their secreted molecules may possess biologically active properties capable of influencing host cell behavior (Hewitson *et al*., 2009).

Therefore, this study aimed to evaluate the antiproliferative effects of ES antigens derived from *Fasciola* spp. and *Paramphistomum* spp. on the HCT116 human colorectal cancer cell line.

Materials and Methods

Collection of Helminths

Adult *Fasciola spp.* and *Paramphistomum spp.* were collected from naturally infected bovines at a licensed abattoir in Shah Alam, Malaysia. Infection was confirmed by post-mortem examination based on the presence of adult flukes in the bile ducts and rumen. Information regarding co-infection with other diseases was not available.

Preparation of ES Antigens

Collected parasites were washed thoroughly with sterile phosphate-buffered saline (PBS, 1×, pH 7.4) containing antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) to remove host contaminants.

Approximately 3 g of parasites were incubated in PBS at 37°C for 24 hours to allow release of excretory–secretory (ES) products.

The incubation medium was centrifuged at 10,000 × g for 15 minutes at 4°C, and the supernatant was collected and filtered using a 0.22 µm syringe filter. The samples were stored at −20°C until further use.

Cell Culture

The human colorectal cancer cell line HCT116 was selected due to its well-characterized genetic profile and widespread use in anticancer studies. Cells were cultured in premixed complete McCoy's 5A medium supplemented with 1% antibiotic solution (penicillin–streptomycin). Cells were maintained at 37°C in a humidified incubator with 5% CO_2 and subculture upon reaching 70–80% confluence.

MTT Assay

Cells were seeded in 96-well plates at a density of 2 × 10^4 cells/well and allowed to adhere overnight.ES antigens were applied at concentrations of 10 µg/mL and 20 µg/mL, selected based on preliminary optimization experiments and previous literature reports.

After 24 and 48 hours of treatment, MTT reagent (0.5 mg/mL) was added and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm.

Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD). Statistical differences between groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test using GraphPad Prism software. A p-value < 0.05 was considered statistically significant.

Results

MTT Assay Results After 24 Hours of Treatment with Fasciola Spp. ES Antigen

Figure 1 presents the absorbance values obtained from the MTT assay after 24 hours of incubation. Cells treated with 10 µg/mL ES antigen showed minimal change in absorbance compared with the untreated control group. However, treatment with 20 µg/mL resulted in reduced absorbance values, indicating decreased metabolic activity and reduced cancer cell viability.

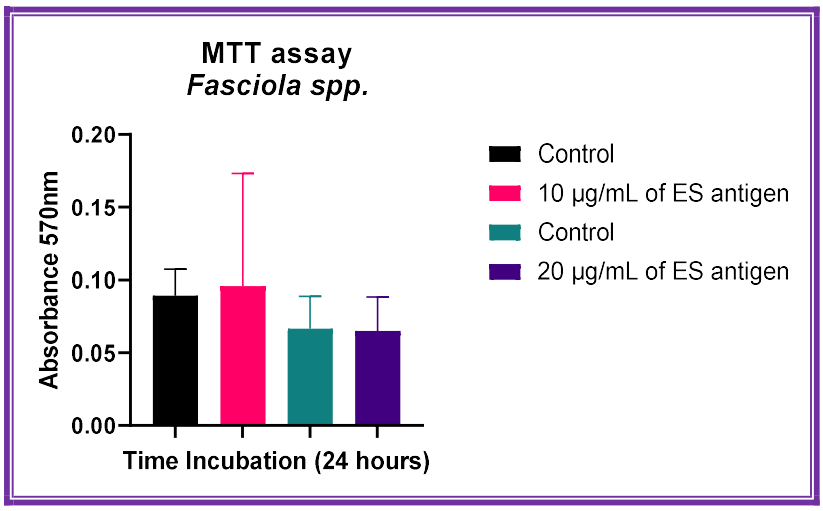

Figure 1: *Effect of Fasciola Spp. ES Antigen on HCT116 Cell Viability After 24 Hours. Cells Were Treated With 10 µg/Ml And 20 µg/Ml. Data Are Presented as Mean ± SD (n = 3), (p > 0.05)*

Figure 2 illustrates the MTT assay results after 48 hours. Cells treated with 20 µg/mL ES antigen continued to exhibit lower absorbance values compared with control cells, demonstrating that the inhibitory effect of the ES antigen persisted over time.

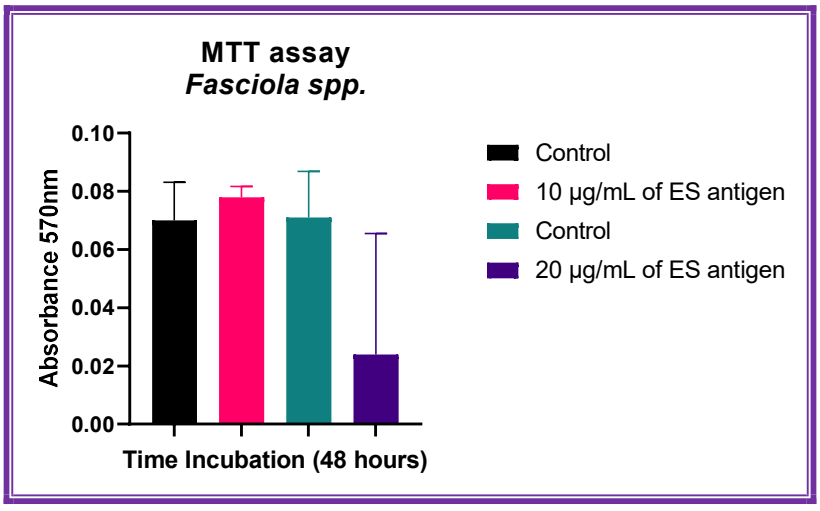

Figure 2: *Effect of Fasciola Spp. ES Antigen on HCT116 Cell Viability After 48 Hours. Cells Were Treated With 10 µg/Ml And 20 µg/Ml. Data Are Presented as Mean ± SD (N = 3), (P > 0.05).*

MTT Assay Results After 24 Hours of Treatment with ParamphistomumSpp

Figure 3 illustrates the MTT assay results after 24 hours of treatment with *Paramphistomum* spp. ES antigen. Cells treated with 20 µg/mL showed reduced absorbance compared to control, indicating decreased metabolic activity.

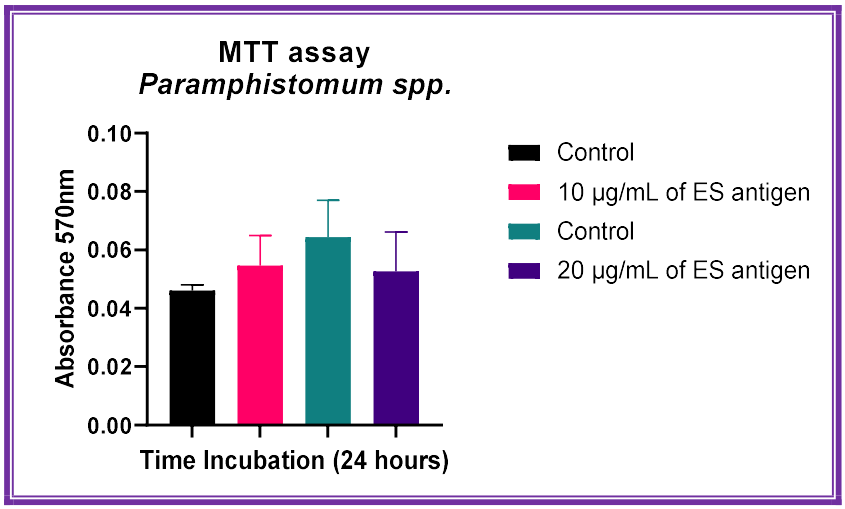

Figure 3: *Effect of Paramphistomumspp ES antigen on HCT116 cell viability after 24 hours. Cells were treated with 10 µg/mL and 20 µg/mL. Data are presented as mean ± SD (n = 3), (p > 0.05).*

MTT Assay Results After 48 Hours of Treatment with ParamphistomumSpp.

Figure 4 shows the MTT assay results after 48 hours of treatment with *Paramphistomum spp.* ES antigen. A further reduction in absorbance was observed at higher concentrations, suggesting sustained inhibitory effects on cell viability**.**

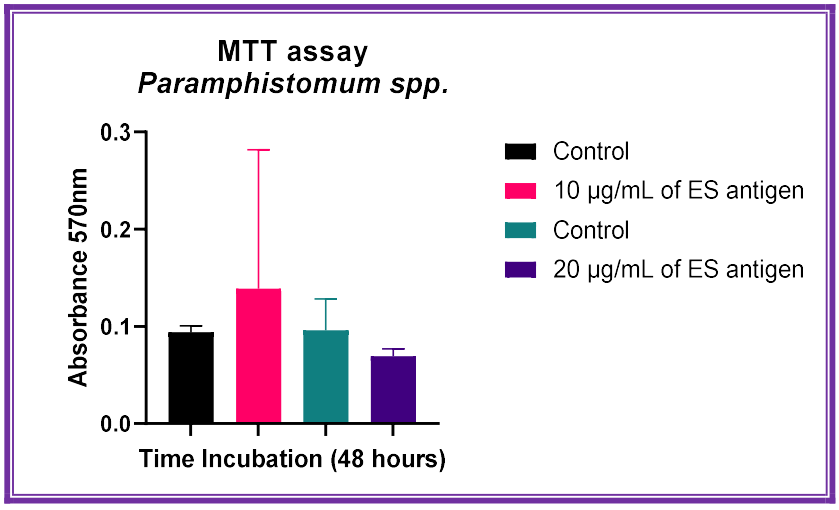

Figure 4: *Effect of Paramphistomumspp ES antigen on HCT116 cell viability after 48 hours. Cells were treated with 10 µg/mL and 20 µg/mL. Data are presented as mean ± SD (n = 3), (p > 0.05).*

Discussion

The present study evaluated the antiproliferative effects of excretory–secretory (ES) antigens derived from *Fasciola* spp. and *Paramphistomum* spp. on the human colorectal cancer cell line HCT116. The results demonstrated reduced cell viability following treatment with ES antigens, particularly at the higher concentration (20 µg/mL) and longer incubation period. Although the reductions observed were not statistically significant (*p*> 0.05), the findings suggest that helminth-derived ES products may influence colorectal cancer cell proliferation.

The reduction in cell viability observed in the MTT assay reflects decreased mitochondrial metabolic activity, which is commonly used as an indicator of cell viability. However, the MTT assay alone cannot distinguish between different mechanisms of growth inhibition such as apoptosis, necrosis, or cell-cycle arrest. Therefore, while the present findings indicate reduced viability of HCT116 cells, the exact biological mechanisms responsible for these effects remain unclear.

Previous studies have demonstrated that helminth-derived molecules possess immunomodulatory and antiproliferative properties capable of influencing host cellular responses. Hewitson *et al*. (2009) reported that helminth excretory–secretory products contain bioactive proteins and enzymes that interact with host immune pathways and cellular signaling mechanisms. Similarly, Maizels*et al*. (2018) described that helminth-secreted molecules can regulate inflammation and immune responses through modulation of host signaling pathways. These findings support the present observations in which exposure to ES antigens reduced the metabolic activity of HCT116 colorectal cancer cells. Previous reports have similarly suggested that helminth-derived molecules may influence inflammatory and tumor-associated signaling pathways involved in colorectal cancer progression (Smallwood *et al*., 2017; Sánchez-Barrera *et al*., 2025).

The findings of the present study are also consistent with previous reports describing the antiproliferative effects of parasite-derived molecules on cancer cells. Callejas *et al*. (2019) demonstrated the impact of helminth-derived compounds in inhibiting active colorectal cancer through the downregulation of proinflammatory and protumorigenic signaling pathways. Sanchez-Barrera *et al*. (2025) present a thorough analysis of the effects of helminths on the macroscopic, histological, immunological, and molecular dimensions of colorectal cancer (CRC). Zhou *et al*. (2026) indicate that *Trichuris suis* ova (TSO) exhibits potential effects on inflammatory bowel disease (IBD) through many routes; however, no significant efficacy has been validated in clinical trials. Jacobs *et al*. (2020) demonstrated that gastrointestinal nematode-derived antigens reduced colorectal cancer cell proliferation and altered the expression of proteins associated with cell-cycle regulation and epithelial–mesenchymal transition. Likewise, Lu *et al*. (2018) reported that biologically active compounds could suppress colon cancer cell viability through apoptosis-related pathways. Although apoptosis and molecular signaling pathways were not investigated in the present study, the reduction in HCT116 cell viability observed after treatment with ES antigens may indicate potential biological activity of helminth-derived molecules. Helminth-secreted proteins are known to modulate host immune responses and cellular pathways that may contribute to altered cancer cell behavior (Hewitson *et al*., 2009; Harnett & Harnett, 2010). The present findings contribute to the growing body of evidence supporting the biological and immunomodulatory potential of helminth-derived molecules in cancer-related research (Tan *et al*., 2018).

Limitations

Several limitations should be considered in interpreting the present findings. First, crude ES antigen preparations were used rather than purified proteins, and therefore the specific active molecules responsible for the observed effects could not be identified. Second, the study utilized only a single colorectal cancer cell line (HCT116), which may limit the generalizability of the findings to other colorectal cancer models. Third, the study relied solely on the MTT assay to evaluate cell viability. Although the MTT assay is widely used to assess cellular metabolic activity, it provides limited information regarding the precise mechanism of cell death or growth inhibition.

Overall, the findings of this study provide preliminary evidence that ES antigens derived from *Fasciola* spp. and *Paramphistomum* spp. may exhibit antiproliferative effects against colorectal cancer cells in vitro. However, further experimental validation is necessary before definitive conclusions regarding their therapeutic potential can be established.

Future Studies and Recommendations

Future studies should therefore focus on detailed proteomic characterization of ES antigens to identify the active bioactive molecules responsible for the observed antiproliferative effects. Additional

investigations involving apoptosis assays, cell-cycle analysis, reactive oxygen species evaluation, and gene expression profiling are also recommended to better understand the molecular mechanisms underlying these effects. Furthermore, the use of multiple colorectal cancer cell lines and in vivo experimental models would provide stronger evidence regarding the therapeutic potential of helminth-derived ES antigens in anticancer research.

Conclusion

This study demonstrates that ES antigens derived from *Fasciola* spp. and *Paramphistomum* spp. exhibit inhibitory effects on the proliferation of HCT116 colorectal cancer cells in a concentration-dependent manner. Although the reductions in cell viability were not statistically significant, the findings suggest the potential biological activity of helminth-derived molecules as candidates for further investigation in anticancer research. The present findings contribute to the growing body of evidence supporting the immunomodulatory and antiproliferative properties of helminth-derived compounds. Further molecular and in vivo studies are required to better understand the mechanisms underlying these effects and to evaluate their potential therapeutic applications in colorectal cancer treatment.

Conflict of Interest

The authors declare that they have no competing interests.

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