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RESEARCH ARTICLE

Preliminary studies on the effect of excretory secretory (ES) *Ascaris lumbricoides* antigens on colorectal cell line viability

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ABSTRACT

Helminth parasites are a group of complex metazoans from various taxonomic families. Excretory secretory (ES) by-products, secreted by living parasites from the surface, appeared to modulate the host immunological response towards helminth infection. This study aims to investigate the effect of ES antigen from helminth parasite on colorectal cell viability. Worm were cultured in phosphate-buffered saline (PBS x1) at 37°C for 24 hours after being rinsed in sterile PBS. Using a mortar and pestle, the worm was crushed vigorously using PBS. The obtained excretory secretory (ES) antigens were extracted and filtered using a 0.22 µM filter and stored at -20°C for further assay. For LCMS, 100 µl of the extract was analysed using Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT. The extraction of ES antigen (10 µg/ml and 20 µg/ml) was used for cell viability studies using CRC cell line HCT 116. Cell viability and MTT assay were conducted as per the protocol mentioned in the MTT kit. The liquid chromatography and mass spectrometry (LCMS) data indicated that the ES antigen contained metabolic compounds, namely fatty acid, amino alcohol, indoles, sterols, glycosides, and sphingoids. For the *Ascaris lumbricoides* LCMS analyses, around 405 metabolic peaks were detected. Out of which, 58 were detected via the database were identified, while several compounds detected have anticancer properties. The MTT assay indicated that after 24 hours and 48 hours of exposure, all treated cells showed a decrease in cell viability compared to the control group. The preliminary studies demonstrated that the ES antigen from *Ascaris lumbricoides* has some ability to decrease the cell viability of the HCT116 CRC cell line. Further studies are needed to examine the cell cycle arrest and apoptosis effect of the ES antigen towards the CRC cell line.

Keywords: Parasitic; helminths; CRC; cell line.

INTRODUCTION

Helminth parasites are a complex group of metazoans from various taxonomic families (Gazzinelli-Guimaraes & Nutman, 2018). Parasitic helminths have co-evolved along with their hosts for millions of years and developed various intricate methods to influence their hosts immune systems to secure their continued survival (Aguayo *et al*., 2019). Furthermore, numerous nematodes release antigenic glycoconjugates (known as ES antigens) by excretion or secretion (Dell *et al*., 1999; Van Die & Cummings, 2010). Excretory/secretory antigens are parasite molecules secreted at the interface between the parasite and immune system cells by various processes, such as active secretion and diffusion from the parasitic body (soma) (Motran *et al.*, 2018). Excretory secretory (ES) products, which are immunomodulatory excretory secretory products, are released by adult worms (Hewitson *et al*., 2013). Previous study indicated that worm parasite excretory secretory (ES) able to modulate the host immune response to helminth infection (Hartmann *et al*., 2013).

Despite ongoing efforts and a decreasing incidence of colorectal cancer mortality over the last twenty-five years, colorectal cancer remains one of the leading causes of cancer-related deaths (Bailey *et al*., 2016). Previous studies published that certain parasites may possess the ability to contribute to anticancer activities (Darani & Yousefi, 2012). In addition, certain helminth species have been identified to have a negative effect on tumour development and improve the life span of the host (Vasilev *et al*., 2015). The antigen derived from gastrointestinal nematode *H. polygyrus* substantially reduced the proliferation of murine and human CRC cells, accompanied by an increase in the expression of p53 and p21 (Jacobs *et al.,*2020). Previous experimental studies indicated that the whole-worm extract of *Ascaris lumbricoides* had a growth-inhibiting effect on tumours of Lewis lung carcinoma cells as it inhibited tumour formation (Yang *et al*., 2013).

Moreover, the coexistence of helminth and bacteria in the stomach suggests that the anti-inflammatory effect of parasite infection may be mediated, at least to some extent, by the microbiota (Shute *et al*., 2021). This preliminary study was carried out to investigate the effect of ES antigen from helminth parasite on colorectal cell viability. The commonly found helminth parasite, *Ascaris lumbricoides* in Orang Asli (indigenous community) in Malaysia, was used in this study.

MATERIALS AND METHODS

Collection of parasites

The adult *Ascaris lumbricoides* worms were collected from the Orang Asli community after deworming activity. The visual gross inspection was used to identify the collected worms, whereas the classification of worms was done using the light microscope technique.

Preparation of worm crude excretion–secretion (ES) products extraction

Worm (n=1 female) were rinsed in sterile and socked in phosphate-buffered saline (PBS) at 37°C prior to ES antigen extraction. The next day, using a mortar and pestle, the worm was crushed vigorously with drops of PBS as diluent. The obtained ES antigen was then filtered using a 0.22 M filters membrane sterile filter to remove bacteria (Oka *et al*., 2014), followed by the supernatant being recovered and centrifuged for 10 minutes at 5000 rpm (Callejas *et al*., 2019; Ebner *et al*., 2020). The ES antigen was stored at -20°C until further use.

Quadrupole Time of Flight (QTOF) LC/MS

The LCMS analysis was performed at the Integrative Pharmacogenomics Institute (iPROMISE), UiTM service laboratory. Basically, 100 µL of the sample was transferred into the new microcentrifuge tube, while 300 µL of methanol was added for the LCMS analysis. The sample was vortexed for 30 seconds and incubated on ice for 30 minutes. The supernatant was transferred to a new microcentrifuge tube after centrifugation at 14 000 RPM for 15 minutes at 4°C. Then, the supernatant was dried using a concentrator. The dried samples were reconstituted with 30 µL mobile phase (H_2O:ACN) followed by 30 seconds of vortex mixing and centrifugation at 14 000 RPM for 15 minutes at 4°C. Twenty (20) µL of the supernatant was transferred into an insert and injected into the LC/MS-QTOF system for analysis.

Chromatographic separation was performed at 40°C using Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (2.1 x 100 mm) 1.8 µm with (A) 0.1% formic acid in dH_20 and (B) 0.1% formic acid in acetonitrile. The gradient elution program was 0.00 – 18.00 min, 5 -95%(B); 18 to 23 minutes, 95% (B). 23.01 minutes; 5% (B). The total run time was 30 minutes. The LC condition was re-equilibrated for 2 minutes before starting the new injection. The sample injection volume was set at 2 µl, and the flow rate of the mobile phase was set at 0.25 mL/min. The mass spectrometer was operated in negative electrospray ionisation (ESI) mode with the optimum gas temperature at 325°C, gas flow at 11 L/min and nebuliser at 35 psi, respectively.

Metabolic Profiling

Database searching of interpreted LC/MS data was carried out using the Agilent Mass Hunter Qualitative Analysis B.05.00 software, PubChem search engine (https://pubchem.ncbi.nlm.nih.gov/) (National Centre for Biotechnology Information, USA) and SwissLipids (http://www.swisslipids.org//) (SIB Swiss Institute of Bioinformatics). Search parameters included mass accuracy and formula. The chromatographic profiles were analysed based on the accurate mass data identified, while the predicted compounds were annotated using the METLIN database.

Cell culture

The human colorectal cancer cell line was HCT 116 obtained from iCell. The cell was cultured in McCoy's 5A medium supplemented with a foetal bovine serum of 0.10% and Penicillin-Streptomycin Solution of 1%. The cells were maintained in culture conditions of gas-phase air at 95%, CO_2 at 5% and incubator temperature of 37°C with humidity at 70% - 80%.

Proliferation assays (MTT assay)

The HCT 116 (colorectal carcinoma cell) cell proliferation was measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. For the cell proliferation test, about 2 000 cells were seeded in each well and triplicated in a 96-well plate. For the control, the cells (untreated) or PBS x1 (Jacobs *et al*., 2018; Jacobs *et al*., 2020). The cells were cultured for 24 hours and 48 hours. Proliferation at different days of culture was determined by incubating cell cultures with different concentrations of *Ascaris lumbricoides* ES antigen extract (10 µg/ml and 20 µg/ml). Then, 50 µL of MTT solution was added to each well and incubated for 4 hours. The supernatant was removed carefully, while 150 µL DMSO was added to each well to dissolve the formazan format. The OD value was measured using the microplate reader at 570 nm.

Statistical analyses

The statistical analyses were conducted using Prism 9 software (GraphPad Software Inc.). The effects of ES antigen extraction and time (incubation) were assessed for in vitro analysis using repeated measures analysis of variance (ANOVA). Significance was determined by ANOVA (p value <0.05). The data presented in this study consists of the mean ± standard deviation (SD) values obtained from three replicate cultures in three different experiments.

RESULTS

LCMS analysis

The LCMS data indicated four significant peaks, as in Figure 1, indicating the existence of diverse metabolic components and a total peak of 405 detected via the analysis. Out of which, 58 were identified via the database (Table 1). In addition, the extract was demonstrated to contain different classes of compounds, including fatty acid, sphingoid, amino alcohol, indoles, and sterols.

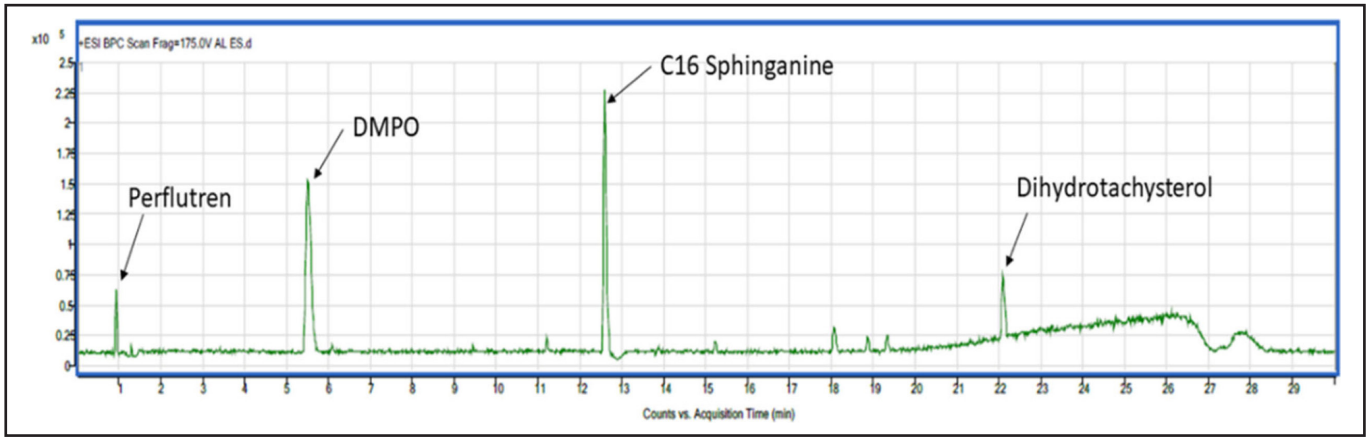


Figure 1. LC-MS analysis identifies several of the highest-peak compound chromatograms of *Ascaris lumbricoides* ES antigen extract. The present analysis found high height scores in C16 Sphinganine, Perflutren, 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), and Dihydrotachysterol.

Table 1. Compounds in the ES antigen extract of *Ascaris Lumbricoides* detected using METLIN database

Name of Compound	Formula	RT (min)	Mass	Diff (DB, ppm)
Perflutren	C3 F8	0.947	187.9874	-0.87
DMPO	C6 H11 N O	5.522	113.0845	-3.67
Octylamine	C8 H19 N	6.104	129.1516	1.3
Lys Gln Ile	C17 H33 N5 O5	7.64	387.2466	4.04
Arg Arg Gln	C17 H34 N10 O5	8.263	458.2714	-0.17
2-(3-Phenylpropyl)pyridine	C14 H15 N	8.387	197.1199	2.74
Decyl acetate	C12 H24 O2	9.46	200.1774	1.19
Halstoctacosanolide A	C48 H76 O12	9.681	844.5353	-1.9
Ganglioside GM3 (d18:1/16:0)	C57 H104 N2 O21	10.385	1152.7188	-4.93
2-ethyl-dodecanoic acid	C14 H28 O2	11.221	228.2086	1.48
NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-Sp	C45 H75 N5 O34	12.299	1229.4303	-0.81
Indinavir	C36 H47 N5 O4	12.513	613.3607	3.4
C16 Sphinganine	C16 H35 N O2	12.599	273.2664	1.48
Xestoaminol C	C14 H31 N O	12.655	229.2398	3.49
Phytosphingosine	C18 H39 N O3	12.704	317.2928	0.46
Riboflavine 2',3',4',5'-tetrabutanoate	C33 H44 N4 O10	12.727	656.3045	1.9
N-ornithinyl-35-aminobacteriohopane-32,33,34-triol	C40 H73 N3 O4	12.738	659.5574	4.09
Taurocholic acid	C26 H45 N O7 S	12.743	515.2934	-3.29
PE(13:0/18:4(6Z,9Z,12Z,15Z))	C36 H64 N O8 P	12.745	669.4349	3.06
DG(20:4(5Z,8Z,11Z,14Z)/22:3(10Z,13Z,16Z)/0:0)[iso2]	C45 H74 O5	12.753	694.5504	4.67
Fucalpha1-2Galbeta1-3GlcNAcbeta1-3Galbeta1-4Glcbeta-Cer(d18:1/18:0)	C68 H124 N2 O27	12.754	1400.8347	3.19
16-hydroxy hexadecanoic acid	C16 H32 O3	12.757	272.2344	2.92
Hexacosanyl oleate	C42 H82 O2	12.765	618.6358	-7.03
Glucosinalbin 4-(4-acetylrhamnoside)	C22 H31 N O15 S2	12.789	613.1069	10.8
Isocaviunin 7-O-gentiobioside	C31 H38 O18	12.795	698.202	5.41
Sporidesmolide I	C33 H58 N4 O8	12.797	638.4237	2.83
Omphalotin B	C74 H123 N13 O18	12.806	1481.9011	6.62
Verteporfin	C41 H42 N4 O8	12.807	718.2951	7.12
Citbismine D	C40 H38 N2 O11	12.813	722.2491	-2.11
PG(14:0/17:0)	C37 H73 O10 P	12.813	708.4922	2.74
14:0 Cholesteryl ester	C41 H72 O2	12.816	596.5497	5.91
PE(21:0/15:0)	C41 H82 N O8 P	12.821	747.5744	4.52
Linoside A	C32 H38 O16	12.854	678.2139	3.03
PG(18:3(9Z,12Z,15Z)/20:5(5Z,8Z,11Z,14Z,17Z))	C44 H71 O10 P	12.867	790.4789	-0.52
PA(P-18:0/13:0)	C34 H67 O7 P	12.871	618.4642	-2.81
Saquinavir	C38 H50 N6 O5	12.872	670.3877	-5.11
TG(13:0/13:0/15:0)[iso3]	C44 H84 O6	12.881	708.6315	-6.63
PE(14:0/20:0)	C39 H78 N O8 P	12.882	719.5442	3.21
Bouillonamide B	C32 H45 N5 O6 S	12.901	627.3069	3.39
Bis(4-methoxybenzoyl)-3a,29-dihydroxy-8-multifloren-7-one	C46 H60 O7	12.905	724.4247	12.71
PE(15:0/20:0)	C40 H80 N O8 P	12.909	733.558	5.62
Epirubicin glucuronide	C33 H37 N O17	12.913	719.2066	-0.63
PA(P-16:0/17:2(9Z,12Z))	C36 H67 O7 P	12.916	642.4538	13.48
PS(O-20:0/15:0)	C41 H82 N O9 P	12.923	763.5695	4.28
Luteolin 7-sulfate-3'-rutinoside	C27 H30 O18 S	12.969	674.1109	6.54
(6R)-vitamin D3 6,19-[4-{2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalinyl)ethyl}-1,2,4-triazoline-3,5-dione] adduct	C42 H59 N5 O6	12.988	729.444	3.41
Enigmol	C18 H39 N O2	13.878	301.2974	2.17
Nb-Palmitoyltryptamine	C26 H42 N2 O	15.232	398.3279	4.63
Polysorbate 60	C22 H42 O8	18.074	434.2869	2.5
Emmotin A	C16 H22 O4	18.873	278.1512	2.22
Linoleamide	C18 H33 N O	20.117	279.2555	2.53
28:2(5Z,9Z)(6Br)	C28 H51 Br O2	20.491	498.3047	5.04
Dihydrotachysterol	C28 H46 O	22.109	398.3557	-2.19
N-stearoyl valine	C23 H45 N O3	22.738	383.339	2.43
Agecorynin C	C22 H24 O9	23.993	432.1404	3.85
3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	C24 H38 O4	25.614	390.2766	1.12
N-Cyclohexanecarbonylpentadecylamine	C22 H43 N O	25.733	337.3338	2.09
Docosanedioic acid	C22 H42 O4	25.748	370.3081	0.6

Cell culture Observation after incubation with ES antigen

The HCT116 colorectal cell line was incubated with 10 µg/ml and 20 µg/ml of ES antigen extract for 24 hours and 48 hours, respectively (Table 2). Table 3 displays various morphology changes in the incubated CRC cell lines.

MTT assay

The viability of cells was analysed by using an MTT assay (Figure 2). According to the results of the MTT assay, after 24 and 48 hours of exposure, treated cells showed a lower level of cell viability when compared to the control cells. The optical density (OD) exhibits a correlation with the number or density of cells. Both of these experiments showed that the viability of HCT 116 cells was decreasing compare to control. Therefore, there might be positive association between increased *Ascaris lumbricoides* ES antigen extract dose and the time of incubation, which shows a reduction in the OD obtained in MTT assay over a longer period of exposure. The results are presented as a comparison of the cell viability and control of three separate experiments carried out in triplicate.

DISCUSSION

Ascariasis, caused by *Ascaris lumbricoides*, is the most prevalent helminth infection in the world, affecting one-fourth of the world population (Barbosa-Valenzuela *et al*., 2021). *A. lumbricoides* live in the ileum and cannot survive in acidic surroundings. This worm survives in the ileum despite intestinal peristalsis due to its high motor activity (Kobayashi & Tsuyuzaki, 2018). *Ascaris* releases digestive enzymes that cause nutrient malabsorption (Al-Tameemi & Kabakli, 2020). Both helminth parasites and their hosts acquire specific adaptations to assist their survival throughout coevolution (Radovic *et al*., 2015).

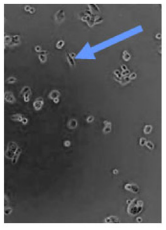
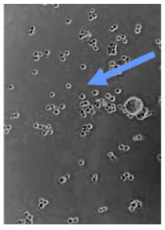
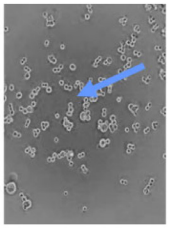
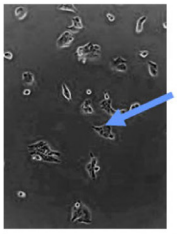
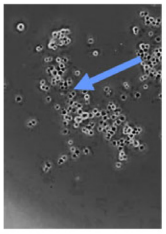
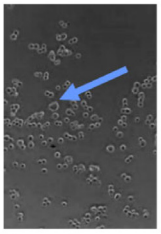
Excretory-secretory products provide various functions during infection, including penetration of host tissues and evasion of host immune responses. In contrast, it also triggers immunological responses (including antibody production) (Mehrdana & Buchmann, 2017). Helminths and microbiota affect immunoregulatory pathways required for immunological tolerance (Radovic *et al*., 2015). Alternatively, discovering that glycosylation patterns of lipids and proteins during the lifecycle of parasitic nematodes that may imitate the host may offer new insights into their potential for immunomodulation, leading to the development of innovative immunotherapies (Bobardt *et al*., 2020).

As reported by previous studies, certain parasite excretory and secretory products might have protective properties in allergy and inflammatory disease (Bobardt *et al*., 2020). For instance, excretory/secretory products from *T. spiralis* adult worms (AES) therapeutic potential might alleviate mice inflammatory colitis (Yang *et al*., 2014), *Heligmosomoides polygyrus* excretory/secretory products (HpES) induced spheroid growth characteristic of fetal epithelium and homeostatic repair (Drurey *et al*., 2021), *Ascaris lumbricoides* protein exposure along multiple stages of the monocyte-macrophage axis

Table 2. Experiment design

	10 µg/ml	20µg/ml
24 Hours	Control and Test	Control and Test
48 Hours	Control and Test	Control and Test

Table 3. The morphology observation of HCT 116 cells without (control) and with *Ascaris lumbricoides* ES antigen extract based on different incubation periods (24 hours and 48 hours) under 10x magnification.

	Control (HCT 116 cell)	*Ascaris lumbricoides* ES antigen extract 10 µg/ml	*Ascaris lumbricoides* ES antigen extract 20 µg/ml
24 hours			
48 hours			

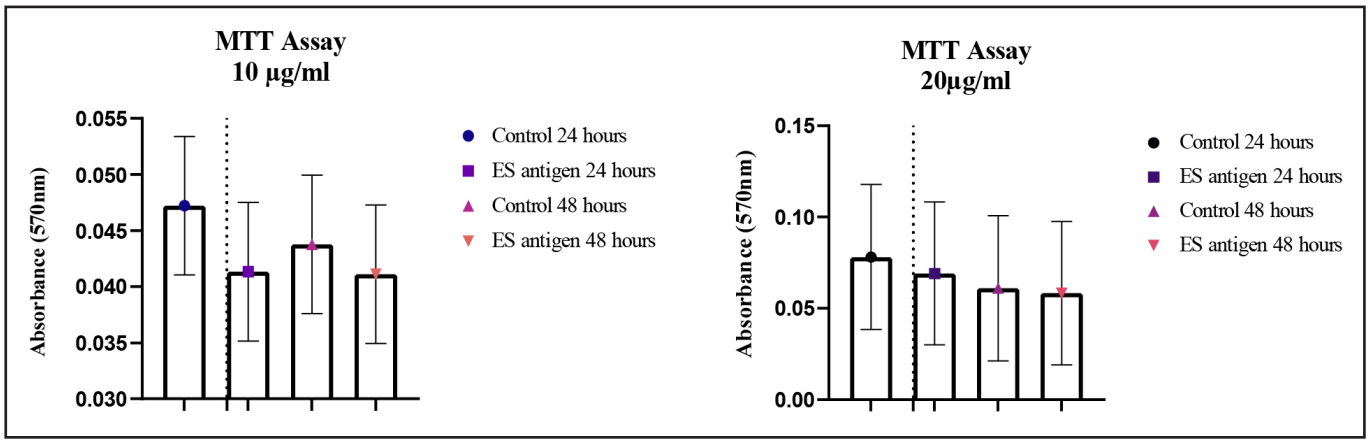


Figure 2. *Ascaris lumbricoides* ES antigen extract decreases in human colorectal cancer cell proliferation compared to control. The HCT 116 cells were incubated with 10µg/ml and 20µg/ml of *Ascaris lumbricoides* ES antigen extract for 24 and 48 hours and measured using microplate at 570nm after the MTT assay was conducted. The results are the mean with error bar. Significance was determined by One Way ANOVA with 10µg/ml ($P < 0.05$) and 20µg/ml (not significant). Both data multiple comparisons also show no significant values.

improved human monocyte-derived macrophage mycobactericidal ability (Togarsimalimath, 2021). On top of that, it has also been previously reported that *Heligmosomoides polygyrus*-derived products could suppress colorectal cancer cell proliferation (Jacobs *et al*., 2020).

Until now, no studies have been conducted to investigate the effect of *Ascaris lumbricoides* infection and ES antigens extract produced from this parasite on the proliferation of malignant cell lines in vitro. On the contrary, there is a growing amount of evidence that parasitic infections and the use of parasite-derived proteins can slow the growth of tumours (Vasilev *et al*., 2015).

Current findings from this study indicated the presence of various metabolic compounds; around 58 metabolic compounds were detected in *Ascaris lumbricoides*. The literature indicated the anticancer potential of these compounds from the extracts (Babahosseini *et al*., 2013). In addition, it has been presented that the extract contains different compounds such as fatty acid, sphingoid, amino alcohol, indoles, and sterols. In the analysis of the helminth metabolic compounds, C16 Sphinganine compounds were detected at high peaks. Additionally, LCMS helminths ES antigen extract also detected with the presence of Xestoaminol C. Reported by previous studies, the Xestoaminol C compound can also inhibit reverse transcriptase and have antiproliferative activity in different human cancer cell lines of A-549, HT-29 and DU-145 (Fabišíková *et al*., 2016).

Following the detection of C16 Sphinganine metabolites compound, this sphingolipid metabolite production has been associated with colon cancer suppression (Sugawara *et al*., 2006). Ceramide is central to sphingolipid metabolism. Sphingolipids consist of a structurally related family of backbones known as sphingoid bases. Many naturally occurring and synthetic sphingoid bases are cytotoxic to cancer cells and pathogenic microorganisms or have other bioactivities (Pruett *et al*., 2008; Giussani *et al*., 2014; Camp *et al*., 2017).

The results are presented as a comparison of the cell viability and control of three separate experiments carried out in triplicate. According to the results of the MTT assay, after 24 hours and 48 hours of exposure, treated cells showed a lower level of cell viability compared to the control cells. Both experiments showed that the viability of HCT 116 cells was decreasing. Consequently, a positive association was observed between increased ES antigen extract exposure and the time of incubation, which showed a reduction in the MTT assay in a longer period of exposure.

Notably, the conducted MTT assay showed cell inhibition with both exposures of ES antigen extraction. Sphingolipids have been reported to exhibit antitumor activity (Li *et al*., 2022). Following studies conducted by Uma Suganya *et al*. (2016) that reported the cell apoptosis induction morphology resulted in structural changes, which were observed to exhibit cellular alterations, including cell-like cell shrinkage, alterations in membrane integrity and suppression of cellular proliferation. These findings are consistent with the current cell morphology observation carried out and comparing to the observation of the control cell. The results of microscopy morphology observation in this study are also comparable to the previous reported findings with evidenced morphology observation of reduction in density and spread of cells, together with shorter diameter and propensity to detach of HCT 116 cells in 24 and 48 hours of incubation with 10µg/ml and 20µg/ml of *Ascaris lumbricoides* ES antigen extract.

The outcome of LCMS analysis on the helminth indicated the presence of several compounds with anticancer properties. Therefore, throughout the study findings, the ES antigen extract of excretory and secretory derived from the helminths can inhibit the colorectal cell proliferation activity. Therefore, further studies are required to identify the cell cycle arrest and apoptosis effect of the ES antigen toward the CRC cell line.

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Conflict of Interests

The authors declare no conflict of interest.

REFERENCES

Aguayo, V., Valdés Fernandez, B.N., Rodríguez-Valentín, M., Ruiz-Jiménez, C., Ramos-Benítez, M.J., Méndez, L.B. & Espino, A.M. (2019). *Fasciola hepatica* GST downregulates NF-κB pathway effectors and inflammatory cytokines while promoting survival in a mouse septic shock model. *Scientific Reports* **9**: 2275. https://doi.org/10.1038/s41598-018-37652-x

Al-Tameemi, K. & Kabakli, R. (2020). *Ascaris lumbricoides*: Epidemiology, diagnosis, treatment, and control. *Asian Journal of Pharmaceutical and Clinical Research* **13**: 8-11. https://doi.org/10.22159/ajpcr.2020.v13i4.36930

Babahosseini, H., Roberts, P. C., Schmelz, E. M., & Agah, M. (2013). Bioactive sphingolipid metabolites modulate ovarian cancer cell structural mechanics. Integrative Biology (United Kingdom), 5(11), 1385–1392. https://doi.org/10.1039/c3ib40121a

Bailey, J.R., Aggarwal, A. & Imperiale, T.F. (2016). Colorectal cancer screening: stool DNA and other noninvasive modalities. *Gut and Liver* **10**: 204-211. https://doi.org/10.5009/gnl15420

Barbosa-Valenzuela, S.C., Nieto, M., Ramɒrez, D., Ariza, J., Montaɒez, W. & Alvarado, L. (2021). Necrotizing pancreatitis due to *Ascaris lumbricoides*: causes, diagnosis, and current treatment. *Revista Colombiana de Gastroenterologia* **36**: 514-518. https://doi.org/10.22516/25007440.671

Bobardt, S.D., Dillman, A.R. & Nair, M.G. (2020). The two faces of nematode infection: virulence and immunomodulatory molecules from nematode parasites of mammals, insects and plants. *Frontiers in Microbiology* **11**: 577846. https://doi.org/10.3389/fmicb.2020.577846

Callejas, B.E., Mendoza-Rodríguez, M.G., Villamar-Cruz, O., Reyes-Martínez, S., Sánchez-Barrera, C.A., Rodríguez-Sosa, M., Delgado-Buenrostro, N.L., Martínez-Saucedo, D., Chirino, Y.I., León-Cabrera, S.A., Pérez-Plasencia, C., Vaca-Paniagua, F., Arias-Romero, L.E. & Terrazas, L.I. (2019). Helminthderived molecules inhibit colitis-associated colon cancer development through NF-κB and STAT3 regulation. *International Journal of Cancer*, *145*(11), 3126– 3139. https://doi.org/10.1002/ijc.32626

Camp, E.R., Patterson, L.D., Kester, M. & Voelkel-Johnson, C. (2017). Therapeutic implications of bioactive sphingolipids: A focus on colorectal cancer. *Cancer Biology and Therapy* **18**: 640-650. https://doi.org/10.1080/15384047.2017.1345396

Darani, H.Y. & Yousefi, M. (2012). Parasites and cancers: parasite antigens as possible targets for cancer immunotherapy. *Future Oncology* **8**: 1529-1535. https://doi.org/10.2217/fon.12.155

Dell, A., Haslam, S.M., Morris, H.R. & Khoo, K.H. (1999). Immunogenic glycoconjugates implicated in parasitic nematode diseases. *Biochimica et Biophysica Acta – Molecular Basis of Disease* **1455**: 353-362. https://doi.org/10.1016/S0925-4439(99)00064-2

Drurey, C., Lindholm, H.T., Coakley, G., Poveda, M.C., Löser, S., Doolan, R., Gerbe, F., Jay, P., Harris, N., Oudhoff, M.J. *et al*. (2021). Intestinal epithelial tuft cell induction is negated by a murine helminth and its secreted products. *Journal of Experimental Medicine* **219**: e20211140. https://doi.org/10.1084/jem.20211140

Ebner, F., Morrison, E., Bertazzon, M., Midha, A., Hartmann, S., Freund, C. & Álvaro-Benito, M. (2020). CD4+ Th immunogenicity of the *Ascaris* spp. secreted products. *Npj Vaccines*, *5*(1), 1–8. https://doi.org/10.1038/s41541-020-0171-z

Fabišíková, M., Martinková, M., Hirková, S., Gonda, J., Pilátová, M.B. & Gönciová, G. (2016). Total synthesis and the anticancer activity of (+)-spisulosine. *Carbohydrate Research* **435**: 26-36. https://doi.org/10.1016/j.carres.2016.09.010

Gazzinelli-Guimaraes, P.H. & Nutman, T.B. (2018). Helminth parasites and immune regulation [version 1; peer review: 2 approved]. *F1000 Research*, **7**(0). https://doi.org/10.12688/F1000RESEARCH.15596.1

Giussani, P., Tringali, C., Riboni, L., Viani, P. & Venerando, B. (2014). Sphingolipids: key regulators of apoptosis and pivotal players in cancer drug resistance. *International Journal of Molecular Sciences* **15**: 4356-4392. https://doi.org/10.3390/ijms15034356

Li, R.Z., Wang, X.R., Wang, J., Xie, C., Wang, X.X., Pan, H.D., Meng, W.Y., Liang, T.L., Li, J.X., Yan, P.Y. & Wu, Q.B. (2022). The key role of sphingolipid metabolism in cancer: New therapeutic targets, diagnostic and prognostic values, and anti-tumor immunotherapy resistance. Frontiers in Oncology. Frontiers Media S.A. https://doi.org/10.3389/fonc.2022.941643

Hartmann, W., Brenz, Y., Kingsley, M.T., Ajonina-Ekoti, I., Brattig, N.W., Liebau, E. & Breloer, M. (2013). Nematode-derived proteins suppress proliferation and cytokine production of antigen-specific T cells via induction of cell death. *PLoS ONE* **8**: e68380. https://doi.org/10.1371/journal.pone.0068380

Hewitson, J.P., Ivens, A.C., Harcus, Y., Filbey, K.J., McSorley, H.J., Murray, J., Bridgett, S., Ashford, D., Dowle, A.A. & Maizels, R.M. (2013). Secretion of protective antigens by tissue-stage nematode larvae revealed by proteomic analysis and vaccination-induced sterile immunity. *PLoS Pathogens* **9**: e1003492. https://doi.org/10.1371/journal.ppat.1003492

Jacobs, B.A., Chetty, A., Horsnell, W.G.C., Schäfer, G., Prince, S. & Smith, K.A. (2018). Hookworm exposure decreases human papillomavirus uptake and cervical cancer cell migration through systemic regulation of epithelial-mesenchymal transition marker expression. *Scientific Reports* **8**: 11547. https://doi.org/10.1038/s41598-018-30058-9

Jacobs, B.A., Prince, S. & Smith, K.A. (2020). Gastrointestinal nematode-derived antigens alter colorectal cancer cell proliferation and migration through regulation of cell cycle and epithelial-mesenchymal transition proteins. *International Journal of Molecular Sciences*, **21**(21), 1–15. https://doi.org/10.3390/ijms21217845

Kobayashi, Y. & Tsuyuzaki, J. (2018). *Ascaris lumbricoides* discharge from the mouth. *Case Reports in Gastroenterology* **12**: 153-157. https://doi.org/10.1159/000488524

Mehrdana, F. & Buchmann, K. (2017). Excretory/secretory products of anisakid nematodes: biological and pathological roles. *Acta Veterinaria Scandinavica* **59**: 42. https://doi.org/10.1186/s13028-017-0310-3

Motran, C.C., Silvane, L., Chiapello, L.S., Theumer, M.G., Ambrosio, L.F., Volpini, X., Celias, D.P. & Cervi, L. (2018). Helminth infections: recognition and modulation of the immune response by innate immune cells. *Frontiers in Immunology* **9**: 664. https://doi.org/10.3389/fimmu.2018.00664

Oka, T., Saif, L.J., Marthaler, D., Esseili, M.A., Meulia, T., Lin, C.M., Vlasova, A.N., Jung, K., Zhang, Y. & Wang, Q. (2014). Cell culture isolation and sequence analysis of genetically diverse US porcine epidemic diarrhea virus strains including a novel strain with a large deletion in the spike gene. *Veterinary Microbiology* **173**: 258-269. https://doi.org/10.1016/j.vetmic.2014.08.012

Pruett, S.T., Bushnev, A., Hagedorn, K., Adiga, M., Haynes, C.A., Sullards, M.C., Liotta, D.C. & Merrill, A.H. (2008). Biodiversity of sphingoid bases ("sphingosines") and related amino alcohols. *Journal of Lipid Research* **49**: 1621-1639. https://doi.org/10.1194/jlr.R800012-JLR200

Radovic, I., Gruden-Movsesijan, A., Ilic, N., Cvetkovic, J., Mojsilovic, S., Devic, M. & Sofronic-Milosavljevic, L. (2015). Immunomodulatory effects of *Trichinella spiralis*-derived excretory-secretory antigens. *Immunologic Research* **61**: 312-325. https://doi.org/10.1007/s12026-015-8626-4

Shute, A., Callejas, B.E., Li, S.H., Wang, A., Jayme, T.S., Ohland, C., Lewis, I.A., Layden, B.T., Buret, A.G. & McKay, D.M. (2021). Cooperation between host immunity and the gut bacteria is essential for helminth-evoked suppression of colitis. *Microbiome*, **9**(1), 1–18. https://doi.org/10.1186/s40168-021-01146-2

Sugawara, T., Zaima, N., Yamamoto, A., Sakai, S., Noguchi, R. & Hirata, T. (2006). Isolation of sphingoid bases of sea cucumber cerebrosides and their cytotoxicity against human colon cancer cells. Bioscience, Biotechnology and Biochemistry, **70**(12), 2906–2912. https://doi.org/10.1271/bbb.60318

Togarsimalimath, S.K. (2021). Erratum: Helminth antigen exposure enhances early immune control of mycobacterium tuberculosis in monocytes and macrophages (Journal of Innate Immunity (2020) DOI: 10.1159/000512279). *Journal of Innate Immunity* **13**: 60. https://doi.org/10.1159/000513971

Uma Suganya, K.S., Govindaraju, K., Kumar, V.G., Prabhu, D., Arulvasu, C., Dhas, S.T., Karthick, V. & Changmai, N. (2016). Anti-proliferative effect of biogenic gold nanoparticles against breast cancer cell lines (MDA-MB-231 & MCF-7). *Applied Surface Science* **371**: 415-424. https://doi.org/10.1016/j.apsusc.2016.03.004

Van Die, I. & Cummings, R.D. (2010). Glycan gimmickry by parasitic helminths: a strategy for modulating the host immune response? *Glycobiology* **20**: 2-12. https://doi.org/10.1093/glycob/cwp140

Vasilev, S., Ilic, N., Gruden-Movsesijan, A., Vasilijic, S., Bosic, M. & Sofronic-Milosavljevic, L. (2015). Necrosis and apoptosis in *Trichinella spiralis*-mediated tumour reduction. *Central European Journal of Immunology* **40**: 42-53. https://doi.org/10.5114/ceji.2015.50832

Yang, X., Yang, Y., Wang, Y., Zhan, B., Gu, Y., Cheng, Y. & Zhu, X. (2014). Excretory/secretory products from *Trichinella spiralis* adult worms ameliorate DSS-induced colitis in mice. *PLoS ONE* **9**: e96454. https://doi.org/10.1371/journal.pone.0096454

Yang, X.J., Yang, J.P., Huang, Y.Q., Liang, H. & Yuan, K. (2013). Anti-tumor effect of the whole worm extract of *Ascaris lumbricoides* on *Lewis* lung carcinoma in mice. (In Chinese). *Chinese Journal of Parasitology & Parasitic Diseases* **31**: 470-472.