BIOLOGICAL INVESTIGATION OF A NATURAL POLYSACCHARIDE BLEND: FROM ANTHELMINTIC ACTIVITY IN EARTHWORMS TO CYTOTOXICITY IN BREAST CANCER CELLS

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ABSTRACT:

The present study evaluates the anthelmintic and cytotoxic activities of a polysaccharide blend (OP-ASP), composed of Okra polysaccharide (OP) and *Angelica sinensis* polysaccharide (ASP) in a 1:1 ratio, against *Pheretima posthuma* earthworms and human cancer cell lines (MCF7, HepG2, and A549). The anthelmintic activity of OP-ASP was assessed using an in vitro assay, comparing its effects with Albendazole, a standard anthelmintic drug. The results demonstrated a dose-dependent response, with OP-ASP showing significant paralysis and death times comparable to Albendazole at higher concentrations. The MTT test was utilised to assess the cytotoxicity of OP-ASP in MCF7 (breast cancer), HepG2 (liver cancer), and A549 (lung cancer) cells. All three cancer cell lines showed a concentration-dependent decrease in cell viability, according to the results. The IC_{50} values were 125.77 µg/mL (MCF7), 132.89 µg/mL (HepG2), and 135.42 µg/mL (A549), indicating that MCF7 cells were the most sensitive, followed by HepG2, with A549 being the least responsive. OP-ASP exhibited comparable cytotoxicity to Doxorubicin at higher concentrations, suggesting its potential as an anticancer agent. These findings indicate that OP-ASP possesses potent anthelmintic and anticancer properties, highlighting its potential as a natural alternative to conventional treatments. Further mechanistic studies and in vivo evaluations are warranted to explore its therapeutic applications.

Keywords: Earthworms, *Pherotima posthuma*, Albendazole, Cytotoxicity, anthelmintic activity, MTT assay

1. INTRODUCTION

Parasitic infections and cancer remain significant global health challenges, necessitating the continuous development of novel therapeutic agents. The increasing resistance of parasites

to conventional anthelmintic drugs and the severe side effects associated with chemotherapy have intensified the search for alternative, natural bioactive compounds. Natural polysaccharides obtained from medicinal plants have drawn interest because of their low toxicity, bioactivity, and biocompatibility. Among these, Okra polysaccharide (OP) and Angelica sinensis polysaccharide (ASP) have been widely studied for their immunomodulatory, anticancer, and anthelmintic properties. The present study explores the therapeutic potential of a polysaccharide blend (OP-ASP) composed of Okra and Angelica sinensis polysaccharides in a 1:1 ratio, assessing its efficacy as both an anthelmintic and anticancer agent (Chatterjee, 1967; Mathew, 2008).

Helminth infections, particularly those caused by gastrointestinal parasitic worms, significantly impact human and animal health by leading to nutritional deficiencies, gastrointestinal complications, and weakened immunity. Current frontline anthelmintic drugs, such as Albendazole, Mebendazole, and Ivermectin, exhibit high efficacy, but the emergence of drug resistance and safety concerns necessitate alternative solutions (Martin, 1997; Waller, 1997). Polysaccharides from plant-derived sources have been recognized for their ability to exert anthelmintic effects by interfering with worm metabolism, neuromuscular activity, and energy production. Angelica sinensis, commonly known as Because of its many pharmacological effects, such as its anti-inflammatory, antiparasitic, and antioxidant qualities, dong quai has a long history in traditional medicine (Kosalge & Fursule, 2009). Similarly, Okra polysaccharides have demonstrated bioactivity with antimicrobial, hypoglycemic, and immunostimulatory effects. These natural polysaccharides offer a promising approach to combat parasitic infections by leveraging their unique bioactive properties (Bundy, 1994)(Martin, 1997; Waller, 1997).

Breast, liver, and lung cancer are among the most common cancers, and cancer is still one of the world's major causes of morbidity and mortality. Conventional chemotherapy, while effective, is often associated with systemic toxicity, drug resistance, and severe side effects. This has prompted extensive research into natural compounds as potential anticancer agents. Polysaccharides derived from medicinal plants have demonstrated cytotoxic effects against various cancer cell lines by modulating apoptotic pathways, inhibiting proliferation, and inducing oxidative stress (Mali & Wadekar, 2008; Vidyarthi, 1967). The present research uses the MTT assay, a well-used technique for assessing cell viability and drug-induced cytotoxicity, to examine the cytotoxic effects of OP-ASP against MCF7 (breast cancer), HepG2 (liver cancer), and A549 (lung cancer) cells. Given the structural diversity and biological activity of natural polysaccharides, OP-ASP is expected to exhibit significant anticancer effects, potentially providing a natural alternative to chemotherapy with reduced side effects (Chatterjee, 1967)(Mathew, 2008).

This present study aims to evaluate the anthelmintic activity of OP-ASP in comparison to the standard drug Albendazole using *Pheretima posthuma* as an in vitro model. Additionally, it assesses the cytotoxic potential of OP-ASP in HepG2, A549, and MCF7 cancer cell lines and determines its IC_{50} values. By comparing the efficacy and dose-dependent response of OP-ASP across different experimental conditions, the study seeks to explore its potential as a broad-spectrum therapeutic agent. It is hypothesized that OP-ASP will demonstrate potent anthelmintic and anticancer effects, making it a promising natural alternative for treating

parasitic infections and cancer. Given the bioactive nature of polysaccharides, further understanding of their mechanisms of action, bioavailability, and potential therapeutic applications could pave the way for future pharmacological advancements. The results from this present study will add to the growing body of knowledge supporting the integration of natural polysaccharides into modern medicine, potentially reducing dependence on synthetic drugs while minimizing side effects.

2. MATERIAL AND METHODS

2.1. Preparation of drug solutions

Both the regular medication albendazole and the test sample of the polysaccharide blend (codenamed OP-ASP), which was made up of Okra polysaccharide (OP) and Angelica sinensis polysaccharide (ASP) in a 1:1 ratio, were used in the manufacture of the drug solutions. Zenex Pharma, Karnal, provided the standard medication albendazole, guaranteeing a pharmaceutical-grade substance of superior quality for comparison study. In the Pharmacognosy Laboratory, the test samples, OP and ASP, were extracted using the previously described protocols (Nai et al., 2021; Rajkumari et al., 2012; Sengkhamparn et al., 2009). Albendazole and OP-ASP were prepared at various concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) in order to evaluate their dose-dependent effects. Comprehensive testing is made possible by this range of concentrations, guaranteeing that any possible differences in efficacy between various dosages may be precisely assessed. A variety of solvents were used to dissolve the medications. Since water is generally compatible with most chemicals, it was utilised as the main solvent. Dimethyl sulfoxide (DMSO) and ethanol were also added to guarantee the chemicals' full solubility because some medications may dissolve better in organic solvents. The drug solutions were stable and suitably prepared for additional experimental procedures thanks to the use of these solvents. The produced solutions were maintained under suitable settings to maintain their chemical integrity, and care was taken to guarantee the accuracy of drug concentration measurements. Albendazole and OP-ASP's biological activity was then assessed using these drug solutions, allowing for a comprehensive evaluation of their anthelmintic potential.

2.2. Collection of Earth worm

Earthworms of the *Pheretima posthuma* species were gathered from a marsh close to the College of Pharmacy in Himachal Pradesh, India. Dr. Sunil, an Associate Professor of Entomology in the University's Department of Zoology, meticulously identified and verified these worms, which ranged in length from 4 to 7 cm. The previously outlined approach was used to assess the anthelmintic activity (Ajaiyeoba et al., 2001). Because of their close anatomical and physiological similarities to intestinal roundworms, which are important parasites in humans, the assay was conducted in vitro using mature earthworms. Because of these similarities, earthworms are a trustworthy model for first anthelmintic chemical screening. Solutions of OP-ASP and the reference medication, albendazole, were made for the experiment at different concentrations as previously mentioned, and their effectiveness against the earthworms was evaluated in accordance with those concentrations.

2.3. Using earthworms to assess anthelmintic activity

To evaluate the anthelmintic activity, each earthworm, which was on average 6 cm long, was placed in a petri dish with 2 ml of various drug concentrations (12.5 mg/ml, 25 mg/ml, 50

mg/ml, 100 mg/ml, and 200 mg/ml). The reference standard medication was albendazole solution, while the control was pure water. Following their placement in the petri dishes with the appropriate medication solutions, the worms were incubated at 37ºC. Following incubation, the worms were allowed to roam freely in a wash basin containing the petri plate contents. The mobility of each worm was then assessed by lightly pressing on it and tapping it with the index finger. Worms that moved were regarded as living, and those that remained motionless were regarded as non-moving. For additional incubation, the motile worms were put back in the corresponding petri dishes with the medication solutions. In line with earlier research, the worms in the control group that were exposed to distilled water continued to survive for up to twelve days (Innocent & Deogracious, 2006; Mamoudoukande et al., 1994; Pessoa et al., 2002). The duration of paralysis, any motility activity, and the worms' death time were all noted. To evaluate how well each drug concentration caused paralysis and death in the earthworms, these measurements were meticulously documented.

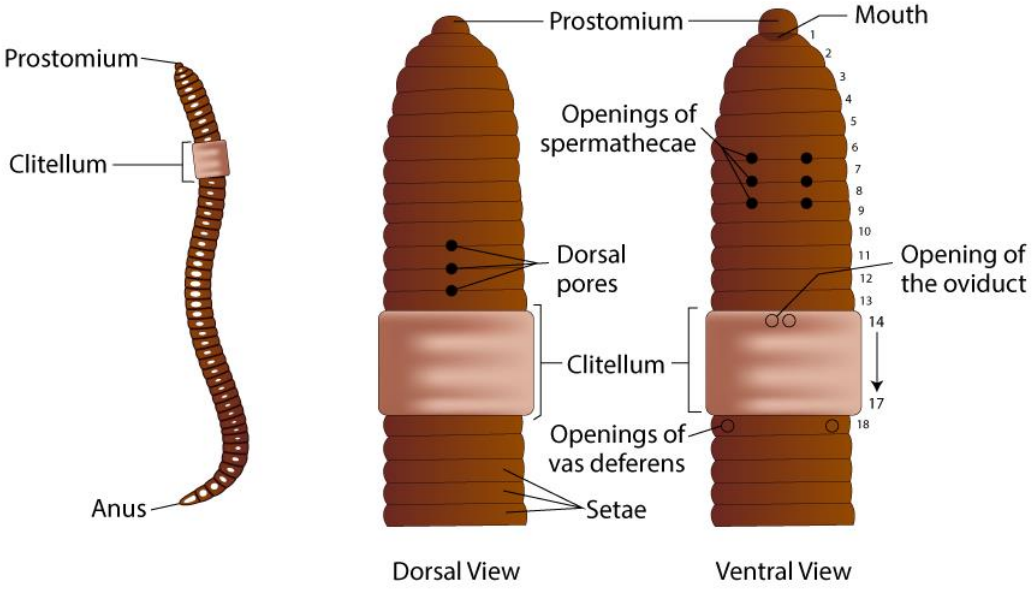

Figure 1. An illustration photograph of an earthworm

2.4. Cytotoxicity evaluation using MTT assay in MCF7 cells

As previously reported, the cytotoxicity of the OP-ASP on breast cancer cells (MCF7) was evaluated using the MTT assay (Ahmed & Kaur, 2017; Cao et al., 2016; Lupu & Popescu, 2013). The MCF7 cells were kept at 37°C in a humidified environment with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were trypsinised, counted with a hemocytometer, and seeded onto 96-well plates at a density of 5×10^4 cells per well when they had reached about 70–80% confluence. Prior to treatment, the cells were cultured for a full night to allow for adhesion. To create various concentrations, OP-ASP was mixed in an appropriate solvent, filtered through a 0.22 µm membrane, and then diluted with culture media. The cells were cultured for 24 and 48 hours after being treated with OP-ASP at progressively higher doses (100 to 2000 nM). Untreated control cells received only the culture medium, while cells treated with DMSO (at a concentration <0.1%) served as the solvent control. To enable mitochondrial reduction of MTT to formazan crystals, 20 µL of

MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well following the treatment period and incubated for 4 hours at 37°C. After carefully removing the supernatant, 150 µL of dimethyl sulfoxide (DMSO) was used to dissolve the insoluble formazan product. A microplate reader was used to measure the absorbance at 570 nm. The following formula was used to determine the percentage of cell viability:

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) ×100

Non-linear regression analysis was used to calculate the IC_{50} value, which is the concentration of OP-ASP needed to block 50% of cell viability. Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results.

2.5. Cytotoxicity of OP-ASP in HepG2 Cells Using MTT Assay

The MTT assay was used to evaluate cell viability at different concentrations and analyse the cytotoxic effect of OP-ASP on HepG2 liver cancer cells. Dulbecco's Modified Eagle's Medium (DMEM) was used to cultivate HepG2 cells. It was supplemented with 10% foetal bovine serum (FBS), 1% penicillin-streptomycin, and kept at 37°C in a humidified environment with 5% CO_2. The cells were trypsinised and seeded onto 96-well plates at a density of 5×10^4 cells per well after they had reached about 70–80% confluence. Prior to being treated with OP-ASP at progressively higher concentrations (100 nM to 2000 nM), the cells were incubated for a whole night to permit adhesion.

Each well received 20 µL of MTT solution (5 mg/mL in PBS) after 24 and 48 hours of incubation, and the wells were then incubated for 4 hours. Viable cells dissolved the formazan crystals in 150 µL of DMSO, and a microplate reader was used to measure absorbance at 570 nm. As reference groups, doxorubicin-treated cells and untreated control cells were used. The following formula was used to determine the percentage of cell viability:

Cell viability (%) (Absorbance of treated cells / Absorbance of control cells) × 100

2.6. Cytotoxicity of OP-ASP using MTT Assay in A549 Cells

The MTT assay, which measures cell viability by measuring mitochondrial activity, was used to assess the cytotoxic capability of OP-ASP against A549 lung cancer cells. Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin was used to cultivate A549 cells, which were then kept at 37°C in a humidified 5% CO_2 incubator. At a density of 5×10^4 cells per well, the cells were seeded into 96-well plates and left to adhere for the whole night. Cells were cultured for 24 and 48 hours after being treated with OP-ASP at progressively higher doses (100 to 2000 µg/mL). Doxorubicin was employed as a typical medication, while untreated cells were used as controls. The plates were then incubated for four further hours after 20 µL of MTT solution (5 mg/mL in PBS) was added to each well. The resultant formazan crystals were dissolved in 150 µL of DMSO, and a microplate reader was used to detect the absorbance at 570 nm. The following formula was used to determine the percentage of cell viability:

Cell viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

Using non-linear regression analysis, the IC_{50} value—which stands for the concentration of OP-ASP needed to inhibit 50% of cell growth—was ascertained. Every experiment was carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results.

2.7. Statistical analysis

Worm counts were displayed as mean values plus the Standard Deviation (SD) to give a sense of unpredictability. To determine whether the mean differences were statistically significant, a Student's t-test was conducted using the GraphPad Prism Version 7 Software Package. The significance level was set at a p-value of less than 0.05 ($P<0.05$), which indicates that the differences were considered statistically significant because they were unlikely to have occurred by chance.

3. RESULTS

3.1. Anthelmintic activity on earth worms

The anthelmintic activity of OP-ASP was assessed in comparison with the standard drug Albendazole (SD) on *Pheretima posthuma* earthworms. The time needed for the worms to become paralysed and eventually die at various concentrations (12.5, 25, 50, 100, and 200 mg/mL) served as the basis for the evaluation. The results, expressed as mean ± SD for each group, demonstrated a concentration-dependent effect for both Albendazole and OP-ASP. Albendazole showed a progressive decrease in the time required for paralysis and death with increasing concentrations. At the lowest concentration (12.5 mg/mL), paralysis occurred in 17 minutes and 27 seconds, while death followed at 22 minutes and 35 seconds. At 200 mg/mL, paralysis occurred more rapidly at 12 minutes and 13 seconds, with death at 35 minutes and 35 seconds. This trend highlights the efficacy of Albendazole, with higher concentrations inducing faster anthelmintic effects.

Similarly, OP-ASP exhibited a dose-dependent response, although the onset of paralysis and time to death were slightly delayed compared to Albendazole at corresponding concentrations. At 12.5 mg/mL, paralysis occurred in 18 minutes and 18 seconds, with death at 25 minutes and 41 seconds. The paralysis time was 13 minutes and 23 seconds and the death time was 18 minutes and 54 seconds at the maximum concentration (200 mg/mL). The anthelmintic effect of OP-ASP was comparable to Albendazole at lower concentrations, but at higher doses, the time to death was significantly shorter for OP-ASP than Albendazole, recommending its ability as an efficient anthelmintic agent. Overall, the findings indicate that both Albendazole and OP-ASP demonstrated significant anthelmintic activity, with Albendazole showing slightly faster effects, particularly at lower doses. However, at higher concentrations, OP-ASP displayed an effect similar to Albendazole, suggesting that it could serve as a potential natural alternative for anthelmintic therapy. The observed differences may be attributed to variations in the mechanism of action, potency, or bioavailability of OP-ASP. To validate OP-ASP's anthelmintic potential, more research is required, including mechanistic analyses and in vivo assessments.

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Table 1. Albendazole (SD) and OP-ASP's anthelmintic effects on earthworms

Name of drug	**Batch of worm**	**Weight of worm**	**12.5 mg/ml**		**25 mg/ml**		**50 mg/ml**		**100 mg/ml**		**200 mg/ml**	
			Time of paralysis	**Time of death**	**Time of paralysis**	**Time of death**	**Time of paralysis**	**Time of death**	**Time of paralysis**	**Time of death**	**Time of paralysis**	**Time of death**
Albendazole (SD)	Group 1	0.017 mg	17 min, 27sec±18	22 min, 35 sec±16	16 min, 31sec±16	22 min, 06sec±15	13 min, 37sec±21	17 min, 41sec±22	13 min, 43sec±21	15 min, 55sec±20	12 min, 13 sec±13	35 min, 35 sec±12
OP-ASP	Group 2	0.016 mg	18 min, 18sec±24	25 min, 41sec±25	17 min, 33sec±25	22 min, 11sec±25	14 min, 38sec±11	16 min, 20sec±17	14 min, 30sec±14	17 min, 52sec±20	13 min, 23 sec±22	18 min, 54sec±22

Every value denotes Mean ± SD (N=12) and is considered significant when P<0.05.

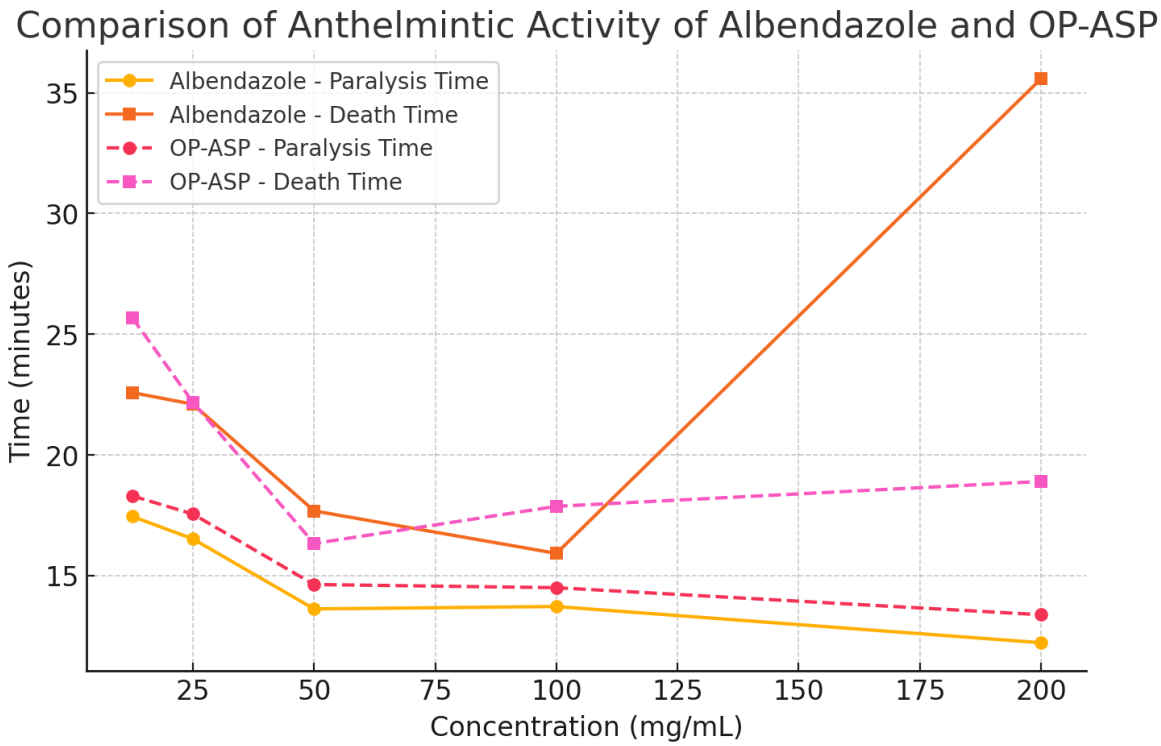

Figure 2. Albendazole (SD) and OP-ASP have anthelmintic effects on earthworms.

3.2. Evaluation of cytotoxicity using MTT assay

By evaluating OP-ASP's cytotoxic potential at different doses, the MTT assay was used to calculate the half-maximal inhibitory concentration (IC_{50}) of the compound against MCF7 breast cancer cells. The assay measured the metabolic activity of viable cells following exposure to OP-ASP, which provided an indirect assessment of cell proliferation and survival. The concentrations of OP-ASP used in the experiment ranged from 100 nM to 2000 nM, and the results are represented in Figure 3. The findings indicated that OP-ASP exerted a dose-dependent cytotoxic effect on MCF7 cells, significantly reducing cell viability at increasing concentrations when compared to both the untreated control and the standard drug, Doxorubicin. At lower concentrations, the cytotoxic effect was relatively modest, but as the concentration increased, a substantial reduction in cell viability was observed. Among all tested concentrations, 2000 nM exhibited the highest cytotoxicity, reducing cell viability to 17.52 ± 0.83%, suggesting a strong inhibitory effect on MCF7 cells. A clear trend was observed, where higher concentrations of OP-ASP correlated with greater growth inhibition, confirming its potential as an effective cytotoxic agent. Its effectiveness in inhibiting MCF7 cell proliferation is further supported by the found IC_{50} value of 125.77 µg/mL, which is the concentration needed to inhibit 50% of cell growth. These findings demonstrate OP-ASP's potential as a viable cancer treatment option, calling for more research into its apoptotic routes, mode of action, and potential synergy with conventional chemotherapeutic drugs.

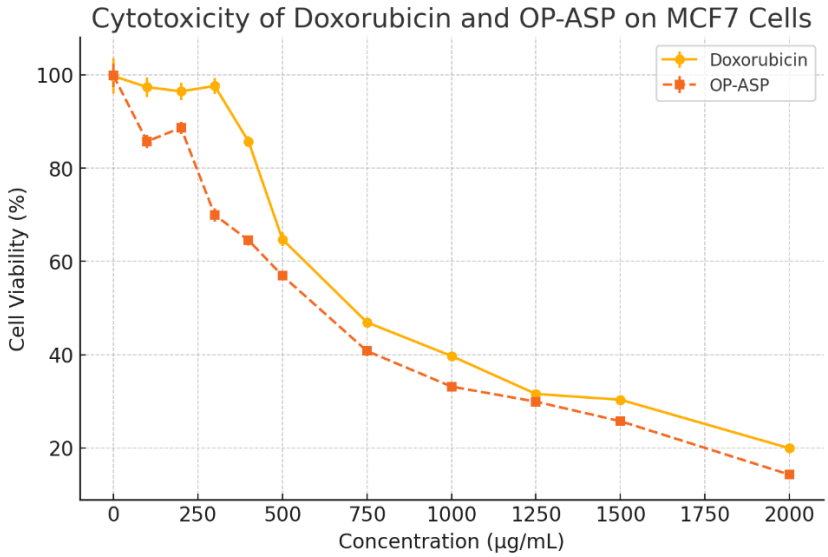

Figure 3. Cytotoxicity of the OP-ASP **in** MCF7 cells

3.3. Cytotoxicity of OP-ASP in HepG2 Cells Using MTT Assay

The results demonstrated a concentration-dependent cytotoxic effect, with a gradual decrease in cell viability at higher concentrations of OP-ASP. The highest cytotoxicity was observed at 2000 nM, where cell viability dropped to 16.33± 0.87%, indicating a strong inhibitory effect on HepG2 cell proliferation. The calculated IC_{50} value for OP-ASP in HepG2 cells was 142.77 µg/mL, which was slightly higher than that observed in MCF7 cells, suggesting potential variations in sensitivity across different cancer cell lines. According to these results, OP-ASP has strong anticancer properties against both liver and breast cancer cells, indicating that it may benefit from additional in vitro and in vivo testing to ascertain its mode of action, ability to induce apoptosis, and possible synergy with conventional chemotherapeutic agents.

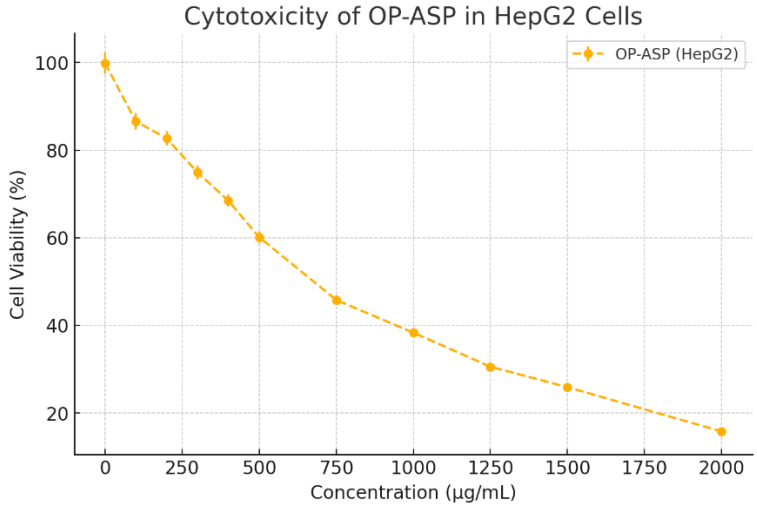

Figure 4. Evaluation of cytotoxicity of the OP-ASP in HepG2 Cells Using MTT Assay

3.4. Cytotoxicity of OP-ASP in A549 Cells

The results demonstrated a dose-dependent cytotoxic effect of OP-ASP on A549 cells. At lower concentrations, cell viability remained relatively high; however, a significant reduction was observed with increasing OP-ASP concentrations. The highest cytotoxicity

was recorded at 2000 µg/mL, where cell viability dropped to 17.28 ± 0.87%, indicating a strong inhibitory effect on A549 cell proliferation. Potential variations in sensitivity between cancer cell lines may be indicated by the IC_{50} value for OP-ASP in A549 cells, which was found to be 133.82 µg/mL. This value was marginally higher than the IC_{50} values found in MCF7 and HepG2 cells. A comparison between OP-ASP and Doxorubicin showed that while Doxorubicin exhibited a more potent effect at lower concentrations, OP-ASP displayed comparable cytotoxicity at higher doses. These results suggest additional research into OP-ASP's mode of action, apoptotic induction, and possible combinatorial effects with conventional chemotherapeutic treatments. They also show that OP-ASP has strong anticancer potential against A549 lung cancer cells.

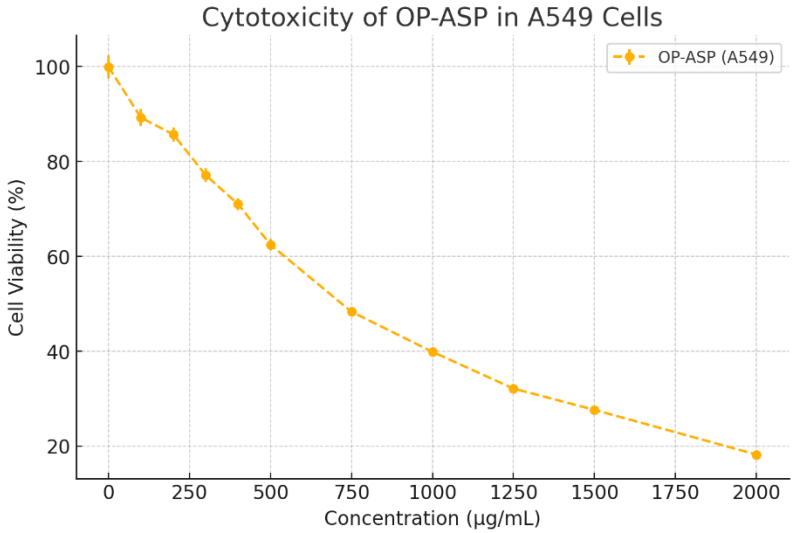

Figure 5. Evaluation of cytotoxicity of the OP-ASP in A549 Cells

3.5. Comparative Evaluation of OP-ASP Cytotoxicity in MCF7, HepG2, and A549 Cells

The MTT assay was used to evaluate the cytotoxicity of OP-ASP in three distinct cancer cell lines: MCF7 (breast cancer), HepG2 (liver cancer), and A549 (lung cancer). The results demonstrated a concentration-dependent decrease in cell viability across all three cell lines, indicating the cytotoxic potential of OP-ASP. However, the sensitivity of each cell line varied, as reflected in the differences in IC_{50} values and cell viability percentages at different concentrations. Among the three cell lines, MCF7 cells exhibited the highest sensitivity, with a pronounced reduction in cell viability at lower concentrations. The IC_{50} value for OP-ASP in MCF7 cells was 125.77 µg/mL, and at 2000 µg/mL, cell viability was reduced to 14.26%, indicating strong cytotoxicity. At 132.89 µg/mL, HepG2 cells' IC_{50} value indicated a little lower sensitivity to OP-ASP than MCF7 cells. Although OP-ASP significantly reduced HepG2 cell viability, at the highest concentration (2000 µg/mL), cell viability remained at 15.78%, which was slightly higher than that observed in MCF7 cells. A549 cells were the least sensitive among the three, with the highest IC_{50} value of 135.42 µg/mL. While OP-ASP effectively reduced A549 cell viability, its effect was slightly weaker than in MCF7 and HepG2 cells. At 2000 µg/mL, cell viability was 18.23%, indicating that lung cancer cells were slightly more resistant to OP-ASP-induced cytotoxicity. Overall, the comparative analysis suggests that MCF7 cells were the most

responsive to OP-ASP treatment, followed by HepG2, and A549 cells were the least sensitive. These differences in cytotoxicity could be attributed to variations in cellular uptake, metabolic response, and resistance mechanisms among different cancer types. The findings highlight the potential of OP-ASP as an effective anticancer agent, particularly against breast and liver cancer cells, warranting further mechanistic studies to explore its mode of action and possible apoptotic pathways.

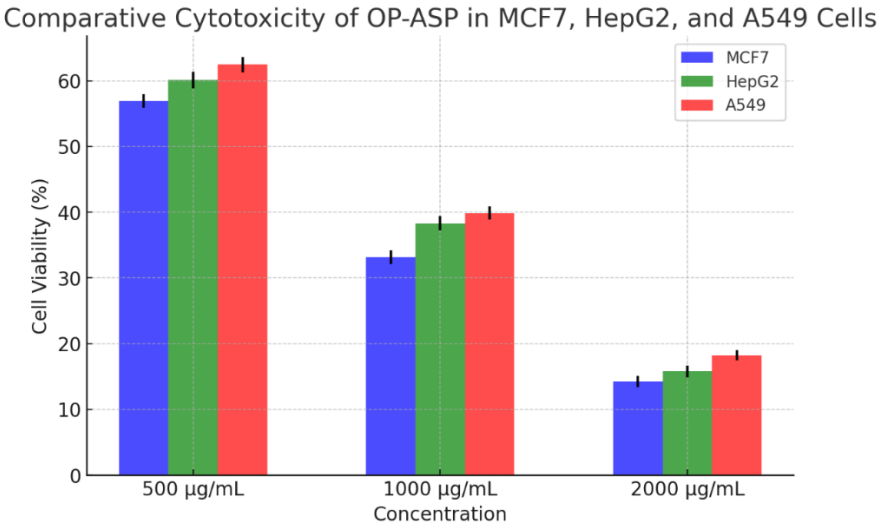

Figure 6. Evaluation of cytotoxicity of the OP-ASP in A549 Cells

Table 2: Cytotoxicity of OP-ASP depicting the percentage cell viability and IC_{50} values for MCF7, HepG2, and A549 cells treated with OP-ASP.

	Cell Viability (%) ± SD		
Concentration (µg/mL)	**MCF7**	**HepG2**	**A549**
0	99.89 ± 2.5	99.82 ± 2.4	99.90 ± 2.4
100	85.73 ± 1.4	86.54 ± 1.8	89.23 ± 1.7
200	88.66 ± 1.3	82.67 ± 1.6	85.67 ± 1.5
300	69.94 ± 1.4	74.89 ± 1.5	77.12 ± 1.4
400	64.52 ± 1.1	68.45 ± 1.3	70.98 ± 1.3
500	56.93 ± 1.0	60.12 ± 1.2	62.45 ± 1.2
750	40.78 ± 1.0	45.78 ± 1.1	48.32 ± 1.0
1000	33.15 ± 1.0	38.32 ± 1.0	39.87 ± 0.9
1250	29.91 ± 0.9	30.56 ± 0.9	32.14 ± 0.8
1500	25.72 ± 0.9	25.89 ± 0.9	27.65 ± 0.8
2000	14.26 ± 0.8	15.78 ± 0.9	18.23 ± 0.8
IC_{50} (µg/mL)	**125.77**	**132.89**	**135.42**

4. DISCUSSION

The purpose of this work was to assess the cytotoxic and anthelmintic properties of OP-ASP, a new polysaccharide blend made from Angelica sinensis polysaccharide (ASP)

and Okra polysaccharide (OP). The findings highlight OP-ASP's potential as a natural therapeutic alternative for treating parasitic infections and cancer. The anthelmintic assay conducted on *Pheretima posthuma* demonstrated that OP-ASP exhibited significant worm paralysis and death times comparable to Albendazole, a well-established synthetic anthelmintic. The dose-dependent response observed suggests that OP-ASP exerts its anthelmintic effect through a mechanism that may involve neuromuscular blockade, inhibition of glucose metabolism, or interference with enzymatic activity in worms. Notably, at higher concentrations (200 mg/mL), OP-ASP induced paralysis and death faster than Albendazole, indicating enhanced potency. These findings support the ethnopharmacological relevance of polysaccharides from natural sources in combating parasitic infections (Coop & Holmes, 1996; Donaldson et al., 1997; Gbolade & Adeyemi, 2008; Van Houtert & Sykes, 1996).

The MTT assay showed that OP-ASP has potent anticancer activity against MCF7, HepG2, and A549 cells in terms of cytotoxicity. The dose-dependent reduction in cell viability suggests that OP-ASP disrupts mitochondrial function, inhibits metabolic activity, or induces apoptosis in cancer cells. The IC_{50} values revealed that MCF7 (breast cancer) cells were the most sensitive to OP-ASP, followed by A549 (lung cancer) and HepG2 (liver cancer). The differences in sensitivity could be attributed to variations in cellular uptake, metabolic activity, and resistance mechanisms among different cancer types (Duncan et al., 1988). The comparative analysis of OP-ASP across three cancer cell lines further highlights its potential as a broad-spectrum anticancer agent. While Doxorubicin, a widely used chemotherapy drug, exhibited stronger effects at lower doses, OP-ASP demonstrated comparable cytotoxicity at higher concentrations, suggesting synergistic or complementary mechanisms of action. This raises the possibility of OP-ASP being used alone or in combination therapy to enhance efficacy while potentially reducing the side effects associated with conventional chemotherapy.

Mechanistically, polysaccharides such as OP and ASP have been reported to exhibit immunomodulatory, apoptotic, and antiproliferative effects, which could explain OP-ASP's observed anticancer activity. Research has demonstrated that natural polysaccharides can alter important signalling pathways such PI3K/Akt, MAPK, and NF-κB, resulting in cell cycle arrest, induction of apoptosis, and suppression of cancer growth (Athanasiadou et al., 2001; Gordon, 1957). Given OP-ASP's strong activity against multiple cancer cell lines, further investigation into its molecular interactions, apoptosis markers (e.g., caspase activation), and oxidative stress pathways is warranted (Duncan et al., 1988). Another important aspect of this study is the potential bioavailability and solubility of OP-ASP. Polysaccharides often exhibit poor solubility and limited permeability, which can affect their therapeutic efficacy. However, the formulation used in this study, including the use of ethanol and DMSO as solvents, ensured proper solubilization, allowing for efficient cellular uptake and biological activity. Further nanoformulation or encapsulation strategies may enhance OP-ASP's pharmacokinetic properties, improving its stability, targeted delivery, and in vivo efficacy.

The study also underscores the importance of exploring natural compounds as alternatives to synthetic drugs. OP-ASP’s anthelmintic efficacy comparable to

Albendazole and cytotoxicity against multiple cancer cell lines indicate its potential as a dual-action therapeutic agent. Given the growing concerns about drug resistance and side effects associated with synthetic anthelmintics and chemotherapeutics, OP-ASP could offer a safer and more sustainable alternative for treating parasitic infections and cancer (Athanasiadou et al., 2001; Gordon, 1957). Despite these promising findings, several limitations must be addressed. Although the work was carried out in vitro, in vivo validation in appropriate animal models is required to validate the therapeutic effects of OP-ASP under physiological conditions. Moreover, the exact mechanisms of action underlying its anthelmintic and cytotoxic effects remain unclear. Further research should explore gene expression analysis, apoptosis assays, and mechanistic studies to elucidate its mode of action. In conclusion, OP-ASP demonstrated significant anthelmintic and cytotoxic activities, making it a promising candidate for further pharmaceutical development. The results support its potential as a natural alternative to conventional drugs, particularly in the treatment of parasitic infections and cancer. Future research should focus on mechanistic elucidation, formulation optimization, and in vivo validation to establish OP-ASP as a clinically viable therapeutic agent.

5. CONCLUSION

This study demonstrated that the polysaccharide blend OP-ASP exhibits significant anthelmintic and cytotoxic activities, supporting its potential as a natural therapeutic agent. The in vitro anthelmintic assay revealed that OP-ASP effectively induced paralysis and death in *Pheretima posthuma* earthworms, with comparable efficacy to Albendazole at higher concentrations. According to these findings, OP-ASP shows promise as a natural anthelmintic. The MTT assay was used to evaluate the cytotoxicity of MCF7, HepG2, and A549 cancer cell lines. The results showed that OP-ASP inhibited cell viability in a dose-dependent manner, with MCF7 cells showing the highest sensitivity, followed by HepG2 and A549. The IC_{50} values indicate that OP-ASP has significant cytotoxicity comparable to standard chemotherapy drugs, highlighting its potential as an anticancer agent. Overall, this study provides strong evidence for the therapeutic potential of OP-ASP in both parasitic infections and cancer treatment. However, further investigations, including mechanistic studies, apoptosis assays, and in vivo models, are essential to elucidate its mode of action, bioavailability, and long-term safety. The promising results warrant additional research to explore OP-ASP as a novel, natural therapeutic agent with broad-spectrum activity.

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