

Fullpaper Oral Presentations

► Fullpaper Oral Presentation 1

In Vitro Investigation of the Antiproliferative Potential of the *Achyranthes aspera* Dichloromethane (DCM) Extract in SH-SY5Y Neuroblastoma Cells

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ABSTRACT

Neuroblastoma is the most common extracranial solid tumor of childhood and arises from the sympathetic nervous system. Even with intensive, multimodal treatment, resistance, relapse, and treatment-related toxicity remain major problems in high-risk cases. Here we examined how the dichloromethane (DCM) extract of *Achyranthes aspera* affects SH-SY5Y neuroblastoma cells. Cells were treated with the extract at 31.25, 62.5, 125, 250, and 500 µg/mL, and measurements were taken at 24, 48, and 72 h. Metabolic activity was assessed with the MTT assay. Untreated cells served as the control and cells exposed to 100 µM cisplatin as the positive control. Wound-healing images were followed qualitatively from D0 to D3. The clearest time-dependent decrease occurred in the 125 µg/mL group, where MTT activity was $92.2 \pm 24.3\%$, $69.5 \pm 5.2\%$, and $61.3 \pm 10.5\%$ at 24, 48, and 72 h, respectively. The reduction in the 250 µg/mL group was more limited. At 500 µg/mL the MTT signal stayed close to the control, yet microscopy showed disrupted cells and a loss of monolayer integrity; we considered that this mismatch might reflect MTT interference. Taken together, our findings suggest that the *A. aspera* DCM extract may have antiproliferative potential in SH-SY5Y cells and that the 125–250 µg/mL range in particular warrants closer study.

Keywords: *Achyranthes aspera*, SH-SY5Y neuroblastoma, Antiproliferative activity, MTT assay, Wound-healing assay

INTRODUCTION

Neuroblastoma is a childhood malignancy that develops from neural crest-derived precursor cells and follows a highly variable clinical course. In high-risk cases, resistance and relapse can occur despite intensive, multimodal treatment, and treatment-related toxicity is a further concern [1]. For this reason, the search for new candidates across different experimental models remains relevant.

Plant extracts are frequently used in such studies because they bring together many compounds from different chemical classes. *Achyranthes aspera*, a member of the Amaranthaceae family, has been reported to contain secondary metabolites such as saponins, flavonoids, alkaloids, and steroids [2]. Various extracts of the plant have been examined in colon, gastric, breast, lung, pancreatic, prostate, and oral carcinoma cells for cell viability and proliferation. These studies have shown that *A. aspera* extracts reduce cell viability and proliferation in the cell lines concerned [3,4,5,6]. A slowing of cell migration has also been reported in COLO-205 colon cancer cells [4]. On reviewing the literature, however, we found no study evaluating the effect of an *A. aspera* extract on the SH-SY5Y neuroblastoma cell line.

In this study we investigated whether the *A. aspera* DCM extract alters metabolic activity and proliferative behavior in SH-SY5Y cells. We tested five concentrations over 24, 48, and 72 h and interpreted the MTT results together with the wound-healing images.

METHODS

Plant Material and DCM Extract

The *A. aspera* DCM extract used here was obtained from the group of Sinan et al. (2020), who had previously prepared and phytochemically characterized it. The plant had been collected in 2019 from Brobo, in the Yamoussoukro region of Côte d'Ivoire, and its aerial parts were used. The material had been macerated in DCM for 24 h at room temperature, then filtered, and the solvent was removed with a rotary evaporator. The resulting dry extract had been stored at 4 °C [2].

Cell Culture

We used the human neuroblastoma–derived SH-SY5Y cell line [7].

MTT Metabolic Activity Assay

SH-SY5Y cells were treated with the *A. aspera* DCM extract at 31.25, 62.5, 125, 250, and 500 µg/mL and incubated for 24, 48, or 72 h. Untreated cells served as the control and cells treated with 100 µM cisplatin as the positive control group. At the end of each period we measured metabolic activity with the MTT assay [8]. The signals obtained were normalized to the mean of the untreated control at the same time point. The control group was taken as 100%, and the remaining groups were expressed as a percentage of the control.

Wound-Healing Model and Microscopic Evaluation

We imaged the control, 100 µM cisplatin, and 31.25–500 µg/mL extract areas at D0, D1, D2, and D3 [9]. The images were assessed qualitatively, taking into account closure of the cell-free area, cell density, detachment of cells from the surface, and monolayer integrity. Because the percentage of closure was not calculated, no statistical test was applied to the wound-healing data.

Statistical Analysis

MTT data are expressed as mean ± standard deviation. The extract and cisplatin groups were compared with the control group at the same time point for each time point. For multiple comparisons we used Dunnett's post hoc test, and $p < 0.05$ and $p < 0.001$ were considered significant.

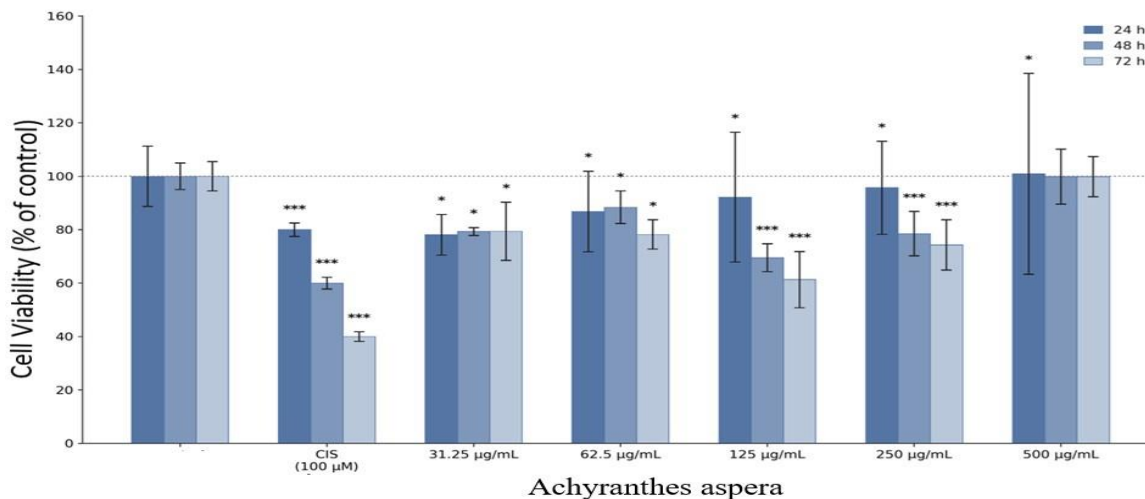


Figure 1. MTT metabolic activity of SH-SY5Y cells across treatment groups at 24, 48, and 72 h, expressed as percentage of the time-matched control. Data are mean ± SD. * $p < 0.05$; *** $p < 0.001$ versus control.

Table 1. MTT metabolic activity (% of control) of SH-SY5Y cells treated with the *A. aspera* DCM extract (31.25–500 µg/mL) and 100 µM cisplatin at 24, 48, and 72 h (mean ± SD). * $p < 0.05$; *** $p < 0.001$ versus control.

	24 h	48 h	72 h
Control	100.0 ± 11.3	100.0 ± 5.0	100.0 ± 5.5
CIS (100 µM)	80.0 ± 2.5***	60.0 ± 2.2***	40.0 ± 1.8***
31.25 µg/mL	78.1 ± 7.6*	79.3 ± 1.5*	79.4 ± 10.9*
62.5 µg/mL	86.8 ± 15.1*	88.4 ± 6.1*	78.2 ± 5.5*
125 µg/mL	92.2 ± 24.3*	69.5 ± 5.2***	61.3 ± 10.5***
250 µg/mL	95.7 ± 17.4*	78.5 ± 8.3***	74.3 ± 9.4***
500 µg/mL	100.9 ± 37.6*	99.9 ± 10.3	99.9 ± 7.5

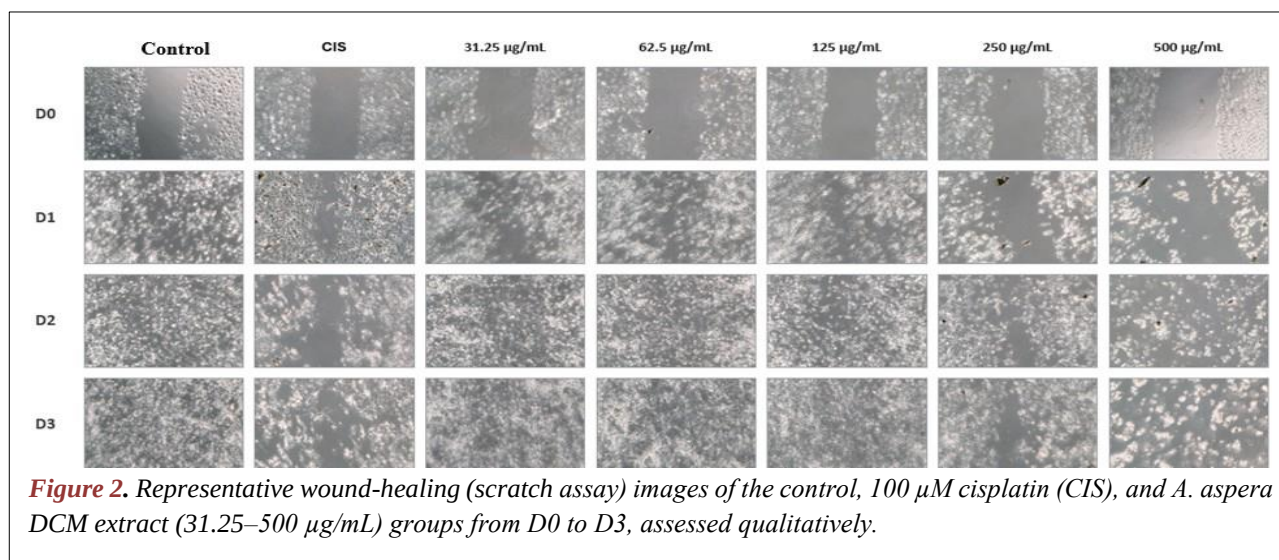
RESULTS

Control groups were normalized to 100% at each time point. In the 100 μ M cisplatin group, relative MTT activity fell steadily across the time course, from $80.0 \pm 2.5\%$ at 24 h to $60.0 \pm 2.2\%$ at 48 h and then $40.0 \pm 1.8\%$ at 72 h; the decrease was significant at all three time points ($p < 0.001$). That the positive control behaved as expected confirmed that our system could register a decline in metabolic activity.

together (Figure 2).

DISCUSSION

The *A. aspera* DCM extract altered the MTT response in SH-SY5Y cells, but increasing the concentration did not produce a steadily stronger dose–response. At 31.25 and 62.5 μ g/mL, MTT values remained below the control, but no consistent decrease was seen over time. The clearest change occurred at 125 μ g/mL, whereas the late decrease



At 31.25 μ g/mL, MTT activity was $78.1 \pm 7.6\%$ at 24 h, $79.3 \pm 1.5\%$ at 48 h, and $79.4 \pm 10.9\%$ at 72 h. Over the same three intervals, the 62.5 μ g/mL group gave $86.8 \pm 15.1\%$, $88.4 \pm 6.1\%$, and $78.2 \pm 5.5\%$. In both groups the means sat below the control, yet without any steady decline over time.

In the 125 μ g/mL group, MTT activity was $92.2 \pm 24.3\%$ at 24 h and then fell to $69.5 \pm 5.2\%$ at 48 h and $61.3 \pm 10.5\%$ at 72 h. The 48 h and 72 h values were significant relative to the control ($p < 0.001$). The 250 μ g/mL group followed a similar course, with $95.7 \pm 17.4\%$ at 24 h, $78.5 \pm 8.3\%$ at 48 h, and $74.3 \pm 9.4\%$ at 72 h; here too the reductions at 48 and 72 h reached $p < 0.001$. Across the extract groups, the lowest mean fell in the 125 μ g/mL, 72 h condition. (Figure 1; Table 1).

At 500 μ g/mL the MTT result was at odds with the microscopic picture. The readings stayed close to control, at $100.9 \pm 37.6\%$ (24 h), $99.9 \pm 10.3\%$ (48 h), and $99.9 \pm 7.5\%$ (72 h), and the 24 h point in particular varied widely. Under the microscope, by contrast, the cells were disrupted and the monolayer had lost its integrity. In the wound-healing images, closure slowed in the 250 μ g/mL group, whereas at 500 μ g/mL the monolayer could not hold

in the 250 μ g/mL group was more limited. For this reason, we think the 125–250 μ g/mL range should be retested using more closely spaced concentrations.

Previous studies have reported antiproliferative effects of *A. aspera* extracts in different cancer cell lines, although the response varies with the cell model and the type of extract used [3,4,5,6]. Our findings are broadly consistent with these reports. However, in SH-SY5Y cells, we did not observe a linear dose–response relationship.

The 500 μ g/mL group showed a different pattern. The MTT signal remained close to the control, while microscopy showed marked cellular disruption and loss of monolayer integrity. The color, turbidity, precipitation, or redox properties of plant extracts can interfere with tetrazolium-based measurements [10,11]. We therefore think that MTT interference may have contributed to the unexpectedly high signal at this concentration. The high variability observed at 24 h also makes this group more difficult to interpret.

In the wound-healing images, the open area in the 250 μ g/mL group closed more slowly (Figure 2). Together with the decrease in MTT activity at the same concentration, this finding suggests that the extract may affect the proliferative

behavior of SH-SY5Y cells. At 500 µg/mL, however, the microscopic changes were not consistent with the MTT signal.

In the next stage, we plan to evaluate the 75–250 µg/mL range using more closely spaced concentrations. This may help define the response around 125 and 250 µg/mL more clearly.

CONCLUSION

The MTT response to the *A. aspera* DCM extract varied with time and concentration. The clearest change was in the 125 µg/mL group, where we measured a relative MTT activity of $61.3 \pm 10.5\%$ at 72 h. The decrease in the 250 µg/mL group was more limited; in the wound-healing images, closure slowed. At 500 µg/mL the MTT result did not agree with the microscopic findings, and we considered that this might stem from MTT interference. Overall, our data suggest that the *A. aspera* DCM extract may show antiproliferative potential in SH-SY5Y cells. In subsequent confirmatory experiments we plan to examine the 125–250 µg/mL range in particular in more detail.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Disclosure

The authors have reported no conflicts of interest in preparing and publishing this article.

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