

## Antineoplastic Properties of Parthenin Derivatives – The Other Faces of a Weed

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### Abstract

*A spiro-isoxazolidine derivative of parthenin namely SLPAR13 was taken up for this study which induced cell death in three human cancer cell lines namely HL-60 (acute promyelocytic leukaemia), SiHa and HeLa (cervical carcinoma) with various inhibitory concentrations. The cytotoxicity test was also done on the normal cells hGF (primary human gingival fibroblast) and the inhibitory concentration was found to be more than 10 times higher than HL-60 cells. The cell death was confirmed by cell cycle arrest exhibited by the test compounds in a concentration dependent manner in HL-60 cells. The nuclear condensation and morphological changes induced by the test compounds further marked the HL-60 cell death which was confirmed to be apoptosis by DNA ladder which is hallmark of apoptosis by the formation of 180bp fragments.*

### Introduction

*Parthenium hysterophorus* (popularly known as Congress weeds, White top, Star weed, Carrot weed, Gajar ghas, Ramphool) is one of the ten worst weeds in the world. As a curse for the bio-diversity, this weed has always been criticized for its ill effects. Sesquiterpene lactones are the active constituents of a variety of medicinal plants used in traditional medicine. However, it has been found to be of interest due to its anti-cancer [1, 2] anti-bacterial [3], anti-malarial [4] and allelopathic properties. The Spiro-isoxazolidine derivative of parthenin have been synthesized [5] and chosen for this study because of the fact that halogen substituted derivatives of most of the natural compounds show higher cytotoxicity [6]. Therefore this compounds has been identified for the present study to determine its potential as a novel anticancer therapeutic.

In cancer, the therapeutic goal is to trigger tumor-selective cell death. One of these events in cell deregulation is obligate compensatory suppression of apoptosis (programmed cell death), which provides support for neoplastic progression. Studies are being

focused towards the induction of apoptosis in the cancer cells but being milder with the adjoining normal cells [7]. For the same reason natural and modifications of these natural compounds have become the centers of attraction of the oncologists and drug discovery groups [8].

### Aim

This study involves evaluation of anticancer potential of SLPAR13. Whatever the mechanisms involved, if the test compound induces apoptosis then that test compound may be the potential candidate for anti-cancer lead optimization.

### Materials and Methods

#### Synthesis of SLPAR13

The synthesis of N-(phenyl)-C-(5-Bromo, 2-methoxy phenyl)-spiro-isoxazolidinyl parthenin (SLPAR13) (Fig.1) was done as described earlier [5, 9].

### Cell Proliferation Assessment by MTT Assay

HL-60, HeLa and SiHa cells were grown in suspension in T-75 flask and centrifuged at 100 g for 5 min. Cell pellet was suspended in RPMI medium and then  $15 \times 10^3$  cells (HL-60) and  $10 \times 10^3$  cells (HeLa, SiHa and hGF) were transferred to each well of Nunclon 96-well flat bottom plate and treated with SLPAR 13 and samples processed as described earlier [10].

### DNA Content and Cell Cycle Phase Distribution

HL-60 cells ( $1 \times 10^6/1.5$  ml/well) treated with different concentrations of SLPAR 13 and incubated for 24h. The preparations were made as described earlier [11] then analyzed for DNA content using BD-FACS CALIBUR. Data were collected in list mode on 10,000 events for FL2-A vs. FL2-W.

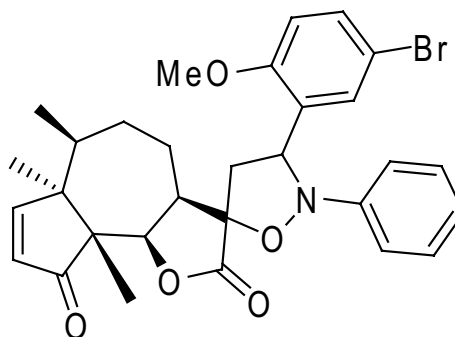


Fig. 1: Structure of SLPAR 13

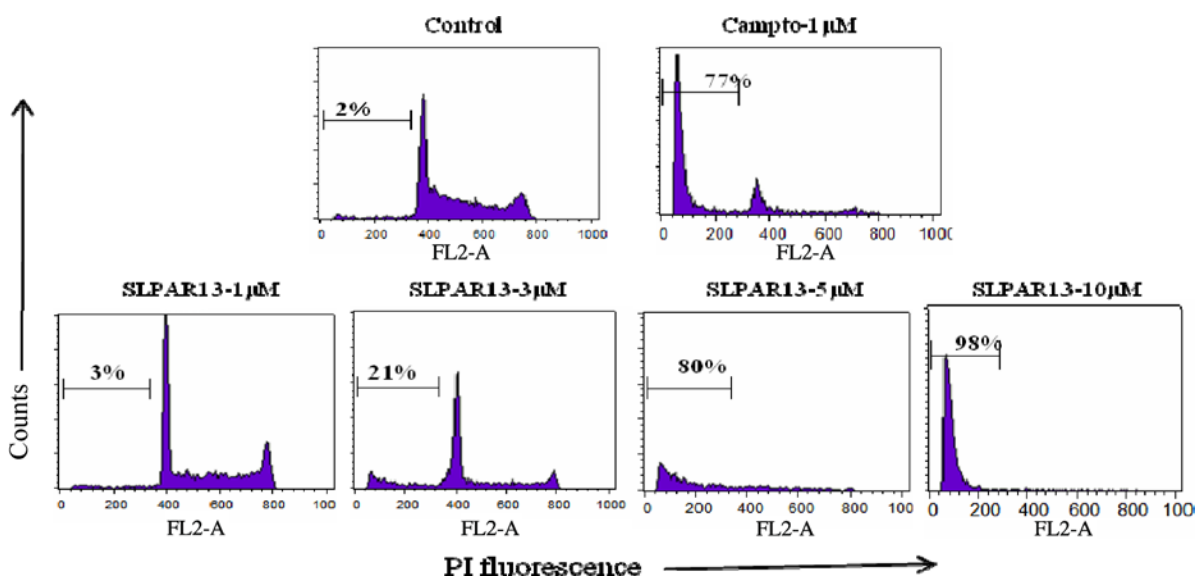
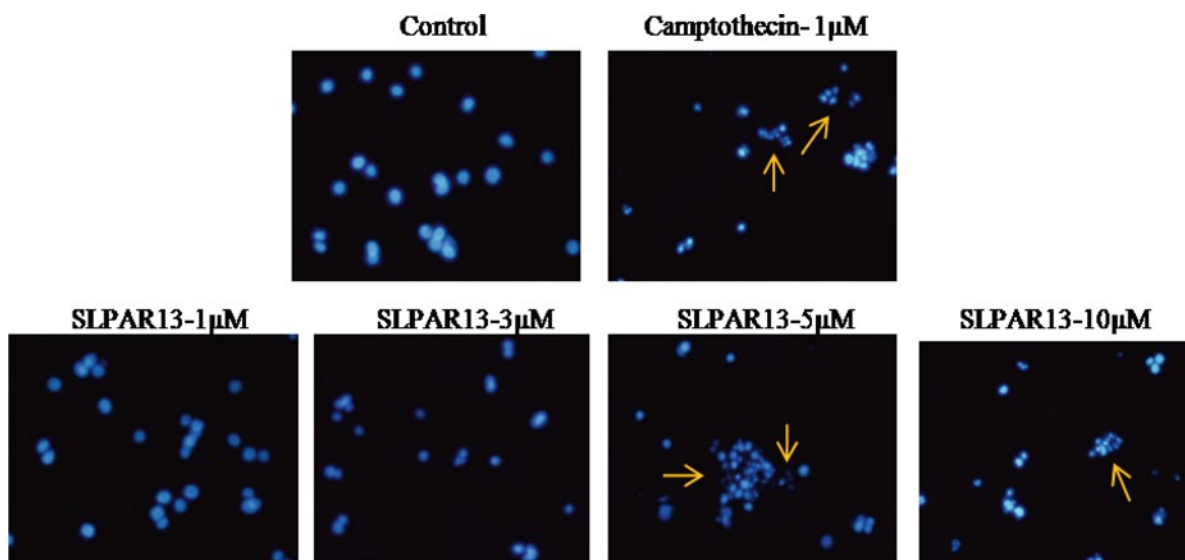


Fig. 2: HL-60 cells treated with SLPAR13, incubated for 24 h, were processed for acquisition in flow-cytometer as described in materials and methods. The compound inhibited cell cycle in a concentration dependent manner with maximum inhibition at 10  $\mu$ M concentration



**Fig. 3:** SLPAR13 induced nuclear condensation stained by Hoechst dye. The nuclei of the control cells are round and uniform while the SLPAR13 treated cells, when stained with Hoechst, exhibited condensed nuclei. The segregation and condensation of nuclei increased with increasing concentrations and was maximum at 10  $\mu\text{M}$  concentration

#### Hoechst 33258 Staining of Cells for Nuclear Morphology

HL-60 cells ( $2 \times 10^6$  cells/3 ml/well) were treated with SLPAR 13 in a concentration dependent manner and incubated for 24h. Cells were treated with Hoechst solution and spread on a clean slide and observed for any nuclear morphological alterations and apoptotic bodies under inverted fluorescence microscope (Olympus 1X70, magnification 60x) using UV excitation [12].

#### Fragmentation of Genomic DNA

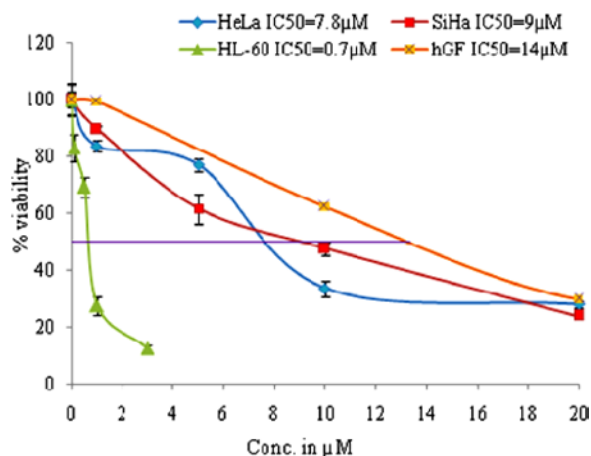
The genomic DNA was extracted from SLPAR 13 treated HL-60 cells. Cells ( $2 \times 10^6$ /3 ml/well) after various treatments, incubated for 24h were centrifuged and processed for electrophoretic analysis as described earlier [13].

## Results

#### Cell Proliferation Assessment by MTT Assay

SLPAR13 inhibits cell proliferation in the three cancer cell lines namely HeLa, SiHa and HL-60 with the  $\text{IC}_{50}$  values 7.8, 9 and  $0.7\mu\text{M}$  respectively (Fig. 4). The

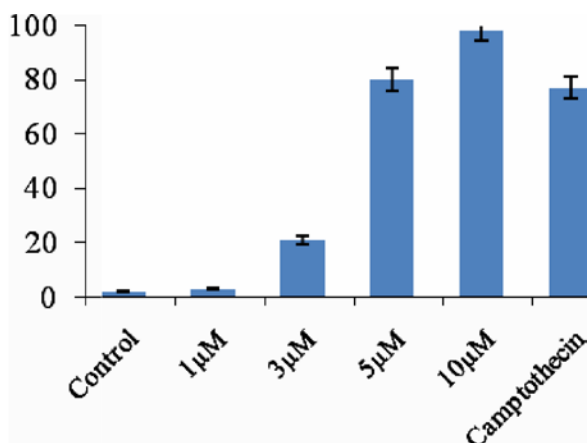
$\text{IC}_{50}$  of SLPAR13 was also calculated in normal cells hGF and was found out to be  $14\mu\text{M}$ .



**Fig. 4:** The  $\text{IC}_{50}$  values of SLPAR13 were calculated by MTT assay against 3 different cancer cell lines HeLa, SiHa and HL-60 and one normal cell line hGF as described in materials and methods

#### DNA Content and Cell Cycle Phase Distribution

The peaks obtained by flowcytometry denoted arrest in cell cycle by SLPAR13 in a concentration dependent manner (Fig. 2). Higher concentration increased the extent of cell cycle arrest (Fig. 5).



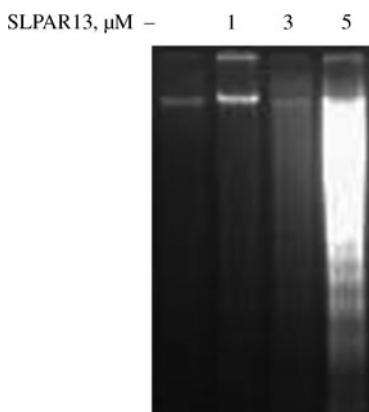
**Fig. 5:** Increasing cell cycle arrest activity of SLPAR13 against HL-60 cells. Camptothecin was taken as positive control

### Hoechst 33258 Staining of Cells for Nuclear Morphology

Condensation of nuclei is observed in the treated HL-60 cells and the nuclear condensation increased with increasing concentration. The condensed nuclei are indicated by arrows (Fig.3).

### Fragmentation of Genomic DNA

Fragments were obtained at 5 µM concentration of SLPAR13 as indicated (Fig. 6).



**Fig. 6:** Fragmentation of genomic DNA induced by SLPAR13. Other details are described in materials and methods

## Discussion

Contemporary research in the anticancer drug development from plants has been focused on investigating the molecular mechanism by which an agent induces cytotoxicity and apoptosis in cancer cells [13]. A spiro-isoxazolidine derivative of parthenin namely SLPAR13 is a semi-synthetic derivative of parthenin. Parthenin is already known for its cytotoxicity but the novelty of this work is that we report for the first time the apoptotic inducing activity of a spiro-derivative of parthenin SLPAR13 in human leukemia and cervical cancer cell lines. The compound was tested in various models, one of them being the MTT assay, which induced cell death in three human cancer cell lines selectively namely HL-60 (acute promyelocytic leukaemia), SiHa and HeLa (cervical carcinoma) with various inhibitory concentrations. This indicated the potent cytotoxicity of the said compound against cancer cell lines at the same time being milder on normal cells. The  $IC_{50}$  of SLPAR13 in the hGF cells was found to be 14 µM. The therapeutic window was more than 10 times when the  $IC_{50}$  values of HL-60 and hGF were compared. The study demonstrated that SLPAR13 is a potential pro-apoptotic agent and hence can be developed into an important anti-cancer lead of therapeutic potential. This is evidenced from measurement of several biological end-points of the apoptosis such as appearance of apoptotic bodies, DNA fragmentation and increase in sub-G0DNA fraction in HL-60 cells. The cell death was confirmed by cell cycle arrest exhibited by the test compound in a concentration dependent manner in HL-60 cells. The arrest marked the termination of series of events that takes place in a cell leading to its division and duplication (replication) which caused the cell death as a result of treatment of SLPAR13. The nuclear condensation and morphological changes induced by the test compound further marked the HL-60 cell death which was confirmed to be apoptosis by DNA ladder. The formation of fragments is hallmark of apoptosis due to the breaking of DNA strand into 180 bp fragments. This created a clear picture of apoptosis induced by SLPAR13 in the HL60 cells. Apoptotic cell death may involve intrinsic mitochondrial signaling pathway [14, 15] or extrinsic signaling cascade emanating through the activation of apical death receptors leading to caspase activation [16] and finally death of

the cell. Successful drug treatment in human disease requires an adequate therapeutic index reflecting the treatment's specific effect on target cells and its lack of clinically significant toxic effect on the host [17]. Whatever the mechanisms involved, if the test compound induces apoptosis then that test compound may be the potential candidate for anti-cancer lead optimization. This study is expected to lead us to identify the active molecule that may have the potential for the treatment and management of cancer.

## Conclusion

This study points towards the fact that natural compounds like parthenin and its halogenated derivatives induce death in human cancer cells. The mode of cell death was confirmed to be apoptosis which is a positive indication of these compounds being taken up for further studies as potential anticancer agents.

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