

ASHWAGANDHA LEAF EXTRACT AND ITS COMPONENTS INDUCE DIFFERENTIATION IN HUMAN GLIOBLASTOMAS

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Introduction Ashwagandha (*Withania somnifera*), also known as Indian Ginseng is widely used in Indian traditional system of medicine, Ayurveda. It has been claimed to have a variety of health promoting effects that range from anti-inflammatory, anti-bacterial, immuno-modulatory, anti-stressor radio-sensitizer and antitumor.

Background We had previously demonstrated that leaf extract of Ashwagandha (i-Extract) has selective killing activity for cancer cells that functions through selective activation of tumor suppressor protein, p53. We also showed by reverse-phase HPLC that i-Extract contains Withaferin A, Withanone and Withanolide A as its main components. Of these, Withanone was mainly involved in tumor suppressor activity. **Purpose** In the present study, we aimed to investigate the growth inhibition and differentiation potential of alcoholic extract of Ashwagandha leaves (i-Extract), its different constituents (Withaferin A, i-Factor and Withanolide A) and their combinations in glioblastomas. **Methods** The effects of i-Extract, its different constituents and their combinations were evaluated for changes in cell morphology, cell proliferation, glial fibrillary acidic protein (GFAP) and mortalin expression by using Trypan blue assay, MTT, immunocytofluorescence, anti-migration assay, Western blotting and cell cycle analyses.

Results We have found that i-Extract and its components markedly inhibited the proliferation of glioma cells in a dose dependent manner. Whereas high doses of i-Extract caused cell killing, low doses caused growth arrest that was accompanied by acquisition of astrocytic type morphology. We anticipated this as a differentiation phenotype and examined it by molecular markers. We found that cells treated with i-Extract had increased expression of GFAP, an astrocyte marker protein. Induction of the growth arrest was marked by changes in immunostaining pattern of mortalin from perinuclear to pancytosolic that suggested that i-Extract treated cells show senescence. Furthermore, NCAM analysis showed that i-Extract treated cells show increased adhesion and decreased migratory activity. We have developed a combinational formula for Ashwagandha leaf-derived phyto-chemicals that caused enhanced differentiation of glioma cells. **Conclusions** We propose that such combinational mixture may serve as a new milder and effective differentiation-based glioma therapeutic reagent.