Folate receptor mediated targeting enhances selective cytotoxicity of Ashwagandha derived drugs to cancer cells

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Background. Folate receptors (FRs) have been shown to be overexpressed on the surface of a variety of cancer cells and their expression are limited in normal cells and tissues. Since FR strongly binds to folic acid (FA), FA-functionalized nanocarriers have been proposed as a reliable strategy for delivery of anticancer drugs. We have earlier reported that the alcoholic extract of Ashwagandha leaves (i-Extract) and its major cytotoxic component, Withaferin A (Wi-A), have cancer cell killing activity. In the present study, we synthesized a FR-targeting i-Extract nanocomplex (FRi-ExNC) and a FR-targeting Wi-A nanocomposite (FRWi-ANC), by conjugating FA to polyethylene glycol and amphiphilic nanoframeworks, respectively. We investigated their anticancer potentials in *in vitro* and *in vivo* assays.

Methods. Selective cellular uptake of FRi-ExNC and FRWi-ANC were evaluated by immunofluorescent microscopy. Cytotoxic effect of FRi-ExNC and FRWi-ANC in cancer cells were detected by assays including cell viability, apoptosis and biochemical determination of proteins involved in these phenotypes. The antitumor efficacy of FRi-ExNC and FRWi-ANC were investigated by *in vivo* tumor formation assays in nude mice.

Results. We found that FRi-ExNC and FRWi-ANC caused stronger cytotoxicity as seen by induction of apoptosis. It was confirmed by cell cycle and protein expression analyses. *In vivo* tumor growth assays for subcutaneous xenografts in nude mice also revealed significantly enhanced suppression of tumor growth in the treated groups.

Conclusions. Our results suggested that these two kinds of nanoparticles serve as useful nanomedical tools for selective targeting of drugs to the cancer cells and enhanced anticancer activity.