ABSTRACT

As research on modern medicines continues to expand, simultaneously use of botanical medicines is also increasing. It is believed that multi-site mechanisms of action of herbal preparations from crude extracts may offer greater chances for success of treatment where conventional single-site agents have been disappointing. Several bioactive compounds have emerged from research in Ayurvedic herbals. *Withania somnifera* (Ashwagandha) is widely used in the Indian traditional system of medicine, Ayurveda and is considered to be a rasayana herb. Ashwagandha, although, shows great potential as a safe and effective antineoplastic agent, but more research is required to determine if Ashwagandha can duplicate this activity in brain and neural cancers. Furthermore, earlier reports have revealed that Ashwagandha is a potent protective agent in neurodegenerative and neuropsychiatric disorders. Also, its constituents induce significant regeneration of axons and dendrites, in addition to reconstruction of pre- and postsynapse in the neurons, therefore it may prove to be an important candidate for treatment of neurodegenerative diseases.

The present study was aimed to evaluate the dual role of *Withania somnifera* in neurooncology and neuroexcitotoxicity. Gliomas and neuroblastomas are the most common primary neural tumors with only limited options for treatment and majority of these tumors develop into malignancy and remain incurable inspite of modern therapeutic modalities. The present study was aimed to investigate whether Ashwagandha leaf water extract (ASH-WEX) can be a potential candidate for differentiation based therapy, which is an attractive alternative therapeutic approach, possibly leading to the treatment of brain and neural tumors. Further it was studied whether Ashwagandha extract has the potential to protect glial and neuronal cells against glutamate induced excitotoxicity. As glutamate is the major excitatory neurotransmitter and commonly involved in neurodegenerative disorders, protection against its excitototoxicity may be a beneficial therapeutic intervention in related neuropathological conditions.

The current study revealed that ASH-WEX induced upregulation of GFAP (in C6 cells), NF200 (in IMR-32 cells), HSP70 and mortalin expression which suggests the induction of differentiation in these cells. The upregulation of NCAM and
downregulation of PSA-NCAM and MMPs may explain the anti-migratory and differentiation inducing properties of ASH-WEX. The decrease in Cyclin D1 and bcl-xl expression and modulation of Akt-P further indicated the arrest of C6 glioma and IMR-32 cell proliferation and their differentiation into mature astrocyte and neuron-like cells, respectively. FACS analysis also provided supporting data that ASH-WEX caused an arrest of the cell cycle in the G0/G1 phase in C6 and IMR-32 cells. ASH-WEX appeared to affect multiple pathways for its anti-cancer and differentiation inducing role in glioma and neuroblastoma cells instead of targeting a single protein or pathway which needs to be further explored. The current study supports the idea that ASH-WEX may have the potential to reduce the malignancy of brain and neural tumors and prove to be suitable candidate for adjunct therapy due to its differentiation inducing activity.

On the other hand, pre-exposure of RA differentiated C6 and IMR-32 cells to ASH-WEX lead to significant increase in their viability against glutamate mediated excitotoxicity thus implicating its cytoprotective role. ASH-WEX treatment rescued the glial and neuronal cells from glutamate induced cytotoxicity by upregulation of plasticity marker proteins such as HSP70, NCAM and PSA-NCAM. ASH-WEX may have therapeutic potential for inducing differentiation of glioblastoma and neuroblastoma cells as well as preventing the neurodegeneration associated with glutamate-induced excitotoxicity, thus emphasizing the role of Ashwagandha in brain cancers as well as neuroprotection. Identification and characterization of the water-soluble active components from ASH-WEX in search of potentially safe neurotherapeutic phyto-reagents is highly warranted.