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Original Research Article

Effect of *Withania Somnifera* (L.) root extract's on PC12 induced by hydrogen peroxide, *in vitro* study

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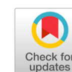

A R T I C L E I N F O

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A B S T R A C T

The *Withania somnifera* L. is also used as a crude medicament that mostly effected in various neurological and immunological disorders. It is also known as Ashwagandha and "Indian ginseng" in Indian region. There is a lack of information on the possible neuroprotective properties of W. somnifera root against H_2O_2- and Ab(1-42)-induced cytotoxicity, which are now targeted for innovative treatments for dementia, particularly dementia of the Alzheimer's type (AD). According to this research, we prepared an aqueous extract of dried roots of W. somnifera that possess the protective effect against Ab- aggregated fibril and H_2O_2 cytotoxicity through MTT assay with the help of differentiated rat pheochromocytoma PC12 cell. The findings indicate that, in a concentration-dependent manner, pretreatment of differentiated PC12 cells with aqueous extracts of W. somnifera root strongly protects differentiated PC12 cells against both H2O2- and Ab(1-42)-induced cytotoxicity. The W. somnifera extract was examined using liquid chromatography-serial mass spectrometry in order to look into the substances that could be responsible for the effects that were seen. Withanolide derivatives, notably withaferin A, were found in abundance. These findings support the hypothesis that W. somnifera may be used ethnopharmacologically to treat oxidative stress-related cognitive and other neurodegenerative illnesses. They also show the neuroprotective activities of an aqueous extract of W. somnifera root.

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1. Introduction

World Health Organization data suggest that neurological and psychiatric disorders are an important and growing cause of morbidity. The magnitude and burden of mental, neurological, and behavioral disorders is huge, affecting more than 450 million people globally.[1] According to the Global Burden of Disease Report, 33 percent of years lived with disability and 13 percent of disability-adjusted life years (DALYs) are due to neurological and psychiatric disorders, which account for four out of the six leading causes of years, lived with disability.[2]

Mental fitness problem and neurological problems is a critical public fitness subject globally with multiple billion patients worldwide.[3] For neurological problems, contemporary-day medicine gives symptomatic remedy this is high priced and related to numerous aspect effects. Natural merchandise had been widely exploited as a critical supply for medicine. Huge ranges of drug treatments are derived from plant-primarily based totally extractions and fractionation and, have awesome importance for humans.[4] nowadays, scientific practitioners are extra willing closer to herbal drug treatments for a straightforward remedy with value effectiveness and decrease occurrence of aspect effect.[5]

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Ayurveda is a famous Indian medicinal system of medicine has a well-advanced path of movement for the control and remedy of mind related disorders. A listing of approximately 450 Ayurvedic medicinal plants, fifty six famous plant or one in all their elements of Ayurvedic preparation are to be had for neurological disorders.[6] One of the conventional well known Indian medicinal plants is Ashwagandha (*Withania somnifera*) that's a common aspect of numerous Ayurvedic formulations advertised for the remedy of neurological disorders.[7]

1.1. *Botanical description*

Table 1: Botanical Description

S No.	Kingdom	Plantae
1	Division	Angiosperms
2	Order	Tubiflorae
3	Family	Solanaceae
4	Synonyms	Indian ginseng, Poison gooseberry, Winter cherry
5	Cultivated region	India- Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan. China. Nepal
6	Temperature	20-37° C
7	Climatic Condition	1500 m above sea level

1.2. *Chemical compositions*

The various chemical components are present in withania somnifera. Some important chemicals are steroidal lactones, withanone, withaferin, withanloids and sitoindoside.[8]

Due to their chemical composition it's useful for neurological disorders like as Parkinson's disease, Alzheimer disease and Huntington disease. All chemicals those presents in crude form of Ashwagandha.[9] changes the brain oxidative stress markers like as catalase, superoxide dismutase and glutathione. The extract of this drugs induce the dendrites and axon outgrowth and its show the neuronal regeneration.[10]

2. Material and Methods

2.1. *Plant materials*

The dried roots of Ashwagandha were purchased from local market of muzaffarnagar authenticated by CCS University Meerut Uttar Pradesh.

2.2. *Preparation of the extracts*

Take a root powder of *W. Somnifera* and grind by electric grinder till fine powder consistency. 1 gm of roots powder was infused in de-ionized hot water (1:50 ratio) for 30 minutes. After infusion, it left and cooled at room temp. and centrifused at 12000 rpm for 20 mint. After centrifugation it's dried at freezing point before used for experimental purpose.

2.3. *Cell culture*

The rat pheochromocytoma (PC12) a type of neuroendorcine tumor cell that developed from chromaffin cells, glutamine, RPMI-1640, penicilline-streptomycin, calf serum, trypan blue and nerve growth factors was purchased from Invitrogen UK and Sigma UK. These PC12 cells were maintained in RPMI media, this media is a nutritive media used to support cell viability in biological samples, and supplemented with 10% heat inactivated bovine serum, 2 mm 1-glutamine, humidified with 5% CO_2 and 95% air at 37° C, 100 IU/ml penicillin-streptomycin. All constituents are cultured in percolated flask. The culture media was changed with every alternate day and examine the viability of every cell with trypan blue (0.5%) dye exclusion methods.

2.4. *Assay of β-amyloid induced cytotoxicity*

The β amyloid is a peptides amino acid that are responsible for oxidative stress, regulation of cholesterol, Kinase enzyme and transcription factors. The in vitro toxicity of Beta amyloid from PC12 cells was measured by cells incubation periods. The toxicity of PC12 cells was measured with increasing concentration of aggregated Aβ in a 96 well plate. The cells viability was measured by MTT assay.

2.5. *Assay of H_2O_2 induced toxicity*

The H_2O_2 is decreased the excitability and action potential of neurons. In this assay, take cells in 96-well plated and plated with at appropriate density (103 cells/100ml) for 24 hrs at 36^0 C. The root extract of W. somnifera and cells were incubated prior to exposure to H_2O2 (Cons- 12.4- 400mm) from a freshly prepared 1000 mm stock solution. After 24 hrs, determine cell viability by MTT assay.

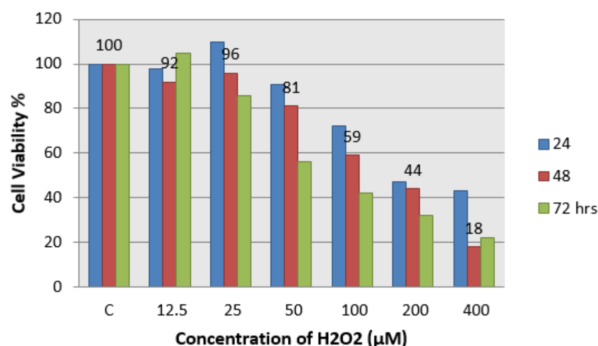

Fig. 1: Concentration-dependent inhibition of cell viability with H_2O_2 in PC12 cells after treated for 24, 48 and 72hrs.

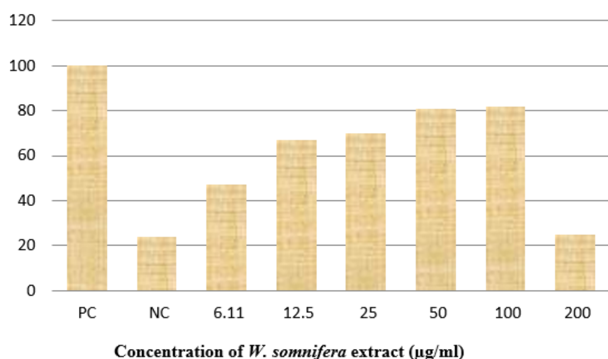

Fig. 2: The beneficial effect of *W. somnifera* extract against H_2O_2 cytotoxicity in deffrentiated PC12. NC- Negative Control, PC- Positive Control (H_2O_2 absent).

2.6. *Liquid chromatography mass spectrometry*

The aqueous extract of *W. somnifera* root was dried at freezing temp. And analyzed by liquid chromatography-serial mass spectrometry (LC-MSn) using HPLC systems. Through this method, we achieved on a 150 mm X 4.6 mm C18 column using a 1ml/min mobile phase of 10% to 100% aqueous acetonitrile which contain 0.1% formic acid. Reduce the ESI source to 200 ml/min with the help of a splitter and this source was operated by the needle voltage of _+4.2 Kv. The heated capillary temp. was 215° C. The device became operated the usage of X calibur 2. zero software program and additives withinside the LC-MSn analyses have been detected the usage of Mass Frontier 4.

3. Result

3.1. *Effect of H_2O_2 on dPC12 cell viability after treatment for 24, 48 and 72 h-*

A concentration-dependent cytotoxicity was detected when dPC12 cells were treated with varied H2O2 concentrations (12.5–400 mm) for 24, 48, and 72 hours (Fig. 1). During a 24-hour incubation period at 200 mm H2O2, cell viability was reduced to 50%, at 200 and 400 mm H2O2 (p 0.001), but less than 50% for 48 and 72 hours (p 0.001). To examine the effects of the *W. somnifera* extract in future trials, 200 mm H2O2 was chosen as a suitable concentration to cause toxicity, with a 24-hour incubation time.

3.2. *The Effect of W. somnifera extract on dPC12 cell against H_2O_2 induced cytotoxicity*

When dPC12 cells were preincubated with W. somnifera extract for 24 hours prior to H2O2 (200 mm) exposure, the aqueous extract effectively protected them against H2O2-induced toxicity, as seen in Fig. 2. Compared to the negative control (H2O2 alone), 50–80 percent cell viability was seen at extract concentrations of 6.11–100 mg/mL after 24 hours of H2O2 exposure, with 50 and 100 mg/mL extract concentrations demonstrating the largest improvement (p 0.001). The cytoprotective effects were lost at the maximum extract concentration (200 mg/mL), possibly due to W. somnifera's direct cytotoxic and antiproliferative activities being expressed at high doses.

3.3. *Effect of W. somnifera extract on viability of dPC12 cells against H2O2-induced cytotoxicity-*

The crude alcoholic extracts of leaf of Ashwagandha were prepared for given to the experimental animal. Take 85% ethanol and mix with leaf powder in a ratio of 1:30 at 85° C for about 2.5 hrs. After reflex system, the extract was collected evaporates at 60°C. After purification the extracted powder was used.

4. Discussion

According to previous published work and research, the W. somnifera is most valuable plant that contain various valuable medically substances which is used in many disease and disorders. In this plant many compounds are present that show the Neuroprotective properties against the neurodegenerative disease/disorders. Neuroprotective treatment approaches designed to block H2O2- and Ab-induced neurotoxicity linked to AD pathology are one area of ongoing pharmacological investigation.

This investigation examined the neuroprotective potential of W. somnifera root aqueous extract against H_2O_2- and Ab-induced toxicity with PC12 cells as an in vitro model. The PC12 cells were selected due to their morphologically and physiologically similarity to living neurons that present in human brain. Instead of human neurons researchers was select this methods because these neuroprotective studies represent the same response to normal human cells.

The results of the current investigation showed that pretreatment of differentiated PC12 cells with a water-based extract of W. In a concentration-dependent manner, somnifera root strongly shielded dPC12 cells against H2O2- and Ab-induced cytotoxicity. Ab is known to enhance the generation of free radicals and lipid peroxidation in PC12 cells, which results in apoptosis and cell death. The presence of substances that scavenge free radicals in the W. somnifera aqueous extract may be responsible for the cytoprotective benefits seen.

5. Source of Funding

None.

6. Conflict of Interest

The authors have declared that there is no conflict of interest.

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