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In Vitro Neuro-Protective Studies of Phyto-Compounds from Azadirachta indica, Centella asiatica and Gloriosa superba

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Abstract

The test substances quercitin dehydrate, asiatic acid and colchicine were evaluated for its in vitro neuro-protective activity against Hydrogen peroxide induced toxicity in HEK-293-(Human embryonic kidney cell line). Firstly Test Substances were estimated for cytotoxicity with different concentrations ranging from 100 μg/ml to 1.56 μg/ml, which resulted high toxicity in HEK-293-(Human embryonic kidney) cell line, and hence the nontoxic concentrations were taken for further studies. In Cytoprotective studies Test Substances were showing significant activity in human embryonic kidney cells against hydrogen peroxide toxicant. These compounds are seen to express their activity in AMPA and SNCA expressed cells in HEK-293 cell line.

Keywords: Quercitin dehydrate; Asiatic acid; Colchicines; Neuro-protective; HEK cell line; Hydrogen peroxide toxicant

Introduction

Since very ancient days various medicinal plants and their phyto-compounds form part and parcel of major populations of India, and other South East Asian countries. For the better management of different Central Nervous System (CNS) disorders, chemical and synthetic drugs studied are not seen to be fully effective; hence different phytomedicinal compounds (phyto-chemicals from herbal sources) are successfully utilized with minimum or without side effects even for long term therapy/treatment [1].

Azadirachta indica (commonly known as neem) have been used in India for over two millennia for its medicinal property. In Ayurveda, Azadirachta indica is reported to have antifungal, antidiabetic, antibacterial, antiviral, antireceptive and sedative properties including neurological property and has been evaluated for a wide spectrum of diseases including cancer, inflammation, ulcer, dementia, immune disorder, hyperlipidemia and liver disease. Phytocompound used in this work is Quercitin dehydrate [2-6].

Centella asiatica (commonly known as thankuni) has been used to treat various disorders and apart from wound healing, the herb is used for the treatment of various skin ailments such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhoea, fever, amenorrhea, diseases of the female genitourinary tract and also for relieving anxiety and improving memory cognition. It is known to rejuvenate the brain and nervous system, increase attention span and concentration and also combat aging. Phytocompound used in this work is Asiatic Acid [7-11].

Gloriosa superba (commonly known as agnisikha or ulatchandal), the alkaloid-rich plant is used as a traditional medicine in many cultures in Indian subcontinent [12]. It has been used in the treatment of gout, snakebite, ulcers, neurological [13], arthritis, chola, colic, kidney problems, typhus, impotence, nocturnal emission, etc. and is in great demand for medicinal use. Phytocompound used in this work is Colchicine [12-14].

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) is a subtype of the ionotropic glutamate receptor coupled to ion channels that modulate cell excitability by gating the flow of calcium and sodium ions into the cell usually seen in schizophrenia and parkinsonism and (SNCA) alpha-synuclein which is a major constituent of Lewy bodies, the protein clumps that are seen of Parkinson’s disease are the neuro-receptors used in this work.

Methodology

Outline of the method

The in vitro cytotoxicity was performed for all four test substances on HEK-293 (Human Embryonic kidney cell) to find toxic concentration of Test Substances to evaluate the cytoprotective activity against hydrogen peroxide.

Preparation of test solution

For cytotoxicity studies, 10 mg of Test Substances were separately dissolved and volume was made up with Ham’s F12 supplemented media containing 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration. Further, sterilized by 0.22 μ syringe filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies. Placebo (Cell culture media with 2% FBS), which demonstrates the suitability of test system to yield a reproducible, appropriate reactive response in the test system [13].

Cytotoxicity studies

The monolayer cell culture was trypsinized and the cell count was adjusted to 100,000 cells/ml using Ham’s F12 containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer once washed with medium and 100 μl of different test concentrations of Test Substances were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 72 h in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were made.

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resulted to be 35.60 ± 0.33, 3.19 ± 0.10, 3.40 ± 0.15 μg/ml for Quercitin dehydrate, Asiatic Acid and Colchicine, and hence the nontoxic concentrations were taken for further studies. In Cytoprotective assay, Quercitin dehydrate, Asiatic Acid, and Colchicine, showed 35.60 ± 0.33, 3.19 ± 0.10 and 3.40 ± 0.15 percentage protection at higher concentration against hydrogen peroxide induced toxicity in human embryonic kidney cell line respectively.

Conclusion

The test substances (phyto-compounds) quercitin dehydrate and asiatic acid are seen to express their activity in AMPA expressed cells in HEK-293 cell line and colchicine is seen to express their activity in SNCA expressed cells in HEK-293 cell line. This result tally with work of in-silico work of Bagchi et al. which established quercitin dehydrate and asiatic acid as novel drug leads for AMPA receptor published in

S. No | Samples | Concentration Tested (μg/ml) | % Protection |
--- | --- | --- | --- |
1. | Quercitin dehydrate | 6 | 65.55 ± 1.0 |
2. | Asiatic Acid | 1 | 54.68 ± 1.1 |
3. | Colchicine | 1 | 60.65 ± 1.1 |

Table 2: Cytoprotective activity of Test Substances is in Human embryonic kidney Cell line against Hydrogen peroxide induced toxicity.

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References


