Medicinal Chemistry
ISSN: 2161-0444

Bagchi et al., Med Chem (Los Angeles) 2018, 8:8
DOI: 10.4172/2161-0444.1000517

Research Article Open Access

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

Preenon Bagchi[1,2*], Ajit Kar[2,3] and Anuradha M[1]

[1]*Padmashree Institute of Management and Sciences, Bangalore, India*
[2]*Sarvasumana Association, Bangalore, India*
[3]*Satsang Herbal Research Laboratory, Deoghar, India*

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, contraceptive 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 rejunivate 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, cholera, 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% CO_2 atmosphere, and microscopic examination was carried out and observations were

***Corresponding author:** Preenon Bagchi, Padmashree Institute of Management and Sciences, Bangalore, Karnataka, India, Tel: +919986274603; E-mail: prithish.bagchi@gmail.com

Received August 13, 2018; **Accepted** August 16, 2018; **Published** August 23, 2018

Citation: Bagchi P, Kar A, Anuradha M (2018) *In Vitro* Neuro-Protective Studies of Phyto-Compounds from *Azadirachta indica, Centella asiatica* and *Gloriosa superba*. Med Chem (Los Angeles) 8: 230-233. doi: 10.4172/2161-0444.1000517

noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the standard formula and concentration of test drug needed to inhibit cell growth by 50% (CTC50) values is generated from the dose-response curves for each cell line.

Cytoprotective study against hydrogen peroxide induced toxicity

The experiment was performed as per the standard protocol, Briefly, exponentially growing HEK-293 (Human embryonic kidney cell line) were trypsinized from the culture flask and 1.5×10^5 cells/ml were seeded in 96 well microtitre plate. After attaining confluency, culture mediums from the wells were discarded and cells were pre-incubated with different non-toxic concentrations of Test Substances were along with the Cell control for 1 h in culture medium. After 1 h pre-incubation, 10 µl of stock H_2O_2 solution was added (stock of 10 mM H_2O_2) to all the wells except control wells. The plates were incubated for 24 h at 37°C with atmosphere of 5% CO_2. After incubation MTT assay was performed to determine the cell viability. From the absorbance values from test and control groups, % protection offered by test substances were against H_2O_2 induced toxicity was calculated [15].

RT-PCR procedure

The mRNA expression level of SNCA and AMPA were carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the HEK-293 cells were cultured in 60 mm petridish and maintained in HAM'S F12 medium for 48 hrs. The DMEM medium was supplemented with FBS and amphotericin. To the dish required concentration of test samples that are phytoconstitute added and incubated for 24 hr. Total cellular RNA was isolated from the untreated (control) and treated cells using Tri Reagent according to manufacturer's protocol. cDNA was synthesized from total isolated RNA by reverse transcriptase kit according to manufactures instructions (Thermo scientific). Then 50 µl of the reaction mixture was subjected to PCR for amplification of hek-293 cDNAs using specifically designed primers procured from Eurofins India and as an internal control the house keeping gene GAPDH was co-amplified with each reaction.

Amplification conditions for SNCA and AMPA gene

SNCA- 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing Tm for 30 seconds and extension at 72°C for 45 seconds. This was followed by final extension at 72°C for 10 min. Product size: 305 bp. AMPA-94°C for 5 min followed by 35 cycles of denaturation at 94°C for 15 seconds, annealing at Tm for 30 seconds and extension at 72°C for 45 seconds. This was followed by final extension at 72°C for 10 min. Product size: 760 bp.

Results

Results of the current study are presented in Tables 1 and 2 (Figures 1-4).

Discussion

Firstly Test Substances were estimated for cytotoxicity with different concentrations ranging from 100 µg/ml to 1.5 µg/ml, which

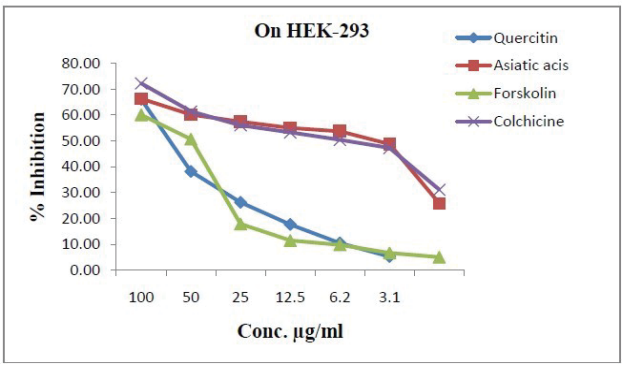

Figure 1: Graph of cytotoxic effect of Test Substances was on HEK-293 Cell line.

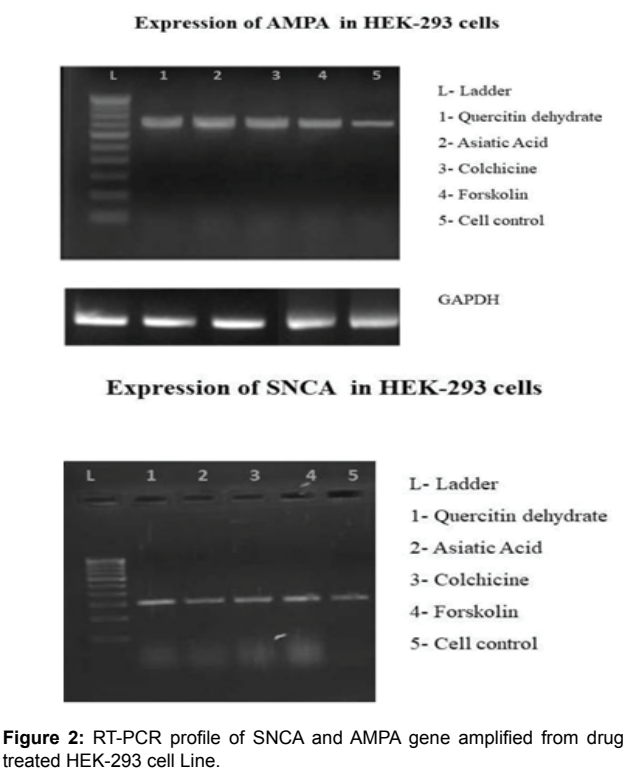

Figure 2: RT-PCR profile of SNCA and AMPA gene amplified from drug treated HEK-293 cell Line.

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

Test Sample	Regulation in Terms of Folds	
	SNCA	AMPA
Quercitin dehydrate	1.31	1.51
Asiatic Acid	1.13	1.48
Colchicine	1.26	1.42.
Forskolin	1.29	1.34
Control	1.00	1.00

*Values shown in term of fold.

Figure 3: The gene expression level of different genes normalized to GAPDH.

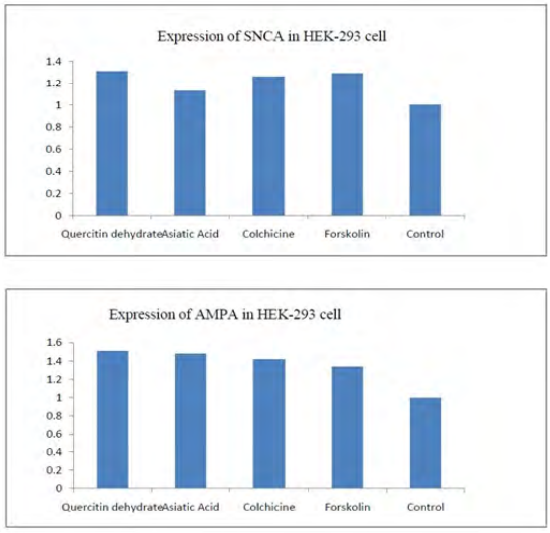

Figure 4: Graph of Densitometric analysis of gene transcripts, the relative level of SNCA and AMPA gene expression is normalized to GAPDH. Values shown depict arbitrary units.

S. No	Name of Test Sample	Test Conc. (µg/ml)	%Cytotoxicity	CTC50 (µg/ml)
1.	Quercitin dehydrate	100	38.17 ± 0.37	35.60 ± 0.33
		50	66.06 ± 0.37	
		25	38.17 ± 0.37	
		12.5	26.24 ± 0.23	
		6.2	17.68 ± 0.38	
		3.1	10.49 ± 0.14	
		1.5	5.25 ± 0.19	
2.	Asiatic Acid	100	66.34 ± 2.14	3.19 ± 0.10
		50	60.20 ± 0.35	
		25	57.46 ± 0.56	
		12.5	55.04 ± 1.07	
		6.2	53.70 ± 0.14	
		3.1	48.77 ± 1.35	
		1.5	25.80 ± 0.14	
3.	Colchicine	100	72.25 ± 0.50	3.40 ± 0.15
		50	61.37 ± 2.28	
		25	56.02 ± 1.33	
		12.5	53.28 ± 1.39	
		6.2	50.40 ± 0.66	
		3.1	47.28 ± 0.72	
		1.5	31.10 ± 4.19	

Table 1: Cytotoxic properties of Test Substances were against HEK-293 cell line.

S. No	Samples	Concentration Tested (µg/ml)	% Protection
1.	Quercitin dehydrate	6	65.55 ± 1.0
		3	34.82 ± 0.7
2.	Asiatic Acid	1	54.68 ± 1.1
		0.5	24.76 ± 2.3
3.	Colchicine	1	60.65 ± 1.1
		0.5	37.50 ± 0.7

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

neuropsychiatry journal [16] and with the *in-silico* work of Bagchi et al. which established colchicine as novel drug lead of SNCA receptor published in Information Systems Design and Intelligent Applications, Advances in Intelligent Systems and Computing, by Springer Nature Singapore Pte Ltd [17].

Acknowledgements

This work forms a part of SERB-NPDF for Preenon Bagchi, author reference number PDF/2015/000047.

References

1. Bagchi P, Somashekhar R, Kar A (2015) Scope of some Indian medicinal plants in the management of a few neuro-degenetative disorders in-silico: A review. International Journal of Public Mental Health & Neurosciences 2: 41-57.
2. Botez SA, Herrmann DN (2010) Sensory neuropathies, from symptoms to treatment. Curr Opin Neurol 23: 502-508.
3. Chattopadhyay R, Bandyopadhyay M (2005) Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. Afr J Biomed Res 8: 101-104.
4. Chattopadhyay RR (1996) Possible mechanism of antihyperglycemic effect of Azadirachta indica leaf extract. Part IV. Gen Pharmacol 27: 431-434.
5. Dasgupta T, Banerjee S, Yadava PK, Rao AR (2004) Chemopreventive potential of Azadirachta indica (Neem) leaf extract in murine carcinogenesis model systems. J Ethnopharmacol 92: 23-36.
6. Kandhare AD, Mukherjee AA, Bodhankar SL (2017) Neuroprotective effect of Azadirachta indica standardized extract in partial sciatic nerve injury in rats: Evidence from anti-inflammatory, antioxidant and anti-apoptotic studies. Excli J 16: 546-565.
7. Vaidya AB (1997) The status and scope of Indian medicinal plants acting on central nervous system. Indian Journal of Pharmacology 29: S340-S343.
8. Diwan PV (1991) Anti-anxiety profile of mandukparni Centella asiatica Linn in animals. Fitoterapia 62: 255-257.
9. Brinkhaus B, Lindner M, Schuppan D, Hahn EG (2000) Chemical, pharmacological and clinical profile of the East Asian medical plant Centella asiatica. Phytomedicine 7: 427-448.
10. Srivastava R, Shukla YN, Kumar S (1997) Chemistry and pharmacology of Centella asiatica: a review. J Med Arom Plant Sci 19: 1049-1056.
11. Gohil KJ, Patel JA, Gajjar AK (2010) Pharmacological Review on Centella asiatica: A Potential Herbal Cure-all. Indian J Pharm Sci 72: 546-556.
12. Lal HS, Mishra PK (2011) Gloriosa superbaa-an endangered plant spotted for the first time from forest of Tpchanchi, Hazaribag (Jharkhand) India. Science Research Reporter 1: 61-64.
13. Bagchi P, Venkatramana DK, Mahesh M, Somashekhar R, Kar A (2014) Identification of Novel Drug Leads for Receptors Implicated in Migraine from

Traditional Ayurvedic Herbs Using in silico and in vitro Methods. Journal of Neurological Disorders 2: 2-6.

14. Ade R, Rai MK (2009) Current Advances in Gloriosa superbaa L. Biodiversitas Journal of Biological Diversity 10.

15. McGaw LJ, Elgorashi EE, Eloff JN (2014) Cytotoxicity of African Medicinal Plants Against Normal Animal and Human Cells. Toxicological Survey of African Medicinal Plants, pp: 181-233.

16. Bagchi P, Anuradha M, Kar A (2018) Pharmacophore Screening and Docking studies of AMPA Receptor Implicated in Alzheimer's Disease with some CNS acting Phytocompounds from Selected Ayurvedic Medicinal Plants. Neuropsychiatry 8: 1101-1114.

17. Bagchi P, Anuradha M, Kar A (2018) Pharmacophore Modeling and Docking Studies of SNCA Receptor with Some Active Phytocompounds from Selected Ayurvedic Medicinal Plants Known for their CNS Activity. Information Systems Design and Intelligent Applications. Springer, pp: 1-9.