

In vitro neuroprotective effect of charantin from *Momordica charantia* against neurotoxin and endoplasmic reticulum stress-induced cell death in SH-SY5Y cells

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Abstract

Aim: The study deals with the evaluation of neuroprotective effect of charantin. Charantin was isolated from *Momordica charantia* (MC) L. (Bitter gourd) and characterized by performing infrared (IR), nuclear magnetic resonance (NMR). As it was earlier proved that amyloid beta deposition and cognition are inter-related, neuroblastoma cell lines SH-SY5Y were utilized in this study to prove the effectiveness of charantin on the against neurotoxin and endoplasmic reticulum (ER) stress-induced cell death. **Materials and Methods:** Charantin was isolated from MC and characterized using IR, NMR spectroscopy studies. The cell viability analysis and IC₅₀ determination for charantin in SH-SY5Y neuroblastoma cell line were performed by MTT assay and neuronal red uptake assay. The neuroprotective effect in SH-SY5Y cells against the MPP+ and ER stress damage was evaluated. The cell viability assays were performed at various concentration ranges from 0.5 to 1 mg/ml. Charantin was added at 0 h, 8 h, and 16 h post the neurotoxin treatment. Brine shrimp lethality assay and MTT assay were also performed to observe the cytotoxicity of charantin at different doses. **Results and Discussion:** Charantin was found to have a significant IC₅₀ value in SH-SY5Y cells. IN case of MPP+ treatment 0.5 mg, 1 mg/ml concentration of charantin showed significant neuroprotection and improvement in cell viability. In tunicamycin-induced damage, the protection was seen at 0 h and not at 8 h and 16 h post-treatment. At the same time, it has less/no toxicity at therapeutic doses; very high doses may pronounce cytotoxicity. **Conclusion:** The present investigation shows the neuroprotective effect of MC and charantin obtained from the plant can be employed as a memory enhancer in Alzheimer's related dementias; further to be evaluated clinically by formulating charantin.

Key words: *Momordica charantia*, MPP+, MTT, neuronal red uptake assay, SH-SY5Y neuroblastoma, tunicamycin

INTRODUCTION

Alzheimer's disease (AD) is defined as the progression in neurodegeneration that is symptomized as memory loss, dementia, and formation of β -amyloid plaques and tangles with amnesia as the striking symptom.^[1] Although AD is characterized and indicated by cognitive impairment, cerebrocortical atrophy, and depletion of cortical and subcortical neurons, it has four stages, namely predementia, early dementia, moderate dementia, and advanced dementia.^[2] It was found that amyloid beta aggregates are even present in the initial stages of AD.^[3,4] Intracerebroventricular administration of amyloid beta to rodents has been a tested method

to analyze the pathology of AD.^[5,6] Various other models such as fruit fly,^[7] *Caenorhabditis Elegans*,^[8] and zebrafish have been innovative models to screen neuroprotective compounds.^[9] It was proved that almost 60–70% of the genes of zebrafish represent the human diseases like AD.^[10] In this study, the isolation and characterization of

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charantin were performed. The neuroprotective effect of the compound against the neurotoxin, MPP⁺, and Tunicamycin induced cell death in SH-SY5Y neuroblastoma cells was evaluated.

In this study, charantin, extracted from *Momordica charantia* (MC) (MC, bitter melon), family Cucurbitaceae^[11] have been evaluated for its role in cognitive improvement and cytotoxicity. It comprises equal amounts of β -sitosterol glucoside [Figure 1a] and 5, 25-stigmasteryl glucoside [Figure 1b] (steroidal saponins) inedible fruits.^[12] Although charantin is obtained from the whole plant of MC, it is highly abundant in the fruits and leaves.^[13,14] Charantin was reported for its efficiency as hypoglycemic^[15,16] and anti-convulsant (high doses like 10–15 mg/kg, p.o).^[17]

Here before, there was no evidence for the neuroprotective role of charantin, and hence, in this study, it was evaluated for its neuroprotective role to prove its efficacy in AD.

MATERIALS AND METHODS

Collection of Plant Material

MC ripened fruits were purchased at a local market; authentication was done (Voucher specimen number-PARC/2017/2894) by Prof. P. Jayaraman. These fruits were dried in the shade, then pulverized into small pieces and sieved. The obtained coarse powder was stored in airtight containers until the beginning of the experiment.

Extraction and Isolation

The coarse powdered charantin was subjected to extraction in a rotary evaporator for 48 h by adding required amounts of 80% ethanol. Potassium hydroxide solution was mixed with the obtained concentrate, diluted with water and further undergone ether (60–80°C) extraction. The obtained ether extract was divided into three equal parts and washed with water, HCl, and water, respectively. Among these, the ether layer was subjected to distillation and recrystallization for 5–6 times with 95% ethanol; charantin obtained as the final required product.

Characterization of Charantin

Fourier transform-infrared (FT-IR) spectrum of charantin

Recording of the IR spectrum was done on FT-IR (Shimadzu). The charantin sample and KBr in 1: 10 ratio were placed in a KBr press to prepare the pellet samples. The spectra were recorded from 4000–400/cm range.

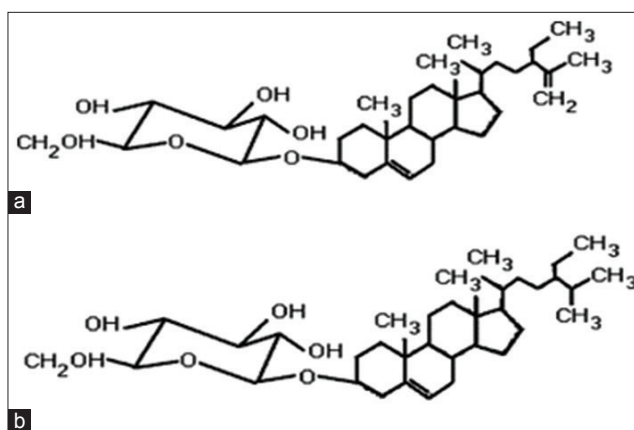


Figure 1: (a) Structure of β -sitosterol glucoside; (b) 5, 25-stigmasteryl glucoside

¹H-nuclear magnetic resonance (NMR) of charantin

¹H-NMR of the compound from MC was performed using BRUKER 300MHz NMR SPECTROMETER with CDCl₃.

Brine shrimp lethality bioassay

This assay is used to assess the toxicity of pure compounds and plant extracts/isolated compounds at different concentrations. The compound/extract was dissolved in a suitable solvent, and several stock solutions were made of 100, 500, 1000, and 2000 μ g/ml concentrations.^[18,19] The cytotoxicity of charantin was evaluated by Brine shrimp lethality assay and the lethality (LC₅₀) was calculated Table 1.

Samples

Dimethylsulfoxide (DMSO) was used to dissolve the samples DMSO and it is diluted with artificial seawater up to 5 ml total volume to get different concentrations (100, 500, 1000, and 2000 μ g/ml) and were transferred to filter paper discs (1/2 inch), dried for 1 h *in vacuo*. DMSO in seawater was used to prepare control discs.

Hatching of shrimps

A small tank having two partitions was filled with seawater, used to culture the shrimp eggs (*Artemiasalina* leach) for 2 days by providing constant oxygen supply for the shrimp to be matured as nauplii. The shell free nauplii were obtained from the hatched shrimps by subjecting them to phototaxis and were utilized for the cytotoxic assay.

Assay procedure

Each sample was transferred to a vial having 10 nauplii using a micropipette, and the volume of the vial was made up to 5 ml using artificial seawater. Each vial was provided with dry yeast suspension (3mg) for the shrimps as a diet. After 6 and 24 h, the numbers of shrimps alive were counted using a $\times 3$ magnifying glass. In the case of 24 h examination, the percentage of deaths for control and test was determined. By probit analysis method, LD₅₀ and 95% confidence intervals

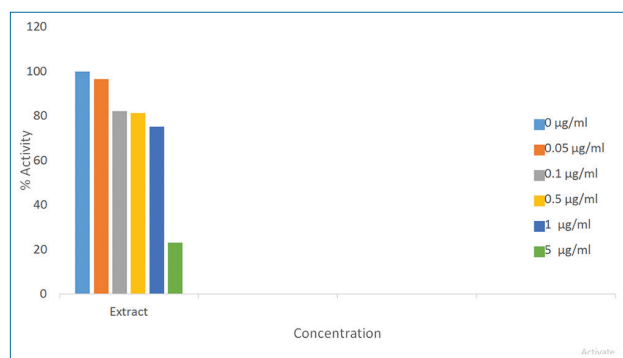


Figure 4: Cell viability in SH-SY5Y cells with charantin

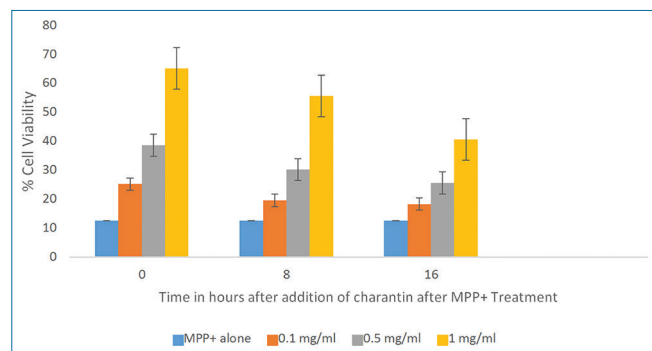


Figure 5: Protective role of charantin against MPP⁺ induced neurotoxicity

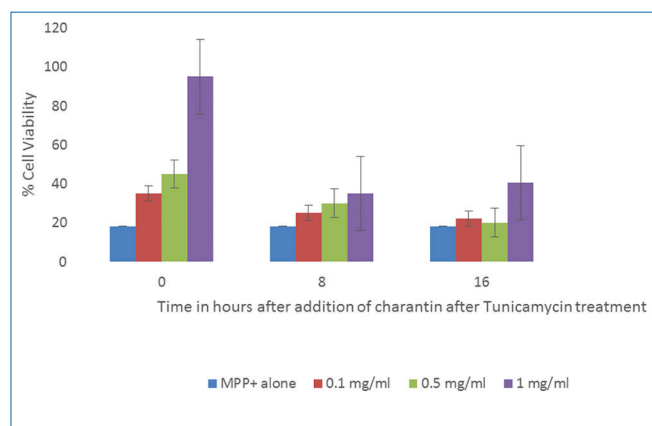


Figure 6: Protective role of charantin against tunicamycin-induced neurotoxicity

24 h. One-way ANOVA was utilized with Turkey's method. The results were represented in the Figure 4 and Table 3.

DISCUSSION

The neurodegeneration process in AD continues until there is a degeneration of the synaptic system, neuronal damage, and formation of amyloid beta plaques in the brain.^[23] Amyloid beta plays a major role in the neuronal dysfunction process.^[24,25] There have been various studies implicating the role of plaque formation in cognitive impairments.^[26] β A-induced

Table 1: Evaluation of cytotoxic effect using brine shrimp lethality assay

Test sample	Concentration (µg/ml)	% mortality
MC extract	12.5	40
	25	40
	50	50
	100	60
	200	80
	400	100
	800	100
CA	0.06	10
	0.125	20
	0.25	30
	0.5	40
	1	50
	5	90
	10	100

CA: Capecitabine, MC: *Momordica charantia*

Table 2: Cell viability assay in SH-SY5Y cells with charantin using MTT assay

Method	Sample	Concentration µg/ml	% cell viability
% cell viability	Extract	0	100
		0.05	96.5
		0.1	82.19
		0.5	81.25
		1	75.14
		5	23.12

Table 3: Cell viability assay in SH-SY5Y cells with charantin neutral red uptake assay

Method	Sample	Concentration µg/ml	% cell viability
% cell viability	Extract	0	100
		0.05	92.5
		0.1	81.41
		0.5	79.23
		1	73.35
		5	21.02

apoptosis is caused in the AD which is due to the activation of mitochondrial pathway leads to cellular death.^[27,28]

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay is a prominent, cytotoxicity testing of compounds or chemicals.^[29] Different concentrations

Table 4: Neuroprotection of charantin against MPP⁺ induced neurotoxicity

Sample	Time in hours % cell viability		
	0 h	8 h	16 h
Extract			
0.1	25.1	19.5	18.2
0.5	38.5	30.1	25.5
1	65.1	55.5	40.5
MPP			
5	12.5	12.5	12.5

Table 5: Neuroprotection of charantin against tunicamycin-induced neurotoxicity

Sample	Time in hours % cell viability		
	0 h	8 h	16 h
Extract			
0.1	35	25	22
0.5	45	30	20
1	95	35	30
Tunicamycin			
5	18.2	18.2	18.2

of the isolated compound preparations were used to culture the shrimp nauplii (larvae) and the lethality (LC_{50}) was calculated by the comparison of mean surviving larvae in the control tube and the treatment tubes. In this study, there was no death of single nauplii within 24 h in any of the tubes. Therefore, LC_{50} was considered as $>2000 \mu\text{g/ml}$. According to the Bastos *et al.* method, the results of brine shrimp lethality assay were interpreted as: LC_{50} values are considered to be non-toxic if more than $1000 \mu\text{g/ml}$, weakly toxic if LC_{50} is between 500 and $1000 \mu\text{g/ml}$, and toxic if the value is below $500 \mu\text{g/ml}$. This analysis can be used to apply the same doses/concentrations to utilize for further *in vivo* studies.

IC50 Determination in SH-SY5Y Cells

SH-SY5Y neuroblastoma cells are an important tool which has been widely used as a neurodegenerative disease model. These cells have the capability to differentiate into neuron-like cells which are morphologically and biochemically similar to neurons.^[30,31] For the present study, the SH-SY5Y cells treated with concentrations of charantin ranging from 0.05 mg/mL to 5 mg/mL showed none of the toxic effects. It may cause some toxic effects only at higher dose ranges.

Neuroprotection of SH- SY5Y cells by Charantin against MPP⁺ Induced Neurotoxicity

MPP⁺ causes neuronal damage in neuronal cell lines by mitochondrial damage and oxidative stress.^[32] The present

study reveals that charantin causes neuroprotection against MPP⁺ induced neuronal damage. Charantin when added at 8 h and 16 h after MPP⁺ treatment at a concentration of 1 mg/ml showed a cell viability of 44% and 36%, respectively. The neuroprotection of charantin may be due to the free radical scavenging properties which have been investigated before.^[15]

Neuroprotection of Charantin in SH- SY5Y cells against Tunicamycin-induced Neurotoxicity

Endoplasmic reticulum (ER) stress response is triggered by the accumulation of misfolded proteins in the ER. These responses are seen in cases of neurodegenerative diseases such as AD and Parkinson's disease which causes neuronal damage and cell death. Tunicamycin is a known bacterial toxin which causes ER stress.^[33] Various ER Stress markers have been evaluated in SH-SY5Y cells when treated with tunicamycin. The present study consists of treating SH-SY5Y cells with charantin which protected against tunicamycin-induced cell death at 0 h. The percentage cell viability was found to be 35, 45, and 95% at 0.1, 0.5, and 1 mg/mL of charantin. After 8 h and 16 h, post-treatment charantin was found to have reduced neuroprotective effect.

Thus, the prevention of neuronal damage and cell death of charantin shows its efficacy of neuroprotection and can be used for treating neuronal damage and cell death seen in cases of neurodegenerative diseases especially for Alzheimer's. Endoplasmic reticulum stress due to a bacteriotoxin, tunicamycin and its protection by charantin was well explained in the Figure 6 and Table 5.

CONCLUSION

The results from the above study indicate the strong neuroprotective potential of MC against MPP⁺ and tunicamycin-induced neurotoxicity. Further investigation can be conducted on the plant and isolated compound charantin for its potential in treating neurodegenerative diseases such as Alzheimer's and Parkinson's disease.

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