International Journal of Chemistry, Mathematics and Physics (IJCMP)
[Vol-8, Issue-1, Jan-Mar, 2024]
https://dx.doi.org/10.22161/ijcmp.8.1
ISSN: 2456-866X

Evaluation of *In-vitro* neuroprotective effect of Ethanolic extract of *Canarium solomonensee* Leaves on H_2O_2 induced toxicity in SH-SY5Y cell line

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Received: 17 Nov 2023; Received in revised form: 21 Dec 2023; Accepted: 03 Jan 2023; Available online: 10 Jan 2024

Abstract— *The ethanolic extract of canarium solomonense leaves (ecsl) was studied for its neuroprotective activity. The neuroprotective activity of ECSL was found to have a significant impact on neuronal cell death triggered by hydrogen peroxide (MTT assay) in human SH-SY5Y neuroblastoma cells. Scopolamine, a muscarinic receptor blocker, is frequently used to induce cognitive impairment in laboratory animals. Injections of scopolamine influence multiple cognitive functions, including motor function, short-term memory, and attention. Using the Morris water maze, the Y maze, and the passive avoidance paradigm, memory enhancing activity in scopolamine-induced amnesic rats was evaluated. Using the Morris water maze, the Y maze, and the passive avoidance paradigm, ECSL was found to have a substantial effect on the memory of scopolamine- induced amnesic rats. Our experimental data indicated that ECSL can reverse scopolamine induced amnesia and assist with memory issues.*

Keywords— ***Canarium solomonense, Neuroprotective Activity, Hydrogen Peroxide, Scopolamine-induced Amnesia, Memory Enhancement***

I. INTRODUCTION

Neurodegeneration is the loss of nerve structure and function caused by a range of illnesses. This deterioration causes a steady loss in cognitive skills such as memory and decision-making. Neurodegeneration is a significant component of a broad spectrum of conditions classed as "neurodegenerative diseases." Neuronal loss in neocortical and allocortical region results in macroscopic grey matter atrophy. In addition to gray matter atrophy, subcortical white matter alters progressively, leading to the loss of physical connections between distant brain regions. (Thomas Jacquemont *et al.* 2017).

Several studies have suggested that polyphenols have anti amyloidogenic effect. Several polyphenol compounds including tannic acid, quercetin, kaempferal, curcumin, catechin and epicatechin were reported to dose-dependently inhibit the formation of Aβ fibrils as well as their stability to Aβ or mature aggregates and impair their stability, as the entire compound tested destabilized preformed Aβ fibrils. Tannic acid was shown to reduce Aβ deposits as well as Aβ species including oligomers in the transgenic AD mouse brain. Curcumin, found in the spice turmeric, has been shown to prevent brain lipid peroxidation in rats and increase gluthathione level and the activity of other detoxifying enzymes. Curcumin was found to prevent protein oxidation and inflammation and reduce the formation of soluble and insoluble Aβ by up to 50% (T.Jayasena *et al* 2013)

The potential of naturally derived coumarin scopoletin as procholinergic and cognition enhancing therapeutic was investigated in a more detailed way, using different experimental approaches like measuring newly synthesized acetylcholine (ACh) in synaptosomes, long term potentiation (LTP) experiments in hippocampal slices and behavior assessment studies. Scopoletin was reported to increase T-maze alternation and ameliorated novel object recognition of mice with scopolamine-induced cholinergic deficit. It also reduce age associated objective memory deficits in 15-18 month old mice. The scopoletin possesses memory improving properties, which are based

on its direct nAhCR agonist activity (A.Hornick *et al.* 2011).

In this study, *Canarium solomonense* leaves were selected for evaluation of in-vitro neuroprotective activity

Fig.1. Canarium solomonense leaves

II. MATERIALS AND METHOD

Collection of plant

The leaves of, *Canarium solomonense*, were collected from Alwar in April 2019. The Botanical Survey of India, Nourthern Regional Center, authenticated the plants.

SH-SY5Y Cell Culture and maintenance

The NCCS, Pune, India, provided the human neuroblastoma SH- SY5Y cell lines. Cells were cultured in DMEM supplemented with 10% heat- inactivated FBS (v/v) and 0.1 % penicillin/streptomycin at 37°C in a humidified environment of 5% CO_2 and 95% air. H_2O_2 was created from a supply just before usage. The ECSL was dissolved in DMSO and the stock solution was immediately added to the growth medium. Control cells were only given DMSO. The final concentration of the solvent was always 0.1 percent (v/v). Substantial cytotoxicity was observed. (Ye-Qing Dua 2019)

Preparation of plant extract

Shade dried at room temperature, leaves of *Canarium solomonense*, were powdered to coarse size 40# using a mixer. The powdered plant leaves were defatted with 90% ethanol and extracted in a Soxhlet apparatus at 60-80ºC for 48 hours to obtain defatted ethanol extract and concentrated dry extract. various extracts of *Canarium solomonense* leaves, were later stored at 4°C.

MTT Assay

Since it is based on the activity of a mitochondrial enzyme, the MTT assay gives a sensitive evaluation of a cell's metabolic condition. SH- SY5Y cells (2.5 x 10^4 cells/well in 96-well plates) were incubated for 24 h at 37ºC with 400 M H_2O_2 with or without extract pretreatment, then treated for 2 h with MTT solution (5 mg/ml). The absorbance at 540 nm was measured using a microplate reader after the dark-blue formazan crystals produced in intact cells were dissolved in DMSO. The results are given as a percentage of MTT decreases, or a change in sample absorbance compared to control cells' absorbance. (Seung-Hwan Kwon 2012; Yongji Lai 2016)

III. RESULTS AND DISCUSSION

The MTT reduction assay is a sensitive assay for assessing cell viability and proliferation that is based on the mitochondrial reduction of tetrazolium salt into insoluble formazan product (G. Wendt 2014). Numerous studies have found that oxidative stress is intimately associated with the pathophysiology of a wide range of illnesses, including neurodegenerative diseases. Oxidative stress generates reactive oxygen species (ROS) such as H_2O_2, and ROS-mediated damage to cellular components causes apoptosis. Thus, by reducing ROS generation, antioxidants may be an effective strategy for avoiding oxidative stress-induced cell death. (2011, Namiko Suematsu)

After 24 hours of treatment with various concentrations of scopoletin and ECSL (2.5, 5, 10, 25, 50, and 100 µg/ml), cell viability was evaluated using the MTT test. Treatment with ECSL and scopoletin alone had no influence on cell growth or toxicity, and no false positive or false negative results were seen. The scopoletin and ECSL dosages were used in the subsequent assays. Cell culture with H_2O_2 at various concentrations (0, 0.05, 0.1, 0.2, 0.5, 0.7, 1, 1.5,2,4, and 10 mM) for 24 or 48 hours to evaluate effective H_2O_2 doses and appropriate incubation time generating a significant percentage of cell loss, as well as the sensitivity profile of each category of cells to oxidative stress generated toxicity; thus, 400 µM H_2O_2 was used to injure cells. (Seung-Hwan Kwon 2012) SH-SY5Y cells were pretreated with ECSL for 30 minutes before being treated with 400 µM H_2O_2 for 24 hours. The vitality of SH-SY5Y cells incubated with H_2O_2 for 24 hours was 90% of the control value, but it increased dramatically to 61, 65, 66, 73, and 77 % (Figure 4.1). Furthermore, ECSL at these doses 1-1000 were not cytotoxic when cells were pretreated with it at 1, 2.5, 5, 10, and 20 µg/ml (Figure 4.2). When cells were pretreated with Scopoletin for 30 minutes before beingexposed to 400 M H_2O_2 for 24 hours, their vitality was 90% of the control value, but it

increased significantly to 51.6 %, 54.6 %, 60.6 %, 69.3 %, and 75.3 % when cells were pretreated with Scopoletin at 1, 2.5, 5, 10, and 20 µg/ml (Figure 4.3). Furthermore, at this doses of 1-1000, Scopaletin was not hazardous (Figure 4.4). The IC_{50} values for ECSL and Scopaletin were 10.50and 10.05 µg/mL, respectively.

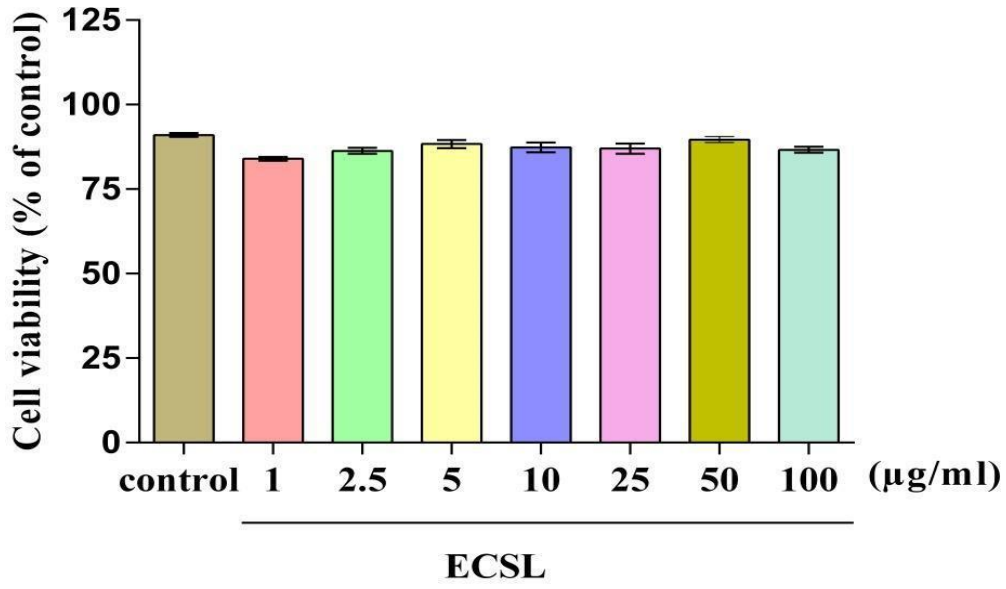

Fig.2 Effect of ECLS on SH-SY5Y cell

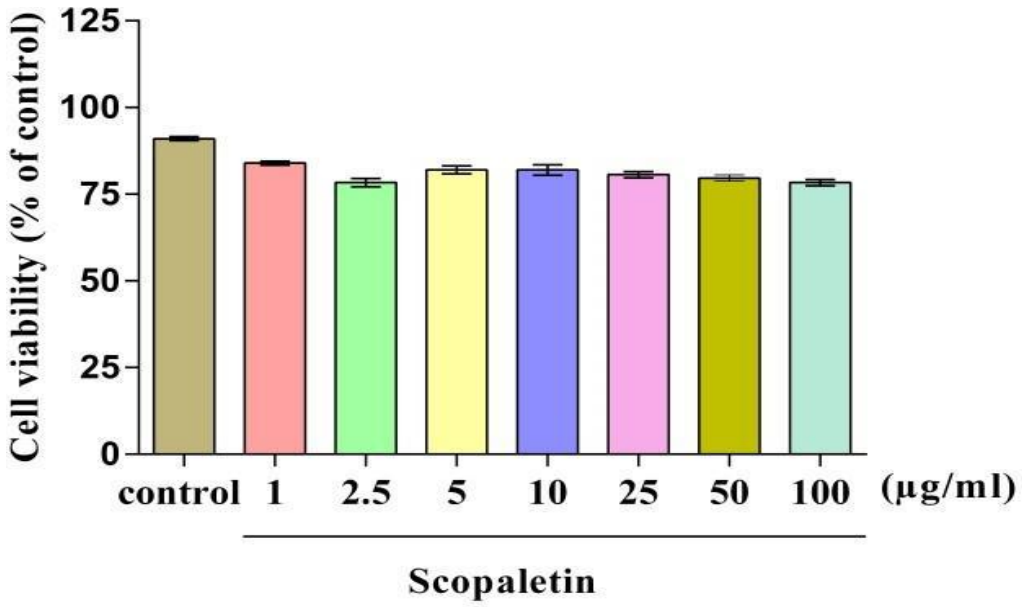

Fig.3 Effect of Scopaletin on SH-SY5Y cell

IV. CONCLUSION

The dried extracts of *Canarium solomonense* were evaluated for neuroprotective property.It showed significant neuroprotective characteristics in the hydrogen peroxide caused cytotoxicity and neuronal cell death in human SH-SY5Y neuroblastoma cells (MTT assay) when compared with other selected plants. The obtained results demonstrated that ECSL displayed substantial inhibitory action. The in vitro results support ECSL's strong anti-acetyl cholinesterase activity and cytotoxicity-induced human SH-SY5Y cell efficiency.

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