SHORT COMMUNICATION

PROTECTIVE EFFECT OF *PORTULACA QUADRIFIDA* ON ROTENONE INDUCED MITOCHONDRIAL DYSFUNCTION IN RAT BRAIN SYNAPTOSOMES

ABSTRACT

Mitochondrial dysfunction as a virtue of oxidative stress leads to neurodegeneration. The neurotoxin rotenone, is widely used to induce mitochondrial dysfunction and hence evaluate the neuroprotective action of a sample drug. For this study and comparison, the hexane extract, chloroform extract, ethyl acetate extract and ethanol extract of the weed *Portulaca quadrifida* (Portulacaceae) were selected to study and compare the neuroprotective activity in rat brain synaptosomes.

Keywords: *Portulaca quadrifida*, Rotenone, MTT, Synaptosomes

INTRODUCTION

Neurodegenerative diseases are one of the major types of disorders affecting mankind. Neurodegeneration primarily causes neuroinflammation and mitochondrial dysfunction[1]. These complications promote the release of free radicals and lead to exacerbated oxidative stress[2]. Mitochondrial toxins such as rotenone and paraquat are commonly used to cause oxidative stress and, as a result, neurodegeneration[3]. Rotenone is a neurotoxin belonging to a pesticide category that disrupts the mitochondrial respiratory chain reaction. The mitochondrial dysfunction induced by rotenone is related to Parkinson's disease[4,5]. Cell viability is determined by the formation of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formazan[6]. The *in vitro* method where the rat brain synaptosomes are exposed to neurotoxins is used to study the neuroprotective activity of a study drug[7].

Portulaca quadrifida is a weed belonging to family Portulacaceae. The plant has been widely utilized as an edible weed. The plant has been reported to have alkaloids, terpenoids, flavonoids, saponins, and tannins as major secondary metabolites. Anticonvulsant, anticancer, antidiabetic, antifungal, antiinflammatory, and gastrointestinal disorders are among the various pharmacological applications attributed to it and reported in the literature[8]. The literature review indicates that the plant shows significant resistance to stress-induced oxidation[9]. The present study was thus designed to evaluate if the weed possesses the neuroprotective activity against rotenone induced mitochondrial dysfunction in rat brain synaptosomes.

MATERIALS AND METHODS

MTT and rotenone were procured from Sigma-Aldrich, Bengaluru, all other chemicals and reagents used were of analytical grade. The whole plant of *P. quadrifida* (Portulacaceae) was collected from the Satpuda region in Maharashtra. The plant material was authenticated by Dr. Rajendra Shinde, Blatter Herbarium, St. Xavier's College, Mumbai with herbarium specimen number 16926, dated 6/8/2018. The whole plant was exposed to successive extraction using Soxhlet apparatus using nonpolar to the polar solvent system to obtain hexane extract (HEPQ), chloroform extract (CEPQ), ethyl acetate extract (EAEPQ), and ethanol extract (EEPQ).

12 Male Wistar albino rats, each weighing approximately 250 g were acclimatized for 7 days before the commencement of the study. The study was carried out in accordance with CPCSEA guidelines issued by the Animal Welfare Division of Ministry of Environment and Forest. The animals were sacrificed, and the brain was excised immediately. The 10% w/V brain homogenate was prepared in an ice-cold 0.3 M sucrose HEPES buffer and centrifuged at 4 °C. The supernatant thus obtained was double diluted with ice cold 1.3 M sucrose HEPES buffer and again cold centrifuged to obtain residual pellets. The pellets were resuspended with ice cold 0.8 M sucrose HEPES buffer to obtain a synaptosome fraction. The MTT assay was used to estimate the cell viability. Briefly, 0.400 mL of the synaptosome fraction was taken. To that, 1 mL of different concentrations of all 4 extracts, i.e., 5, 10, 20, 50 and 100 µg mL^{-1}, were added. In addition, 0.1 mL of 1 mM rotenone was added, followed by a 2 h incubation at 37 °C. 10 µL of 5 mg mL^{-1} MTT was added and incubated for 24 h. The absorbance was read at 570 nm[10,11,12]. All the experiments were performed in sextuplet.

Table I: Effect of extracts of *Portulaca quadrifida* on cell viability in MTT assay

	Percent cell viability			
Concentrations (µg mL^{-1})	**HEPQ**	**CEPQ**	**EAEPQ**	**EEPQ**
5	31.88±5.19	28.78±4.23	25.02±3.11	22.13±2.40
10	42.18±4.19*	38.97±3.09*	31.42±1.85	25.60±1.98
20	62.37±3.29*	48.63±4.94*	32.88±3.31	31.37±2.33
50	68.70±3.29*	65.80±9.34*	47.78±2.52*	34.98±2.40*
100	81.02±2.00*	77.50±2.79*	49.37±6.20*	37.75±2.87*

*The experiment was performed in sextuplet and the values are expressed as mean ± SD. The data were analyzed by one-way ANOVA followed by Dunnett's test, where *indicates significant difference (P < 0.05) when compared with rotenone control*

RESULTS AND DISCUSSION

Synaptosomes from the brain are rich in synaptic protein and are used to study neuronal disruption[10]. The synaptosomal viability is an important factor in determining neuroprotection. Rotenone is a pesticide and has very high lipophilicity. It is one of the most used neurotoxins in various models of Parkinson's disease. Rotenone is the potent inhibitor of the respiratory chain in mitochondria. It acts via inhibition of complex I i.e., NADH-ubiquinone oxidoreductase[3,4]. Thus, the microtubule is disrupted, leading to cell death. The viable cells have the mitochondrial dehydrogenase enzyme which reduces the MTT into its violet formazan salt[13,14]. The MTT reduction is found to be lowered in the non-viable cells[6]. Thus, the greater the intensity of the violet color indicates the maintained viability of the synaptosomes and hence neuroprotection. In our study, the percent viability in the rotenone control group was found to be 22.70 ± 5.19. The HEPQ and CEPQ showed a significant increase ($P < 0.05$) in cell viability at 10, 20, 50, and 100 µg mL^{-1}, while EAEPQ and EEPQ showed a significant increase ($P < 0.05$) in cell viability at 50 and 100 µg mL^{-1} (Table I). Thus, the hexane and chloroform extracts of *P. quadrifida* exerted better neuroprotective activity in comparison to ethyl acetate and ethanolic extract in rat synaptosomal preparation.

REFERENCES

1. Fischer R. and Maier O.: Interrelation of oxidative stress and inflammation in neurodegenerative disease: Role of TNF. **Oxid. Med. Cell. Longev.**, 2015, Available at https://doi.org/10.1155/2015/610813.
2. Andersen J. K., Davies K. J. and Forman H. J.: Reactive oxygen and nitrogen species in neurodegeneration. **Free Radic. Biol. Med.**, 2013, 62, 1–3.
3. Ayala A., Venero J. L., Cano J. and Machado A.: Mitochondrial toxins and neurodegenerative diseases. **Front. Biosci.**, 2007, 12, 986–1007.
4. Li N., Ragheb K., Lawler G., Sturgis J., Rajwa B., Melendez J. A. and Robinson J. P.: Mitochondrial Complex I Inhibitor Rotenone Induces Apoptosis through Enhancing Mitochondrial Reactive Oxygen Species Production. **J. Biol. Chem.**, 2003, 278, 8516–8525.
5. Campos F., Carvalho M. M., Cristovao A. C., JE G., Baltazar G., Salgado A. J., Kim Y. S. and Sausa N.: Rodent models of Parkinson's disease: beyond the motor symptomatology. **Front. Behav. Neurosci.**, 2013, Available at https://doi.org/10.3389/fnbeh.2013.00175.
6. Berridge M. V. and Tan A. S.: Characterization of MTT Berridge. **Arch. Biochem. Biophys.**,1993, 2, 474–482.
7. Cruz V. P., Cort C. G., Chaverr J. P., Maldonado D. P., Mart L. A. and Santamar A.: Protective effect of S-allylcysteine on 3-nitropropionic acid-induced lipid peroxidation and mitochondrial dysfunction in rat brain synaptosomes. **Brain Res. Bull.**, 2006, 68, 379–383.
8. Dhande S. R. and Patil V. R.: An overview of Protulaca quadrifida. **Int. J. Veg. Sci.,** 2020, 26, 450–456.
9. Shahwan M. J. and Gacem S. A.: Neuroprotective antioxidant effect of portulaca quadrifida linn. Leaves extract on immobilization stress-induced changes in rat's brain. **Asian J. Pharm. Clin. Res.**, 2018, 11, 378–381.
10. Kamat P. K., Kalani A. and Tyagi N.: Method and validation of synaptosomal preparation for isolation of synaptic membrane proteins from rat brain. **MethodsX**, 1, 2014, 102–107.
11. Makhija D. T. and Jagtap A. G.: Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: Comparison with rat model. **J. Pharmacol. Pharmacother.**, 2014, 5, 39–46.

12. Santamaría A., Salvatierra R., Roman B. V., Lopez D. S., Hernandez J. V., Arzate S. G., Capdeville M. J. and Ali S. F.: Protective effects of the antioxidant selenium on quinolinic acid-induced neurotoxicity in rats: *In vitro* and *in vivo* studies. **J. Neurochem.**, 2003, 86, 479–488.
13. Mitra, I., Mukharjee S., Reddy V. P., Dasgupta S., Bose J. C. Mukherjee S., Linert W. and Moi S. C.: Benzimidazole based Pt(II) complexes with better normal cell viability than cisplatin: Synthesis, substitution behavior, cytotoxicity, DNA binding and DFT study. **RSC Adv.**, 2016, 6, 76600–76613.
14. Mosmann T.: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. **J. Immunol. Methods,** 1983, 65, 55–63.

[a] Department of Pharmacology, Bharati Vidyapeeth's College of Pharmacy, Sector-8, CBD Belapur, Navi Mumbai - 400 614, Maharashtra, India

Swati Dhande[a]* and Vijay Patil[b]

[b] Department of Pharmacognosy, TVES's HLMC College of Pharmacy, Faizpur, Jalgaon - 425 503, Maharashtra, India

***For Correspondence: E-mail: dswatir@gmail.com**

(Received 02 March 2022) (Accepted 01 November 2022)

https://doi.org/10.53879/id.60.03.13394