CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3593564

Available online at: http://www.iajps.com Research Article

NEUROPROTECTIVE ACTIVITY OF HESPERIDIN AGAINST MPTP (1-METHYL-4-PHENYL-1, 2, 3, 6-TETRAHYDROPYRIDINE) INDUCED NEUROTOXICITY: *IN VITRO* APPROACHES

G. Devala Rao[2], Hanumanthu Penchalaiah[1]* S Praveen Begum[1]

1 Research Scholar, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.

2K.V.S.R. Siddhartha College of Pharmaceutical Sciences, Vijayawada.

Abstract:

Aim: In the present study, we examined the neuroprotective activity of hesperidin on the accumulation of neuronal oxidative stress induced by MPTP by in vitro. Method: After decapitation, healthy rat brain was removed rapidly from the skull and rinsed with cold artificial cerebrospinal fluid (ACSF) which has been equilibrated with 95% O2/5 % CO2 gas mixture. Group I brain slices was incubated in ACSF serve as normal, Group II brain Slices was incubated in ACSF and DMSO (10%) serve as disease, Group III brain was incubated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (1 ng/ml) Group IV brain was incubated in MPTP with Bromocriptine (10 µg/ml) as a standard, Group V brain was incubated in MPTP with hesperidin (10 µg/ml) Group VI brain was incubated in MPTP with hesperidin (20 µg/ml). After 1hr of incubation brain slices were homogenized in PBS buffer, pH 7.4 and supernatant subjected estimation of for protein content, lipid peroxidase and reduced glutathione. Results and conclusion: MPTP incubated brain LPO activity was significantly ($p<0.001$***) increased and GSH activity decreased ($p<0.001$***) compared to normal group. Hesperidin incubated group brain LPO* activity was significantly ($p<0.05$*) decreased and GSH activity was increased ($p<0.01$**), The research results were concluded that the hesperidin exhibited significant neuroprotectivite effect against MPP+ free radicals due to their antioxidant activity.*

Keywords: *Hesperidin, Cerbrospinal fluid, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), Brain slices, Lipid peroxidase (LPO*),Glutathione (GSH)*

Corresponding author:

Hanumanthu Penchalaiah,

Research Scholar,

University College of Pharmaceutical Sciences,

Acharya Nagarjuna University, Nagarjuna Nagar,

Guntur. Andhra Pradesh

Mail. Id: hanumanthu1423@gmail.com

QR code

Please cite this article in press Hanumanthu Penchalaiah et al., ***Neuroprotective activity of Hesperidin against MPTP (1-Methyl-4-Phenyl-1, 2, 3, 6-Tetrahydropyridine) Induced Neurotoxicity: In Vitro Approaches.,*** *Indo Am. J. P. Sci, 2018; 05(12).*

INTRODUCTION:

Glias, a specialized type of non-neuronal cell, regulate the neuronal microenvironment and provide support to the nervous system [1,2]. Amongst the glial cells, astrocytes are abundantly present with a close connection to neurons in the brain and spinal cord regulating various physiological and pathological conditions [3]. Astrocyte metabolism is a key feature on which the neurons are functionally dependent, including its role in energy metabolism and synthesis of neurotransmitters by maintaining the amino acid homeostasis [4]. Astrocytes play a dynamic role in the brain and is associated with apoptosis, ischemia and various neurodegenerative disorders [5,6,7]. The brain's vulnerability towards oxidative stress is highly dependent on astrocytes and thus astrogliosis may critically impair the survival of neurons [8, 9]. Astrocyte activation releases neuroinflammatory molecules like the proinflammatory cytokines tumor necrosis factor α (TNFα); interleukin (IL-1β and IL-6) [10, 11]. These neuroinflammatory cytokines modulate the astroglia dependent apoptosis resulting in malignant glioma development. Reactive astrocytes are a key feature for formation of the 'glial scar' expressing the glial fibrillary acidic protein and ultimate consequence of neuronal death [12].

Hesperidin is an abundant and inexpensive by-product of Citrus cultivation and is the major flavonoid in sweet orange and lemon. The bioflavonoids, formerly called 'vitamin P', were found to be the essential components in correcting this bruising tendency and improving the permeability and integrity of the capillary lining. These bioflavonoids include hesperidin, citrin, rutin, flavones, flavonols, catechin and quercetin [13]

Studies reported that it hesperidin was protect against various diseases such as diabetes, chronic venous insufficiency, hemorrhoids, scurvy, various ulcers and bruising [14]. Hesperidin has been reported to possess antioxidant properties [15], hepatoprotective activity [16], Anti inflammatory [17], Anti hypercholesterolemia, Anti atherosclerosis [18]. In present study was evaluated the potential neuroprotective activity of hesperidin on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced neurotoxicity using *in vitro* model.

MATERIAL AND METHODS:

Animals

Wistar albino rats of either sex, weighing 200±25 g, were procured from Mahaveer enterprises, Hyderabad. Animals were consumed a commercial diet for 1 week. The experimental protocol was approved by Institutional Animals Ethics Committee (253/IAEC/SICRA/PhD/2017) and animal care was taken as per the guidelines of CPCSEA (1821/PO/RE/S/15/CPCSEA).

Experimental procedure for neuroprotective effect of hesperidin by in *in vitro* [19]

Preparation of artificial cerebrospinal fluid (ACSF), pH 7.4 Contains sodium chloride (122 mM), potassium chloride (3.1 mM), calcium chloride (1.3 mM), magnesium sulfate (1.2 mM), glucose (10 mM), and glycyl glycine (30 mM). All the above chemicals were dissolved in 200 ml of distilled water. The solution of salts can be prepared and kept in refrigerator and glucose with glycyl glycine can be added later on the day of the experiment.

Isolation of brain: After decapitation, the brain was removed rapidly from the skull and rinsed with cold artificial cerebrospinal fluid (ACSF) which has been equilibrated with 95% O2/5 % CO2 gas mixture. Study was approved by Institute animal ethical committee

Treatment was followed:

Group I Brain slices was incubated in ACSF serve as normal

Group II Brain Slices was incubated in CSF and DMSO (10%) serve as disease,

Group III Brain was incubated with MPTP (1 ng/ml)

Group IV Brain was incubated in MPTP with Bromocriptine (10 µg/ml)

Group V Brain was incubated in MPTP with Hesperidin (10 µg/ml)

Group VI Brain was incubated in MPTP with Hesperidin (20 µg/ml)

After incubation, brain slices were homogenized in PBS buffer, pH 7.4 and estimated for protein content, GSH, LPO* as per the procedures.

Statistical analysis

All data are expressed as the means ± SEM. Statistical differences among the experimental groups were tested by using a one way analysis of variance (ANOVA) and Dunnet test was employed for multiple comparisons. P-values less than 0.05 were accepted as significant.

RESULTS:

Table: 1 Effects of hesperidin on selected biomarkers tested on sagittal brain slices in MPTP solution

Groups	Protein content [µg/mg tissue]	LPO* [nmoles TBARS / mg protein]	GSH [µmoles/ mg protein]
Normal control	18.1±1.3	4.5±0.8	0.04±0.003
DMSO control	16.2±0.2	4.2±0.3	0.04±0.002
MPTP (1 ng/ml)	5.6±0.3***	10.3±0.7***	0.008±0.0001**
Bromocriptine (10 µg/ml)	12.3±0.8	6.5±0.2**	0.034±0.005
10 µg/ml of hesperidin	6.5±0.3	8.2±0.3**	0.015±0.004
20 µg/ml of hesperidin	10.6±0.3**	7.2±0.4*	0.038±0.002**

All values are expressed in Mean± SEM. Statistical analysis determined by ANOVA followed by Dunnet's method of comparison. b denotes treated groups were compared against MPTP group. while the a denotes MPTP control group was compared against the DMSO control.

Fig:1 Effect of hesperidin on protein and LPO* on sagittal brain slices in MPTP solution

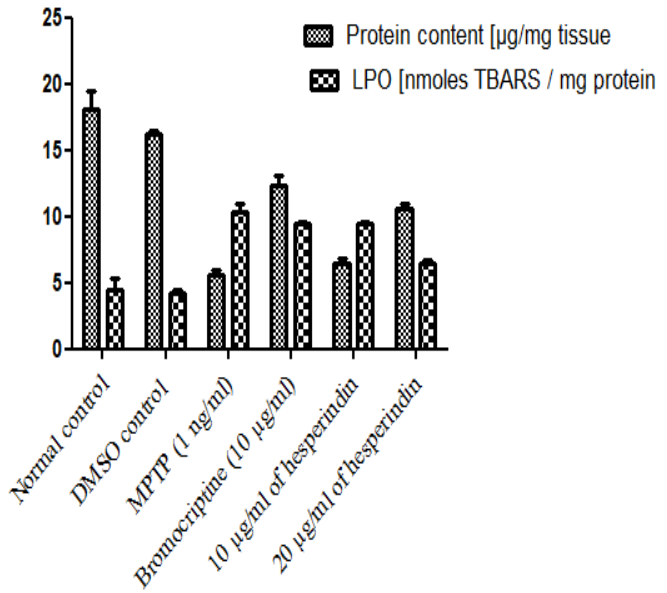
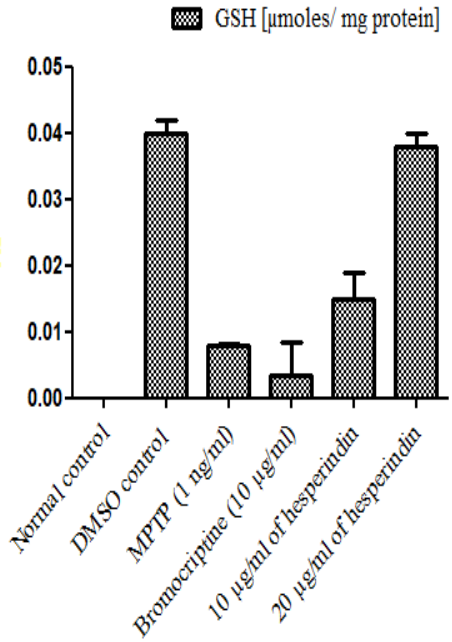


Fig: 2 Effect of hesperidin on GSH on sagittal brain slices in MPTP solution



Effects of hesperidin on protein content, GSH and LPO on brain slices

The effects of Hesperidin on biochemical parameters in the sagittal brain slices are tabulated as follows. There was a considerable decrease in the protein content (5.6±0.3µg/mg tissue, P < 0.001***) and GSH content (0.008±0.0001 µmoles/ mg protein, P < 0.01**) while increase in the lipid peroxidation products was observed (10.3±0.7 nmoles TBARS / mg protein, P < 0.001***) in the MPTP group. Hesperidin at 20 µg/ml showed considerable neuroprotective properties in term of restored GSH levels of 0.038±0.002 µmoles/ mg protein; P < 0.01**) and decreased LPO levels of 7.2±0.4 nmoles TBARS/ mg protein P < 0.05*) and improved protein content 10.6±0.3**. Thus, Hesperidin showed better results than bromocriptine in terms of GSH and LPO, while it improved (Table.1; Fig1 and 2)

DISCUSSION:

In human and nonhuman primates MPTP produces clinical, biochemical, and neuropathologic changes analogous to those observed in idiopathic Parkinson's disease. The neurotoxic effects of MPTP are thought to be initiated by MPP+ , which is a metabolite formed by the monoamine oxidase (MAO) B-mediated oxidation of MPTP [20]. MPP+ is selectively taken up by high-affinity dopamine and noradrenaline uptake systems and is subsequently accumulated within mitochondria of dopaminergic neurons. There it disrupts oxidative phosphorylation by inhibiting complex I of the mitochondrial electron transport chain [21]. The interruption of oxidative phosphorylation results in decreased levels of ATP [22], which may lead to partial neuronal depolarization and secondary activation of voltage-dependent NMDA receptors, resulting in excitotoxic neuronal cell death [23]. Although excitotoxic neuronal damage has been linked to Ca" influxes, the subsequent crucial steps that lead to cell death remain unknown. Recent evidence has implicated both oxygen free radicals and nitric oxide (NO'). The entry of calcium through NMDA receptor channels into cells stimulates nitric oxide synthase (NOS) activity by binding to calmodulin, a cofactor for NOS. Studies in dissociated cell cultures showed that NOS inhibitors effectively blocked NMDA-induced cell death [24]. Furthermore, NO' may react with superoxide to generate peroxynitrite, which may promote nitration of tyrosine and produce hydroxyl radicals, lipid peroxidase free radicals (LPO*) that decrease reduced antioxidant activity [19, 25, 26]. In the present study results revealed that Hesperidin exhibited significant neuroprotection against MPP+ free radicals due to neutralization of LPO* free radicals and enhance GSH activity. Although the mechanism by which Hesperidin regulates MPTP induced oxidative stress remains to be determined, there are several possible explanations. Firstly, as a polyphenolic flavonoid, Hesperidin has strong free radical scavenging activity [27]. Hesperidin reacts with a damaging free radical and forms a flavonoid radical, which has greater stability, and then breaks the free radical chain reaction [28]. It is possible that Hesperidin prevents oxidative damage directly by scavenging free radicals.

The results from the present study confirm that Hesperidin could alleviate the neurotoxicity induced by MPTP *in vitro* method. The effect of Hesperidin may be attributed to the prevention of oxidative damage, measured in terms of the amount of peroxidized lipid and the level of GSH. Therefore, Hesperidin is a potential candidate for further preclinical study aimed at the treatment of neutotoxicity.

Conflict of interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

ACKNOWLEDGEMENTS:

The authors thank to University College of Pharmaceutical Sciences, for provided necessary facilities.

REFERENCES:

1. Jessen KR, Mirsky R. Glial cells in the enteric nervous system contain glial fibrillary acidic protein. Nature. 1980; 286(5774):736-737.
2. Swaminathan N. Glia—The other brain cells. Jan-Feb: Discover Magazine. 2011.
3. Tiwari V, Guan Y, Raja SN. Modulating the delicate glial-neuronal interactions in neuropathic pain: promises and potential caveats. Neuroscience and biobehavioral reviews. 2014; 45:19-27.
4. Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. Journal of neurochemistry. 2006; 98(3):641-53.
5. Liebner S, Czupalla CJ, Wolburg H. Current concepts of blood-brain barrier development. International Journal of Developmental Biology. 2011; 55(4-5):467-476.
6. Burke RE, Antonelli M, Sulzer D. Glial cell line-derived neurotrophic growth factor inhibits apoptotic death of postnatal substantia nigra dopamine neurons in primary culture. Journal of neurochemistry. 1998; 71(2):517-525.

7. Barreto G, E White R, Ouyang Y, et al. Astrocytes: targets for neuroprotection in stroke. Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents). 2011; 11(2):164-173.
8. Feeney CJ, Frantseva MV, Carlen PL, et al. Vulnerability of glial cells to hydrogen peroxide in cultured hippocampal slices. Brain research. 2008; 1198:1-15.
9. Lu M, Hu L-F, Hu G, et al. Hydrogen sulfide protects astrocytes against H2O2-induced neural injury via enhancing glutamate uptake. Free Radical Biology and Medicine. 2008; 45(12):1705-1713.
10. Sun W, Depping R, Jelkmann W. Interleukin-1β promotes hypoxia-induced apoptosis of glioblastoma cells by inhibiting hypoxia-inducible factor-1 mediated adrenomedullin production. Cell death & disease. 2014; 5(1):1020.
11. Castigli E, Arcuri C, Giovagnoli L, et al. Interleukin-1β induces apoptosis in GL15 glioblastomaderived human cell line. American Journal of Physiology-Cell Physiology. 2000; 279(6):C2043- C2049.
12. Salewski R, Emrani H, G M. Neural Stem/Progenitor Cells for Spinal Cord Regeneration. 2013.
13. Tomas Barberán FA, Clifford MN. Flavanones, chalcones and dihydrochalcones–nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture. 2000 15; 80(7):1073-80.
14. Garg A, Garg S, Zaneveld LJ, Singla AK. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytotherapy Research. 2001; 15(8):655-69.
15. Wilmsen PK, Spada DS, Salvador M. Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. J Agric Food Chem. 2005; 53(12):4757-61.
16. Balakrishnan A, Menon VP. Protective effect of hesperidin on nicotine induced toxicity in rats. Indian J Exp Biol. 2007; 45(2):194-202.
17. Emim JA, Oliveira AB, Lapa AJ. Pharmacological evaluation of the anti-inflammatory activity of a citrus bioflavonoid, hesperidin, and the isoflavonoids, duartin and claussequinone, in rats and mice. Journal of pharmacy and Pharmacology. 1994;46(2):118-22.
18. Cha JY, Cho YS, Kim I, Anno T, Rahman SM, Yanagita T. Effect of hesperetin, a citrus flavonoid, on the liver triacylglycerol content and phosphatidate phosphohydrolase activity in orotic acid-fed rats. Plant Foods Hum Nutr. 2001; 56(4):349-58.
19. Sriram K, Pai KS, Boyd MR, Ravindranath V. Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice. Brain research. 1997 Feb 21;749(1):44-52.
20. Tipton KF, Singer TP. Advances in our understanding of the mechanisms of the neurotoxicity of MPTP and related compounds. Journal of neurochemistry. 1993 ;61(4):1191-206.
21. Gluck M. R., Krueger M. J., Ramsey R. R., Sabin S. O., Singer T. P., and Nicklas W. J. (1994) Characterization of the inhibitory mechanism of 1-methyl-4-phenylpyridinium and 4-phe nylpyridine analogs in inner membrane preparation. J. Biol. Chem. 269, 3167-3174.
22. Chan P, DeLanney LE, Irwin I, Langston JW, Di Monte D. Rapid ATP loss caused by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine in mouse brain. Journal of neurochemistry. 1991; 57(1):348-51.
23. Beal MF. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses?. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 1992; 31(2):119-30.
24. Dawson VL, Dawson TM, London ED, Bredt DS, Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proceedings of the National Academy of Sciences. 1991; 88(14):6368-71.
25. Beckman JS, Ischiropoulos H, Zhu L, van der Woerd M, Smith C, Chen J, Harrison J, Martin JC, Tsai M. Kinetics of superoxide dismutase-and iron-catalyzed nitration of phenolics by peroxynitrite. Archives of Biochemistry and Biophysics. 1992; 298(2):438-45.
26. Van der Vliet A, O'Neill CA, Halliwell B, Cross CE, Kaur H. Aromatic hydroxylation and nitration of phenylalanine and tyrosine by peroxynitrite. Febs Letters. 1994; 339(1-2):89-92.
27. Trouillas P, Marsal P, Svobodová A, Vostalova J, Gažák R, Hrbacˇ J, Sedmera P, Křen V, Lazzaroni R, Duroux JL, Walterova D. Mechanism of the antioxidant action of silybin and 2, 3-dehydrosilybin flavonolignans: a joint experimental and theoretical study. The journal of physical chemistry A. 2008; 112(5):1054-63.
28. Weber KC, Honório KM, Bruni AT, da Silva AB. The use of classification methods for modeling the antioxidant activity of flavonoid compounds. Journal of molecular modeling. 2006; 12(6):915-20.