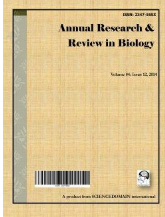

Annual Research & Review in Biology

24(2): 1-9, 2018; Article no.ARRB.39256
ISSN: 2347-565X, NLM ID: 101632869

Neuroprotection Effects of *Celastrus paniculatus* Seed Oil against Monosodium Glutamate in Human IMR-32 Cells

Naumita Shah[1], Ankit Nariya[1], Ambar Pathan[1], Alpesh Patel[2], Shiva Shankaran Chettiar[2] and Devendrasinh Jhala[1*]

[1]Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India.
[2]GeneXplore Diagnostics and Research Centre Pvt. Ltd., B-301 & 302, Tulip Corpus, Opp. V. S. Hospital, Above Pakwan Dinning Hall, Ellisbridge, Ahmedabad-380006, Gujarat, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author NS carried out the experiments and wrote the first draft of the manuscript. Authors AN and Amber Pathan helped in sample analysis and in proof reading of manuscript. Authors Alpesh Patel, SSC and DJ were involved in study design and statistical analysis. Author DJ was also involved in overall management of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/39256
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Ioana Stanciu, University of Bucharest, Romania.
(2) Ilesanmi Omotayo Babatundde, Federal University, Nigeria.
(3) Bhaskar Sharma, Suresh Gyan Vihar University, India.
(4) Dharma Lindarto, University of North Sumatra, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/23225

Original Research Article

Received 7th December 2017
Accepted 12th February 2018
Published 16th February 2018

ABSTRACT

Aims: The aim of this study is to evaluate the neuroprotective effects of *Celastrus paniculatus* seed oil (CPO) against monosodium glutamate in human IMR-32 cells.
Study Design: *Celastrus paniculatus* seed oil used historically in Indian subcontinent for its neuro-enhancement property and also considered to have free radical scavenging activity.
Methodology: In present study we have employed IMR-32, a neuroblastoma cells as our model system and utilized monosodium glutamate (MSG) a widely used food additive and proven inducer

**Corresponding author: E-mail: ddjhala@gmail.com;*

of free radicals to study the ameliorative effect of CPO against induced oxidative stress in neuronal cells.

Results: Results showed that CPO ameliorates total protein level, decreases protein carbonyl and lipid peroxidation levels ($p<0.001$) as well as enhances the activity of superoxide dismutase and catalase ($p<0.001$) under oxidative stress conditions. Further we found that CPO increases the free radical scavenging capacity of cell by enhancing ($p<0.001$) glutathione level and help its regeneration by revitalizing the activity of glutathione peroxidase, glutathione S-transferase and glutathione reductase enzymes.

Conclusion: It can be concluded that CPO has antioxidant property and proved to have ameliorative role against free radicals induced neuronal impairment.

Keywords: Celastrus paniculatus; monosodium glutamate; neuroprotection; free radicals.

1. INTRODUCTION

Neurodegenerative diseases are one of the major health challenges especially in elderly population and problems related to it has been enlarged many fold with extended average life expectancy. Neurodegenerative diseases are group of more than 100 diseases characterized by progressive loss of neurons [1]. These diseases lead to several complications such as behavioral abnormalities, speech alteration etc. and finally lead to the state of total debilitation. This causes a tremendous amount of emotional, financial, physical and social burden to the patients and their family. Both genetic and environmental factors have been attributed as risk [2]. One of the major hypotheses proposed for the causation and progression of these diseases is oxidative stress mediated injury to the nerve cells. Studies have showed that alteration in reactive oxygen species (ROS) scavenging machinery may lead to neuro-degeneration [3]. Many food additives responsible for ROS production and monosodium glutamate (MSG) is among one of them [4]. ROS causes formation of lipid peroxides which further react with proteins leading to oxidative modifications of amino acid residue causes protein fragmentation [5,6]. This altered protein and lipid molecules may leads to apoptosis. Similarly, hydrogen peroxide, which is normally produced in the brain in small amounts leading to generate free radicals. Its toxicity is due to its reaction with amino acids, nucleic acids, phospholipids and sugars, eventually causing cell death [7]. It is postulated that a proper management of the ROS produced in the patients could help to avoid the complications related to neurodegeneration and elevate behavioral abnormalities [3]. Many therapeutic drugs used for treatment of neurodegenerative disorders for longer duration are costly and also are associated with many side effects. Hence there is need of alternative medicinal approaches that have less or no side effects and are cost effective. In this regard, use of medicinal plant product could be one of the strategic options. Medicinal plants are rich sources of phytochemicals along with antioxidant properties that could be helpful to manage diseases by slowing their progressions and complications and have reduced or no side effects [8].

Celastrus paniculatus (CP), commonly known as Malkangni or Jyotishmati in India is a rich source of phytochemicals with medicinal properties [8]. Parts of these plants have been used as treatment to several disorders such as pain, depression, arthritis etc. [9,10]. The ROS scavenging activity of CP seed oil (CPO) and its different organic extracts has been studied in human immortalized fibroblast cell by Russo and coworkers in 2001 [11]. Further, Godkar and coworkers in 2006 has shown that CPO has antioxidant activities in rat embryonic forebrain neuronal cells [12]. There is paucity of data regarding the role of CPO in human neuronal cellular systems. Deciphering the role of CPO in human neuronal cell lines and estimating the potential dose will be worth to elucidate the therapeutic approach related to neurodegenerative diseases. So, the hypothesis of the present study is to check the neurotoxic effects of MSG on human neuronal cells IMR-32 and its amelioration by CP seed oil. Hence, in this study, using neuroblastoma cell line IMR-32 as model test system and stimulating the free radical production in it by MSG, we have investigated the role of CPO as a free radical scavenger and neuroprotector.

2. MATERIALS AND METHODS

Monosodium glutamate (MSG) was procured from HiMedia, Mumbai and *Celastrus paniculatus* Oil (CPO) was obtained from Shree Narayan Ayurvedic Pharmacy, Ahmedabad, India.

2.1 Phytochemical Analysis of CPO

The absence or presence of various phytocomponents like saponins, alkaloids, tannins, phenolic compound, flavonoids, Glycosides, fats, steroids and triterpenoids in the CPO was determined by the method as described by Harborne [13].

2.2 Free Radical Scavenging Capacity of CPO

Free radical scavenging activity of CPO was evaluated by DPPH assay [14] and NO inhibition assay at different concentrations ranging from 0.1 to 10 mg/ml [15].

2.3 IMR-32 Cell Culture and Treatment

IMR-32 cells were obtained from National Centre for Cell Sciences, Pune, India. Cell line was maintained in MEM medium supplemented with 2 mM L-glutamine, 0.1 mM nonessential amino acids and 10% fetal bovine serum (HiMedia, Mumbai, India) in CO_2 incubator at 37°C with 5% CO_2. After reaching 80% confluency in culture medium, IMR-32 cells were trypsinized and seeded in 96 as well as 12 well plates according to the requirement of the experimental protocol.

2.4 Dose Selection of CPO and MSG by MTT Assay

The CPO was solubilized in mixture of 1% Dimethyl sulfoxide (DMSO) and 0.4% Tween 20 to obtain sufficient solubility for experimental procedure. Various concentrations of MSG (1 – 10 mM) and CPO (0.25 – 2 µg/ml) were studied separately for its cytotoxicity analysis for 24 hrs exposure using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay [16]. IMR-32 cells were harvested and seeded in 96 well plate with 200 µl MEM media for the MTT assay. This method is based on the reduction of the tetrazolium salt MTT into formazan. The amount of formazan produced was calculated and it represented the number of viable cells. After 24 hrs of treatment, the culture medium was removed and 20 µl MTT (5 mg/ml) was added in each well with serum free media. After 4 hrs of incubation media was removed and the resulting formazan was solubilized in 200 µl of DMSO and the optical density was obtained at 595 nm using microplate reader (Epoch, BioTek, USA). Based on this study, the IC_{50} value of MSG was determined to be 7 mM/10^5 cells. The ameliorative effect of CPO on MSG (7mM) induced cytotoxicity were analyzed on IMR-32 cells using various doses (0.25 – 2 µg/ml) of CPO. However, based on the results obtained from this study total three concentrations of CPO (0.25, 0.5 and 1 µg/ml) were selected for the free radical toxicity study against 7 mM MSG.

2.5 Experimental Groups

To check protective effect of CPO against MSG induced free radical toxicity, the study was divided into seven groups as listed in Table 1.

Table 1. Experimental groups

NO	Groups
I	Control
II	Vehicle Control (0.4% Tween 20 + 1% DMSO)
III	*Celastrus paniculatus* seed oil (CPO) (1µg/ml)
IV	Monosodium glutamate (MSG) (7mM)
V	MSG (7mM) + CPO (0.25µg/ml)
VI	MSG (7mM) + CPO (0.5µg/ml)
VII	MSG (7mM) + CPO (1µg/ml)

2.6 Cell Lysate Preparation

After 24 hrs of treatment, cells were used to prepare cell lysate according to the experimental groups listed in Table 1. After trypsinization, cells were treated using 1 ml of lysis buffer containing 1% Triton X-100, 130 mM NaCl, 10 mM Tris-HCl and 10mM NaH_2PO_4 to prepare cell lysate [17] The pH was maintained at 7.5 and the mixture was incubated for 30 min at 4°C. The supernatant was used for biochemical assays that were obtained from centrifugation at 2000 rpm for 2 min.

2.7 Free Radical Toxicity Study

To evaluate the free radical toxicity of MSG and its amelioration by CPO, the supernatant obtained after centrifugation of cell lysate was used to perform various assays like Total protein [18], Lipid peroxidation [19], Protein carbonyl [20], Catalase [21], Superoxide dismutase [22], Total glutathione [23], Glutathione peroxidase [24], Glutathione S-transferase [25] and Glutathione reductase [26].

2.8 Statistical Analysis

The results were expressed as Mean±S.E. The statistical significance was evaluated by Analysis of Variance (ANOVA) and Graph Pad Prism 5. The individual comparison was obtained by

Tukey's multiple comparison test and by student's t-test. Value of $p<0.05$ was considered to indicate significance difference.

3. RESULTS

3.1 Phytochemical Analysis and Free Radical Scavenging Capacity of CPO

The study of phytochemical analysis showed the presence of various phytochemical components except steroid in the CPO as mentioned in Table 2. The reactive oxygen species scavenging assays or the DPPH assay was based on the fact that when the antioxidant molecules present in the test samples would react with DPPH radical, it converts deep purple colour of DPPH in to yellow colour solution according to the capacity of extract to react with DPPH radical and decrease in absorbance resulting in scavenging potential. The 100% inhibition of DPPH radical was obtained by the CPO at 0.9mg/ml concentration (Fig. 1). Results of NO scavenging assay, the nitric oxide ions were produced by using sodium nitroprusside, which interacts with oxygen to generate nitrite, which can be estimated by the use of Griess illosvoy reagent. In the present experiment, NO inhibition was increasing with the increase in CPO concentration. At 10 mg/ml concentration of CPO the NO inhibition was found to be 95.12 ± 0.92% (Fig. 2).

Table 2. Phytochemical analysis of CPO

Constituents	CPO
Saponin	+
Alkaloids	+
Tannin and phenolic compounds	+
Flavonoid	+
Glycosides	+
Fats	+
Steroid	-
Triterpanoids	+

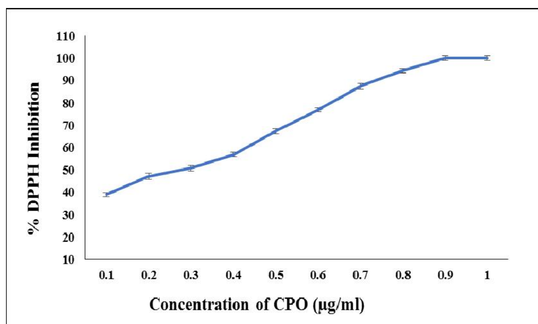

Fig. 1. Percent DPPH inhibition by various concentrations of CPO

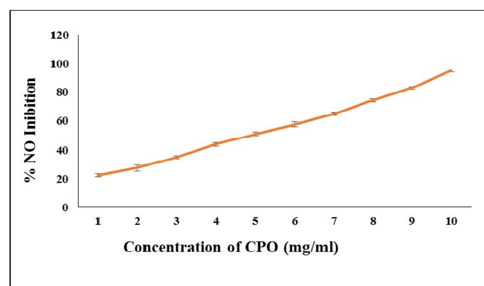

Fig. 2. Percent Nitric oxide ions inhibition by various concentrations of CPO

3.2 CPO Mitigated MSG Induced Cytotoxicity

Cultures were treated with various doses of MSG (1 – 10 mM) and CPO (0.25 – 2 µg/ml) separately. Cultures were treated for 24 hrs to determine the cytotoxicity of MSG and CPO if any on IMR-32 cells. MTT assay on CPO treated cells showed no adverse effect on viability (Fig. 3) whereas MSG treated cells revealed dose dependent substantial increase in cytotoxicity (Fig. 4). The 7mM MSG exposure induced 50% IMR-32 neuronal cell death. Treatment of 1 µg/ml dose of CPO for 24 hrs with 7 mM MSG inhibited this effect and protect IMR-32 cells (Fig. 5). Based upon MTT assay three doses of CPO (0.25, 0.5 and 1 µg/ml) against 7 mM MSG were selected for determinative free radical toxicity study.

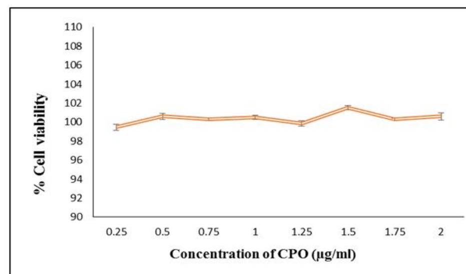

Fig. 3. Percent cell viability by MTT assay against various doses of CPO on IMR-32 cells

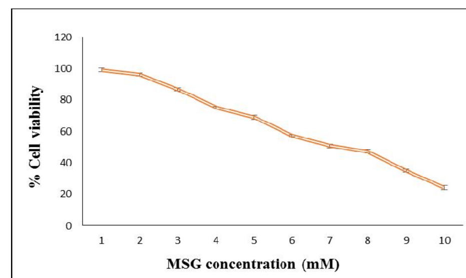

Fig. 4. Percent cell viability by MTT assay against various dose of MSG on IMR-32 cells

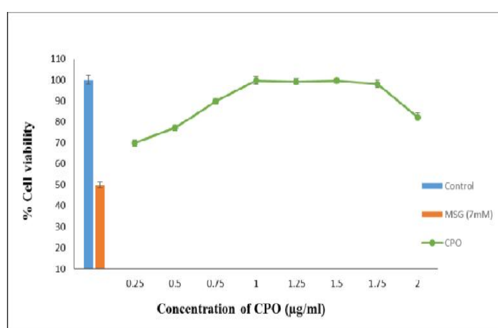

Fig. 5. Effect of different concentrations of CPO on 7mM MSG induced toxicity in IMR-32 cells

3.3 Protein Carbonyl and Lipid Peroxidation

The treatment of 7 mM MSG showed significant increase (P<0.001) in protein carbonyl as compared to control and significantly ameliorated (p<0.001) at only 1µg CPO treatment (Table 3). Malondiadehyde (MDA) level was measured to record the lipid peroxidation (LPO) after MSG and CPO treatment. MSG treatment showed highly significant increase (p<0.001) in LPO, which may be responsible for neurodegeneration. While, CPO was able to reduce the LPO level significantly (p<0.001) at doses of 0.5 and 1 µg/ml. The amelioration was more pronounced at 1 µg/ml dose of CPO for both protein carbonyl and lipid peroxidation (Table 3).

3.4 Catalase and Superoxide Dismutase

The treatment of 7 mM MSG on IMR-32 cells decreased catalase and SOD activity significantly (p<0.001) as compared to control. There was no significant recovery found in catalase and SOD activity at low concentration (0.25 µg/ml) of CPO but recovery was significant (p<0.001) at 1 µg/ml dose of CPO, whereas, 0.5 µg/ml of CPO dose showed less significant recovery (p<0.05 for catalase and p<0.01 for SOD) as compared to alone MSG treated group (Table 3).

3.5 Glutathione (GSH) Level and Activity of Glutathione Peroxidase (GPx), Glutathione S-transferase (GST) and Glutathione Reductase (GR)

There was no adverse effect found on activity of GPx, GST, GR and level of GSH by treatment of 1 µg/ml CPO alone but significant decline (p<0.001) was observed by 7 mM MSG treatment as compare to control. The recovery against 7 mM MSG treatment was nonsignificant at 0.25 µg/ml of CPO (Group V) for level of GSH and activity of GPx as well as GR. The GST activity showed significant recovery (p<0.05) at 0.25 µg/ml of CPO dose. Significant level of amelioration at 0.5 µg/ml CPO (Group VI) was more discrete among various parameter (p<0.05 for GSH level; p<0.01 for GPx and GR; p<0.001 for GST). However highly significant amelioration (p<0.001) was observed at 1 µg/ml CPO dose (Group VII) as compared to 7 mM MSG treatment (Group IV) in all studied parameters (Table 3).

4. DISCUSSION

Present study deals with investigation of protective effects of CPO against MSG induced alterations in IMR-32 human neuronal cells. In this study, the presence of phytocomponents in CPO were analyzed. Results showed presence of different phytochemicals like alkaloids, flavonoids and phenolic compounds, which are good source of antioxidants. Arora and Pandey-Rai [27] also reported similar results in the study carried out on phytocomponents of CPO. Natural antioxidants have demonstrated beneficial effects in maintenance of health, management of age related diseases and ameliorating the harmful effects of toxic agents [28].

The result of this study showed that CPO significantly stabilized reactive molecules of DPPH by bleaching it at a concentration of 0.9 mg/ml. The effect of ROS scavenging capacity of CPO, based on converting the reactive molecule of DPPH into a stable DPPH was also reported by other researchers [8,11]. Like DPPH, the nitric oxide or reactive nitrogen species are also formed during their metabolic reaction with oxygen or with superoxide ions. This may be reduced due to the antioxidant properties of the CPO, which compete with oxygen to react with nitric oxide and thereby inhibiting the generation of nitrite. Nitric oxide (NO) ions mediator are generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes [29]. On the other hand, excess concentration of NO is associated with several diseases [30]. The NO scavenging assay performed using Griess illosvoy reagent, showed high percentage of NO inhibition at 10 mg/ml concentration of CPO and hence, it's potential NO scavenging property can be noted.

Table 3. Effect of MSG and CPO individually and in combination, on the IMR-32 cells

No.	Groups	Protein carbonyl (nM pro carbonyl /mg protein)	LPO (Nm MDA Formed /60min /mg protein)	Catalase (nM H_2O_2 consumed /min/mg protein)	SOD (U SOD/mg protein)	GSH (mM GSH/mg protein)	GPx (mM GSH consumed/ min/mg protein)	GST(µmols of CDNB-GSH conjugate formed/min /mg protein)	GR (nM of NADPH oxidized/min/mg protein)
I	Control	4.2 ±0.1	1.3 ±0.02	150.4 ±0.9	32.2 ±0.6	1.3 ±0.03	2.1±0.07	3.5 ±0.03	18.7 ±0.2
II	VC	4.2 ±0.1ns	1.3 ±0.1ns	146.6 ±2.8ns	33.0 ±0.6ns	1.3 ±0.03ns	2.1 ±0.08	3.5 ±0.05ns	19.56 ±0.4ns
III	CPO 1 µg/ml	4.2 ±0.1ns	1.2 ±0.01ns	148.2 ±2.6ns	33.7 ±0.3ns	1.3 ±0.07ns	2.1 ±0.03	3.3 ±0.1ns	20.16 ±0.8ns
IV	7 mM MSG	9.1 ±0.1***	4.2 ±0.005***	117.9 ±0.3***	25.0 ±0.5***	1.1±0.02***	1.6 ±0.03***	1.5 ±0.1***	8.66 ±0.2***
V	7 mM MSG +0.25 µg/ml CPO	9.0 ±0.004NS	3.9 ±0.02#	119.9 ±0.2NS	26.0 ±0.5NS	1.0 0.02NS	1.6 ±0.03NS	1.9 ±0.02#	9.27 ±0.8NS
VI	7 mM MSG +0.5 µg/ml CPO	8.9 ±0.02NS	3.6±0.01###	127.8 ±0.2#	29.3 ±0.1##	1.0 ±0.04#	1.8 ±0.05##	2.7 ±0.1###	14.21 ±0.7##
VII	7 mM MSG +1 µg/ml CPO	7.9 ±0.01###	2.6±0.01###	138.9 ±0.2###	33.1±0.5###	1.2±0.03###	1.9 ±0.02###	3.3 ±0.05###	19.18 ±1.2###

** = p <0.05; ** = p <0.01; *** = p<0.001 and ns = non-significant when Group II, III and VI compared with Group I. # = p< 0.05; ## = p< 0.01; ### = p< 0.001 and NS = non-significant when Group V, VI and VII compared with Group IV. MSG= Monosodium glutamate; CPO = Celastrus paniculatus oil*

Since, free radicals are main culprit for several neurological disorders, this finding of extraordinary free radical scavenging capacity of the CPO can be used as alternative medicine for neurological disorder. Moreover, the wider use of CPO against versatile disorders since ancient time without any noticeable adverse effects proposes that it as a major source for discovery of alternative medicine. The data of MTT assay on IMR-32 cell with CPO also showed no adverse effect or cytotoxicity and results were comparable to control. In support to our findings [12] Godkar and coworkers (2006) also showed antioxidant effect of CPO on rat embryonic forebrain neuronal cells but there is paucity of data available regarding its antioxidant property in human. Henceforth in the present study, we have tried to show neuroprotective effect of CPO on human cell lines against widely used food additive monosodium glutamate induced oxidative damage.

Neurodegenerative disorders have been shown to have protein accumulation as amyloid body in neuronal cells. These amyloid bodies are resulted from and furnished with protein oxidations and related products [31]. The product of oxidation of protein side chain especially at amino acid such as Proline, Arginine, Lysine, and Threonine results in to aldehyde or ketone containing species commonly called as protein carbonyls. They are the most commonly quantified markers for protein oxidation estimation [20]. In the present study we observed that the level of total protein deceased and protein carbonyls were increased after MSG treatment. Level of total protein and protein carbonyls showed significant amelioration in dose dependent manner after CPO co-treatment with MSG, as compared to alone MSG treatment. This suggests that CPO can be effective in regressing the complications of neurological disorder by avoiding the accumulation of amyloidal body in the neurons.

Lipid peroxidation is an important parameter presenting toxicity inside the cells and represents for peroxidation of many important molecules such as lipid layer present in cell membranes. The end product of lipid peroxidation i.e. 4-hydroxynonenal may act as secondary messenger for apoptosis and cell death [32]. For this herbal remedies have been consumed from past many centuries as a source of antioxidants which reduces the lipid peroxidation. Co-treatment of CPO along with MSG also decreased the level of LPO which was increased by alone MSG treatment in present study. Similar result was also observed by [12] Godkar and coworkers (2006) in rat embryonic cells. This observation suggests that CPO can be helpful in neuroprotection by reducing the organic peroxide induced toxicity such as membrane damage in neurons and effective in preventing neuronal death due to membrane lipid peroxidation.

Free radical scavenger enzymes such as superoxide dismutase and catalase are important especially for superoxide ions produced from metal reductions as well as under normal/abnormal metabolic conditions and have been implicated in several complications related to ROS generation [33]. They are the primary enzymes of cells provide protection against H_2O_2 and O_2^- toxicity. Present study showed alteration in level of these free radical scavenging enzymes that are involved in protection against elevated ROS level after MSG treatment. Co-treatment of CPO along with MSG showed dose dependent protective effect of CPO against adverse effects of MSG. This indicates that CPO may scavenge O_2^-/H_20_2 and help in maintaining the activity of SOD as well as catalase and defend the neuronal cells against free radical toxicity.

Along with impairment of enzymatic activity which work against free radical toxicity the most robust and significant alteration in the antioxidant defense is a decrease in GSH concentration. The depletion in glutathione and glutathione disulfide is indicator of total oxidative stress in the cellular system and has been implicated in cell survival, immunity, signal transduction and detoxification [34]. This study showed decreased in the level of glutathione and activity of glutathione related enzymes (GPx, GST and GR) after MSG treatment, which was ameliorated in dose dependent manner after co-treatment of CPO. Increase in GR level suggests that there may be induction of regeneration of glutathione triggered by GR from its oxidized form glutathione disulfide when CPO is used along with MSG. Amelioration of glutathione related antioxidant activity suggests that CPO may prevent neuronal cell damage by inhibiting xenobiotics that are common risk factors for neurodegeneration. [12] Godkar and coworker (2006) also showed that CPO substantially provided protection against H_2O_2 and glutamate induced toxicity.

5. CONCLUSION

In conclusion, this study showed the protective effects due to antioxidant properties of CPO on

human neuronal cell line IMR-32 and its efficacy in preventing toxicity of MSG. It is also concluded that CPO could be used as alternative medicine against free radical toxicity related neuronal disease and useful to decrease symptoms and complications in neurodegenerative disorders.

ACKNOWLEDGEMENT

This work is supported by the DST in the form of INSPIRE Fellowship programme (IF130664), New Delhi, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dugger BN, Dickson DW. Pathology of neurodegenerative diseases. Cold Spring Harbor Laboratory Press. Cold Spring Harbor Persp Biol. 2017;1-22.
2. Al-Chalabi A, Hardiman O. The epidemiology of ALS: A conspiracy of genes, environment and time. Nat Rev Neurol. 2013;9:617-628.
3. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neuro-degenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Cur Neuropharmacol. 2009;7:65-74.
4. Shabbir B, Fatima Z, Ganesh S. Is mono sodium glutamate salt (MSG) harmful to living systems? International Journal of Bioassays. 2014;3:3303-3306.
5. Hong L, Dixit Sanjaya M. Advances in Oligoprotection. Neurosci & Med. 2011;2: 93-103.
6. Ernst A, Stolzing A, Sandig G, Grune T. Antioxidants effectively prevent oxidation-induced protein damage in OLN 93 cells. Arch of Biochem and Biophys. 2004; 421:54-60.
7. Mann H, McCoy MT, Subramaniam J, Van Remmen H, Cadet JL. Overexpression of superoxide dismutase and catalase in immortalized neural cells: Toxic effects of hydrogen peroxide. Brain Res. 1997;770: 163-168.
8. Bhanumathy M, Harish MS, Shivaprasad HN, Sushma G. Nootropic activity of *Celastrus paniculatus* seed. Pharmaceutical Bio. 2010;48:324-327.
9. Kulkarni YA, Agarwal S, Garud MS. Effect of Jyotishmati (*Celastrus paniculatus*) seeds in animal models of pain and inflammation. J of Ayurveda and Integrative Med. 2015;6:82-88.
10. Valecha R, Dhingra D. Behavioral and biochemical evidences for antidepressant-like activity of *Celastrus paniculatus* seed oil in mice. Basic and Clin Neurosci. 2016;7:49-56.
11. Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomed. 2001;8:125-132.
12. Godkar PB, Gordon RK, Ravindran A, Doctor BP. *Celastrus paniculatus* seed oil and organic extracts attenuate hydrogen peroxide-and glutamate-induced injury in embryonic rat forebrain neuronal cells. Phytomed. 2006;3:29-36.
13. Harborne JB. Methods of plant analysis. In Phytochemical Methods. Springer Netherlands.1984;1-36.
14. MacDonald-Wicks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant capacity *in vitro*: A review. J of the Sci of Food and Agri. 2006;86:2046-2056.
15. Parul R, Saha P, Kundu SK. *In vitro* nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants. The Pharma Int. 2013;1:83-88.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J of Immuno Meth. 1983;65:55-63.
17. Shen HM, Yang CF, Ding WX, Liu J, Ong CN. Superoxide radical–initiated apoptotic signalling pathway in selenite-treated HepG 2 cells: Mitochondria serve as the main target. Free Radical Bio and Med. 2001;30:9-21.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem. 1979;95:351-358.
20. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymol. 1990;186:464-478.
21. Sinha AK. Colorimetric assay of catalase. Analytical Biochem. 1972;7:389-394.

22. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys. 1984;21:130-132.
23. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70-77.
24. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179:588-590.
25. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J of Biol Chem. 1974;249:7130-7139.
26. Carlberg I, Mannervik B. Glutathione reductase. Meth Enzymol. 1985;113:485-490.
27. Arora N, Pandey-Rai S. GC–MS analysis of the essential oil of *Celastrus paniculatus* willd seeds and antioxidant, anti-inflammatory study of its various solvent extracts. Indust Crops and Prod. 2014;61:345-351.
28. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicology. 2003;189:1-20.
29. Lata H, Ahuja GK. Role of free radicals in health and disease. Ind J of Physiol and Allied Sci. 2003;57:124-132.
30. Ialenti A, Moncada S, Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. British J of Pharmacol. 1993;110: 701-706.
31. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 2003;29:23-38.
32. Houglum K, Filip M, Witztum JL, Chojkier M. Malondialdehyde and 4-hydroxynonenal protein adducts in plasma and liver of rats with iron overload. J of Clin Invest. 1990;86:1991-1998.
33. Rahman T, Hosen I, Islam MT, Shekhar HU. Oxidative stress and human health. Adv in Biosci and Biotech. 2012;3:997-1019.
34. Schulz JB, Lindenau J, Seyfried J, Dichgans J. Glutathione, oxidative stress and neurodegeneration. Euro J of Biochem. 2000;267:4904-4911.

© 2018 Shah et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23225