World Journal of Agricultural Research, 2018, Vol. 6, No. 3, 87-93
Available online at http://pubs.sciepub.com/wjar/6/3/3

DOI:10.12691/wjar-6-3-3

Mitigation by Aqueous Extract of *Celastrus Paniculatus* Seeds against Monosodium Glutamate Induced Impairments in Human Neuronal cells IMR-32

Naumita Shah[1], Ankit Nariya[1], Ambar Pathan[1], Alpesh Patel[2], Shiva Shankaran Chettiar[2], 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., Ellis Bridge, Ahmedabad-380006, Gujarat, India
*Corresponding author: ddjhala@gmail.com

Received August 23, 2018; Revised September 29, 2018; Accepted October 16, 2018

Abstract The free radicals are considered as primary culprit for many multifactorial diseases. These free radicals scavenging remains a foremost challenge in most neurological disorders, which can be subjected with least collateral damage by herbal extracts. In this study, *Celastrus paniculatus* (CP) seeds aqueous extract (AE) (0.25, 0.5 and 1.0 µg/ml) was used to evaluate the neuroprotective efficacy against adverse effects of monosodium glutamate (MSG) (7 mM) in neuroblastoma cell line IMR-32. Preliminary pharmacological investigations and free radical scavenging capacity were evaluated for AE. Cytotoxicity and oxidative stress were studied using MTT assay and some biochemical parameters (total protein and glutathione level as well as activity of superoxide dismutase and catalase). Moreover, genotoxicity due to free radicals was also assessed using comet assay in IMR-32 cells. Results showed presence of various phytochemicals in AE and its significant inhibition of DPPH and NO radicals. AE was not only enhancing the activity of antioxidant enzymes but also reduced the free radical mediated cytotoxicity of MSG in IMR-32 cells. The DNA damage found in neuronal cells due to free radical toxicity of MSG was reduced in presence of free radical inhibitory phytochemical present in AE. From these results it can be concluded that AE of CP seeds is an effective antioxidant agent and potent neuroprotective herb to mitigate MSG induced neuronal impairments in IMR-32 cells.

Keywords: *celastrus paniculatus, genotoxicity, monosodium glutamate, neurodegeneration, oxidative stress*

Cite This Article: Naumita Shah, Ankit Nariya, Ambar Pathan, Alpesh Patel, Shiva Shankaran Chettiar, and Devendrasinh Jhala, "Mitigation by Aqueous Extract of *Celastrus Paniculatus* Seeds against Monosodium Glutamate Induced Impairments in Human Neuronal cells IMR-32." *World Journal of Agricultural Research*, vol. 6, no. 3 (2018): 87-93. doi: 10.12691/wjar-6-3-3.

1. Introduction

Neurodegenerative diseases affect the central nervous system causing progressive loss of neuron function. These debilitating and incurable conditions are characterized by loss of neuronal cell function and are often associated with atrophy of the affected parts of nervous system [1]. Neurons normally don't regrow or replace themselves, so when they become damaged or die they cannot be replaced by the body [2]. Neurodegenerative diseases associated with glutamatergic dysfunction, share a common pathogenesis mechanism involving, impairment of cellular calcium homeostasis, activation of nitric oxide synthesis, generation of free radicals and programmed cell death which leads to progressive neurodegeneration [3]. It also documented that, generation of high levels of reactive oxygen species (ROS) and down regulation of antioxidant mechanisms result in neuronal cell death of neurodegenerative diseases [4]. There is compelling evidence from a multitude of studies indicating that food borne excitotoxin additives can cause adverse health effects like neurodegeneration [5]. It is well established that an excitotoxic mechanism plays a role in many neurologic disorders [6]. Excitotoxins destroy neurons by excessive stimulation of postsynaptic excitatory membrane receptors [7], whereas the under stimulation of such receptors during the developmental period triggers apoptosis [8]. The well-known flavor enhancers like L-glutamic acid (monosodium glutamate) and aspartame (1-methyl N-L-alpha aspartyl-phenylalanine) etc., has been examined extensively and concerns have been expressed over their excitotoxic effects was that, they are potential for destroying neuronal cells [7]. Monosodium glutamate (food additive number E621) is one of the world's most used flavor enhancer [9]. It increases the sapidity of food. It elicits a taste described in Japanese as umami, which is translated to ''savory'' [10]. MSG was demonstrated for Chinese restaurant syndrome that causes symptoms such as numbness, weakness, flushing, sweating, dizziness and headache [9]. It is evident that glutamate is related to the generation of free radicals produced as a consequence of activation of enzymes such as nitric oxide synthase, Xanthine oxidase

and by oxidative dysfunction in mitochondria [9,11]. Reduction in brain antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase contribute to the development of a wide range of neurodegenerative diseases through the generation of free radical species under pathological conditions [12]. To mitigate this effect, *Celastrus paniculatus* (CP) seeds aqueous extract (AE) was selected for this study. CP is an unarmed woody climbing shrub and decoction of its seeds prescribed as brain tonic, used in headache, depression, swooning and as a laxative [13]. Seeds powder of CP mixed with water is taken orally to treat nervous disorders [14]. It stimulates intellect and sharpens the memory. Godkar and his coworkers 15] evaluated studies of various extracts of CP seeds in which the AE of CP seeds was found to be neuroprotective against hydrogen peroxide induces toxicity in rat forebrain neuronal cells. There is a paucity of scientific data regarding the effect of aqueous extract of CP seeds on protection against cytotoxicity and genotoxicity due to ROS generated by food additives like MSG on human neuronal cells. The study was therefore carried out to explore mitigative efficacy of aqueous extract of CP seeds against MSG induced toxicity.

2. Material and Method

All chemicals used in this study were procured from Merck, USA; Sigma Aldrich, Mumbai, India (AR Grade) and HiMedia, Mumbai (Culture Grade). The *Celastrus paniculatus* (CP) seeds were purchased from Ahmedabad and authenticated by Department of Botany, Gujarat University, Ahmedabad, Gujarat, India.

2.1. Extract Preparation

The CP seeds were washed with distilled water and dried under shade to prepare aqueous extract (AE). Seeds were powdered using mechanical grinder after drying. Soxhlet apparatus was used to prepare AE of CP seeds (400 ml of distilled water and 50 g of CP seeds powder). After 18 hrs the solution obtained was filtered through Whatman filter paper No. 1 and kept for drying in an incubator at 37℃. The percentage yield of AE was measured using given formula.

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of product after evaporation}}{\text{Weight of powder used}} \times 100.$$

2.2. Phytochemical Analysis of Extracts

Phytocomponents present in aqueous extract of CP seeds were analyzed by standard method of Harborne [16].

2.3. FTIR Analysis

To identify the presence of functional groups, AE of CP seeds was applied to alpha fourier transform infrared attenuated total reflection (FTIR-ATR) (Bruker, Germany) spectroscopy and the result was analyzed using OPUS spectroscopy software.

2.4. Antioxidant and Free Radical Scavenging Capacity

Free radical scavenging capacity of AE was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging assay [17] and nitric oxide (NO) scavenging assay [18].

2.5. Neuronal Cell Culture

IMR-32 cell line was obtained from National center for cell science (NCCS), Pune, India. The cells were maintained at 37℃ with 5% CO_2 in minimum essential medium (MEM) supplemented with 10% FBS; 100 mg/ml streptomycin and 100 U/ml penicillin. Cells were sub-cultured or passaged on attaining 80% confluency in 96 well/ 12 well / T flask and used for experimental studies.

2.6. Cytotoxicity Analysis

Cell viability were analyzed by MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay [19]. IMR-32 cells (10^5 cells/well) were seeded in 96 well plate to analyzed mitigative effects of AE against MSG in this assay. Based on our previous study [20], various concentration of AE was tested to check cytoprotective effects against LC50 of MSG (7 mM) on IMR-32 cells.

2.7. Experimental Groups

To evaluate the effect of various treatment on oxidative stress and genotoxicity, this study was divided in seven different groups (Table 1).

Table 1. Experimental groups and doses.

GROUPS	TREATMENTS
I	Control
II	Vehicle Control (1% DMSO + 0.4% Tween 20)
III	1 µg Aqueous Extract (AE)
IV	7 mM MSG
V	7 mM MSG + 0.25 µg AE
VI	7 mM MSG + 0.5 µg AE
VII	7 mM MSG + 1 µg AE

Group I was untreated control. Group II was vehicle control. Group III was alone 1 µg/ml AE treatment. Group IV was alone 7 mM MSG treatment. Whereas, Groups V, VI and VII were treated with three different doses (0.25, 0.5 and 1 µg/ml respectively) of AE along with 7 mM of MSG. Cells were cultured in 12 well culture plate for exposing different compounds as per experimental groups.

2.8. Cell Lysate Preparation

After 24 hrs of treatment cells were used to make cell lysate. After trypsinization, cells were treated with 1 ml of lysis buffer [21] containing 1% Triton X-100, 130 mM NaCl, 10 mM Tris-HCl and 10 mM NaH_2PO_4 to prepare cell lysate. The pH was maintained at 7.5 and mixture was incubated for 30 min at 4℃. The supernatant was used for biochemical assays after centrifugation at 4℃, 2000 rpm for 2 min.

2.9. Oxidative Stress Related Parameter

The supernatant obtained after centrifugation of cell lysate was used to perform assays like total protein [22] and glutathione [23] level as well as superoxide dismutase [24] and catalase [25] activity to evaluate the mitigative effect of AE against oxidative stress generated by MSG.

2.10. Comet Assay

Slides prepared for comet assay [26] were observed under fluorescent microscope (BX 53F, Olympus) and total 100 comets were scored from each group by comet score software (comet Assay IV^{TM}) to obtained percentage DNA in tail as well as percentage DNA in head.

2.11. Statistical Analysis

Various parameter was performed in triplicate and results were expressed as Mean±S.E. The statistical significance was evaluated by Analysis of Variance (ANOVA) and GraphPad Prism 6. 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.

3. Results and Discussion

3.1. Pharmacological Investigation

Antioxidant properties from vegetables, fruits and medicinal herbs are candidates, which can be used for the prevention of oxidative damage caused by free radicals [27,28]. Our attention has been focused on *Celastrus paniculatus* (CP) seeds, which have been found to improve memory and cognition with excellent antioxidant as reported in literature [29]. The aqueous extract (AE) of CP seeds was prepared using Soxhlet apparatus. The percentage yield of this extract was 54%. The phytochemical analysis revealed presence of phytocomponents like flavonoids, alkaloids, saponin, tannins, phenols, fats and oil whereas carbohydrates, steroids, triterpenoids and glycosides were absent in the AE of CP seeds.

3.2. FTIR Analysis

Based on results of the FTIR spectrum, it was obvious that nitro compounds, amides, alcohols, carboxylic acids, alkenes, aliphatic, carbonyl, ether and phenyls carbonyl compounds as well as alkyl halides functional groups present in the aqueous extract of CP seeds (Figure 1).

In support to our findings the report of Kulkarni and co-workers [30] showed presence of sesquiterpenes, alkaloids, polyalcohol, triterpenoid, sterols as well as polyol esters present in CP seeds. Other scientists [31,32] also described that AE of CP seeds were found to have various bioactive components, which have ability to scavenge free radicals. Thus, based on phytochemical and FTIR analysis of present study and literature review on AE of CP seeds, it can be used as a good antioxidative agent.

3.3. Free Radical Scavenging Activity

The aqueous extract of CP seeds was able to bleach the 50% of DPPH radicals at 0.2 mg/ml and the 100% inhibition of DPPH was observed at a dose of 0.6 mg/ml (Figure 2). Nitric Oxide (NO) is also classified as a free radical that plays an important role as an effector molecule in diverse biological systems. Persistent exposure to nitric oxide radical is associated with neurodegeneration [33].

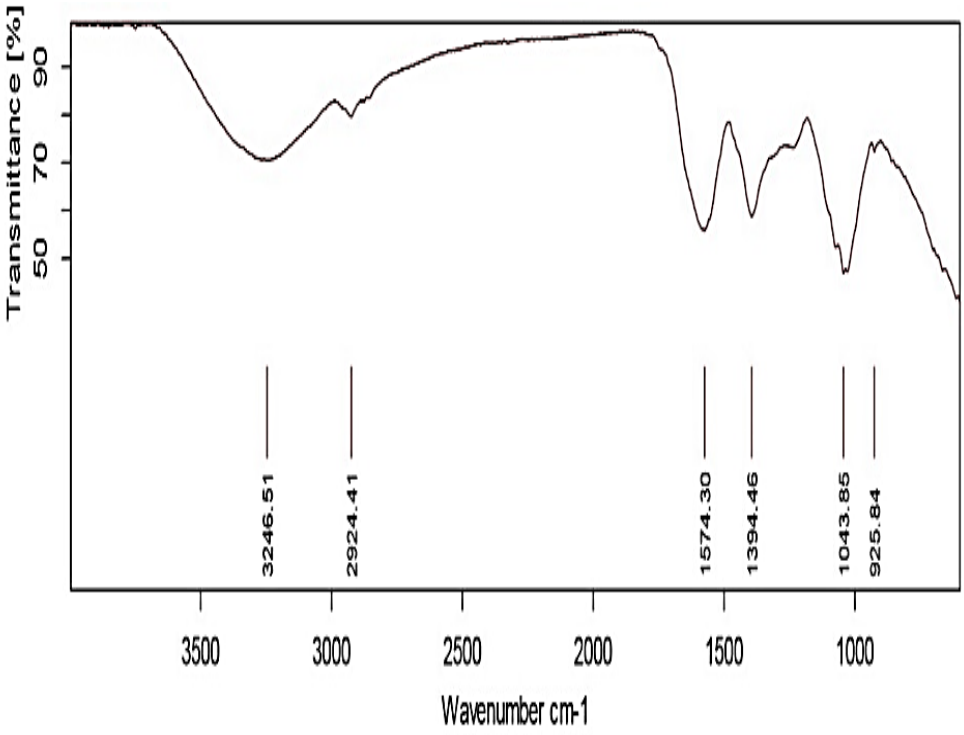

Figure 1. FTIR spectroscopic analysis of AE of CP seeds

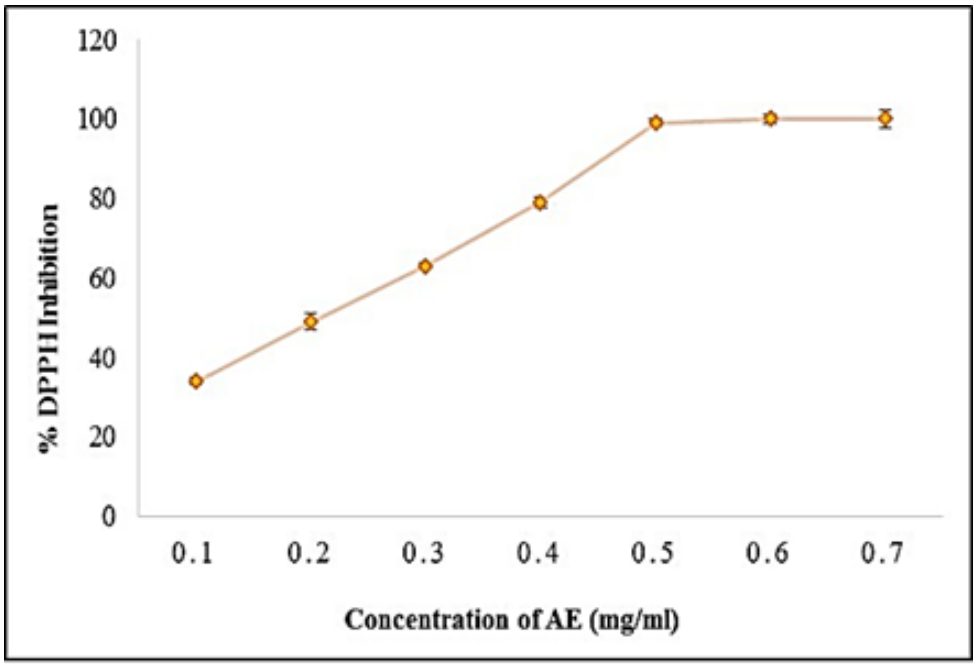

Figure 2. Free radical scavenging activity in the form of percentage DPPH inhibitions by various concentrations of CP seeds aqueous extract

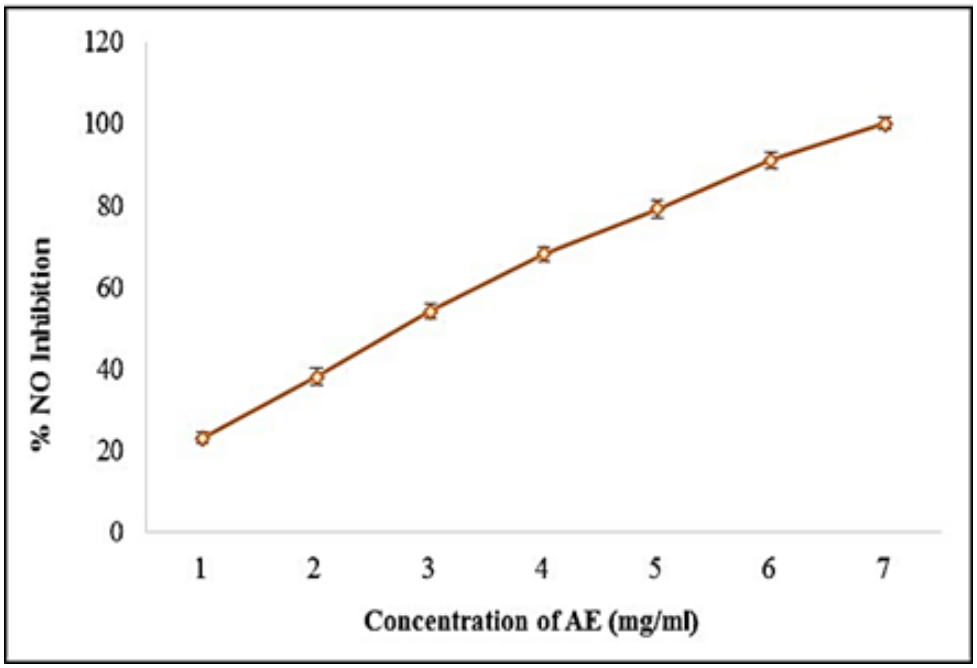

Figure 3. Free radical scavenging activity in the form of percentage NO inhibitions by various concentrations of CP seeds aqueous extract

The toxicity of NO increases greatly when it reacts with the superoxide radical present in excitotoxins, forming the highly reactive peroxynitrite anion (ONOO−) [34]. The NO scavenging activity of AE was 50% at 2.91 mg/ml and 100% at 7 mg/ml in this study (Figure 3). The DPPH and NO scavenging activity of CP was also demonstrated by other researchers [35,36]. Based on phytochemical analysis as well as DPPH and NO scavenging activity study it can be concluded that AE of CP seeds can be used as protective agent against MSG induced free radical toxicity.

3.4. Cytotoxicity Study on IMR-32 Cells

In the MTT assay, various concentrations (0.25 to 2 µg/ml) of aqueous extract of CP seeds alone and in combination with 7 mM MSG were added in cultured IMR-32 cells for 24 hrs to evaluate mitigative effects of AE against cytotoxicity of MSG. Alone AE did not show decreased in the percentage cell viability at any concentration in MTT assay (Figure 4) indicating no cell death at even highest dose. On the other hand, when AE was used to check the protective effect against MSG, it showed dose dependent protection.

At low concentration of AE (0.25 µg/ml) along with MSG the IMR-32 cell viability was 73% whereas 100% viability was observed at 1 µg/ml concentration (Figure 5). Similar result was also found on rat forebrain neuronal cells by other researchers [37] which depict the protective effect of AE of CP seeds against cytotoxicity of MSG in neuronal cells.

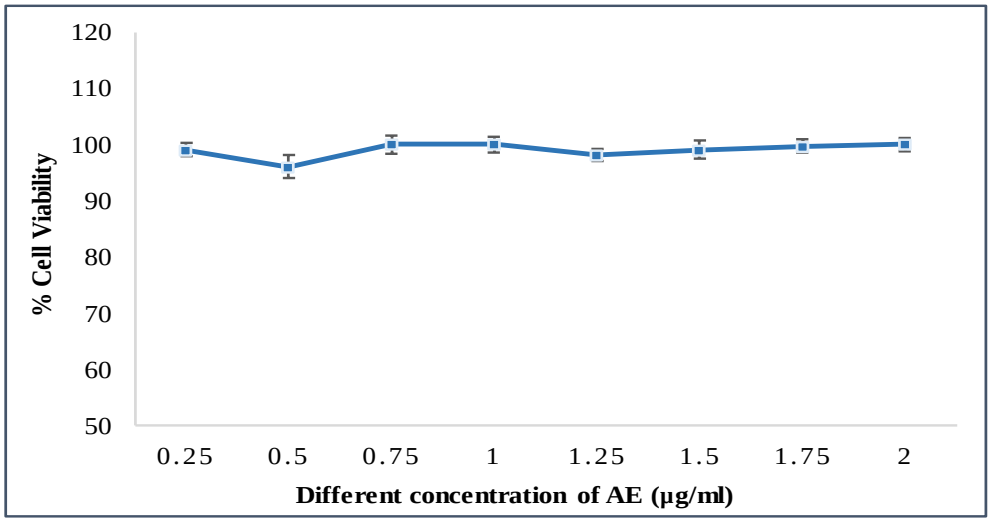

Figure 4. MTT assay showed percent cell viability after treatment of various concentrations of CP seeds aqueous extract

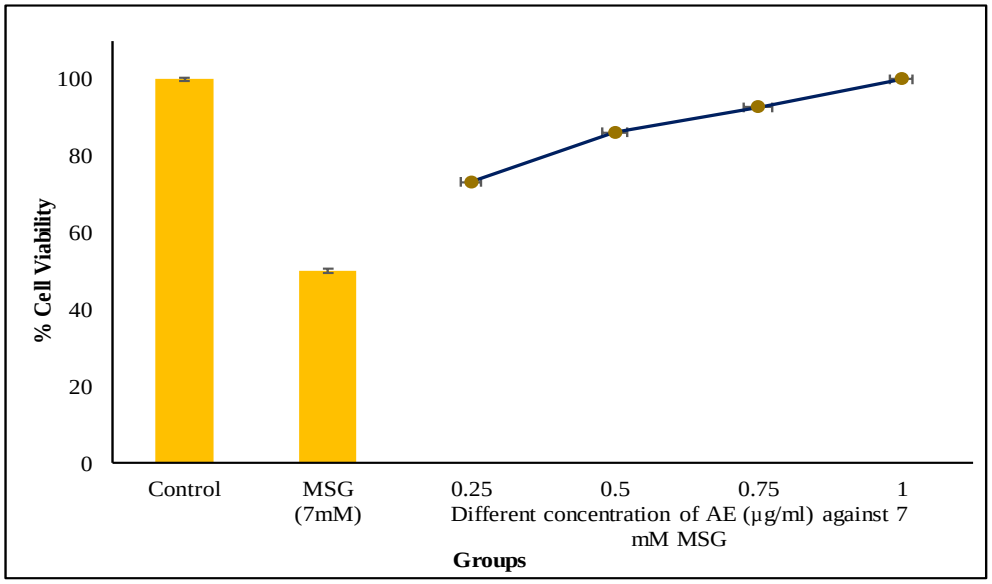

Figure 5. Percent cell viability after treatment of various doses of AE along with 7 mM MSG in MTT assay

3.5. Oxidative Stress Parameters

The cells possess a variety of primary and secondary defense against oxidative damage which includes the presence of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). SOD is the only enzyme that uses the superoxide anions as a substrate and produces hydrogen peroxide as a metabolite, which is more toxic than O_2 radical and must be removed by CAT as well as GSH [38,39]. In this study the level of GSH as well as activity of SOD and CAT were significantly ($p<0.001$) decreased in IMR-32 cells after treatment of 7 mM MSG. The administration of AE of CP seeds along with 7 mM MSG showed dose dependent significant increase in the level of GSH and activity of SOD and CAT as compared with alone 7 mM MSG treated group (Table 2).

Table 2. Result of oxidative stress related parameters in control and all treated groups

Gr.	GSH (mM GSH/mg protein)	SOD (U SOD/mg protein)	CAT (nM H_2O_2 consumed/min/mg protein)	Total protein (mg protein/ 10^5 cells)
I	1.767±0.028	41.65±0.12	150.4±1.87	0.418±0.023
II	1.681±0.014^{ns}	41.67±0.18^{ns}	146.6±3.11^{ns}	0.416±0.012^{ns}
III	1.632±0.063^{ns}	41.62±0.41^{ns}	154.6±2.87^{ns}	0.423±0.034^{ns}
IV	1.009±0.031*	34.83±0.71*	115.4±1.23*	0.385±0.045*
V	1.120±0.021^{NS}	35.88±0.45$^{\#}$	125.6±2.34^{NS}	0.409±0.011$^{\#}$
VI	1.279±0.012$^{\#}$	38.73±0.39$^{\#\#}$	130.7±1.23$^{\#}$	0.416±0.034$^{\#}$
VII	1.539±0.014$^{\#\#}$	40.56±0.28$^{\#\#}$	138.1±1.86$^{\#\#}$	0.420±0.041$^{\#}$

Values are Mean ± S.E.

* = $p<0.001$ and ns = non-significant when groups II, III and IV compared with group I. # = $p<0.01$; ## = $p<0.001$ and NS = non-significant when groups V, VI and VII compared with group IV.

I=Control; II=Vehicle control; III=1 µg/ml AE; IV=7 mM MSG; V=7 mM MSG + 0.25 µg/ml AE; VI=7 mM MSG + 0.5 µg/ml AE; VII=7 mM MSG + 1 µg/ml AE.

The result of this study is in accordance with the findings of Kumar and Gupta [29], who showed that antioxidant properties of AE of CP seeds significantly increased glutathione level as well as increase activity of SOD and catalase in male Wistar rats.

Along with antioxidant defense system the total protein content of the cell also equally important to maintain normal homeostasis of the cells. The total protein level found decreased significantly ($p<0.01$) at 7 mM MSG treatment as compared to control. When AE treatment was given along with 7 mM MSG to IMR-32 cells, the dose dependent significant amelioration (for 0.25 µg/ml AE-$P<0.05$; for 0.5 and 1 µg/ml AE-$p<0.01$) observed in total protein content (Table 2). Hence, based on pharmacological investigation, free radical scavenging activity study and evaluation of oxidative stress related parameters it can be concluded that due to presence of various phytocomponents and its free radical scavenging activity AE of CP seeds showed protective effects against MSG induced oxidative stress in neuronal cells.

3.5. Genotoxicity Study

Excessive amount of MSG can enhance the production of free radicals, which may cause several perturbations to cellular integrity, including DNA modification [40,41]. Damage to DNA is one of the important markers of genotoxicity which plays a pivotal role in apoptosis and ultimately leading to the cell death [42,43].

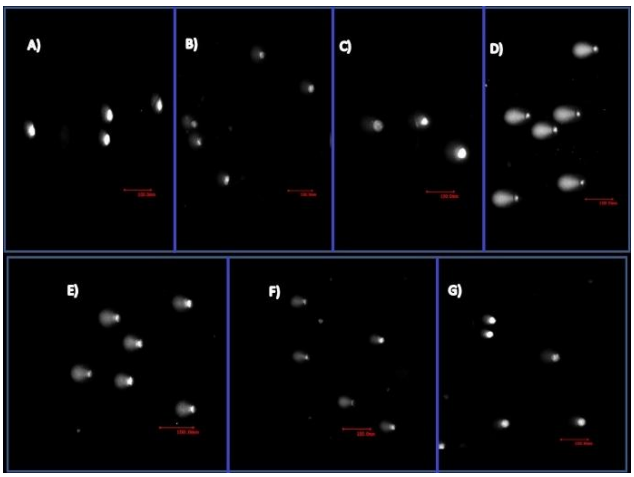

Figure 6. Comet assay (Cell DNA migration pattern) in control and all treated groups (I=Control; II=Vehicle control; III=1 µg/ml AE; IV=7 mM MSG; V=7 mM MSG + 0.25 µg/ml AE; VI=7 mM MSG + 0.5 µg/ml AE; VII=7 mM MSG + 1 µg/ml AE).

Comet assay is a sensitive method to detect DNA strand breaks in cells [44]. Dead or dying cells may undergo rapid DNA fragmentation, which is expected to increase DNA migration in the comet assay [45]. Hence, MSG induced DNA damage and its mitigation by AE of CP seeds was further confirmed using the comet assay. Aqueous extract showed highly significant ($p<0.001$) protective capability at 1 µg/ml against MSG induced DNA damage, which is evidenced by dose dependent significant reduction in the % DNA in tail with increase in the % DNA in head when both MSG and AE together added to the culture of IMR-32 cells (Figure 6, Table 3).

Table 3. Study of DNA damage by comet assay

Groups	% DNA in tail	% DNA in head
I	1.073±0.12	99.62±0.08
II	1.101±0.21 ^{ns}	99.71±0.12 ^{ns}
III	1.078±0.13 ^{ns}	99.29±0.11 ^{ns}
IV	2.314±0.28 *	89.44±0.14 *
V	1.59±0.18 ^{NS}	91.69±0.23 ^{NS}
VI	1.294±0.11 $^{\#}$	95.17±0.12 $^{\#\#}$
VII	1.104±0.09 $^{\#\#}$	98.93±0.16 $^{\#\#}$

Values are Mean ± S.E.
* = $p<0.001$ and ns = non-significant when groups II, III and IV compared with group I. # = $p<0.01$; ## = $p<0.001$ and NS = non-significant when groups V, VI and VII compared with group IV
I=Control; II=Vehicle control; III=1 µg/ml AE; IV=7 mM MSG; V=7 mM MSG + 0.25 µg/ml AE; VI=7 mM MSG + 0.5 µg/ml AE; VII=7 mM MSG + 1 µg/ml AE.

In corroboration with present study Russo and co-workers (2001) [46] also demonstrated reduction in DNA damage in human fibroblast cells by AE of CP seeds. So, the genotoxicity induced by 7 mM MSG in IMR-32 neuroblastoma cells was mitigated by aqueous extract of CP seeds in dose dependent manner.

4. Conclusion

On basis of present study, it can be postulated that the free radical mediated cellular impairment and DNA damage cause by monosodium glutamate in neuronal cells can be ameliorate by the presence of phytoconstituents present in aqueous extract of *Celastrus paniculatus* seeds.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgments

This work was supported by DST inspire fellowship program (IF130664), New Delhi, India.

References

[1] Giacoppo, S., Mandolino, G., Galuppo, M., Bramanti, P. and Mazzon, E., "Cannabinoids: new promising agents in the treatment of neurological diseases", Molecules, 19. 18781-18816. 2014.

[2] Kandale, V. V., Mujawar, S. N., Welasly, P. J. and Nimbalkar, J. M., "Development of integrated database of neurodegenerative diseases (IDND)", Review of Research, 2. 1-5. 2013.

[3] Lee, O. H., Lee, B. Y., Lee, J., Lee, H. B., Son, J. Y., Park, C. S. and Kim, Y. C., "Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities", Bioresource Technology, 100. 6107-6113. 2009.

[4] Farooqui, T. and Farooqui, A. A., "Aging: an important factor for the pathogenesis of neurodegenerative diseases", Mechanisms of Ageing and Development, 130. 203-215. 2009.

[5] Blaylock, R. L., "A possible central mechanism in autism spectrum disorders, part 3: the role of excitotoxin food additives and the synergistic effects of other environmental toxins", Alternative Therapies in Health and Medicine, 15. 56-60. 2009.

[6] Lipton, S. A. and Rosenberg, P. A., "Excitatory amino acids as a final common pathway for neurologic disorders", New England Journal of Medicine, 330. 613-622. 1994.

[7] Rothman, S. M. and Olney, J. W., "Excitotoxicity and the NMDA receptor-still lethal after eight years", Trends in Neurosciences, 18. 57-58. 1995.

[8] Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vöckler, J., Dikranian, K., Tenkova, T. I., Stefovska, V., Turski, L. and Olney, J. W., "Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain", Science, 283, 70-74. 1999.

[9] Husarova, V. and Ostatnikova, D., "Monosodium glutamate toxic effects and their implications for human intake: a review", JMED Research, 2013. 1-12. 2013.

[10] Veni, N. K., Karthika, D., Devi, M. S., Rubini, M. F., Vishalini, M. and Pradeepa, Y. J., "Analysis of monosodium l-glutamate in food products by high-performance thin layer chromatography", Journal of Young Pharmacists, 2. 297-300. 2010.

[11] Federico, A., Cardaioli, E., Da Pozzo, P., Formichi, P., Gallus, G. N. and Radi, E., "Mitochondria, oxidative stress and neurodegeneration", Journal of the Neurological Sciences, 322. 254-262. 2012.

[12] Uttara, B., Singh, A. V., Zamboni, P. and Mahajan, R. T., "Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options", Current Neuropharmacology, 7. 65-74. 2009.

[13] Deodhar, K. A. and Shinde, N. W., "Celastrus paniculatus: Traditional uses and Ethnobotanical study", Indian Journal of Advances in Plant Research, 2. 18-21. 2015.

[14] Karuppusamy, S. and Rajasekaran, K. M., "High throughput antibacterial screening of plant extracts by resazurin redox with special reference to medicinal plants of Western Ghats", Global Journal of Pharmacology, 3. 63-68. 2009.

[15] Godkar, P. B., Gordon, R. K., Ravindran, A. and Doctor, B. P., "Celastrus paniculatus seed oil and organic extracts attenuate hydrogen peroxide-and glutamate-induced injury in embryonic rat forebrain neuronal cells", Phytomedicine, 13. 29-36. 2006.

[16] Harborne, J. B., Methods of plant analysis. Springer, Dordrecht. 1984.

[17] Macdonald‐Wicks, L. K., Wood, L. G. and Garg, M. L., "Methodology for the determination of biological antioxidant capacity in vitro: a review", Journal of the Science of Food and Agriculture, 86. 2046-2056. 2006.

[18] Parul, R., Saha, P. and Kundu, S. K., "In vitro nitric oxide scavenging activity of methanol extracts of three Bangladeshi medicinal plants", The Pharma Innovation, 1. 83-88. 2013.

[19] Mosmann, T., "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays", Journal of Immunological Methods, 65. 55-63. 1983.

[20] Shah, N., Nariya, A., Pathan, A., Patel, A., Chettiar, S. S. and Jhala, D., "Neuroprotection Effects of Celastrus paniculatus seed oil against monosodium glutamate in human IMR-32 cells", Annual Research & Review in Biology, 24. 1-9. 2018.

[21] Shen, H. M., Yang, C. F., Ding, W. X., Liu, J. and Ong, C. N., "Superoxide radical–initiated apoptotic signalling pathway in selenite-treated HepG2 cells: mitochondria serve as the main target", Free Radical Biology and Medicine, 30. 9-21. 2001.

[22] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., "Protein measurement with the Folin phenol reagent", Journal of Biological Chemistry, 193. 265-275. 1951.

[23] Ellman, G. L., "Tissue sulfhydryl groups", Archives of Biochemistry and Biophysics, 82. 70-77. 1959.

[24] Kakkar, P., Das, B. and Viswanathan, P. N., "A modified spectrophotometric assay of superoxide dismutase", Indian Journal of Biochemistry and Biophysics, 21. 130-132. 1984.

[25] Sinha, A. K., "Colorimetric assay of catalase", Analytical Biochemistry, 47. 389-394. 1972.

[26] Singh, N. P., Mccoy, M. T., Tice, R. R. and Schneider, E. L., "A simple technique for quantitation of low levels of DNA damage in individual cells", Experimental Cell Research, 175. 184-191. 1988.

[27] Kumar, G. P. and Khanum, F., "Neuroprotective potential of phytochemicals", Pharmacognosy Reviews, 6. 81-90. 2012.

[28] Sofowora, A., Ogunbodede, E. and Onayade, A., "The role and place of medicinal plants in the strategies for disease prevention", African Journal of Traditional, Complementary and Alternative Medicines, 10. 210-229. 2013.

[29] Kumar, M. H. V. and Gupta, Y. K., "Antioxidant property of Celastrus paniculatus willd.: A possible mechanism in enhancing cognition", Phytomedicine, 9. 302-311. 2002.

[30] Kulkarni, Y. A., Agarwal, S. and Garud, M. S., "Effect of Jyotishmati (Celastrus paniculatus) seeds in animal models of pain and inflammation", Journal of Ayurveda and Integrative Medicine, 6. 82-88. 2015.

[31] Gamlath, C. B., Gunatilaka, A. L., Tezuka, Y., Kikuchi, T. and Balasubramaniam, S., "Quinone-methide, phenolic and related triterpenoids of plants of Celastraceae: further evidence for the structure of Celastranhydride", Phytochemistry, 29. 3189-3192. 1990.

[32] Venkataramaiah, C. and Rajendra, W., "Phytochemical screening of bioactive compounds present in the seed of Celastrus paniculatus: Role in traditional medicine", Indo American Journal of Pharmacology Research, 3. 9104-9112. 2013.

[33] Wink, D. A., Hines, H. B., Cheng, R., Switzer, C. H., Flores‐Santana, W., Vitek, M. P., Ridnour, L. A. and Colton, C. A., "Nitric oxide and redox mechanisms in the immune response", Journal of Leukocyte Biology, 89. 873-891. 2011.

[34] Hetrick, E. M. and Schoenfisch, M. H., "Analytical chemistry of nitric oxide" Annual Review of Analytical Chemistry, 2. 409-433. 2009.

[35] Zohera, F. T., Habib, M. R., Imam, M. Z., Mazumder, M. E. H. and Rana, M. S., "Comparative antioxidant potential of different extracts of Celastrus paniculatus wild Seed", Stamford Journal of Pharmaceutical Sciences, 3. 68-74. 2010.

[36] Lakhanpal, P. and Rai, D. K., "Quercetin: a versatile flavonoid", Internet Journal of Medical Update, 2. 22-37. 2007.

[37] Godkar, P. B., Gordon, R. K., Ravindran, A. and Doctor, B. P., "Celastrus paniculatus seed water soluble extracts protect against glutamate toxicity in neuronal cultures from rat forebrain", Journal of Ethnopharmacology, 93. 213-219. 2004.

[38] Imlay, J. A., "Cellular defenses against superoxide and hydrogen peroxide", Annual Review of Biochemistry, 77. 755-776. 2008.

[39] Kim, G. H., Kim, J. E., Rhie, S. J. and Yoon, S. "The role of oxidative stress in neurodegenerative diseases", Experimental Neurobiology, 24. 325-340. 2015.

[40] Dugan, L. L. and Choi, D. W., "Excitotoxicity, free radicals, and cell membrane changes", Annals of Neurology, 35. S17-S21. 1994.

[41] Ataseven, N., Yuzbaşioglu, D., Keskin, A. C. and Unal, F., "Genotoxicity of monosodium glutamate", Food and Chemical Toxicology, 91. 8-18. 2016.

[42] Kannan, K. and Jain, S. K., "Oxidative stress and apoptosis", Pathophysiology, 7. 153-163. 2000.

[43] Circu, M. L. and Aw, T. Y., "Reactive oxygen species, cellular redox systems and apoptosis", Free Radical Biology & Medicine, 48. 749-762. 2010.

[44] Liao, W., Mcnutt, M. A. and Zhu, W. G., "The comet assay: a sensitive method for detecting DNA damage in individual cells", Methods, 48. 46-53. 2009.

[45] Hartmann, A., Schumacher, M., Plappert‐Helbig, U., Lowe, P., Suter, W. and Mueller, L., "Use of the alkaline in vivo Comet assay for mechanistic genotoxicity investigations", Mutagenesis, 19. 51-59. 2004.

[46] Russo, A., Izzo, A. A., Cardile, V., Borrelli, F., and Vanella, A., "Indian medicinal plants as antiradicals and DNA cleavage protectors", Phytomedicine, 8. 125-132. 2001.