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Research Article

The Study of Pycnogenol Protective Effect on Glutamate Induced Neurotoxicity: In Vitro Evaluation

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Abstract

Excessive release of the excitatory neurotransmitter glutamate is thought to be a major contributor to the pathophysiology of numerous neurodegenerative including epilepsy and Alzheimer's disease. Therefore, it is important to investigate for compounds that protecting neuronal cells against glutamate-induced cytotoxicity. Pycnogenol®, a standardized French maritime pine bark extract, has strong antioxidant activity which primarily comprises phenolic compounds and flavonoids. Therefore, in the present study, inducing glutamate toxicity in primary cultured cortical neurons, we studied the neuroprotective effects of bioflavonoid compound Pycnogenol an extract of Pinus maritime bark. The cortical neuron cells were exposed to 10^{-5} mM glutamate for 30 min to induce excitotoxicity. Then, different concentrations (10^{-1} - 10^{-5}) of PYC were added to the cells for 24 hours. The cell viability was determined using MTT assay. To investigate oxidative damage, the total antioxidant status (TAS)- total antioxidant status (TOS) analysis were used. According to MTT analysis results, it was found that 10^{-1} and 10^{-2} pycnogenol groups significantly attenuated the glutamate excitotoxicity induced cell damage. Furthermore, TAS-TOS analysis showed a correlation with MTT result. According to the results of this study, pycnogenol has the potential to be used as a therapeutic agent against glutamate excitotoxicity.

Keywords: Glutamate excitotoxicity, cell viability, cortical neurons, oxidative stress, pycnogenol.

Glutamat Kaynaklı Nörotoksisite Üzerinde Pycnogenol'un Koruyucu Etkisinin Araştırılması: İn Vitro Analizi

Öz

Eksitatör bir nörotransmitter olan glutamatın aşırı salınımı epilepsi ve Alzheimer gibi birçok nörodejeneratif hastalığın patogenezinde yer almaktadır. Bu nedenle nöronal hücreleri glutamata bağlı toksisiteye karşı koruyan bileşiklerin araştırılması oldukça önemlidir. Pinus maritima'dan elde edilen piknogenol® içerdiği fenolik bileşikler ve flavonoidlerden dolayı güçlü antioksidan özelliğe sahiptir. Bu çalışmada Pinus maritime'den elde edilen bioflavonoid yapısında olan pignogenolün nöroprotektif etkilerini glutamat toksisitesi oluşturulan kortikal nöron kültüründe araştırmayı amaçladık. Kortikal nöron hücreleri 30 dakika süre ile 10^{-5} mM glutamata maruz bırakılarak glutamat eksitotoksisitesi indüklendi. Sonrasında faklı konsantrasyonlarda (10^{-1} - 10^{-5}) PYC ile 24 saat boyunca inkübe edilmiştir. Hücre canlılık testi MTT yöntemi ile belirlendi. Oksidatif hasarı göstermek için total antioksidan seviyesi (TAS)- - total oksidan seviyesi (TOS) analizleri kullanılmıştır. MTT sonuçlarına göre 10^{-1} ve 10^{-2} piknogenol konsantrasyonlarının glutamat eksitotoksisitesine bağlı hücre ölümünü azalttığı görüldü. Aynı zamanda TAS-TOS sonuçları MTT sonuçları ile korelasyon saptandı. Bu çalışma sonuçlarına göre piknogenolün glutamat eksitotoksisitesine karşı teropatik ajan olarak kullanılabileceği düşünülmüştür.

Anahtar Kelimeler: Glutamat eksitoksisitesi, hücre canlılığı, kortikal nöron, oksidatif stres, piknogenol.

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1. Introduction

Throughout the past century, the prevalence of age-related neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson disease (PD), Huntington's disease (HD), and others continues to increase dramatically (Stephenson et al., 2018; Ehrenberg et al.,.2020) The characteristic features of neurodegenerative diseases are progressive dysfunction and loss of neurons caused by certain neurological deficits (Muddapu et al.,2020). During recent years, research has indicated that neurons containing excitatory amino acids have pivotal roles in psychological functions such as learning, memory, neuronal outgrowth, survival and plasticity. On the other hand, disturbances of the excitatory amino acid system may have a role in the pathogenesis of neurodegenerative diseases (Dong et al.,2009). Glutamate is the main excitatory neurotransmitter in the central nervous system and is participated all activities of the nervous system. There is 6 – 7 μmol/g wet weight of glutamate in human brain. Consequence of this, glutamate together with glutamine is the very abundant free amino acid in the brain (Muddapu et al.,2020; Yudkoff, 2017). Even though glutamate concentration in the nervous system is maintained milimolar range, the extracellular glutamate concentration is in the low micromolar range (Mahmoud et al., 2019). Excess extracellular glutamate induces the overstimulation of glutamate receptors, which lead to neuron depolarization and extreme influx of calcium, giving rise to in reactive oxygen species (ROS) generation, oxidative stress responses and following injury or death of neuronal cells (Elmann et al.,2017). Growing evidence suggests that oxidative stress due to glutamate excitotoxicity is associated with both acute and chronic neurodegenerative disorders. Therefore, searching for agents targeting glutamate excitotoxicity related mechanisms are good strategy for potential treatments of neurodegenerative diseases (Elmann et al.,2017; Prasansuklab et al., 2020).

An increasing number of ongoing studies have proposed that dietary intake or supplementation of herbal extracts can suppress or retardation the onset of neurodegenerative disorders. These natural products have a wide range of pharmacological actions, comprising ROS scavenging, antioxidant and neuroprotective features (Solanki et al., 2015; Elufioye et al., 2017). Pycnogenol® (PYC) is a combination of bioflavonoids extracted from the bark of the Pinus pinaster Aiton (previously known as Pinus maritime Mill) (Simpson et al., 2019). PYC is comprised of many compounds including polyphenols, flavonoids and phenolic acids, however; its main ingredient is procyanidin. Procyanidin is contain strong antioxidants which enable it used extensively as a dietary food supplement (Malekahmadi et al., 2020). Due to its antioxidant features, PYC possess much beneficial effects in preventing neurotoxicity by a) being a potent free radical scavenger, b) increasing of antioxidant enzymes synthesis, c) protecting DNA and endogenous antioxidants such as vitamin C, vitamin E and glutathione against damage from oxidative stress (Malekahmadi et al., 2020; Maritim et al., 2003; Rohdewald et al. 2002).

Given the elevation levels of free radicals that are connected with glutamate toxicity, we hypothesized that PYC supplementation may be protective effects in oxidative stress damages in consequence of toxic injury of excessive glutamate. This study was designed to investigate the neuroprotective effect of PYC against glutamate-induced excitotoxicity in the primary cultured cortical neurons.

2. Materials and Methods

2.1. Cell culture and treatment

Frozen primary rat cortical neuronal cells obtained from the Pharmacology Department of Medical Faculty of Ataturk University (Erzurum, Turkey). Primary neuronal cells were resuspended in Neurobasal medium contain B27, 10% fetal bovine serum (FBS) and 0.1% antibiotic (penicillin–streptomycin-amphotericin B). The cells were maintained at 37 °C and 5% CO_2. Cells were grown for 24 h to allow cell adhesion and recovery to occur. Cells were treated for 30 min with L-glutamic acid (10^{-5} mM) to induce excitotoxicity insult (Taghizadehghalehjoughi and Naldan, 2019). Then, in order to evaluate the neuroprotective effect of PYC on excitotoxicity, cells were treated with different concentrations of PYC (10^{-1} - 10^{-5}) for 24 h (Mojžišová etal., 2009). Cells were then tested with the relevant experimental protocols.

2.2. Cell viability assay

Cell viability was estimated by using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The viable cells include NAD(P)H-dependent oxidoreductase enzymes which reduce the MTT to formazan. At the end of the treatment, the medium was removed and 20 µl MTT solution (5 mg/ml; Sigma-Aldrich) was added to each well and incubated for 4 h at 37 °C. Then the solution was removed and 100 µl DMSO was added to the wells. The absorbance was read spectrophotometer at a wavelength of 490 nm with an automatic micro plate reader (µQuant, Biotek, Germany) (Varmazyari et al., 2020)

2.3. Oxidative stress markers analysis

Total antioxidant status (TAS) and total oxidant status (TOS) were determined using the the Erel method that is a novel automated colorimetric measurement (Erel, 2004; Erel, 2005). The measurement of TAS levels was depends on the ability of antioxidants to inhibit the formation of 2,2'-azino-di-3-ethylbenzthiazoline sulfonate $(ABTS)^+$ from the oxidation of ABTS at wavelength 660 nm by spectrophotometrically. TOS levels are based on the measurement of color intensity at 530 nm by oxidation of ferrous ioneo-dianisidine complex to ferric ion in the presence of various oxidative species under acidic condition. TAS results was shown as mM Trolox equivalent per liter (Trolox Equiv/mmol L^{-1}) whereas TOS results was shown in terms of micromolar hydrogen peroxide equivalent per liter (mmol H_2O_2 Equiv/mmol L^{-1}).

2.4. Statistical Analysis

Statistical analyses were employed using by one-way analysis of variance (ANOVA) following by Tukey's post hoc test according to the statistical program IBM SPSS Statistics 22.0 (IBM SPSS, Turkey). The significance values between the groups were considered at the levels of $P<0.05$ and $P<0.001$.

3. Results

3.1. Cell Viability – MTT assay

The MTT assay was used to quantify cell viability in response to glutamate and PYC alone and in combined (Fig1.). According to MTT analysis results, the cell viability ratio significantly decreased by 10-5 mM glutamate in 24 h (61,48%) when compared with the control group (P<0.001). It was found that 10-1 and 10-2 PYC groups significantly attenuated the glutamate excitotoxicity induced cell damage (85 and 77.35%, respectively). On the contrary, PYC applied at a dose range of 10-3 - 10-5 were found to have cytotoxic effects on cell viability compared to control group (P<0.05, P<0.001; respectively). In the data obtained with cell vitality rate, the dose of 10-3, 10-4 and 10-5 PYC levels were 73.69, 70.40 and 70.14%, respectively.

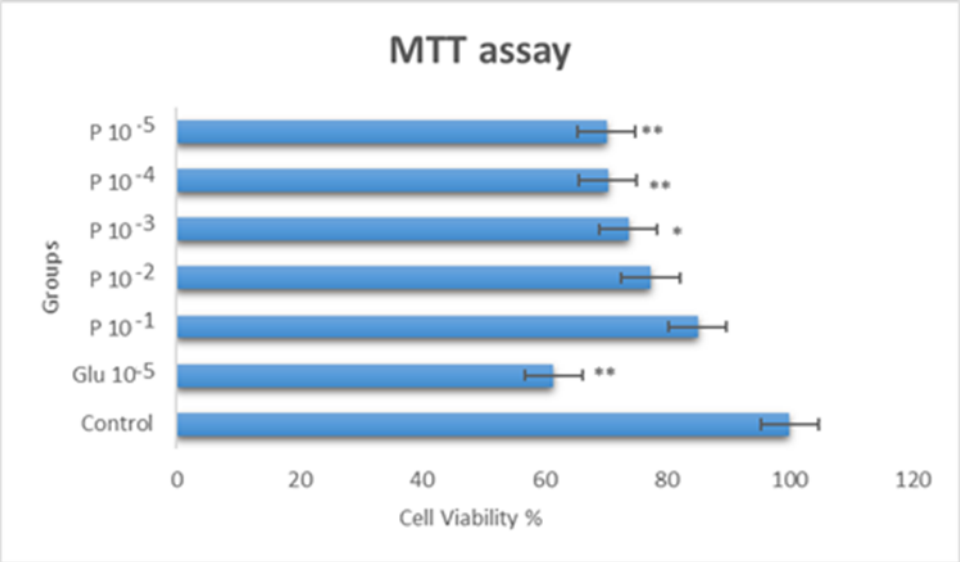

Figure 1. The cytotoxicity effect of PYC in primary cortical neuron cultures (Mean±standart deviation; * *P*<0.05; ** *P*<0.001 difference from glutamate control; C: control (untreated cells), G: cells treated with glutamate, P: cells treated with pycnogenol)

3.2. Oxidative stress markers

Fig 2. and 3. reflects the oxidant–antioxidant profile of PYC on primary rat cortical neuronal cells exposed to glutamate excitotoxicity. The results demonstrated that administration of 10^{-5} mM glutamate to cells lead to significant decreases in the TAS level as compared to control group (*P*<0.05, Fig.2). Applications of the lower concentrations (10^{-5} and 10^{-4}) of PYC did not lead to any alterations in TAS level compared to glutamate control group. In contrast, highest PYC concentration significantly increased TAS level and this value was determined as 4.9 Trolox Equiv/mmol L^{-1}. These increases were clearly in a concentration-dependent manner of PYC (Fig.2).

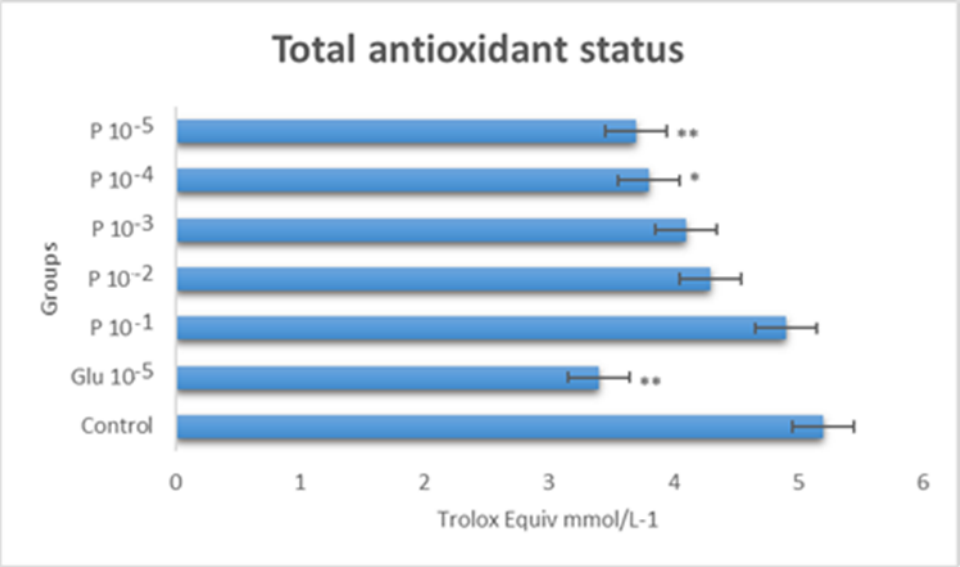

Figure 2. TAC levels of primary cortical neuron exposed to different concentrations of PYC after 24 h (Mean±standart deviation; * P<0.05 difference from glutamate control; C: control (untreated cells), G: cells treated with glutamate, P: cells treated with pycnogenol)

In all treatment groups, the TOS level was the highest in glutamate control group and and this value was detected as 5.1 mmol H_2O_2 Equiv/mmol L^{-1} (Fig. 3). Although administration of 10^{-5} and 10^{-4} PYC increased TOS activities compared to control (P<0.05), 10^{-3} - 10^{-1} concentrations of PYC were not statistically significant compared to control groups.

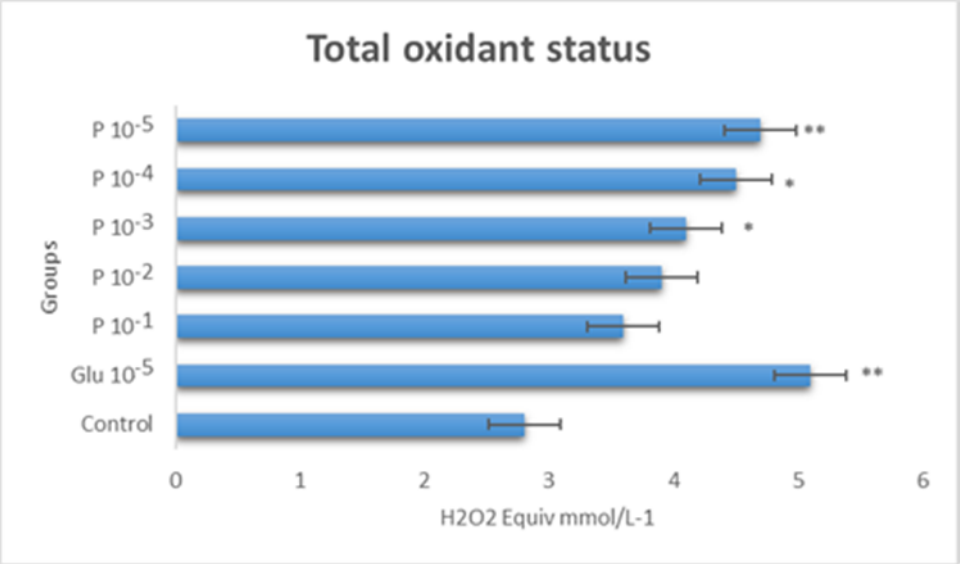

Figure 3. TOS levels of primary cortical neurons exposed to different concentrations of PYC after 24 h (Mean±standart deviation; * P<0.05; ** P<0.001 difference from glutamate control; C: control (untreated cells), G: cells treated with glutamate, P: cells treated with pycnogenol)

4. Discussion and Conclusions

Glutamate excitotoxicity induces a part of neurodegenerative events, like calcium overload in the cytosol, and ROS production causing neuronal cell death (Prentice et al., 2015; Chávez-Castillo et al., 2017). Substances that protect neuronal cells from glutamate toxicity are crucial in treatment of neurodegenerative disease such as Parkinson and Alzheimer's diseases. Although flavonoid deficiency does not cause any disease, exogenous antioxidants can affect cellular health and protect against inflammatory and degenerative diseases (Frandsen and Narayanasamy, 2018). In this study, we demonstrate that PYC, a nutritional supplement and as a bioactive remedy for several chronic diseases, possess protective effects against glutamate toxicity in dose dependent manner. To date, no study has explored the effect of PYC on neuronal glutamate toxicity in the nervous system; therefore, this report is the first to present such findings.

Neuronal cell death caused by glutamate is associated with excessive ROS production in neurons (Elmann et al., 2017; Cui et al., 2019). In an animal study of glutamate toxicity involved in brain ischemia, increased superoxide production and Ca^{2+} overload was observed (Gottlieb et al., 2006). Increased production of reactive oxygen species and decreased antioxidant compound GSH, antioxidant enzymes SOD, GSH-Px levels were reported in neuronal cultures treated with glutamate (Shimmyo et al., 2008; Yu et al.,2005). In accordance with the previous data, in this study, we determined significantly elevated TOS levels and decreased TAS levels in glutamate exposed neuronal cells. All these alterations were successfully prevented by higher concentrations of Pycnogenol administration to the glutamate toxicity.

The data about the effects of natural antioxidant substances in preventing glutamate toxicity induced ROS production is limited in literature (Frandsen et al., 2018). Very recently, Rajabian et al. (2018) reported that an antioxidant plant, Rheum turkestanicum, was significantly effective in vitro models of rat pheochromocytoma (PC12 cells) and mouse neuroblastoma (N2a) cell lines in preventing glutamate induced toxicity (Rajabian et al., 2018). Also selenium, an antioxidant agent, was demonstrated to alleviated glutamate-induced superoxide production and suppressed mitochondria-initiated cell death (Ma et al., 2017). Pycnogenol specific concoction of bioflavonoids having increasing the synthesis of antioxidant enzymes and oxidative damage alleviating functions (Ozoner et al., 2019; Taghizadehghalehjoughi and Cicek, 2018). In our study we determined that in highest concentration of pycgnogenol (10^{-1}) administered group, increased TAS levels and reduced TOS levels presenting the anti-oxidant effects of pycnogenol.

It has been reported that pycnogenol has neuroprotective effects in some different previous studies (Khan et al.,2013; Dvořáková et al.2006). Khan et al. (2013) reported the neuroprotective effects of pycgnegenol following MPTP-induced parkinson disease models most probably by increasing antioxidant enzyme activity including GPx, GR and SOD (Khan et al.,2013). Also, Dvoráková et al. (2006) reported that treatment of children suffering from attention deficit hyperactivity disorder with pycgnogenol normalises TAS and enhances the redox state of the organism through an important reduce of oxidized glutathione levels and a highly significant increase of reduced glutathione levels in comparison to a group taking placebo (Dvořáková et al.2006).

In conclusion, we indicated that pycgnegenol pretreatment attenuated oxidative stress caused by glutamate toxicity in neuron cells dose-dependently. Our study demonstrated that the highest concentration of pycgnegenol increased TAS levels and reduced TOS levels against glutamate toxicity. Regarding these findings, it can be suggested that, pycgnegenol may be a promising candidate for neuroprotection in the glutamate toxicity involved in pathogenesis of many neurodegenerative diseases. Further in vitro and in vivo studies are warranted to clarify the exact mechanism of pycnogenol in prevention of glutamate toxicity.

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References

Chávez-Castillo, M., Rojas, M., Bautista, J., 2017. "Excitotoxicity: an organized crime at the cellular level", Archıvos De Medıcına, 8(3), 193.

Cui, Y., Amarsanaa, K., Lee, J. H., Rhim, J. K., Kwon, J. M., Kim, S. H., Eun, S. Y., 2019. "Neuroprotective mechanisms of dieckol against glutamate toxicity through reactive oxygen species scavenging and nuclear factor-like 2/heme oxygenase-1 pathway", The Korean Journal of Physiology & Pharmacology, 23(2), 121-130.

Dong, X. X., Wang, Y., Qin, Z. H., 2009. "Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases", Acta Pharmacologica Sinica, 30(4), 379-387.

Dvořáková, M., Sivoňová, M., Trebatická, J., Škodáček, I., Waczuliková, I., Muchová, J., Ďuračková, Z., 2006. "The effect of polyphenolic extract from pine bark, Pycnogenol® on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD)", Redox Report, 11(4), 163-172.

Ehrenberg, A. J., Khatun, A., Coomans, E., Betts, M. J., Capraro, F., Thijssen, E. H., Paterson, R. W. 2020. "Relevance of biomarkers across different neurodegenerative diseases", Alzheimer's research & therapy, 12, 1-11.

Elmann, A., Telerman, A., Ofir, R., Kashman, Y., 2017. "Glutamate toxicity to differentiated neuroblastoma N2a cells is prevented by the sesquiterpene lactone achillolide A and the flavonoid 3, 5, 4′-trihydroxy-6, 7, 3′-trimethoxyflavone from Achillea fragrantissima", Journal of Molecular Neuroscience, 62(1), 99-105.

Elufioye, T. O., Berida, T. I., Habtemariam, S., 2017. "Plants-derived neuroprotective agents: cutting the cycle of cell death through multiple mechanisms", Evidence-Based Complementary and Alternative Medicine, 25(1), 1-28.

Erel, O., 2004. "A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation", Clinical biochemistry, 37(4), 277-285.

Erel, O., 2005. “A new automated colorimetric method for measuring total oxidant status”, Clinical biochemistry, 38(12), 1103-1111.

Frandsen, J. R., Narayanasamy, P., 2018. “Neuroprotection through flavonoid: Enhancement of the glyoxalase pathway”, Redox biology, 14, 465-473.

Gottlieb, M., Leal-Campanario, R., Campos-Esparza, M. R., Sánchez-Gómez, M. V., Alberdi, E., Arranz, A., Matute, C., 2006. “Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia”, Neurobiology of disease, 23(2), 374-386.

Khan, M. M., Kempuraj, D., Thangavel, R., Zaheer, A., 2013. “Protection of MPTP-induced neuroinflammation and neurodegeneration by Pycnogenol”, Neurochemistry international, 62(4), 379-388.

Ma, Y. M., Ibeanu, G., Wang, L. Y., Zhang, J. Z., Chang, Y., Dong, J. D., Jing, L., 2017. “Selenium suppresses glutamate-induced cell death and prevents mitochondrial morphological dynamic alterations in hippocampal HT22 neuronal cells”, BMC neuroscience, 18(1), 1-14.

Mahmoud, S., Gharagozloo, M., Simard, C., Gris, D. 2019. “Astrocytes maintain glutamate homeostasis in the CNS by controlling the balance between glutamate uptake and release”, Cells, 8(2), 184.

Malekahmadi, M., Moghaddam, O. M., Islam, S. M. S., Tanha, K., Nematy, M., Pahlavani, N., .Norouzy, A., 2020. “Evaluation of the effects of pycnogenol (French maritime pine bark extract) supplementation on inflammatory biomarkers and nutritional and clinical status in traumatic brain injury patients in an intensive care unit: A randomized clinical trial protocol”, Trials, 21(1), 1-9.

Maritim, A., Dene, B. A., Sanders, R. A., Watkins III, J. B., 2003. “Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats”, Journal of Biochemical and Molecular Toxicology, 17(3), 193-199.

Mojžišová, G., Šarišský, M., Mirossay, L., Martinka, P., & Mojžiš, J. 2009. “Effect of Flavonoids on Daunorubicin-induced Toxicity in H9c2 Cardiomyoblasts”, Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 23(1), 136-139.

Muddapu, V. R., Dharshini, S. A. P., Chakravarthy, V. S., & Gromiha, M. M., 2020. “Neurodegenerative Diseases–Is Metabolic Deficiency the Root Cause?”, Frontiers in Neuroscience, 14, 1-19.

Ozoner, B., Yuceli, S., Aydin, S., Yazici, G. N., Sunar, M., Arslan, Y. K., Suleyman, H., 2019. “Effects of pycnogenol on ischemia/reperfusion-induced inflammatory and oxidative brain injury in rats”, Neuroscience letters, 704, 169-175.

Prasansuklab, A., Brimson, J. M., Tencomnao, T., 2020. “Potential Thai medicinal plants for neurodegenerative diseases: A review focusing on the anti-glutamate toxicity effect”, Journal of Traditional and Complementary Medicine, 10(3), 301-308.

Prentice, H., Modi, J. P., Wu, J. Y., 2015. “Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases”, Oxidative medicine and cellular longevity, 20, 1-8.

Rajabian, A., Sadeghnia, H. R., Moradzadeh, M., Hosseini, A. 2018. Rheum turkestanicum reduces glutamate toxicity in PC12 and N2a cell lines. Folia neuropathologica, 56(4), 354-361.

Rohdewald, P., 2002. “A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology”, International journal of clinical pharmacology and therapeutics, 40(4), 158-168.

Shimmyo, Y., Kihara, T., Akaike, A., Niidome, T., & Sugimoto, H., 2008. “Three distinct neuroprotective functions of myricetin against glutamate-induced neuronal cell death: involvement of direct inhibition of caspase-3”, Journal of neuroscience research, 86(8), 1836-1845.

Simpson, T., Kure, C., Stough, C., 2019. “Assessing the efficacy and mechanisms of Pycnogenol® on cognitive aging from in vitro, animal and human studies”, Frontiers in pharmacology, 10, 694-702.

Solanki, I., Parihar, P., Mansuri, M. L., Parihar, M. S., 2015. “Flavonoid-based therapies in the early management of neurodegenerative diseases”, Advances in nutrition, 6(1), 64-72.

Stephenson, J., Nutma, E., van der Valk, P., Amor, S. 2018. “Inflammation in CNS neurodegenerative diseases”, Immunology, 154(2), 204-219.

Taghizadehghalehjoughi, A., Cicek, B., 2018. “Momordica and Pycnogenol Can Tolerate Imazamox Induced Toxicity in L929 Cells Line: In Vitro Study”, In Multidisciplinary Digital Publishing Institute Proceedings, 2 (25), 1584.

Taghizadehghalehjoughi, A., Naldan, M. E., 2019. “Is ketamine suitable for use in glutamate toxicity conditions?: An in vitro study”, Journal of Investigative Surgery, 1-8.

Varmazyari, A., Taghizadehghalehjoughi, A., Sevim, C., Baris, O., Eser, G., Yildirim, S., Mezhuev, Y. 2020. “Cadmium sulfide-induced toxicity in the cortex and cerebellum: in vitro and in vivo studies”, Toxicology Reports, 7, 637-648.

Yu, D., Duan, Y., Bao, Y., Wei, C., An, L., 2005. “Isoflavonoids from Astragalus mongholicus protect PC12 cells from toxicity induced by L-glutamate”, Journal of ethnopharmacology, 98(1-2), 89-94.

Yudkoff, M., 2017. “Interactions in the metabolism of glutamate and the branched-chain amino acids and ketoacids in the CNS”, Neurochemical research, 42(1), 10-18.