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CENTELLA ASIATICA NEUROPROTECTIVE EFFECT ON 6-OHDA-STIMULATED OXIDATIVE STRESS IN DIFFERENTIATED SH-SY5Y CELLS[1-2]

CENTELLA ASİATİCA'NIN, FARKLILAŞMIŞ SH-SY5Y HÜCRELERİNDE 6-OHDA KAYNAKLI OKSİDATİF STRES ÜZERİNDEKİ NÖROPROTEKTİF ETKİSİ

Yeşim YENİ[1], Betul ÇİCEK[2],
[1]Malatya Turgut Ozal University, Faculty of Medicine, Department of Medical Pharmacology, Malatya / Turkiye
[2]Erzincan Binali Yildirim University, Faculty of Medicine, Department of Physiology, Erzincan / Turkiye
ORCID NO: 0000-0002-6719-7077[1], 0000-0003-1395-1326[2]

Abstract: Aim: Parkinson's disease is qualified by advancing the loss of dopaminergic neurons and depletion of dopamine. However, the pathophysiological mechanisms need new perspectives for therapeutic strategies that alleviate and abolish neurodegenerative. The last searches have demonstrated that Centella Asiatica is commonly used in conventional medicine. The aim of our study is to reveal the neuroprotective effect of Centella Asiatica, which we will use in the treatment, on the neurotoxicity stimulated by 6 OHDA.

Method: First, the SH-SY5Y cell line was grown in prepared media. Then, 25-50-75 and 100 µg/ml concentrations of Centella Asiatica were supplemented to the wells that reached 85% confluence 2 hours before (except for the control and 6-OHDA groups). Afterward, 200 µM 6-OHDA was added to the wells (except for the control group) and incubated for 24 hours. Then, IL-1β, GSH, MTT, LDH, GPx, TNF-α, SOD, MPO, CAT, and MDA analyses were performed. One-way analysis of variance was performed using the IBM SPSS 22.0 package program. The results were compared with the control and 6-OHDA groups, and values below p<.05 were considered statistically significant.

Results: It was found that Centella Asiatica demonstrated a dose-dependent rise in the vivacity rate and the cell vivacity was 92% at the highest concentration. Moreover, antioxidant parameters (GSH, GPx, SOD, CAT) correlated with MTT and LDH assay. In IL-1β, TNF-α, MPO, and MDA activities, it was observed that the oxidant amount reduced depending on the concentration.

Conclusion: These findings revealed that Centella Asiatica exerts a neuroprotective effect opposite 6-OHDA induction by rising cell viability and reducing oxidative stress.

Keywords: Centella Asiatica, Parkinson Model, SH-SY5Y Cell Line

Öz: Amaç: Parkinson hastalığı, dopaminerjik nöronların progresif kaybı ve dopamin tükenmesi ile karakterizedir. Bununla birlikte, patofizyolojik mekanizmalar nörodejeneratifi hafifleten ve ortadan kaldıran terapötik stratejiler için yeni perspektiflere ihtiyaç duymaktadır. Son araştırmalar, Centella Asiatica'nın geleneksel tıpta yaygın bir şekilde kullanıldığını göstermiştir. Çalışmamızın amacı, tedavide kullanacağımız Centella Asiatica'nın 6-OHDA ile oluşturulan nörotoksisite üzerindeki nöroprotektif etkisini ortaya koymaktır.

Yöntem: Başlangıçta SH-SY5Y hücre hattı hazırlanmış besiyerlerinde büyütüldü. Ardından, %85 konflense ulaşan kuyucuklara 2 saat öncesinden Centella Asiatica'nın 25-50-75 ve 100 ug/ml dozları eklendi (kontrol ve 6-OHDA grubu hariç). Sonrasında kuyucuklara (kontrol grubu hariç) 200 µM 6-OHDA ilave ederek 24 saat boyunca inkübasyona bırakıldı. Ardından IL-1β, GSH, MTT, LDH, GPx, TNF-α, SOD, MPO, CAT ve MDA analizleri yapıldı. Elde edilen veriler IBM SPSS 22.0 paket programı kullanılarak tek yönlü varyans analizi yapıldı. Sonuçlar kontrol ve 6-OHDA grupları ile karşılaştırılarak p<.05'in altındaki değerler istatiksel olarak anlamlı kabul edildi.

Bulgular: Canlılık oranında Centella Asiatica'nın konsantrasyona bağlı olarak bir artış gösterdiği ve en yüksek konsantrasyonda hücre canlılığı % 92 oranında bulundu. Ayrıca antioksidan parametreleri (GPx, SOD, GSH, CAT), MTT ve LDH testi ile benzerlik gösterdi. IL-1β, TNF-α, MPO ve MDA aktivitelerinde ise doza bağlı olarak oksidan miktarının azaldığı gözlendi.

Sonuç: Bu bulgular, Centella Asiatica'nın hücre canlılığını artırarak ve oksidatif stresi azaltarak 6-OHDA indüksiyonuna karşı nöroprotektif bir etki gösterdiğini ortaya koydu.

Anahtar Kelimeler: Centella Asiatica, Parkinson Model, SH-SY5Y Hücre Hattı

[1] Sorumlu Yazar, Corresponding Author: Yeşim YENİ, Malatya Turgut Ozal University, Faculty of Medicine, Department of Medical Pharmacology, Malatya / Türkiye, yesim.yeni@ozal.edu.tr, *Geliş Tarihi / Received: 06.04.2023, Kabul Tarihi / Accepted: 19.09.2023, Makalenin Türü: Type of Article: (Araştırma – Uygulama; Research - Application) Çıkar Çatışması, Yok – Conflict of Interest, None, Conflict of Interest, None, Etik Kurul Raporu veya Kurum İzin Bilgisi Ethical Board Report or Institutiınal Approval, Yok / None "Hücre hattı kullanılması sebebiyle etik kurul izni gerektirmeyen çalışmalar arasında yer aldığını yazarlarca beyan edilmiştir."*
[2] Çalışma, araştırma ve yayın etiğine uygun olarak hazırlanmıştır. / The study was prepared in accordance with research and publication ethics.

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INTRODUCTION

Parkinson's disease (PD) is the second best common neurodegenerative illness after Alzheimer's illness, qualified by progressive loss of dopaminergic neurons and depletion of dopamine (Masato et al., 2019; Del Din et al., 2021). However, the pathophysiological mechanisms are not yet fully understood to pave the way for new perspectives for therapeutic strategies that alleviate or even abolish the neurodegenerative phenomenon (Ioghen et al., 2023). An accessible, inexpensive, and widely used in vitro model for PD is culturing immortalized cell lines (Cetin et al., 2022). Neurotoxicity-induced SH-SY5Y models serve as a starting point to study the protective effect of different compounds (Ioghen et al., 2023). Since 6-OHDA is similar to catecholamines, it enters cells using their transporters. Thus, it induces mitochondrial dysfunction, increases oxidative stress, and produces neuronal cell death (Falkenburger et al., 2016; Xicoy et al., 2017). It has also been used in an in vivo model of PD by inducing cell death of dopaminergic neurons (Zeng et al., 2018).

Many studies show that mostly natural phenols have a certain antioxidant effect, offering big occasions in the prevention and treatment of neurodegenerative phenomena because of their security and low side effects (Ioghen et al., 2023). This effect of phenols maintains the oxidation/redox balance in the nervous system and fights opposite the oxidative injury (Park & Ellis, 2020).

Centella Asiatica (CA) Urban, (Apiaceae), familiar as Gotu Kola is used in traditional Chinese and Ayurvedic medicine to enhance memory, cure cognitive function, and inverse cognitive impairments (Shinomol et al., 2011). The neuroprotective and cognitive enhancing effects of CA extracts have been validated in human works (Dev et al., 2009; Tiwari et al., 2008) as well as in preclinical model systems (Veerendra-Kumar & Gupta, 2003; Defillipo et al., 2012). Previous studies have shown that CA can prevent Aβ toxicity in vitro (Gray et al., 2014), without changing plaque load (Soumyanath et al., 2012), and reduce cognitive impairments in a transgenic mouse model of Aβ accumulation. Though the mechanism is unknown, studies in other models of neurotoxicity suggest that CA has antioxidant activity and may change mitochondrial function (Shinomol & Muralidhara, 2008; Prakash & Kumar, 2013). In addition to these effects, CA reduced the neurobehavioral and neurochemical effects of stroke in rodents (Tabassum et al., 2013), accelerated nerve regeneration, protected against oxidative neurotoxicity, and showed anti-inflammatory and antioxidant effects (Haleagrahara & Ponnusamy, 2010).

In this study, we researched the mechanism by which CA preserves opposite 6-OHDA toxicity using the neuroblastoma cell line (SH-SY5Y). These cells are widely used to model the effects of catecholamine 6-OHDA treatment. We investigated the effects of CA on cytotoxicity and antioxidant response in this cellular system.

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METHOD

Cell Culture and Treatment

For our study, SH-SY5Y (ATCC® CRL-2266™) cell line was bought from ATCC. The suspended cells were cultured in a Dulbecco-modified eagle medium containing 10% fetal bovine serum and 1% antibiotic (Thermo Fisher, Germany) and kept at 37°C with 5% CO_2. The medium was refreshed every 2-3 days. The cells were seeded in 96-well plates and stored in an incubator. For toxicity assessment, various dosages (25-50-75, and 100 µg/mL) of CA were added to the medium two hours before 6-OHDA (Sigma-Aldrich) application. After two hours, cells were exposed to 200 µM 6-OHDA for 24 hours. MTT and LDH assays were used to determine cytotoxicity after 24 hours.

Determination of Cytotoxicity

To evaluate the therapeutical potential of CA opposite 6-OHDA toxicity, we assessed the cell vivacity by measuring mitochondrial activity in alive cells by 3-4,5-dimetil-tiyazolil-2,5 difeniltetrazolyum bromür (MTT) quantitative colorimetric analysis. For this, cells were incubated with MTT for 4 hours. Then the medium was removed and the cells were solved with dimethyl sulfoxide. Its absorbance was read at 570 nm. Cell vivacity was stated as a percentage of the worth in the control.

Lactate dehydrogenase (LDH) activity is a test used to measure the leakage of LDH into the cell medium when the plasma membrane integrity of cells is disrupted. LDH activity was appointed in a colorimetric way using an LDH analysis kit (Elabscience, USA) according to the kit procedure. The absorbance was measured at 450 nm in the plate reader.

Measurement of Oxidative Stress Markers

Glutathione peroxidase (GPx), interleukin-1β (IL-1β), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), superoxide dismutase (SOD), myeloperoxidase (MPO), catalase (CAT), and glutathione (GSH) were determined by ELISA kits (Elabscience, USA). The oxidative injury assays were performed based on the manufacturer's instructions. The absorbance was specified by a spectrophotometer at 450 nm.

Statistical Analysis

The quantitative data were stated as the mean ± standard deviation (SD). Assays were performed by one-way assays of variance with post hoc Tukey's test (IBM SPSS 22.0) ($p<.05$).

RESULTS

Pretreatment with CA Reduced 6-OHDA Stimulated Cytotoxicity SH-SY5Y Cells

We specified whether pretreatment with CA had prophylactic effects opposite 6-OHDA stimulated cell demise by MTT test. After the cells were treated with 25-50-75, and 100 µg/mL CA for two hours, 200 µM 6-OHDA was supplemented for culture for 24 hours. CA stopped 6-OHDA stimulated cytotoxicity in a dose-dependent manner. Cell viability was recovered at 64% (25-CA), 72% (50-CA), 83% (75-CA), and 92% (100-CA) ($p<.001$) according to 6-OHDA (Figure 1).

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LDH activity, which is a metabolic marker of cell viability, is demonstrated in Figure 1. As a result of exposure of cells with simply 6-OHDA 200 µM, LDH activity raised in correlation with the reduction in cell liveliness and it was figured out to be significant compared to the control group (*p<.05*). For neuroprotective activity, LDH levels gradually reduced depending on the concentration in the groups that were administered CA before the 6 -OHDA application (*p<.001*). These data demonstrate that CA significantly decreases the cytotoxic effect of 6-OHDA.

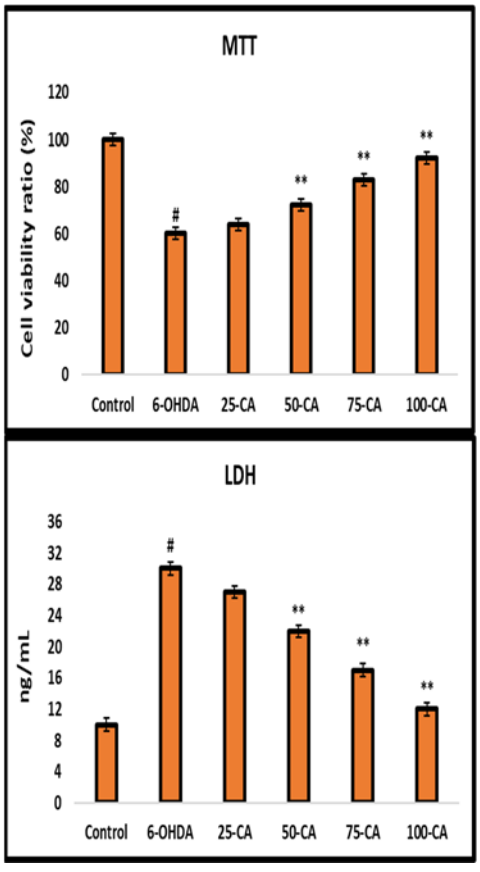

Figure 1: MTT and LDH results of application groups. Data are determined as the means±SD. #*p<.05* values are significant for control group; **p<.05*, ***p<.001* for 6-OHDA.

Pretreatment with CA Suppresses Oxidative Stress in 6-OHDA-Induced SH-SY5Y Cells

As seen in Figure 2, SOD, GPx, GSH, and CAT activities reduced importantly in the 6-OHDA group compared to the control group, while MPO, MDA, IL-1, and TNF-α levels increased significantly (*p<.05*). Due to the raised concentration in the 6-OHDA group, SOD, GSH, GPx, and CAT levels were raised in the CA group, while MDA, MPO, IL-1, and TNF-α was significantly decreased (*p<.001*). The findings support MTT and LDH data (Figure 1).

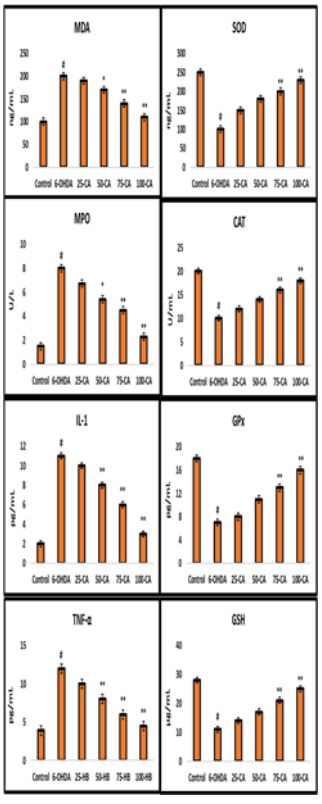

Figure 2: GPx, SOD, GSH, CAT, TNF-α, MPO, MDA, and IL-1 results of thenapplication group. Data are determined as the means±SD. #*p<.05* values are significant for control group; **p<.05*, ***p<.001* for 6-OHDA.

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DISCUSSION

6-OHDA is considered an experimental toxicant to study the mechanisms of PD in vitro (Bove et al., 2005). The results of the current study demonstrated that 24 hours of 6-OHDA treatment reduced differentiated SH-SY5Y cell viability by approximately 60%. The neuroprotective effect of CA in the MTT test paralleled that of the LDH assay.

Mitochondrial dysfunction and oxidative stress play a crucial role in the pathogenesis of PD (Seaton et al., 1997). In our study, the rising levels of MDA, MPO, IL-1, and TNF-α activity observed in the group exposed to 6-OHDA alone showed that mitochondrial dysfunction and oxidative stress were stimulated by 6-OHDA. The mitochondrial respiratory chain is one of the most significant sites of reactive oxygen types manufacture, and a relatively small level of inhibition is enough to rise reactive oxygen species production. Meanwhile, mitochondria themselves are vulnerable to reactive oxygen species, and an excess of reactive oxygen species can cause mitochondrial damage (Bueler, 2009; Yao & Wood, 2009). CA pretreatment significantly reduced reactive oxygen species production in 6-OHDA-induced SH SY5Y cells, indicating its potential to clean free radicals and protect dopaminergic cells opposite 6-OHDA-stimulated damage.

The neuroprotective effects of CA are well documented, but these effects are generally attributed to the bioactive triterpenes found in the plant (Xu et al., 2012; Zhang et al., 2012). Though the etiology of PD is not exactly figured out, oxidative stress (Trist et al., 2019) and inflammatory responses (Gao et al., 2022) are significant risk links causing PD. Current findings have shown that CA enhances the antioxidant features of the PD pattern by rising CAT, GSH, SOD, and GSH-Px activities and decreasing MDA, MPO, IL-1, and TNF-α levels. SOD, CAT, GSH Px, and GSH activities of the antioxidant defensive system are relatively poor during PD, and improving their activities is a significant approach to preventing PD advance and development (Wang et al., 2018; Chen et al., 2021). SOD is a strong endogenous antioxidant enzyme for Superoxide radicals. It was determined that PD models created with 6-OHDA were significantly reduced both in vitro and in vivo (Soto-Otero et al., 2000). MDA is an oxidative stress signal related to PD damage and is a potential avenue for its clinical treatment (Tamtaji et al., 2019). CA will show beneficial effects in controlling the development of PD by decreasing the injury led to 6-OHDA.

In the brain of PDs, the secretion of proinflammatory cytokines is nearly associated with the exterminate of neurons (Chen et al., 2018). In a model of PD stimulated by the bacterial endotoxin lipopolysaccharide, dopamine neurons suggested a significant role of inflammation in the degeneration of the nigrostriatal path (Milde et al., 2021). Therefore, it is important to control the three proinflammatory cytokines (TNF-α, IL 1β, and IL-6) to prevent PD advancement. (Chen et al., 2021). The current result is that CA decreased the

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inflammatory cytokine (IL-1β and TNFα) levels.

CONCLUSION

CA exhibited protecting effects on 6-OHDA-stimulated toxicity in SH-SY5Y cells. These effects were related to the capability to decrease oxidative stress and preserve mitochondrial membrane potential. Therefore, CA can be thought of as a potential agent for the treatment of neurodegenerative disorders like PD, alone or in combination with other now-used anti-parkinsonism agents.

Conflict of Interest

The authors report that there is no conflict of interest.

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