Abant Tıp Dergisi

Abant Medical Journal

Abant Med J 2022;11(3):xx-xx, doi:10.47493/abantmedj.1123424

In Vitro Evaluation of The Protective Effects of Curcumin and Resveratrol Against U87 Cells Induced by Beta-Amyloid

Beta-Amiloid Tarafından İndüklenen U87 Hücrelerine Karşı Kurkumin ve Resveratrol'ün Koruyucu Etkilerinin İn Vitro Değerlendirilmesi

Ares ALİZADE [1*], Gülüzar ÖZBOLAT [2]

[1] Van Yüzüncü Yıl University Faculty of Medicine, Department of Pharmacology, Van, Türkiye

[2] Sinop University, Faculty of Health Science, Sinop, Türkiye

Geliş Tarihi (*Received*): 30.05.2022 **Kabul Tarihi (*Accepted*):** 08.09.2022 **Yayın Tarihi (*Published*):** 30.12.2022

Abstract

Objective: In this study we aimed to investigate the effects of curcumin and resveratrol antioxidant enzymes in β-amyloid (Aβ)-induced in vitro Alzheimer's Disease (AD) cell model.

Materials and Methods: Three groups were created in this study; The control group consisted of U87 cells, the Aβ group which was the in vitro AD model formed from the β-amyloid-induced U-87 cell lines, and the Aβ+ Curcumin+ Resveratrol group by adding curcumin and resveratrol to the Aβ group. Cell viability in groups was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test. Total Antioxidant Level (TAS), Superoxide dismutase (SOD), Total Oxidant Level (TOS), and Catalase (CAT) enzyme levels, results were analyzed in order to evaluate the antioxidant levels.

Results: When cell viability was evaluated, it was determined that curcumin and resveratrol did not have cytotoxic effects. TAS levels were statistically higher in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$) and TOS levels were found to be significantly low in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$). However, when compared to the control group, CAT and SOD enzyme levels were statistically and significantly low in the Aß group. In contrast, these enzyme levels were found significantly higher in the Aβ+ curcumin and resveratrol group in comparison with the Aß group ($p<0.05$).

Conclusion: In conclusion, it has been shown that curcumin and resveratrol support antioxidant activity.

Keywords: Curcumin, Resveratrol, β-amyloid-induced U-87

&

Öz

Amaç: Çalışmamız, β-amiloid (Aβ) ile indüklenen U87 hücrelerin in vitro Alzheimer Hastalığı (AH) modeli olarak kurkumin ve resveratrol'un antioksidan enzimler üzerindeki etkilerini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntemler: Bu çalışmada; U87 hücrelerden oluşan kontrol grubu, β-amiloid ile indüklenen U-87 hücre dizilerini içeren in vitro AD modelinin oluşturulduğu Aβ grubu ve Aβ grubuna kurkumin ve resveratrol eklenerek Aβ+ Curcumin+ Resveratrol grubu olmak üzere üç grup oluşturuldu. Grupların hücre canlılığı 3-(4,5-dimetiltiazol-2-il)-2, 5-difeniltetrazolyum bromür (MTT) testi ile değerlendirildi. Total Antioksidan Seviyesi (TAS), Süperoksit dismutaz (SOD), Total Oksidan Seviyesi (TOS) ve Katalaz (CAT) enzim seviyeleri, antioksidan durumunu değerlendirmek için sonuçlar analiz edildi.

Bulgular: Hücre canlılığı değerlendirildiğinde kurkumin ve resveratrolün sitotoksik etkilerinin olmadığı belirlendi. Aβ+ kurkumin ve resveratrol grubunda Aβ grubuna göre TAS düzeyleri istatistiksel olarak daha yüksek ($p<0,05$) ve TOS düzeyleri Aβ+ kurkumin ve resveratrol grubunda Aβ grubuna göre istatistiksel olarak anlamlı derecede düşük bulundu($p<0,05$). Ancak kontrol grubu ile karşılaştırıldığında Aß grubunda CAT ve SOD enzim düzeyleri istatistiksel olarak anlamlı düzeyde düşük bulunurken, bu enzim düzeyleri Aβ+ kurkumin ve resveratrol grubunda Aß grubuna göre istatistiksel olarak anlamlı düzeyde yüksek bulundu. ($p<0,05$).

Sonuç: Sonuç olarak, kurkumin ve resveratrolün apoptoza bağlı nörodejenerasyonu azalttığı ve antioksidan aktiviteyi desteklediği gösterilmiştir.

Anahtar Kelimeler: Kurkumin, Resveratrol, β-amiloid kaynaklı U-87

Atıf/Cite as: Alizade A. , Özbolat G. In vitro evaluation of the protective effects of Curcumin and Resveratrol against U87 Cells Induced by Beta-Amyloid. Abant Med J. 2022; 11(3): 304-311, doi:10.47493/abantmedj.1123424

[*]**Sorumlu Yazar (Corresponding Author):** Ares Alizade, **e-mail**: arash.alizadeh82@gmail.com

Introduction

Alzheimer's disease (AD) is most characterized by progressive loss of cognitive capacity as well as severe neurodegeneration leading to dementia (1). It is characterized by memory loss and behavioral and mental dexterity disorders. Amyloid (senile) plaques originating from Amyloid ß (Aß) peptides and neurofibrillary tangles containing tau protein, degeneration, and neuronal loss are seen in Alzheimer's disease (1-4). Although there are many hypotheses (amyloid cascade, glutamatergic, cholinergic, oxidative stress, and tau effects) trying to explain the pathophysiology of AD, nevertheless none of these hypotheses can fully explain the pathophysiology of the disease (5). Since approved drugs available today are insufficient in the treatment of the disease and in relieving symptoms, Scientists have turned to finding new molecules that can be an alternative treatment option. In new therapeutic pursuits today, plants are recognized as one of the main sources of biologically active substances. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a naturally occurring polyphenol. Clinically, resveratrol has exhibited significant anti-inflammatory, antioxidant, anti-cancer, and anti-viral, properties. Experimental studies suggest that resveratrol is active against AD pathogenesis (6-8). Curcumin is a pigment found in the spice castor, also known as turmeric. Curcumin is a compound with powerful antioxidant and anti-inflammatory properties. It has been used in medicine as an anti-inflammatory agent to treat gas, chest pain aches, toothaches, and menstrual difficulties. In a study, the Curcumin-Cu2+ complex was examined in two different segments of the β-amyloid protein to prevent the formation of Curcumin-binding Aβ fibrils. As a result of this study, it was determined that Curcumin binds to Cu2+ and Aβ and acts as a chelator and binding partner to Aβ. Thus, it has been shown that it is possible to use Curcumin in the treatment of AD (9-11).

Oxidative stress also plays an important role in the formation of neurodegenerative diseases such as Alzheimer's by inducing mitochondrial abnormalities. Antioxidants are an important component that plays a protective role in eliminating ROS and protecting from neuronal damage caused by ROS. Antioxidants provide first-line defense against damage from free radicals and are critical in maintaining optimal health and well-being. Enzymatic and low molecular weight antioxidants create the balance between ROS and anti-oxidative mechanisms. While the enzymatic antioxidants are SOD, CAT, and GPx, glutathione (GSH) is one of the low molecular weight antioxidants that are effective in AD (11-13).

We planned this study to investigate the possible effects of curcumin and resveratrol, which we predicted to be used for the treatment of Alzheimer's disease, on the U87 cell line. In accordance with this purpose, we investigated SOD and CAT activities to evaluate antioxidant status, TAS and TOS protein levels, and activities to see their effect on the apoptosis process. In addition, in present study, we examined whether curcumin and resveratrol have a cytotoxic effect on the U87 cells and its effect on cell proliferation by using the MTT test. Before starting experimental animal model studies, determining how the cytotoxic effect depends on the concentration will make a contribution to the drug development stages in the future.

Materials and Methods

U87MG Cell Culture and In vitro Experiments:

The study has been conducted in the laboratory of Cukurova University, Faculty of Medicine, Department of Pharmacology. The materials used in this study were obtained from the laboratory and the chemicals used are listed below. In the study, Aß1-40, Dulbecco's Modification of Eagle's Medium (DMEM), penicillin/streptomycin antibiotic (PSA), fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), U87 MG cell line, 18β-GA from Sigma Aldrich; SOD, KAT, and GPx ELISA kits were obtained from R&D Systems. Literature studies were used to create an in vitro model of AD in U87 MG cells (12).

Culture Medium was created by adding 1% (PSA) and 10% FBS to DMEM broth. Cells were cultured in a 5% humidified and CO2 at 37°C to adhere to the surface in culture flasks. Before proceeding with the assays, the frequency of the cell was at 80%, during which time old media were replaced with new culture media twice a week. The cells, which reached 80% frequency, were washed with serum-free medium after

removing the used medium and kept in serum-free medium for 2 hours. Differentiated U87 MG cells obtained by cell passage were incubated with 5 micromolar (μM) Aß for 24 hours. In vitro groups were formed by performing the corresponding procedures, Control group (U87 MG cells + medium), Aß Group (Aβ-treated U87 MG cells), Aß+18ß GA Group (U87 MG cells treated with 5 μM 18ß-GA 18β-GA + Aβ) with each group having 5x104 cells in each well of the 96-well plate with 6 replicates.

MTT Test:

The MTT assay test was used according to the protocols of manufacturer's instructions to examine cell viability and cytotoxic effects of compounds. Briefly, three groups (Control group, Aβ group, Aß+18ßGA group) were inoculated with a new FBS-free medium containing 10% MTT (5 mg/ml concentration) solution in well plates. The cells were incubated for 24 hours in a dark incubator at 370C and 5% CO2. After incubation, the medium was removed and in order to dissolve the formazan crystals 100 microliters (μl) of DMSO were added to the cells. In order for the formed crystals to dissolve, they were kept in a CO2 (5%) incubator at 37°C for 10 minutes. After all, procedures were completed carefully, absorbance values were recorded by reading each section at 570 nm wavelength.

ELISA Test:

SOD, CAT, and GPx levels, which are antioxidant enzymes, were determined in the control, Aß, and Aß+18ß-GA groups with the ELISA test. The relevant ELISA test procedures are different for each kit and were performed according to the protocols specified in the relevant kits.

Statistical Analysis:

Statistical analyzes of results were performed in the Statistical Package for the Social Sciences program called IBM SPSS 25. As the first step in data analysis, the assumption of normality was checked with the Shapiro Wilk test. ANOVA test for examining the difference between the means of variables with normal distribution and more than two independent groups; Kruskal Wallis test was used to examine the difference between the means of variables that did not have a normal distribution and had more than two independent groups. Bonferroni analysis, one of the post hoc tests, was performed to reveal the group or groups that made the difference. $p<0.05$ was considered statistically significant.

Results

MTT Test:

Change of U87 cell viabilities according to the β-amyloid, Curcumin, and resveratrol applied are shown in Fig.1. It was determined that Curcumin and resveratrol, applied to the human brain cell line (U87), increased the viability and proliferation of the cells and Curcumin applied on cells caused less increase in cell proliferation than resveratrol ($p<0.05$), (Figure 1). IC50 values were calculated as the concentration of the Inula viscosa extract that causes 50% inhibition in cell proliferation.

ELISA Test:

Application of Aβ to the U87 cells reduced the activity of SOD enzymes significantly. Curcumin and resveratrol administration significantly increased this decrease (Figure 2). The application of curcumin and resveratrol significantly increased catalase activity in cells that were treated whit Aβ (Figure 3). Application of Aβ to cells increased the amount of total oxidant (TOS) significantly, and treatment whit Curcumin and resveratrol significantly decreased this increase (Figure 4). Application of Aβ to the cells reduced the amount of TAS, Curcumin, and resveratrol administration significantly increased this decrease (Figure 5).

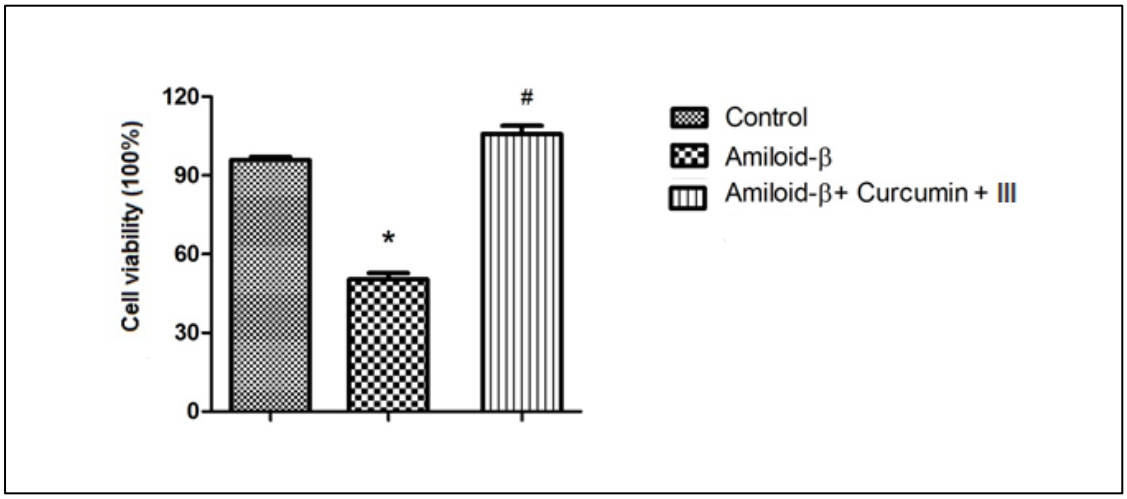

Figure 1. Effect of experimental groups on cell proliferation. p<0.05 relative to control

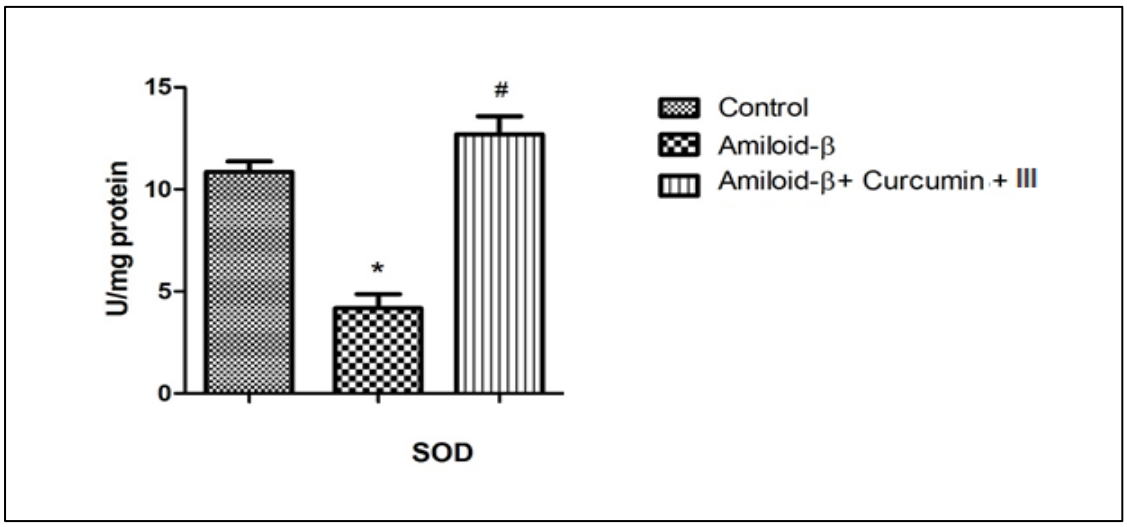

Figure 2. The effect of curcumin and resveratrol administration on SOD activity (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

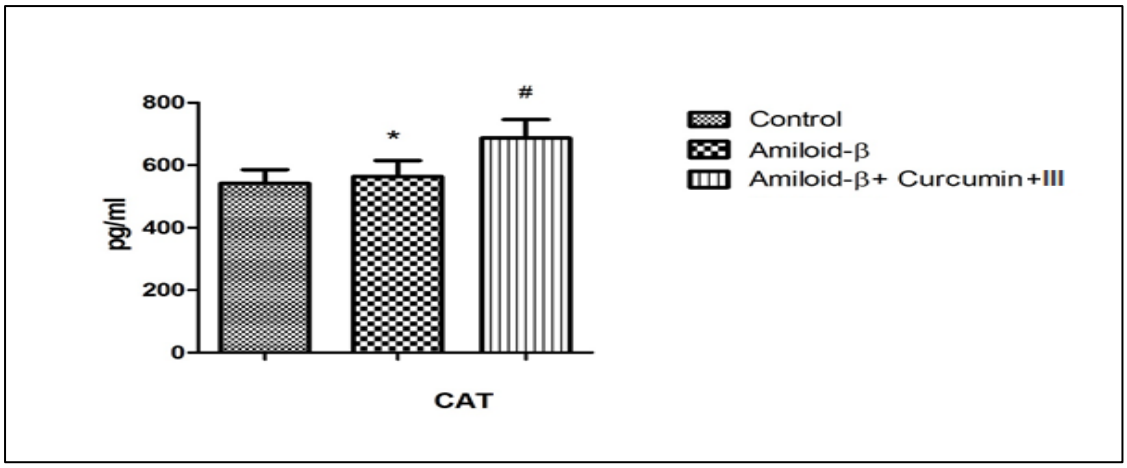

Figure 3. Effect of curcumin and resveratrol application on catalase activity (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

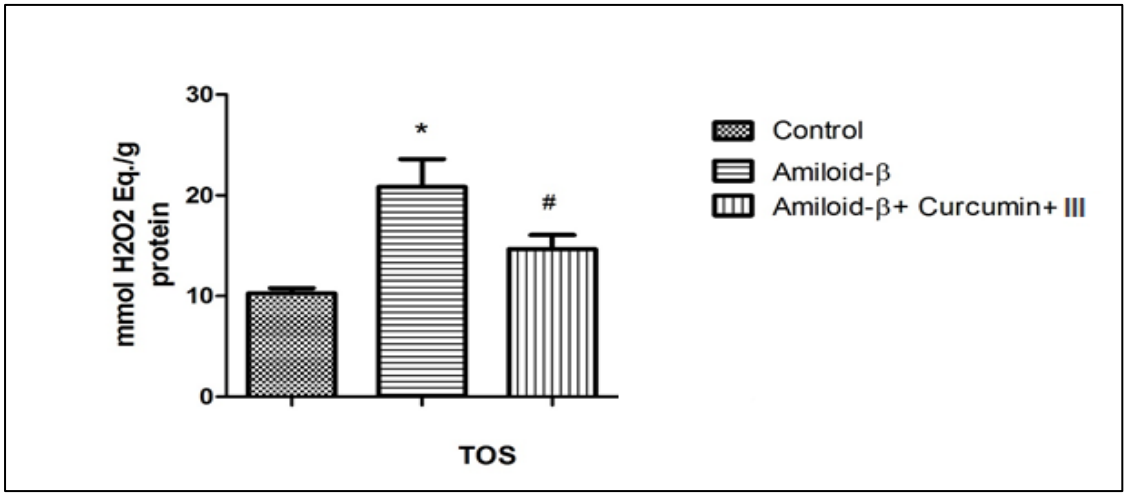

Figure 4. The effect of curcumin and resveratrol application on the amount of oxidant (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

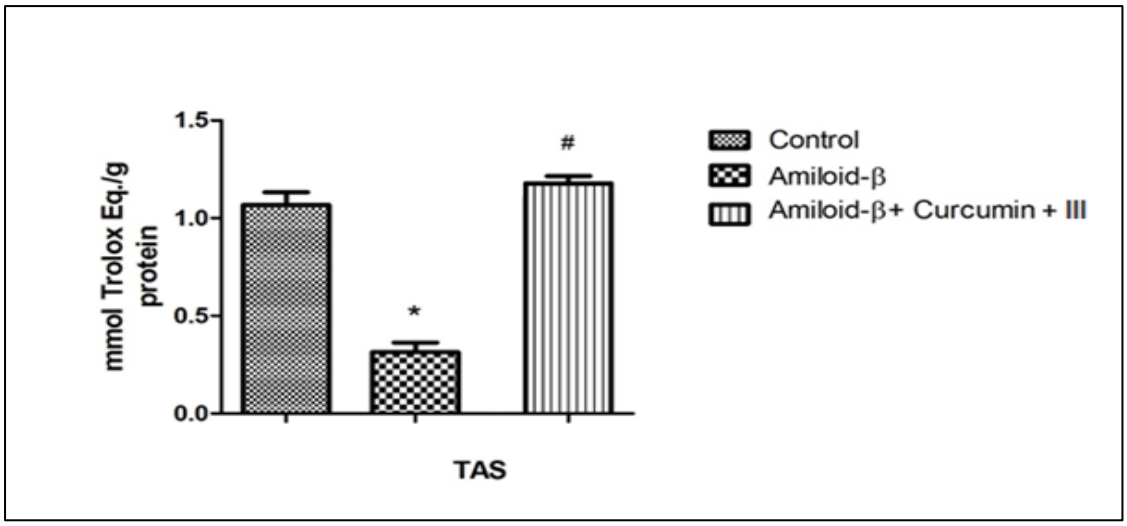

Figure 5. Effect of curcumin and resveratrol application on antioxidant content (n=6). Statistical analysis: one-way anova posthoc: benferroni. (* : p<0.05 compared to control. # compared to amyloid-B).

Discussion

AD is the most common type of dementia in the elderly and is characterized by progressive loss of cognitive capacity as well as severe neurodegeneration. the most important targets in treatment of AD is inhibition of amyloid precursor protein (APP) and Aβ production by blocking Aβ aggregation and inhibiting the inflammatory response and Aβ-induced neurotoxicity (14-16).

Although the relationship between Alzheimer's disease and ROS is not fully understood, it has been reported in the literature that another cause of pathological changes in Alzheimer's disease is the imbalance between ROS and antioxidative mechanisms. While there is indirect evidence showing that oxidative stress mechanisms are increased in AD, ROS has been found to have a potential role in amyloid plaque deposition. It was found that SOD was localized in the brain amyloid plaques of the patients, there was a significant decrease in plasma GPx activity throughout the clinical evolution of the disease, and It has been reported that the role of CAT is in the second defense against ROS in the antioxidant defense system by somehow helping the GPx role (17-19).

In this study, we investigated the effectiveness of curcumin and resveratrol in the Alzheimer's model cell line. the effects of curcumin and resveratrol on cell viability and proliferation was examined by MTT test, and the antioxidant effects of those were evaluated by measuring the levels of SOD, TAS, TOS, and CAT by ELISA.

According to our data, amyloid-B administration on the human brain cell line U87 significantly reduced SOD activity compared to the control. Curcumin and resveratrol administration increased this decrease when compared to the control groups ($p<0.05$). Application of Aβ to the U87 cells increased the amount of total oxidant curcumin and resveratrol application decreased this increase significantly ($p<0.05$ compared to control). The application of Aβ to cells reduced the number of antioxidants significantly. Curcumin and resveratrol administration increased this decrease significantly ($p<0.05$ compared to control). TAS levels were found to be statistically higher in the Aβ + curcumin and resveratrol group compared to the Aβ group ($p<0.05$) and TOS levels were found to be statistically lower in the Aβ+ curcumin and resveratrol group compared to the Aβ group ($p<0.05$). The increase in ROS in the cell and accordingly the defense mechanism activity of the antioxidant may have caused an increase in cell proliferation. Accordingly, we can argue that the application of curcumin and resveratrol increases the activity of SOD enzymes, which constitute the defense mechanism of the cell, together with increased cell proliferation. ROS is effectively cleared by a detoxification defense system such as SOD, and CAT to maintain redox homeostasis in the cellular system. When ROS production is above the cell's detoxification capacity, over-produced ROS causes severe damage to DNA, proteins, and lipids of the cell membrane. It can be suggested here that ROS could act as a second messengers in cell proliferation, possibly through activation of protein kinases and transcriptional factors. Curcumin and resveratrol treatments reduced amyloid-B-induced cell death. Proliferation and antioxidant enzyme activity studies on U87 cell lines, a human brain cell line in vitro, showed significant differences compared to the control groups. Curcumin has been shown to have beneficial effects on Alzheimer's in many studies with its antioxidant, anti-inflammatory, and anti-amyloid effects (20-22).

Conclusion

In our study, we invastigated the effects of Curcumin and resveratrol on the Alzheimer's cell line model. We predict that resveratrol may be more beneficial to Alzheimer's than curcumin. Curcumin and resveratrol can be used as a potential compound that can be an alternative to the few drugs used in the field of AD treatment today and are preferred during treatment.

Ethics Committee Approval: Ethics committee approval was not obtained because it was a cell culture study.

Informed Consent: Not applicable.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

Acknowledgement: None.

References

1. Dinamarca MC, Cerpa W, Garrido J, Hancke JL, Inestrosa NC. Hyperforinprevents beta-amyloidneurotoxicity and spatial memory impairments by disaggregation of Alzheimer's amyloid-beta-deposits. MolPsychiatry, 2006, 11(11):1032--1048.
2. Dos Santos Picanco, LC, Ozela PF, Fatima de Brito Brito M, Pinheiro AA. Padilha E. C, Braga FS Silva Hage-Melim, LI. Alzheimer's disease: A review from the pathophysiology to diagnosis, new perspectives for pharmacological treatment. Current medicinal chemistry. 2018; 25 (26): 3141-3159

3. Gol M, Ghorbanian D, Soltanpour N, Faraji J, Pourghasem M. Protective effect of raisin (currant) against spatial memory impairment and oxidative stress in alzheimer disease model. Nutritional neuroscience. 2019; 22 (2): 110-118.
4. Baluchnejadmojarad T, Mohamadi-Zarch SM, Roghani M. Safranal, an active ingredient of saffron, attenuates cognitive deficits in amyloid β-induced rat model of alzheimer's disease: Underlying mechanisms. Metabolic Brain Disease. 2019; 34 (6): 1747-1759.
5. Topçuoğlu ES, Selekler K. Alzheimer disease. Turkish Journal of Geriatrics, 1998;1(2), 63-7.
6. A. Baur, D.A. Sinclair. Therapeutic potential of resveratrol: the in vivo evidence Nat Rev Drug Discov, 5 (2006), pp. 493-506
7. Takaoka, M. (1940). The phenolic substances of white hellebore (Veratrum Grandiflorum Loes. Fill). V. Nippon Kagaku Kaishi 61.
8. Tadolini, B., Juliano, C., Piu, L., Franconi, F., and Cabrini, L. (2000). Resveratrol inhibition of lipid peroxidation. Free Radic. Res. 33, 105–114. doi: 10.1080/10715760000300661
9. J. A. Baur and D. A. Sinclair, "Therapeutic potential of resveratrol: the in vivo evidence," Nature Reviews Drug Discovery, vol. 5, no. 6, pp. 493–506, 2006.Picciano AP. Vaden TD. Complexation between Cu(II) and curcumin in the presence of two different segments of amyloid β. Biophysical Chemistry.2013; 184, 62--67.
10. JA. Baur and D. A. Sinclair, "Therapeutic potential of resveratrol: the in vivo evidence," Nature Reviews Drug Discovery, vol. 5, no. 6, pp. 493–506, 2006.Picciano AP. Vaden TD. Complexation between Cu (II) and curcumin in the presence of two different segments of amyloid β. Biophysical Chemistry.2013; 184, 62--67.
11. Shankar GM, Walsh, DM. Alzheimer's disease: Synaptic dysfunction and abeta. Molecular neurodegeneration. 2009; 4: 48.
12. Gol M, Ghorbanian D, Soltanpour N, Faraji J, Pourghasem M. Protective effect of raisin (currant) against spatial memory impairment and oxidative stress in alzheimer disease model. Nutritional neuroscience. 2019; 22 (2): 110-118.
13. Sherki YG, Melamed E & Offen D. Oxidative Stress İnduced-Neurodegenerative Diseases: The Need For Antioxidants That Penetrate The Blood Brain Barrier. Neuropharmacology. 2001. 40(8): 959–975, PMID: 11406187
14. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: The Indian solid gold. Adv Exp Med Biol 2007; 595:1–75.
15. Youssef KM, El-Sherbeny MA. Synthesis and antitumor activity of some curcumin analogs. Arch Pharm (Weinheim) 2005; 338:181–9
16. Lin C.W., Yang L.Y., Shen S.C., Chen Y.C. IGF-I plus E2 induces proliferation via activation of ROS-dependent ERKs and JNKs in human breast carcinoma cells. J Cell Physiol, 2007; 212(3): 666-74.
17. Kılınç E, Var A, Mavioğu H, et al. Cırculatıng Manganese Superoxıde Dısmutase (Mn-SOD, SOD2) Levels And Its Ala16val Polymorphısm In Patıents Wıth Alzheımer's Dısease. Turkish Journal Of Geriatrics. 2012. 15(1): 1-6.
18. Altıner A. Atalay H, Bilal T. Serbest Radikaller ve Stres İle İlişkisi. Balıkesir Sağlık Bilimleri Dergisi. 2017. 7 (1):51-55, DOİ: 10.5505/bsbd.2018.38243
19. Sherki YG, Melamed E, Offen D. Oxidative Stress İnduced-Neurodegenerative Diseases: The Need For Antioxidants That Penetrate The Blood Brain Barrier. Neuropharmacology. 2001. 40(8): 959–975, PMID: 11406187.
20. Joanna T. Maciejczy M, Konopka T, Zalewska A. Total Oxidant and Antioxidant Capacity of Gingival Crevicular Fluid and Saliva in Patients with Periodontitis: Review and Clinical Study, Antioxidants 2020, 9(5), 450.
21. Rong W. Feng J. Yang Y. Dai C. Lu A. et al., Significance of Serum Total Oxidant/Antioxidant Status in Patients with Colorectal Cancer, 2017.

22. El-Edel Rawhia H, Abou-Elela Dalia H, Sonbol Ahmed A, Shalaby Amr S, Fouaad Mariam, Total antioxidant capacity as an aging marker in Egyptian patients with Alzheimer disease, A: 2020; 33 (3): 914-919.