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ARAŞTIRMA / RESEARCH

Antioxidant activities of inula viscosa extract and curcumin on U87 cells induced by beta-amyloid

Beta-amiloid ile indüklenen U87 hücrelerinde inula viscosa ekstresi ve kurkuminin antioksidan aktiviteleri

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Abstract

Purpose: The aim of this study was to investigate the effects of Inula viscosa extract and Curcumin on the U87 (human astrocytoma cell line) treated with amyloid-beta (Aβ), which is the Alzheimer's disease (AD) model cell line.
Materials and Methods: Firstly, the cytotoxic potential of ınula and curcumin was investigated in the U87 cells by the colorimetric MTT (3-4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide) assay. Then, the amount of Total Glutathione, Malondialdehyde (MDA), Glutathione reductase (GR) activities were investigated. ELISA test was used to examine the expression and activity of cleaved Bax and Bcl-2 proteins in the Inula viscosa and Curcumin treated U87 cell lines.
Results: Inula viscosa and Curcumin treatment reduced cell death caused by amyloid-B in cells. It also reduced the oxidative stress caused by amyloid-B, while reducing the activation of the proapoptotic protein Bax, and Bcl-2.
Conclusion: Our results suggest that inula viscosa may represent a new approach in the treatment of Alzheimer's.

Keywords:. Inula viscosa, curcumin, Alzheimer, U87, Bax, Bcl-2

Öz

Amaç: Bu çalışmada, Inula viscosa ekstresi ve Kurkumin'in Alzheimer hastalığı (AD) model hücre dizisi olan amiloid-beta (Aβ), ile tedavi edilen U87 (insan astrositom hücre dizisi) üzerindeki etkilerini araştırmayı amaçladık.
Gereç ve Yöntem: İlk olarak, U87 hücrelerinde inula ve kurkuminin sitotoksik potansiyeli kolorimetrik MTT (3-4,5-dimetil-tiyazolil-2,5-difeniltetrazolyum bromür) testi ile araştırıldı. Daha sonra Total Glutatyon miktarı, Malondialdehit (MDA), Glutatyon redüktaz (GR) aktiviteleri araştırıldı. Inula viscosa ve Curcumin ile muamele edilmiş U87 hücre hatlarında bölünmüş Bax ve Bcl-2 proteinlerinin ekspresyonunu ve aktivitesini incelemek için ELISA testi kullanıldı.
Bulgular: Inula viscosa ve Kurkumin muamelesinin, hücrelerde amiloid-B'nin neden olduğu hücre ölümünü azalttı. Ayrıca, proapoptotik protein Bax ve Bcl-2'nin aktivasyonunu azaltırken amiloid-B'nin neden olduğu oksidatif stresi de azalttı.
Sonuç: Sonuçlarımız, inula viscosa'nın Alzheimer tedavisinde yeni bir yaklaşımı temsil edebileceğini göstermektedir.

Anahtar kelimeler: Inula viscosa, curcumin, Alzheimer, U87, Bax, Bcl-2

INTRODUCTION

Today, plants are preferred as one of the main sources of biologically active materials in search of new treatment methods. Many medicinal plants have been described in traditional medicine for the treatment of dementias such as Alzheimer's disease (AD). Alzheimer's disease (AD) is a neurodegenerative disease affecting people globally, mainly the elderly. During the twentieth century, while the number of Alzheimer's patients increases, scientists are looking for treatments for the disease, even if the cause and pathology of the disease remain unclear. There is currently no cure for AD, but some

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symptomatic treatments are available. Effective treatments are greatly needed. Currently, the most prominent targets for therapeutic intervention include the inhibition of amyloid precursor protein (APP) and Aβ production by blocking Aβ aggregation and the resulting inflammatory response and inhibiting Aβ-induced neurotoxicity. In the new therapeutic quests, plants are regarded as one of the main sources of biologically active substances [1-4].

Curcumin is one of these plants used in traditional medicine. As an antioxidant, anti-inflammatory, and lipophilic effect, curcumin has been shown in studies that improve cognitive functions in patients with AD. Curcumin has been administered in different doses and times in many neurodegenerative rat model studies and has been found to prevent the increase in MDA level, decrease GSH release, and SOD activity. In a study conducted by Lim et al. on transgenic Alzheimer mice, it was reported in their studies curcumin reduced oxidative stress and decreased Aβ accumulation rate in mice receiving low dose curcumin. It has been shown that Aβ plaques and their accumulation decrease significantly, inhibit phosphorylation, and reduce oxidative damage and alleviate inflammation as a result of curcumin administration in transgenic AD mice[5-8].

Inula viscosa (Linnaeus) is widely accessible in the Mediterranean region, is a constantly green and self-growing plant. It has been used as a medicine since ancient times due to its anti-inflammatory, antipyretic, antiseptic, anthelmintic, antiphlogistic activities. It has been stated that the chemical elements that are identified and isolated from the plant are flavonoids, azulenes, sesquiterpenes, and essential oils, especially the essential oils and sesquiterpenes (carboxyeudesmadiene) have antifungal activity[9-14].

The anti-inflammatory activity of the compounds isolated from the Inula viscosa plant was investigated in the study conducted by Manez et al. In the light of the obtained findings, it is stated that the inuviskolite compound isolated from the plant shows activity by inhibiting the secretion of elastase, cyclooxygenase 1 enzymes and phospholipase A2 enzyme. In addition, it has been emphasized that this compound shows anti-inflammatory activity by reducing skin leukocyte infiltration in murine dermatitis[15] In the study of Talib et al., The antiproliferative and antimicrobial effects of the compounds obtained from Inula viscosa were evaluated. Methylated quercetins isolated from Inula viscosa have been found to have more anticancer and antimicrobial properties than other flavonoids[16].

Inula viscosa's antioxidant activity and ability to cleanse reactive oxygen species (ROS) show the potential use of this compound to treat oxidative damage associated with increased ROS in some degenerative diseases. Conducting Inula viscosa-based research, which we foresee to be used as a potential drug for the treatment of AD due to these effects, reveals the possibility of developing a new drug treatment. There are no studies in the literature regarding the effect of Inula viscosa extract on Alzheimer's disease which used in our study[1,2]. On the other hand, there are many studies with Curcumin as a potential drug among treatments for AD today. Curcumin has proven to be a potential role in the prevention and treatment of AD today, in many studies. Therefore, the beneficial effects of Inula viscosa and Curcumin were investigated by creating an invitro AD model[17].

Inula viscosa is one of these plants used in traditional medicine because it's anti-inflammatory, antioxidant, and anti-cancer effect[10-12]. The ability of Inula viscosa to clear reactive oxygen species (ROS) and antioxidant activity, age-related, and the potential use of this compound to treat oxidative damage containing ROS such as some degenerative diseases. we anticipate that these effects can be used as potential drugs for Alzheimer's Disease (AD) treatment, studies based on Inula viscosa can suggest better drug development. At the same time, there are many studies with Curcumin as a potential drug for treatments of AD. It has been determined that specifically inhibits pathways involved in apoptosis and cell invasion also has strong antioxidant effects. In this study, we aimed to investigate the effects of Inula viscosa and curcumin on Alzheimer's disease cell line model U87 (glioblastoma astrocytoma). First of all, we aimed to evaluate cell growth and/or cell death indirectly by MTT assay. Then to investigate the effect of total glutathione, Malondialdehyde (MDA), Glutathione reductase and the effect of apoptosis on the caspase-3 protein levels by ELISA in the cell line administered with Inula viscosa and Curcumin.

MATERIALS AND METHODS

Extract preparation of Inula viscosa

Inula viscosa leaves were collected from the Hatay Mustafa Kemal University campus, Turkey. The

leaves of the Inula viscosa plant were dried in the oven for 24 hours, then the dried plant leaves were filtered after extraction in 70% ethyl alcohol (v/v) solution and incubated overnight with a shaker. The extract was filtered (Whatman filter paper) and evaporated under vacuum at 35°C to remove the ethanol and water to dryness. The extracts were kept at -20°C till use[18].

Cell culture

U-87 (ATCC number HTB-14) is a glioblastoma, astrocytoma cell line were kept in an incubator at 37° C, 5% CO2, and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. The cell line was replicated in flasks using this medium. The U87 cell line density in the flask, which was kept in the incubator for 48 hours, was examined under a light microscope and the cells were passage from the intense flasks[19].

Cell viability assay

Cellular proliferation was recorded by using MTT following the manufacturer's protocol. The U87 cells were seeded at a density of 3x104 cells each well of 96-well plate and cultured with 10 µM Aβ1-42 at 37° C for overnight. Then, the cells were incubated with 0.25% IV extract for 48 h. After incubation, MTT (0.5 mg/ml) reagent was added. The supernatants were removed and 150 µl of DMSO (Sigma-Aldrich) was added to each well to dissolve the formazan crystals for 20 min. The absorbance quantity was measured at 590 nm and reading UV spectrophotometer.

ELISA (Enzyme-linked immunosorbent assay) test

Total Glutathione amount, Malondialdehyde (MDA), glutathione reductase activities were examined by the ELISA method in order to evaluate the antioxidant effects of inula viscosa and Curcumin in applied cell line and also Bax, Bcl-2 protein levels were examined to determine its effect on apoptosis. ELISA kits test (Shenzhen Genesis Technology, Guangdong, China) protocols were done according to the manufacturer's protocols.

Statistical analysis

Student's t-test was applied to the variables to reveal the difference between the groups. The values of $p < 0.05$ were considered statistically significant in the interpretation of the results. The results are presented as mean ± standard deviation (SD) or min-max.

RESULTS

Application of amyloid-B to the cells significantly increased Bax activity. Inula viscosa (IV) and curcumin application reduced this increase (Figure.1).

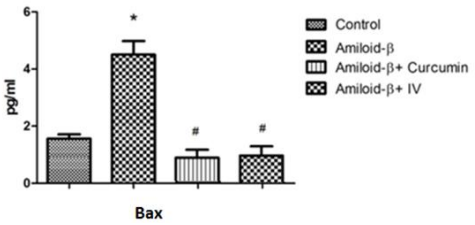

Figure 1. The effect of inula viscosa (Amiloid-β+IV) and curcumin application on Bax activity.
Statistical analysis: Student's t test. (* According to control P <0.05. # According to amyloid-B).

Application of amyloid-B to cells significantly increased Bcl-2 activity. Inula viscosa (Amiloid-β+IV) and curcumin applications reduces this increase (Figure. 2).

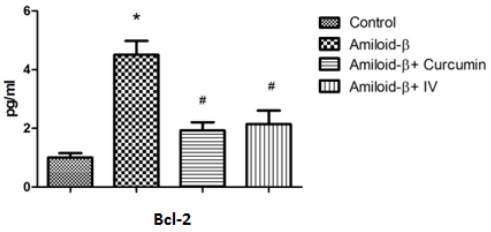

Figure 2. The effect of Inula viscosa and curcumin application on Bcl-2 activity (n = 6).
Statistical analysis: Student's t test. (*According to control, P <0.05. # According to amyloid-B).

Administration of amyloid-B to cells significantly reduced MDA activity. Inula viscosa (Amiloid-β+IV) and curcumin application increased this increased significantly (Figure 3).

Application of amyloid-B to cells significantly reduced Glutathione reductase activity. Inula viscosa (IV) and curcumin application increased this increased significantly (Figure.4).

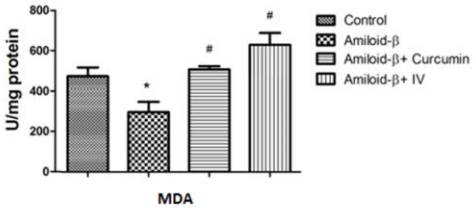

Figure 3. The effect of Inula viscosa and curcumin application on MDA activity (n = 6).

Statistical analysis: Student's t test. (*According to control, P <0.05. # According to amyloid-B).

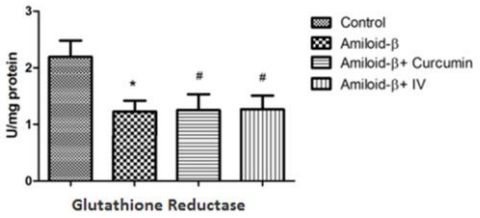

Figure 4. Effect of Inula viscosa and curcumin application on Glutathione reductase activity. Student's t test. (*According to control, P <0.05. # According to amyloid-B).

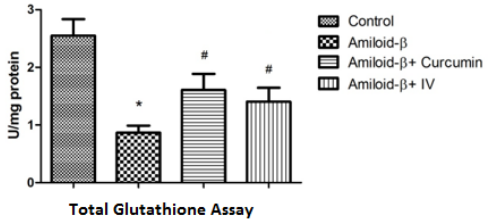

Figure 5. The effect of Inula viscosa and curcumin application on total glutathione activity.

Statistical analysis: Student's t test. (* According to control, P <0.05. # According to amyloid-B).

Application of amyloid-B to cells significantly reduced total glutathione activity. Inula viscosa (IV) and curcumin application increased this increase significantly (Figure.5).

In our study, the viability and proliferation of the cell decreased in U87 cells treated with amyloid-B compared to the control group, but Inula viscosa (IV) and Curcumin increased cell viability and proliferation when compared with the group treated with amyloid-B (p <0.05), (Figure.6).

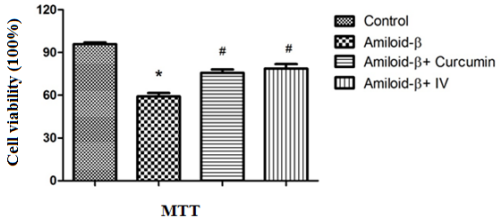

Figure 6. The effect of experimental groups on cell proliferation.

a: p <0.05 according to the control(* According to control, P <0.05. # According to amyloid-B).

DISCUSSION

In this study, we aimed to investigate the effects of Inula viscosa and curcumin on Alzheimer's disease cell line model U87 (glioblastoma astrocytoma). First of all we aimed to evaluate cell growth and / or cell death indirectly by MTT assay. MTT method was used to examine the effects on cell proliferation and viability, in order to investigate the effects on the antioxidant defense system, Total Glutathione amount, Malondialdehyde (MDA), glutathione reductase activities were determined and Bax, Bcl-2 protein levels were examined by ELISA method to examine its effect on apoptosis. Reactive oxygen species (ROS), including hydroxyl radicals (OH.), Superoxide anion and hydrogen peroxide (H2O2), are considered to be important in the formation of cancer and various diseases by acting on cell metabolism. Excessive ROS production and increased oxidative stress have been reported to be associated with various degenerative diseases such as atherosclerosis, Alzheimer's disease, and Parkinson's disease. Reactive oxygen species (ROS) are created during aerobic cellular reactions and are effectively cleansed by the detoxification defense system of the ROS cell, such as Malondialdehyde (MDA), Glutathione reductase and total Glutathione activity, to maintain redox homeostasis. However, when ROS production exceeds cell detoxification capacity, overproduced ROS can directly replace lipid, protein, or DNA and signal transduction pathways, resulting in an irreversible oxidative modification [20-25].

Based on our data, amyloid-B administration significantly reduced MDA activity compared to control in U87 cells. This decrease in inula viscosa and curcumin application increased significantly (P <0.05 according to the control). Amyloid-B

administration to cells significantly reduced glutathione reductase activity. Inula viscosa and curcumin application significantly increased this decrease (P <0.05 compared to control).In another study, age-related memory disorders were associated with decreased brain and plasma antioxidant mechanisms. Intracellular Glutathione, which has an antioxidant function, has been shown to decrease with increasing age in brain regions of different animal models. In a study on transgenic mice with amyloid precursor protein mutation, it was understood that oxidative stress could not explain AD pathology alone. The interaction of senile plaques and Aβ 42 with free radicals suggests that they may play a role in the pathogenesis of AD by causing oxidative stress. Amyloid β 42 enters the lipid layer and begins lipid peroxidation[26,27]. Two different Cu (II) complexes of Curcumin were synthesized by using different solvents. CT- DNA interactions with both complexes were investigated and It was determined that it interacts with CT-DNA in both complexes. In addition, complexes were found to show better cytotoxicity than cisplatin when the cytotoxicity examination was performed on the A549, MCF-7 ve HCT-116 tumors cell line under the same experimental conditions. Another important enzyme in the antioxidant defense system is the GPx enzyme. In Alzheimer's patients, while glutathione reductase and GPx activities were found to be elevated in the hippocampus, amygdala and piriform cortex, GPx activity did not change in measurements of the frontal, temporal and cerebellar cortex regions in the same individuals. In another study, they emphasized that one of the factors involved in Aβ toxicity is H2O2 and therefore the protective function of CAT enzyme activity in cells is important. PC12 cells resistant to Aβ toxicity were identified, and it was found that resistant cells contained high amounts of CAT and GPx in these studies[28-30].

Bcl-2 and Bax are members of the Bcl-2 family. Anti-apoptotic effects bcl-2 protein family in many cancer types levels were determined to be induced[33]. To our knowledge, the present study is the first study in the literature to show the increase in the expression of Bax, and Bcl-2 protein in U87 cell lines after inula treatment. In this study, inula significantly increased the proapoptotic proteins (Bax and Bcl-2). The activity of Bcl-2 was observed to decrease significantly. In addition, the current study demonstrated that inula is as effective to induce apoptosis. Bcl-2 and Bax are members of the Bcl-2 family. Anti-apoptotic effects bcl-2 protein family in many cancer types levels were determined to be induced[31,32]. The results of the present study revealed that inula promoted Bax expression and reduced Bcl-2 protein expression, in U87 cells. In this study, the complex significantly increased the pro-apoptotic proteins (Bax). The activity of Bcl-2 was observed to decrease significantly. In addition, it reduced oxidative stress caused by amyloid-B and decreased caspase activation by proapoptotic protein. In conclusion, our study revealed that Inula viscosa and Curcumin applications may be beneficial for Alzheimer's disease.

Yazar Katkıları: Çalışma konsepti/Tasarımı: AA; Veri toplama: AA, GÖ; Veri analizi ve yorumlama: GÖ; Yazı taslağı: AA; İçeriğin eleştirel incelenmesi: GÖ; Son onay ve sorumluluk: AA, GÖ; Teknik ve malzeme desteği: AA, GÖ; Süpervizyon: AA; Fon sağlama (mevcut ise): yok.
Etik Onay: Ticari olarak temin edilebilen bu insan hücre dizisi (U87 (insan astrositom hücre dizisi) üzerindeki Etik Çalışmalar, bir etik kurul onayı gerektirmez.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
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Author Contributions: Concept/Design : AA; Data acquisition: AA, GÖ; Data analysis and interpretation: GÖ; Drafting manuscript: AA; Critical revision of manuscript: GÖ; Final approval and accountability: AA, GÖ; Technical or material support: AA, GÖ; Supervision: AA; Securing funding (if available): n/a.
Ethical Approval: Studies on this commercially available human cell line (U87 (human astrocytoma cell line) do not require an ethics committee approval.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
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