JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2021; 3: 213- 217
https://doi.org/10.30621/jbachs.990503
RESEARCH ARTICLE

IN VITRO STUDIES ON THE PROTECTIVE EFFECT OF TANNIC ACID OF U87 CELLS INDUCED BY BETA-AMYLOID

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Received: 03.09.2021; **Accepted:** 13.09.2021; **Available Online Date:** 20.09.2021

Cite this article as: Ozbolat G, Alizade A. In Vitro Studies on The Protective Effect of Tannic Acid of U87 Cells Induced by Beta-Amyloid. J Basic Clin Health Sci 2021; 3: 213- 217.

ABSTRACT

Background: While the prevalence of Alzheimer's disease continues to increase throughout the twentieth century, the cause and pathology of the disease are not well understood and scientists are seeking treatments for the disease. Tannic acid can be used effectively to treat Alzheimer's disease and seems to be one of the alternative therapeutic strategies in medicine. In this study, we aimed to investigate the effects of tannic acid 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: In the study; three groups were formed as the control group, the Aβ group, and the Aβ + tannic acid group obtained by adding tannic acid to the Aβ group. Firstly, the cytotoxic potential of TA in U87 cells was investigated by the colorimetric MTT (3-4,5-dimethyl-thiazolyl-2,5 diphenyltetrazolium bromide) test. To determine the antioxidant status in the cell line treated with tannic acid, to examine the effects of total oxidant status (TOS), superoxide dismutase (SOD), total antioxidant status (TAS) and catalase (CAT) activities, were measured by the ELISA method.

Results: In our study, the viability and proliferation of the cell decreased in U87 cells treated with amyloid-B compared to the control group, but tannic acid increased cell viability and proliferation when compared with the group treated with amyloid-B. When compared to the control group, the TAS, SOD, and CAT levels were significantly decreased in the U87 cell line exposed to Aβ; TOS levels were found to increase significantly.

Conclusions: In *in vitro* experiments, we determined that tannic acid has a protective effect by increasing antioxidant parameters in the amyloid beta-induced cell line.

Keywords: Tannic acid, Amyloid-beta, U87, Alzheimer's

INTRODUCTION

Alzheimer's disease (AD) is a chronic disease characterized by insidious cognitive impairment. Alzheimer's disease takes place among the most common causes of dementia (1,2,3). No approved drugs can either revert or arrest the progression of AD. Effective treatments are greatly needed. The amyloid-cascade hypothesis has, since its introduction in 1991, provided the dominant framework for understanding the pathogenesis of AD. Aβ peptides are at the root of the pathology of Alzheimer's disease (AD), one of the devastating diseases of an increasingly aging society (4,5).

The 37–43 amino acid amyloid β-peptide (Aβ) is produced by the proteolytic cleavage of a membrane protein called β-amyloid precursor protein (APP).The most prominent targets for therapeutic intervention include the inhibition of APP (6-9).Age-related memory impairments have been depicted to be associated with decreased antioxidant mechanisms in the brain and plasma. The interaction of Aβ42 plaques with free radicals and the oxidative stress as a result of it may play a pivotal role in AD pathogenesis (10,11).

Tannic acid has antimutagenic and antioxidant activities. Tannic acid (TA) is a naturally occurring plant-derived polyphenol found in several plants. TA has been well studied for its antimutagenic, anticarcinogenic and antioxidant activities. Notably, the high content of polyphenols present in some plants may positively influence the pathology of AD (12-15). It is thought that tannic acid could be utilized effectively for the treatment of Alzheimer's Disease and we anticipated that it could be evaluated among alternative treatment strategies in the field of medicine.

MATERIALS AND METHODS

Working Groups

Control group: 50 µl of saline was added to the medium of differentiated U87 cells and incubated for 48 hours. Dimethyl Sulfoxide (DMSO) was added to the medium for 24 hours in the incubator.

Aβ group: Aβ1-42 was added to the medium of the differentiated U87 cells at a concentration of 5 µM and incubated for 48 hours. After, DMSO was added to the medium of the cells.

Aβ + tannic acid group: The differentiated U87 cells were added to the culture medium at a final concentration of 5 µM, 48 hours after Aβ1-42 application. 300 µmol/L TA was added and the mixture was incubated.

Cytotoxicity: At the end of the 48 hours, U87 cell line cells were transferred to 96 well plates with 100 µL of cell suspension per well and 10µL of MTT solution was added. The MTT solution was prepared by dissolving it in PBS as 5 mg / mL and transferring it to a bottle with sterile filtration. Afterward, 100 µL of DMSO was added to each well and the well was kept in a CO2 incubator at 37 ° C for 10 minutes at 37 ° C to dissolve the formazan crystals formed by MTT. Their cytotoxicity levels were calculated employing the following formula.

1- (the absorbance of the test pad - the absorbance of the control pad / the absorbance of the test pad) x 100 the concentration with 50% cytotoxic effect relative to the control was accepted as the cytotoxic dose.

ELISA (Enzyme-Linked Immunosorbent Assay) Test: To evaluate the antioxidant status in the cell line treated with TA, SOD, CAT, TOS, and TAS activities were measured based on ELIZA method to examine its effect on the apoptosis process. Experimental protocols of ELIZA kits vary for each kit.

RESULTS

MTT Test

The effect of TA human brain cell line U87 (glioblastoma astrocytoma) on cell viability and proliferation was also investigated. Following the application of Aβ1-42, the number of viable cells decreased in all the groups except for the control group ($p < 0.05$). After the application of the TA on U87 cells, there was a significant statistical increase in the number of cells in the Aβ + TA group ($p < 0.05$) (Fig.1). According to these results, it was determined that the TA increased cell survival in the in vitro AH model.

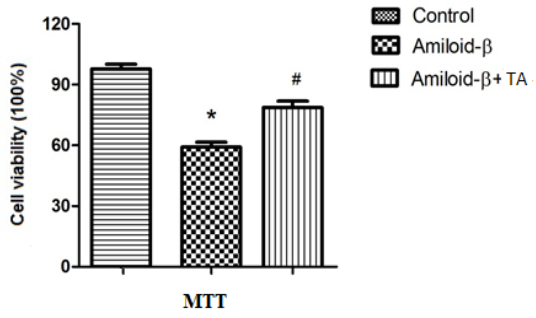

Figure 1. Cell viability of the groups with TA added versus Aβ1-42 application.

CAT Activity

Our findings indicated that CAT enzyme level (pg/ml) significantly decreased after Aβ1-42 administration in all the groups except for the control group ($p < 0.05$). We observed a significant statistical increase in CAT enzyme level in the Aβ + TA group after the TA was applied on the U87 cell ($p < 0.05$) (Figure 2). Moreover, the results implied that the TA triggers the antioxidant enzyme mechanism.

SOD Activity

The application of amyloid-B to the cells significantly decreased the SOD (U / mg protein) enzyme level in all the groups except for the control group ($p < 0.05$). The SOD enzyme level increased significantly in the Aβ + TA group after Timoquinone was applied on the U87 cell ($p < 0.05$) (Figure 2). It has been determined that TA triggers the antioxidant enzyme mechanism.

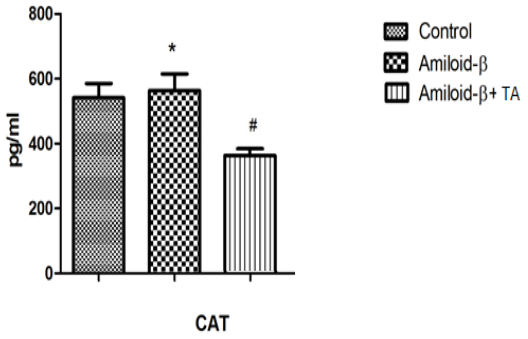

Figure 2. CAT enzyme (pg / ml) level values in in vitro AH model cell lines.

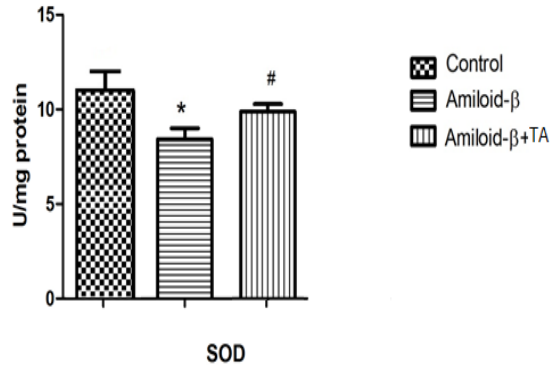

Figure 3. SOD enzyme (U/mg) level values in the in vitro AH model cell lines

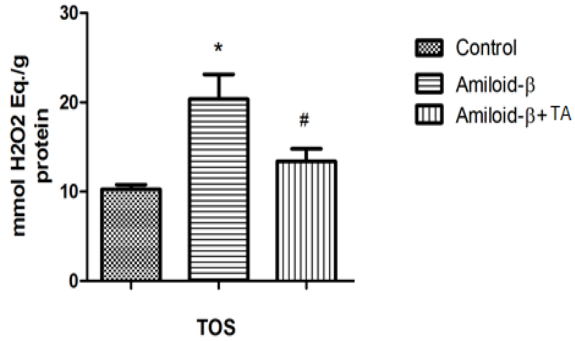

Figure 4. TOS level values in the in vitro AH model cell lines

TOS Activity

Our findings indicated that TOS enzyme level significantly decreased after Aβ1-42 administration in all the groups except for the control group ($p < 0.05$). We observed a significant statistical increase in TOS enzyme level in the Aβ + TA group after TA was applied on the U87 cell ($p < 0.05$) (Figure 4).

TAS Activity

Our findings indicated that TOS enzyme level significantly increased after Aβ1-42 administration in all the groups except for the control group ($p < 0.05$). We observed a significant statistical decrease in TOS enzyme level in the Aβ + TA group after TA was applied on the U87 cell ($p < 0.05$) (Figure 3).

DISCUSSION

Alzheimer's disease (AD) is characterized by the accumulation in the brain of extracellular amyloid β (Aβ) plaques. AD is one of the leading causes of dementia affecting millions of people worldwide.Oxidative stress is the most important factor in the pathogenesis of Alzheimer's and occurs when reactive oxygen and nitrogen species increase, or the antioxidant defense system decreases. TAC represents DNA oxidation and provides data on the interpretation of the pathogenesis and treatment of AD (16-18).

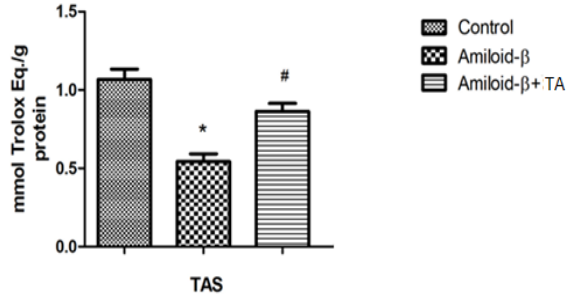

Figure 5. TAS level values in the in vitro AH model cell lines

In our study, we analyzed TOS and TAS values to evaluate the total oxidant and antioxidant effect. We observed a significant statistical decrease in TOS and TAS enzyme level in the Aβ + TA group after TA was applied to the U87 cell. Arslan et al. showed TAC level was significantly increased after farnesene exposure also; there was a significant difference in TOS levels compare to β-amyloid applied cell culture (19,20).

In some studies, a difference has been reported between AD and controls in terms of SOD, yet it was not statistically significant. On the contrary, other studies have shown that the SOD level increased significantly. According to the obtained data herein, amyloid-B administration significantly reduced SOD activity on human brain cell line U87 (glioblastoma astrocytoma) cells compared to the controls. We found that the TA application significantly augmented this decrease and implied an antioxidant effect. In the studies in the literature, different results were obtained with the change of SOD expression and activity in patients with Alzheimer's Disease. On top of the findings showing a significant decrease in SOD activity in the hippocampus, there are researches indicating no differences in SOD activity compared to controls. It has been determined that TA triggers the antioxidant enzyme mechanism (21-23).

In addition, the increase in the level of antioxidant enzyme CAT in the in vitro AD group compared to the control and its decrease with TA application, along with the increase in the free radicals in AD, is indicative of an increase in enzymatic antioxidant activation in order to eliminate the toxic effects of these radicals. The oxidative stress induced by Aβ and the antioxidant defense mechanism associated with them are accepted as basic mechanisms in the etiology and pathogenesis of Alzheimer's. As a result of the disruption of the balance between antioxidant and oxidant systems, free radicals emerge. It protects the cell against oxidative stress by eliminating these radicals, which are SOD, GSH-Px, CAT; they are endogenous enzymes involved in the antioxidant defense mechanism (23-26).

U87 supports the antioxidant defense system in the Alzheimer's cell line model, significantly increasing the levels of TAS, CAT and SOD while decreasing the level of TOS. TA represented antioxidant activity and decreased neurodegeneration due to apoptosis. Therefore, the treatment with TA could be suggested as a novel therapeutic approach to the prevention and symptomatic treatment of Alzheimer's.

Author contributions: All authors discussed the results and contributed to the final manuscript. G.Ö. and A.A.carried out the experiment. G.Ö and A. A. wrote the manuscript.

Funding statement: All funding of the study was supported by the authors.

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