YAKIN DOĞU ÜNİVERSİTESİ
DESAM ARAŞTIRMA ENSTİTÜSÜ

Alzheimer Araştırmalarında Deneysel Modeller: *In vitro* Modeller

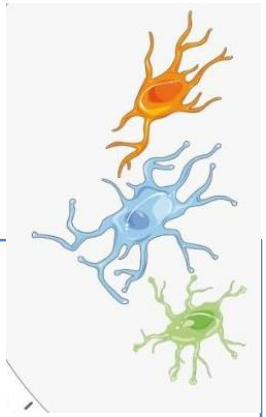
Dr. Dyt. Müjgan KUŞİ

Yakın Doğu Üniversitesi

Sağlık Bilimleri Fakültesi

Beslenme ve Diyetetik Bölümü

In vitro Alzheimer Modeli



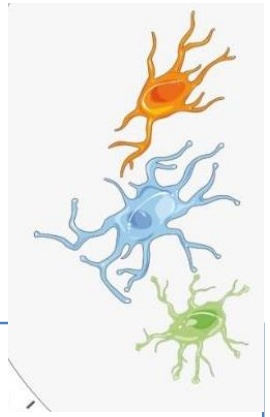
- **Kanser hücre hattı modelleri**
  - SH-SY-5Y
  - **Düşük maliyetli**
  - Kültürleri, iPSC ve NPC'lere kıyasla oldukça **kolay ve iyi tanımlı**
  - Kanser hücrelerinden geliştirilen modeller, sağlıklı olgun nöronlardan yapısal farklılıklar göstermektedir

- **İndüklenebilir pluripotent kök hücre modelleri**
  - Alzheimer ilişkili mutasyonları taşıyan bireylerden alınan hücrelerden elde edilen iPSC'ler sayesinde **genetik manipülasyonlara ihtiyaç duyulmaz**

  Bir kişiden elde edilen iPSC'ler beyinde bulunan tüm hücre tiplerine farklılaşma kapasitesi taşır
  - iPSC kültürlerinin geliştirilmeleri ve devamlılıklarının sağlanmasına yönelik standardize **protokoller eksik**
  - Kanser hücre hatları ve NPC kökenli kültürlere nazaran **daha maliyetlidir**

Journal of Health Institutes of Turkey 2023;6(1):54-77

In vitro Alzheimer Modeli



- **Nöronal öncül hücre modelleri**
  - NPC'ler, nöronlara farklılaştırılana dek AH ilişkili proteinleri düşük seviyede ifade ederler
  - Deneysel serebral organoid modellerine göre **görece daha kolay ve düşük maliyetli** olup
  - Beynin farklı bölgelerinde bulunan farklı tiplerde nöronların farklılaştırılmalarına yönelik **detaylı protokoller mevcut değildir**

- **Serebral organoidler**
  - Farklı beyin bölgelerine özgü serebral organoidler **hücresel bileşimi, yapısal özellikleri ve moleküler süreçleri simüle etme kapasiteleri** ile modellenen beyin bölgesini yansıtmakta **daha başarılı**
  - Görece **yüksek maliyetlidir** ve yüksek seviyede **yetkinlik gerektirir**

Journal of Health Institutes of Turkey 2023;6(1):54-77

In vitro Alzheimer Modeli

- **β-amiloid**
  - Oksidatif stresi indukler
  - $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{25-35}$
- **Gen modifikasyonları**
  - Presenilin 1 (PSEN1), PSEN 2
  - Amiloid Prekürsör Protein (APP)

Regenerative Research 2013;2(2):8-13

*Alzheimer Deneysel Modelinde Hesperidin ve Narinjin Bileşenlerinin Nöroprotektif Etkisi

JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
2023, VOL. 42, NO. 4, 418–426
https://doi.org/10.1080/07315724.2022.2062488

Neuroprotective Effects of Hesperidin and Naringin in SK-N-AS Cell as an *In Vitro* Model for Alzheimer's Disease

Müjgan Kuşi[a], Eda Becer[b,c], Hafize Seda Vatansever[c,d], and Sevinç Yücecan[e]

[a]Faculty of Health Sciences, Department of Nutrition and Dietetics, Near East University, Nicosia, Mersin 10 Turkey; [b]Faculty of Pharmacy, Department of Biochemistry, Near East University, Nicosia, Mersin 10 Turkey; [c]DESAM Institute, Near East University, Nicosia, Mersin 10 Turkey; [d]Faculty of Medicine, Department of Histology and Embryology, Manisa Celal Bayar University, Manisa, Turkey; [e]Faculty of Health Sciences, Department of Nutrition and Dietetics, Lokman Hekim University, Ankara, Turkey

ABSTRACT

Objective: Hesperidin and naringin are flavonoids that are found in citrus fruits. Our aim was to create an *in vitro* model of Alzheimer's disease (AD) and to evaluate the neuroprotective effects of hesperidin and naringin in SK-N-AS and AD model cells.

Methods: $A\beta_{25-35}$ was used to create an AD model in SK-N-AS cells. The cytotoxicity of hesperidin and naringin was evaluated using MTT. β-amyloid, tau and α-synuclein distributions were analyzed using indirect immunoperoxidase staining to investigate the neuroprotective effects of hesperidin and naringin.

Results: The AD model was created by 1 µM of $A\beta_{25-35}$ for 48 hours after ThT staining. The intensity of β-amyloid was reduced through both hesperidin and naringin treatment in AD model cells. Both flavonoids significantly decreased the intensity of α-synuclein in SK-N-AS and AD model cells.

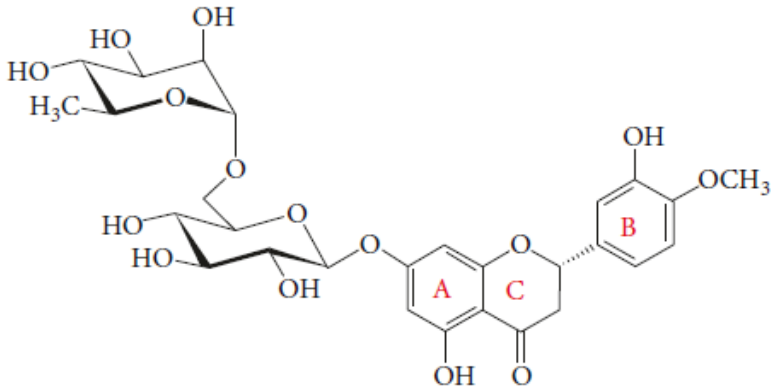
Conclusions: Hesperidin and naringin can be potentially used as neuroprotective agents. Naringin may be more effective than hesperidin in the accumulation of β-amyloid and tau proteins.

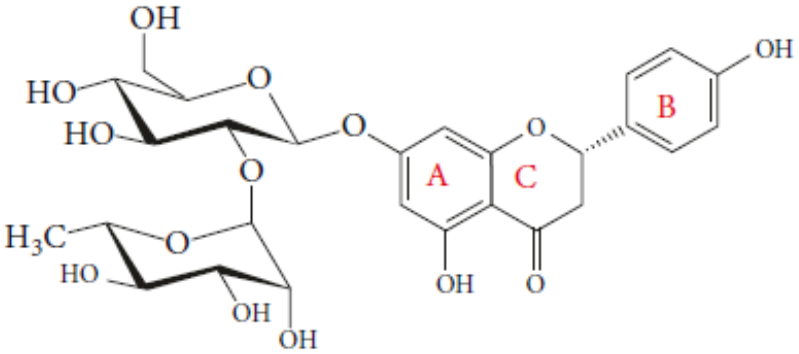
ARTICLE HISTORY
Received 22 February 2022
Accepted 1 April 2022

KEYWORDS
Alzheimer's disease; β-amyloid; Tau; Hesperidin; Naringin

Çalışmanın Amacı

SK-N-AS ve *In vitro* Alzheimer model hücrelerinde;





Hesperidin ve **Narinjin**

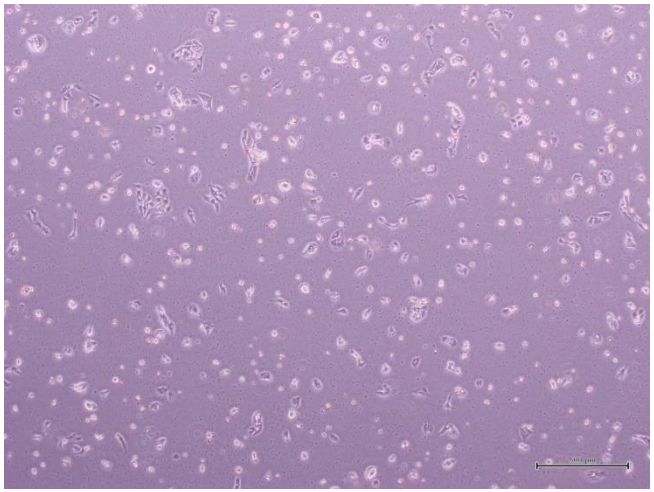
nöroprotektif etkilerinin araştırılması

Hesperidin

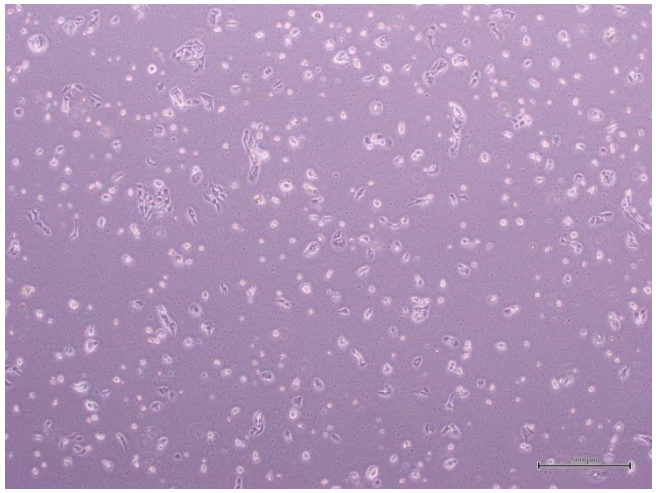
Narinjin

SK-N-AS Hücreleri

İnsan nöroblastom hücre hattı



Hücre Kültürü



DMEM (*Dulbecco's Modified Eagle Medium*)

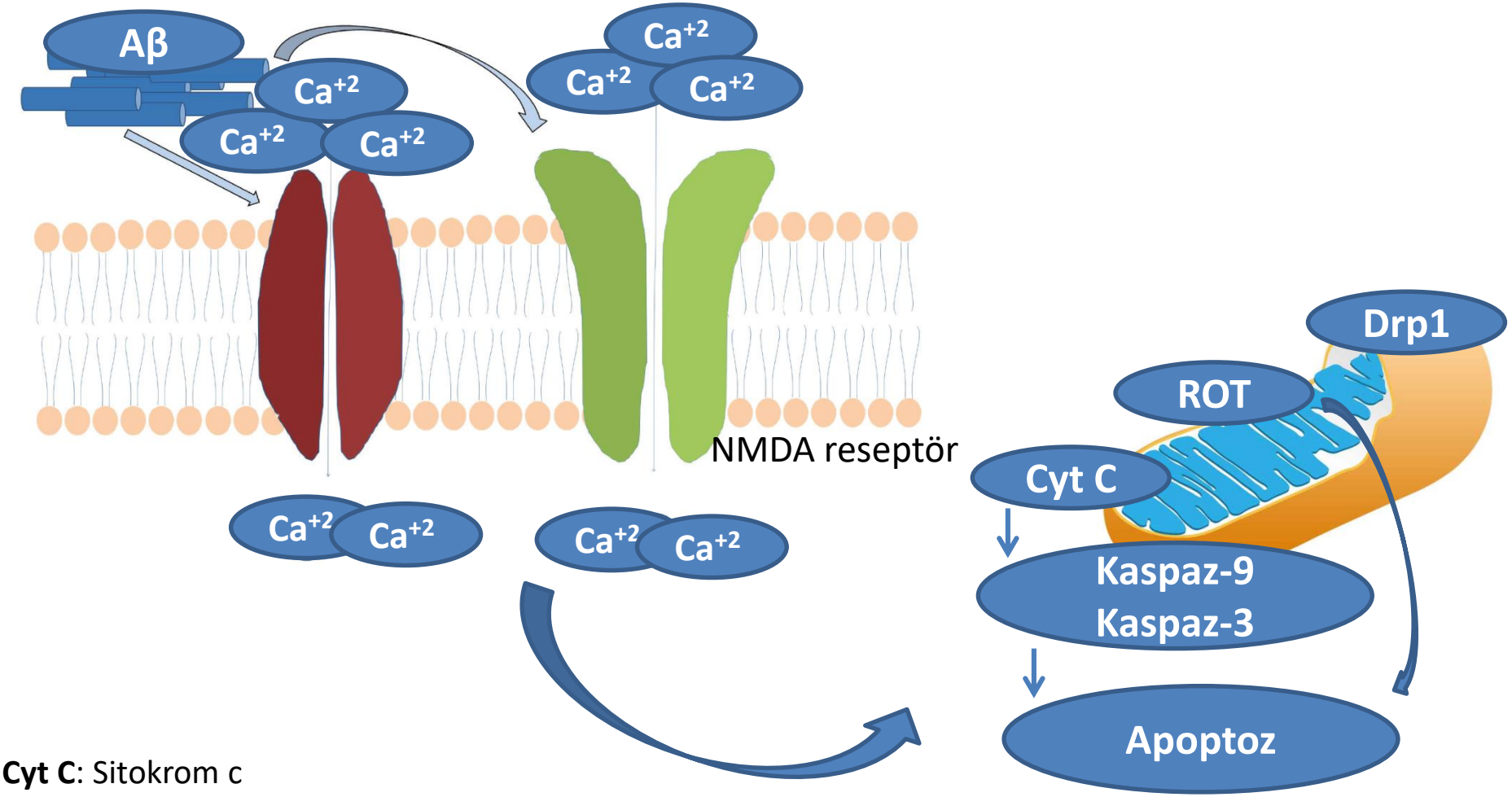
%10 FBS (Fetal Bovine Serum)

%1 Penisilin-streptomisin

%1 L-glutamin

Kültür vasatı 2 günde bir değiştirildi

Aβ'nın Nöron Üzerindeki Etkisi



Cyt C: Sitokrom c
Drp1: Dinamin bağımlı protein-1
NMDA: N-metil D-aspartik asit
ROT: Reaktif oksijen türleri

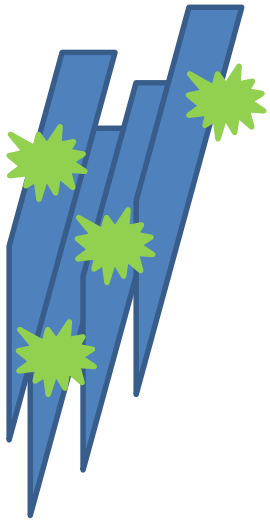
Reviews in the Neurosciences 2018;29(6):613-627
International Journal of Biological Sciences 2021;17(9):2181
International Journal of Alzheimer's Disease 2012;2012:1-7

In vitro Alzheimer Modeli

Amiloid β-peptit (25-35) ($A\beta_{25-35}$ peptit)

Model Kontrolü:

Tiyoflavin T (ThT)



In vitro Alzheimer Modeli

Amiloid β stok solüsyonu hazırlandı

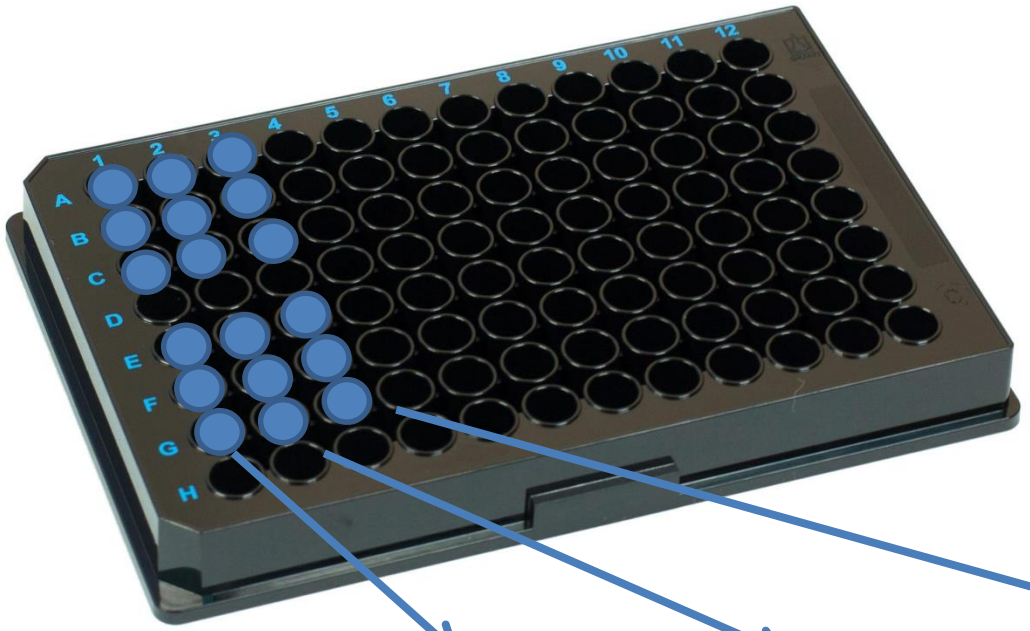
$A\beta_{25-35}$ peptit

+

Distile su

❖ 37^{o}C inkubatörde 5 gün agregasyona bırakıldı

In vitro Alzheimer Modeli



24 saat ve 48 saat

1 µM

5 µM

$A\beta_{25-35}$ peptit

Kontrol : 100 µL vasat

1 µM $A\beta_{25-35}$

5 µM $A\beta_{25-35}$

In vitro Alzheimer Modeli

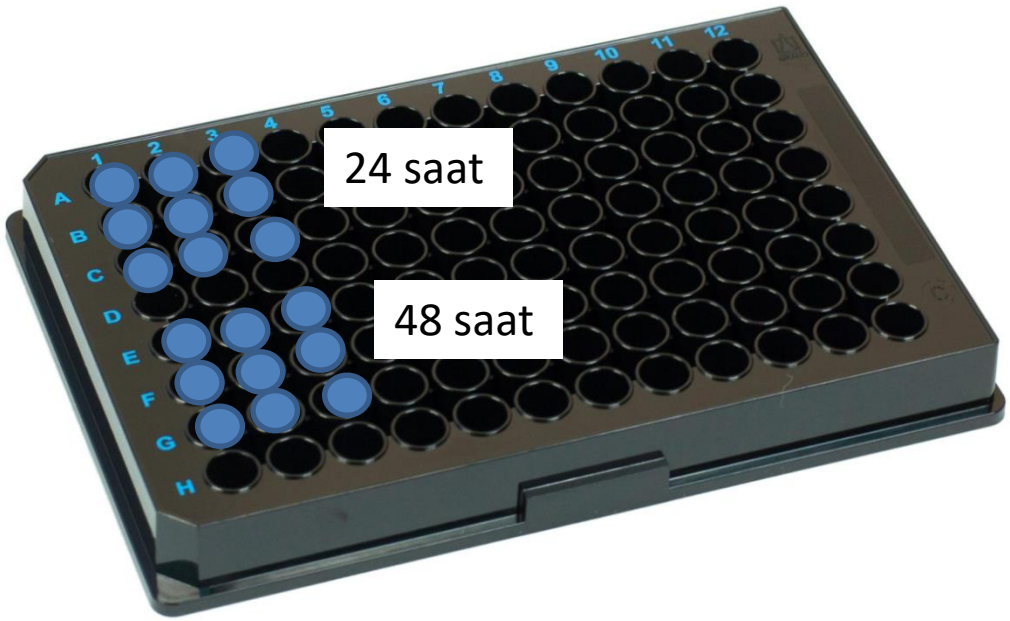
Model kontrolü

ThT + PBS çözdürülüp

Her göze 200 µL ekildi

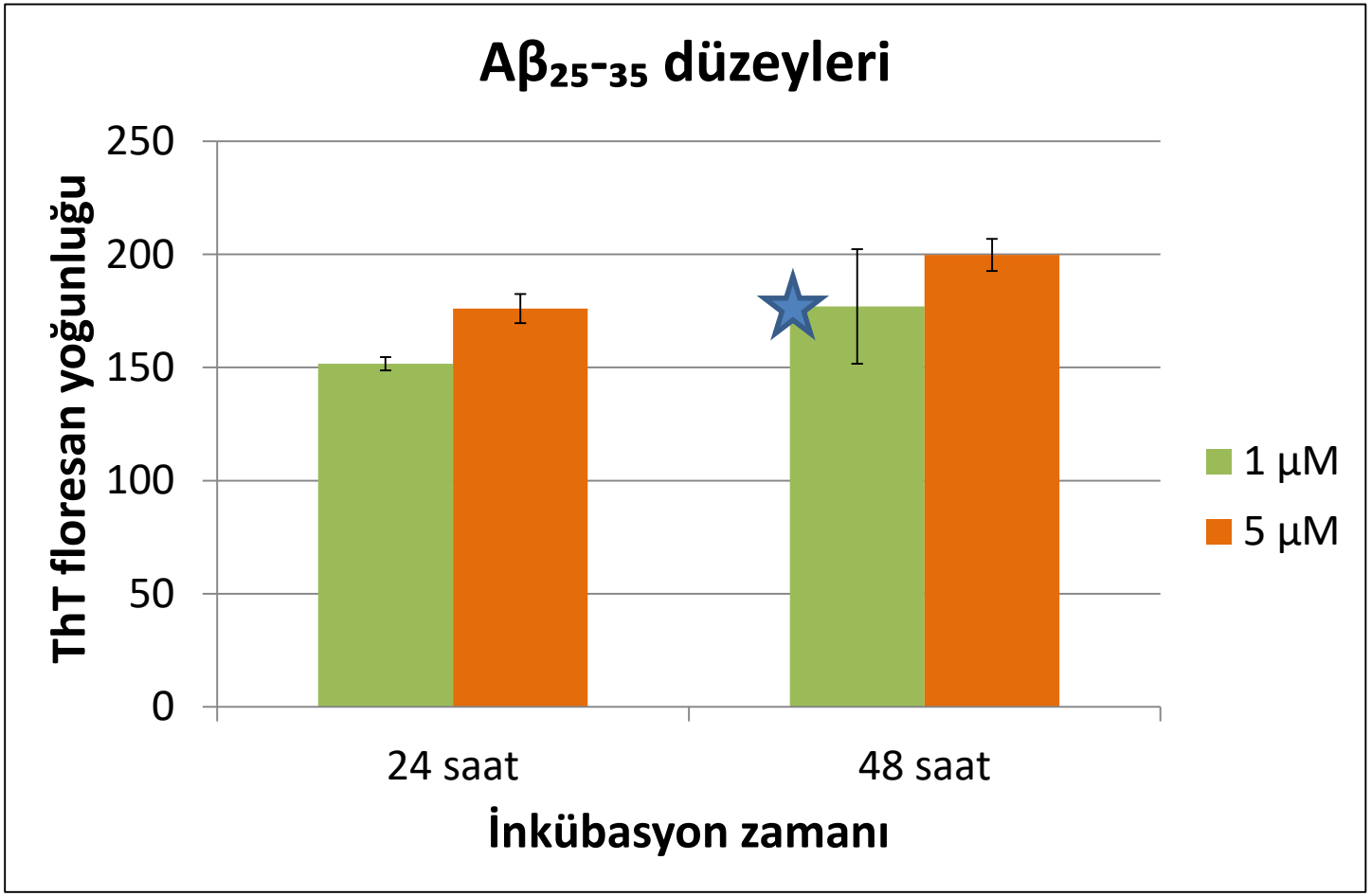
↓

60 ^{o}C'de 30 dakika inkübasyon

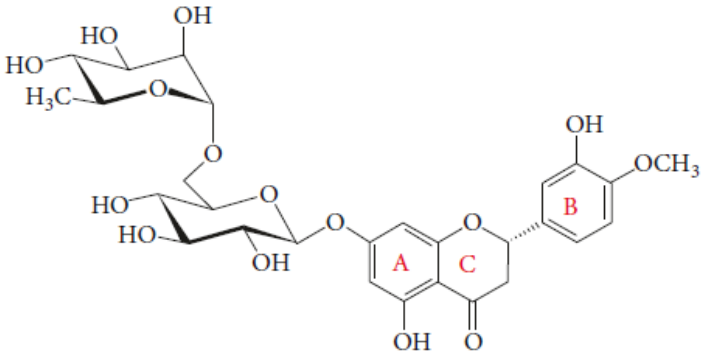


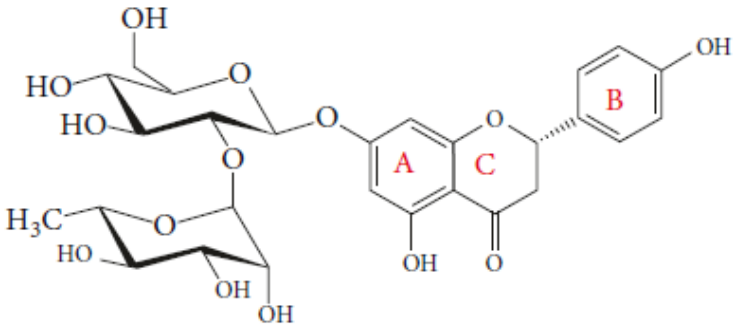
440 – 482 nm ölçüm yapıldı

In vitro Alzheimer Modeli



MTT Analizi





Hesperidin ve Narinjin

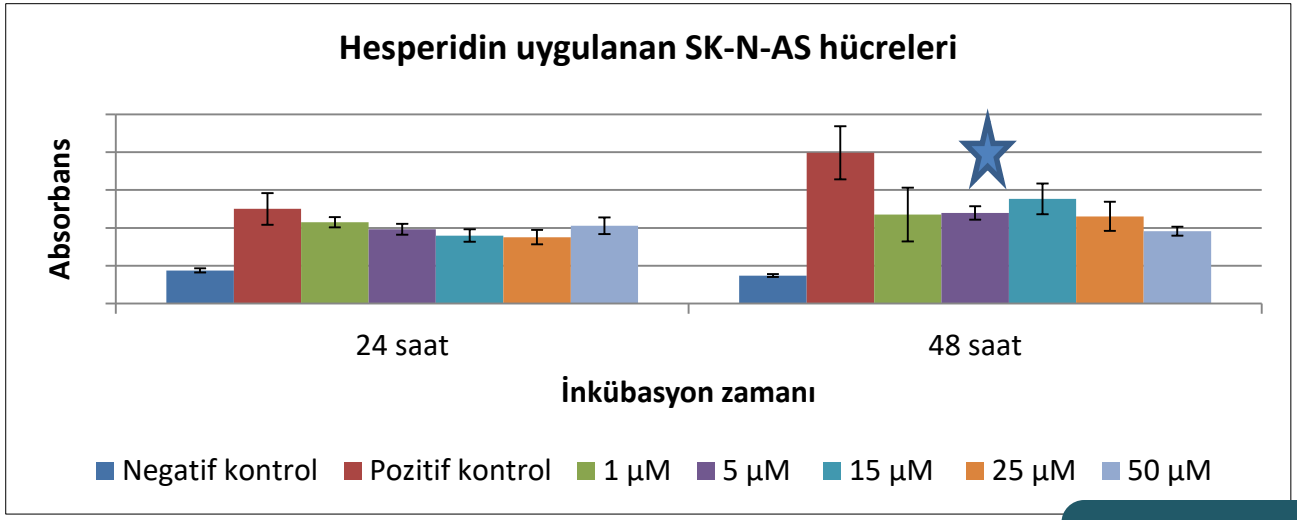
DMSO'da çözdürüldü

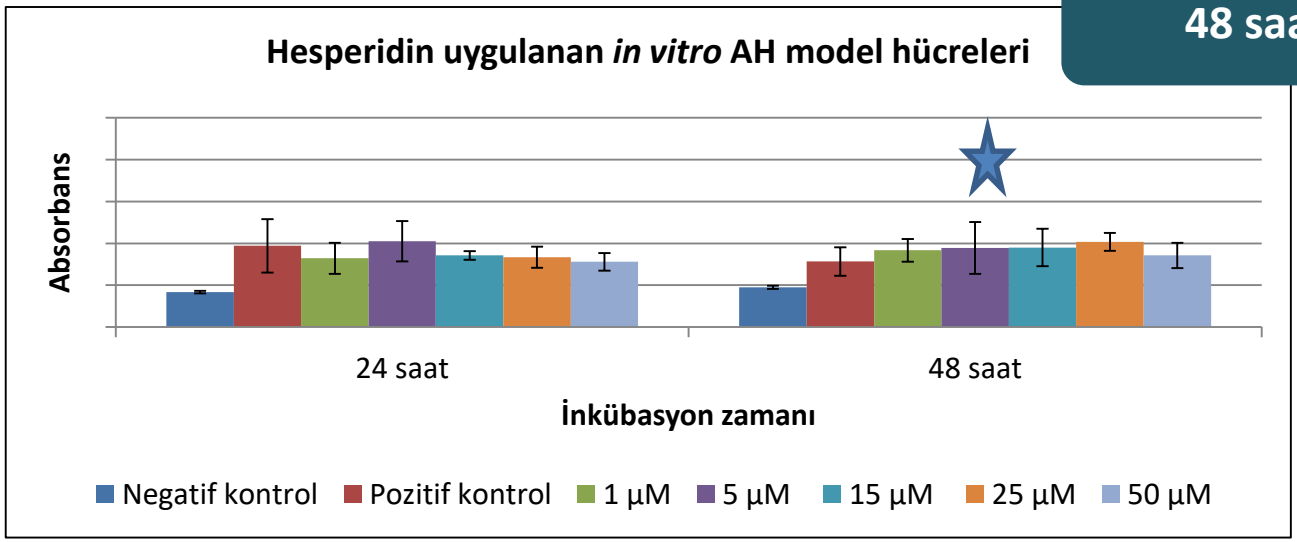
24 ve 48 saat

SK-N-AS ve *In vitro* Alzheimer hücreleri

1 µm, 5 µM, 15 µM, 25 µM, 50 µM

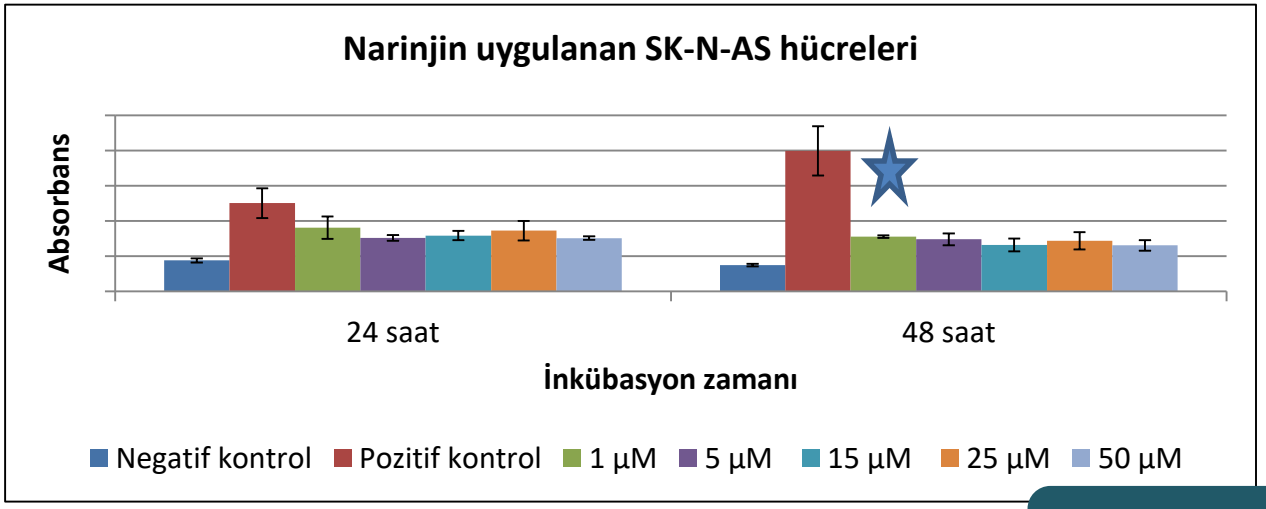
MTT Analiz Sonuçları



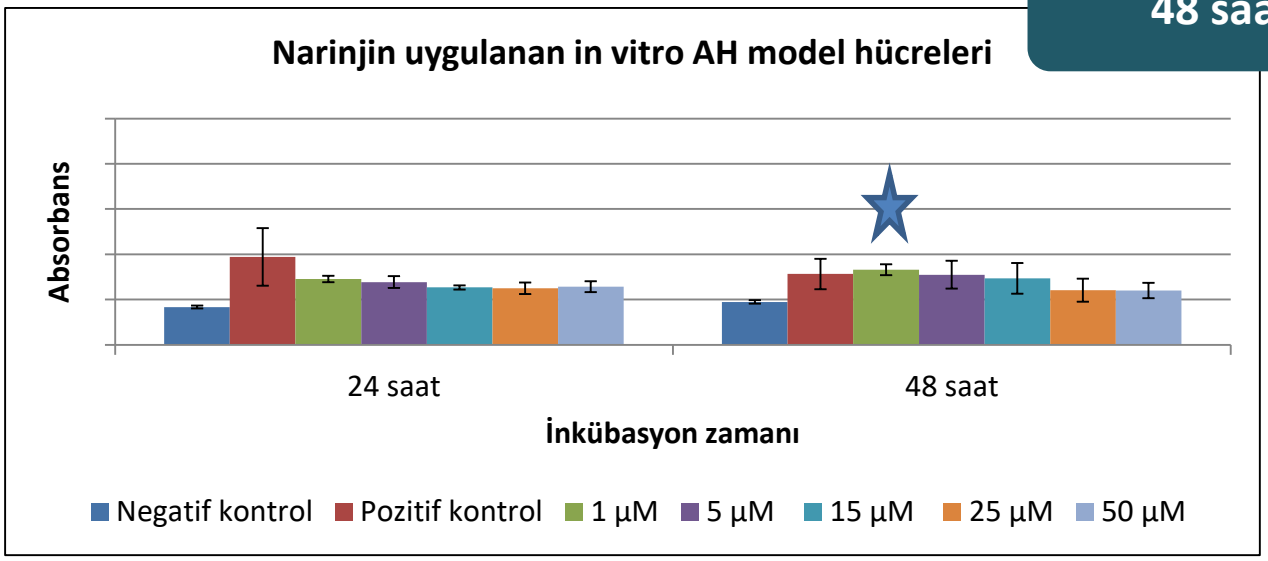


48 saat 5 µM

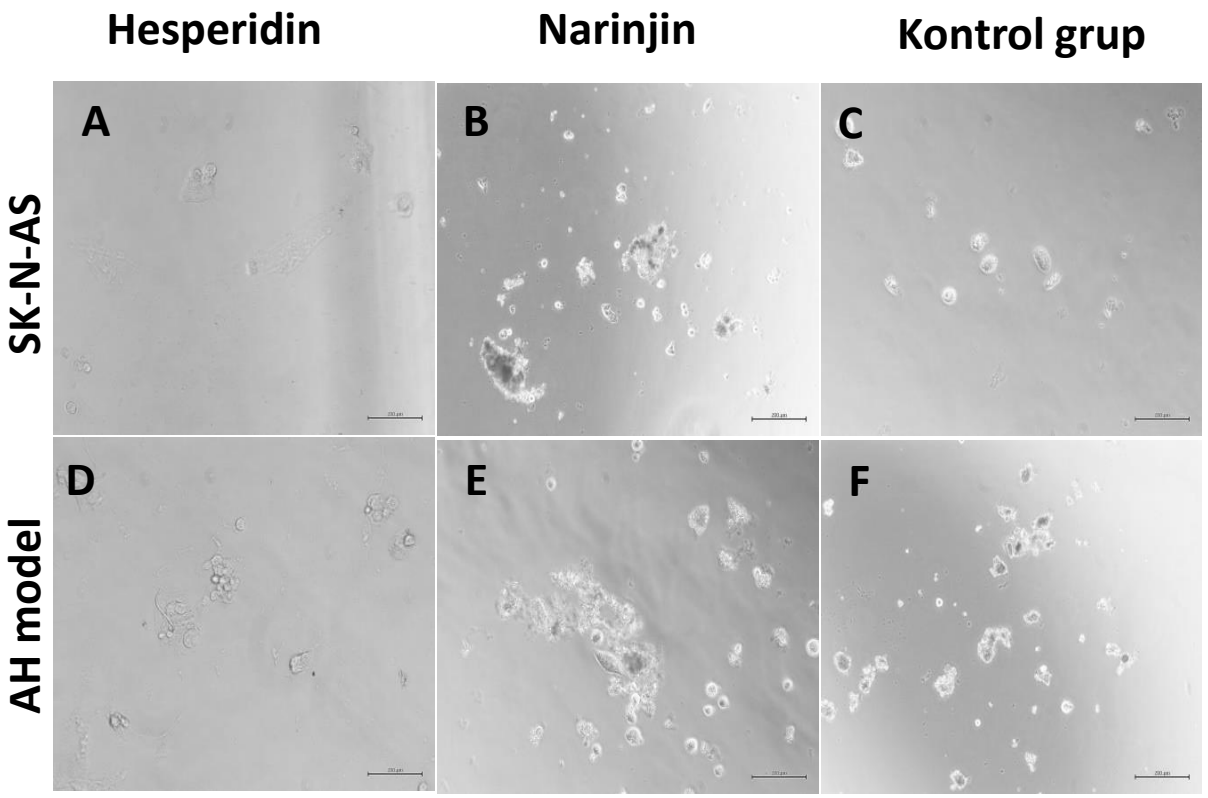
MTT Analiz Sonuçları

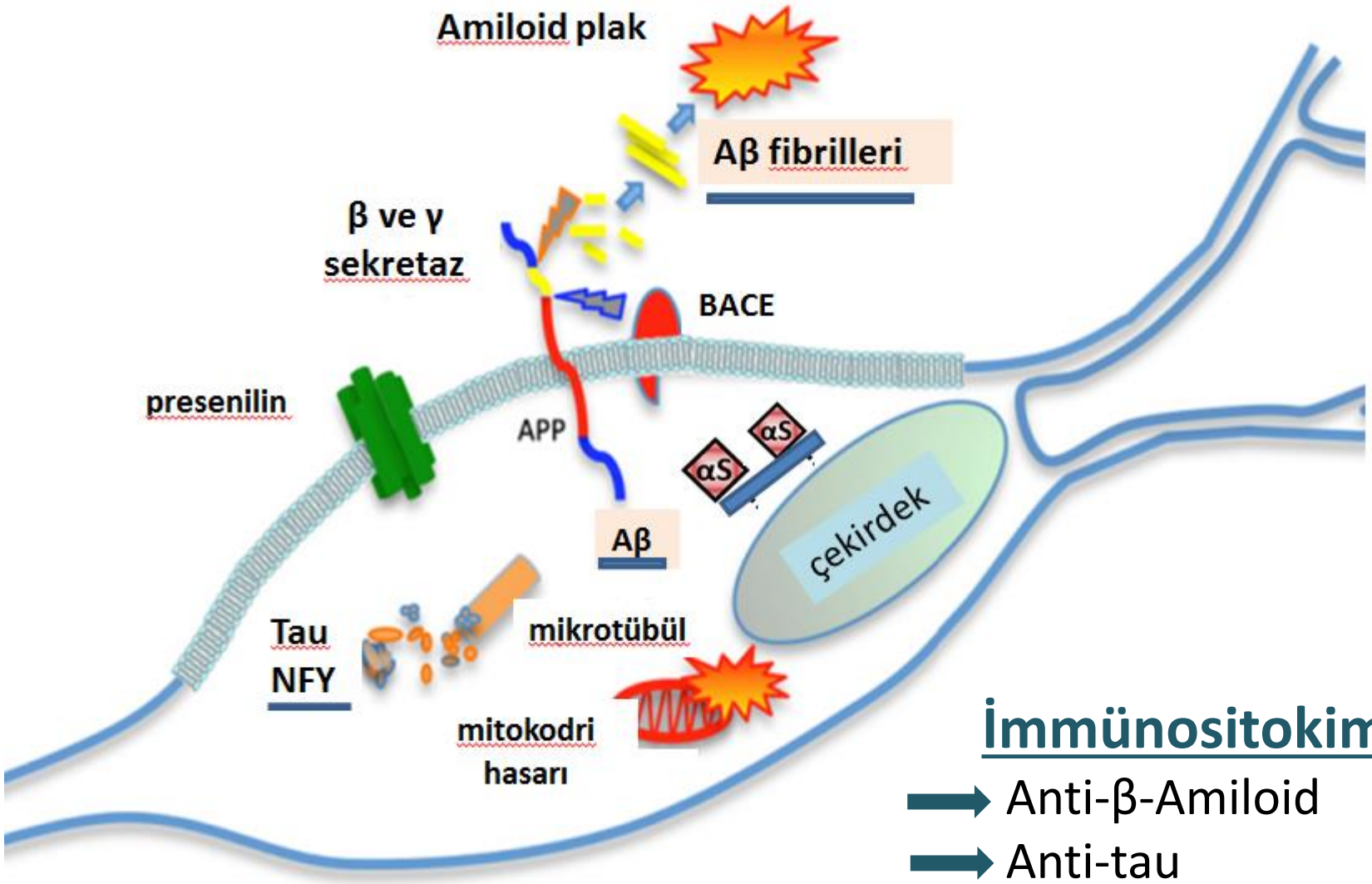


48 saat 1 µM



Hücre Morfolojisi





İmmünositokimya

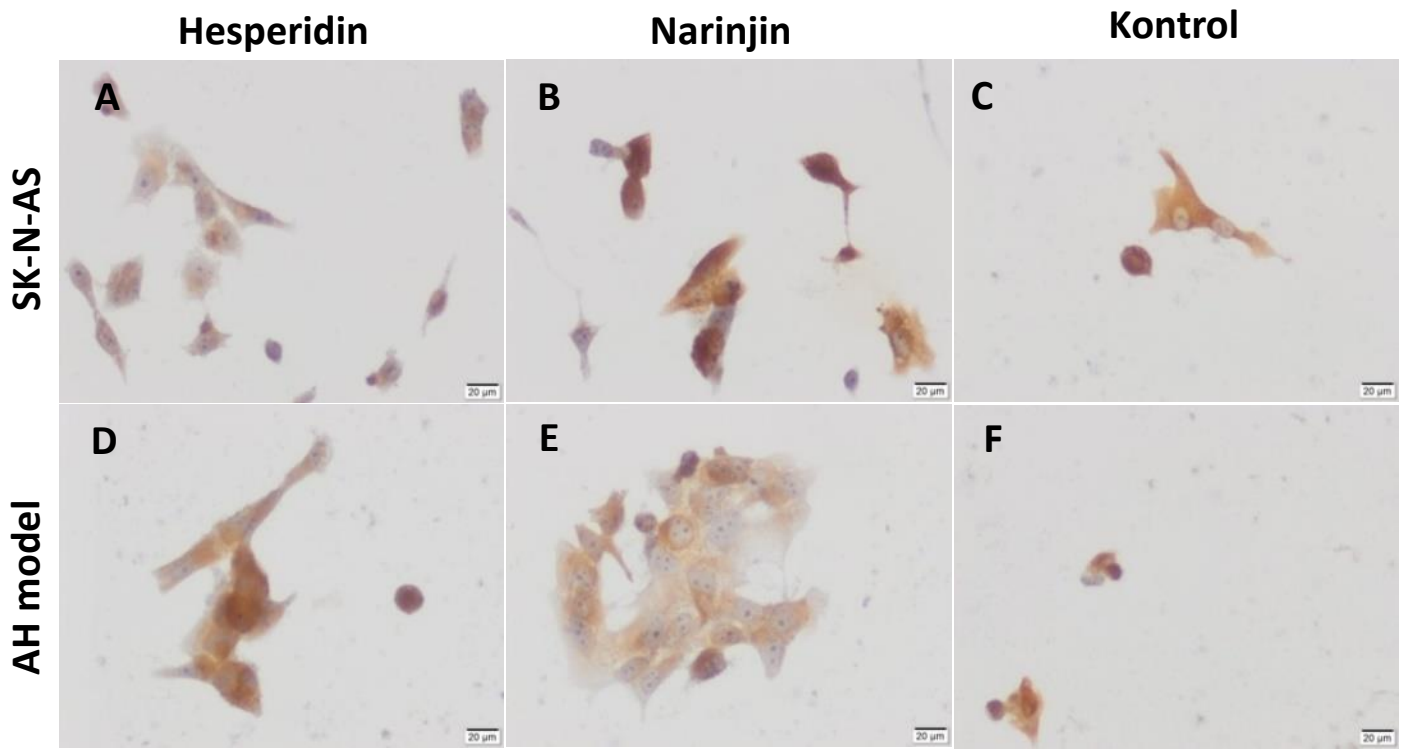
- Anti-β-Amiloid
- Anti-tau
- Anti-α-sinüklein

APP: Amiloid prekürsör protein
BACE: β-sekretaz

Immunity & Ageing 2012;9(1):1-8
Molecular Neurodegeneration 2019;14(1):1-19

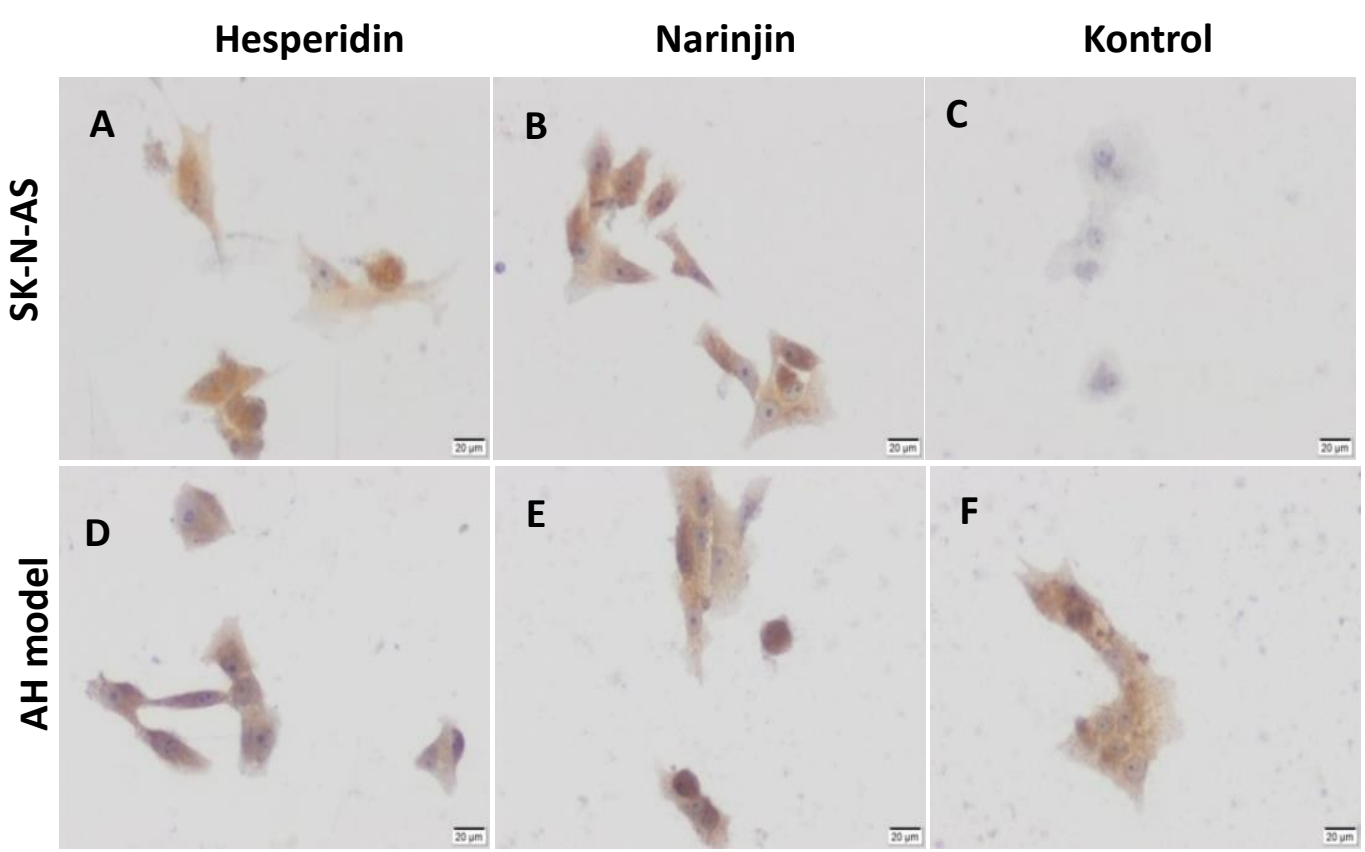
Tau

	Hesperidin	Narinjin	Kontrol grup	p[1]
SK-N-AS	286.16±20.22	309.09±37.37	338.33±53.64	>0.05
AH model	314.81±45.67	285.41±27.53	308.33±28.86	>0.05
p[2]	0.645	0.763	0.612	



β-Amiloid

	Hesperidin	Narinjin	Kontrol grup	p[1]
SK-N-AS	326.38±60.41[a]	276.31±18.32[a]	120.83±25.00[b]	<0.05*
AH model	293.05±52.77	280.83±28.33	305.41±48.36	>0.05
p[2]	0.619	0.998	<0.0001*	

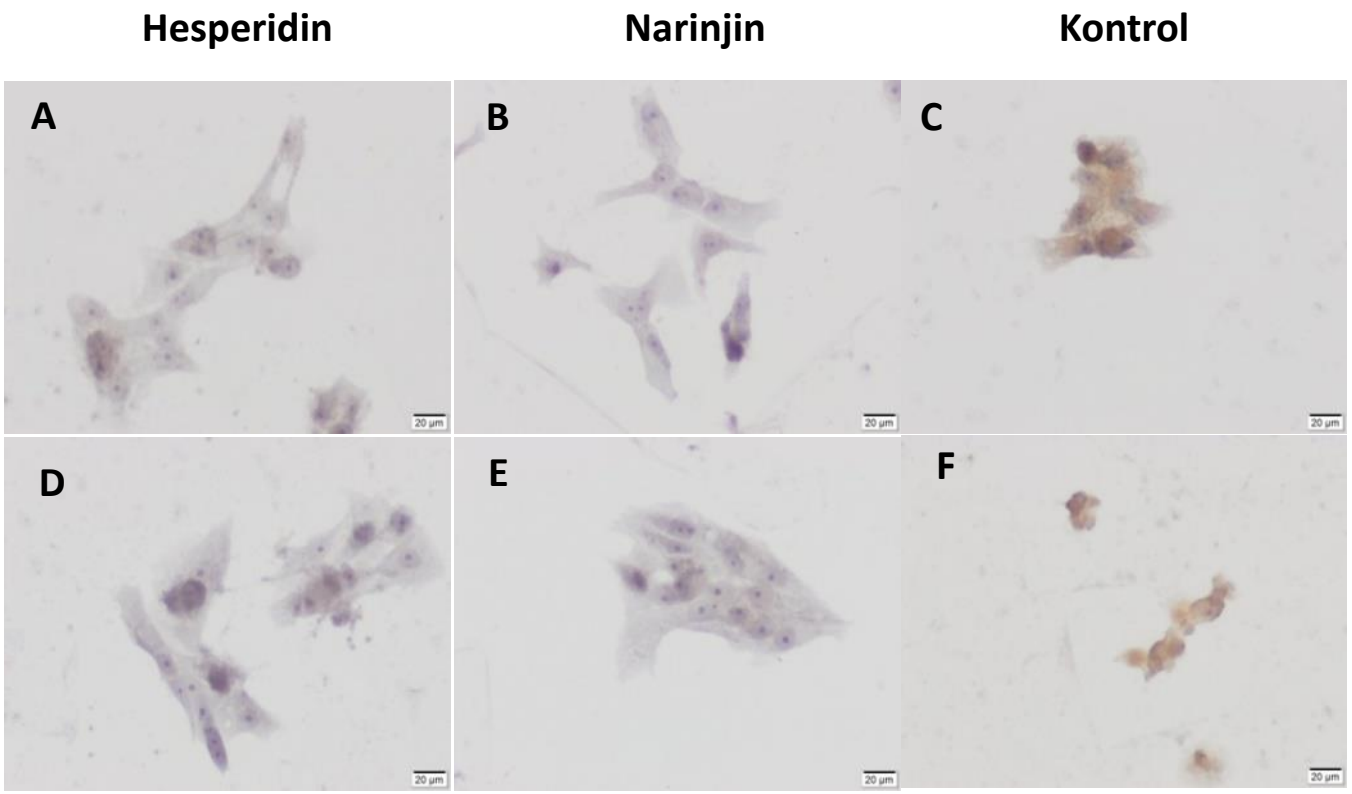


p[1]:
Hesperidin-Kontrol: <0.001
Narinjin-Kontrol: < 0.001

α-sinüklein

	Hesperidin	Narinjin	Kontrol grup	p[1]
SK-N-AS	177.97±13.07[a]	115.00±12.24[a]	291.87±56.76[b]	<0.05*
AH model	122.91±16.82[a]	128.12±26.65[a]	235.25±65.48[b]	<0.05*
p[2]	0.997	0.951	0.146	

p[1]: SK-N-AS
Hesperidin-Kontrol: <0.001
Narinjin-Kontrol: < 0.001
p[1]: AH model
Hesperidin-Kontrol: 0.001
Narinjin-Kontrol: 0.002



Sonuç ve Öneriler

- SK-N-AS hücrelerinde tau immünoreaktivitesi hesperidin uygulanan grupta daha düşükken; AH model hücrelerinde narinjin uygulanan grupta tau immünoreaktivitesinde azalma gözlenmiştir

- AH model hücrelerinde narinjinin Aβ immünoreaktivitesinin hesperidine göre daha düşük olduğu saptanmıştır

- α-sinüklein immünoreaktivitesinin SK-N-AS hücrelerinde narinjinde; AH model hücrelerinde de hesperidin de daha zayıf olduğu gözlenmiştir

Sonuç ve Öneriler

- Narinjinin hesperidine göre daha etkili olabileceği sonucuna varılmıştır
- Her iki bileşenin de Alzheimer hastalığı için potansiyel terapötik etkilerde bulunabileceği öngörülmektedir
- Sinyal yolaklarının belirlenebilmesi için *in vivo* çalışmalara ve klinik çalışmalara ihtiyaç vardır

JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION
2023, VOL. 42, NO. 4, 418–426
https://doi.org/10.1080/07315724.2022.2062488

Neuroprotective Effects of Hesperidin and Naringin in SK-N-AS Cell as an *In Vitro* Model for Alzheimer's Disease

Müjgan Kuşi[a], Eda Becer[b,c], Hafize Seda Vatansever[c,d], and Sevinç Yücecan[e]

[a]Faculty of Health Sciences, Department of Nutrition and Dietetics, Near East University, Nicosia, Mersin 10 Turkey; [b]Faculty of Pharmacy, Department of Biochemistry, Near East University, Nicosia, Mersin 10 Turkey; [c]DESAM Institute, Near East University, Nicosia, Mersin 10 Turkey; [d]Faculty of Medicine, Department of Histology and Embryology, Manisa Celal Bayar University, Manisa, Turkey; [e]Faculty of Health Sciences, Department of Nutrition and Dietetics, Lokman Hekim University, Ankara, Turkey

ABSTRACT

Objective: Hesperidin and naringin are flavonoids that are found in citrus fruits. Our aim was to create an *in vitro* model of Alzheimer's disease (AD) and to evaluate the neuroprotective effects of hesperidin and naringin in SK-N-AS and AD model cells.

Methods: $A\beta_{25\text{-}35}$ was used to create an AD model in SK-N-AS cells. The cytotoxicity of hesperidin and naringin was evaluated using MTT. β-amyloid, tau and α-synuclein distributions were analyzed using indirect immunoperoxidase staining to investigate the neuroprotective effects of hesperidin and naringin.

Results: The AD model was created by 1 µM of $A\beta_{25\text{-}35}$ for 48 hours after ThT staining. The intensity of β-amyloid was reduced through both hesperidin and naringin treatment in AD model cells. Both flavonoids significantly decreased the intensity of α-synuclein in SK-N-AS and AD model cells.

Conclusions: Hesperidin and naringin can be potentially used as neuroprotective agents. Naringin may be more effective than hesperidin in the accumulation of β-amyloid and tau proteins.

ARTICLE HISTORY
Received 22 February 2022
Accepted 1 April 2022

KEYWORDS
Alzheimer's disease; β-amyloid; Tau; Hesperidin; Naringin

Sözel Bildiri

3rd International Eurasian Conference on Science, Engineering and Technology (EurasianSciEnTech 2021) - Sözel Bildiri

İn vitro Alzheimer modelinde hesperidin ve narinjinin hücre canlılığına etkisi

Müjgan Kuşi[1*] (*ORCID: https://orcid.org/0000-0001-8361-7206*), Eda Becer[2,3] (*ORCID: http://orcid.org/0000-0002-2378-128X*), Hafize Seda Vatansever[3,4] (*ORCID: http://orcid.org/0000-0002-7415-9618*), Sevinç Yücecan[5] (*ORCID: http://orcid.org/0000-0003-4751-0924*)

[*1]Yakın Doğu Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Lefkoşa, Kıbrıs

[2]Yakın Doğu Üniversitesi, Eczacılık Fakültesi, Biyokimya Anabilim Dalı, Lefkoşa, Kıbrıs

[3]Yakın Doğu Üniversi

[4]Manisa Celal B

Dalı, Manisa, Türkiye

[5]Lokman Hekim Üniv

Bölümü, Ankara, Türk

*Sorumlu yazar e-mai

Sözel Bildiri-Tam Metin

4. Uluslararası Katılımlı Hücre Ölümü Araştırma Derneği Kongresi – Sözel Bildiri-Tam Metin

İn Vitro Alzheimer Model Hücrelerinde Narinjinin Nöroprotektif Etkilerinin Değerlendirilmesi

Müjgan Kuşi[1,*], Eda Becer[2,3], Hafize Seda Vatansever[3,4], Sevinç Yücecan[5]

[1]Beslenme ve Diyetetik Bölümü, Sağlık Bilimleri Fakültesi, Yakın Doğu Üniversitesi, Lefkoşa, Kıbrıs

tesi, Yakın Doğu Üniversitesi,

si, Lefkoşa, Kıbrıs

ı Fakültesi, Manisa Celal Bayar

nleri Fakültesi, Lokman Hekim

becer@yahoo.com,

.com

ı; Tel:05428820945

Sözel Bildiri

2. International Mediterranean Scientific Research And Innovation Congress-Sözel Bildiri

Hesperidinin Alzheimer Deneysel Modelindeki Nöroprotektif Etkileri

Müjgan KUŞİ
Near East University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Nicosia, Cyprus
ORCID: 0000-0001-8361-7206
Eda BECER
Near East University, Faculty of Pharmacy, Department of Biochemistry, Nicosia, Cyprus
Near East University, DESAM Institute, Nicosia, Cyprus
ORCID: 0000-0002-2378-128X
Hafize Seda VATANSEVER
Near East University, DESAM Institute, Nicosia, Cyprus
Manisa Celal Bayar University, Faculty of Medicine, Department of Histology and Embryology, Manisa, Turkey
ORCID: 0000-0002-7415-9618
Sevinç YÜCECAN
Lokman Hekim University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Turkey
ORCID: 0000-0003-4751-0924

aştırma Derneği Kongresi, Online,