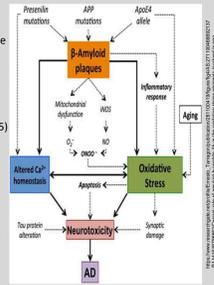


Underlying Potential Mechanism of Anti-Alzheimer's Disease Using Maysin Derivative Isoorientin 2-O-a-L-rhamnoside using in Vitro Assay System

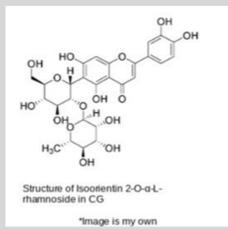
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Introduction

- Alzheimer's Disease- progressive mental deterioration of the brain (6)
 - Confusion and dementia increase progressively overtime
 - Most common in those ages 65 and older
 - Life expectancy after diagnosis is 4-15 years
 - Result in tangles and plaque build up in the Brain
 - Unregulated amyloid beta and tau lead to cell death(3,5)
- Amyloid Beta-** Also known as Aβ (5)
 - Protein pieces in the plaque
 - Chemically "sticky" and clumps into groups
 - Groups outside nerve cells
 - Destroys synapses before and while clumping in the brain
 - Cannot effectively be cleared from brain
- Amyloid beta plaques is believed to cause complications**
- Oxidative Stress-** Imbalance in reactive oxygen and the bodies ability to detoxify
 - Stimulates neuronal cell dysfunction in the development of AD pathogenesis
 - Aβ fibrils

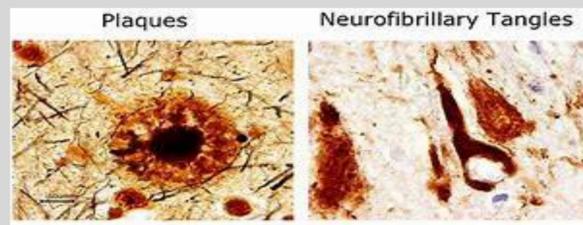
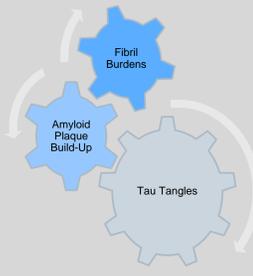


- Maysin** is a flavone C-glycoside from corn silks and maize (1,3)
 - Shown to be protective against amyloid beta.
 - Seen to limit plaque build-up and tangles
 - Immune responses observed
 - Mitigates formation of plaques outside the neurons by enhancing the immune response associated with Th2-skewed cytokine response (1)
- Isoorientin 2-O-rhamnoside (IR)** is a flavonoid compound
 - Similar to Maysin
 - Found in Centipede grass



Literature Review

- Citron et. al. in 1992
- Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production
 - More plaques
 - Higher cell death rates
- Murphy et Al. (2010)
- Studied if AD is driven by the production and deposition of amyloid beta protein.
- Sabri et Al. (2015)
- Used PET imaging to scan for amyloid plaques in AD patients
- TL Spire-Jones et AL. (2014)
- Studied the intersection of Amyloid beta plaques and tau at synapses in AD models
- Sevigny et Al. (2015)
- Studied an anti-amyloid beta antibody and its affects as a prospective treatment for AD
- Song et al. (2017)
- Found Maysin significantly reduced beta
- Lee et al. (2014)
- Found Maysin enhanced early innate immunity



Gap in the Research

Minimal research has previously investigated IR compounds such as Maysin or Isoorientin to be utilized as a potential anti-AD measure.

Hypothesis and Objectives

Objective

To test and identify the effects of Maysin and its derivatives such as Isoorientin 2-O-a-L-rhamnoside using in Vitro Assay System for its anti-Alzheimer's disease affects in transgenic mouse models.

Hypothesis

We hypothesize that IR flavonoid compounds can exert anti-AD properties such as an inhibitor of amyloid beta oligomerization process and the ability to counteract BACE activation.

Methodology

Chemical Preparation-

- IR compounds extracted from CG and analyzed according to modifications of the methods described in a previous study [17]
- Chromatography performed with UV detector
- The standard substances to generate calibration curve used from Lee et al. (2010)
 - * For monitoring purpose, Maysin isolated from maize was used as the standard substance.

Aβ oligomeric and fibrillary form and Visualization onto SDS-PAG-Western Blot

- The peptide, synthetic Aβ1-42 was dissolved in 1 mM hexafluoroisopropanol
- Removed under vacuum in a Speed Vac
- Residual peptide re-suspended in dry dimethyl sulfoxide
- By adding phenol red F-12 medium to the re-suspended peptide, the concentration was made to 100 μM and the peptide was kept at 4°C for 24 h.
- The samples were diluted in NuPage sample buffer

Oxidative Stress

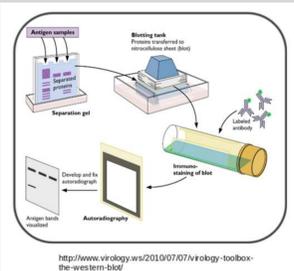
Measured the ROS at cellular level using PC12 neuronal cells as supplemental data
Used dichlorofluorescein diacetate (DCF-DA) assay
Seeded in 96-well plates & incubated overnight
Samples treated for 24 then samples & H2O2 groups but not the control group was treated with H 2 O 2 for 3 h.
DCF-DA (10 μM) added to the pretreated cells for 50 min
*Produced DCF contents were quantified using a at 485 nm excitation wavelength and 535 nm emission wavelength

Statistical Analysis-

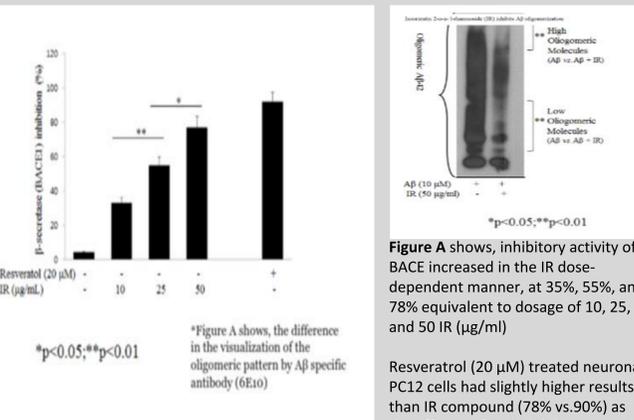
The experimental data were represented as the means ± S.E.M.

Used neuronal cell based assay by SigmaPlot software & analysis of variance (ANOVA) and two-tailed Student's t-test.

A value of *p < 0.05 was considered statistically significant; n.s. mean not significant.



Results



Graph 1 shows that using neuronal PC12 cells, we determined the inhibitory of Aβ oligomerization in vitro process in the presence or absence of IR (50 μg/ml) for incubate 24 hr. in 4oC and separation them onto SDS-PAGE by electrophoresis, and detected finally the difference in the visualization of the oligomeric pattern by Aβ specific antibody (6E10).

Discussion

IR flavonoid compound could play a key role in counteracting oxidative stress by reducing Aβ oligomeric forms as seen in figure one.

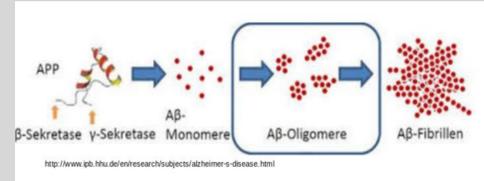
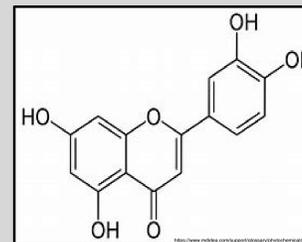


Figure 2

- BACE inhibitory ability at a cellular level was increased on neuronal PC12 cells.
- Anti-BACE effect seen
- Oxidative stress mediated BACE enzyme activity could be counteracted by CG flavonoid, IR in neuronal PC12 cells.

The results support the hypothesis and agree with past studies with the use of Isoorientin 2-O-a-L-rhamnoside and other flavonoid compounds.

Limitations

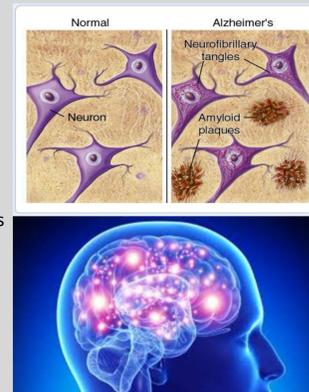


Limitations of the study are due to low yield of byproducts separated in the centipede grass.

Low yield of chemicals such as Maysin and other derivatives limited the effects of the brains of the mice studied. Effects of Maysin were not able to be extensively studied because of the minute amounts of chemicals able to be extracted.

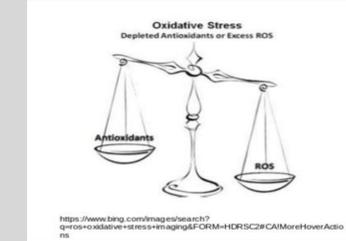
Significance

- The results show possibilities for application in anti-Alzheimer's purposes.
- Maysin and its compounds show significance in Alzheimer's intervention and applications in the clearing of fibrils and plaques which are believed to cause AD.
- Promising research into possible therapeutic intervention and treatment.



Conclusion

- Oxidative stress mediated BACE enzyme activity could be counteracted by Centipede grass flavonoid Isoorientin compounds in neuronal PC12 cells
- Insight into the link between these compounds and possible treatment for potential Alzheimer's disease mechanisms
- Supported the hypothesis regarding IR compounds anti-AD mechanisms
- IR compounds could potentially have future pharmaceutical applications

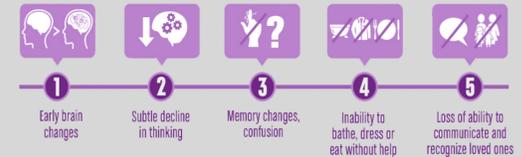


The goal was to test and identify the effects of Maysin and its derivatives such as Isoorientin 2-O-a-L-rhamnoside using in Vitro Assay System for its anti-Alzheimer's disease affects in transgenic mouse models

Future Research

- Further research into the link between these compounds and possible treatment for potential Alzheimer's disease mechanisms
- Focus further into the components relating to ROS & Oxidative stress
- Future research should focus on anti-AD like functions are associated with prevention of Aβ accumulation or mitigate beta amyloid plaque in brain.
- Increased yield of maysin and flavonoid compounds will benefit future studies.

THE ALZHEIMER'S TIMELINE



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