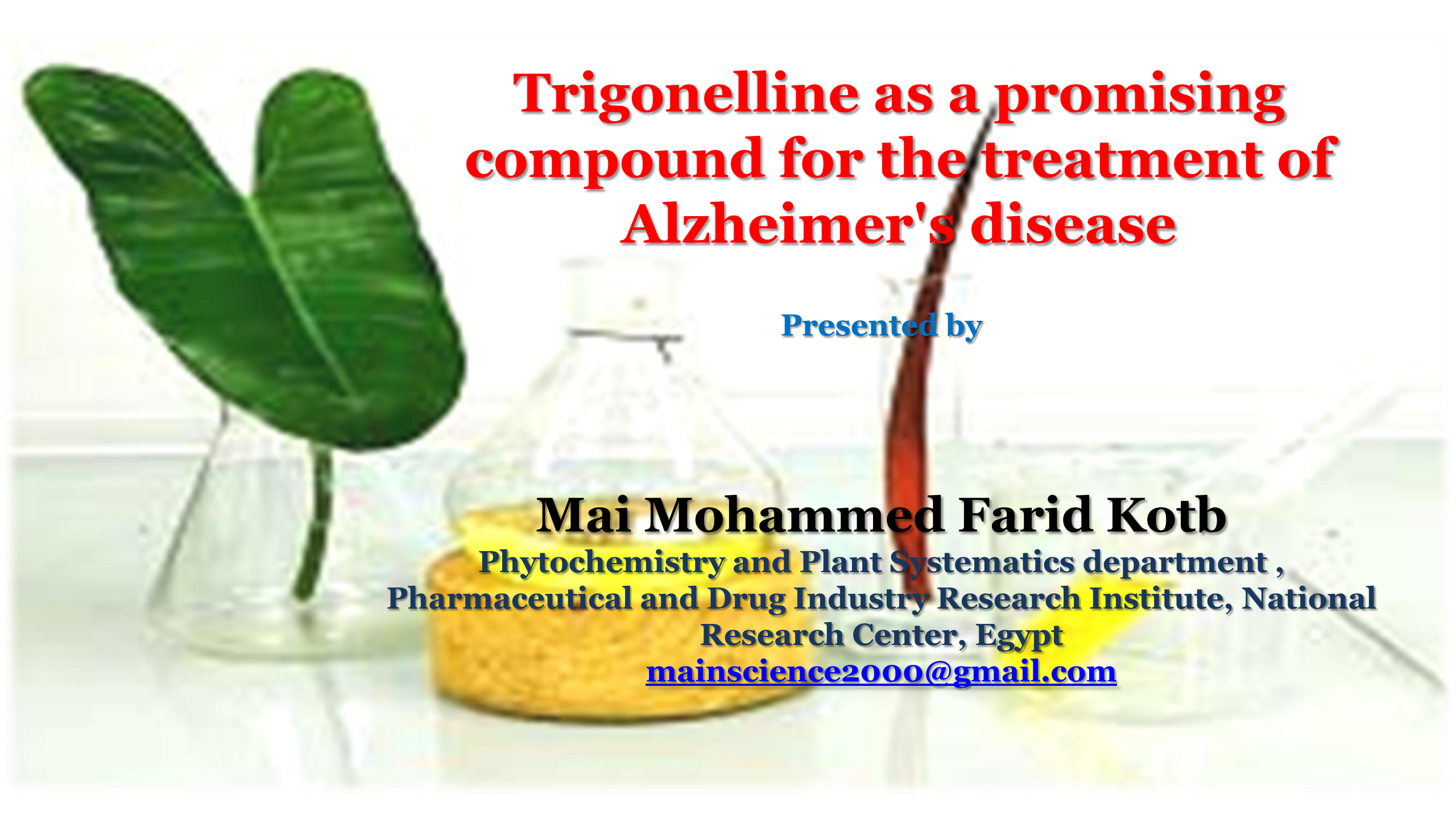

Trigonelline as a promising compound for the treatment of Alzheimer's disease

Presented by

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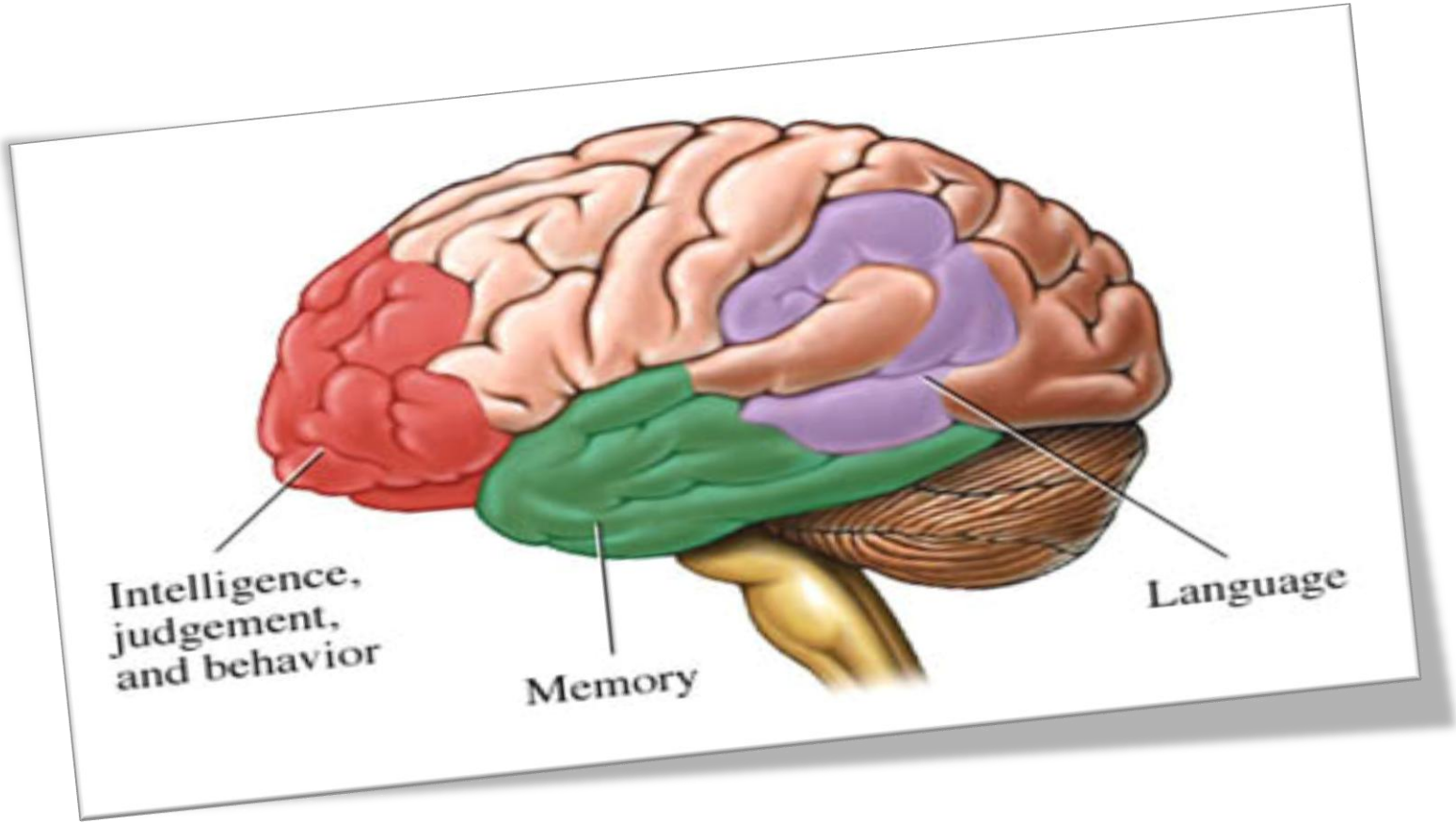
Alzheimer's disease

Resulting symptoms

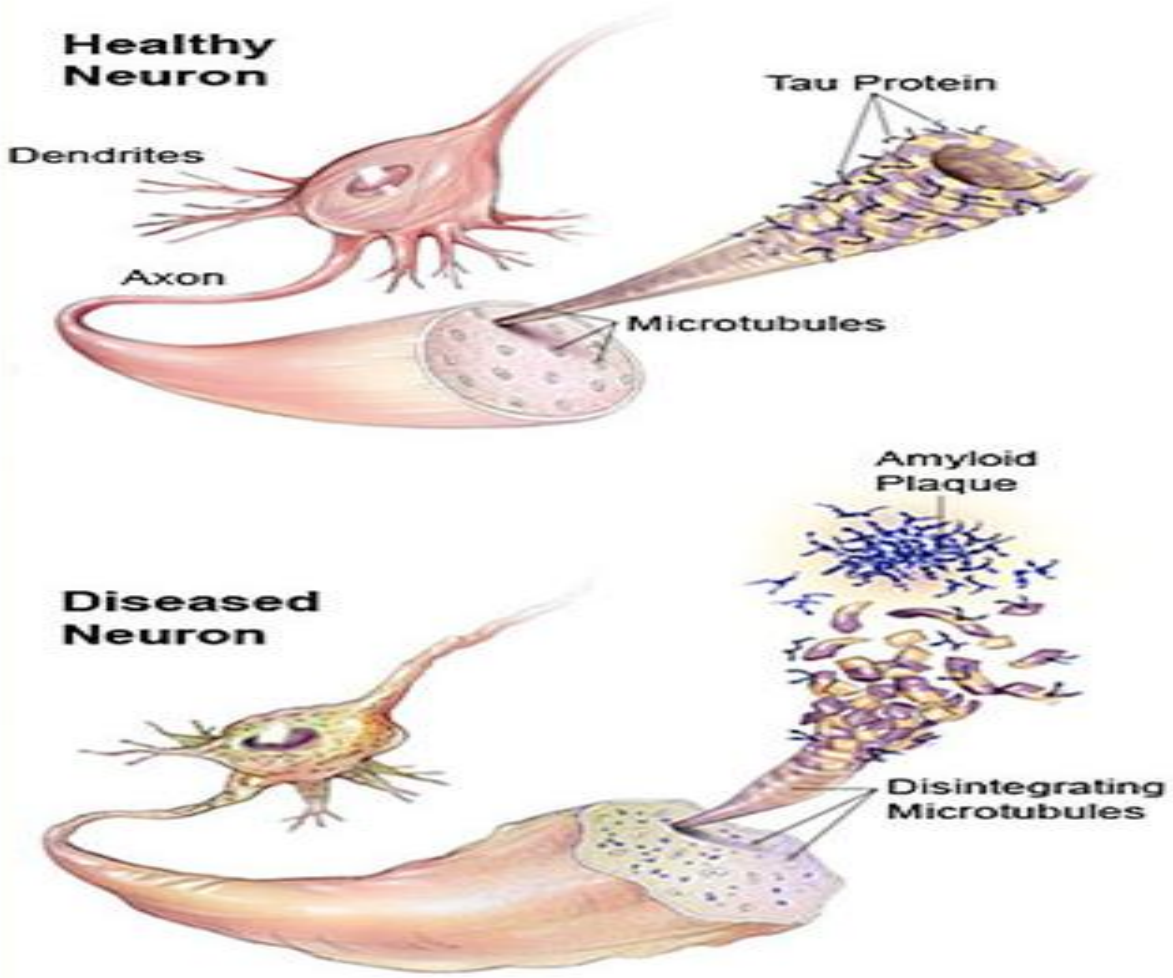
Memory impairment

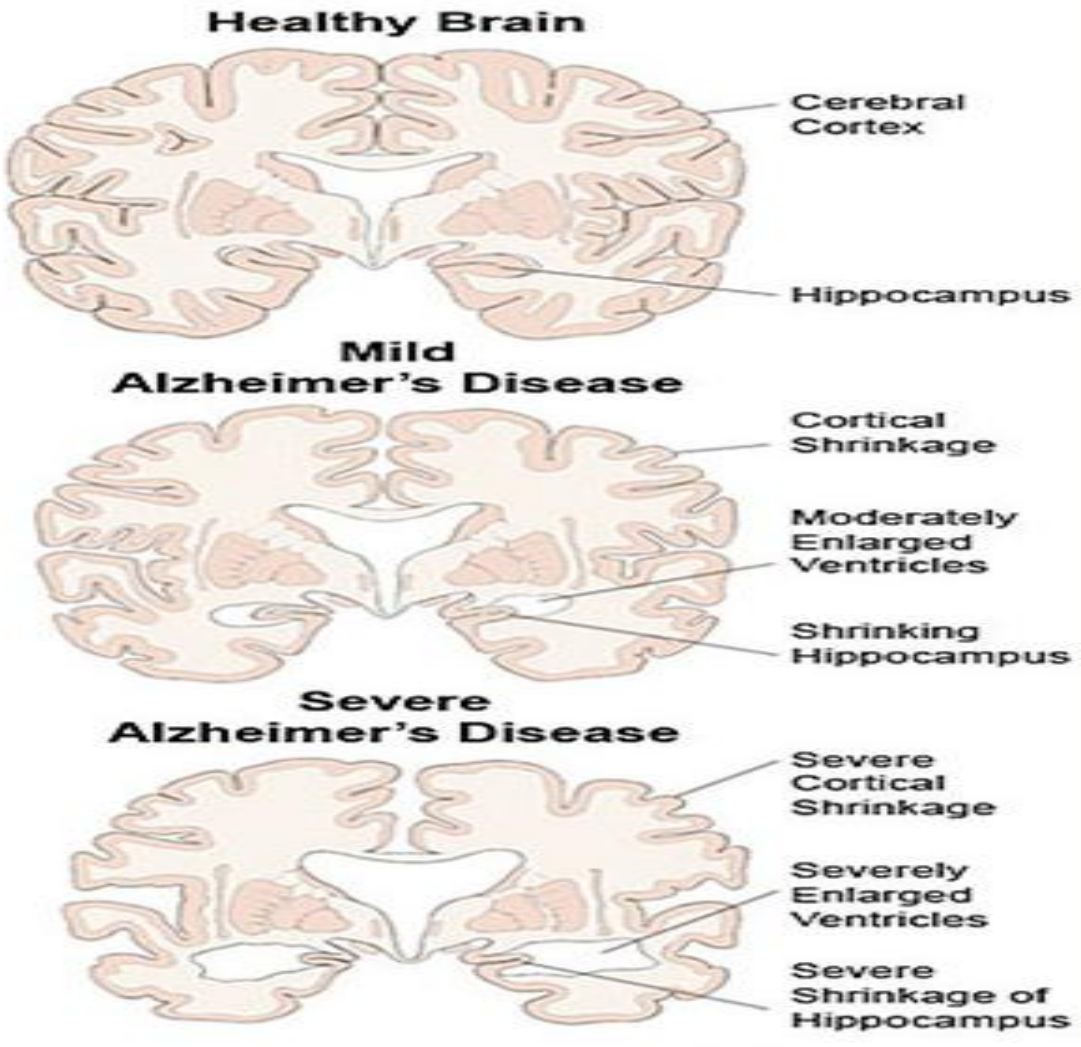
Inability to think by themselves

Inability to function independently



How Brain And Nerve Cells Change During Alzheimer's Disease



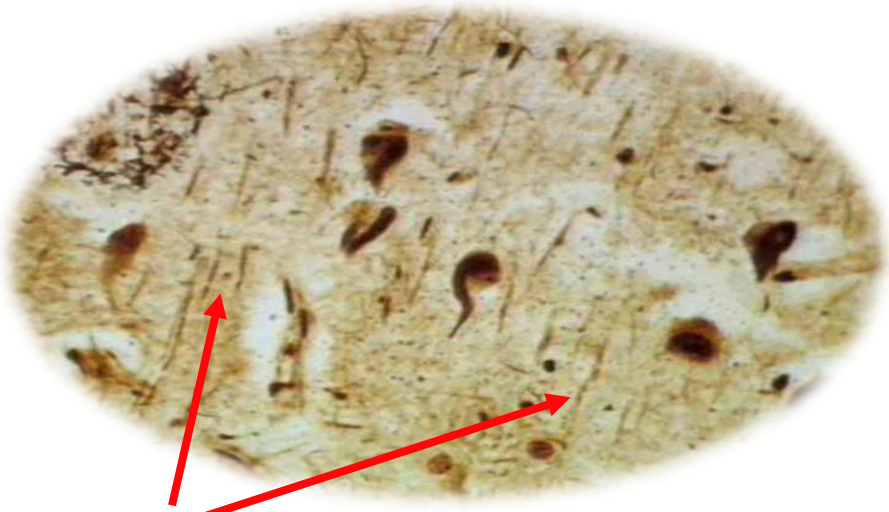


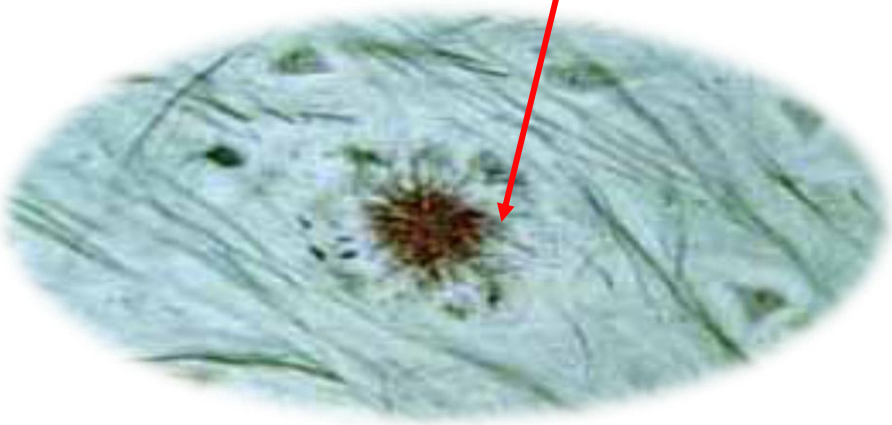
The Anatomical Hallmark of Alzheimer's

❖ Amyloid Plaques contain large amounts of a 42 amino acid peptide termed **"b-amyloid"**, or **Ab42**

❖b-amyloid itself is the initial cause of the pathophysiology that leads to dementia.

❖Amyloid plaques probably contribute to the later stages of pathology





Neurofibrillary tangles rich in cytoskeletal proteins, especially the microtubule-associated protein, "**tau**".
In the tangles: heavily phosphorylated proteins which may cause aggregation and precipitation of the cytoskeleton.

❖ **Also generally reduced brain volume, especially in entorhinal cortex and hippocampus**

Therapeutic approaches for AD

Five drugs are approved for use

❖**Acetylcholinesterase inhibitors** (because cholinergic basal forebrain neurons are among the first to die in AD

- Tacrine
- Donepezil
- Galantamine
- Rivastigmine
- The fifth drug **NMDA inhibitors**: memantine

Still in development : β-secretase inhibitors

Failed: latrepirdine = dimebolin (unknown mechanism)

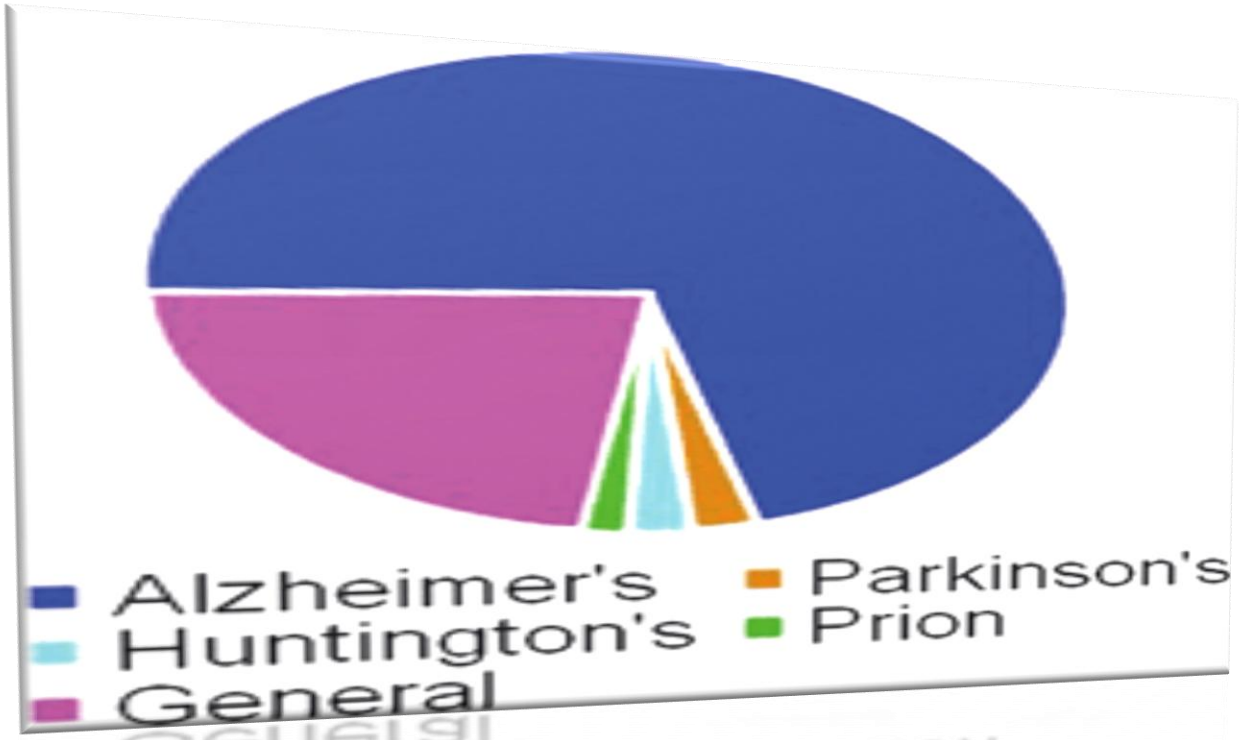
γ-secretase inhibitors such as semagecestat have **failed** so far

Antibodies against β-APP or amyloid-β have given **disappointing** results

Natural products for the treatment of neurodegenerative diseases

Considered as

- ❖ Safe
- ❖ Effective
- ❖ Less toxic than traditional drugs

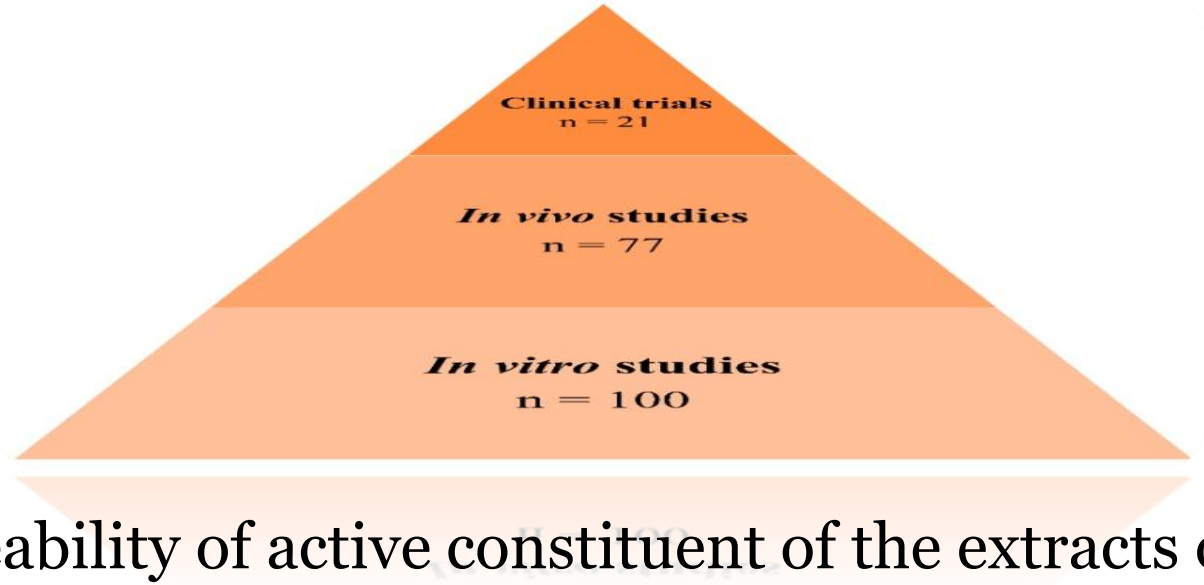


➢Number of bioactive natural products related to specific neurodegenerative diseases. Data are presented as a percentage of the total number of bioactive compounds identified for neurodegeneration (Alzheimer's disease: 71.9%, general neurodegenerative diseases: 21.8%, Parkinson's disease: 2.4%, Huntington's disease: 2.2%, prion diseases, 1.7%).

Major Concerns

- The use of inappropriate model systems
- Only few natural products have been examined in clinical trials
- The need of clear data to support the neuroprotective effect of most of the plant extracts and their active components, not only from *in vitro* experiments or single studies

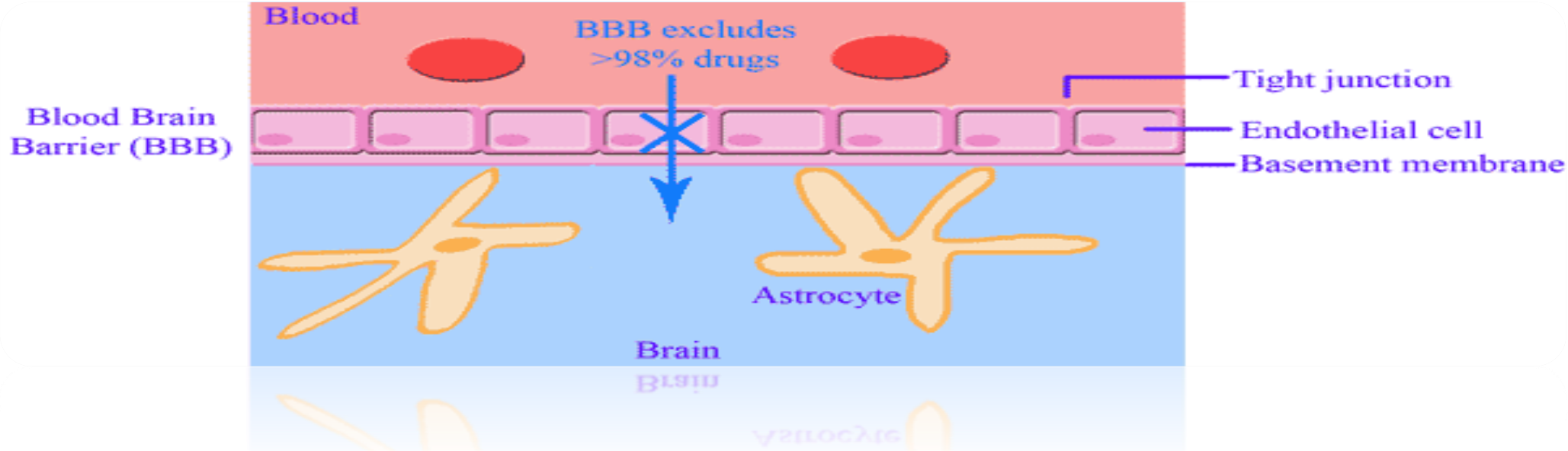
❖ Figure illustrate the Number of natural products studied in different development phases for the treatment of AD



- Ignoring the blood brain barrier permeability of active constituent of the extracts or single isolated compounds and the metabolization process inside the body

Blood Brain Barrier (BBB)

- A physiological mechanism that alters the permeability of brain capillaries, so that some substances, such as certain drugs, are prevented from entering brain tissue, while other substances are allowed to enter freely.
- The separation of the brain, which is bathed in a clear cerebrospinal fluid, from the bloodstream. The cells near the capillary beds external to the brain selectively filter the molecules that are allowed to enter the brain, creating a more stable, nearly pathogen-free environment.



Background

Fenugreek (*Trigonella foenum-graecum*) is a plant in the family Fabaceae. Fenugreek is used both as a herb (the leaves) and as a spice (the seed). It is cultivated worldwide as a semi-arid crop. It is frequently used in curry.

- Traditionally used to treat diabetes – insulin like effect.
- Widely cultivated in other parts of the world for treatment of diabetes.
- To date, trials with humans have been small and are inconclusive.
- Common problems:
  - diarrhea and gas
  - may absorb oral medications
  - blood thinning potential
  - sold online for 12.25€

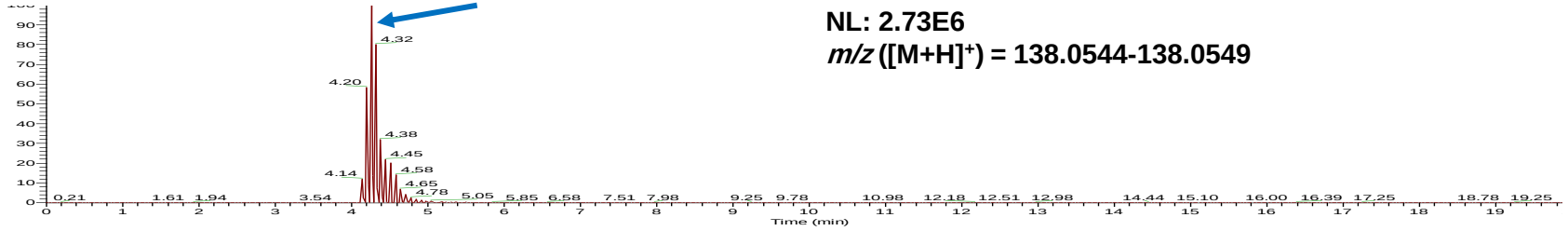
Fenugreek contains at least diosgenin and trigonelline. Although several reports **showed that trigonelline and fenugreek extract might be effective memory deficits in model mice, those data were not clear cut and used model mice were not established as Alzheimer's disease model.**

- **Pyridine alkaloids**

Trigonelline - fenugreek (*Trigonella foenum – graecum*, Fabaceae)

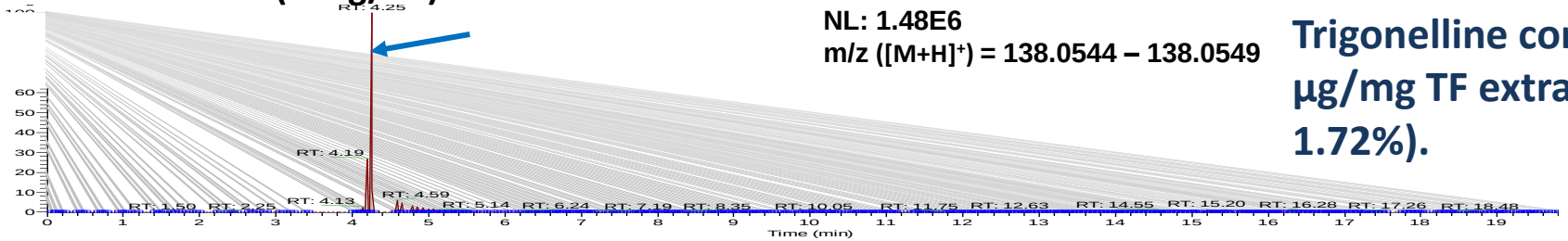
trigonelline

A) Standard Trigonelline (10 µg/ml)



NL: 2.73E6
m/z ([M+H]⁺) = 138.0544-138.0549

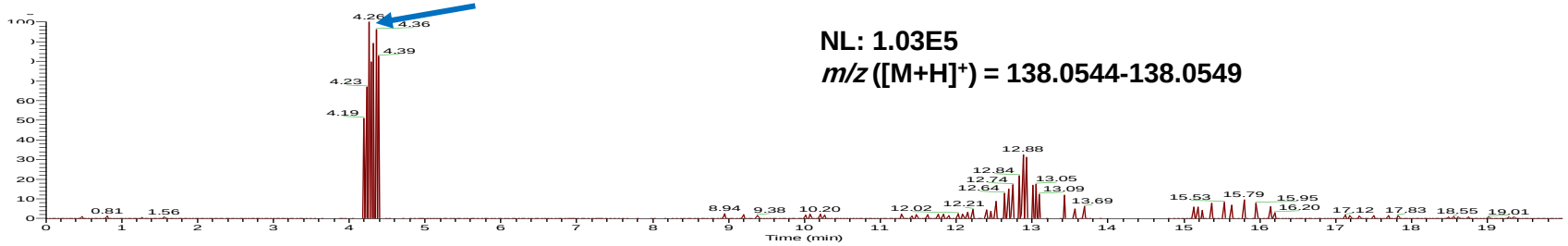
B) Trigonelline in TF extract (1 mg/ml)



NL: 1.48E6
m/z ([M+H]⁺) = 138.0544 – 138.0549

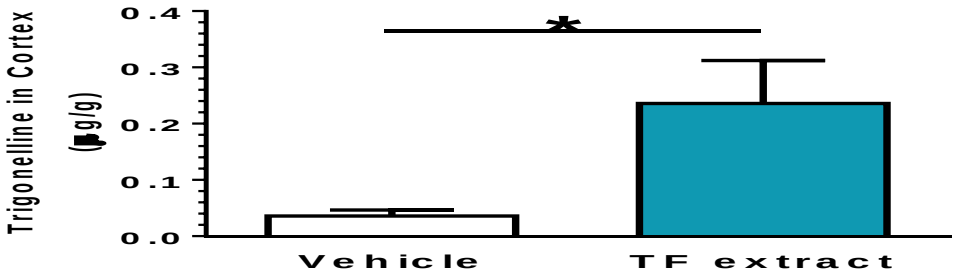
Trigonelline concentration was 17.22 µg/mg TF extract (content: 1.72%).

C) Trigonelline in the cerebral cortex after oral TF extract administration



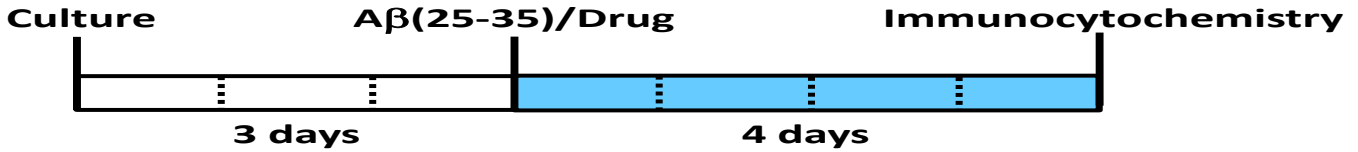
NL: 1.03E5
m/z ([M+H]⁺) = 138.0544-138.0549

D)

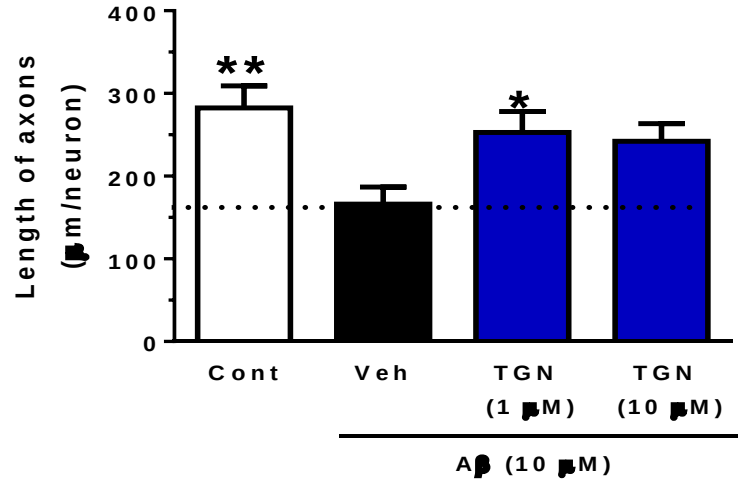


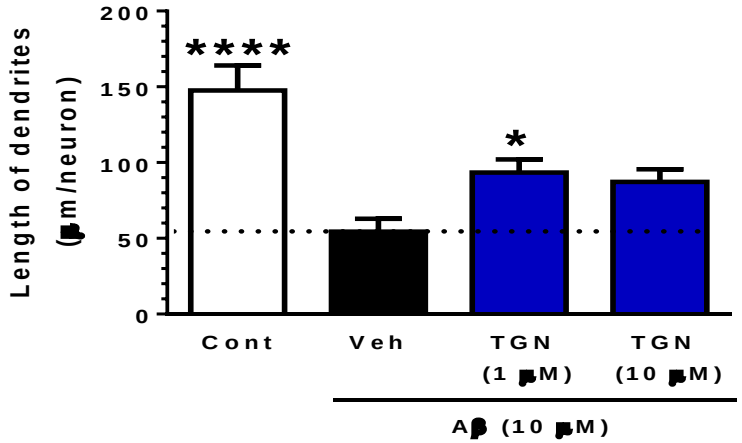
Trigonelline Significantly Ameliorated Axonal and Dendrite Atrophies in Amyloid *B*-treated Cortical Neurons

- Time course of the experiment



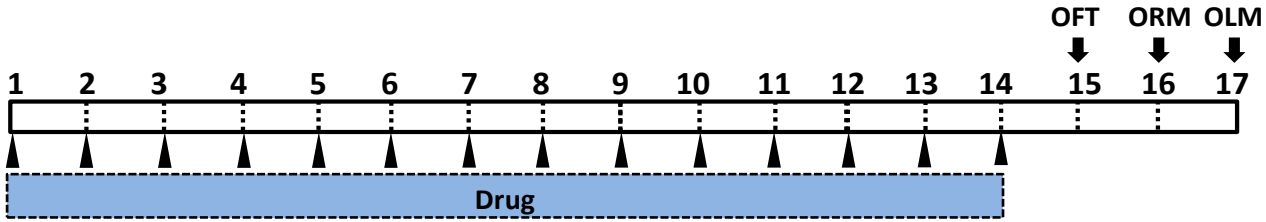
- Mouse cortical neurones were cultured for three days and then treated with Aβ25-35 (10 μM) with or without TGN (1 or 10 μM) or vehicle solution. After 4 days of treatment, the axons and dendrites were immunostained. Phosphorylated neurofilament-H (pNF-H) and microtubule-associated protein 2 (MAP2) were stained as axonal and dendritic markers.

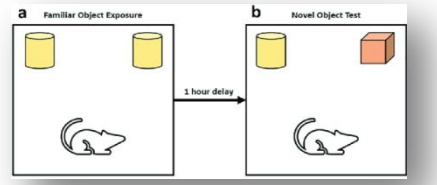




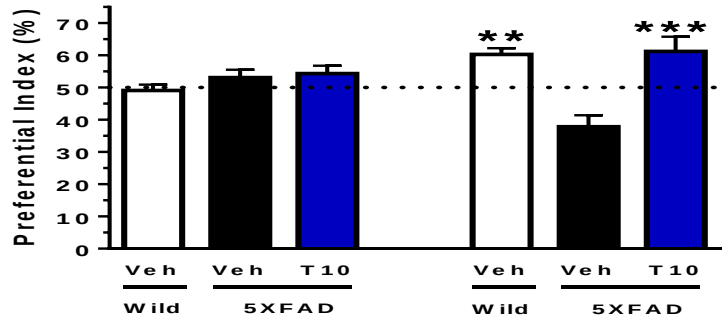
- Effects of trigonelline (TGN) on axonal and dendrite growth in amyloid β (Aβ)-treated cortical neurones

Improvement of object recognition and object location memories by Trigonelline in 5XFAD mice

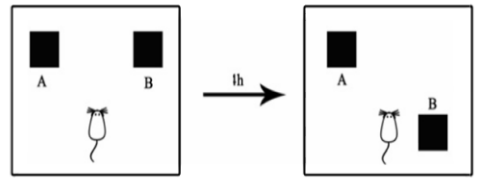




Object recgnition memory

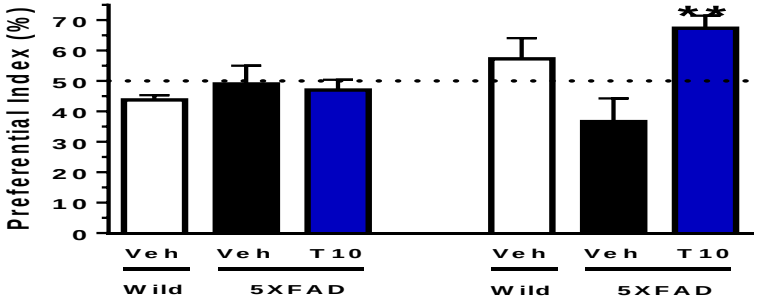


➢In the object recognition memory test. In the test session performed after a 1-h interval, wild-type mice showed a significant increase in recognition of the novel object. In contrast, vehicle solution-treated 5XFAD mice could not recognize the object. However, treatment with TGN significantly increased their object recognition memory.



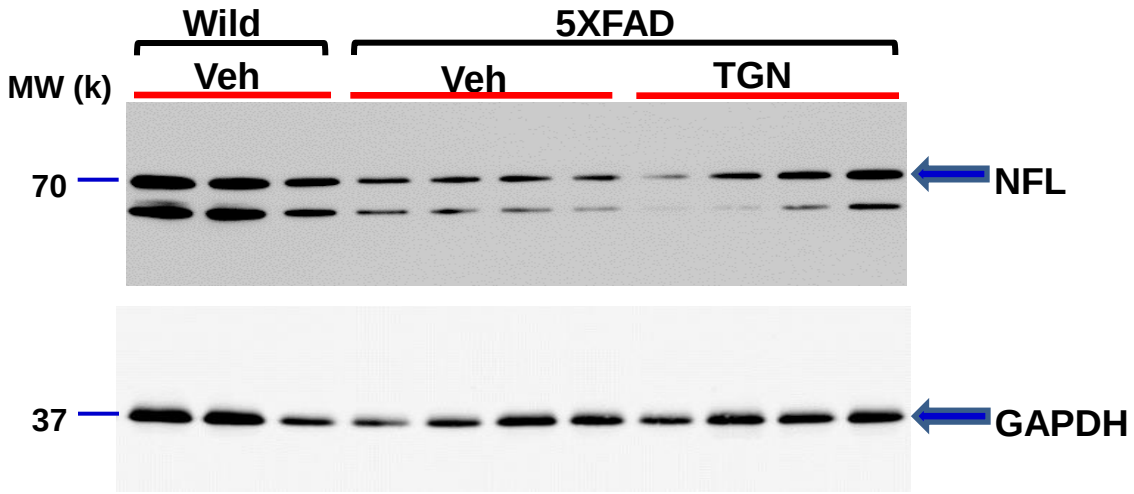
➢An object location memory test was performed to test spatial memory. The results indicated that TGN-treated 5XFAD mice significantly increased their exploratory behaviour toward the object in a novel location.

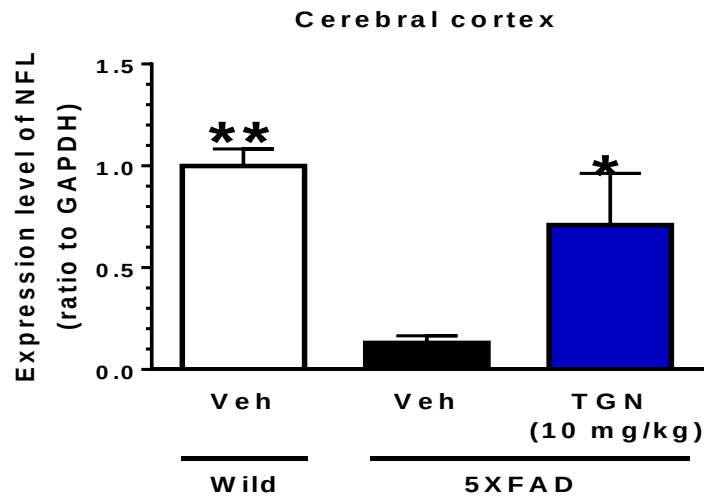
Object location memory



A significant increase in Neurofilament-L protein expression by oral administration of Trigonelline

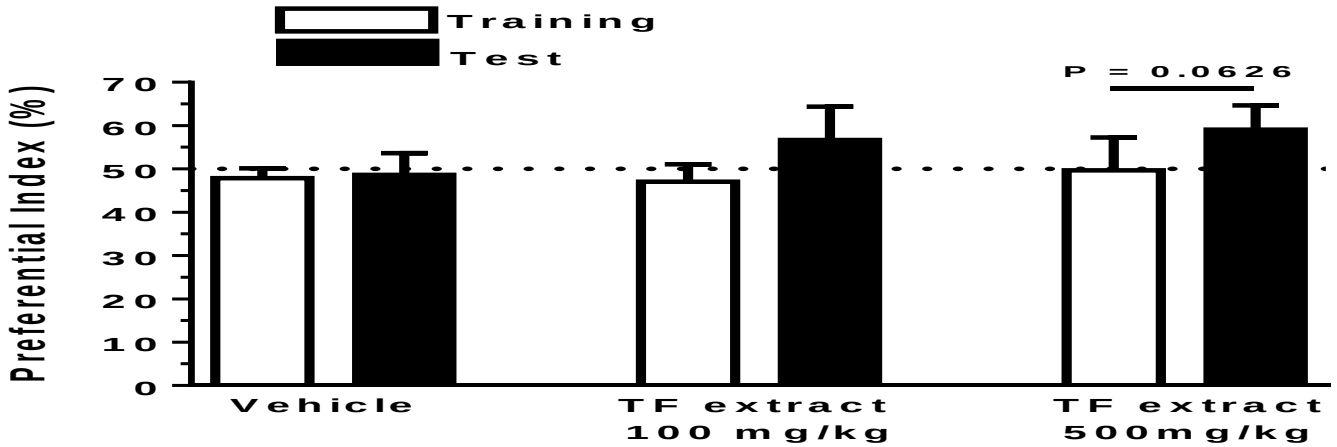
Neurofilament- L (NF-L) is located mainly in myelinated axons, and a marker of large- caliaber axonal degeneration.

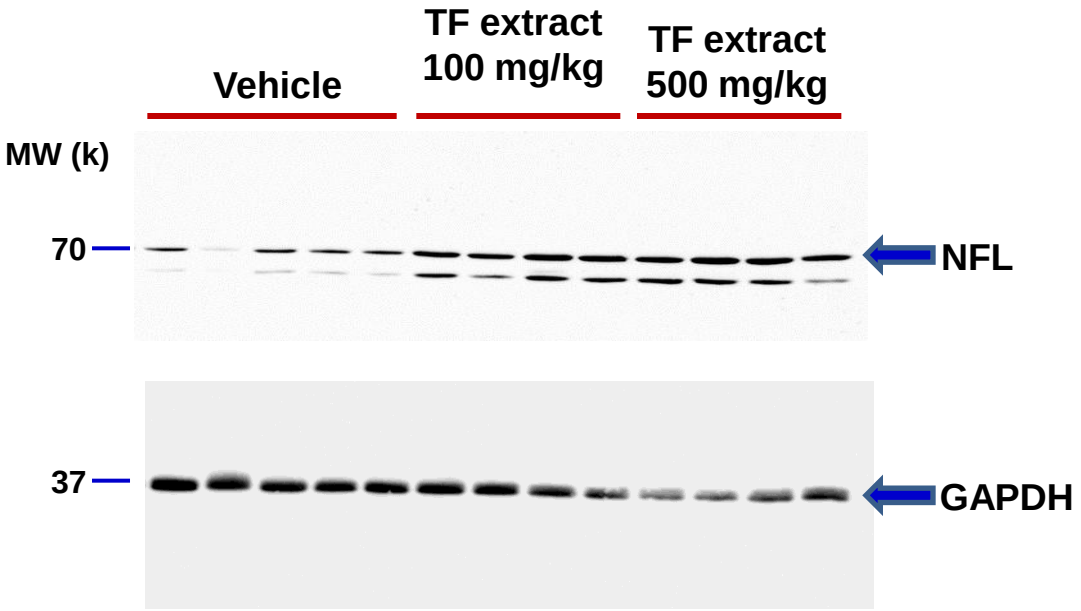


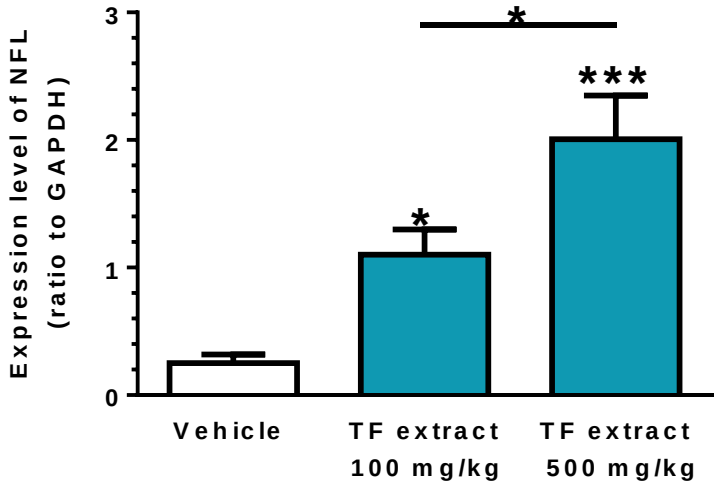


➢The level of NFL is decreased in the brain associated with axonal disruption, NFL levels were low in the vehicle solution-treated 5XFAD mice compared to the wild-type mice. The TGN-treated group showed a significant increase in NFL levels in the cerebral cortex and a trend of an increase in the hippocampus

Improvement of object recognition memory and Neurofilament-L protein expression by *Trigonella foenum graceum* in 5XFAD mice oral administration

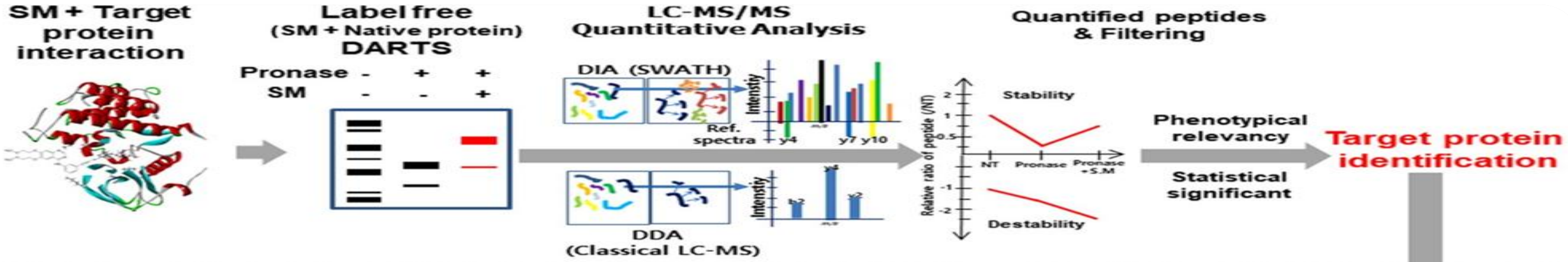


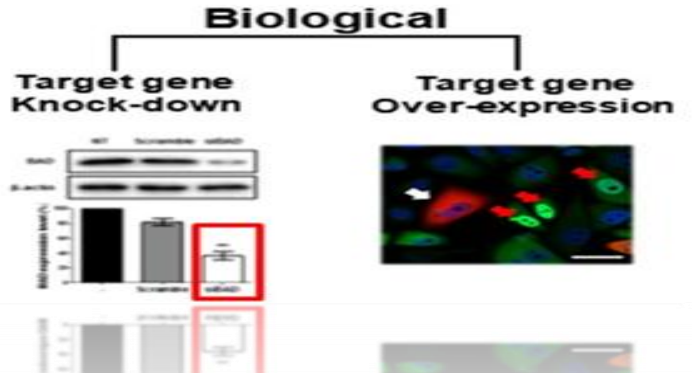




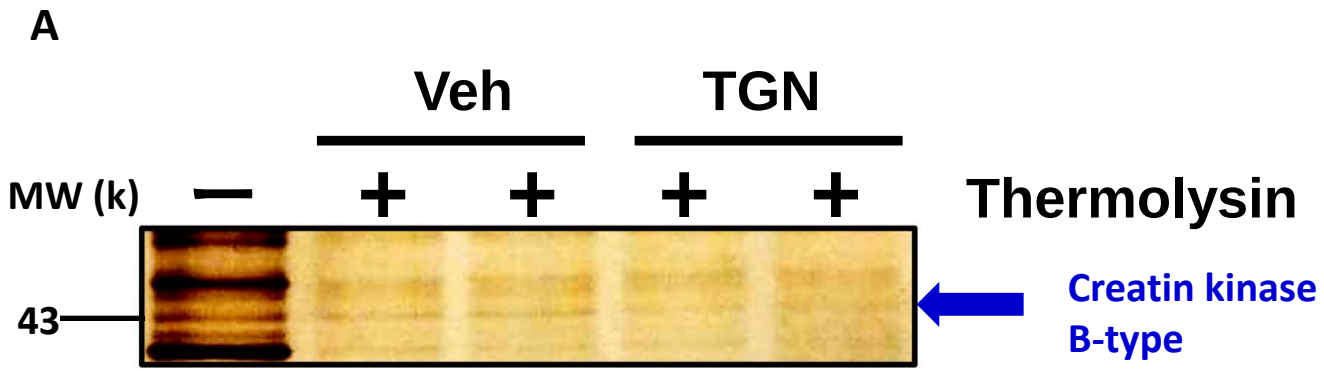
Drug affinity responsive target stability (DART) analysis for drug discovery

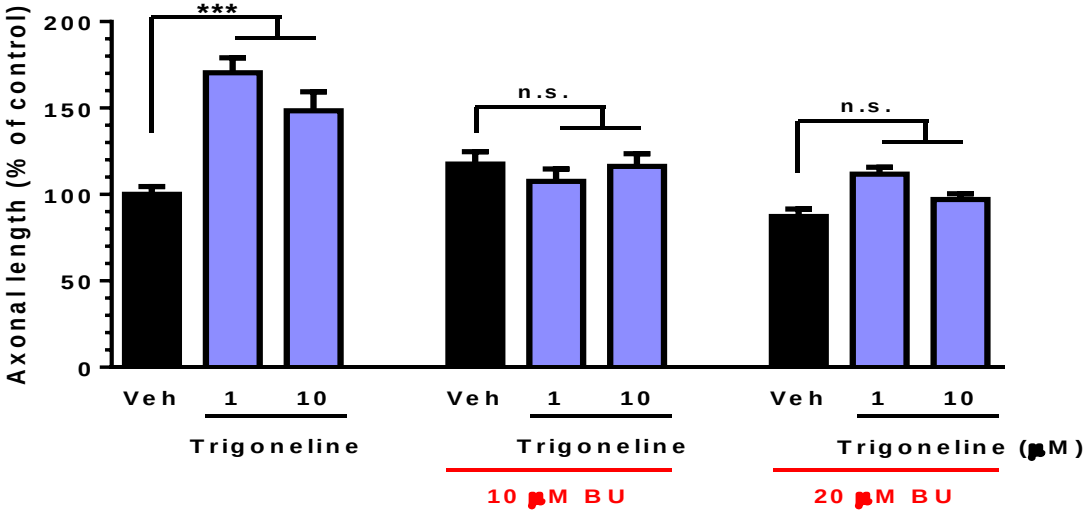
➢DARTS combined with LC-MS/MS considered recent a proteomic approach. This method has a great advantage to identify multiple protein targets

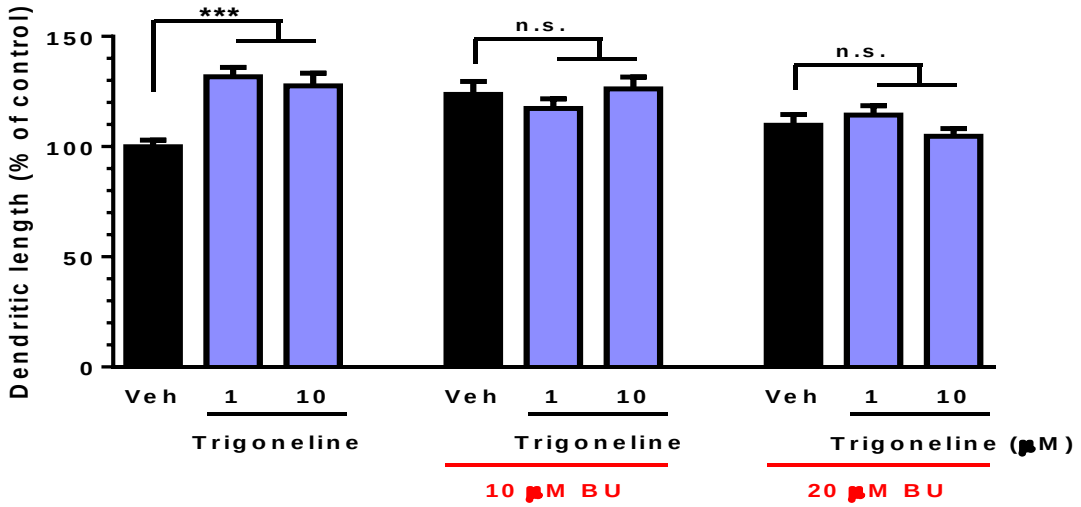




Creatine kinase B-type was identified as a binding target of trigonelline (TGN)

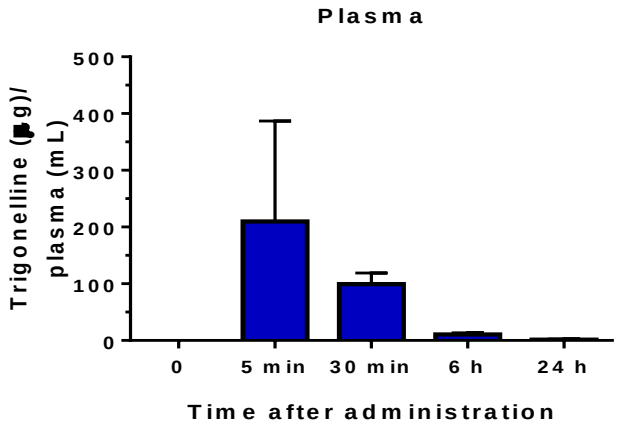




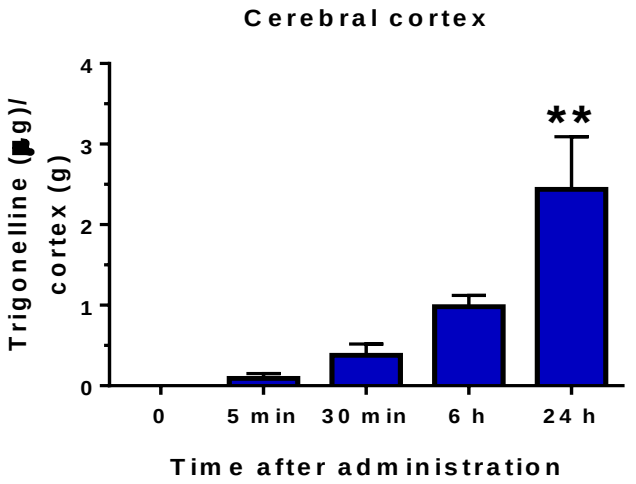


Quantification of Trigonelline in Both Blood and Brain By LC-MS/MS Suggests Good Penetration of Trigonelline Into The Brain After Oral Administration

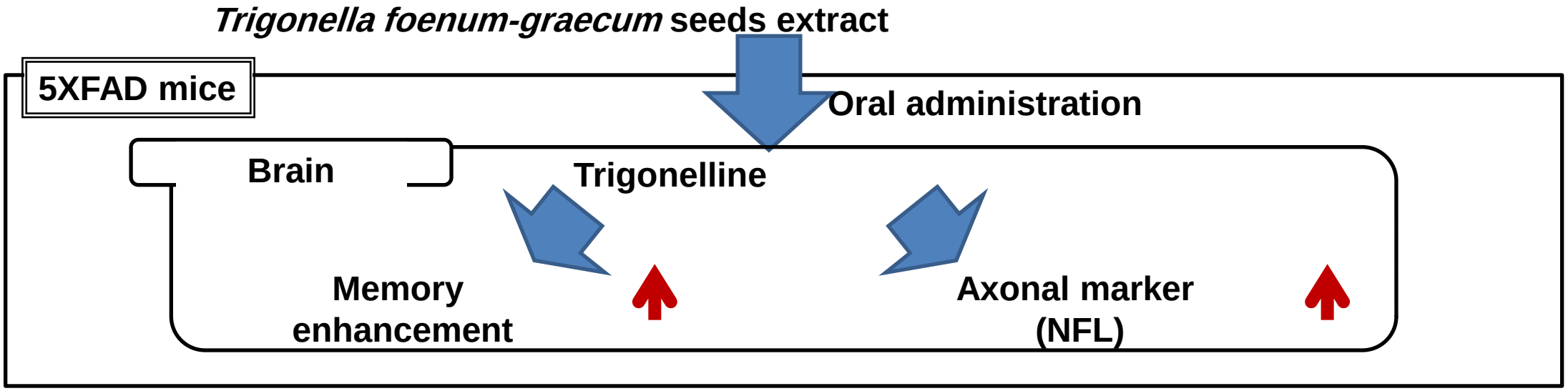
➢ We used LC–MS/MS to determine whether TGN could be detected in the blood and brain 5 min, 30 min, 6 h, and 24 h after the oral administration of 500 mg/kg TGN, we detected TGN by comparing the MS–MS data and fragmentation patterns with a reference standard. The TGN molecular weight was 137.05



➢We confirmed that TGN was detected in the plasma and cortex from 5 min. After this, the TGN concentration in the plasma peaked 5 min after administration and then gradually decreased at 30 min, 6 h, and 24 h. In the cerebral cortex, the amount of TGN started to increase from 5 min and continuously accumulated to 24 h.



Conclusion



TGN penetrates the brain and may activate CKB, leading to axonal formation. This study shows the potential of TGN as a new drug candidate, and a new target molecule, CKB, in memory recovery signaling

Publications

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Trigonelline recovers memory function in Alzheimer's disease model mice: evidence of brain penetration and target molecule

Mai M. Farid[1,2], Ximeng Yang[1], Tomoharu Kuboyama[1] & Chihiro Tohda[1]

Publications

Regular article

日本食品化学学会誌、Vol. 28(2), 63-70(2021)
Japanese Journal of Food Chemistry and Safety (JJFCS)

Effects of *Trigonella foenum-graecum* seeds extract on Alzheimer's disease transgenic model mouse and its potential active compound transferred to the brain

(Received February 9, 2021)
(Accepted March 15, 2021)

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c) Laboratory of Pharmacognosy, Daiichi University of Pharmacy

Acknowledgment

Neuromedical Science Division, Institute of Natural Medicine, University of Toyama, Japan


Thank you!