EVALENCE OF ALZHEIMER'S DISEA

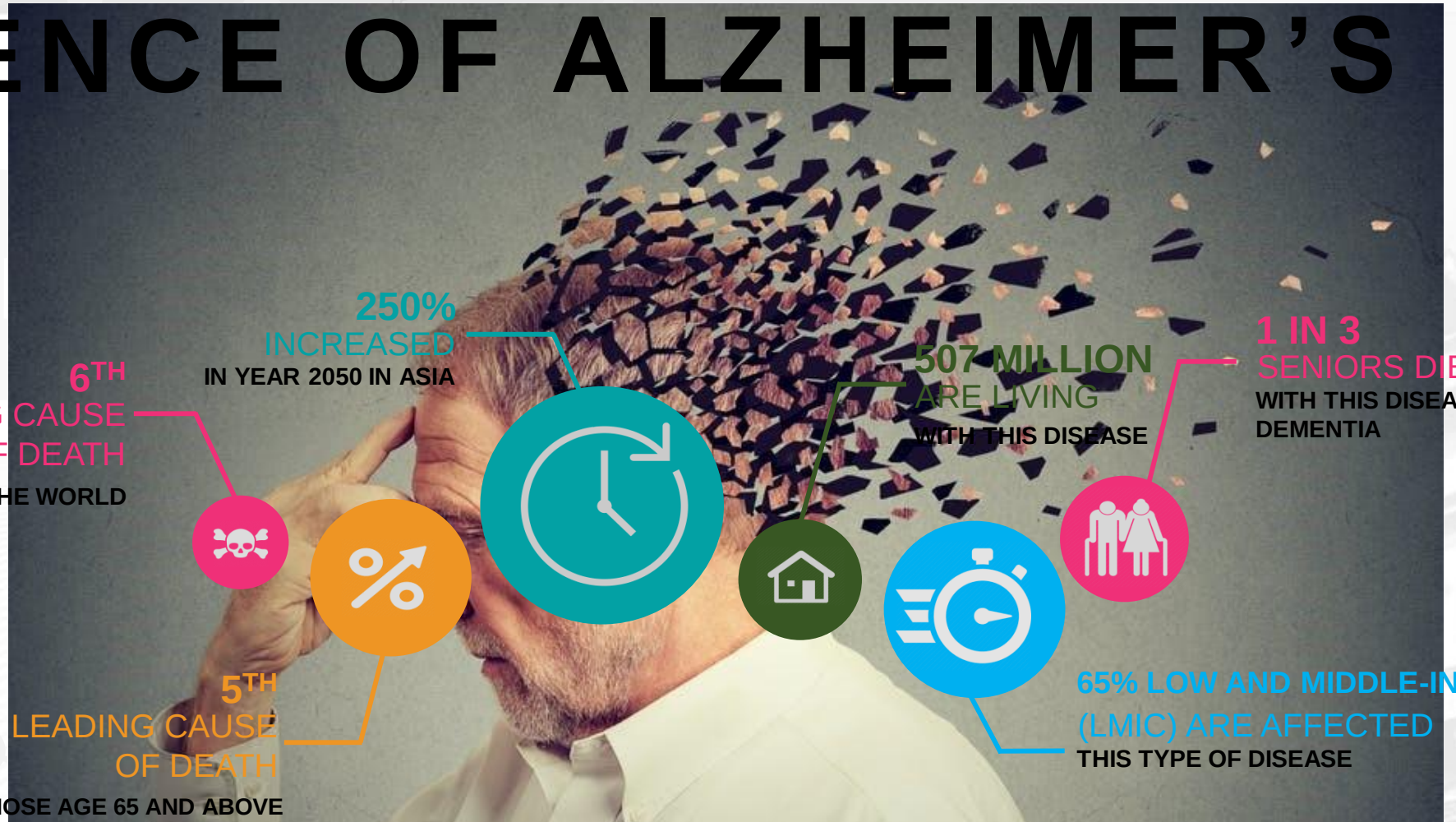


Figure 1. Statistics of Alzheimer's Disease Prevalence

Source: NHS Choices. (2016). *Alzheimer's Disease.* Retrieved from NHS Choices: www.nhs.uk/conditions/alzheimers-disease/symptoms/

Dominguez et al., (2018). Prevalence of Dementia and Associated Risk Factors: A Population-Based Study in Philippines. *Journal of Alzheimer's Disease*. 63. pp. 1065-73.

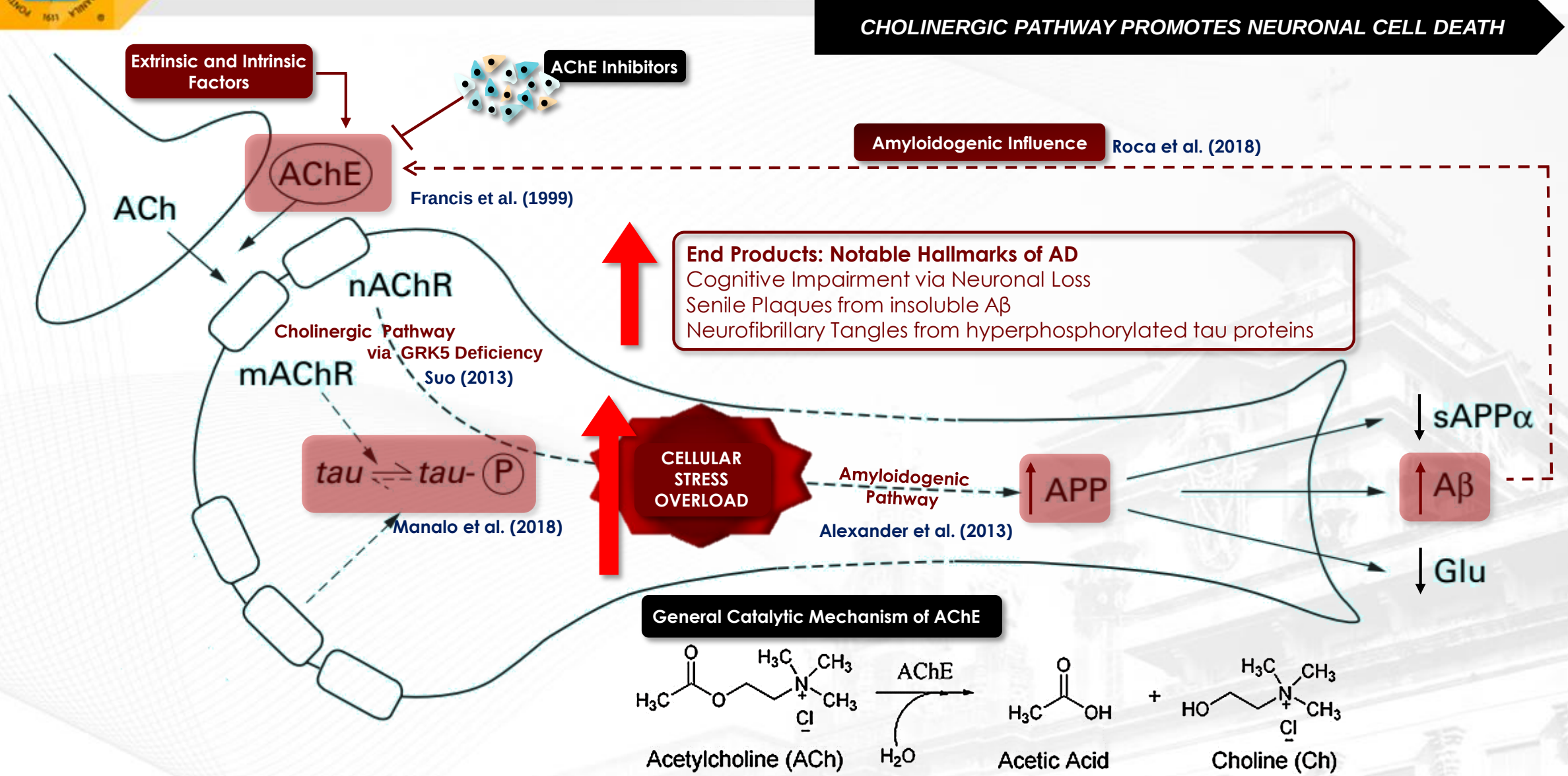


Figure 2. General Pathway of Cholinergic Hypothesis towards Alzheimer's Disease (AD) Progression

Cholinesterase Inhibitor	Current Price
Donepezil	Php 62.50-162.00 (1 tablet= 10 mg)

Source: https://www.rosepharmacy.com/product-category/pharmacy/generic/d/donepezil-hcl/

Advantages of Peptides-Based Therapeutics

- Naturally occurring biologics conferring **safety**
- **Greater efficacy, specificity and selectivity**
- Coordinates and harbors physiological processes
- **Degrades into non-toxic metabolites**
- Diverse pharmacological functions
- Lower manufacturing costs, higher stability and **greater activity**

Source: https://www.gesundheitsindustrie-bw.de/en/article/news/the-growing-significance-of-peptide-therapeutics

AD-Related Studies	Peptide	Peptide Source	Pharmacological Activities
Cholinergic/ Neuroprotection	PhTx3-1	Phoneutria nigriventer	Memory improvement (Gomes et. Al, 2013)
	PhKv		Anti-AChE activity (Rigo et. al, 2017)
	PhTx4-5-5		Neuroprotective action against NMDA receptors (Silva et. Al, 2016)

Source: de Souza et al .(2018). Animal toxins as therapeutic tools to treat neurodegenerative diseases. *Frontiers in Pharmacology, 9*(145), pp. 1-25. doi: 10.3389/fphar.2018.00145

UNIVERSITY OF SANTO TOMAS

13TH PNHRS Week Celebration: Achieving Health-Related Sustainable Development Goals (SDGs) through Research and Innovation

Cholinergic Inhibitory Characterization of Philippine Spider Venom from *Phlogiellus bundokalbo* for Purported Applications against Alzheimer's Disease

Presented by

SIMON MIGUEL M. LOPEZ

In collaboration with:

August 13, 2019

Limketkai Luxe Hotel, Cagayan de Oro City

RESEARCH OBJECTIVES & SIGNIFICANCE

MAIN OBJECTIVE

To assess the cholinergic impact of Philippine spider venom from *Phlogiellus bundokalbo*

FRACTIONATE

CHARACTERIZE

EVALUATE CHOLINERGIC PROPERTIES

- *IN VITRO*
- *IN SILICO*
- *IN VIVO*

SIGNIFICANCE

Research to Enhance and Extend Healthy Lives

- Raising awareness on AD as NCD
- Establishing and integrating several factors leading to AD

Global Competitiveness and Innovation in Health

- Exploring for novel bioactive compounds for drug discovery and development
- Improve potency via OMICs technologies

Research in Equity and Health

- Spider venom for geriatric care

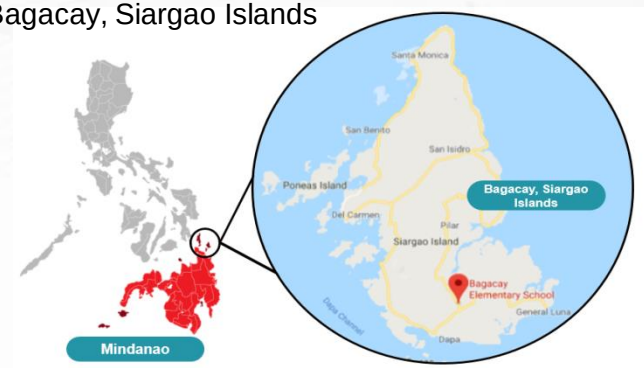
Conservation of Natural Resources

- Protection of spiders
- Education and transformation to become citizen scientists

RESEARCH METHODOLOGY

1 SPIDER COLLECTION AND IDENTIFICATION

Location: Bagacay, Siargao Islands



2 SPIDER VENOM EXTRACTION

3 C18 RP-HPLC AND FRACTIONATION

4 PROTEIN AND PEPTIDE DETERMINATION

5 ACHE INHIBITION ASSAY

6 ACHE DOCKING AND VISUALIZATION

7 PHYSIOLOGICAL EVALUATION

8 DATA ANALYSIS AND INTERPRETATION

Figure 4. Schematic Diagram of the Study

P. bundokalbo contains several peaks indicating the presence of peptide-based compounds

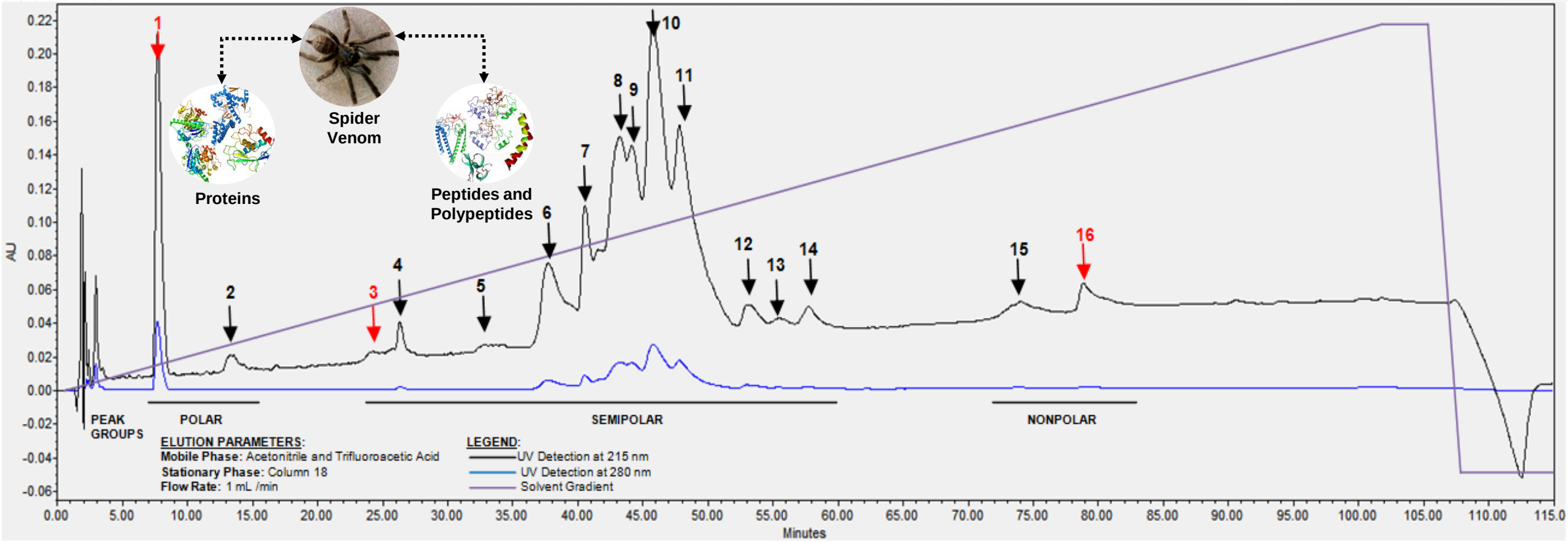
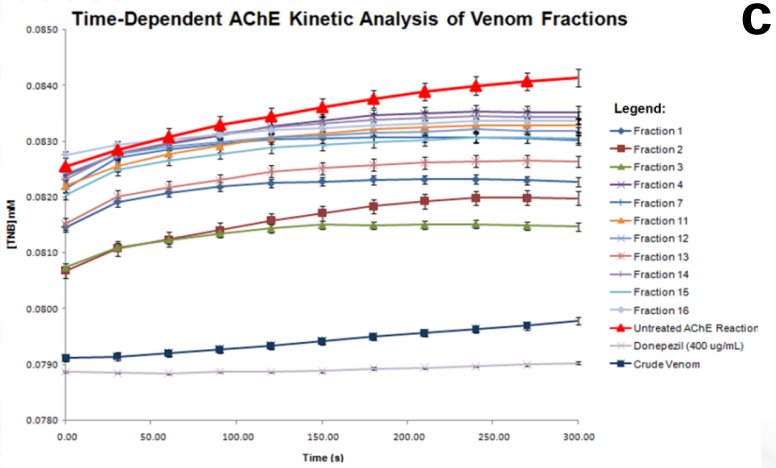


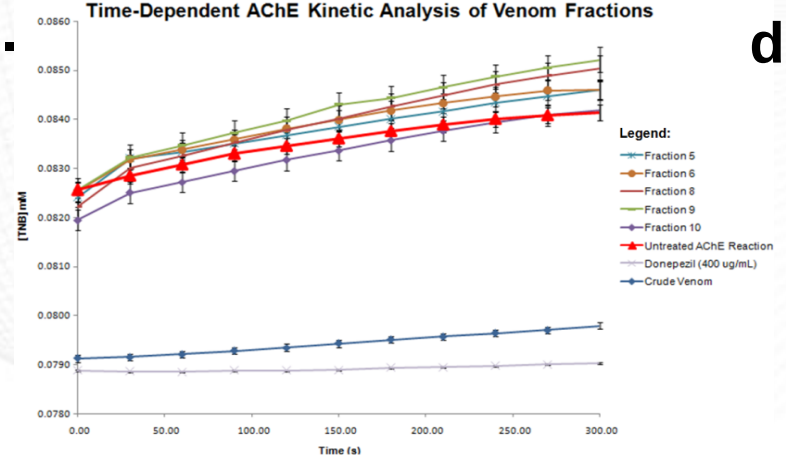
Figure 5. The C18 RP-HPLC Profile of the crude spider venom suggests the presence of peptide-based compounds according to UV detection at 215 nm and 280 nm. Intense and faint purple colorations from crude and fractionated spider venom respectively. The crude venom concentration is equal to 5.73 mg/mL (± 0.338) (R^2= 0.992).

Crude and fractionated spider venom contains compounds manifesting anti-AChE activities which exhibits competitive and uncompetitive inhibition

a.



b.



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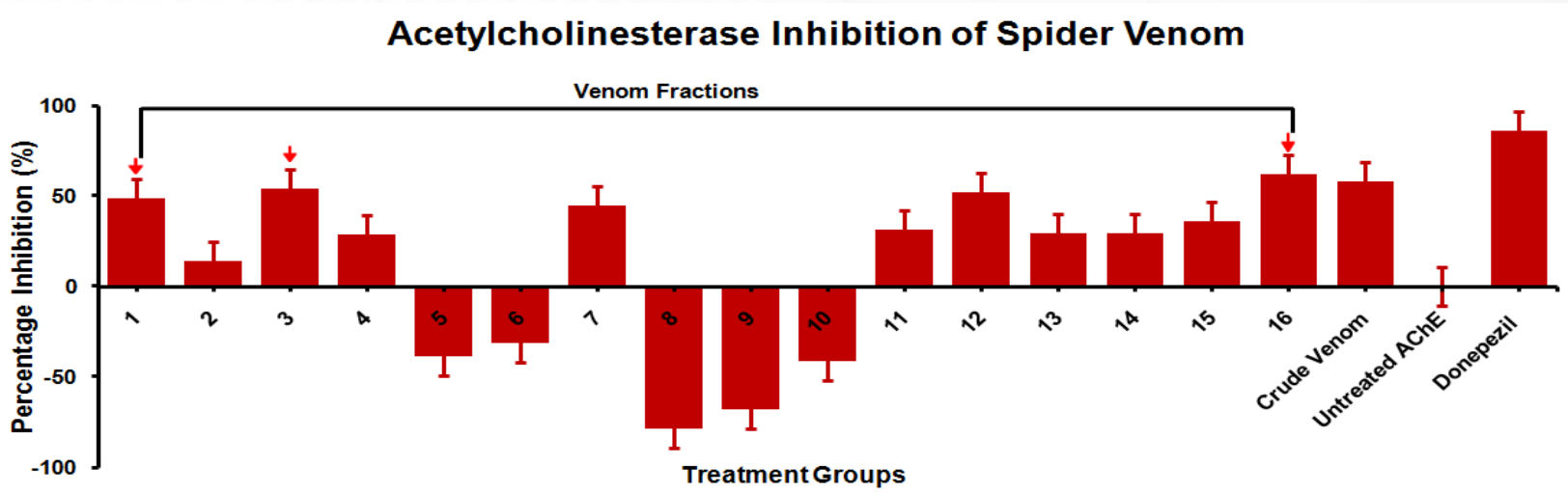

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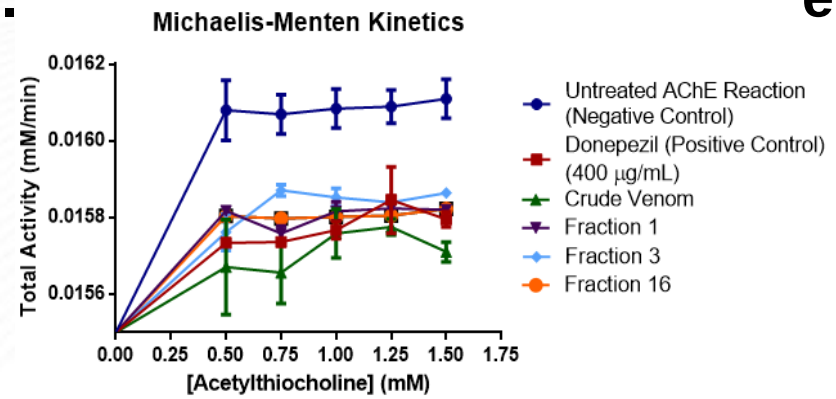


e.

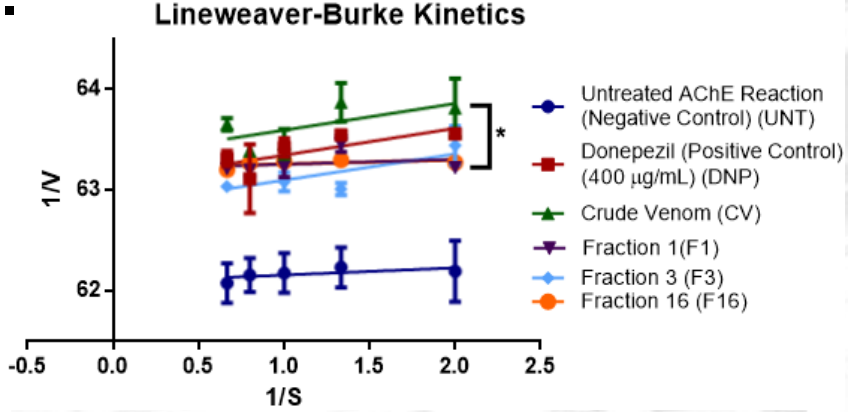
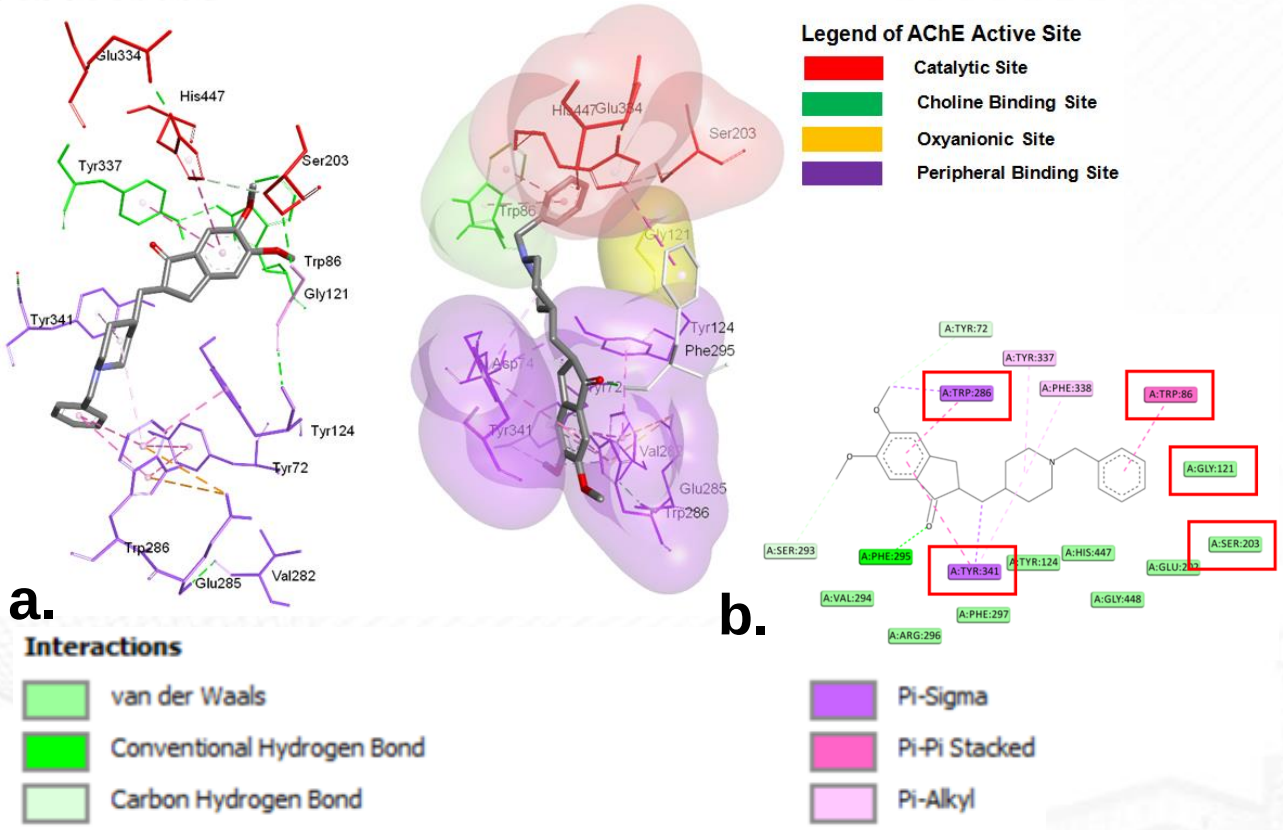


Figure 6. (a) Fractions exhibited anti-AChE show a kinetic curve below the untreated AChE reaction. (b) Fractions exhibited pro-AChE show a kinetic curve above the untreated AChE reaction. (c) Generally weak inhibition was observed of crude and fractionated spider venom in comparison to donepezil. (d) and (e) F1,F3,F16, crude venom and donepezil exhibited (un)competitive inhibitions (n=3).

AChE Docking and Visualization Studies: Insights Towards Rational Drug Design of Venom-Peptide Inhibitors

Donepezil docking reveal certain binding interactions with the AChE active site.



Visualization of putative inhibition sites of spider venom peptides on AChE

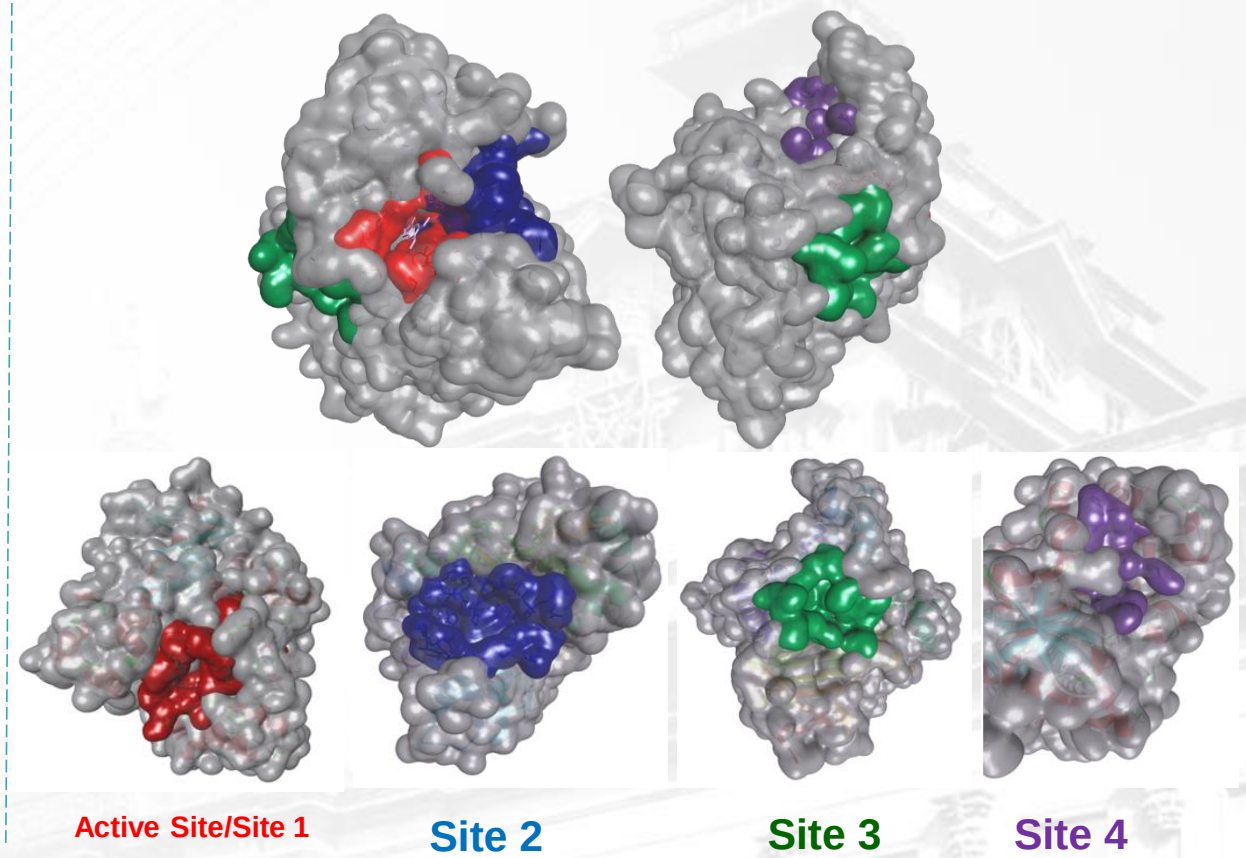
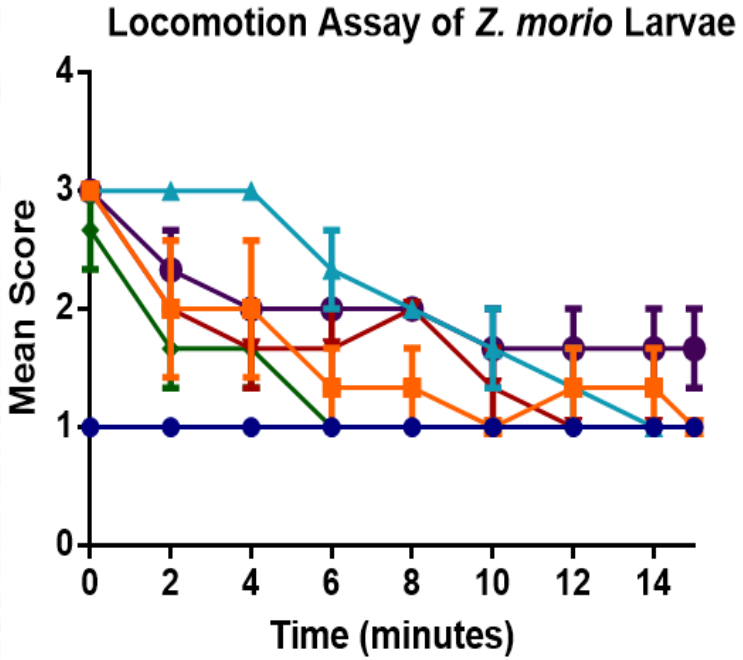


Figure 7. (a) 3D and (b) 2D interaction diagram have shown that the drug has preferential binding in peripheral binding site and oxyanionic site of the AChE active site.

Figure 8. (a) Overlayed and (b) individual visualizations reveal certain putative regions of AChE where the binding of spider venom peptides may happen to exhibit conformational change-induced enzyme inhibition (Roca et al., 2018).

Different degrees of locomotion were observed after administration of donepezil and spider venom on *Zophobas morio* larvae.



Parameters Observed

Rate of forward and backward movements

Scoring:
4 - Severe continuous movements, larvae dies
3 - Continuous, rapid movements
2 - Sporadic movements
1 - Little to no movements

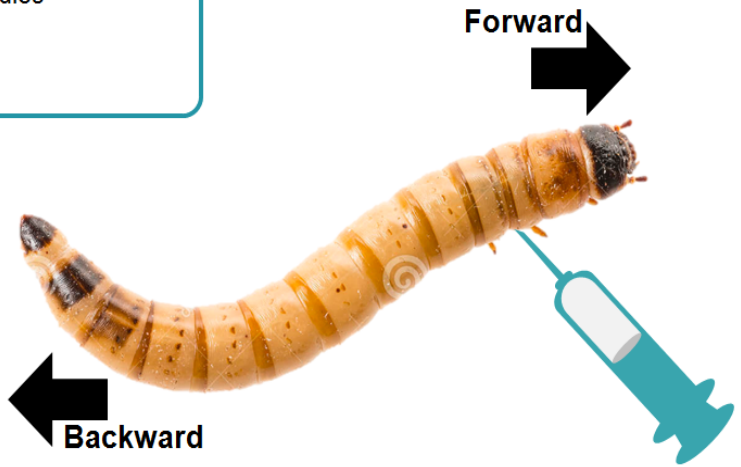
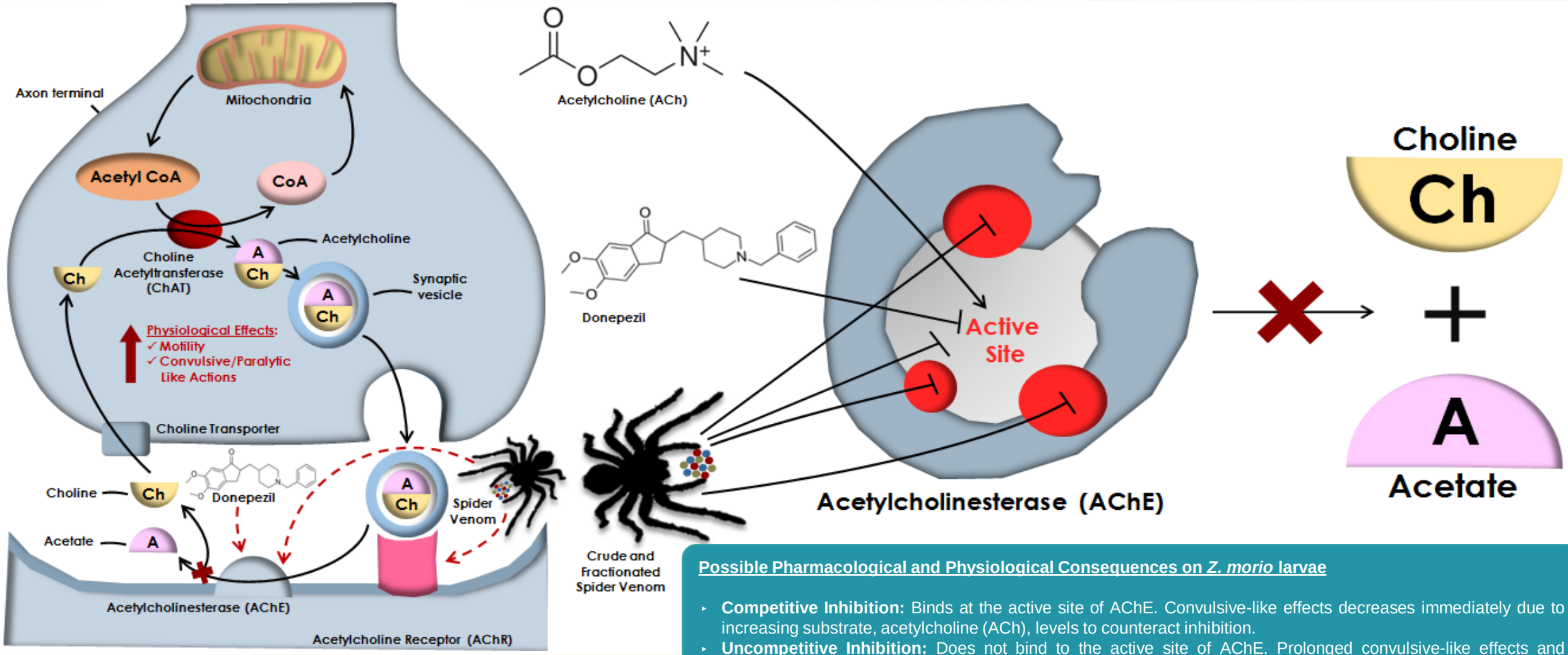


Figure 9. Downward trend was observed on donepezil and spider venom upon metathoracic administration on *Z. morio* larvae in comparison with 0.9% NSS. This could be attributed to cholinergic inhibition which is manifested on different degrees of motility on the larvae (n=3).

Proposed Cholinergic Action of Donepezil and Spider Venom from *P. bundokalbo*



Possible Pharmacological and Physiological Consequences on *Z. morio* larvae

- **Competitive Inhibition:** Binds at the active site of AChE. Convulsive-like effects decreases immediately due to increasing substrate, acetylcholine (ACh), levels to counteract inhibition.
- **Uncompetitive Inhibition:** Does not bind to the active site of AChE. Prolonged convulsive-like effects and increasing ACh levels cannot counteract inhibition.

Figure 10. Cholinergic Role of Spider Venom to Cholinergic Nervous System

Conclusions and Recommendations

- Crude and fractionated **spider venom from *P. bundokalbo*** contains **various peptides** which exhibited **cholinergic impact** as supported by *in vitro*, *in silico* and *in vivo* studies.
- May exhibit therapeutic potential against AD **as an AChE inhibitor.**

Dose-dependent evaluation of the crude and fractionated venom

Further optimization and purification of the peaks which cannot by separated by one round of RP-HPLC

Structural elucidation of the venom fractions exhibiting AChE inhibitory activities for purported used against AD.

Integrate the AChE inhibitory results of spider venom ***in vitro*** to **transgenic model** organisms preferably with hallmarks of **Alzheimer's disease *in vivo***

Acknowledgement:

ProVe Proteomics and Venomics Group