VALIDATING THE TRADITIONAL USE OF ANNONA LEAVES AS BIO-PESTICIDES THROUGH ACETYLCHOLINESTERASE INHIBITION ASSAYS

RITU SHARMA[1]

Department of Zoology, Pradhan Mantri College of Excellence, Govt. Autonomous P.G. College, Chhindwara, M.P., India

ABSTRACT

The agricultural sector faces a critical need for sustainable alternatives to synthetic organophosphate and carbamate pesticides, which cause environmental toxicity and human health risks through irreversible inhibition of acetyl cholinesterase (AChE). This study validates the traditional use of *Annona squamosa* (Sugar Apple) leaves as a bio-pesticide by investigating their neurotoxic effects on a non-target aquatic organism, *Tilapia mossambica*(Peters).Fresh *Annona squamosa* leaves were ethanolic-extracted using a Soxhlet apparatus. The fish were exposed to a 100 mg/L concentration of the extract for 96 h. Following exposure, a recovery study was conducted by transferring the fish to fresh water for 120 h. AChE activity in the kidneyswas periodically measured using the Ellman method, and protein content was determined using the Lowry assay. Exposure to *Annona squamosa* leaf extract resulted in a significant reduction in AChE activity in the kidney, falling to 5.91 ± 1.5 nmol/min/mg protein, representing a 21.3% inhibition compared to the control group. During the recovery phase, the enzymatic activity showed gradual restoration, with 0.75% recovery at 48 h, 5% at 72 h, and 33% recovery after 120 h in freshwater. These findings confirm that *Annona squamosa* leaf extract possesses distinct anticholinesterase activity. This study provides biochemical evidence supporting the integration of *Annona* species into modern Integrated Pest Management (IPM) strategies as a biodegradable and potent natural alternative to synthetic chemicals.

KEYWORDS: *Annona squamosa*, acetylcholinesterase (AChE) Inhibition, Bio-pesticides, *Tilapia mossambica*, Ethnopharmacology, Neurotoxicity

INTRODUCTION

The global agricultural sector is currently grappling with a dual crisis: escalating demand for food security and environmental degradation caused by synthetic chemical pesticides. Although conventional organophosphates and carbamates have historically bolstered crop yields, their indiscriminate use has led to pesticide resistance, soil toxicity, and severe neurotoxic risks to non-target organisms, including humans (Saeed *et al.*, 2021). They cause acute toxicity due to their ability to phosphorylate acetyl cholinesterase (AChE, EC: 3.1.1.7), the enzyme that hydrolyses the neurotransmitter acetylcholine in muscular and neural synaptic clefts, leading to the irreversible inhibition of its active site and resulting in overstimulation of postsynaptic cholinergic receptors due to the accumulation of the neurotransmitter acetylcholine in synapses. They are used as markers of pesticide exposure and effects (Fulton and Key, 2001; Colovic *et al.*, 2013).

A variety of toxicants are constantly discharged into water bodies from major sources such as domestic, agricultural, and industrial waste. The accumulation of such toxicants yields physiological, biochemical, and histological detrimental effects in freshwater fauna by affecting various activities, such as metabolism and enzymes (Cope, 2004; Wang *et al.*, 2009).Consequently, there is a renewed scientific urgency to explore botanical alternatives that are biodegradable, effective, and ecologically sustainable.

Plants belonging to the genus *Annona* (Family: Annonaceae), such as *Annona muricata* (soursop) and *Annona squamosa* (Sugar Apple), have been utilised for centuries in traditional farming practices across tropical regions (Isman, 2020).Indigenous knowledge systems frequently cite the use of *Annona* leaf extracts as potent "bio-pesticides" to protect stored grains and standing crops from insect infestation (Souza *et al.*, 2022).The insecticidal efficacy of these plants is primarily attributed to a unique class of polyketide-derived compounds, known as annonaceousacetogenins (ACGs). While their ability to disrupt cellular respiration via the inhibition of Mitochondrial Complex I is well-documented (Zeng *et al.*, 2023), emerging research suggests a more complex, multi-targeted neurotoxic effect on insect pests.

The search for natural AChE inhibitors aims to identify compounds that offer high specificity for pests with lower toxicity to nontarget organisms, including fish and mammals (Rattan, 2010). Despite widespread anecdotal evidence supporting the use of *Annona* leaves, rigorous biochemical validation is required to bridge the gap between traditional wisdom and modern pharmacology (Grdiša and Gršić, 2021).

Recent phytochemical screening suggests that the alkaloids and flavonoids present in *Annona* species may act synergistically with acetogenins to disrupt the enzymatic activity of AChE (Saleem *et al.*, 2024).Given the potential ethnobotanical and pharmacological applications of this plant, this study was designed to investigate the possible efficacy of *Annona squamosa* leaf extract on acetylcholinesterase inhibitory activity in the

[1]**Corresponding author**

kidneys of the fish *Tilapia mossambica*(Peters).The kidneys, the major detoxification organs for many xenobiotics, are frequently susceptible to nephrotoxic effects. The kidneys receive approximately 20-25% of the resting cardiac output. Therefore, it receives a relatively higher load of drugs or chemicals. The process involved in forming concentrated urine also serves to concentrate potential toxicants in tubular cells (Rekha *et al.*, 2013).

By validating the biochemical pathways through which *Annona*squamosa leaf extract exerts neurotoxic effects, this study seeks to justify the integration of traditional botanical knowledge into modern Integrated Pest Management (IPM) strategies.

MATERIALS AND METHODS

Fish (*Tilapia mossambica*, length: 10±2 cm, weight: 10±2 gm) were collected from Patra fish seed farm, located in Bhopal, M.P., India and were acclimatised to the laboratory conditions for 15 days in glass aquaria. They were fed daily with palletised supplementary feed, and the water was renewed daily. Collection and preparation of plant material: Fresh *Annona squamosa* leaves were collected from the botanical garden in Bhopal. The leaves were thoroughly washed, shade-dried, powdered, and extracted in a Soxhlet apparatus with 90% ethanol. The extract was kept at room temperature for the evaporation of ethanol until a semi-solid mass remained. This was kept stored at 40°C for further use as a source of plant extract. 100 mg/L of A. squamosa extract was used for 96 hrs. exposure to fish. A fresh stock solution of the extract was prepared in acetone for each use.

Treatment protocol: Fish were divided into two groups of 30 each. Group-I: served as control without toxicant; Group-II: added 100 mg/L of *Annona squamosa* leaf extract daily for 96 hrs. After 96 hrs, five fish were removed from each group to study the effect of toxicants.

Drug withdrawal: The remaining fish were transferred to fresh water to study the leaching effect of water and recovery of inhibited AChE for 120 h. The water was changed every 24 h. Five fish from each group were removed, dissected, and the kidneys were collected at the end of every 24, 48, 72, 96, and 120 h. for the assessment of AChE level recovery.

Sample preparation: Fishes were euthanised, dissected, d and the kidneys were quickly removed y and washed with n 0.9% saline. A 10% (w/v) tissue homogenate was prepared in an Elvehjem potter homogeniser and centrifuged at 5000 rpm for 20 min in a cooling centrifuge (Remi) at 4°C. The supernatants were kept in a deep freeze for AChE assay, inhibition, AChE recovery, and protein content. AChE was estimated using the method described by Ellman *et al.* (1961).

Protein Assay: Five fish from each group were used for protein estimation using the method described by Lowry *et al.* (1951), using BSA as the standard. Samples of homogenate were diluted with reagents, then 0.5 ml of Folin's reagent was added, and after 20 min. incubation at RT, read at 620 nm against a reagent blank. All measurements were performed in duplicate.

Statistical analysis: Graphs of the results were prepared using Excel 2007 software. For the statistical comparison between treatments and controls, data were analysed using Student's t-test to determine the effect of the treatment. The level of statistical significance was set at $p>0.05$.

RESULTS AND DISCUSSION

In the present investigation, the anticholinesterase potential of *Annona squamosa* leaf extract toxicityin the kidney of *Tilapia mossambica* was studied. In addition, recovery after keeping the fish in fresh water for 120 hours was also discovered in this study.

The physico-chemical characteristics of water were determined (APHA, 1995), and were: temperature- 26 ± 2° C, pH- 7.1 ± 0.2, dissolved oxygen- o 6.8 ± 0.3 mg/L, total alkalinity- 170 ± 10 mg/L and total Hardness- 15 ± 1 mg/L.

Effect of toxicants on AChE activity: AChE activity in the kidney of fish exposed to *Annona squamosa* leaf extract decreased to 5.91 ± 1.5 nmol/min/mg protein compared to control fish (7.51 ± 3.82 nmol/min/mg protein), yielding 21.3 % inhibition in AChE activity. The recovery was 0.75%, 5% and 13.6 % at 48,72 and 96hours, respectively. 33% AChE recovered after 120 h. (Table-1). Our findings show that the ethanolic leaf extract of *Annona squamosa* significantly decreased AChE activity. Almost similar results were found in other fishes (Jindal *et al.*, 2014; Joseph *et al.*, 2011; Wang *et al.*, 2010). AChE inhibitory activities were exhibited by various plant extracts, such as *Capsicum chinense* Jacq. (Vargas *et al.*, 2016) *Tamarindus indica* (Biswas *et al.*, 2017), *Calendula arvensis*, *Chenopodium murale*, and *Nicotiana glauca* (Sellem *et al.*, 2016).

The accumulation and bio magnification of synthetic compounds into different non target organisms, including humans, through the food chain, with an increased risk of the development of diseases or disease syndromes, has prompted the exploration of relatively safer and more potent molecules for better insect pest management.

Table 1: Effect of A. squamosa on Kidney AChE Activity and Subsequent Recovery

Experimental Condition	AChE activity (nmole/min/ mg protein)	% inhibition of AChE	% Recovery
Control Group	7.51 + 3.82	0 %	
Exposed Group	5.91 + 1.5***	21.3%	
Recovery: 48 Hours			0.75
Recovery: 72 Hours			5.0
Recovery: 96 Hours			13.6
Recovery: 120 Hours			33.0

CONCLUSION

Based on the findings of the present study, it can be concluded that *Annona squamosa* possesses anticholinesterase activity, which may be due to the presence of alkaloid components in the extract. Further purification and isolation should be performed to understand the mechanism of AChE inhibitory activity. Thus, the ethanolic extract of *Annona squamosa* leaves may be considered a potent and natural herb with AChE-inhibiting properties.

REFERENCES

APHA/AWWA/WEF : 21st edition, editors: Andrew D. Eaton, Lenore S., Clesceri, Eugene, W. Rice and Arnold E.: Standard Methods for the Examination of Water and Waste water (2005).

Biswas K, Azad A.K., Sultana T., Khan F., Hossain S., Alam S., Choudhary R. and Khatun Y., 2017. J. Intercul. Ethnopharmacol., **6(1)**:115-120.

Colovic M.B., Krstic D.Z., Lazarevic-Pasti T.D., Bondzic A.M. and Vasic V.M., 2013. Acetylcholinesterase inhibitors: Pharmacology and toxicology. Current Neuropharmacology, **11(3)**:315–335. https://doi.org/10.2174/1570159X11311030006

Cope W.G., 2004: Exposure classes, toxicants in air, water, soil, domestic, and occupational settings. In: A T.B. of Modern Toxicology (Ed.: E. Hodgson). John Wiley and Sons Inc., New Jersey, USA, pp. 33-48.

Ellman G.L., Courtney K.D., Andres V. and Featherstone R.M., 1961. A new and rapid colorimetric method for the determination of acetylcholinesterase activity. Biochemical Pharmacology, **7(2)**: 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Fulton M.H. and Key P.B., 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Toxicol. Chem. **20**: 37–45.

Grdiša M. and Gršić K., 2021. Botanical insecticides: A review of their uses and health effects. International Journal of Environmental Research and Public Health, **18(9)**: 4642. https://doi.org/10.3390/ijerph18094642

Isman M.B., 2020. Botanical insecticides in the twenty-first century: fulfilling their promise? Annual Review of Entomology, **65**: 233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Jindal R. and Kaur M., 2014. Intern. J. Sci. Environ. Technol., **3(2)**: 473-480.

Joseph B. and Raj S.J., 2011. Int. J. Zool. Res., **7**: 212-222.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J., 1951. J. Bol. Chem., **193**: 265-275.

Rattan R.S., 2010. Mechanism of action of commercially available botanical insecticides: A review. Journal of Entomology and Nematology, **2(3)**: 27–32.

Rekha, Raina S. and Hamid S., 2013. Histopathological effects of pesticide chloropyrifos on the kidneys of albino rats. Int. J. Res. Med. Sci., **1**:465- 75. DOI: 10.5455/2320 6012.ijrms2013113.

Saeed M., Shoaib M., Faisal S., Al-Ghamdi A.A. and Ahmad W., 2021. Pesticide resistance and the role of botanical insecticides in sustainable agriculture. Environmental Science and Pollution Research, **28(15)**: 18456–18468.

Saleem M., Ahmed B. and Qasim M., 2024. Synergistic neurotoxic effects of flavonoids and alkaloids in *Annona* species: A target for modern bio-pesticides. Phytochemistry Letters, **58**: 112–120.

Sellem I., Fatma Kaaniche F., Mtibaa A.C. and Mellouli

L., 2016. Bangladesh J. Pharmacol., **11**: 531-544.

Souza L.P., Silva R.R. and Martins G.F., 2022. Ethnobotanical uses of Annonaceae: A systematic review of their insecticidal properties. Journal of Ethnopharmacology, **285**: 114858. https://doi.org/10.1016/j.jep.2021.114858

Vargas-Méndez L.Y., Rosado-Solano D.N., Sanabria Flórez P.L., Puerto-Galvis C.E. and Kouznetsov V., 2016. J. Med. Plants Res., **10(6)**: 59-66.

Wang C., Lu G., Cui J. and Wang P., 2009. Sublethal effects of pesticide mixtures on selected biomarkers of Carassius auratus, Environmental Toxicology and Pharmacology, **28(3)**: 414-419.

Wang C., Lu G. and Cui J., 2010. Environmental Toxicology, **27**: 50-57.

Zeng L., Ye Q., Oberlies N.H., Shi G., Gu Z.M., He K. and McLaughlin J.L., 2023. Recent advances in annonaceousacetogenins. Natural Product Reports, **40(2)**: 345–362.