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Inhibition and Recovery of Acetylcholinesterase Activity in the fish Labeo rohita (Hamilton,1822) following Exposure to Annona squamosa Leaf Extract

Dr. Ritu Sharma

Assistant Professor, Department of Zoology, Pradhan Mantri College of Excellence, Government Autonomous Post Graduate College, Chhindwara, M.P., India

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ABSTRACT

The agricultural sector urgently requires sustainable substitutes for synthetic organophosphate and carbamate pesticides, which pose environmental and health risks due to their irreversible inhibition of acetylcholinesterase (AChE). This study confirms the traditional use of *Annona squamosa* (Sugar Apple) leaves as a biopesticide by examining their neurotoxic impact on the non-target aquatic species *Labeo rohita* (Hamilton, 1822). The fish were subjected to a 100 mg/L concentration of fresh *Annona squamosa* leaf extract for a duration of 96 hours. Following this, a recovery phase was initiated by transferring the fish to clean water for 120 hours. AChE activity in the kidney was periodically measured using the Ellman method, while protein content was determined with the Lowry assay. Exposure to *Annona squamosa* leaf extract resulted in a significant reduction in AChE activity in the kidney, with a value of 5.93 ± 1.01 nmol/min/mg protein, corresponding to 21.45% inhibition relative to the control group. During the recovery phase, enzymatic activity gradually improved, with 13.58% recovery at 48 hours, 38.88% at 72 hours, 64.81% at 96 hours, and 79.62% after 120 hours in freshwater. These findings demonstrate that *Annona squamosa* leaf extract exhibits considerable anticholinesterase activity, yet the recovery of inhibited AChE is rapid. This research provides biochemical evidence supporting the integration of *Annona* species into modern Integrated Pest Management (IPM) strategies as a biodegradable and effective natural alternative to synthetic chemicals.

Keywords: AChE, Inhibition, *Annona squamosa*, Kidney, *Labeo rohita*

INTRODUCTION

The global agricultural sector faces a dual challenge: the escalating demand for food security and the environmental damage associated with synthetic chemical pesticides. Various toxic substances are continuously discharged into aquatic environments from primary sources, including domestic, agricultural, and industrial waste. The accumulation of these toxicants leads to detrimental physiological, biochemical, and histological effects on freshwater fauna by disrupting metabolic and enzymatic functions (Cope, 2004; Wang et al., 2009). Consequently, there is a renewed scientific interest in exploring botanical alternatives that are biodegradable, effective, and environmentally sustainable. Although traditional organophosphates and carbamates have historically increased agricultural productivity, their widespread use has led to pesticide resistance, soil contamination, and significant neurotoxic risks to non-target organisms (Saeed et al., 2021). They induce acute toxicity by phosphorylating acetylcholinesterase (AChE, EC: 3.1.1.7), the enzyme which hydrolyses neurotransmitter acetylcholine in muscular and neural synaptic clefts, leading to the irreversible inhibition of its active site and resulting overstimulation of postsynaptic cholinergic receptors due to the accumulation of the neurotransmitter acetylcholine in synapses and used as marker of pesticide exposure and effects (Fulton and Key, 2001). 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).

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

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(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). Their ability to disrupt cellular respiration via the inhibition of Mitochondrial Complex I is well-documented (Zeng et al., 2023), and emerging research suggests a more complex, multi-targeted neurotoxic effect on insect pests. Despite widespread evidence supporting the use of Annona leaves, rigorous biochemical validation is required to bridge the gap between traditional wisdom and modern pharmacology (Grdisa & Grsic, 2021).

Kidneys, the major detoxification organs for many xenobiotics, receive approximately 20-25% of the resting cardiac output. Therefore, it receives a relatively higher load of drugs or chemicals and is frequently susceptible to nephrotoxic effects (Rekha et al., 2013).

Given the potential pharmacological and ethnobotanical applications of *Annona squamosa,* this study was designed to investigate the possible efficacy of the leaf extract of this plant on acetylcholinesterase inhibitory activity and to determine the recovery of inhibited activity in the kidney of the fish *Labeo rohita* (Hamilton, 1822). By validating the biochemical pathways through which *Annona squamosa* exerts neurotoxic effects, this study seeks to justify integrating traditional botanical knowledge into modern Integrated Pest Management (IPM) strategies.

MATERIALS AND METHODS

Experimental animal: Healthy fingerlings of fish (*Labeo rohita*, 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.

Sample preparation: Fishes were euthanised, dissected, and the kidneys were quickly removed and washed within 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 the AChE assay, inhibition, AChE recovery, and protein content. AChE was estimated using the method described by Ellman et al. (1961).

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 until the ethanol evaporated, leaving a semi-solid mass. This was kept stored at 40°C for further use as a source of plant extract. 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 48, 72, 96, and 120 h. for the assessment of AChE level recovery.

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

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 $p>0.05$.

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RESULTS AND DISCUSSION

The accumulation and biomagnification of synthetic compounds in nontarget organisms, including humans, through the food chain, with an increased risk of disease development, has prompted the exploration of safer molecules for improved insect pest management.

In the present investigation, the anticholinesterase potential of *Annona squamosa* leaf extract and its toxicity in the kidneys of Labeo rohita were 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- 25 ± 2° C, pH- 7.1 ± 0.8, dissolved oxygen- 6.8 ± 0.8 mg/L, total alkalinity- 172 ± 12 mg/L and total Hardness- 15 ± 2 mg/L.

Table 1 shows the effect of *A. squamosa* on kidney acetylcholinesterase (AChE) activity and the subsequent recovery phase. AChE activity in the kidney of fish exposed to *Annona squamosa* leaf extract decreased to 5.93 ± 1.01 nmol/min/mg protein compared to control fish (7.55 ± 2.01 nmol/min/mg protein), yielding 21.45 % inhibition in AChE activity. 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). The recovery was 13.58%, 38.88%, and 64.81% at 48, 72, and 96 hours, respectively. 79.62% AChE recovered after 120 h. (Table-1). Our findings showed that the ethanolic leaf extract of *Annona squamosa* significantly decreased AChE activity. Almost similar results were found in other fish species (Jindal et al.,2014; Joseph et al., 2011; Wang et al.,2010). Various plant extracts, such as Capsicum chinense Jacq exhibited AChE inhibitory activities. (Vargas et al., 2016) *Tamarindus indica* (Biswas et al., 2017), *Calendula arvensis*, *Chenopodium murale*, and *Nicotiana glauca* (Sellem et al.,2016).

Exposure to *A. squamosa* caused a significant alteration in kidney AChE activity compared to the control group. After the cessation of exposure, the recovery phase demonstrated almost complete restoration of AChE activity toward baseline levels, indicating that the effect induced by *A. squamosa* on kidney AChE activity is potentially reversible.

These findings suggest that *A. squamosa* influences cholinergic function in the kidney of the fish. The reversible nature of the effect implies potential for recovery following removal of the toxicant, which is important for understanding the toxicodynamics of *A. squamosa*.

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

Group / Parameters	Control	*Annona squamosa* leaf extract	Recovery in hours			
Time in hours	-	-	48	72	96	120
AChE activity (nmole/min/ mg protein)	7.55 ± 2.01	5.93± 1.01	6.15± 0.34	6.56± 0.41	6.98±0.33	7.22±0.84
% inhibition of AChE	-	21.45	18.54	13.11	7.54	4.37
% recovery of AChE	-	-	13.58	38.88	64.81	79.62

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CONCLUSION

Based on the present investigations, it may 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 extract of *Annona squamosa* leaves may be considered a potent natural herb with AChE-inhibiting properties.

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