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Preliminary Investigation into Expression and characterization of Acetylcholinesterase from *Bulinus globosus* as a potential target for control of Schistosomiasis

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Abstract
Bulinus globosus, a freshwater snail, serves as the intermediate host for schistosomiasis, a disease that affects millions globally. Vector control remains a key strategy for disease elimination, and the emergence of resistance highlights the urgent need for novel molluscicides. AChE is a crucial enzyme that could be exploited as a target for controlling this vector.
In this study, AChE was extracted from the hepatopancreas, visceral mass, and foot muscle of *B. globosus* using established protocols. Partial purification was achieved through ion exchange chromatography using CM-Sephadex C-50 followed by DEAE-Sephadex A-25 chromatography. Kinetic parameters and physicochemical properties of the partially purified enzyme were determined.
Among the tissues analyzed, the hepatopancreas exhibited the highest specific AChE activity (0.591 ± 0.0134 units/mg protein), followed by the foot muscle (0.199 ± 0.0143 units/mg protein) and visceral mass (0.1908 ± 0.0113 units/mg protein). AChE purification folds were 6, 4, and 3 for the hepatopancreas, foot muscle, and visceral mass, respectively. The optimal pH for AChE activity was 8.0 for both the hepatopancreas and visceral mass, and 7.0 for the foot muscle. The Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) values for AChE were 0.72 mM ± 0.13 and 0.32 ± 0.003 units/mg protein (hepatopancreas), 0.18 mM ± 0.01 and 0.20 ± 0.01 units/mg protein (foot muscle), and 0.18 mM ± 0.014 and 0.20 ± 0.005 units/mg protein (visceral mass). The differential expression and physicochemical properties of AChE in various tissues of *B. globosus* suggest its potential as a target for molluscicidal intervention.

Keywords: Acetylcholinesterase; *Bulinus globosus*; Schistosomiasis; Kinetic properties.

INTRODUCTION

Acetylcholinesterase (AChE; EC 3.1.1.7) is an essential enzyme that has been extensively investigated across vertebrate and invertebrate species, with particular focus on its functions in nerve and muscle tissues (Lazarevic-Pasti *et al*., 2017). AChE, a serine hydrolase enzyme, is present in synaptic and non-synaptic regions, where it efficiently degrades acetylcholine (ACh) a neurotransmeter to halt signaling at cholinergic synapses and neuromuscular interfaces (Morrison, 2021). Understanding the catalytic mechanism of AChE at the molecular level is vital for interpreting the actions of its inhibitors, which are widely applied in medicine (as therapeutic agents), agriculture (as pesticides), and, regrettably in chemical warfare. (Colović *et al*., 2013). Beyond its primary role in nerve impulse transmission, AChE also has "non-classical" functions. It's known to be involved in several key biological processes, including

regulating cell growth and specialization, and helping cells manage the effects of oxidative stress (Morrison, 2021).

AChE exists in both hydrophobic and hydrophilic forms, each fulfilling distinct roles. Hydrophilic AChE operates within cells to degrade excess intracellular ACh, while lipid-linked (hydrophobic) AChE functions at the synaptic cleft or neuromuscular junction, ensuring the rapid inactivation of ACh by breaking it into acetate and choline. The hydrophobic forms are embedded within post-synaptic membranes, strategically positioned near receptor molecules to facilitate the quick termination of nerve impulses (Trang and Khandhar, 2025).

AChE is widely distributed in nervous tissues, including the brainstem, cerebellum, and both the peripheral and autonomic nervous systems. In skeletal muscles, its localization appears to be linked to muscle type, distinguishing between fast- and slow-twitch fibers, as well as their specific roles. While AChE is well known for its function in the nervous system and muscles, its presence in red blood cells is less frequently acknowledged. Blood group antigens, found on the outer lipid bilayer of red blood cells, enable antibody recognition, and similarly, AChE is also integrated into red blood cell membranes (Sam and Bordoni, 2023).

AChE plays an important role in maintaining homeostasis. However, it can be inhibited by potent toxins, including insecticides, snake venom, and chemical warfare agents. Due to its widespread distribution in tissues, functional and molecular diversity, understanding the structure and physicochemical properties of AChE can provide insights into designing specific drugs for treating neurodegenerative diseases associated with AChE dysregulation (Walczak-Nowicka and Herbet, 2021).

B. globosus is a freshwater snail that acts as the intermediate host for *Schistosoma haematobium*, the parasite that causes the human disease schistosomiasis. (Mkize *et al*., 2016). Schistosomiasis, a water-transmitted parasitic infection, impacts around 258 million individuals worldwide, with sub-Saharan Africa's poorest regions bearing approximately 90% of global cases (Colley *et al*., 2014, WHO, 2019). Nigeria is the most affected country globally, with an estimated 20 million people requiring annual treatment for the disease. (Hastings, 2016). The disease schistosomiasis arises from infection with five principal *Schistosoma* species. Intestinal forms are caused by *S. mansoni*, *S. intercalatum*, *S. japonicum*, and *S. mekongi*, while *S. haematobium* is responsible for urogenital complications. Within sub-Saharan Africa, *S. mansoni* and *S. haematobium* are the most prevalent. (Salawu and Odaibo, 2016).

Schistosomiasis control in developing regions remains difficult due to financial limitations and environmental challenges, with current strategies emphasizing snail population management, treatment with Praziquantel, and improved sanitation, etc (Limpanont *et al*., 2020). Among these, the use of molluscicides remains an important approach (Zheng *et al*., 2021). Understanding the kinetic and physicochemical behaviour of AChE in *B. globosus* may contribute to innovative approaches in vector control, considering the pivotal role of snails in schistosomiasis transmission. This study aims to partially purify AChE from *B. globosus*, determine its kinetic parameters (K_m and V_{max}), and assess the effect of pH on enzyme activity. These findings may contribute to the development of novel schistosomiasis control strategies.

MATERIALS AND METHODS

Organism

Live freshwater snails (*B.s globosus*) were collected from a running drainage within Obafemi Awolowo University (OAU), Ile-Ife, Nigeria.

Chemicals

2-mercaptoethanol, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide, boric acid, bovine serum albumin (BSA), citric acid, CM-Sephadex C-50, DEAE- Sephadex A-25, disodium hydrogen phosphate (Na_2HPO_4), ethylene-diamine tetraacetic acid (EDTA), glycerol,

glycine, sodium acetate, sodium borate, sodium chloride, sodium citrate, sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide pellets, Tris acid, and Tris base were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals used were of analytical grade.

Preparation of crude AChE tissue homogenates

Live freshwater snails were sacrificed, and the hepatopancreas, foot muscle, and visceral mass were dissected, kept on ice, and weighed immediately. A 30% (w/v) tissue homogenate was prepared in 10 mM phosphate buffer (pH 8.0) for each tissue. The homogenates were centrifuged at 12,000 × g for 30 min at 4 °C to obtain clear supernatants. The supernatants were filtered through cheesecloth to remove floating lipids, and aliquots were stored at -20 °C for subsequent AChE assays and protein concentration determination.

AChE activity assay and protein determination

The activity of AChE was determined in both crude and partially purified enzyme preparations using the method of Ellman *et al*. (1961). The reaction mixture (1.2 ml total volume) contained 0.1 M phosphate buffer (pH 8.0), 8 mM DTNB in 0.1 M phosphate buffer (pH 7.0), and 48 mM acetylthiocholine iodide. The enzyme solution was added to initiate the reaction. A reaction mixture without enzyme served as a blank to estimate the rate of spontaneous substrate hydrolysis. Absorbance of the reaction product, 2-nitro-5-thiobenzoate anion, was monitored at 412 nm every 15 s for 4 min. A blank without substrate was also used to evaluate thiol interaction with DTNB. One unit of AChE activity was defined as the amount of enzyme catalyzing the hydrolysis of 1.0 µmol of acetylthiocholine per minute under the assay conditions. The extinction coefficient (ε) of the product was 1.36×10^4 mM^{-1} cm^{-1}. The protein concentrations were routinely measured at all purification steps following the method of Bradford (1976) as described by Agunbiade *et al*., (2021) using bovine serum albumin as the standard.

Enzyme purification

Purification on CM-Sephadex C-50 column

Crude extract obtained from different tissues of *B. globosus* was layered on packed CM-Sephadex C-50 resin column. The unbound proteins were washed with equilibration buffer at a flow rate of 15 ml/hr, while bound proteins were subsequently eluted at the flow rate of 22 ml/hr with 0.1 M phosphate buffer, pH 8.0 (linear gradient 0–1 M in NaCl). Each fraction was assayed for AChE activity, and the protein profile of the fractions was determined at 280 nm. Active fractions were pooled together, concentrated and assayed for AChE activity and protein concentration.

Further purification by ion exchange chromatography on DEAE-Sephadex A-25 column

A column containing DEAE-Sephadex A-25 was equilibrated with 0.1 M Tris buffer (pH 8.0). The concentrated enzyme obtained from post-CM-Sephadex C-50 was applied to the column. The unbound proteins were washed with equilibration buffer at a flow rate of 15 ml/hr, while bound proteins were subsequently eluted at the flow rate of 22 ml/hr with elution buffer containing NaCl in a linear gradient (0–1 M). Forty fractions (2.0 ml each) were collected and active fractions were pooled and stored at 0 °C.

Determination of kinetic parameters

The apparent Michaelis–Menten constant (K_m^{ATCI}, K_m^{DTNB}) in the partially purified extracts of the different tissues was determined by measuring esterase activity at a fixed concentration of DTNB (1.0 mM) and varying concentrations of ATCI from 0.2 to 5.0 mM. The assay was repeated at a fixed concentration of ATCI (1.0 mM) and varying concentrations of DTNB (0.2 – 5.0 mM). The apparent K_m was determined from a non-linear regression curve on Graphpad Prism 8.0.

Effect of pH on AChE activity

The effect of pH on the activity of purified *B. globosus* AChE was evaluated using various buffer systems over a pH range of 5.0 to 10.0. The following buffers were employed for the assays: 0.1 M acetate buffer (pH 3.0 - 5.0), 0.1 M citrate buffer (pH 5.5 - 6.5), 0.1 M phosphate buffer (pH

7.0 - 8.5), and 0.1 M borate buffer (pH 9.0–10.0). Enzyme activity was measured as previously described for AChE.

Statistic

All experiments are carried out at least in triplicate unless otherwise stated. Data were expresses as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 8.0 software.

RESULTS

Distribution of AChE activity in B. globosus

The specific activities of AChE in the crude extracts of different tissues from *B. globosus* are presented in Figure 1. AChE activity was detected in the hepatopancreas, foot muscle, and visceral mass, with specific activities of 0.591 ± 0.0134 units/mg protein, 0.199 ± 0.0143 units/mg protein, and 0.1908 ± 0.0113 units/mg protein, respectively. The highest specific AChE activity was observed in the hepatopancreas, while the lowest activity was recorded in the foot muscle.

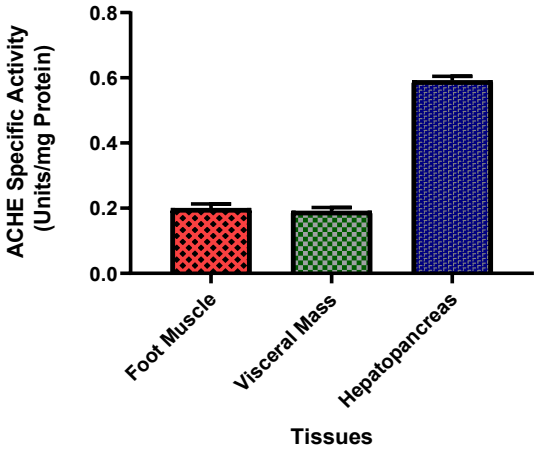

Figure 1: Specific Activity of AChE in Crude Extracts from Different Tissues of *B. globosus*

AChE purification

The purification of AChE from the hepatopancreas, foot muscle, and visceral mass of *B. globosus* was achieved through two consecutive ion exchange chromatography steps, as illustrated in Figures 2 and 3. Final purification on DEAE-Sephadex A-25 column resulted in a single distinct peak of activity for each tissue type (Figure 3). Final recovery of AChE activity following purification on the DEAE-Sephadex A-25 column was 67%, 63%, and 41% for the hepatopancreas, foot muscle, and visceral mass, respectively. The corresponding purification folds were 5.7 for hepatopancreas, 3.8 for foot muscle, and 3.0 for visceral mass.

Kinetic parameters of AChE

The apparent kinetic constants (K_m) for partially purified *B. globosus* AChE were estimated from Michaelis-Menten plots using non-linear regression as shown in Figure 4. Apparent K_m ^{ACTI} varied between 0.211 mM for the protein from the visceral mass and foot muscle, to as high as 1.053 mM for extracts from the hepatopancreas. Apparent V_{max} varied from 0.2 units/mg protein for the extract from visceral mass and foot muscle to 0.32 units/mg protein for that from the hepatopancreas. The summary of the kinetic parameters is presented in Table 2.

Effect of pH on B. globosus AChE activity

Figure 5 illustrates the effect of pH on partially purified *B. globosus* AChE activity. The optimum pH values were 8.0, 7.0, and 8.0 for the purified AChE activity from hepatopancreas, foot muscle, and visceral mass, respectively.

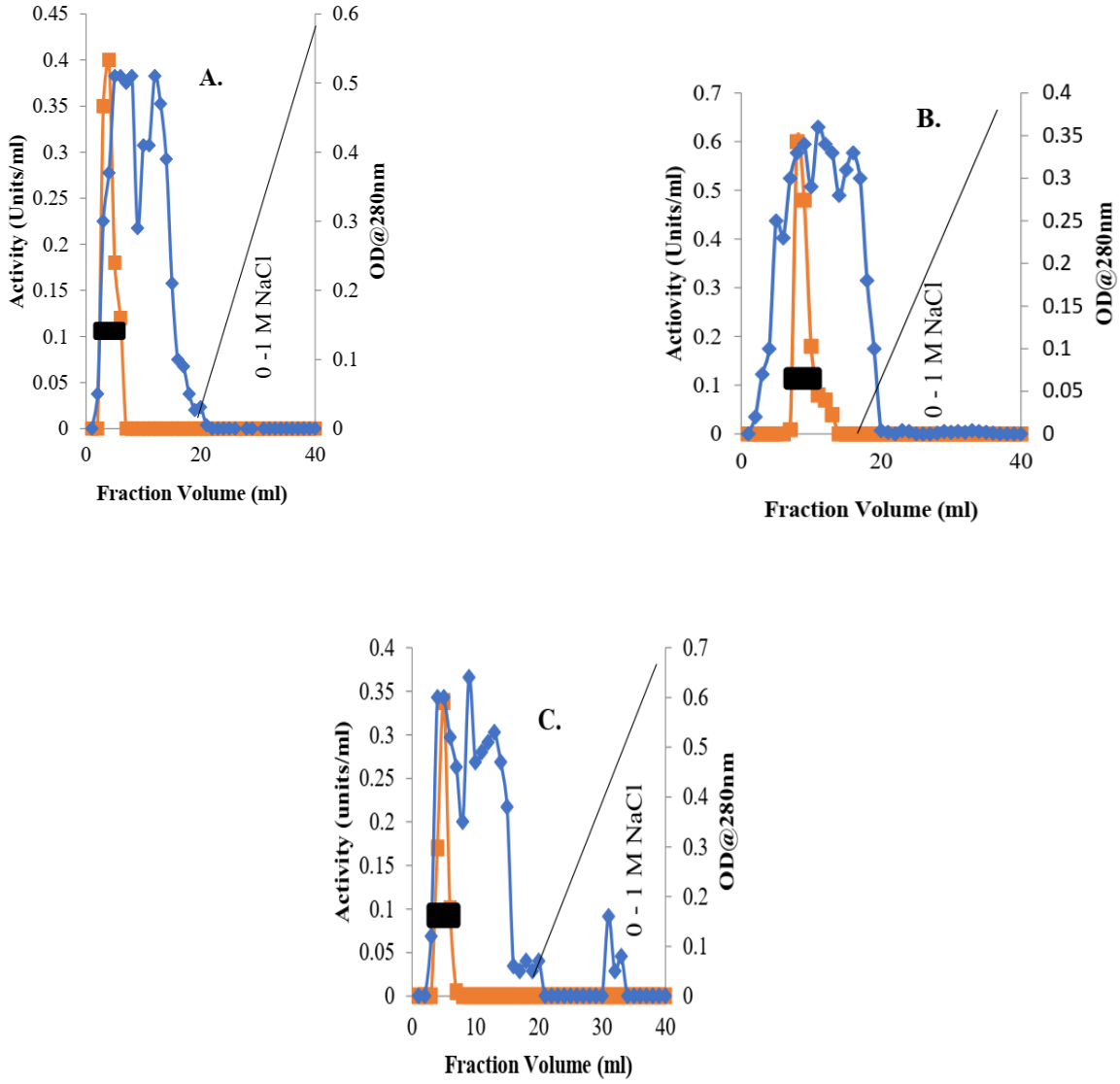

Figure 2: **Elution Profile of Crude AChE on CM-Sephadex C-50 Column**
The extract obtained from (A) hepatopancreas (B) foot muscle (C) visceral mass were layered on CM-Sephadex C-50 column (1.5 cm x 14.0 cm). The unbound proteins were washed with equilibration buffer at a flow rate of 15 ml/hr, while bound proteins were subsequently eluted at the flow rate of 22 ml/hr with elution buffer containing NaCl in a linear gradient (0–1 M). Fractions of 2 ml each were collected and active fractions were pooled and concentrated. Fractions pooled (▬), Absorbance @ 280 nm (—●—), AChE activity (units/ml) (—▲—).

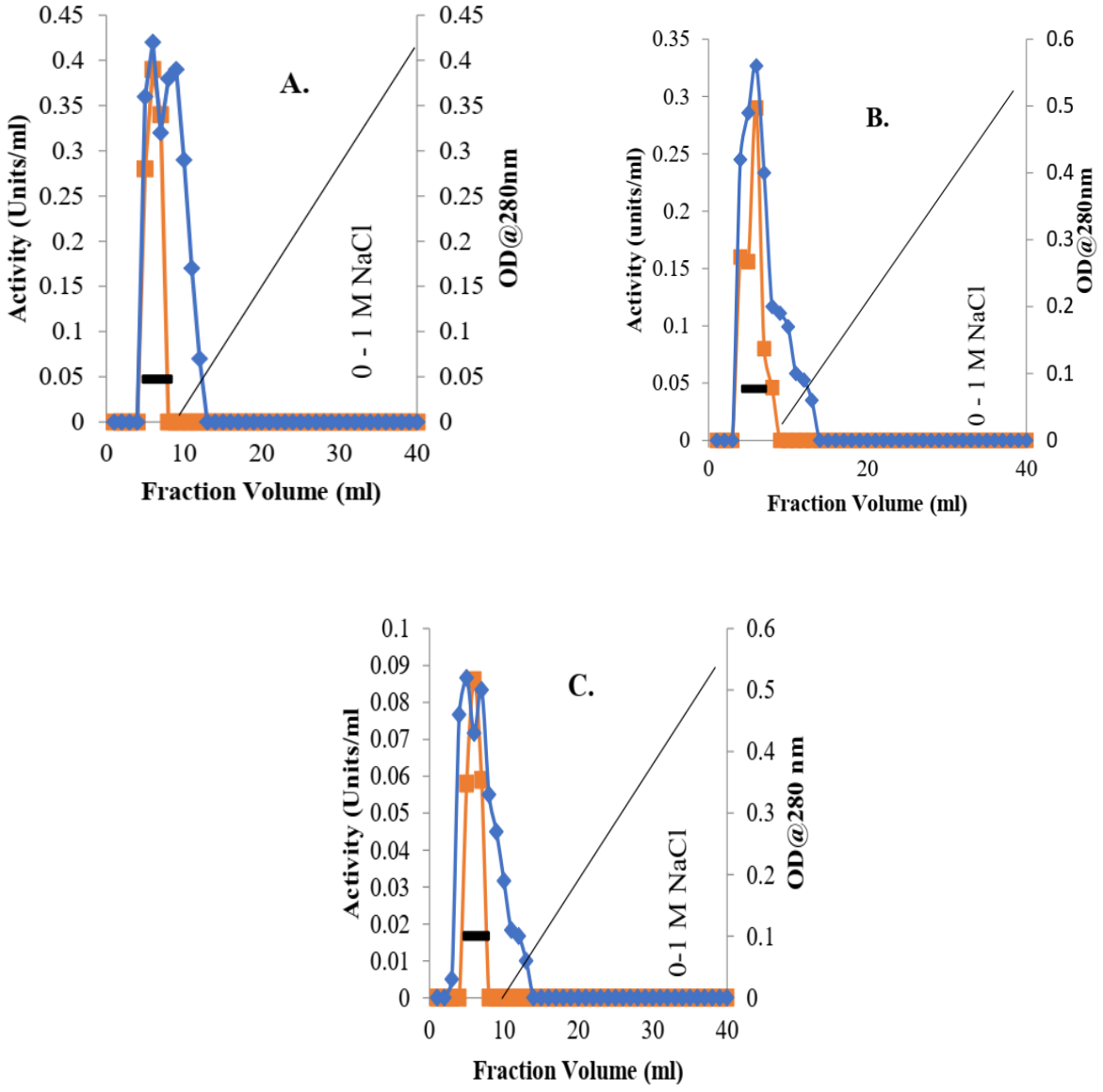

Figure 3: **Elution Profile of Partially Purified AChE on DEAE-Sephadex A-25 Column**
The post CM-Sephadex C-50 pool obtained from (A) hepatopancreas (B) foot muscle (C) visceral mass were layered on DEAE-Sephadex A-25 (1.5 cm x 15.0 cm). The unbound proteins were washed with equilibration buffer at a flow rate of 15 ml/hr, while bound proteins were subsequently eluted at the flow rate of 22 ml/hr with elution buffer containing NaCl in a linear gradient (0–1 M). Fractions of 2 ml each were collected and active fractions were pooled and concentrated. Fractions pooled (▬), Absorbance @ 280 nm (—●—), AChE activity (units/ml) (—▲—).

Table 1: **Purification summary of AChE from Different Tissues of *B. globosus***

Sample	Steps	Total Activity (Units)	Total Protein (mg)	Specific Activity (Units/mg protein)	Yield	Purification Fold
Hepatopancreas	Crude	4.4	42.3	0.1±0.001	100	1
	CM-Sephadex C-50	4.4	34.4	0.13±0.03	100	1.3
	DEAE-Sephadex A-25	2.9	4.97	0.58±0.06	65.9	5.8
Foot Muscle	Crude	2.9	54.3	0.05±0.007	100	1
	CM-Sephadex C-50	2.8	26.1	0.11±0.002	97.4	2.2
	DEAE-Sephadex A-15	1.8	9.2	0.2±0.01	63.1	4
Visceral Mass	Crude	2.3	35.8	0.06±0.006	100	1
	CM-Sephadex C-50	1.4	21.3	0.07±0.081	60.9	1.2
	DEAE-Sephadex A-25	0.9	4.97	0.18±0.011	39.1	3

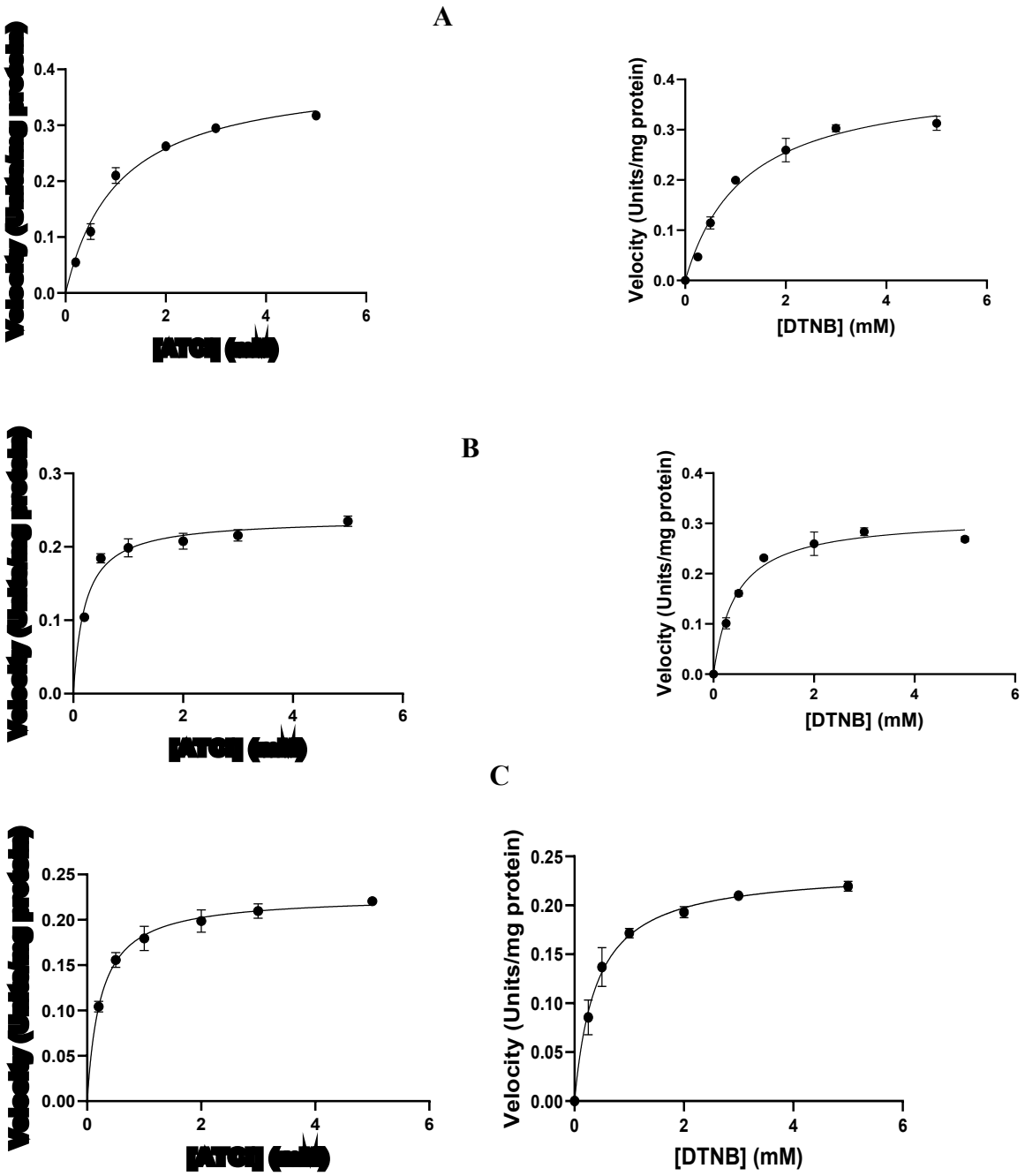

Figure 4: **Non-linear regression curves for the determination of kinetic parameters (K_m and *Vmax*) of partially purified AchE activity from *B. globosus* in (A) hepatopancreas, (B) foot muscle, and (C) visceral mass**

Table 2: Summary of Kinetic Parameters for Partially Purified AChE Activity from Tissues of *B. globosus*

Tissue	K_m ^{ACTI}(mM)	K_m ^{DTNB}(mM)	V_{max} (units/mg protein)
Hepatopancreas	1.053 ± 0.131	0.923± 0.131	0.395 ± 0.003
Foot Muscle	0.211 ± 0.008	0.345±0.062	0.238 ± 0.006
Visceral Mass	0.238 ± 0.013	0.267±0.081	0.226 ± 0.005

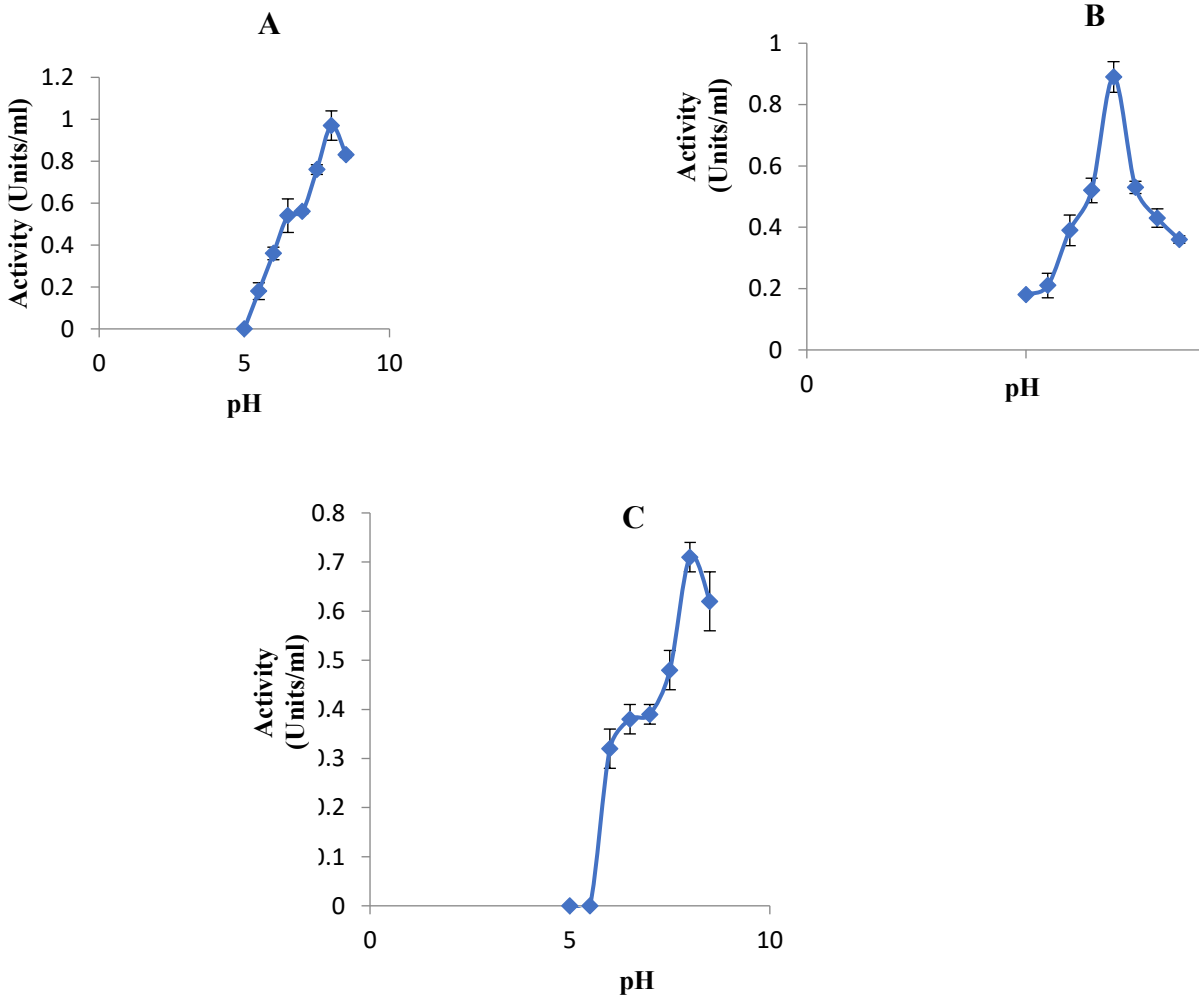

Figure 5: Effect of pH on the Partially Purified AChE Activity from (A) Hepatopancreas, (B) Foot Muscle and (C) Visceral Mass of *B. globosus*

DISCUSSION

This study presents an in-depth biochemical exploration of AChE derived from various tissues of *B. globosus*, a key intermediate host of *Schistosoma haematobium*. Through a comparative analysis of enzymatic distribution, purification behavior, kinetic parameters, and pH optima, the findings illuminate the distinct functional roles of AChE across tissues. These insights not only advance our mechanistic understanding of AChE's physiological relevance but also highlight promising strategies for targeted snail control interventions.

The crude extract from the hepatopancreas exhibited the highest specific AChE activity (0.591 ± 0.0134 units/mg protein), compared with the foot muscle (0.199 ± 0.0143 units/mg protein) and the visceral mass (0.1908 ± 0.0113 units/mg protein). The markedly elevated activity in the hepatopancreas suggests that AChE may play an important role in metabolic regulation and detoxification within this organ. As the hepatopancreas is central to digestion, nutrient assimilation, and the biotransformation of xenobiotics, the presence of AChE could support cholinergic signaling processes that influence enzyme secretion and maintain metabolic balance (Chang *et al*., 2019). Given that the hepatopancreas is a primary site of detoxification in molluscs, the high AChE activity may also play a protective role by facilitating the degradation of neurotoxic compounds. This is particularly relevant in environments contaminated with organophosphate and carbamate insecticides, which are potent AChE inhibitors (Kaushal *et al*., 2021). Such elevated activity might therefore represent an adaptive response, enabling the organism to cope with environmental neurotoxicants, a phenomenon previously observed in aquatic species exposed to polluted habitats (Assis *et al*., 2010). In contrast, AChE activity in the foot muscle was lower, but still likely essential for efficient neuromuscular transmission. Here, AChE ensures the rapid hydrolysis of ACh at neuromuscular junctions, preventing prolonged muscle contraction and allowing smooth locomotion (Sam and Bordoni, 2023). The visceral mass showed the lowest AChE activity among the three tissues examined, which may indicate a lesser dependence on cholinergic neurotransmission. This reduced activity could reflect alternative modes of regulation or lower metabolic demand in these visceral organs.

The partial purification strategy using sequential cation- and anion-exchange chromatography yielded purification folds of 5.7, 3.8, and 3.0 for hepatopancreas, foot muscle, and visceral mass, respectively. Interestingly, the weak binding of AChE to both CM-Sephadex C-50 and DEAE-Sephadex A-25 columns suggests a zwitterionic nature under the experimental pH, consistent with AChE isoforms possessing a balanced distribution of charged residues on their surfaces (Lazarevic-Pasti *et al*., 2017). This physicochemical property could influence enzyme localization in tissues, interaction with membrane lipids, and susceptibility to inhibitors. Similar charge-balanced properties have been observed in invertebrate AChEs adapted to aquatic environments, potentially facilitating stability across variable ionic conditions (Silva *et al*., 2013).

Kinetic analysis further explained the tissue-specific differences in the properties of partially purified AChE. The apparent Michaelis-Menten constants (K_m $^{\text{ACTI and DTNB}}$) were 1.053 ± 0.1313; 0.923 ± 0.131 mM for the hepatopancreas, 0.2112 ± 0.0077; 0.345 ± 0.062 mM for the foot muscle, and 0.2377 ± 0.0136; 0.262 ± 0.081 mM for the visceral mass respectively. The much higher Km value observed in the hepatopancreas indicates that AChE in this tissue has a lower affinity for its substrate. This aligns with its likely function in broader metabolic regulation rather than in fast neurotransmission. In contrast, the foot muscle and visceral mass showed lower Km values, reflecting a stronger substrate affinity. This pattern is consistent with the role of AChE in rapid cholinergic signaling, where quick breakdown of acetylcholine is essential for efficient neuromuscular activity. The variation in Km across tissues may therefore suggest a functional adaptation: in the hepatopancreas, AChE may be tuned for broader substrate tolerance and reduced sensitivity to acetylcholine fluctuations, supporting its metabolic functions, while in muscle and visceral tissues, it remains specialized for rapid neurotransmission. (Walczak-Nowicka and Herbet, 2021).

The maximum enzyme activity (Vmax) showed clear tissue-specific differences, with the hepatopancreas displaying the highest value (0.3945 ± 0.0028 units/mg protein), while the foot muscle (0.2383 ± 0.0064 units/mg protein) and visceral mass (0.2264 ± 0.0046 units/mg protein) exhibited comparatively lower activities. The elevated Vmax observed in the hepatopancreas likely reflects higher enzyme abundance or enhanced catalytic capacity, enabling it to process larger substrate loads during periods of active digestion and detoxification. Similar patterns have been reported in other aquatic species, including *Colossoma macropomum* and *Cichla ocellaris*, where

distinct tissue-specific AChE isoforms exhibit catalytic properties adapted to physiological requirements (Assis *et al*., 2010; Silva *et al*., 2013).

The optimum pH for the partially purified AChE activity varied between 7.0 – 8.0 for all the tissues, aligns with slightly alkaline conditions common in molluscan hemolymph and aquatic environments. This suggests evolutionary adaptation of *B. globosus* AChE to function efficiently under natural habitat conditions, as also observed in marine and freshwater teleost AChEs (Silva *et al*., 2013). Importantly, this pH range overlaps with conditions under which certain molluscicides (e.g., nicotinic ACh receptor agonists) are most stable, indicating potential synergy in control strategies (Zheng *et al*., 2021). These values are in agreement with those reported for AChE in other species, such as *Colossoma macropomum*, *Cichla ocellaris*, and *Scomber scombrus*, where brain AChE exhibited similar optimal pH ranges (Assis *et al*., 2010; Silva *et al*., 2013). The slightly alkaline pH optimal observed in this study are in agreement with the role of AChE's in cholinergic neurotransmission, as the enzyme typically exhibits peak activity under such conditions.

Given the enzyme's critical role in both neuromuscular and metabolic functions, AChE represents a promising molecular target for novel molluscicidal agents. Inhibiting AChE could impair locomotion, feeding, and reproduction in *B. globosus*, reducing transmission potential. Moreover, tissue-specific differences in kinetic parameters suggest that inhibitors could be optimized to preferentially disrupt neuromuscular function (low K_m tissues) or metabolic regulation (high Km tissues), depending on desired outcomes. This aligns with broader efforts to develop species-specific, environmentally safe snail control measures (Limpanont *et al*., 2020).

CONCLUSION

The high activity of AChE in the hepatopancreas shows that the organ is involved in a number of metabolic processes, and detoxification, where cholinergic signalling pathways may be implicated. Also, the lower AChE activity, together with its kinetic properties in the foot muscle and visceral mass, suggests that these tissues rely on neuromuscular coordination and alternative physiological mechanisms, respectively. These findings provide a better understanding of the role of AChE in the different tissues of invertebrates and may inform novel strategies for vector control, particularly by exploring AChE-targeting molluscicides or inhibitors to disrupt the life cycle of *B. globosus* and reduce schistosomiasis transmission. Future work should focus on structural characterization of *B. globosus* AChE isoforms, screening of selective inhibitors, and in vivo validation of molluscicidal efficacy.

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