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THE ASSESSMENT OF CHOLINESTERASE FROM THE BRAIN OF *Anabas testudineus* AS DETECTION OF METAL IONS

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PENGESAHAN

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ABSTRACT

Anabas testudineus (A. testudineus) is a freshwater fish that belongs to the family of Anabantidae and its local name in Malaysia is 'Ikan Puyu'. It can be used as a bioindicator for heavy metal contamination as it is quite sturdy and ideally suited for experimentation in laboratory for a longer period. The present study aimed to partially purify cholinesterase (ChE) from the brain extract of *A. testudineus* and to study the optimization of assay conditions and inhibition effect towards ChE. The result showed that ChE was successfully partially purified from the brain extract of *A. testudineus* using DEAE-cellulose through ion exchange chromatography. The serial purification method gave 2.80 fold of purification with a recovery of 17.38%. ChE was successfully purified in protein analysis through non-denaturing polyacrylamide gel electrophoresis (native-PAGE) and it proved that DEAE-cellulose matrix can serve as an effective medium to separate protein molecules. The optimum conditions for ChE assay was found to be at pH 9 in Tris-HCl and temperature of 35°C. Substrate specificity profile showed acetylcholinesterase (AChE) as the predominant enzyme which resides in partially purified samples because it indicated the highest V_{max} and lowest K_m when using acetylthiocholine iodide (ATC) as substrate. Inhibition study showed mercury as a strong inhibitor because it gave the highest percentage of inhibition effect (68.1%) towards ChE. Secondary screening found that half maximal inhibitory concentration (IC_{50}) value for mercury was 1.8 mg/L. These finding suggested that partially purified ChE from brain extract of *A. testudineus* is suitable to be applied as biosensor to detect the presence of heavy metal in the environment.

ABSTRAK

Anabas testudineus (A. testudineus) merupakan sejenis ikan air tawar yang berasal dari kumpulan Anabantidae dan nama tempatannya di Malaysia ialah ikan puyu. Ia boleh dijadikan sebagai biopenanda untuk pencemaran logam berat kerana ia agak kuat dan sesuai untuk tujuan eksperimen dalam jangka masa yang lama. Kajian ini bertujuan untuk melakukan separa penulenan terhadap kolinesterase (ChE) yang diekstrak dari otak *A. testudineus* dan untuk mengkaji keadaan asai optimum dan kesan perencatan terhadap ChE. ChE telah berjaya disepara tulenkan melalui kromatografi pertukaran ion menggunakan DEAE-selulosa. Hasil penulenan bersiri menghasilkan faktor penulenan 2.80 dengan nilai pulangan sebanyak 17.38%. ChE telah ditulenkan dalam analisa protein melalui poliakrilamid gel elektroforesis natif (native-PAGE) dan telah membuktikan DEAE-selulosa boleh digunakan sebagai media yang efektif untuk mengasingkan molekul-molekul protein. Keadaan optimum untuk ChE telah ditentukan pada pH 9 dengan menggunakan penimbal Tris-HCl dan 35°C sebagai suhu optimum. Profil substrat yang spesifik menunjukkan asetilkolinesterase (AChE) sebagai enzim utama yang berada di dalam sampel separa tulen. Hal ini disebabkan oleh hasil nilai V_{max} yang tertinggi dan nilai K_m yang terendah apabila menggunakan asetiltiokolin iodide (ATC) sebagai substrat. Kajian perencatan menunjukkan merkuri sebagai perencat yang kuat kerana menghasilkan peratusan tertinggi dalam kesan perencatan (68.1%) terhadap ChE. Pengesanan kedua mendapati nilai kepekatan separa perencatan (IC_{50}) bagi merkuri ialah 1.8 mg/L. Dapatan ini menyarankan bahawa ChE separa tulen dari otak *A. testudineus* sangat sesuai diaplikasi sebagai bahan biopengesan untuk mengesan pencemaran logam berat di persekitaran.

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LIST OF ABBREVIATIONS

%	Percent
°C	Degree celsius
µl	Microlitre
ACh	Acetylcholine
AChE	Acetylcholinesterase
ATC	Acetylthiocholine iodide
BCh	Butyrylcholine
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin
BTC	Butyrylthiocholine iodide
CBB	Coomasie brilliant blue
ChE	Cholinesterase
DEAE-cellulose	Diethylaminoethyl-cellulose
DTNB	5,5-dithio-bis-2-nitrobenzoate
et al.,	And friends
HCl	Hydrochloric acid
L	Litre
M	Molar
mg	Milligram
min	Minute
ml	Millilitre
mM	Milimolar
native-PAGE	Non-denaturing polyacrylamide gel electrophoresis
nm	Nanometre
PCh	Propionylcholine
PChE	Propionylcholinesterase
PMSF	Phenylmethylsulfonyl flouride
PTC	Propionylthiocholine iodide

CHAPTER 1

INTRODUCTION

Cholinesterase (ChE) is a group of enzymes pivotal for nerve response and function. It is involved in a reaction where a cholinergic neuron must return to its resting state after activation. ChE, which is abundant in brain tissue, plays its role as a vital catalyst that hydrolyses the neurotransmitter, acethylcholine (Sabullah *et al.*, 2014). Thus, ChE directly helps in signal transmission at neuromuscular junction and brain cholinergic synapses. ChE can be classified into three classes of enzymes according to their affinity to a specific substrate and to their susceptibility to selective inhibitors (Gomes *et al.*, 2014). There are three main substrates namely acetylcholine (ACh), butyrylcholine (BCh) and propionylcholine (PCh) that are preferentially hydrolysed by acethylcholinesterase (AChE, EC 3.1.1.7), butyrylcholinesterase (BChE, EC 3.1.1.8) and propionylcholinesterase (PChE, EC 3.1.1.8) respectively (Nunes, 2011).

Heavy metals are usually present in the form of metal ions and in trace amounts. They are important in the biological system because they play crucial roles in a variety of biological reaction such as maintaining homeostasis and for cell growth (Cohen *et al.*, 2000). However, heavy metals can disturb the physiological function at high concentrations due to their accumulation in vital organ (Singh *et al*., 2011). Fish are one of the aquatic organisms that can be affected by those heavy metals. Waser *et al.*, (2009) in their previous study have mentioned that heavy metal such as copper could decrease the performance of fish swimming activity. In addition, the preliminary studies used fish as a biomarker tool for the

detection of pollutant contamination in aquatic environment because fish are more susceptible to the bioaccumulation of pollutant (Assis *et al*., 2011).

ChE activity has been popularly used as a marker of neural changes in an organism. The use of ChE in the detection of metal ions is appropriate because ChE has broad distribution across several types of tissue including serum, liver, heart, vascular endothelia and nervous system (Valbonesi *et al.,* 2011). Besides, ChE is one of the main targets of heavy metals that block the function of this enzyme, which leads the excessive accumulation of neurotransmitter in synaptic cleft (Gomes *et al.,* 2014; Rakhi *et al.,* 2013). Thus, the current study was designed to study the characteristic and behavior of ChE as a biomarker for heavy metals.

In this study, crude ChE was extracted from the brain of local freshwater fish *Anabas testudineus (A. testudineus),* also known as climbing perch or 'Ikan Puyu'. The crude ChE was then partially purified through ion exchange chromatography and analysed for various parameters. Native polyacrylamide gel electrophoresis (native-PAGE) was conducted for the separation and analysis of protein in the sample. Besides, inhibition study was carried out to evaluate the effect of metal ions towards ChE activity and to verify its viability as a biomarker in pollutant detection.

The objectives of the study are as follow:

i. To extract and purify ChE from *A. testudineus* brain by using ion exchange chromatography (DEAE-cellulose).
ii. To determine the specific synthetic substrate and optimum assay conditions from purified ChE by using Ellman's method.
iii. To evaluate the effect of metal ions towards ChE activity in freshwater fish.

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