Determination of Acetylcholinesterase Activity and Brain Histology of Cyclophosphamide-Induced Neurotoxicity in Rat Treated with Protein Hydrolysate from *Arthrospira platensis*

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ABSTRACT

Cyclophosphamide is an immunosuppressant agent commonly used as a chemotherapeutic drug. However, it induces the production of reactive oxygen species, leading to neurotoxicity and cognitive impairment. The search for bioactive compounds from natural sources with neuroprotective properties has gained increasing attention among researchers. Therefore, this study aimed to evaluate the neuroprotective efficacy of protein hydrolysate from *Arthrospira platensis* (PS) in CP-induced neurotoxicity in rats. Male rats were divided into four groups: a normal control, a negative control, and two treatment groups. neurotoxicity was induced by administering CP at a dose of 200 mg/kg. The normal and negative control groups received distilled water orally for 14 days, while the treatment groups received PS at doses of 3 and 6 mg/kg. Acetylcholinesterase (AChE) activity in hippocampus, cerebral cortex and serum were measured, and brain histopathology was examined. The results showed a significant ($p<0.05$) decreases in AChE activities in the cerebral cortex and hippocampus of CP-induced rats treated with PS at 3 mg/kg compared to the normal and negative controls. However, PS at both doses significantly increased ($p<0.05$) AChE activity in the serum. PS at all doses did not alter the normal structure of the cerebral cortex but inhibited CP-induced pyknotic cell death in CA1, CA2, and CA3 of the hippocampus. However, PS at all doses decreased the thickness of the pyramidal cell layer in CA2 and CA3 of the hippocampus in CP-induced rats. Therefore, treatment with protein hydrolysate at 3 and 6 mg/kg for 14 days did not effectively exhibit neuroprotective effects against CP-induced neurotoxicity in rats.

INTRODUCTION

The nervous system plays a crucial role in regulating the activities and functions of our body. When central nervous system is damaged, it can lead to various diseases that often affect daily life. These diseases include vascular diseases, Alzheimer's disease, Parkinson's disease, headaches, and migraines. Risk factors contributing to the pathogenesis of neurological disorders include alcohol consumption, smoking, and the use of medications for the treatment of dizziness, immunosuppression, and cancer, especially cyclophosphamide (CP) [1]. Memory decline occurs due to a decrease in acetylcholine (Ach), a neurotransmitter crucial for learning and memory. When acetylcholine levels are reduced, the ability to learn and remember new information also declines, which is a hallmark of Dementia. This can be treated with acetylcholinesterase inhibitors, such as donepezil and galantamine, which help prevent the breakdown of acetylcholine [2]. However, these medications have potential side effects, including nausea, vomiting, diarrhea, loss of appetite, bloating, headaches, dizziness, and insomnia. Although medical drugs are more accessible today, due to their high cost, dangerous side effects, and the belief in herbal remedies, the development of herbal supplements has increased. Therefore, this research aims to study the effects of hydrolyzed protein extracted from *Arthrospira platensis*, cultivated in the Applied Algae Laboratory at the Department of Biology, Faculty of Science, Chiang Mai University, in preventing neural damage in rats induced by cyclophosphamide.

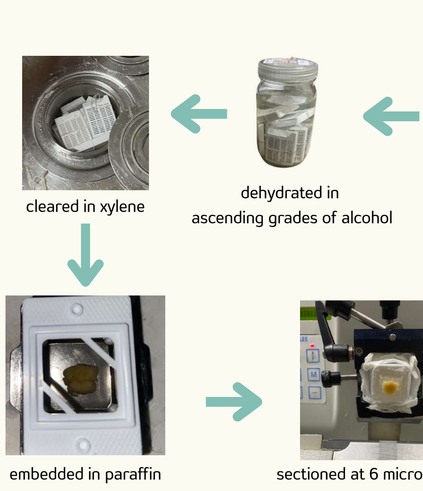
MATERIALS AND METHODS

EXPERIMENTAL TREATMENT

Normal control (NC)	Negative control (NEG)	Low dose	High dose
			
Distilled water	Distilled water	protein hydrolysate at 3 mg/kg	protein hydrolysate at 6 mg/kg

Treated for 14 days, orally.

Intraperitoneally injected CP at days 7 and 14 to induce neurotoxicity.



RESULTS

Table 1 AChE activity in the cerebral cortex, hippocampus, and serum of rats.

Groups	AChE activity (unit/min/mg)		
	Cerebral cortex	Hippocampus	Serum (unit/min/µl)
NC	83.22±10.89[b]	206.72±40.58[b]	1,318.98±150.49[b]
Neg	77.70±11.98[b]	189.07±42.15[b]	502.40±112.46[a]
Low	62.53±7.29[a]	117.71±17.64[a]	1,615.08±257.49[bc]
High	83.22±6.11[b]	189.07±60.92[b]	1,442.82±49.25[c]

Values are expressed as mean ± S.D. The superscript letters indicate statistically significant differences ($p<0.05$) between the experimental groups when tested with Duncan test.

Table 2 Thickness of neuronal layer in different area of hippocampus.

Groups	Neuron layer thickness (µm)		
	CA 1	CA 2	CA 3
NC	45.50±3.26[a]	39.00±2.85[c]	52.50±5.00[c]
Neg	42.50±4.68[a]	31.50±3.35[b]	45.00±3.95[b]
Low	42.00±4.47[a]	24.00±2.24[a]	41.00±5.18[b]
High	44.00±3.79[a]	20.50±3.26[a]	36.50±5.76[a]

Values are expressed as mean ± S.D. The superscript letters indicate statistically significant differences ($p<0.05$) between the experimental groups when tested with Duncan test.

Cerebral cortex

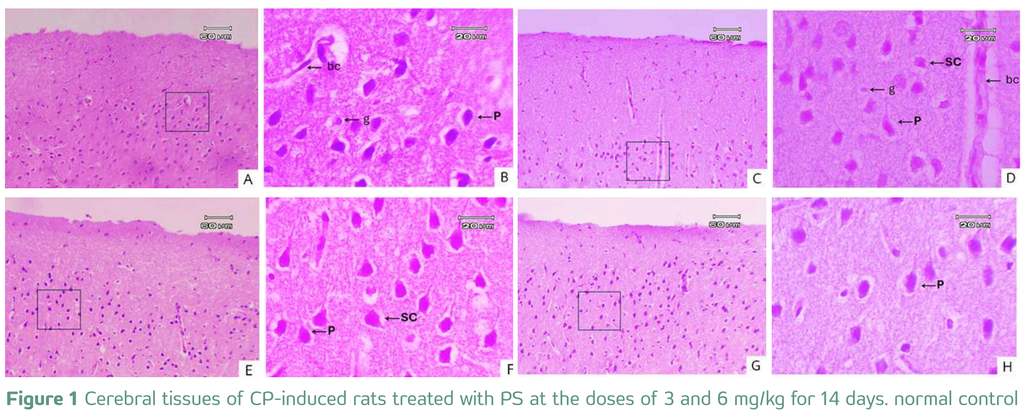


Figure 1 Cerebral tissues of CP-induced rats treated with PS at the doses of 3 and 6 mg/kg for 14 days. normal control (A, B), negative control (C, D), PS at 3 (E, F) and 6 (G, H) mg/kg. H&E. 10x and 40x. pyramidal cell (P), stellate cell (SC), glia cell (g) and blood capillary (bc)

Hippocampus

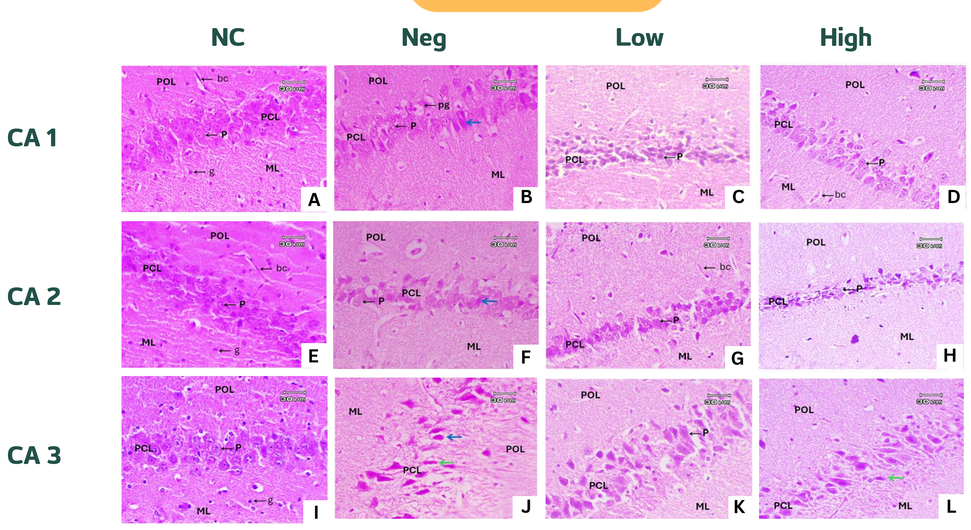


Figure 2 Hippocampal tissues of CP-induced rats treated with PS at the doses of 3 and 6 mg/kg for 14 days. H&E. 20x. polymorphic layer (POL), pyramidal cell layer (PCL), molecular layer (ML), pyramidal cell (P), glia cell (g), blood capillary (bc), perineural glia (pg), pyknotic cell (blue arrow), shrunken cell (green arrow)

CONCLUSION

Administration of protein hydrolysate from *Arthrospira platensis* at a dose of 3 mg/kg significantly reduced AChE activity in the cerebral cortex and the hippocampus and restored AChE activity in the serum of CP-induced neurotoxicity rats. Although protein hydrolysate at all doses inhibited pyknotic cell death, it also decreased the thickness of the neuronal layer in the Cornu Ammonis of the hippocampus. Therefore, administration of protein hydrolysate at 3 and 6 mg/kg for 14 consecutive days did not effectively exhibit neuroprotective effects against CP-induced neurotoxicity in rats.

ACKNOWLEDGEMENTS

The author would like to thank the Department of Biology, Faculty of Science, Chiang Mai University for providing facilities. I would also thank all members of laboratory animal building for helping, supporting and giving suggestions.

REFERNCES

[1] Baker, M., Markman, M. and Niu, J. 2014. Cyclophosphamide-induced severe acute hyponatremic encephalopathy in patients with breast cancer: report of two cases. Case Rep Oncol. 7(2): 550-4.

[2] Philpot, R.M., Ficken, M., Johns, B.E., Engberg, M.E. and Wecker, L. 2019. Spatial memory deficits in mice induced by chemotherapeutic agents are prevented by acetylcholinesterase inhibitors. Cancer Chemother Pharmacol.(3):579-589.