



Inhibitory Effect of *Papaver laevigatum* on Acetylcholinesterase Enzyme using HPLC-Based Activity Profiling Method

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ABSTRACT

Introduction: Alzheimer's disease (AD) is one of the most significant neurological disorders globally. Damage to acetylcholine production and increased oxidative stress are potential contributors to this condition. Therefore, agents that influence these two factors may be effective in treating AD. Research indicates that various species of the *Papaver* genus contain alkaloids with therapeutic potential control against AD through multiple mechanisms, including the inhibition of the acetylcholinesterase enzyme. In the current study, we investigated the inhibitory effects of *Papaver laevigatum* species on the acetylcholinesterase enzyme and identified the active alkaloids using liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) techniques. In addition, we assessed the cell damage and protective effects of the fractions through lactate dehydrogenase (LDH) assays, hydrogen peroxide (H₂O₂) toxicity tests, and reactive oxygen species (ROS) evaluations. The plant was collected from Mount Binalud in Mashhad, Iran.

Methods and Materials: The dried plants were extracted using methanol as a maceration method. The alkaloidal fraction was obtained using the acid-base extraction approach. The antioxidant and anti-acetylcholinesterase inhibitory activity of fractions were tested at non-cytotoxic concentrations. LC-ESIMS investigated the phytochemical profile of the alkaloidal fraction. Purification of the compounds in the alkaloid fraction was established using different types of chromatography, and the pure compounds were identified using NMR and HRMS (High-resolution mass spectrometry) techniques. The fractions were not toxic at 50, 20, 10, and 5 µg/ml concentrations. In the H₂O₂ test, the alkaloidal and aqueous fractions showed a protective effect compared to the control group at 50, 20, 10, and 5 µg/ml concentrations.

Results: In the ROS test, all three fractions effectively reduced ROS compared to the control group at concentrations of 50, 20, 10, and 5 µg/ml. In the LDH release test, all fractions were able to reduce LDH release by more than 50% at concentrations of 10 and 20 µg/ml compared to the control group. In the acetylcholinesterase enzyme inhibition test, the alkaloidal fraction reduced the activity of acetylcholinesterase enzymes by 6.43% and 15.83%, respectively, at concentrations of 20 and 40 µg/ml. LC-MS data showed that the alkaloidal fraction contains mainly aporphines and protoporphyrins, including rophine, membrane, nuciferine, and N-methylasimilobine.

Conclusion and Discussion: According to the NMR data, nuciferine is one of the main purified compounds. The findings indicate that the alkaloid fraction of *Papaver laevigatum* exhibits significant antioxidant properties and inhibits acetylcholinesterase activity. The literature suggests that the antioxidant activity of this extract may be attributed to nuciferine, while its acetylcholinesterase inhibitory effects can be linked to the alkaloids rophine and N-methylasimilobine.

Keywords: Alkaloids, Alzheimer's disease, Aporphines, Papaveraceae, Pronuciferine

Citation:

Alinezhad F, Akaberi M, Mashani A, Tayarani Najaran Z, Emami SA, Nesměrāk K, Štícha M. Inhibitory Effect of *Papaver laevigatum* on Acetylcholinesterase Enzyme using HPLC-Based Activity Profiling Method. *Iranian biomedical journal. Supplementary* (12-2024): 399.

