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 important neurological disorders in the world. Damage to the production of acetylcholine and increased oxidative stress can be one of the causes of this disease. Therefore, agents influencing these two factors can be effective in AD treatment. Studies show that different species of the Papaver genus have alkaloids with therapeutic potential control against AD through various mechanisms, such as inhibiting the acetylcholinesterase enzyme. Thus, in the current study, we investigated the inhibitory effects of Papaver laevigatum species on acetylcholinesterase enzyme and identified the active alkaloids using LC-MS and NMR techniques. In addition, the fractions' cell damage and protective effects were investigated by lactate dehydrogenase (LDH), H_2O_2 toxicity, and ROS tests. The plant was collected from Binalud Mount. (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 method. The fractions' antioxidant and anti-acetylcholinesterase inhibitory activity 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 techniques. The fractions were not toxic at 50, 20, 10, and 5 µg/ml concentrations. In the H_2O_2 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 proapoporphines, including romaine, membrane, nuciferine, and N-methylasimilobine.

Conclusion and Discussion: According to the NMR data, nuciferine was identified as one of the main purified compounds. The results showed that the alkaloid fraction of *Papaver laevigatum* has significant antioxidant and anti-acetylcholinesterase inhibitory effects. According to the literature, the antioxidant activity of this extract can be related to nuciferine, and its acetylcholinesterase inhibitory activity can be related to the alkaloids romaine and N-methylasimilobine.