Journal of Biological Science 3 (11): 1046-1049, 2003
ISSN 1727-3048

In vitro Inhibition of Acetylcholinesterase, Butyrylxcholinesterase and Lipoxygenase by Crude Extract of *Myricaria elegans* Royle

[1]Waqar Ahmad, [2]Bashir Ahmad, [3]Manzoor Ahmad,
[2]Zafar Iqbal, [2]Muhammad Nisar and [1]Mansoor Ahmad
[1]Research Institute of Pharmaceutical Sciences,
University of Karachi, Karachi-75270, Pakistan
[2]Department of Pharmacy, University of Peshawar, Peshawar-25120, Pakistan
[3]H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Abstract: The 80% methanolic extract of ***Myricaria elegans*** Royle was investigated for ***in vitro*** acetylcholinesterase (ACHE), butyrylcholinesterase (BCHE) and lipoxygenase enzyme inhibition activities. The crude extract was found to have significant acetylcholinesterase inhibitory activity (74.8%) and remarkable butryrylcholinesterase inhibitory activity (96.0%). However, no activity was observed against the enzyme lipoxygenase (0.0%).

Key words: Acetylcholinesterase, butyrylcholinesterase, lipoxygenase, ***Myricaria elegans***

Introduction

According to the cholinergic hypothesis, the memory impairment in the patients with senile dementia of Alzheimer's type results from a deficiency in cholinergic function in the brain. Hence the most promising therapeutic strategy for activating central cholinergic functions has been the use of cholinomimetic agents. The enzyme acetylcholinesterase (ACHE) has long been an attractive target for the rational drug design and discovery of mechanism-based inhibitors. Because of its role in the hydrolysis of the neurotransmitter acetylcholine (Taylor, 1998). The inhibition of the enzyme is considered as a promising approach for the treatment of Alzheimer's disease (AD) and for other possible therapeutic applications in the treatment of Parkinson's disease, ageing and myasthenia gravis. The role of butyrylcholinesterase (BChE) in the normal ageing and diseased brain is still unknown. However, recently it has been found that BCHE inhibition may also be an effective tool for the treatment of AD and related dementias (Yu ***et al.***, 1999).

Arachidonate 5-lipoxygenase (5-LO) is the key enzyme in the leukotriene (LT) biosynthesis and catalyzes the initial steps in the conversion of arachidonic acid to biologically active leukotrienes (LTs). Leukotrienes are considered as potent mediattors of inflammatory and allergic reactions which are released by leukocytes and other 5-LO expressing cells Archidonic acid metabolism through lipoxygenase (LOX) pathway generates various biologically active lipids that play important role in inflamation (Steinhilber, 1999). Angiogenesis, the formation of new capillary

vessels from pre-existing ones, underpins a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer (Nie, 2002). Lipoxygenases are therefore potential targets for the rational drug design and discovery of machanism based inhibitors for the treatment of a variety of disorders including bronchial asthma, inflammation, cancer and autoimmune disease. Thus, search for new lipxygenase inhibitors appears to be a promising approach for the development of new drugs.

Materials and Methods

Plant material

Myricaria elegans Royle. was collected in July 2002 at the hills of Swat, NWFP., Pakistan and was identified by Prof. Jehandar Shah (Vice Chancellor, University of Malakand, Chakdara, NWFP, Pakistan).

Extraction

The shade dried plant material was powdered and extracted with 80% methanol by percolation at room temperature. The pharmacological activities were performed by using different concentrations of the crude extract as per requirement of the individual assay methods.

In vitro cholinesterase inhibition assay

Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by slightly modifying the spectrophotometric method developed by Ellman ***et al.***, 1961. Electric-eel ACHE (type VI-S, Sigma) and horse-serum BChE (Sigma) was used as source of both the cholineserases. Acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were used as substrate of the reaction. The 5,5 -dithiobis (2-nitrobenzoic) acid (DTNB, Sigma) was used for the measurement of cholinesterase activity. 140 µL of 0.1 mM sodium phosphate buffer (pH 8.0), 10 µL of DTNB, 40 µL of crude extract and 20 µL of acetylchoilnesterase/butyrylcholonesterase were mixed and incubated for 5 min at 25°C. The reaction was then initiated by the addition of 10 µL acetylcholinesterase/butyrylcholinesterase. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of yellow 5-thio-2-nitrogenzoate anion as the result of the reaction of DNTB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine or butyrylthiocholine at a wavelength of 412 nm. The crude extract and control were dissolved in 50% ethanol and all the reactions were repeated in triplicate.

In vitro lipoxygenase inhibition assay

Lipoxygenase inhibiting activity was undertaken by slightly modifying the spectrophotometric method developed by A.L. Tappel (Tappel, 1962). Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from sigma (St. Louis, MO). All other chemicals were of analytical grade. 160 µL of 100 mM sodium phosphate buffer (pH 8.0), 0 µL of crude extract solution and 20 µL of lipoxygenase solution were mixed and incubated for 10 min at 25°C. The reaction was then

initiated by the addition of 10 µL linoleic acid solution (substrate) with the formation of (9Z, 11E)-(13S)-13-hydroperoxyoctadeca-9, 11-dienoate, the change in the reaction was followed for 10 min.

Crude extract and the control were dissolved in MeOH. All the reactions were performed in triplicate in 96 well plates.

Results and Discussion

To the best of our knowledge and from the literature search it was revealed that no earlier scientific study has been undertaken to evaluate the ***M. elegans*** for its therapeutic potential in phytomedicines or its value for the isolation of bioactive chemical constituents. Therefore, the current study was designed and undertaken to screen ***M. elegans*** to confirm and provide scientific basis for its use in transitional system of medicine (Watt, 1972) and also to explore some new biological and pharmacological activities of this plant. Moreover, the present study will provide a base for the researchers to carry out research work for the isolation of biologically active compounds.

Table 1: *In vitro* enzyme inhibition studies of *M. elegans* against different enzymes

Enzymes	% inhibition (at 0.2 µg mL^{-1})
Acetylcholinesterase	74.8
Butyrylcholinesterase	96.0
Lipoxygenase	0.0

In the present study, the crude extract of ***Myricaria elegans*** exhibited remarkable activity against butyrylcholinestrase and has inhibited the enzyme by 96% at a concentration of 0.2 µg mL^{-1}. The crude extract has also shown good activity against the enzyme acetylcholinesterase and has inhibited the enzyme by 74.8% at the same concentration. However the crude extract was ineffective against the enzyme lipoxygenase Table 1.

From the results shown in the table, it can be postulated that the crude extract of ***Myricaria elegans*** has a strong potential to inhibit the cholinesterase enzymes and can therefore, be a potential target for activity guided isolation of active chemical constituents for the treatment of Alzheirmer's disease (AD) and for possible therapeutic applications for the treatment of Parkinson's disease.

References

Ellman, G.L., K.D. Courtney, V.Jr. Andres, R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol., 7:88-95.

Nie, D., K.V. Honn, 2002. Cyclooxygenase, lipoxygenase and tumor angiogenesis, Cell. Mol. Life Sci., 59: 799-807.

Steinhilber, D., 1999. 5-Lipoxygenase, A target for anti inflammation drugs Revisited Current Med. Chem., 6: 71-85.

Taylor, P., 1998. Development of acetylcholinesterase inhibitors in the therapy of Alzheimer's disease, Neurol., 51: 30-35.

Yu, Q.S., H.W. Holloway, T. Utsuki, A. Brossi, N.H. Greig, 1999. Synthesis of novel Phenserine-based selective inhibitors of butyrylcholinesterase for Alzheimer's disease, J. Med. Chem., 42: 1855-1861.

Watt, G., 1972. The Dictionary of Economic Products of India, Vol. V, Part I, Cosmo Publications, New Delhi, India, 310-11.