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Research Article

Inhibition of acetylcholinesterase and NADH oxidase by *Acacia farnesiana*

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Abstract

Physiological role of NADH oxidase is to reduce oxygen and to catalyse different reactions in the living system. Acetylcholinesterase catalyzes the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses. Absence of evidence on acetylcholinesterase and NADH oxidase inhibitory activity of *Acacia farnesiana* let us embark on this study with an aim to scientifically prove the traditional claim of this plant. Acetylcholinesterase and NADH oxidase inhibitory activity was determined using *in vitro* methods. Results show that the methanolic extract of areal parts of *Acacia farnesiana* plant exhibit significant acetylcholinesterase and NADH oxidase inhibitory activity. The AChE inhibitory and NADH oxidase inhibitory activity of this plant in this study make it an important target for the isolation and characterization of the phytocompounds responsible for this biological activity.

Introduction

Acetylcholinesterase (AChE) comprises a family of enzymes which include serine hydrolases. They share about 55% of amino acid sequence identity and have similar catalytic properties. The enzyme system is responsible for the termination acetylcholine at cholinergic synapses.[1] The major function of AChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses.[2] Cholinesterase inhibitors are considered to be an important and ongoing strategy to introduce new drug candidates for the treatment of Alzheimer's disease and other possible therapeutic applications in the treatment of Parkinson's disease, senile dementia and ataxia.[3] AChE inhibitors as eserine, tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of Alzheimer's disease however, these drugs are known to have limitations for clinical use due to their short half lives and/or unfavorable side-effects.[4] NADH oxidase catalyzes the two-electron reduction of oxygen to peroxide or the four electron reduction of oxygen to water. Physiological role of NADH oxidase is to reduce oxygen and to catalyze different reactions.[5] NADH oxidases are becoming a potential target for therapeutic interventions in vascular disease.[6]

Acacia farnesiana is also known aroma and sweet acacia grown in various parts of world. The bark of this plant is used as astringent and demulcent. The leaves and roots are used for medicinal purposes. Woody branches used in India as tooth brushes. The gummy roots also chewed for sore throat. The roots of this plant are also used for the antispasmodic, aphrodisiac, astringent, demulcent, diarrhea, febrifuge, rheumatism, and stimulant.[7] It is also used for dyspepsia and neuroses. Mexicans sprinkle powdered dried leaves onto wounds. The flowers are added to ointment, rubbed on the forehead for headache. Green pods are decocted for dysentery and inflammations of the skin and raucous membranes. Colombians bathe in the bark decoction for typhoid. Panamanians and Cubans used the pod to treat conjunctivitis. Cubans use the pod decoction for sore throat. For rheumatic pains, West Indians bind bark strips to the afflicted joint. The root decoction has been suggested as a folk remedy for tuberculosis. The decoction of the root, used in hot baths, is said to help stomach cancer. A plaster, made from the pulp, is said to alleviate tumors.[8] In preliminary phytochemical investigations it has found that the leaves contain

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lipids, carotenoids, alkaloids, flavonoids and reducing and non-reducing sugars and seven polyphenols (gallic acid, ellagic acid, m-digallic acid, methyl gallate, kaempferol, atomadendrin, and narigenin). Also found narigenin-7-glucoside and naringenin-7-rhamnoglucoside (naringin), as well as naringenin, glucose, and gallic acid.[9] Another phytochemical compound quercetin, of this plant is found to be shown antioxidant activity.[10] Absence of evidence on acetylcholinesterase and NADH oxidase inhibitory activity of *Acacia farnesiana* let us embark on this study with an aim to scientifically prove the traditional claim of this plant.

Experimental

Materials

Acetylthiocholine iodide, 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB) and nicotinamide adenine dinucleotide, reduced form ($NADH^{+2}$) were purchased from Sigma Chemical Inc., USA. All chemicals used were of analytical grade.

Plant extract preparation

The whole plant of *Acacia farnesiana* was collected from local costal area and was authenticated. The areal parts of the plant were shade dried and reduced to coarse powder. The powdered material obtained was then subjected to successive extraction by hot percolation method using methanol (95%) in a soxhlet apparatus. The extract obtained was evaporated at 45 ºC to get a semisolid mass. The extract thus obtained was subjected to phyto-chemical analysis.

Phytochemical screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out on extract using standard procedure studies.

Preparation of enzyme source

Fresh chicken liver (1 g) was purchased from the local market, washed with 50 mM Tris-HCl buffer pH 7.4 and homogenized in 10 ml extraction buffer (50 mM Tris-HCl buffer pH 7.4, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.32 M sucrose) in a homogenizer for 15 s each after 10 s intervals. Test tube was placed in ice bucket to avoid heating. Contents were filtered through 3 layers of miracloth and centrifuged at 15000 rpm for 15 min at 4 ºC. The supernatant was used as a source of enzyme. Enzyme source was made fresh everyday and used within 4 hours. Protein was determined by Bradford method and 40-60 µg protein (10 µl) was used per assay.[11]

Determination of AChE inhibitory activity

The methanol extract of plant was examined for AChE inhibitory activities at concentration of 250 mg/l and were dissolved in a base-tris (0.05 M) buffer, following the spectrophotometric method developed by Ellman et al. (1961). In this method, to a 1 cm path length glass cell, were added in order, 200 µl of acetylthiocholine iodide (15 mM), 1000 µl of DTNB (3 mM) and 200 µl of each test extract solution at the different concentrations evaluated, which were mixed and incubated for 15 min at 30 ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200 µl of AChE (0.3 U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined. Control contained all components except the tested extract. As positive control eserine (2.75 mg/l) was used. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

$\% IA = [(C_c - C_e)/C_c] \times 100$

where: C_c is the control kinetic (containing all reactants, except the AChE enzyme) and C_e is the experimental kinetic for each sample concentration. All treatments were performed in triplicate with two replicates.

The concentrations of the tested extracts that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC_{50}) were determined by a linear regression analysis between the inhibition percentages against the extract concentrations by using the Excel program.[12]

Determination of NADH oxidase inhibitory activity

NADH oxidase inhibitory activity was determined in total volume of 200 µl reaction mixture which consisted of 160 µl 50 mM Tris HCl buffer, pH 7.4 containing 1 mM EDTA, with or without plant extract followed by the addition of 10 µl enzyme (40-60 µg protein) from fresh chicken liver homogenate. The contents were mixed and pre-incubated for 10 min at 25 ºC. The reaction was initiated by the addition of 10 µl of 3 mM $NADH^{+2}$. After 45 min incubation at 25 ºC, absorbance was measured at 340 nm using 96-well plate reader. All experiments were carried out with their respective controls. Results are mean of three independent determinations.[11]

Results and Discussion

The percentage yield of methanolic extract was found to be 34.2% w/w. In preliminary phytochemical investigations it has found that the extract contains lipids, carotenoids, alkaloids, flavonoids, reducing and non-reducing sugars and polyphenols. The plant extract that inhibited the enzymes activity presented in the Table 1. The IC_{50} value for inhibition of AChE was found out to be 173 mg/l.

Table 1. Inhibitory effect of *Acacia farnesiana* methanol extract on acetylcholinesterase and NADH oxidase.

Activity	Percent inhibition (Mean ± S.E.M., n=3)
AChE inhibition	58.75 ± 1.84
NADH oxidase inhibition	74.29 ± 2.11

Enzymes are the primary targets for the development of new drugs because of the simplicity of enzyme based assays. The inhibitor interacts with the enzyme or enzyme-substrate complex with a decreasing in the rate of reaction. The enzyme inhibition assays have prompted us to carry out a primary screening of the methanolic extract of this plant commonly used in traditional medicine. Therefore these studies were conducted to investigate the presence of inhibitors of some therapeutically important enzymes. The phytochemical analysis on the methanol extract of the plant showed the presence of polyphenols, which can generate false-positives effects.[13] The AChE inhibitory activity of *Acacia farnesiana* have never been reported before and its AChE inhibitory activity could be attributed to their alkaloidal contents, this correlates with the statement established by Roddick (1989).[14] The AChE inhibitory and NADH oxidase inhibitory activity of this plant in this study make it an important target for the isolation and characterization of the phytocompounds responsible for this biological activity.

Conclusion

In summary, *Acacia farnesiana* methanol extract possess moderate levels of inhibitory activity against NADH oxidase and AChE. Thus, the plant extract should be further subjected to chromatographic separations and phytochemical analyses and the purified AChE and NADH oxidase should be used to carry out more inhibition studies.

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