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Inhibitory activity of Acetylcholinesterase (AChE) and Antioxidant activity of methanolic extract of *Desmodium gangeticum* (L.)

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Abstract: Acetylcholinesterase (AChE) inhibition and antioxidant activity are considered to be highly correlated with Alzheimer's disease treatment. The present study was designed to investigate the antioxidant and acetylcholinesterase inhibitory activity of *Desmodium gangeticum* L. Properly identified powdered plant material was extracted successively using methanol as a solvent. Acetylcholinesterase inhibitory activity was measured with modified Ellman method at 405 nm and antioxidant activity measured based on 1, 1- diphenyl-2-picrylhydrazil (DPPH) free radical scavenging test at 517 nm. Percentage inhibition for AChE ranged from 28.78±0.12 to 40.83±0.05 whereas DPPH radical scavenging percentage ranged from 24.68±0.72 to 42.22±0.67.

Key words: Antioxidants; Acetylcholinesterase inhibition; Alzheimer's disease; DPPH; *Desmodium gangeticum* L.

Introduction

Desmodium gangeticum (L.) commonly known as Salparni, belongs to family Papilionaceae. It is widely distributed mainly in the Himalayan territory at elevations upto 5,000 feet. It is also distributed in the China, Philippine and tropical Africa[1]. Traditionally, the plant has been used as antipyretic, diuretic, astringent, anthelmintic, laxative, and in the treatment of dementia[2]. The plant has been reported to exhibit anti-inflammatory, antibacterial, antidiabetic, hepatoprotective, antiulcer, locomotor and wound healing activities. *D. gangeticum* has been reported to contain alkaloids, flavonoids, steroids and terpenoids[3].The aqueous extract of *Desmodium gangeticum* has been shown to reverse scopolamine induced amnesia by decreasing whole brain acetylcholinesterase activity[4].

Inhibition of Cholinesterases, mainly Acetylcholinesterase (AChE) and therefore prevention of acetylcholine degradation in synapses of cholinergic system is one of the most accepted palliative therapy opportunities for Alzheimer's disease (AD) today[5]. Since the introduction of the first cholinesterase inhibitor in 1997, most clinicians would consider the cholinergic drugs, donepezil, rivastigmine[6], and galantamine[7], to be the first line pharmacotherapy for mild and moderate AD. The most that these drugs could achieve is to modify the manifestations of AD. Due to a lack of selectivity of cholinesterase inhibitor drugs on the market, AD-patients suffer from side effects like nausea or vomiting.

The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the ester bond of acetylcholine (ACh) to terminate the impulse transmitted action of ACh through cholinergic synapses[8]. Although the basic reason of Alzheimer's disease (AD) is not clear so far, AD is firmly associated with impairment in cholinergic transmission. A number of AChE inhibitors have been considered as candidates for the symptomatic treatment of AD as the most useful relieving strategy [9].

Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years. Mostly, herbs and spices contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing antimicrobial effects. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavoniods inhibit the mechanism that leads to degenerative diseases [10].

Materials and Methods

Plant material: *D. gangeticum* roots were procured from Niraj Traders, Jhansi (Uttar Pradesh), India in July, 2016. The plant was identified by Dr. Manorma Kumari, University Professor, Department of Botany, A N College, Patna (M.U.), India.

Preparation of various extracts: *D. gangeticum* roots were dried and powdered in a grinder. The plant material was exhaustively extracted successively using methanol. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator. Various extracts were screened for detection of acetylcholinesterase and antioxidant activity.

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Chemicals: Acetylcholinesterase (EC 3.1.1.7) from Electrophorus electricus (*electric eel*); acetylthiocholine iodide (ATChI); 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB); 1, 1- diphenyl-2-picrylhydrazil (DPPH) and Ascorbic acid were purchased from Alfa-Aesar. Tris-HCl buffer from Rankem; Phosphate buffer and methanol were obtained from Merck Laboratories Pvt. Ltd., India.

Acetylcholinesterase (AChE) inhibition assay: AChE inhibiting activity was measured by the spectrophotometric method developed by Lopez *et al.,* (2002)[11] inspired from Ellman *et al.,* (1961)[12] method. The enzyme activity was determined by observing the increase of a yellow colour produced from thiocholine (resulting from acetylthiocholine hydrolysis by enzyme) when it reacts with DNTB (5, 5'-dithiobis-2-nitrobenzoic acid) ion. This can be detected at 405 nm [13]. Ten percent methanol in buffer was used as negative control (enzyme activity without extract), Tris-HCl buffer 50 mM, pH 8, 0.1% BSA as enzyme blank and Galanthamine as reference standard. The substrate ATCI (Acethylthiocholine Iodide) 15 mM was prepared in water and enzyme (0.22 U/mL) in Tris-HCl buffer 50 mM, pH 8, 0.1% BSA. Kinetic reaction was followed for 3 min. The percentage of enzyme inhibition (I %) of the enzymatic reaction was determined by the following equation:

$$I\% = (E - S) / E \times 100$$

where,

E: The substrate hydrolysis kinetic by enzyme without test compound

S: The substrate hydrolysis kinetic by enzyme with test compound

Antioxidant activity by DPPH Assay: Free radical scavenging activity of different extracts was tested against a methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1-1-diphenyl -2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. The samples of different extracts were prepared in various concentrations viz. 50, 100, 150, 200, 250 µg/ml in methanol. 1 ml samples of above concentrations were mixed with equal volume of 0.1mM methanolic solution of DPPH (0.39mg in 10 ml methanol). An equal amount of methanol and DPPH was added and used as a control. Ascorbic acid solutions of various concentrations viz. 50, 100, 150, 200, 250 µg/ml in distilled water were used as standard. After incubation for 30 minutes in dark, absorbance was recorded at 517 nm. Experiment was performed in triplicates. Percentage scavenging was calculated by using the following formula:

$$\text{Scavenging effect (\%)} = (1\text{-As/Ac}) \times 100$$

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

A graph was plotted with concentration (µg/ml) on X axis and % scavenging on Y axis and IC^{50} values were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization[14].

Results

The inhibitory activity of AChE by *D. gangeticum* is presented in Table 1 at a final concentration of 50-250 µg/mL. Percentage inhibition of *D. gangeticum* ranged from 28.78±0.12 to 40.83±0.05.

Table 1: AChE Inhibition and DPPH radical scavenging of *D. gangeticum* L.

S.No.	Conc. (µg/ml)	% Scavenging DPPH		%Inhibition AChE
		MeOH extract	Ascorbic acid	MeOH extract
1	50	24.68±0.72	88.66±0.43	28.78±0.12
2	100	34.48±0.36	91.26±0.66	31.60±0.52
3	150	38.35±0.56	93.27±0.56	34.30±0.42
4	200	40.28±0.51	93.08±0.39	40.22±0.20
5	250	42.22±0.67	89.17±0.43	40.83±0.05

All values in the table represent mean ± SD (n=3)

Discussion

The inhibition might come from the presence of phenolic acids, flavonoids and other antioxidant compounds. Antioxidant compounds might be implicated in AChE inhibition[15]. Recent studies bound Alzheimer's disease to an inflammatory process induced by reactive oxygenated substances[16]. The oxidative stress intervenes, for a share, in the physiopathology of the neuronal degeneration.

In vitro tests of methanolic extract of *D. gangeticum* L. evaluated for its antioxidant property revealed DPPH activity. The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-diphenyl–2-picryl hydrazine. The ability to scavenge the stable free radical DPPH was measured by decrease in the absorbance at 517 nm. A concentration dependent assay was carried out with these extracts and the results are presented in Table 1. The amount of extract needed for 50% inhibition of DPPH free radical is known as IC^{50} value of the extract. Lower the IC^{50} value shows better scavenging ability of the sample.

Conclusion

AChE enzyme is considered to be related to the mechanism of memory dysfunction as Alzheimer's disease (AD). Galanthamine was used as standard AChE inhibitor and showed at 25 µg/mL inhibition amount 52.85%.

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. DPPH stable free radical method is an easy, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts[17]. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of desmodium extracts[18]. The result of the rapid radical scavenging screening confirmed their high

radical scavenging activity. Plants have been used as a source of new bioactive compounds for drug discovery since ages and have many advantages in relation to efficacy[19]. However, the search for potent long-acting anti-cholinesterase (AChE) inhibitors is still ongoing.

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