Antistress activity of methanolic extract of *Ficus benghalensis*

M. Vignesh, V. Vishnu Priya*, R. Gayathri

**ABSTRACT**

**Background:** Stress is a pattern of physiological reaction that prepares an organism for action. It varies from person to person. Prolonged stress is a potent contributor to psychosocial and physical pathological conditions in humans. For the relief and prevention of stress, a wide variety of medications can be used. **Materials and Methods:** Methanolic extract of *Ficus benghalensis* was prepared. Methanolic extract was tested for acetylcholinesterase inhibitory activity against SHSY5Y cells lines. **Results:** The acetylcholinesterase inhibition activity of methanolic extract of *Ficus benghalensis* was measured. IC50 value was found to be 228.3 (µg/ml).

**KEY WORDS:** *Ficus benghalensis*, Acetylcholinesterase, Stress

**INTRODUCTION**

Stress is “a condition of psychological and physiological imbalance resulting from exceeded demand and a person’s ability to meet those needs.” Stressful circumstances are faced by every human in our day-to-day life and it is characterized by physiologic, behavioral, neuroendocrine, as well as emotional responses to threatening stimuli. Stress disturbs the body’s normal homeostasis; furthermore, overstress affects cognitive functions and contributes to the development of disorders such as depression, anxiety, Alzheimer’s disease, and Parkinson’s disease.[1] The World Health Organization defined stress as “The pattern of physiological reaction that prepares an organism for action.” It varies from person to person. When the hypothalamus senses stress, chain of reactions is initiated which produces general adaptation syndrome and the stimuli that produce the syndrome are called stressors.[1] Stress is a potent contributor to psychosocial and physical pathological conditions in humans. For the relief and prevention of stress, a wide variety of medications can be used. There is no one specific family of medicines that are used to decrease stress. A range of medications may be prescribed for stress-related symptoms. Many of the useful medications relieve stress such as sedative (central nervous system depressant) medications; beta-blockers are also addictive. Using such substances may possess serious behavioral and health problems, unless care is exercised. In spite of the great advances in modern medicine, plants still make an important contribution to health care. Pharmaceutical companies are showing renewed interest in investigating higher plants as sources for new lead structures and also for the development of efficacious and safe phytomedicines. The ecosystem of *Ficus* species is one of the important ecosystems and has great economic and medicinal values. *Ficus benghalensis* (FB) commonly known as Vad in Marathi is a rich source of medicinal value having multidimensional curative properties. Different parts of the tree have been found to possess medicinal properties; leaves are used for treating ulcers, aerial roots for gonorrhea, whereas seeds and fruits are cooling and tonic. The roots of FB are given for obstinate vomiting and infusion of its bark is considered as a tonic and is also used in dysentery, diarrhea, and diabetes. In India, milky juice (latex) of stem bark of FB is used for the treatment of rheumatism and other inflammatory diseases. Phytochemical investigation of FB explored a wide variety of constituents which are responsible for its wide range of pharmacological activities. They include ketones, flavonoids, flavonols, sterols, pentacyclic triterpenes and triterpenoids, furocoumarin, tiglic acid ester, and some other esters.[2] From this interest of study, stress is life killing disease which has to be eradicated. Stress may be considered an extension of homeostasis and all homeostatic pathways potentially can contribute to the integrated stress system.[3] Various neurotransmitters

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are mainly responsible for this stress-related modalities and the cholinergic neurotransmission is mainly responsible for the mammalian stress response. One of the neurotransmitters is acetylcholine which is mainly responsible for the stress.[8] Mainly bark of this plant is used as traditional medicine. Bark of Ficus benghalensis exhibits astringent, diuretic, antidysenteric and antidiabetic properties.[5] Quercetin 3-galactoside and rutin were detected from leaves.[6] Delphinidin-3-O--L-rhamnoside, pelargonidin-3-O--L-rhamnoside, leucocyanidin-3-O--D-galactosyl-celllobioside, methyl ester of leuconthycyanidin, 20-tetratriacontane-2-one, pentatriacontane-5-one, 6-heptatriacontane-10-one, -sitosterol--D-glucoside, and meso-inositol were identified from stem bark.[7] It is also used as a treatment for diarrhea, dysentery, and piles[8-9] and teeth disorder.[10] rheumatism, skin disorders like sores and to boost immune system.[12]

MATERIALS AND METHODS

Cell Culture

SHSY5Y cells were cultured in minimal essential medium with Earle’s salts supplemented with 10% FBS, 1% NEAA, 2 mM L-glutamine, and 1% Penicillin-Streptomycin solution (PEST). The cells were plated at a density of s500 cells/mm² in 96-well plates overnight in the culture medium. The medium was replaced with the differentiation medium (Dulbecco’s modified medium with Ham’s F12 medium [1:1], 1% N₂ supplement, and 1% PEST) containing 1 μM RA. The cells were differentiated for 3–6 days. Half of the medium per well was changed every 48 h.

Preparation of MeOH Extract

FB was purchased and the samples were ground with grinder, and the powders were stored in a freezer at –20°C until use. The FB (500 g) was refluxed with MeOH (1 L) for 3 h, and the filtrates were concentrated to dryness in vacuo at 40°C to render the MeOH extracts (175.0 g). These MeOH extracts were separately suspended in distilled H₂O.

Cells Treatment

The cells were pretreated with different concentrations of FBE (100–400 µg/ml) for 4 h and then incubated with MPP⁺ (1 mM) for 2 h. The effective dose of sample extract was used to identify potential neuroprotective effects against MPP⁺ toxicity.

Acetylcholinesterase Inhibitory Activity

Different concentrations (100–400µg/ml) of the sample were incubated with 10µL of AChE for 45min at room temperature. To the reaction mixtures, 125µL of 3mM DTNB was added and the total volume was made up to 1mL with Tris-HCl buffer (pH 8.0). Subsequently, 25µL of 15mM acetylcholine iodide was added to the reaction mixtures to initiate the enzyme activity. The formation of 5-thio-2-nitrobenzoate anion was detected by yellow coloration and the absorbance was detected in the wavelength of 405nm using ultraviolet-visible spectrophotometer. The experiments were done in triplicates with Donepezil as standard drug control.

\[
\text{Percentage of inhibition} = \left( \frac{\text{Enzyme activity without sample} - \text{Enzyme activity with sample}}{\text{Enzyme activity without sample}} \right) \times 100
\]

Statistical Analysis

Results were expressed as mean ± SD. Statistical significance was determined by one-way analysis of variance and post hoc least significant difference test. P < 0.05 was considered statistically significant.

RESULTS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance at 405 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHSY5Y untreated cells</td>
<td>0.315±0.29</td>
<td></td>
</tr>
<tr>
<td>FBE 100</td>
<td>0.215±0.15*</td>
<td></td>
</tr>
<tr>
<td>FBE 200</td>
<td>0.175±0.13*</td>
<td></td>
</tr>
<tr>
<td>FBE 300</td>
<td>0.108±0.10*</td>
<td></td>
</tr>
<tr>
<td>FBE 400</td>
<td>0.089±0.09*</td>
<td></td>
</tr>
<tr>
<td>Donepezil (µM)</td>
<td>1.5</td>
<td>0.056±0.04*</td>
</tr>
</tbody>
</table>

The acetylcholinesterase inhibition activity was measured. IC₅₀ value is 228.3 (µg/ml).

Results were expressed as mean ± SD (n=3). *P < 0.05 significantly different as compared with SH5YHY control.
DISCUSSION

Acetylcholine esterase is found in the many types of conducting tissue: Nerves and muscles and central and peripheral tissues, motor and sensory fibers, and chlorogenic and non-chlorogenic fibers. The first neurotransmitter Ach is neurotransmitter at all autonomic ganglia, at many automated innervated organ at neuromuscular junction and at the many synapses in the central nervous system.[13] In peripheral nervous system, Ach is also the neurotransmitter at the neuromuscular junction between the motor nerve and skeletal muscle.[13] Acetylcholinesterase is the enzyme responsible for activating the Ach expression gene and thus shifting the pre-mRNA slicing pattern during stress.[14] FB extract is proved to be more efficient to inhibit acetylcholinesterase compared to the competitive inhibitor donepezil.

CONCLUSION

Thus, the FB extract reduces the stress more effective than any drugs and used as the natural stress reliever without any side effects.

REFERENCES

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