PHCOG RES. ORIGINAL ARTICLE

Acetylcholine and memory-enhancing activity of *Ficus racemosa* bark

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Submitted: 02-04-2011 Revised: 21-05-2011 Published: 15-11-2011

ABSTRACT

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder resulting in dementia and enhancement of acetylcholine (Ach) levels in brain using acetylcholinesterase inhibitors is one of the most important approaches for the treatment of AD. **Methods:** In this study, aqueous extract of *Ficus racemosa* Linn. (Moraceae) bark having anti-infl ammatory, antioxidant, and anticholinesterase activity was evaluated for its ability to enhance Ach levels, and to ascertain its antidementia activity in rats. This work was carried out under the assumption that the *F. racemosa* extract may show combination of actions which could be beneficial in the treatment of AD, such as neuroprotection, attributed to antioxidant and anti-infl ammatory property and may elevate levels of Ach like *Ficus hispida* extract reported earlier. **Results:** Administration of the extract at two levels viz., 250 and 500 mg/kg signifi cantly raised ($P \leq 0.05$) Ach levels in hippocampi of rats compared to control. The percentage enhancement in Ach levels was found to be 22% and 38%, respectively. Further, the extract at both dosage levels elicited signifi cant reduction ($P \leq 0.05$) in transfer latency on elevated plus-maze, which was used as an exteroceptive behavioral model to evaluate memory in rats. **Conclusion:** It is inferred that it would be worthwhile to explore the potential of *F. racemosa* in the management of Alzheimer disease.

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.89744

Quick Response Code:

Key words: Acetylcholine, Alzheimer disease, memory, plus-maze, transfer latency

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas resulting in dementia.[1,2] The central cholinergic pathways play a prominent role in memory processes.[3] The allopathic system of medicine is yet to provide a radical cure for AD which principally relies on nootropic agents such as piracetam, aniracetam, fosracetam, and anticholinesterases such as donepezil, metrifonate, and rivastigmine.[4-10] One of the most important approaches for the treatment of this disease involves the enhancement of acetylcholine (Ach) levels in brain using AChE inhibitors.[11,12] Several studies have reported anticholinesterase activity of the plant extracts and drugs.[13-16] Certain reports have claimed that a few herbal extracts can act on the central nervous system, thereby enhancing the faculties of learning and memory.

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In our earlier report, cold and hot aqueous extracts of *Ficus racemosa* stem bark showed dose-dependent inhibition of rat brain acetylcholinesterase with IC_{50} values of 1813 and 1331 μg/mL, respectively.[17]

It is postulated that anti-inflammatory and antioxidant drugs are useful in controlling the progression and inflammatory damage to brain tissue as immunohistochemical studies have shown chronic inflammatory changes in AD.[18,19] In view of the above, *F. racemosa* bark having anti-inflammatory, antioxidant, and anticholinesterase activity[17,20,21] was evaluated for its ability to enhance ACh in rat brain and its effect on cognitive function using elevated plus-maze as the exteroceptive behavioral model to evaluate memory in rats.

MATERIALS AND METHODS

Chemicals and plant material

All the reagents and chemicals used in the study were of extra pure analytical grade. *F. racemosa* stem bark was identified by Dr. Shivprasad Hudeda, JSS Ayurvedic

Medical College, Mysore, and the voucher specimen (BOT-001/2008) was deposited at the herbarium of Department of Studies in Botany, University of Mysore, Mysore, India. The bark was cut into small pieces, dried (50°C) and powdered, passed through 60 mesh sieve (BS) and stored in an air tight container at 4°C till further use.

Preparation of the extracts

Aqueous extract (FR) was prepared by extracting *F. racemosa* bark powder with distilled water (1:8 w/v) at 70°C in a temperature controlled mechanical shaker for 24 h, filtered and freeze dried (yield: 12% w/v).

Acetylcholine enhancing activity

Healthy male Wistar rats of 6 weeks were divided into the following three groups ($n = 6$).

Group I: Served as control and received 1 mL normal saline p.o.

Group II: FR group received *F. racemosa* extract dissolved in 1 mL distilled water (250 mg/kg p.o.).

Group III: FR group received *F. racemosa* extract dissolved in 1 mL distilled water (500 mg/kg p.o.).

The rats were housed in polyacrylic cages, maintained at 27 ± 2 °C, 45–60% RH, and 12-h photoperiod. They were provided with a standard pellet diet (Amrut feeds, Pune, India) and water *ad libitum*. The animals were maintained with the above treatment for a period of 4 weeks. After 4 weeks, the rats were euthanized by decapitation, brains were rapidly excised, hippocampi were dissected out on ice and placed in chilled NaCl solution (0.9%).[22] The hippocampi were then homogenized using a Teflon–glass homogenizer in freshly prepared 10% trichloroacetic acid (1:5 w/v) in ice cold conditions. The homogenates were centrifuged at 4°C (8000 × *g*; 10 min) and the supernatant was collected and immediately used for the estimation of Ach by the fluorimetric method.[23]

Elevated plus-maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in rats. The apparatus for rats consisted of a central platform (10 × 10 cm^2) connected to two open arms (50 × 10 cm^2) and two covered (enclosed) arms (50 × 40 × 10 cm^3), and the maze was elevated to a height of 50 cm from the floor (Parle and Singh, 2004). On the first day (i.e., seventh day of drug treatment), each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into any one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The rat was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h after the first day trial. Significant reduction in the TL value of retention indicated improvement in memory.[24]

Statistical analysis

All values are expressed as mean ± SD. Data were analyzed by ANOVA followed by Tukey's multiple comparison tests for significant differences using SPSS 16.0 software. The values were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Acetylcholine plays a central role in basic nerve transmission, concentration, memory, and learning. The leading pharmaceutical drugs used for senility are, in fact, aimed at elevating Ach levels in the brain.[25] Age-related neurodegenerative disease like AD often associated with decreased level of neurotransmitter, primarily Ach in the hippocampal region, the area which performs the major memory task. Defectiveness of the Ach in the cholinergic forebrain ultimately leads to dementia.[26] The reduction of cholinergic activity in the CNS of AD patients correlates with their deterioration in scores on dementia rating scales.[27]

This study reports the Ach and memory enhancing activity of aqueous extract of *F. racemosa* bark in rats. Administration of the extract at two levels namely 250 and 500 mg/kg significantly enhanced ($P \leq 0.05$) Ach levels in hippocampi of rats compared to control [Figure 1]. ACh levels were increased from 42 nM/g in control to 53.9 and 67.7 nM/g by 250 and 500 mg/kg of the extracts, respectively. The percentage raise in Ach levels were found to be 22% and 38% at 250 and 500 mg/kg dose of the *F. racemosa* extract, respectively. These findings are in good agreement with an earlier study, wherein the leaf extract of *Ficus hispida* at 200 and 400 mg/kg increased Ach

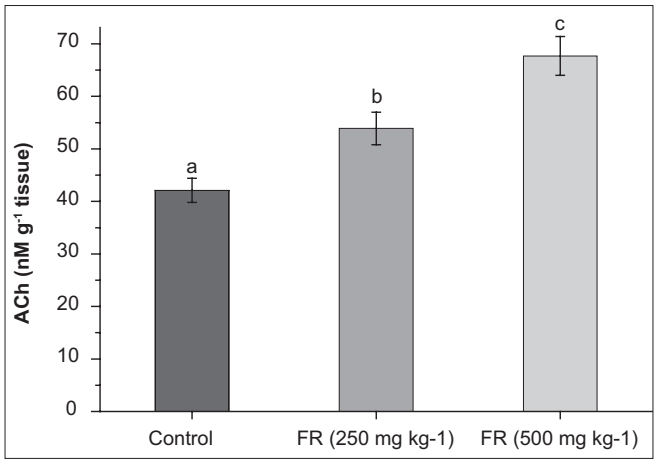

Figure 1: Acetylcholine concentration in the hippocampi of various groups. Values are mean ± SD ($n = 6$), bars with different letters a, b, and c differ significantly ($P \leq 0.05$)

concentration by 21% and 31% in neonatal rats and by 48% and 55%, respectively, in adult rats.[28] The elevated levels of ACh in the hippocampal region could be attributed to the inhibition of acetylcholinesterase by *F. racemosa* bark extract.[17]

Memory refers to the storage, retention, and recollection of information including past experiences, knowledge, and thoughts.[24,29] Drugs that enhance acquisition and recall of associative memory represent important goals in the therapy of cognitive disorders. The changes in TL with the administration of *F. racemosa* extracts (250 and 500 mg/kg) are presented in Figure 2. The extract at both dosage levels showed significant reduction ($P \leq 0.05$) in TL. The extract at 500 mg/kg showed significantly a higher reduction in TL compared to a 250 mg/kg dosage level. It is opinioned that anti-inflammatory, antioxidant, and cholesterol lowering drugs are useful in controlling the progression of AD[18] as chronic inflammation and abnormal accumulation of cholesterol increases β-amyloid (Aβ) plaques resulting in dementia.[30] Therefore, the memory enhancing activity of *F. racemosa* extract could be attributed to its anti-inflammatory, antioxidant, and hypocholesterolemic activity.[20,21,31]

The chemical composition of *F. racemosa* bark is widely reported and is known to contain steroids such as stigmasterol, β-sitosterol, β-sitosterol-D-glucoside, tripenoids such as lupeol, lupeol acetate, α-amyrin acetate, gluanol acetate, glycosides such as racemosic acid, leucocyanidin-3-*O*-β-D-glucopyrancoside, leucopelargonidin-3-*O*-β-D-glucopyranoside, leucopelargonidin-3-*O*-α-L-rhamnopyranoside, ceryl behenate, phenoloic compounds such as quercetin, kaempherol, catachin, epicatachin, and other compounds including friedelin, bergenin, bergapten, psoralens, alkaloids, and tannins.[32-39] Of these compounds phenolic compounds and racemosic acid are known to exhibit excellent anti-inflammatory and antioxidant activity. Phenolic compounds are also known to inhibit acetylcholinesterase *in vitro*.[40] Thus, the ACh and memory enhancing activity of *F. racemosa* bark extract can be attributed to the various antioxidant phenolic compounds and the glycoside; racemosic acid.

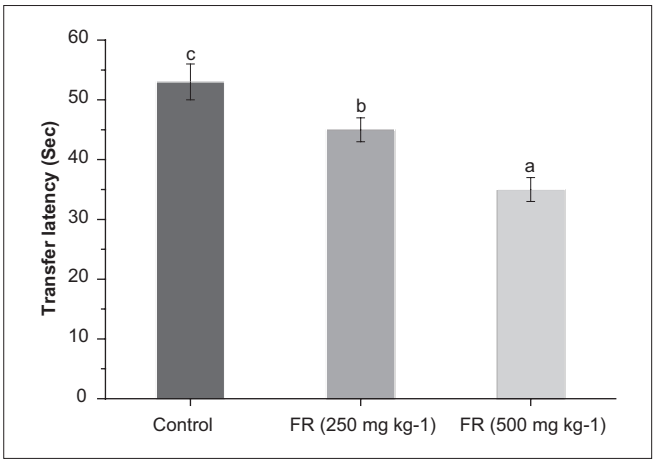

Figure 2: Effect of *Ficus racemosa* bark extract on transfer latency. Values are mean ± SD ($n = 6$), bars with different letters a, b, and c differ significantly ($P \leq 0.05$)

CONCLUSION

AD is a complex neurodegenerative disorder, leading to accelerated cognitive decline and dementia. The clinical treatment strategy of AD mainly involves elevation of cholinergic hypofunction and administration of anti-inflammatory drugs, antioxidants, and life style management. The plant extract selected for investigation shown to have antioxidant and anti-inflammatory activity from previous studies. It is evident from our study that administration of this extract elevated Ach levels and improved memory in rats. The collective pharmacological actions attributed by *F. racemosa* extract may serve as beneficial and supporting agent in the treatment of AD.

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Cite this article as: Ahmed F, Narendra Sharath Chandra JN, Manjunath S. Acetylcholine and memory-enhancing activity of Ficus racemosa bark. Phcog Res 2011;3:246-9.

Source of Support: Nil, **Conflict of Interest:** None declared.