Cholinergic Basis of Memory Improving Effect of *Ocimum tenuiflorum* Linn.

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Dementia is one of the age-related mental problems and a characteristic symptom of Alzheimer's disease. Nootropic agents are used in situations where there is organic disorder in learning abilities. The present work was undertaken to assess the potential of *Ocimum tenuiflorum* Linn. as a nootropic and anticholinesterase agent in mice. Ethanol extract of dried whole plant of *O. tenuiflorum* Linn. ameliorated the amnesic effect of scopolamine (0.4 mg/kg) and aging-induced memory deficits in mice. Passive avoidance paradigm served as the exteroceptive behavioural model. *O. tenuiflorum* extract increased step-down latency and acetyl cholinesterase inhibition significantly. Hence, *O. tenuiflorum* can be employed in the treatment of cognitive disorders such as dementia and Alzheimer's disease.

In Ayurveda, *Ocimum tenuiflorum* Linn. (*O. sanctum* – Lamiaceae) is popularly known as the sacred *tulsi* (holy basil) and has been in clinical use for centuries; leaves possess anthelmintic, expectorant, diaphoretic, stimulant effects; infusion of the plant is given in arthritis, toothache, ringworm infections, and piles; decoction of the root is given in genitourinary disorders and malaria[1]. It is reported to possess chemo preventive[2], antistress[3], anticonvulslant[4], antiulcer[5], antidiabetic[6], analgesic[7], antioxidant[8], anticancer[9], immunomodulatory[10], and antiinflammatory[11] activity. The present study was undertaken to assess the potential of ethanol extract of *Ocimum tenuiflorum* Linn. as a memory strengthening and anti cholinesterase agent.

The whole plant of *Ocimum tenuiflorum* Linn. was collected from the local areas of Bangalore, identified and authenticated at Department of Pharmacognosy, M. S. Ramaiah College of Pharmacy, Bangalore. A voucher specimen (OT/HS-235) has been deposited in the department. One kilogram powder of *O. tenuiflorum* was extracted by Soxhlet method using ethanol (90%). The crude extract was filtered and concentrated by rotavapour flash evaporator. The yield of the extract from crude powder of *O. tenuiflorum* was 17%. A suspension was prepared using Tween 80.

Swiss mice of either sex weighing around 18 g (younger ones, aged 3 months) and around 25 g (older ones, aged 7 months) were used in the present study. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol, and care of animals was taken as per guidelines of CPCSEA (Reg. No. 220/CPCSEA).

Exteroceptive behavioural model (passive avoidance paradigm) and Interoceptive behavioural models (scopolamine-induced amnesia and ageing-induced amnesia) were employed[12]. Passive avoidance behaviour is based on negative reinforcement and is used to examine the long-term memory. Step-down latencies (SDL) were recorded. The whole brain acetyl cholinesterase (AChE) activity was measured using the method reported by Ellman *et al*[13]. The data were expressed as mean±SEM. The normally distributed data were subjected to one-way ANOVA, followed by unpaired 't' test using SPSS-computer software. Kruskal Wallis[14] one-way ANOVA, followed by multiple range tests, was used for the analysis of non-normally distributed data. $P < 0.05$ was considered significant.

Normal ageing is known to deteriorate memory in human beings[15]. *O. tenuiflorum* increased SDL in both young and aged mice when subjected to passive avoidance paradigm, indicating its potent antiamnesic activity (Table 1). Central cholinergic system plays an important role in learning and memory[16]. Phenytoin is known to reduce hippocampal ACh concentration[17]. In our study, phenytoin *per se* (12 mg/kg, p.o.) significantly elevated brain AChE activity, whereas piracetam (250 mg/kg, p.o.) and *O. tenuiflorum*

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TABLE 1: EFFECT OF *O. TENUIFLORUM* LINN. ON STEP-DOWN LATENCY

Mice	Group	Treatment	Dose (Kg^{-1})	SDL after 24 h (s)
Young	I	Control (DW)	10 ml	112.1±3.2
Young	II	OE	50 mg	248.1±6.4*
Young	III	OE	100 mg	191±2.36*
Young	IV	OE	200 mg	284.2±4.62*
Young	V	OE	0.4 mg	16.2±2.19*
Young	VI	OE+ Scopolamine	200 mg 0.4 mg	253.62±3.21*[a]
Aged	VII	Control (DW)	10 ml	42.46±6.31
Aged	VIII	OE	50 mg	48.18±6.29[b]
Aged	IX	OE	100 mg	62.51±4.31[b]
Aged	X	OE	200 mg	98.19±1.96[b]

Each group consisted of 5 animals, except control group (n=6), Values are each Mean ± SEM, *Indicates $p<0.05$ compared to Control (for young mice), [a]$p<0.05$ compared to Scopolamine-treated group alone; [b]$p<0.05$ compared to Control (aged mice alone) OE: *Ocimum tenuiflorum* extract

TABLE 2: EFFECT OF *O. TENUIFLORUM* LINN. AND PIRACETAM ON AChE ACTIVITY IN AGED MICE

Treatment	Dose (mg/kg, p.o.)	AChE (µ moles)
Control	10 ml/kg	118.45±6.20
Phenytoin	12	192.21±1.84*
Piracetam	250	90.55±8.68*
OE	50	111.23±6.21*
OE	100	93.27±8.52*
OE	200	79.71±8.10*

Each group consisted of 5 animals, except control group (n=6), Values are mean ± SEM; AChE- whole brain AChE activity; $p<0.05$ *vs.* control (multiple range test), OE: *Ocimum tenuiflorum* extract-0-

(50, 100, and 200 mg/kg, p.o.) lowered this activity significantly (P <0.05) (Table 2). Hence *O. tenuiflorum* may be useful as a nootropic agent in the early management of various cognitive disorders.

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