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NOOTROPIC EFFECT OF *IPOMOEA AQUATICA* FORSK IN RAT HIPPOCAMPUS

Dhanasekaran.Sivaraman* and Palayan.Muralidaran

*Department of Pharmacology and Toxicology,C.L.Baid Metha College of Pharmacy,Jyothi Nagar,Thoraipakkam,Chennai 97,Tamil Nadu,India.

*Corres. Author : sivaramand83@gmail.com
Mobile: 09841575334
Telephone: 04425522874

ABSTRACT: Alzheimer's disease (AD) is a primary degenerative disease of the central nervous system. The progression of disease will ultimately lead to dementia, behavioral and cognitive impairments.AD is characterized by selective neuronal cell death, the presence of extra cellular amyloid deposits in the core of neuritic plaques and the formation of intraneuronal neurofibrillary tangles in the brain of affected individuals.AD affects up to 15% of people over age 65 years and nearly half of people age 85 years. Prevalence rates for more than 20 million people worldwide including 4.5 million Americans. In AD, the severe loss of cholinergic neurons in the nucleus basalis and associated areas that form the cholinergic forebrain area and their projections to the cerebral cortices are marked with decreased levels of acetylcholine (ACh). The aim of the study is to elvaluate the acetylcholine enhancing activity of methanol leaf extract of *Ipomoea aquatica* Forsk (MEIA), is known to posses various therapeutic properties.We found that treatment with 200 and 400 mg/kg of MEIA, for 30 days in neonatal and young adult age groups of rat, significantly increased acetylcholine (ACh) content in their hippocampus as compared to age matched controls. Increase in ACh content in their hippocampus may be the neurochemical basis for their improved learning and memory.
Key words: Nootropic effect, Ipomoea aquatica Forsk.

1. INTRODUCTION

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care[1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. *Ipomoea aquatica* Forsk (IA) belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in the region. Many of the waters where IAF grows serve as recipients for domestic and other types of waste water. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka[2]. O nly a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases3, constipation[4].IA is considered a tonic the species contains several vitamins, including A, B, C, E, and "U" (S-methyl-methionine), and is used to treat gastric and intestinal disorders[5,6,7].The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, β-sitosterol and its glycosides, prostaglandin, leukotrine, N-trans- and N-cis feruloyltyramines [8,9,10,11,12,13,14]. It is runner type plant with numerous small flowers[15, 16, 17,18]. The current study was undertaken to evaluate the acetyl choline enhancing activity of methanolic leaf extract of IA by, till now no pharmacological evaluation has been done on IA especially in leaf for its neuroprotective and

rejuvenating activity. This prompted us to pursue the activity and was examined for their efficacy and for determination of their possible mechanism of action.

2. MATERIALS AND METHODS

2.1. Plant material.

The fresh leaf's of IA were collected from (Changlepet,Tamilnadu,India) western Ghats of South India during March 2008 . The plant was identified and authenticated by Dr.Sasikala Ethirajulu.Captain srinivasan research Foundation,Chennai,Tamil nadu,India.The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L.Baid Metha College of Pharmacy, Chennai,Tamil nadu,India.

2.2. Preparation of the Methanolic Extract of IA.

The fresh leaf of IA was collected and washed with running water. It was shade dried at room temperature and 1 kg of the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The weight of extract obtained was 19.6 %.

2.3. Phytochemical screening:

The freshly prepared leaf extract of IA was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's,Fehling's and Benedict's reagent; proteins and amino acids with Millon's,Biuret, and xanthoprotein test.Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red;Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures[19].

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The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids + ve; Gums and mucilage + ve; Glycosides + ve; Saponins + ve; Terpenes + ve and Tannins – ve,

Where + ve and – ve indicates the presence and absence of compounds.

2.4. Animals

Swiss albino rats of either sex of two age groups, (i) neonatal pups (7 days old) and (ii) young adults (60 days old) were used for the study, were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai, Tamil nadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy .The animals were maintained in colony cages at 25±2 ◦C, relative humidity 50–55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd.banglore, India) and water ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conducted between 10-00 and 17.00 h and were in accordance with the ethical guidelines of the International association for Study of Pain[20].All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The study was approved by the Institutional Animal Ethical Committee (Ref No: IAEC/XIII/02/CLBMCP/2008-2009 Dated 16/06/08).

2.5. Acute toxicity studies

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD)[21].

2.6. Treatment schedule

Seven-day-old neonatal rat pups and 60-day-old young adult rats were further subdivided into two groups.

Group A (neonatal group)

Consists of Seven-day-old neonatal rat pups, further subdivide in to Group I (control group) received 10ml/kg normal saline,p.o. Group II and Group III (Treatment groups) animals received MEIA 200 and 400 mg/kg,p.o. for 30 days (from 8th day to 37th day in neonatal group).

Group B (Adult group)

Consists of 60-day-old young adult rats, further subdivide in to Group I (control group) received 10ml/kg normal saline,p.o. Group II and Group III (Treatment groups) animals received MEIA 200 and 400 mg/kg,p.o. for 30 days (from 61st day to 90th day in young adult rats).

2.7. Tissue preparation

After the period of treatment, both neonatal and young adult rats (Group A and B) were killed by quick decapitation. Their brains were rapidly removed and placed on ice.Hippocampi were then dissected out on ice and placed in chilled 0.9% NaCl solutions[22]. Hippocampi from rats were pooled for estimation of ACh (n=6 pooled samples in each group).

2.8. ACh estimation

ACh was estimated by fluorimetric method[23] .The pooled hippocampi were weighed and homogenized using a Teflon–glass homogenizer in freshly prepared cold 10% trichloroacetic acid.The homogenates were centrifuged at 10 000 rev./min for 10 min at 4 8C.The supernatant was collected and processed immediately for estimation of ACh.

2.9. Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

3. RESULTS

The effect of MEIA on hippocampal Ach level in rats is shown in Fig. 1. Oral treatment with 200 and 400 mg/kg of MEIA during neonatal period and young adult age in rats enhanced ACh content significantly ($p < 0.05$) in their hippocampus when compared with control group. Interestingly, treatment with MEIA at the dose of 200 and 400 mg/kg shown significant increase in Ach levels (65.40±2.1 and 71.52±1.8nmol/g tissue) in neonatal rats. Adult group rats administrated with MEIA showed a significant increase in the Ach level (71.68±0.9 and 84.12±2.2 nmol/g tissue) at the dose 200 and 400 mg/kg, but MEIA at the high dose 400mg/kg dose, showed much significant increase when compare to 200mg/kg dose.

Hippocampal ACh content was found to be significantly less in 90-day-old control rat's 38.33±1.1 nmol/g tissue as compared to 37-day-old control rat's 51.30±1.8 nmol/g tissue. On the contrary, hippocampal ACh content was found to be higher in 90-day-old MEIA 400 mg/kg treated rats than in 37-day-old MEIA treated rats shown in Table 1.

4. DISCUSSION

We studied the Acetylcholine enhancing activity of methanol leaf extract of *Ipomoea aquatica* Forsk in rat hippocampus. 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 acetylcholine levels in the brain [24]. Age related neurodegenerative disease like Alzheimer's disease (AD) often associated with decreased level of neurotransmitter, primarily Ach in the hippocampal region, the area which perform the major memory task. Defectiveness of the Ach in the cholinergic forebrain ultimately leads to dementia[25]. The reduction of cholinergic activity in the CNS of AD patients correlates with their deterioration in scores on dementia rating scales [26].Preliminary phytochemical investigations of MEIA revealed the presence of carbohydrates, Flavonoids, sterols, saponins, phenols and terpenes. The Ach enhancing effect of MEIA might therefore be due to any one or combination of these phytochemical. Since MEIA exhibited Ach enhancing activity, it might be clinically useful in the control of age related memory disorders like AD. Thus, successive studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

5. CONCLUSION

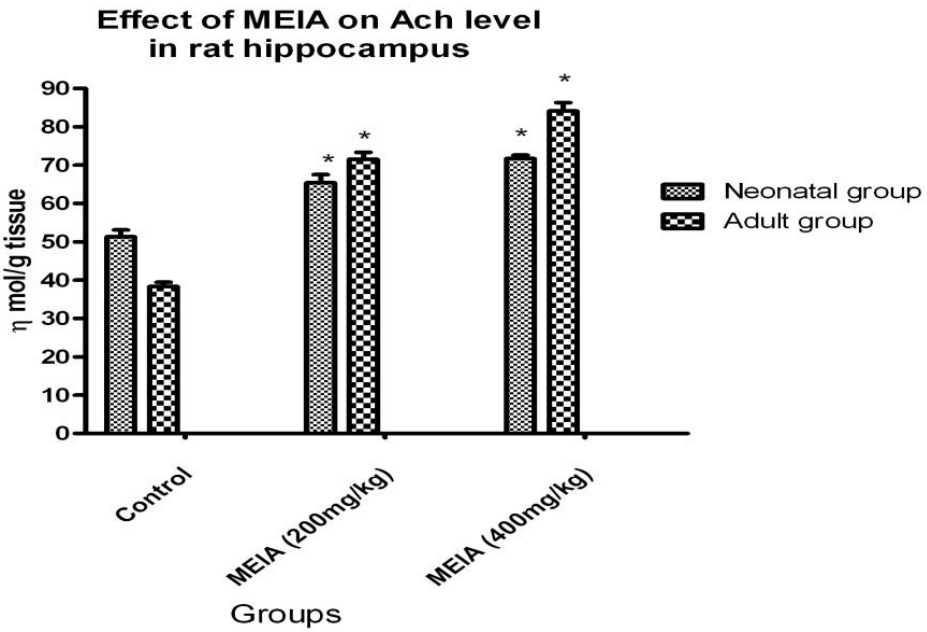
In conclusion, we suggest that MEIA markedly improves brain Ach level. The ability of MEIA treatment in rats to increase Ach content suggests that this extract treatment may be useful as a memory enhancer, even it may be concluded that MEIA treatment may be of value for reinforcing depressed cholinergic transmission in certain age related memory disorders and to improve learning and memory in normal individuals.

Table 1: Effect of MEIA on hippocampal Ach level in rats.

Treatment (mg/kg) (nmol/g tissue)	Hippocampal Ach level.	
	Neonatal group mean ± S.E.M.	Adult group mean±S.E.M
Control: NS (10ml/kg, p.o.)	51.30±1.8	38.33±1.1
MEIA (200mg/kg, p.o.)	65.40±2.1*	71.52±1.8*
MEIA (400mg/kg, p.o.)	71.68±0.9*	84.12±2.2*

NS: normal saline; MEIA: Methanol extract of *Ipomoea aquatica* Forsk.

- Significant $P < 0.05$ compared to control, ANOVA; n =6.



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