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Nootropic Activity of Leaf Extract of *Anogessius latifolia*

K. HEMAMALINI, N. LAKSHMI NARASIMHA REDDY, S. PRAVEEN KUMAR REDDY, K.V. LOKESH* and G. MAALU

Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Saroornagar, Meerpet, Hyderabad-500 097, India

*Corresponding author: E-mail: lokesh.kv12@gmail.com

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Nootropic effect of methanolic and chloroform extracts of *Anogessius latifolia* was evaluated by using elevated plus maze and diazepam induced amnesia models. Piracetam was used as the standard drug. A significant increase in inflexion ratio was recorded in elevated plus maze and diazepam induced amnesia models. The results indicate that nootropic activity observed with methanolic extract and chloroform extract of leaf of *Anogessius latifolia* could be through improved learning and memory either by augmenting the noradrenaline transmission or by interfering with 5-hydroxytryptamine (5-HT) release. Further, the extracts neither facilitated nor blocked release of the dopamine. Thus methanolic extract and chloroform extract elicited significant nootropic effect in mice and rats by interacting with cholinergic, GABAnergic, adrenergic and serotonergic systems. Phyto constituents like flavonoids have been reported for their nootropic effect and these are present in both methanolic extract and chloroform extract of leafs of *Anogessius latifolia* and these active principles may be responsible for nootropic activity.

Key Words: Antiamnesic, *Anogessius latifolia*, Leaf extracts.

INTRODUCTION

Dementia is described as a syndrome due to chronic or progressive disease of the brain, leading to disturbances of multiple functions of higher cortical centers including memory, orientation, comprehension, calculation, learning capacity, language and judgment without altering consciousness. Further deterioration of emotional control, social behaviour or motivation may accompany or proceed to cognitive impairment.

Alzheimer's disease is a incurable, progressive brain disorder that causes dementia and abnormal phosphorylation of the intracellular tan proteins, causing abnormalities of microtubules assembly and collapse of the cytoskeleton affected, particularly pyramidal cells of the cortex and subcortex[1]. Hypo function of various neuronal systems particularly the cholinergic neuronal systems with decrease in several cholinergic parameters, *e.g.* choline acetyl transferase and cholinesterase activity, choline acetyl transferase mRNA and acetyl choline receptors were observed[2].

A protein called casein kinase-I could slow the formation of β-amyloid protein, the main constituents of the amyloid plaques found in the brain's of alzheimer's disease thought to cause the debilitating effects of the disease. β-amyloidprotein is toxic to mitochondria found in cells and ultimately causes nerve cell death[3]. The dementia in the elderly *i.e.* about 5 million people in the united states are estimated to be affected by this disorder and the percentage approximately doubles with every 5 years of age with about 1 % of 60 years old and about 30 % of 85 year old having the disease[4].

In the present study a plant *Anogessius latifolia* popularly used in folklore medicine was selected as it is reported for its nerve tonic, galactogogue, antiinflammatory, brain tonic and as a mind power syrup in ayurvedic formulation.

Earlier the plant has been studied for the antifungal, anti-inflammatory, wound healing, antioxidant[5] activities.

EXPERIMENTAL

Drugs: Piracetam (B & B Pharmaceuticals Ltd), Diazepam (Ranbaxy Laboratories Ltd.,) were used.

Animals: Albino mice and rats of either sex weighing between 18-22 g and 150-200 g respectively procured from Shri Venkateswara Enterprises, Bangalore were used. After procuring, all the animals were acclimatized for 4 days and housed in groups of six under standard husbandryconditions[6,7] (26 ± 2 ºC, 45-55 % RH) and 12:12 h light/dark cycle. All the animals were fed with synthetic standard diet (Amrut Laboratories Pranava Agro Industries Ltd., Sangli, Bangalore) and water provided *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethical Committee (IAEC) of Nizam Institute of Pharmacy, Deshmukhi, Pochampally (Mandal), Nalgonda, animal studies were performed as per rules and regulations and in accordance to the

guidelines of CPCSEA with registration number 1330/ac/10/ CPCSEA. All the experiments were carried out during the light period between 08.00-16.00 h.

Preparation of different extracts with leaves of *Anogessius latifolia*

Plant extract[8]: The leaf powder was extracted with chloroform and methanol in soxhlet apparatus at 60-80 ºC for 18 h, the methanolic and chloroform extract was subjected to evaporation in a beaker on a water bath maintained at 50 ºC till a thick paste of extract remained in the beaker and was kept in refrigerator below 4 ºC till the experimental study.

Pharmacological activities

Determination of acute toxicity (LD_{50}): The acute toxicity of methanolic extract and chloroform extract of leaves of *Anogessius latifolia* was determined in albino mice of either sex (16-25 g) maintained under standard husbandry conditions. The animals were fasted 3 h prior to the experiment. Up and down method (OECD guidelines no: 425) of CPCSEA was adopted for toxicity studies[9]. Animals were administered with single dose of extract and observed for mortality during 48 h study period (short term toxicity). Based on the short term toxicity profile of extract the dose for the next animals was determined as per the OECD guideline No: 425.

Laboratory models for testing learning and memory-elevated plus maze (exteroceptive behaviour model)[10]. Eight groups of mice each having 6 animals, weighing between 18-22 g were used. Group-I was maintained as normal control, which was given distilled water (10 mL/kg, p.o) only once daily for 7 days, group-II with piracetam (200 mg/kg, p.o) which served as standard and groups III & IV were treated orally with different doses of methanolic extract (75 & 100 mg/kg) and chloroform extract (200 and 400 mg/kg) of leaf extract of *Anogessius latifolia* respectively once daily for 7 days.

On the 7th day, 1.5 h after treatment of last doses each mouse was placed at the end of an open arm of elevated plus maze facing away from the central platform.

Transfer latency was recorded *i.e.* the time taken by mouse to move into one of the enclosed arms with all its four legs. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into any of the two enclosed arms and the transfer latency was assigned as 90 s. The mouse was allowed to explore the maze for next 10 s and then to be returned to its home cage. Retention of this learned task was examined 24 h after the 7th day trial. The inflexion ratio was calculated by the formula as follows[11]:

$$IR = L_O - L_1/L_O$$

where L_O is the initial transfer latency (S) on first day and L_1 is the transfer latency (S) on the 2nd day.

***Diazepam-induced amnesia* (Interoceptive behaviour model)[12]:** Nine groups of mice each comprising 6 animals and weighing between 18-22 g were used. Group-I was maintained as normal control, which was given with distilled water (10 mL/kg p.o), once daily for 7 days and on 7th day 1.5 h after treatment (distilled water) transfer latency was recorded on elevated plus maze and retention (memory) of learned task was examined 24 h later. Group-II was administered with diazepam (5 mg/kg p.o) alone on 1st day only and after 45 min transfer latency was recorded on elevated plus maze and retention (memory) of learned task was examined 24 h later. Group-III was treated with different doses of methanolic extract (75 & 100 mg/kg. p.o) and chloroform extract (200 & 400 mg/kg, p.o) of leaf extracts of *Anogessius latifolia* respectively once daily for 7 days. On 7th day 1.5 h after administration of Piracetam, methanolic extract, chloroform extract amnesic agent diazepam was administered.

Then 45 min later transfer latency was recorded on elevated plus maze and retention (memory) of learned task was examined 24 h later. The inflexion ratio was calculated as described in earlier study.

Statistical analysis: The data were subjected to statistical analysis by one way analysis of variance (ANOVA) followed by Dannet's 'T' test and $P<0.05$, 0.01 and 0.001 were considered as significant.

RESULTS AND DISCUSSION

Pharmacological investigations

Acute toxicity studies: Acute toxicity of methanolic extract and CH extracts were determined in mice as per OECD guidelines No: 425. LD_{50} of methanolic extract was found to be 1500 mg/kg while chloroform extract even in greater than 2000 mg/kg dose did not produce any mortality.

Laboratory models for testing learning and memory: Effect of extracts on inflexion ratio in mice with elevated plus maze model-piracetam (200 mg/kg) and leaf extracts at different dose levels of methanolic extract (75 & 100 mg/kg) and chloroform extract (200 & 400 mg/kg), treated groups had shown increased inflexion ratio . Statistically significant reduction in transfer latency was observed with piracetam and both methanolic extract and chloroform extract except with chloroform extract (100 mg/kg)dose (Table-1).

TABLE-1
NOOTROPIC EFFECT OF LEAF EXTRACT OF ANOGESSIUS LATIFOLIA IN MICE WITH EPM MODEL

Group No.	Treatment	Dose (per kg)	Inflexion ratio
I	Control vehicle	10 (mL p.o.)	0.7690 ± 0.084
II	Piracetam	200 (mg p.o.)	2.3451 ± 0.286**
III	Methanolic extract	75 (mg p.o.)	2.6836 ± 0.104**
IV	Methanolic extract	100 (mg p.o.)	3.1890 ± 0.292**
V	Chloroform extract	200 (mg p.o.)	1.5910 ± 0.085**
VI	Chloroform extract	400 (mg p.o.)	2.5070 ± 0.164**

Significance at $P<0.05^{*}$ $<0.01^{**}$ and ns-not significant *vs.* control (Vehicle). (Values are mean ± SE from 6 animals in each group).

Effect of extracts on inflexion ratio in diazepam induced amnesia model-diazepam has induced dose dependent amnesia in this amnesic model, a decrease in inflexion ratio was observed as compared to normal control group. Piracetam and all the doses of methanolic extract and chloroform extract treated groups had shown an increase in the inflexion ratio and a significant reduction in transfer latency observed on elevated plus maze and diazepam induced amnesia was reversed (Table-2).

Nootropics popularly referred as smart drugs, which boost human cognitive abilities. Typically these are alleged to work by increasing the brain's supply of neurochemicals, improving brain's oxygen supply or by stimulating nerve growth.

Despite the extensive experimental and clinical studies, the neurochemical basis for learning and memory remains controversial but a predominant role of cholinergic mechanism has long been emphasized in learning and memory processes. The role of the central cholinergic system in fairly well established and its deficiency being implicated in memory deficits[12]. Though a large number of other receptor systems too are now reported to be involved in the behavioural expression of dementia in animals and human beings as well the role of these neurotransmitter systems cannot be ignored[13].

TABLE-2
NOOTROPIC EFFECT OF LEAF EXTRACT OF ANOGESSIUS LATIFOLIA ON DIAZEPAM INDUCED AMNESIA IN MICE.

Group no.	Treatment	Dose (per kg)	Inflexion ratio
I	Normal control	10 (mL p.o.)	0.7690 ± 0.0844
II	Diazepam control	5 (mg i.p.)	-0.046 ± 0.070
III	Piracetam	200 (mg p.o.)	1.574 ± 0.07**
IV	ME	75 (mg p.o.)	1.182 ± 0.115**
V	ME	100 (mg p.o.)	1.224 ± 0.137**
VI	CHE	200 (mg p.o.)	0.896 ± 0.114**
VII	CHE	400 (mg p.o.)	1.127 ± 0.103**

Significance at P<0.05* <0.01** and ns-not significant *vs*. diazepam control. (Values are mean ± SE from 6 animals in each group).

A simplistic generalization cannot be made in view of the controversial reports available about the role of the central catecholaminergic neurotransmitter system in general and noradrenaline system in particular, in learning and memory. If is well known that amphetamines, which markedly augment central noradrenergic activity, leads to mental confusion and retards memory consolidation. The amnesic effect of electroconvulsive shock, which is attenuated by piracetam, is known to produce marked increase in the turnover of noradrenaline in rat brain[14]. The peripheral as well as central administration of noradrenaline was found to suppress avoidance behaviour but was facilitatory in some experiments[15].

The present study revealed the methanolic extract and chloroform extract of leaf of *Anogessius latifolia* contained flavonoids, which might be exhibited nootropic activity in view of its facilitating effect on retention (memory) of acquired learning in mice as these are reported with nootropic activity[15].

This observation has been supported by the findings with methanolic extract (75 & 100 mg/kg) and chloroform extract (200 & 400 mg/kg) of leaf of *Anogessius latifolia* that they have shortened the transfer latency in the elevated plus maze model indicating an improvement in the memory, which is in accordance with the hypothesis proposed by Itoh *et al*.[16].

Diazepam, GABA mimetic agent induces memory impairment and the subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory[17,18]. Diazepam (5 mg/kg) prolonged transfer latency from the open arm to the closed arm *i.e.*, decreased inflexion ratio [19]. The methanolic extract (75 & 100 mg/kg) and chloroform extract (200 mg/kg) of leaf of *Anogessius latifolia* have decreased transfer latency from the open arm to the closed arm *i.e.*, increased inflexion ratio thus confirms their nootropic activity. This protective effect offered against diazepam induced amnesic model may be due to indirect facilitation of ach in the brain[20].

It has been indicated that an increase in serotonergic transmission in the median raphe of mid brain will interfere with learning acquisition and memory consolidation.

Controversial reports are available on the involvement of dopamine activity in learning and memory as learning and memory storage can proceed normally despite depletion of brain dopamine[21]. Piracetam is known to augment dopaminergic activity[22] and in the present study piracetam (200 mg/kg) potentiated and prolonged the duration of catalepsy. Involvement of chronic inflammation in certain regions of brain and or free radicals has been implicated in the pathogenesis of alzheimer's disease. Antiinflammatory drugs have been found to be effective in enhancing cognitive function in this condition. Antiinflammatory action of *Anogessius latifolia* reported earlier might be contributing to the memory-enhancing activity observed in the present study. The phytochemical studies with the leaf extract of *Anogessius latifolia* revealed the presence of most of the chemical constituent and one of the important chemical constituent like diadzein, a natural isoflavone found in natural tea plant also reported with this plant, which will activate the choline acetyltransferases (chat), an enzyme responsible for biosynthesis of acetyl choline in the cholinergic neuronal cells.

Several studies had shown that the use of estrogen can cause improvement in performance on memory and cognition tests and the earlier studies with this plant had reported that it possess good estrogenic activity. Thus the combined effects *i.e.*, activation of choline acetyl transferases, estrogenic, anti-inflammatory, adaptogenic and neuroprotective roles of leaf extracts of *Anogessius latifolia* could be leading to the net memory enhancing effect.

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