

EFFECT OF *PIPER BETEL* AND *ARGYREIA SPECIOSA* EXTRACTS ON BRAIN ACETYLCHOLINESTERASE AND MONOAMINES CONCENTRATIONS IN MICE

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Abstract: In present investigation effects of ten days administration of two doses (200 mg and 400 mg/kg) of hydroalcoholic extracts of leaves of *Piper betel* (PB) and roots of *Argyrea speciosa* (AS), on brain acetylcholinesterase, dopamine, serotonin (5-HT) and noradrenaline levels was explored in mice. Both these plants are traditionally claimed as nootropic plants and have effect on learning and memory. Present study showed that the brain AChE level was unaltered with both the extracts. The brain dopamine concentration was found to be reduced significantly with both the doses of AS and higher dose of PB. The lower dose of PB increased level of dopamine in brain. Serotonin level was significantly increased with PB and decreased with AS, both with lower and higher doses. Contrary to these, noradrenaline was decreased in all the cases. The significance of these alterations has been discussed in the contribution.

Key words: *Piper betel*, *Argyrea speciosa*, AChE, Monoamines

INTRODUCTION

Mind altering drugs have always occupied man's attention, especially those derived from the plants. Since age immemorial the plant products have been used to change human behavior, thoughts, cognition and mood. Several such plants have been investigated more extensively in past and being searched presently too. However, understanding of the mechanism of action of such plant products is still a major challenge to the researcher [1]

The nootropics are the drugs that belong to the class of psychotropic agents with selective facilitatory effect on intellectual performance [2]. A variety of plants have been mentioned in the Indian traditional system of medicine for their nootropic activity [3]. These claims have attracted the researchers due to increased complaints of cognition in today's life of stress and strain [4]. Some of these plants have been validated scientifically with diverse neurochemical basis of their action [2, 5, 6].

The complexity of chemical transmission in normal neuronal communication and in various neurodegenerative disorders (like Alzheimer's disease) affecting learning and memory have suggested functional significance of specific neurotransmitters. Amongst more than 60 neurotransmitters speculated, central cholinergic system and biogenic amines specially noradrenaline, dopamine, serotonin have been found to be more important [7].

We have validated traditional nootropic claim of leaves of *P. betel* (PB) [8] and roots of *A. speciosa* (AS) [9] using different paradigms of learning and memory. The present investigation is an attempt to establish the neurochemical basis of its action. The objective of the present study was to investigate the effect of hydroalcoholic extract of leaves of *Piper betel* and roots of *Argyrea speciosa* on brain acetylcholinesterase (AChE) activity and biogenic amines such as noradrenaline (NA), dopamine (DA) and serotonin (5-HT) concentrations.

MATERIALS AND METHODS

Preparation of extract: The hydro-alcoholic (50%) extracts of leaves of *Piper betel* (PBT-4031) and roots of *Argyrea speciosa* (ARG-4019), prepared by the following procedure, were received as a gift sample from Green Chem. Herbals, Bangalore, India. Leaves of *P. betel* and roots of *A. speciosa* were extracted with 50% alcohol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. Then the mass was diluted with 25% aqueous alcohol, filtered, concentrated and dried to get the powdered form of the extract.

Preparation of drug solution: Accurately weighed quantities of the powdered extract were dissolved individually in distilled water to prepare an appropriate stock solution of the drug i.e. 20 mg/ml and 40 mg/ml respectively. The doses were administered orally.

Animals: Male Swiss albino mice of 18 to 22 g were used. They were maintained at $25 \pm 2^\circ\text{C}$, relative humidity of 45 to 55 % and under standard environmental conditions (12 h light 12 h dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water *ad libitum*. Research protocol was approved by Institutional Animal Ethics Committee. All estimations were carried out between 12:00- 16:00 h.

Drug treatment schedule: Fifty mice were divided into five groups. Each group consisted of ten mice. Animals of group 1 were kept as control and treated with vehicle. Mice of group 2 and 3 were treated with 200 mg and 400 mg/kg of leaves extract of *Piper betel*. Like wise, mice of group 4 and 5 were treated separately with 200 mg and 400 mg/kg of roots of *Argyrea speciosa*. All the treatments were continued for 10 days. The details of the treatments are summarized in Table 1.

Table 1. Details of treatment of mice

Animal groups	Plant extracts	Doses mg/kg		Days of treatment	Day of scarify
Group 1 (control)	vehicle	vehicle		10	10 th day
Groups 2 & 3	leaves of <i>P. betel</i>	200	400	10	10 th day
Groups 4 & 5	roots of <i>A. speciosa</i>	200	400	10	10 th day

Estimation of AChE: On the 10th day, 1 h after the last dose, the mice were decapitated and their heads were placed on ice. The brains were rapidly removed and suspended into the 0.1 M ice cold phosphate buffer (pH 8). The brains were dried on the blotting paper, weighed and homogenized individually in a tissue homogeniser making 20 mg of the tissue per ml of 0.1 M phosphate buffer, followed by centrifugation (3000 RPM) for 3 minutes at 0°C. 0.4 ml supernatant was added to a cuvette containing 2.6ml of phosphate buffer. 100 μ lit of Ellaman's reagent was added and absorbance was observed at 412 nm till the increasing absorbance became stable. This stable absorbance was set to zero and 20 μ lit of acetylthiocholine iodide (substrate) was added and changes in absorbance per minute were recorded till 10 minutes. The mean change in absorbance was considered for calculation [10].

Estimation of monoamines: For the estimation of monoamines {dopamine (DA), serotonin (5-HT) and noradrenaline (NA)}, the brains were homogenized in 2 ml of (0.1 M) PCA containing isoproterenol at concentration of 30 ng/ml. After centrifugation at 3000 rpm for 15 min at 4 °C, the supernatant obtained was filtered through a 0.2 μ membrane filter and 100 μ l of the filtrate was injected onto a HPLC column. After separation NA, DA, and 5-HT were detected at the wavelength of 280 nm and an emission wavelength of 315 nm. The slit width was kept at 10/10 for excitation/emission respectively [11].

Statistical analysis: The results are expressed as mean \pm SEM. Comparison between the groups was made by one way Analysis of Variance (ANOVA) followed by Dunnett's test. [12]

RESULTS

Changes in brain AChE concentration: The treatment of mice with both the herbal extracts (*Piper betel* and *Argyrea speciosa*) did not show any significant change in AChE activity in the brain with any of the doses (Table 2).

Changes in brain dopamine concentration: The concentration of dopamine in the brain of control mice was 108.12 ± 6.25 . After *Piper betel* extract treatment, it was increased significantly with low dose doses of 200 mg/kg to 133.17 ± 5.86 , thus revealing about 23.01% increase. Contrary to this, the higher dose of 400 mg decreased the dopamine by 25.08

Biomolecules	Control	<i>Piper betel</i> extract doses				<i>Argareia spiciosa</i> extract doses			
		200 mg/kg	% changes	400 mg/kg	% changes	200 mg/kg	% changes	400 mg/kg	% changes
AChE	3.78 ± 0.09	4.01 ± 0.11	6.04	4.05 ± 0.05	7.14	4.13 ± 0.07	9.25	3.88 ± 0.09	2.64
DA	108.12 ± 6.25	133.17 ± 5.86	23.16 *	81.02 ± 3.76	25.07**	86.92 ± 5.04	19.61*	87.00 ± 6.45	19.54*
5-HT	58.01 ± 3.91	72.89 ± 2.45	25.65*	73.29 ± 4.39	26.34*	28.10 ± 1.97	51.57**	44.17 ± 2.17	23.86*
NA	216.79 ± 12.37	162.93 ± 13.52	24.85*	94.88 ± 7.83	56.24**	95.87 ± 12.3	55.78**	164.60 ± 15.94	24.08 *

Table 2 reveals concentration of AChE and monoamines in the brain of control mice and the fluctuations after herbal treatments. * Significant P <0.01, ** P <0.05

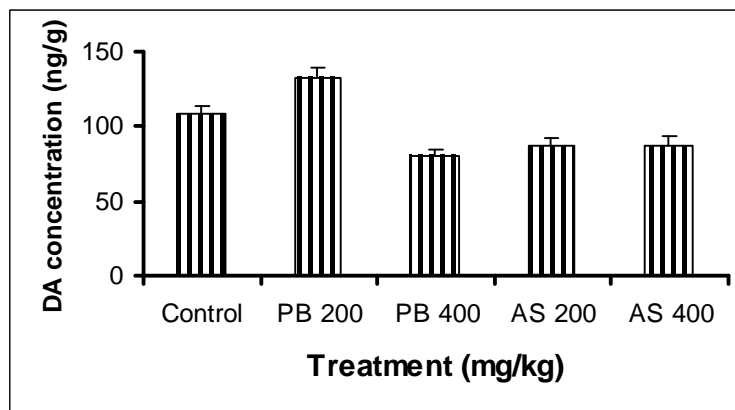


Fig. 1: Effect of PB and AS extracts on brain dopamine concentration in mice. *P<0.05, **P< 0.01

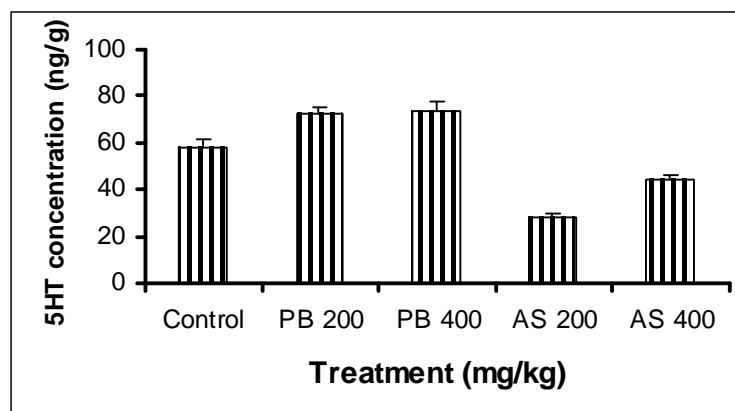


Fig 2: Effect of PB and AS extracts on brain serotonin concentration in mice.*P<0.05, **P< 0.01

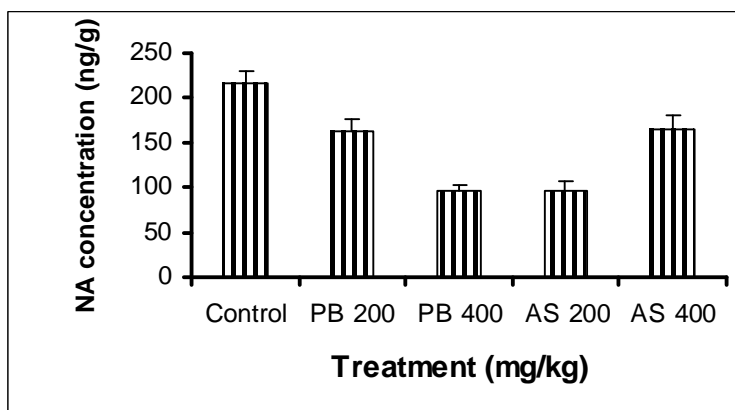


Fig 3: Effect of PB and AS extracts on brain noradrenaline concentration in mice.*P<0.05, **P< 0.01

%. Both the changes are significant ($P < 0.05$). *Argyrea speciosa* extract treatment for 10 days with both the doses showed almost same effect, where brain dopamine content decreased significantly by 20.45 % and 19.53 % levels respectively (Fig. 1).

Changes in brain serotonin concentration: The brain serotonin (5-HT) concentration in control mice was found to be 58.01 ± 3.91 . Interestingly enough, this concentration was increased significantly with both the doses of *Piper betel* extract treatment by 24.13 % and 25.84 % respectively. The increase was statistically significant ($P < 0.05$). In Sharp contrast to this, *Argyrea speciosa* extract treated animals demonstrated a sharp and significant decrease and the level of 5-HT in brain, collapse to 28.10 ± 1.97 , 44.17 ± 2.17 with 200 mg/kg and 400 mg/kg doses respectively. Thus 51.73% and 24.14% decrease of this monoamine was noticed (Fig. 2).

Changes in brain noradrenaline concentration: The trend of noradrenaline alteration in brain of mice treated with *Piper betel* and *Argyrea speciosa* extracts is exactly similar. In both the cases the concentration decreased sharply. However, the higher dose of *Piper betel* exhibited more decrease than lower dose, while reverse is true during *Argyrea speciosa* extracts treatment (Fig. 3). All the changes are statistically significant.

DISCUSSION

An interesting observation of the present study is that all the biomolecules showed different trends of alterations in brain during *Piper betel* and *Argyrea speciosa* extracts applications for both the doses. While AChE was not affected, the dopamine level increased with lower dose and decreased with higher dose of *Piper betel* extract, also dopamine level decreased with both the doses of *Argyrea speciosa* extract. 5-HT level increased with *Piper betel* extract and decreased with *Argyrea speciosa* extract irrespective of the doses. Contrary to this, noradrenaline decreased in all of the cases.

P. betel leaves and *A. speciosa* roots have been claimed for its nootropic effect in Indian traditional system of medicine [3]. *A. speciosa* is also one of the constituents in a marketed preparation "Geriforte" promoted to enhance memory especially in geriatric patients [13,14]. The leaves extract of *P. betel* has been known for facilitation of spatial learning, memory

retention and attenuation of electroshock induced amnesia [15]. We have also reported the nootropic activity of *P. betel* [8], and *A. Speciosa* extract [9] using various paradigms of learning and memory.

Cholinergic system involving acetylcholine and acetylcholinesterase activity is believed to affect the learning abilities. Disturbances in cholinergic system in various diseases of central nervous system like Alzheimer's, Parkinson's and dementia, wherein impaired learning abilities and loss of memory are commonly observed manifestations [16]. Though in the present investigation, the extracts did not show any significant alteration of AChE, it does not rule out the role of cholinergic system. Diverse reports are available suggesting inverse and direct relationship between ACh content and AChE activity [17]. In addition, direct cholinergic action without involvement of AChE may be responsible to enhance cognitive performance [18].

Diverse reports, available on the involvement of dopamine in learning and memory, have been receiving greater attention. Piracetam, an established nootropic, is reported to augment and reduce the DA levels [4,6]. While another study has shown that learning and memory can proceed normally despite depletion of brain DA [6]. It was also argued that dopamine enhances learning process but interferes with memory processes [19].

In the present investigation, dopamine inhibition was observed with AS. This was again in accordance with the behavioral results [9] indicating possible role of dopamine. Administration of 200 mg/kg dose of PB resulted in significant increase, whereas 400mg/kg showed significant reduction in brain dopamine concentration. Such dose dependent variations were not observed in behavioral studies [8] suggesting no involvement of dopamine in its nootropic activity.

The reports regarding the role of serotonin in the learning and memory are controversial. Studies have revealed that serotonin modulates cognitive processes although it is unclear at present, the manner and the exact site at which serotonergic system is involved. It has been reported that serotonin (5HT)-1A receptors are involved in learning and memory processes and its agonist 8-hydroxy 2-(di-n-propilamino) tertaralin (8-OH-DPAT) improved consolidation of a conditioned response when injected after training [20]. Another study reported

improvement in cognitive performance of rats and patients suffering from Alzheimer's disease by the 5HT-1A receptor agonist [21]. On the other hand, increase in serotonergic transmission was found to interfere with learning in acquisition and memory consolidation. Similar results were obtained for methanolic extract of *C. ternatea*, a reputed Indian nootropic plant [6]. In the present investigation, serotonin concentration was found to be increased with PB (200 and 400 mg/kg) showing good agreement with behavioral observations as well as our previous report [8]. On the contrary, AS showed inverse reduction with respect to dose. This does not show correlation with behavioral results, wherein 400 mg/kg was found to be more effective dose. These findings suggest the role of serotonergic transmission in the action of PB and not that of AS.

It is well known that, amphetamine, which markedly augments 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 rat brain NA. [6]. The peripheral as well as central administration of NA was also found to suppress avoidance behavior in laboratory animals [4]. These reports implicate the inverse role of noradrenergic transmission in the learning and memory. The reduced levels of noradrenaline with PB and AS support aforementioned previous reports. This is further strengthened by prevention of electroshock induced amnesia by the PB [15], amphetamine antagonism and inability of the extracts to reverse clonidine induced hypothermia by the PB and AS [8]. Thus it is concluded from the study that extract of *Piper betel* reduces noradrenergic transmission and facilitates serotonergic transmission, while extract of *Argyrea speciosa* inhibits the noradrenergic and dopaminergic transmission.

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