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Effect of Aqueous *Piper betle* Leaf Extract against Scopolamine Induced Amnesia on Albino Rats

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ABSTRACT

The current study deals with effect of aqueous extract of *Piper betel* leaves for testing of memory enhancing activity on scopolamine induced amnesia in rats. Nootropics are the smart drugs that gained importance in treating dementia, a major disorder in aged due to stress and also in Alzheimer disease. Cholinesterase inhibitors like, Rivastigmine and Donepezil Nootropics drugs like, Piracetam are commonly used for improving memory, mood and behaviour but their adverse effects have made their use limited. To treat memory disorders, in recent years, new therapeutic agents, such as the steady impairment of memory in aging or in neurodegenerative pathology and even lifestyle factors. In the treatment of various cognitive disorders, an effective therapy for the resolution of the cognitive deficits is still needed and to discover the utility of traditional medicines is worthwhile. *Piper betel* leaves appears as glossy and heart shaped, having a tremendous potential as a potent source for novel herbal drugs. It contains active constituents mainly allylpyrocatechol, Chavibetol, Eugenol and Hydroxychavicol; which has many therapeutic uses and supports the folklore. Because of the presence of wide range of flavonoids in the leaves of *Piper betel*, the aqueous extract of the leaves was tested for the study of memory enhancing effect, against scopolamine induced amnesia in rats.

KEY WORDS: *Piper betel*, Nootropics activity, Y-maze.

1. INTRODUCTON

Learning is a progression which involves acquiring knowledge about the world whereas memory is the consequence of learning. From transient experiences, it reflects the enduring changes in the nervous system that are resulted in the brain of the individual. Simply, memory is an individual ability to store, encode, preserve and successively evoke past experiences and information. From the previous experiences, it provides an individual the capacity and ability to learn and makes them more adaptable (Vivek, 2014; Srithi Srinath, 2014). It provides the power to recall such previously learned facts like skills or habits. According to the WHO 2001, approximately 460 million people are suffering from either a mental or behavioural disorder, but only few of them are receiving even the basic treatment (Hafsa Ahmad, 2016; Rajat Ghosh, 2014). This accounts to 12.4% of the worldwide problem of this disease and by 2020 it is expected to rise to 15%. Age, stress, emotions are conditions that may lead to amnesia, anxiety, memory loss, dementia, high blood pressure and further posing threats like Alzheimer's diseases and schizophrenia (Chopra, 1956; Tijani 2012; 2013; Krishna Mohan, 2014; Yusuf Sadiq, 2009).

Drugs acting on the central nervous system are still among the most widely used group of pharmacological agents and were the first to be revealed by aboriginal man. Therapeutically the value of CNS drugs is immeasurable, and can produce specific psychological and physiological effects. Till now, so many plants have been stated from the indigenous system to have activity against CNS disorders and thus for alleviating human suffering they act as worthwhile remedies. One such herb is piper betel. *Piper betle* L. has been use in Indian, Chinese traditional medicine for eras. It is known as Pan belonging to the family Piperaceae. It is widely grown in India, Taiwan, Sri Lanka, Thailand and other South-east Asia countries. *Piper betle* is a rich source of flavonoids and terpenoids which are responsible for various activities of P*iper betel* (Ma, 2007; Masaya Miwa, 2011; Hasafa Ahmed, 2012; Abhishek pal, 2010; Huaying Sun, 2011; Barbara Thompson, 2006).

2. MATERIALS AND METHODS

Plant material: *Piper betle* leaves were collected from a field located in GUDIVADA. The leaves of *Piper betle* were authenticated by Dr.P. Satyanarayana Raju, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar Guntur, Andhra Pradesh.

Preparation of Aqueous Extract of *Piper betle* Linn: After collection, to remove dust particles and adherent sand the leaves were cleaned. It was dried under shade and to obtain a powder of preferred particle size leaves are treated to size reduction. In the ratio of 1:3 the dried powder and the water was taken and it is treated to aqueous extraction. In a Clevenger apparatus Extraction was done for 3-4 hrs at (60 – 70^{0}C). After the effective extraction, the solvent were distilled off and the extract was then concentrated on a hot plate by evaporating the solvent. Over calcium carbonate this concentrate was desiccated and kept in the room temperature.

Animals: From Mahaveer Enterprises, Male Wistar rats (120-150g body weight) were obtained. Animals were maintained at room temperature and housed five to a cage under a 12 h dark /light cycle with free accessibility to water, food and maintained under laboratory conditions of relative humidity (14±1%), temperature (22 ± 1°C) and

12 h light and dark cycle. Experiments were held between 9:00 and 15:00 h and as adopted by the CPCSEA in accordance with the Guide for the Care and Use of Laboratory Animals. The experimental protocol including treatment was accepted by Institutional Animal Ethical Committee IAEC with approval no: 003/IAEC/VPC/2015. Before behavioural studies they are randomly assigned with 6 rats per group to 6 main experimental groups and were familiarized to the laboratory conditions for 7 days.

Chemicals and drugs: Scopolamine (Mankind pharmaceuticals Ltd.), Piracetam (Ampus life sciencs Ltd).

Behavioural Apparatus: To evaluate acquisition of spatial memory in rats the Y-maze served as the exteroceptive model. The apparatus was built of plain wood and consists of three identical arms. The arms were arbitrarily designated as start arm, in which the rat tends to explore, novel arm; which was open during the second trial but, blocked during the first trial, and the other arm was always open. Each arm was 35 cm width x 6 cm height x 15 cm length. The maze consists of equilateral triangular centre, each arm and each side of the Y beginning from of the triangle and at an angle of 120° extending radially away from the centre, forming the letter Y shape of the maze. When introduced into the maze, it was important that the three arms be made alike to prevent preference on the part of the animal. In order to eliminate olfactory stimuli saw dust was spread all over the floor of the maze. This sawdust was mixed or changed after each trial.

Y-maze Test: To assess spatial recognition memory the Y-maze test consisted of two trials separated by an inter-trial interval. With the third arm being blocked the first trial had 10 min duration and allowed the mouse to explore only two arms of the mazes. After 1 h of ITI the second trial was conducted, during which all three arms were accessible and by comparing behaviour in all three arms novelty vs. familiarity was analysed. For the second trial, in the same start arm the mouse was placed back in the maze, to all three arms for 5 min with free accessibility. All trials were recorded on a VCR by using a ceiling-mounted CCD camera. Later Video recordings were analysed and the time spent in each arm and number of entries were analysed. Data was expressed as percentage of total time spent in arms during the total 5 min.

Experimental Procedures: The animals were treated with various test and standard doses for thirty minutes before the first trial. These include control (vehicle) (Group I), scopolamine (Group II), and scopolamine with aqueous *Piper betel* extract (200 mg/kg) (Group III). Scopolamine with aqueous piper betel extract (400 mg/kg) (Group IV), Piracetam 200 mg/kg (Group V). For the first trial, the novel arm was blocked with a block while the rats were placed inside the start arm. Therefore, rats were capable to explore the start and other arms, but not the novel arm through 20min period. After the first trial of 1 h, Memory retrieval was carried out and evaluated in a test session. For this trial, with free access to all three arms for 20min, trained animals were placed back in the maze in the same starting arm. TL was recorded for each animal in each trial and expressed as inflexion ratio (IR). IR was calculated by the formula; Inflexion ratio= $(L_1-L_0)/L_0$

Where, L_1 is the initial TL (sec) and L_0 is the TL (sec) after 24 h.

In order to measure spatial recognition memory, the time spent in each arm and number of entries of the maze by each rat was recorded and the behaviour of each rat in all three arms was compared by from which novelty versus familiarity was analysed. The number of arms visited was taken as an indicator of exploratory and locomotor activity.

Spontaneous Alteration: To measure the spatial working memory through the spontaneous alternation behaviour Y-maze task is used. It is made of black painted wood. Each arm is 13 cm high, 40 cm long, 3 cm wide at the bottom, 10 cm wide at the converges and top at an equal angle. Through the maze each mouse is allowed to move freely and placed at the end of one arm. Ratentering each arm in turn tend to explore the maze systematically. The ability to alternate requires that the rat to have knowledge of already visited arm. The series of arm entries, including possible returns into the same arm are recorded visually. In Y-maze immediate working memory performance was assessed by recording spontaneous alternation behaviour in a single session. Alternation is defined as the number of successive entries into the three arms (A, B, C) on overlapping triplet sets i.e., ABC, CAB, or BCA but not BAB. Percentage of alternation was calculated as % Alternation = {(No. of alternations) / (Total arm entries– 2)} X 100.

Statistical Analysis: Entire data was expressed as Mean ± SEM and analysed by One-way ANOVA followed by Tukey's multiple comparison test as post hoc test. A 'p' value of <0.001 was measured as statistically noteworthy. Data was analysed by using Graph Pad Prism software.

3. RESULTS AND DISCUSSION

Result of transfer latency in Y-maze by aqueous extract of *Piper betle* leaves: The results for initial transfer latency, retention latency and inflexion ratio (Table.1) showed that the preliminary transmission latency of the animals recorded during training in Y maze was found to be not significant when matched to the control group (Fig.1). Treatment with scopolamine 1 hour after the training exhibited the substantial increase ('c'= P<0.001) in the retention latency of control group when equalled with the normal group (Fig.1).

Treatment of groups IV and V doses of 200 mg/kg body weight and 400 mg/kg body weight with aqueous extract of *Piper betle* leaves after the scopolamine treatment revealed the momentous decrease (***P<0.001) in the retention latency when equalled with the control group (Fig.2).

The inflexion ratio of the control group showed a significance decrease ('b' = P<0.01) and the group treated with 200 mg//kg of AEPBL showed a major decrease at p<0.05, 400 mg/kg at p<0.01 and positive control showed a noteworthy increase at p<0.001 (Fig.3).

Effect on Arm entries (Novelty vs Familiarity) in Y-mazeby aqueous extract of *Piper betle* leaves: The arm entries were noted and analysed to compare novel and familiar arm entries in the current study (Table 2). The familiar arm entries were increased but not significantly when compared the scopolamine treated group with normal group. The familiar arm entries of AEPBL subjected groups unveiled a substantial fall (** P<0.01) against scopolamine subjected control group (Fig.4). Novel arm entries of control group decreased significantly ('a' P<0.05) when compared with normal group. Novel arm entries of the AEPBL treated groups showed a steady rise (*P<0.05) when matched with control group (Fig.5).

Effect on % spontaneous alterations in Y-maze by aqueous extract of *Piper betle* leaves (AEPBL): The spontaneous alterations of control group were significantly lessened ('c' P<0.001) when compared to the normal group as listed in (Table.3). AEPBL treated groups showed a significant rise (***P<0.001) in the % spontaneous alteration when compared with control group (Fig.6).

Effect on duration of time spent in each arm of Y-maze by aqueous extract of *Piper betle* leaves: The extent of time spent in familiar arm and start arm were increased at 'b' P<0.01 significantly and'd' not significant when the scopolamine subjected control group was treated with normal group respectively (Fig.7) and the extent of time spent in novel arm declined significantly ('c' P<0.001) when equalled with normal group (Fig.8). The duration of time spent in start arm by the AEPBL treated groups decreased significantly (***P<0.001) when compared with the control group (Fig.9).The period of time spent in familiar arm by the AEPBL treated groups presented a substantial fall (***P<0.001) when related with control group (Fig.7).The length of time spent in novel arm by AEPBL treated groups increased significantly (***P<0.001) when equalled with normal group (Fig.8).

Table.1. Effect on transfer latency in Y-maze by aqueous extract of *Piper betle* leaves

Group	Treatment	Initial transfer latency	Retention latency	Inflexion Ratio
I	Normal	12.00 ± 0.577	3.00 ± 0.258	3.17 ± 0.405
II	Control (Scopolamine 0.5 mg/kg)	12.33 ± 0.803^{d}	7.50 ± 0.342^{c}	0.67 ± 0.088^{b}
III	Standard (Piracetam 200 mg/kg)	12.50 ± 0.428	3.00 ± 0.516	3.68 ± 0.623
IV	Scopolamine + AEPBL (200 mg/kg)	12.17 ± 0.307^{ns}	3.33 ± 0.333***	2.90 ± 0.460*
V	Scopolamine + AEPBL (400 mg/kg)	12.67 ± 0.421^{ns}	3.17 ± 0.167***	3.05 ± 0.203**
VI	Scopolamine + Piracetam	12.50 ± 0.428^{ns}	3.00 ± 0.365***	3.55 ± 0.602***

Values were expressed as Mean ± SEM; n=6 and are significant when analysed by one way ANOVA with Tukey's post hoc test. ns = not significant, *P<0.05, **P<0.01and ***P<0.001 when compared with Control group. 'b'P<0.01 'c'P<0.001 and'd' = not significant when compared with the Normal group.

Table.2. Effect on Arm entries (Novelty vs Familiarity) in Y-maze by aqueous extract of *Piper betle* leaves

Group	Treatment	Number of Arm entries	
		Novel Arm	Familiar Arm
I	Normal	3.50 ± 0.224	3.17 ±0.307
II	Control (Scopolamine 0.5 mg/kg)	2.00 ± 0.258^{a}	3.67 ±0.211^{d}
III	Standard (Piracetam 200 mg/kg)	4.33 ± 0.422	1.50 ±0.224
IV	Scopolamine + AEPBL (200 mg/kg)	3.33 ± 0.422*	2.33 ±0.211**
V	Scopolamine + AEPBL (400 mg/kg)	3.50 ± 0.224*	2.17 ±0.167**
VI	Scopolamine + Piracetam	3.83 ± 0.167**	2.00 ±0.258***

Values were expressed as Mean ± SEM; n=6 and are significant when analysed by one way ANOVA with Tukey's post hoc test. **P<0.01 and ***P<0.001 when compared with Control group. 'a' = 'd' = not significant when compared with the Normal group

Table.3. Effect on spontaneous alterations by AEPBL

Group	Treatment	% Spontaneous alterations
I	Normal	30.10 ± 0.918
II	Control (Scopolamine 0.5 mg/kg)	16.90 ± 1.242^{c}
III	Standard (Piracetam 200 mg/kg)	32.78 ± 0.954
IV	Scopolamine + AEPBL (200 mg/kg)	29.76 ± 0.809***
V	Scopolamine + AEPBL (400 mg/kg)	31.04 ± 0.758***
VI	Scopolamine + Piracetam	30.85 ± 0.620***

Values were expressed as Mean ± SEM; n=6 and are significant when analysed by one way ANOVA with Tukey's post hoc test. ***P<0.001 when compared with Control group. 'c' P<0.001 when compared with the Normal group.

Table.4. Effect on duration of time spent in each arm of Y -maze by AEPBL

Group	Treatment	Duration of time spent		
		Start	Familiar	Novel
I	Normal	73.00 ± 3.130	86.00±2.745	88.00±0.931
II	Control (Scopolamine 0.5 mg/kg)	91.00 ± 1.862[b]	99.00±0.730[c]	56.00±1.033[c]
IIIs	Standard (Piracetam 200 mg/kg)	40.00±4.442	58.00±2.436	96.00±0.577
IV	Scopolamine + AEPBL (200 mg/kg)	69.00±3.120[***]	64.00±0.730[***]	93.00±1.414[***]
V	Scopolamine + AEPBL (400 mg/kg)	54.00±1.844[***]	61.00±1.713[***]	148.00±1.673[***]
VI	Scopolamine + Piracetam	49.00±1.528[***]	67.00±0.730[***]	92.00±1.390[***]

Values were expressed as Mean ± SEM; n=6 and are significant when analysed by one way ANOVA with Tukey's post hoc test. ***P<0.001 when compared with Control group. 'b'P<0.01 'c' P<0.001 when compared with the Normal group.

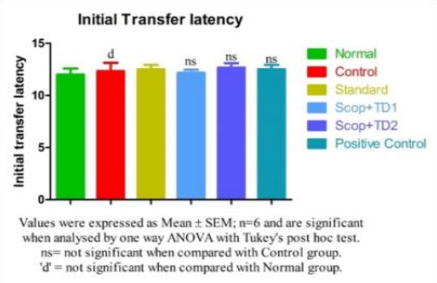

Figure.1. Effect of AEPBL on Intial Transfer Latency

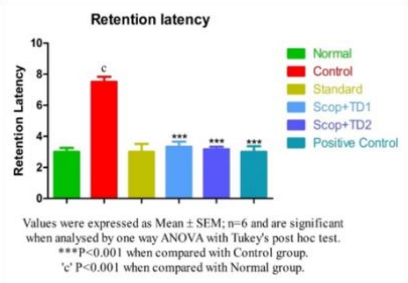

Figure.2. Effect of AEPBL on Retention Latency

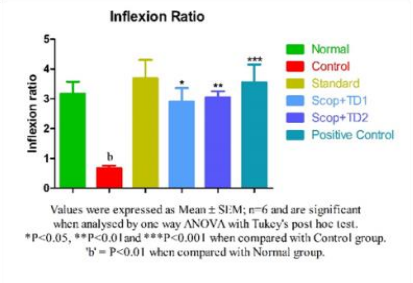

Figure.3. Effect of AEPBL on Inflexion Ratio

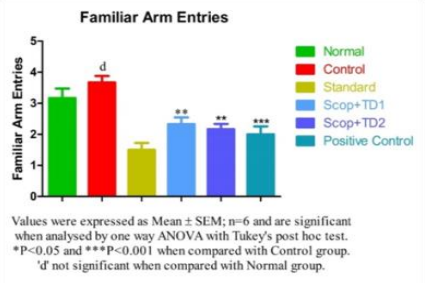

Figure.4. Effect of AEPBL on Familiar arm entries

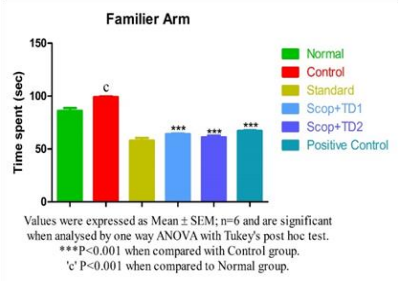

Figure.5. Effect of AEPBL on Novel Arm Entries

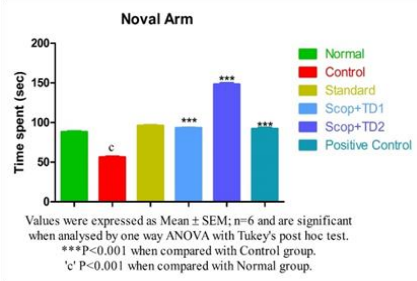

Figure.6. Effect of AEPBL on % Spontaneous Alterations

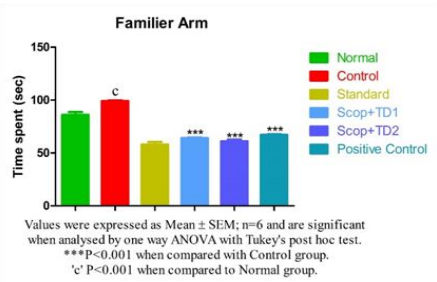

Figure.7. Effect of AEPBL on time spent in familiar arm

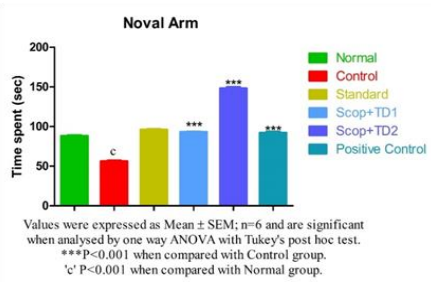

Figure.8. Effect of AEPBL on time spent in noval arm

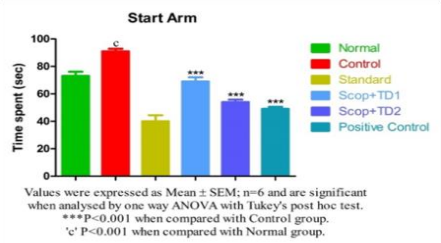

Figure.9. Effect of AEPBL on time spent in start arm

4. CONCLUSION

In the current study, pre-treatment with aqueous extract of *Piper betle* (200mg and 400mg/kg body weight) significantly improved the memory when administered with scopolamine. Among the (200mg/kg and 400mg/kg) doses of *Piper betle*, the dose 400mg/kg of body disclosed significant reversal effect on scopolamine induced amnesia. The aqueous extract of *Piper betle* contains flavonoids and Terpenoids. These compounds were proved to have reversal effect against scopolamine induced amnesia. Based on these earlier reports and results of present study it was concluded that the presence of above compounds in aqueous *Piper betle* extract might be responsible for its reversal role in scopolamine induced amnesia in albino rats.

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