Effect of Piper Betle Leaf Extracts on Enhancement of Memory and Learning in Rodents

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ABSTRACT

BACKGROUND

Medicinal plants have long been used for treating various diseases. *Piper betle*, an evergreen and perennial plant, is valued for its medicinal properties from ancient times. The purpose of this study was to assess the effect of aqueous extract and ethanolic extract of *Piper betle* leaf on animal models of learning and memory and compare it with piracetam.

METHODS

Piper betle leaves aqueous extract (PBAE) and ethanolic extract (PBEE) were administered to the Swiss albino mice at a dose of 100, 200 mg/kg body weight orally for 14 days to evaluate their effect on spatial learning and memory using the Hebb–William's maze (HWM) and elevated plus maze (EPM). The study group included a control (distilled water) and a standard nootropic agent (Piracetam 150 mg/kg).

RESULTS

One-way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test (P = 0.05) were used for comparison of groups and tabulated as mean ± SE. PBEE at a dose of 100 mg/kg and 200 mg/kg has shown significant memory enhancing activity, as indicated by a decrease in the TRC (time taken to reach the reward chamber) and TL (transfer latency) in both the screening tests of learning and memory.

CONCLUSIONS

PBEE possess potent memory enhancing property and can be considered as a potential drug for improving memory in the medical management of dementia, Alzheimer's disease, and neurodegenerative disorders.

KEY WORDS

Piper betle L, Swiss Albino Mice, Hebb–Williams Maze (HWM), Elevated Plus Maze (EPM), Piracetam.

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BACKGROUND

An organism's ability to store and retain the sensory stimuli and recall the information and events as and when required is referred as the memory. Retention of the acquired experience or information could be considered as indicator of memory.[1] Learning is the act of gaining new or altering and boosting long lost existing knowledge, behaviours, skills, morals, or preferences. Memory is one of the most common cognitive ability lost with dementia. Cognition is the set of all mental abilities and processes related to memory, learning, comprehension, evaluation, reasoning and decision making. Human cognition is said to be intuitive and as well as conceptual. Areas of the brain that perform a vital role in learning and memory are hippocampus, amygdala and striatum. Disorders of cognition, therefore, can impact personal, occupational, and social functioning. Elderly are the demographic group most at risk of cognitive disorders. The proportion of the general population with dementia who are aged 60 and over is between 5 - 8 %.[2] One of the common causes of adult-onset dementia is Alzheimer's disease. It is progressive irreversible neurodegenerative disorders and contributes 60 - 70 % of cases. The management includes nootropics like piracetam and inhibitors of cholinesterase (tacrine, donepezil). However, these drugs are not able to contain the progression of the disease. Moreover, the improvement is modest and high morbidity exists. Side effects like abdominal cramps, nausea, vomiting and diarrhoea are dose-limiting. Thus, memory enhancers could be of great significance in the future.[3,4]

To treat psychotropic and behavioural conditions such as anxiety, depression, seizures, poor memory, dementia, insomnia, and drug intoxication, the medicinal plant products are being used in many communities. Some of these natural herbal medicines have been investigated and the benefits derived from using herbal treatments have been proved very promising for disorders like Alzheimer's and dementia.[5] The medicinal plants may therefore provide a new source of beneficial neuropsychotropic drugs when subjected to appropriate tests and deciphering the mechanism of action.[6]

Piper betle Linn. (betel leaf), commonly known as the betel vine is an important medicinal herb to be considered in this context. It belongs to Piperaceae family and is rich in phenols and flavonoids.[7,8] Studies have reported phenolics to be neuroprotective.[9,10] Hence, it would be worthwhile to screen the effect of *Piper betle* leaves by using its aqueous and ethanolic extract on learning and memory enhancement in Swiss albino mice.

METHODS

Experimental Animals

Healthy Swiss albino mice of both sexes, aged between 3 – 4 months, weighing 25 - 30 gms were used in the present study. They were housed under standard conditions and were having free access to food and water. The study was conducted in the Ethnopharmacology laboratory of the Department of Pharmacology, Yenepoya Medical College, Mangalore, from December 2018 to March 2019 after obtaining the approval (1 / 9.6.2016) from the Institutional Animal Ethics Committee (IAEC) clearance.

Drugs

Piracetam (procured from Yenepoya Hospital Pharmacy, Mangalore) was used in the present study.

Plant Material

Fresh betel leaves were purchased from a local market at Kasaragod, Kerala. The collected leaves were authenticated and certified by a botanist from Mangalore University, were used in this study.

Aqueous Extract of Piper Betle Leaves (PBAE)

The leaves were shade dried for two months, then coarse powdered using a mechanical grinder. The Soxhlet extraction method was applied using distilled water (1000 ml) as solvent at 70^{o}C - 80^{o}C for 23 hours. Rota-vapour apparatus was used to concentrate the obtained extract.[11,12] A brownish extract was collected in the paste form.

Ethanolic Extract of Piper Betle Leaves (PBEE)

Extract is prepared from dried powdered betel leaves using 95 % ethanol v/v in Soxhlet apparatus maintained at around 60^{o}C - 70^{o}C for a period of 3 days and concentrated using the Rota-vapour.[11,13] Aromatic greenish extract was obtained.

Acute Toxicity Study of Test Drugs - PBEE and PBAE

The organisation for economic co-operation and development (OECD) guidelines 425 limit test at the dose of 2000 mg/kg was followed to conduct the acute oral toxicity study of *Piper betle* leaf extracts.[14] Accordingly, two sets of 5 female Swiss albino mice fasted overnight were administered with a single dose of 2000 mg/kg of PBEE and PBAE. Each animal was individually observed for 48 hours from the time of drug administration and found that there were no behavioural changes or mortality. Hence the dose was fixed at 100 mg/kg and 200 mg/kg.[15]

Experimental Design

A total of 36 mice were used. Animals were divided into 6 different groups of equal number at (6 animals in each group). The study groups are

1. Control– distilled water (10 ml/kg);
2. PBAE100 mg/kg;
3. PBAE200 mg/kg;
4. PBEE100 mg/kg;
5. PBEE200 mg/kg; and
6. Standard– Piracetam (150 mg/kg[16]. The aforesaid six treatments (i.e., control / extracts / standard) were orally administered for 14 days. Two laboratory models –

1. Hebb–Williams maze, and
2. Elevated plus maze were used for testing the learning and memory in experimental animals.

Hebb–Williams Maze (HWM)

One of the commonly used behavioural models to assess the spatial memory in rodents is Hebb-Williams maze. The HWM consists of an exploratory chamber connected its either side to a start chamber and a reward chamber where the food is kept. These three chambers are provided with guillotine removable doors. One hour after oral administration of drug on 14th day, the mouse was placed in the start chamber and the door was opened so that the animal can enter into the next chamber. Once the animal entered to the exploratory chamber, the door of start chamber is closed to prevent back-entry and time taken by the animal to reach reward chamber (TRC) from the start box was recorded. TRC observed in this training session is considered as learning index of the animal. The animal is then allowed to explore the maze for 3 minutes with all the doors opened and then taken back to its home cage. Retention memory was examined 24 hours after the training session. A significant decrease in TRC (in seconds) time was taken as improvement in memory.[17]

Elevated Plus Maze (EPM)

An exteroceptive behaviour model to assess learning and memory in rodents is the Elevated plus maze. It has a central platform of size 5 x 5 cm extended with two open arms (16 X 5 cm) and two closed arms (16 × 5 × 12 cm). The maze is elevated to a height of 50 cm from the floor. In the training session, the experimental animal is placed at the end of one of the open arms facing away from the central platform and the time taken by it to enter one of the closed arms with all its legs inside is recorded. This time is referred as transfer latency (TL). The mouse was allowed to explore the maze for another 2 minutes before taking back to its home cage. After 24 hours, the experiment is repeated to assess the animal's ability to retention of the learned-task. A significant decrease in transfer latency time was considered as enhancement in memory.[18]

Statistical Analysis

Data was analysed using one-way ANOVA and the statistical comparisons among the groups were performed with Tukey Kramer test with level of significance P = 0.05. For every group, mean ± standard error was provided. Dunnett's pair wise multiple comparison t - tests was used for inferring advantage of treatment groups overcontrol. For data analysis, Statistical Package for Social Sciences (SPSS) v23.0 was used.

RESULTS

Hebb–Williams Maze (HWM)

The TRC (measured in seconds) on the learning day (14th day) in HWM for all groups was observed to be not significantly different (122.2 ± 10.01 to 65.2 ± 26.8) except for the group PBEE 200 (78.0 ± 11.1) as seen from the ANOVA followed by multiple range test. Retention of the learned task that was measured on next day (15th day), subjected to one-way ANOVA revealed significant difference among the groups (P = 0.001). Least time was recorded in standard (25.0 ± 5.38) while maximum in control (107.7 ± 12.30). The TRC on 15th day for the groups PBEE100 and PBEE200 was not significantly different from that of standard (Table 1). Dunnett's pair wise multiple comparison t-test for lower group means than the control indicated significantly lower values for standard (P < 0.01) and for PBEE100 and PBEE200 (P < 0.05). The reduction in TRC (in seconds) in the groups PBEE100 and PBEE200 clearly indicates improvement in memory.

Groups	TRC (s) on 15th day[1]	Test of Significant for Lower Than Control[2]
Control	107.7 ± 12.30^{d}	-
Standard	25.0 ± 5.38^{a}	.006
PBAE100	83.8 ± 21.5^{cd}	.990
PBAE200	71.8 ± 15.9^{bcd}	.687
PBEE100	52.0 ± 3.8^{abc}	.049
PBEE200	40.3 ± 7.63^{ab}	.017

Table 1. Comparison of Test Groups in Hebb–Williams Maze

[1]Homogeneous group means based on Tukey Kramer multiple range test are indicated with common alphabets.
[2]Level of significance of paired comparison with control – single tailed test for group mean < control.

Elevated Plus Maze (EPM)

On 14th day, the TL (measured in seconds) did not show any significant difference among the groups. It varied between 12.5 ± 3.5 (PBEE100) to 21.8 ± 2.8 (control). On the other hand, TL measured on 15th day was significantly different among the groups (P < 0.0005). The average value recorded for the standard was the lowest (4.17 ± 0.7) which is significantly lower to the control (16.0 ± 2.0) (Table 2). Further TL for the two doses was not significantly different for both types of extracts. The TL for standard, PBEE100 and PBEE200 was significantly lower than the control according to single tailed Dunnett's pair wise multiple comparison t-test (Table 2). The result of EPM suggests the memory enhancement effect of PBEE100 and PBEE200.

Groups	TL (s) on 15th day[1]	Test of Significant for Lower than Control[2]
Control	16.0 ± 2.0^{d}	-
Standard	4.17 ± 0.7^{a}	.010
PBAE100	10.7 ± 0.8^{bc}	.254
PBAE200	14.3 ± 1.5^{cd}	.982
PBEE100	6.8 ± 1.6^{ab}	.045
PBEE200	6.3 ± 1.6^{ab}	.035

Table 2. Comparison of Test Groups in Elevated Plus Maze

[1]Homogeneous group means based on Tukey Kramer multiple range test are indicated with common alphabets.
[2]Level of significance of paired comparison with control – single tailed test for group mean < control

DISCUSSION

Currently, the cognitive disorders are a major obstacle which affects the quality of life. A decline in cognitive and neural systems including memory and learning are associated with normal ageing. Other causes of cognitive impairment affecting memory and learning include tumours in brain, cerebrovascular accidents, head injuries, infections of the central nervous system, exposure to neurotoxins (i.e., drugs & substances of abuse), genetic factors, and diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and Huntington's disease.[19]

Nootropics drugs are commonly used to enhance memory (e.g., piracetam, pramiracetam, aniracetam and cholinesterase inhibitors like donepezil). However, the effectiveness of nootropic substances, in utmost cases, has not been decisively determined. Moreover, at present, there are many herbal remedies as substitutes for the treatment of cognitive disorders.

In the present study, *Piper betle* leaf extracts (aqueous and ethanolic) were used to screen learning and memory in normal mice using exteroceptive behaviour models: Hebb–William maze and elevated plus maze. Here, mice treated with graded doses of ethanolic extract of *Piper betle* leaf showed a significant enhancement in cognition in the animal models of learning and memory as revealed by a reduction in TRC and TL compared to control.

Maximum reduction in TRC and TL was observed in the group with piracetam which is significantly higher than the groups with aqueous extracts. Aqueous extract of *Piper betle* leaves was reported to improve learning and memory function in rats and thus suggested to be useful for treating Alzheimer's disease.[20] The result from the present study indicates advantage of ethanolic extract over aqueous extract.

The probable mechanism of memory-enhancing activity of the *Piper betle* could be attributed to the presence of phytochemicals such as flavonoid and phenolic compounds like chavibetol, chavicol and eugenol. Recent reports mentioned about the neuroprotective action of eugenol. Its action is by plummeting the neurotoxicity induced in vitro by amyloid-beta peptides (Aβ) and also by suppressing Aβ-activated enormous influx of calcium ion into neurons that results in neuronal death.[21,22]

Whereas flavonoids have been shown to enhance synaptic plasticity, reverse age-related deficits in spatial memory and increases in hippocampal BDNF [brain - derived neurotrophic factor] protein levels.[23] In an earlier study with aqueous extract of *Piper betle*, it was mentioned that presence of flavonoids and terpenoids might be responsible for its reversal role in scopolamine induced amnesia in albino rats.[24]

However, the use of *Piper betle* in cognitive disorders needs further detailed biochemical and phytochemical investigations concerning the possible active ingredients.

CONCLUSIONS

From this study it can be concluded that the ethanolic extract of *Piper betle* leaf has memory-enhancing properties in the selected doses 100 and 200 mg/kg. This may be due to flavonoid and phenolic compounds. Nonetheless, further studies are needed to know its exact mechanism of action as an effective nootropic agent.

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REFERENCES

[1] Kosuri KC, Ramakrishna A. Beneficial effect of aqueous root extract of Glycyrrhiza glabra on learning and memory using different behavioral models: an experimental study. J Nat Sci Biol Med 2013;4(2):420-5.
[2] World Health Organization. Dementia. Geneva: World Health Organisation, 2019. https://www.who.int/news - room/fact - sheets/detail/dementia.
[3] Gupta SK. Drug screening methods. 3rd edn. New Delhi: Jaypee Brothers Medical Publishers Pvt. Ltd., 2016: p. 460.
[4] Musial A, Bajda M, Malawska B. Recent developments in cholinesterases inhibitors for Alzheimer's diseasetreatment. Curr Med Chem 2007;14(25):2654-79.
[5] Prabhu J, Prabhu K, Chaudhuri A, et al. Neuro-protective effect of Ayurveda formulation, Saraswatharishtam, on scopolamine induced memory impairment in animal model. Pharmacog J 2020;12(3):465-72.
[6] Saraf MK, Prabhakar S, Khanduja KL et al. Bacopamonniera attenuates scopolamine-induced impairment of spatial memory in mice. Evidence Based Complement Alternat Med 2011;2011:236186.
[7] Shah SK, Garg G, Jhade D, et al. Piper betle: phytochemical, pharmacological and nutritional value in health management. Int J Pharm Sci Rev Res 2016;38(2):181-9.
[8] Dwivedi V, Tripathi S. Review study on potential activity of Piper betle. J Pharmacogn Phytochem 2014;3(4):93-8.
[9] Spagnuolo C, Napolitano M, Tedesco I, et al. Neuroprotective role of natural polyphenols. Curr Top Med Chem 2016;16(17):1943-50.
[10] Krishna U, Nayak RP, Chaitra SR. Preclinical evaluation of antidepressant activity of aqueous extract of Piper betle leaves in Swiss albino mice. Int J Basic Clin Pharmacol 2019;8(1):143-6.
[11] Evans WC. Trease & Evan's Pharmacognosy. 16th edn. Edinburg: Saunders Elseveir 2009: p. 121-5.
[12] Sherikar AS, Mahanthesh MC. Evaluation of aqueous and methanolic extract of leaves of epipremnumaureum for radical scavenging activity by DPPH method, total phenolic content, reducing capacity assay and FRAP assay. J Pharmacogn Phytochem 2015;4(4):36-40.
[13] Ganguly S, Mula S, Chattopadhyay S, et al. An ethanol extract of Piper betle Linn. mediates its anti-inflammatory activity via down-regulation of nitric oxide. J Pharm Pharmacol 2007;59(9):711-8.
[14] Organisation for Economic Co-operation and Development. Guidance document on acute oral toxicity testing. Paris: Organisation for Economic Co-operation and development, 2001 [cited 2020 Jun 6]. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2001)4&doclanguage=en
[15] Ghosh MN. Fundamentals of experimental pharmacology. 7th edn. Kolkata: Hilton & Company, 2019: p. 165.
[16] Vyawahare NS, Bodhankar SL. Effect of Argyreiaspeciosa extract on learning and memory paradigms in mice. Pharmacogn Mag 2009;5(17):43-8.
[17] Wangkhem BD, Sengottuvelu S, Haja SS, et al. Memory enhancing activities of Ficus religiosa leaves in rodents. Int J Res Ayurveda Pharm 2011;2(3):834-8.
[18] Alshabi AM, Shaikh IA, Chetan S. Nootropic and neuroprotective effects of ethanol extract of Vateria

indica L bark on scopolamine - induced cognitive deficit in mice. Trop J Pharm Res 2020;19(3):587-94.
[19] Niraj A. A Short Textbook of Psychiatry. 7th edn. New Delhi: Jaypee Brothers Medical Publishers Pvt. Ltd., 2011: p. 1-3.
[20] Upadhyaya S, Gangachannaiah S, Pallavi LC. Effect of piper betel leaf extract in Alzheimer's disease. Biomedical & Pharmacology Journal 2019;12(3):1425-31.
[21] Yoshifumi I. Effects of eugenol on the central nervous system: its possible application to treatment of Alzheimer's disease, depression and Parkinson's disease. Curr Bioact Compd 2006;2(1):57-66.
[22] Taheri P, Yaghmaei P, Tehrani HS, et al. Effects of Eugenol on Alzheimer's disease - like manifestations in insulin and Aβ-induced rat models. Neurophysiology 2019;51:114-9.
[23] Catarina R, David V, Marcus R, et al. Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain - derived neurotrophic factor. PLoS One 2013;8(5):e63535.
[24] Saka VP, Srinivasa BP, Himaja V, et al. Effect of aqueous piper betle leaf extract against scopolamine induced amnesia on albino rats. Journal of Chemical and Pharmaceutical Sciences 2017;10(1):116-20.