ORIGINAL ARTICLE

Memory enhancing effect of various polar and non-polar extracts of *Plumbago zeylanica* Linn. roots

Vaibhav Uplanchiwar[1], M. K. Gupta[1], Rupesh K. Gautam[2]

[1]Department of Pharmacy, Oriental University, Indore – 453 555, Madhya Pradesh, India, [2]Department of Pharmacology, MM School of Pharmacy, Maharishi Markandeshwar University, Sadopur, Ambala – 134 007, Haryana, India

Abstract

Aim: The main aim of our study is to evaluate the memory enhancing effect of various extracts of *Plumbago zeylanica* Linn. roots in suitable animals models. **Materials and Methods:** Roots were extracted by successive solvent methods by petroleum ether, chloroform, methanol, butanol, and finally water. All the extracts were subjected to phytochemical screening for the presence of various active phytoconstituents. All the extracts were evaluated by Morris water test, and brain acetylcholinesterase level was measured. **Result:** The chloroform extract showed the presence of alkaloids. Among all the extracts, chloroform extract significantly decreased the escape latency and increased the time spent in target quadrant. Chloroform extract significantly lowers the level of acetylcholinesterase level as compared to all other extracts. **Conclusion:** Among all the extracts, chloroform extract significantly increased the learning and memory in Morris water test and lowered the brain acetylcholinesterase level.

Key words: Chloroform extract, cholinesterase level, Morris water test, Plumbagin, *Plumbago zeylanica* Linn

INTRODUCTION

Progressive neurodegenerative disorder was characterized by the steady onset of dementia. It is characterized by gradually progressive refuse in cognitive function, with deficits, especially in memory retrieval.[1,2] The primary reason of neurodegenerative disorder appears to be reduction in cholinergic activity.

Nootropic agents such as piracetam,[3] pramiracetam, aniracetam,[4] and cholinesterase inhibitors like donepezilare presently used for humanizing memory, mood, and behavior. However, the resulting adverse effects linked with these agents have limited their use,[5] and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders.

Plumbago zeylanica (Plumbaginaceae) is an old age Rasayana herb in traditional Ayurveda. *Plumbago zeylanica* Linn. is distributed as a weed throughout the tropical and subtropical countries of the world native to south Asia and cultivated throughout India and Sri Lanka.[6] Its roots bark and seed are used in diversity of alignments. Paste made from roots of the plant is useful to the skin to treat abscesses, fever or malaria, rheumatism, and intestinal parasites; anemia due to "stagnant blood," internal and external trauma, toxic swelling, and memory can be treated with this plant.[7]

In Indian system of medicine, various plants are reported to have memory enhancing activity. *Plumbago zeylanica* is one of the well-documented plants has memory enhancing activity. In absence of any scientific report for its memory enhancing activity, our main aim is to evaluate its memory enhancing activity by suitable animal model since there is lack of satisfactory drugs in allopathic system of medicine with fewer side effects.

Address for correspondence:
Vaibhav Uplanchiwar, Department of Pharmacy, Oriental University, Indore–453555, Madhya Pradesh, India.
Phone: +91-7987759722.
E-mail: vaibhavuplanchiwar@gmail.com

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MATERIALS AND METHODS

Plant Material

The roots of *P. zeylanica* purchased from the local market. The roots were taxonomically identified and authenticated by senior botanist. A voucher specimen is preserved in the department for the further reference.

Successive Solvent Extraction Method

The roots (250 g) were dried in shade and sliced into small pieces and pulverized using a mechanical grinder for the coarse powder. The coarse powder of root was subjected to Soxhlet extraction using petroleum ether to remove all fats. The marc was dried and then extracted using chloroform, methanol, butanol, and finally with water for 72 h. After exhaustive extraction, the extracts were filtered, concentrated, and dried. All the extracts were subjected for the preliminary phytochemical screening for the presence of alkaloids, fatty acids, terpenoids, steroids, flavonoids, glycosides, etc.

Experimental Animals

Disease-free Swiss male albino mice, weighing around 25–35 g, were purchased from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved vendor. Animals were housed separately in polycarbonate cage in groups of 6–8 per cage under proper laboratory conditions with alternating light and dark cycle of 12 h each. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India.

Pharmacological Evaluation of Extracts

Morris water maze

The procedure and parameters for testing learning and memory of mice using Morris water maze were followed as reported earlier.[8-10]

Animals were divided into 10 groups and six animals were placed in each group. Group 1 served as control and Group 2 as standard drug (physostigmine, 0.1 mg/kg i.p.) treated. Groups 3–10 were treated by different extracts (chloroform, methanol, butanol, and water) in a dose of 200 and 400 mg/kg, respectively, were administered for 15 successive days. Escape latency (EL) was recorded 120 min after drug administration from 11th day to 14th day. On the 15th day, time spent in target quadrant (TSTQ) was noted 120 min after the drug administration. In case of animals administered with physostigmine, EL and TSTQ were noted after 30 min of drug administration.

Biochemical Estimation

Collection of brain sample

After the 15th day using Morris water maze, the animals were sacrificed on the 16th day by cervical dislocation. Whole brain was carefully removed from the animals. The fresh whole brain was weighed first and then homogenized in 10 volumes of 0.1 M phosphate buffer (pH 8) using a glass homogenizer. The homogenate was centrifuged at 3000 rpm for 10 min at 4°C using refrigerated centrifuge (Remi, Mumbai). The resultant cloudy supernatant liquid was used for the estimation of brain acetylcholinesterase activity.[11]

Estimation of Acetylcholinesterase Activity

About 0.4 ml of brain homogenate was added into a test tube containing 2.6 ml of phosphate buffer. 5,5-dithiobis-2-nitrobenzoic acid reagent (0.1 ml) was added to the above mixture and absorbance was noted at 412 nm. Then, 0.02 ml of acetylcholine iodide solution was added and again absorbance was noted 15 min thereafter. Change in absorbance per minute was calculated.[11]

Statistical Analysis

All the results are expressed as mean ± standard error of the mean. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's *post hoc* test in GraphPad Prism. $P < 0.05$ was considered as statistically significant.

RESULTS

Effect of Different Extracts on EL and TSTQ of Mice using Morris Water Maze

As per results showed in Tables 1 and 2, learning and memory are associated with EL and TSTQ. Decline of EL and augment of TSTQ by mice in Morris water maze indicates improvement of learning and memory and *vice versa*. Different extracts and physostigmine (0.1 mg/kg, *i.p.*) administered for 15 successive days significantly decreased EL of mice from 11th to 14th day and increased TSTQ by mice on the 15th day as compared to the control, thus showed significant improvement of learning and memory. Among all the extracts, chloroform extract showed a highly significant effect on EL and TSTQ. Chloroform extracts significantly decreased EL and significantly increased TSTQ as compared to vehicle-treated control.

Effect of Different Extracts on Brain Acetylcholinesterase Activity of Mice

Administration of different extracts and physostigmine for 15 consecutive days produced a significant decrease in brain

Table 1: Effect of different extract on EL of mice using Morris Water Maze

Treatment schedule	EL (s) day 11	EL (s) day 12	EL (s) day 13	EL (s) day 14
Control	93.22±1.11	94.21±1.13	94.48±1.66	93.12±2.28
Physostigmine, 0.1 mg	92.32±1.21	89.11±1.24*	85.18±1.41**	78.32±2.19***
Chloroform extract, 200 mg/kg	92.41±1.09	88.15±1.21*	83.51±1.16**	78.31±1.58***
Chloroform extract, 400 mg/kg	92.11±1.29	85.25±1.31*	81.30±1.41**	80.52±1.32***
Methanol extract, 200 mg/kg	92.42±1.08	93.41±1.22	94.18±1.47	93.11±1.78
Methanol extract, 400 mg/kg	92.31±1.42	92.51±1.78	94.58±1.90	93.47±1.87
Butanolic extract, 200 mg/kg	93.67±1.57	90.31±1.22	88.28±1.77*	85.32±1.70**
Butanolic extract, 400 mg/kg	92.56±1.35	92.11±1.27	86.32±1.88*	84.18±1.76**
Water extract, 200 mg/kg	94.12±1.34	93.41±1.21	93.28±1.88	93.10±1.28
Water extract, 400 mg/kg	93.46±1.76	93.20±1.83	94.18±1.72	94.13±1.66

Values are expressed as mean±SEM, *n*=6 in each group; **P*<0.05 compared to disease control ***P*<0.01 compared to disease control. ****P*<0.001 compared to disease control. SEM: Standard error of the mean, EL: Escape latency

Table 2: Effect of different extract on TSTQ of Morris Water Maze

Treatment schedule	TS (s) TQ (15th day)
Control	45.42±2.23
Physostigmine, 0.1 mg	98.21±2.15***
Chloroform extract, 200 mg/kg	99.31±2.45***
Chloroform extract, 400 mg/kg	101.45±2.98***
Methanol extract, 200 mg/kg	58.35±1.26*
Methanol extract, 400 mg/kg	60.21±1.78*
Butanolic extract, 200 mg/kg	62.42±2.87**
Butanolic extract, 400 mg/kg	65.36±2.64**
Water extract, 200 mg/kg	63.89±1.74**
Water extract, 400 mg/kg	65.68±1.64**

Values are expressed as mean±SEM, *n*=6 in each group; **P*<0.05 compared to disease control ***P*<0.01 compared to disease control. ****P*<0.001 compared to disease control. TSTQ: Time spent in target quadrant, SEM: Standard error of the mean

Table 3: Effect of different extracts on brain acetylcholinesterase activity of mice

Treatment schedule	Acetylcholinesterase activity (mol/l/min×10–6/g of tissue)
Control	0.061±0.010
Physostigmine, 0.1 mg	0.019±0.012***
Chloroform extract, 200 mg/kg	0.021±0.045***
Methanol extract, 200 mg/kg	0.050±0.026*
Butanolic extract, 200 mg/kg	0.045±0.087**
Water extract, 200 mg/kg	0.041±0.074**

Values are expressed as mean±SEM, *n*=6 in each group; **P*<0.05 compared to disease control ***P*<0.01 compared to disease control. ****P*<0.001 compared to disease control. SEM: Standard error of the mean

acetylcholinesterase activity as compared to control group. Chloroform extract showed a highly significant decreasing effect on brain acetylcholinesterase activity compared to all other extracts. Results were expressed in Table 3.

DISCUSSION

In the present study, memory enhancing activity of *P. zeylanica* roots in suitable animal model. Various extracts were prepared by successive solvent extraction methods and solvents were selected on the basis of polarity. Preliminary phytochemical studies showed the presence of alkaloids, steroids, flavonoids, and glycosides in various extracts. All the extracts in a dose of 200 and 400 mg/kg were administered for 15 successive days significantly improved learning and memory of mice. Memory improving effects of extracts were comparable to physostigmine. Morris water maze was employed as a behavioral model for evaluation of learning and memory. Chloroform extract significantly decreased EL during training and it significantly increased TSTQ during retrieval, indicating improvement of learning and memory.

Preliminary phytochemical study showed the presence of alkaloids in chloroform extract and in previous literature survey Plumbagin; well-known alkaloids were present in chloroform extract. Hence, the presence of Plumbagin was employed for elucidating its probable mechanisms of memory improving activity for chloroform extract.

Acetylcholine is measured as the most important neurotransmitter involved in the regulation of cognitive functions.[12] Selective defeat of cholinergic neurons or decreased synthesis of acetylcholine was reported to be an attribute feature of neurodegenerative disorder.[13] Drugs that increase the overall quantity of acetylcholine was considered as memory enhancing drug.[14] In the present

study, chloroform extracts significantly impaired memory of mice in Morris water maze test. As we have previously discussed that Plumbagin which is main active constituents in chloroform extract may be responsible for the memory enhancing effect due to inhibition of acetylcholinesterase, leading to increase in brain acetylcholine levels. Cognitive dysfunction has been shown to be linked with impaired cholinergic transmission and the facilitation of central cholinergic transmission resulting in enhanced memory. The deterioration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of Alzheimer's disease.[13,15,16] Thus, the drugs which enhance cholinergic function can be used for the treatment of dementia intimately related to Alzheimer's disease.

CONCLUSION

Plumbagin is the main active constituents in chloroform extract and may be responsible for improved learning and memory of mice probably through inhibition of brain acetylcholinesterase activity. Further, detailed studies are required on bioactivity-guided isolation and characterization of active constituent and explore the other possible mechanisms for nootropic activity of Plumbagin and its worth in the treatment of cognitive and neurodegenerative disorders.

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