



International Journal of Medical Sciences and Pharma Research

Open Access to Medical and Research

Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited



Open Access

Research Article

Nootropic and Free Radical Scavenging Activity of *Plumbago zeylanica* Linn. By Different In-Vivo & In-Vitro Methods

Sakina Akbarali Chandanwala*, Neetesh Kumar Jain, Nitu Singh

Department of Pharmacology, Faculty of Pharmacy, Oriental University Indore-India

Article Info:

Article History:

Received 02 April 2022
Reviewed 29 May 2022
Accepted 05 June 2022
Published 15 June 2022

Cite this article as:

Chandanwala SA, Jain NL, Singh N, Nootropic and Free Radical Scavenging Activity of *Plumbago zeylanica* Linn. By Different In-Vivo & In-Vitro Methods, International Journal of Medical Sciences & Pharma Research, 2022; 8(2):12-18

DOI: <http://dx.doi.org/10.22270/ijmspr.v8i2.31>

*Address for Correspondence:

Sakina Akbarali Chandanwala, Department of Pharmacology, Faculty of Pharmacy, Oriental University Indore-India

Abstract

AIM- The aim of the present investigation is to evaluate *Plumbago zeylanica* L. (Plumbaginaceae) for evaluation of memory enhancing activity. **MATERIAL & METHODS-** All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of roots of *Plumbago zeylanica* Linn. extracted using petroleum ether, chloroform, methanol, butanol and finally water by soxhlet apparatus for 72 h. Qualitative chemical tests of chloroform, methanol, butanol and water extracts were subjected to various chemical tests to detect various Phytoconstituents. Briefly, 6.0 mg β -carotene was dissolved in 10 ml of chloroform, and then 1 ml of solution pipette to glass filled of 20 mg linoleic acid. 5 ml of mixture then pipette to reaction tube filled of extract in a range of concentration, mixed homogenously. Briefly, 1 mL of 0.3 mM of DPPH solution was added to 1 mL each of the test solutions, and was incubated in the dark at room temperature for 30 min. Escape latency (EL) was recorded 120 min after drug administration from 11th day to 14th day. On 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 was noted after 30 min of drug administration. 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. **RESULTS-** The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 70% inhibition at 30 minutes which was reduced to 45% at the time of 120 minutes. Hydroxyl radical scavenging ability calculated as IC_{50} reveals that chloroform; methanol, butanol and water extracts have IC_{50} values of 47 μ g/mL, 100 μ g/mL, 200 μ g/mL and 150 μ g/mL, respectively. Among all the extracts, chloroform extract showed a highly significant effect on EL and TSTQ. Chloroform extracts significantly decreased ($P<0.001$) EL and significantly increased TSTQ as compared to vehicle treated control. **CONCLUSION-** The deterioration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of Alzheimer's disease.

Keywords: Alzheimer's disease, Vitamin E, DPPH solution, *Plumbago zeylanica* Linn., Plumbaginaceae, EL and TSTQ

Email: akbaralisakeena@gmail.com

INTRODUCTION

The mean age at commencement of AD is around 75 years of age and the overall occurrence is about 1% in most urbanized nations. If AD prevalence is divided into dissimilar age bands then we see a rapid augment from 1% in the 60-64 year age group to greater than 25% in individuals over 85 years of age. The National Institute of Health predicts, if the in progress trend continues, there will be more than 8.5 million AD patients by the year 2013 in USA alone (Joshi & Parle, 2006).

Formation of memory is the most complex procedure and involves multiple neuronal pathways and neurotransmitters. It is well recognized that the cholinergic neuronal system plays an important responsibility in learning and memory in humans and animals. This is the remedial rational at the back the use of nootropic agents such as Piracetam, various Piracetam analogues like oxiracetam, aniracetam, and metrifonate. However, the adverse effects connected with these agents have limited their use (Kriti et al., 2010).

Nootropic are agents that augment the cognitive skills, and amnestics are agents that disrupt the learning and memory process (Kshirsagar, 2011). Nootropic agents such as Piracetam, Aniracetam, and cholinesterase inhibitors like Donepezil are presently used for humanizing memory, mood and behavior (Joshi & Parle, 2006). The cholinergic neural system theater an important role in learning and memory in humans and animals.

The *Plumbago zeylanica* L. roots (Plumbaginaceae) is included in "Rasayana"- an Ayurvedic ancient system of medicine for rejuvenation. It also contains Plumbagin as active constituent which is an alkaloid which may act as nervine tonic. Hence, by keeping in view, *Plumbago zeylanica* L. (Plumbaginaceae) are selected for evaluation of memory enhancing activity.

MATERIAL & METHODS

Procurement of Plant Materials & Authentication

The roots of *Plumbago zeylanica* Linn. were purchased from the local market. The roots were taxonomically identified by Dr. Gynendra Tiwari, Senior Scientist at Horticulture College. The herbarium sheets were submitted in Department of Pharmacognosy, under voucher specimen.

Preparation of Extract by Successive Solvent Method

All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of roots of *Plumbago zeylanica* Linn. extracted using petroleum ether, chloroform, methanol, butanol and finally water by soxhlet apparatus for 72 h. The extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined (Kumar *et al.*, 2009).

Preliminary Phytochemical Tests

Qualitative chemical tests of chloroform, methanol, butanol and water extracts were subjected to various chemical tests to detect various Phytoconstituents (Kokate, 2003; Khandelwal, 2006).

Evaluation of Antioxidant Activity of Different Extracts

β-carotene inhibition method

This assay is based on the capacity of β-carotene oxidative bleaching in β-caroten/linoleic acid mixture with and without the addition of different extract of both plants, the method described by Kikuchi and Kitamura (1987) with a slight modification. Briefly, 6.0 mg β-carotene was dissolved in 10 ml of chloroform, and then 1 ml of solution pipette to glass filled of 20 mg linoleic acid. 5 ml of mixture then pipette to reaction tube filled of extract in a range of concentration, mixed homogenously. Sample absorptions were conducted before and after incubation at 50°C for 30, 60, and 120 minutes.

β-carotene bleaching inhibition percentage was calculated by the following formula:

$$\% \text{ Inhibition} = [1 - (AA(120) - AC(120)) / (AC(0) - AC(120))] \times 100$$

AA(120): sample absorbance at t = 30, 60 or 120 minute

AC(120): control absorbance at t = 30, 60 or 120 minute

AC(0): control absorbance at t = 0 min

Hydroxyl radical (OH•) scavenging activities

One mL of the reaction mixture contained 100 μL of 2.8 mM 2-deoxyribose (dissolved in phosphate buffer (10 mM), pH 7.4), 500 μL solution of various concentrations of the extract (500n1000 μg/mL), 200 μL of 200 μM FeCl₃ and 1.04 μM EDTA (1:1 v/v), 100 μL of H₂O₂ (1.0 mM) and 100 μL of ascorbic acid (1.0 mM). After incubation time of 1 hour at 37°C, the amount of deoxyribose degradation was measured by TBA reaction (Badmus *et al.*, 2011; Halliwell *et al.*, 1987).

The % inhibition of hydroxyl radical was calculated by using following formula.

$$\% \text{ Inhibition} = \frac{(100 - A_{\text{Sample}})}{A_{\text{Control}}} \times 100$$

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity

When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The modification in color from

deep violet to golden/light yellow can be measured at 518 nm. Briefly, 1 mL of 0.3 mM of DPPH solution was added to 1 mL each of the test solutions, and was incubated in the dark at room temperature for 30 min (Badmus *et al.*, 2011). The absorbance values were read at 518 nm, and converted into percentage antioxidant activity, using the below mentioned formula:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Preliminary In-Vivo Evaluation of different extracts

Selection of animals

Wistar albino mice of either sex between 1 and 2 months of age weighing 25-35 g were used which were procured from the central animal house, India. The animals were allowed free to access commercial rat pallet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum*. All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments. The study designs were approved by the Institutional Animal Ethical Committee.

Acute toxicity studies

The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 423.

One tenth and one fifth of the lethal dose was taken as effective dose (therapeutic dose) and cut off value was selected as 200 and 400 mg/kg to evaluate the dose dependent action for the evaluation (OECD guidelines, 2001).

Evaluation of Memory Enhancing Activity by Different Models

Morris Water test

The procedure and parameters for testing learning and memory of mice using Morris water maze were followed as reported earlier (Domange *et al.*, 2013; Parle & Singh, 2007).

Animals were divided in ten groups and six animals were placed in each group. Groups 1 served as control and group 2 as standard drug (physostigmine, 0.1 mg/kg *i.p.*) treated. Group 3 to 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 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 was noted after 30 min of drug administration.

The treatment schedule was as follows-

Group-1 Served as control

Group-2 As standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3 Animals were treated by chloroform extract of *P.zeylanica* in dose of 200 mg/kg

Group-4 Animals were treated by chloroform extract of *P.zeylanica* in dose of 400 mg/kg

Group-5 Animals were treated by methanolic extract of *P.zeylanica* in dose of 200 mg/kg

Group-6 Animals were treated by methanolic extract of *P.zeylanica* in dose of 400 mg/kg

Group-7 Animals were treated by butanolic extract of *P.zeylanica* in dose of 200 mg/kg

Group-8 Animals were treated by butanolic extract of *P.zeylanica* in dose of 400 mg/kg

Group-9 Animals were treated by water extract of *P.zeylanica* in dose of 200 mg/kg

Group-10 Animals were treated by water extract of *P.zeylanica* in dose of 400 mg/kg

The procedure and parameters for testing learning and memory of mice using Morris water maze were followed as reported earlier (Domange *et al.*, 2013; Morris, 1984; Parle & Singh, 2007).

Elevated Plus-Maze Test

To assess plus maze test, the mouse was individually positioned on the central platform facing towards open arm. The percentage of time spent (duration) in open arms and frequency of open arm entries were counted for a period of 5 min. All precaution was taken to ensure that no external stimuli, other than the height of plus maze could invoke anxiety in the animals. The percentage of time spent in the open arms and number of open arm entries were calculated using the formulas $[100 \times \text{open} / (\text{open} + \text{enclosed})]$ and $(100 \times \text{open} / \text{total entries})$, respectively.

After 1 h of oral administration of vehicle, diazepam and different extracts of *P. zeylanica* (chloroform, methanol, butanol and water) were assessed for memory enhances or behavior studies using elevated plus-maze test. Test extracts of both plants were used in a dose of 200 & 400 mg/kg.

Biochemical Estimation

Collection of Brain Sample

After 15th day using Morris water maze, the animals were sacrificed on 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 acetyl cholinesterase activity (Ellman *et al.*, 1961).

Estimation of Acetyl cholinesterase Activity

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 (Ellman *et al.*, 1961).

Statistical Analysis

The values are expressed in mean $\pm$ SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p < 0.05$ was chosen as the level of significance.

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

Antioxidant Activity of Different Extracts of *Plumbago zeylanica* Linn.

β -carotene inhibition method

In this activity, we found that chloroform extract among all extracts showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 70% inhibition at 30 minutes which was reduced to 45% at the time of 120 minutes.

Table 1: Effect of different extracts on β -carotene inhibition method

S. No.	Extracts	Concentration	Time		
			30 Min	60 Min	120 Min
1	Chloroform Extracts	20 $\mu\text{g/ml}$	52.33 $\pm$ 4.49	38.94 $\pm$ 3.33	29.33 $\pm$ 3.33
		40 $\mu\text{g/ml}$	60.14 $\pm$ 3.55	49.33 $\pm$ 3.55	30.91 $\pm$ 3.55
		60 $\mu\text{g/ml}$	65.22 $\pm$ 3.49	50.39 $\pm$ 2.56	42.33 $\pm$ 3.11
		80 $\mu\text{g/ml}$	70.10 $\pm$ 1.22	59.31 $\pm$ 1.44	45.21 $\pm$ 3.33
2	Methanolic Extracts	20 $\mu\text{g/ml}$	40.11 $\pm$ 2.33	18.22 $\pm$ 3.13	16.22 $\pm$ 3.71
		40 $\mu\text{g/ml}$	42.33 $\pm$ 3.23	20.54 $\pm$ 3.71	18.33 $\pm$ 3.31
		60 $\mu\text{g/ml}$	45.77 $\pm$ 3.33	22.35 $\pm$ 2.44	21.33 $\pm$ 3.48
		80 $\mu\text{g/ml}$	49.30 $\pm$ 1.13	25.31 $\pm$ 3.36	22.25 $\pm$ 3.68
3	Butanolic Extracts	20 $\mu\text{g/ml}$	33.13 $\pm$ 2.44	15.22 $\pm$ 3.12	12.23 $\pm$ 3.44
		40 $\mu\text{g/ml}$	35.22 $\pm$ 3.35	18.33 $\pm$ 3.33	14.11 $\pm$ 3.55
		60 $\mu\text{g/ml}$	38.71 $\pm$ 3.45	20.25 $\pm$ 2.66	18.32 $\pm$ 2.48
		80 $\mu\text{g/ml}$	40.41 $\pm$ 2.13	22.44 $\pm$ 3.66	21.27 $\pm$ 2.77
4	Water Extracts	20 $\mu\text{g/ml}$	30.22 $\pm$ 2.33	15.33 $\pm$ 3.33	13.43 $\pm$ 3.55
		40 $\mu\text{g/ml}$	32.44 $\pm$ 3.44	16.77 $\pm$ 3.88	15.66 $\pm$ 2.98
		60 $\mu\text{g/ml}$	35.44 $\pm$ 3.55	19.23 $\pm$ 3.77	17.32 $\pm$ 3.55
		80 $\mu\text{g/ml}$	38.43 $\pm$ 2.44	21.58 $\pm$ 3.44	20.64 $\pm$ 3.68
5	Vitamin E	4 $\mu\text{g/ml}$	30.34 $\pm$ 2.10	33.45 $\pm$ 2.22	15.45 $\pm$ 2.21
		6 $\mu\text{g/ml}$	37.88 $\pm$ 2.56	32.86 $\pm$ 3.95	20.45 $\pm$ 2.33
		8 $\mu\text{g/ml}$	69.113 $\pm$ 2.35	58.21 $\pm$ 2.22	40.26 $\pm$ 3.63
		10 $\mu\text{g/ml}$	84.21 $\pm$ 3.11	73.21 $\pm$ 3.55	60.22 $\pm$ 3.44

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging ability calculated as IC₅₀ reveals that chloroform; methanol, butanol and water extracts have

IC₅₀ values of 47 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, respectively. Since chloroform extract had very lowest IC₅₀ as compared to other extracts so it was considered as best extract for the further evaluation.

Table 2: Effect of different extracts on Hydroxyl radical scavenging activity

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	50	53.78±2.33	48.22±2.44	40.22±2.44	48.38±2.34
2.	100	57.21±3.21	50.24±3.34	42.21±3.56	49.11±3.11
3.	150	65.78±3.45	55.44±3.78	46.28±3.31	50.78±3.51
4.	200	74.34±3.66	58.34±3.99	50.56±3.55	53.21±3.87
5.	250	82.76±3.55	62.31±3.51	53.76±3.48	60.99±3.53

Values are expressed as the mean of triplicate

DPPH scavenging activity

DPPH scavenging ability calculated as IC₅₀ from Tables shows that chloroform extract has IC₅₀ of 36 µg/mL followed by

ascorbic acid (3.2 µg/mL), gallic acid (3.5µg/mL), methanol (40µg/mL), butanolic (47µg/mL) and water (45µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability.

Table 3: Effect of different extracts on Percentage DPPH scavenging activities

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	10	28.23±2.11	22.55±2.12	25.01±2.42	26.33±2.31
2.	20	33.11±3.32	26.26±3.15	28.11±3.22	29.11±2.14
3.	30	41.78±3.45	36.14±3.78	32.28±3.11	33.12±2.51
4.	40	55.37±3.55	50.44±3.33	42.56±3.24	45.31±3.44
5.	50	62.76±3.12	52.31±2.11	53.11±3.22	54.17±2.66

Values are expressed as the mean of triplicate

Table 4: Percentage (%) DPPH radical scavenging effect (standard)

S. No.	Concentration (µg/mL)	Vitamin C (%)	Gallic Acid (%)
1.	2	47.98±2.33	43.66±3.56
2.	3	49.22±3.67	48.77±3.77
3.	4	62.33±3.28	55.22±3.11
4.	5	70.38±3.78	60.78±3.59

Pharmacological Evaluation of Different Extracts for Memory Enhancing Activity**Morris Water Test For Different Extracts of *Plumbago zeylanica* Linn.**

Different extracts and Physostigmine (0.1mg/ kg, i.p.) administered for 15 successive days significantly decreased EL

of mice from 11th to 14th day and increased TSTQ by mice on 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 (P<0.001) EL and significantly increased TSTQ as compared to vehicle treated control.

Table 5: Effect of different extract on escape latency (EL) of mice using Morris Water Maze

Treatment Schedule	Escape Latency (Sec) Day 11	Escape Latency (Sec) Day 12	Escape Latency (Sec) Day 13	Escape Latency (Sec) 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

Table No 6: Effect of different extract on time spent in target quadrant (TSTQ) of Morris Water Maze

Treatment Schedule	Time spent (sec) in target quadrant (15 th 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

Table No 7: Effect of different extracts on brain Acetyl cholinesterase activity of mice

Treatment Schedule	Acetyl cholinesterase activity (mol/l per min × 10-6/g of tissue)
Control	0.061 ± 0.010
Physostigmine, 0.1 mg	0.019±0.002***
Chloroform Extract, 200 mg/kg	0.021±0.005***
Chloroform Extract, 400 mg/kg	0.020±0.008***
Methanol Extract, 200 mg/kg	0.050±0.006*
Methanol Extract, 400 mg/kg	0.046±0.006*
Butanolic Extract, 200 mg/kg	0.045±0.007**
Butanolic Extract, 400 mg/kg	0.042±0.005**
Water Extract, 200 mg/kg	0.041±0.004**
Water Extract, 400 mg/kg	0.038±0.002**

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

Elevated Plus Maze Test for *Plumbago zeylanica* Linn.

Oral administration of chloroform extract of *P. zeylanica* in 200 and 400 mg/kg, respectively exhibited significant ($P <$

0.01) increase in the percentage of number of open arm entries and time spent in open arm whereas, in the closed arm number of entries and time spent was significantly ($P < 0.01$) reduced as compared to vehicle-treated group.

Table No 8: Effect of different extracts on open and closed entries

S. No.	Treatments	No of Entries		Time Spent (Sec)	
		Open Arm	Closed Arm	Open Arm	Closed Arm
	Vehicle	6.2 ± 1.2	22.2 ± 2.4	25.7 ± 3.8	198.3 ± 4.8
	Diazepam	12.5 ± 1.1***	14.2 ± 1.3***	37.7 ± 2.7**	140.2 ± 4.6
	Chloroform extract, 200 mg/kg	11.3 ± 1.4**	10.2 ± 1.8**	44.2 ± 3.8**	132.7 ± 3.6**
	Chloroform extract, 400 mg/kg	11.6 ± 1.6 **	11.3 ± 1.6**	50.2 ± 3.1**	148.5 ± 3.9**
	Methanolic extract, 200 mg/kg	8.4 ± 1.1 *	11.5 ± 1.8**	30.3 ± 3.8*	111.6 ± 4.2**
	Methanolic extract, 400 mg/kg	9.2 ± 1.3 *	11.6 ± 1.1**	35.2 ± 3.4 *	118.7 ± 4.6**
	Butanolic extract, 200 mg/kg	8.5 ± 1.2 *	11.2 ± 1.4**	44.3 ± 3.4**	126.5 ± 3.6**
	Butanolic extract, 400 mg/kg	9.2 ± 1.2 *	11.4 ± 1.2**	47.9 ± 3.3 **	130.3 ± 5.9**
	Water extract, 200 mg/kg	10.9 ± 1.7 *	11.2 ± 1.8**	48.5 ± 3.2**	136.8 ± 4.6**
	Water extract, 400 mg/kg	11.2 ± 1.1 **	11.6 ± 1.5**	49.3 ± 3.6 **	140.9 ± 4.9**

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

DISCUSSION

Lipid peroxidation is an accumulated effect of reactive oxygen species (ROS), which leads to worsening of biological systems (Dzingiral *et al.*, 2007). Oxygen radicals and other reactive kind are generated in biological systems either as by-products of oxygen reduction or by xenobiotic catabolism (Chance *et al.*, 1979). These ROS such as superoxide anion (O_2^-), hydroxyl radicals (OH.), nitric oxide (NO) and peroxy radical ($ROO\cdot$) are unbalanced and can attack key biomolecules such as lipids, proteins and nucleic acids (Halliwell & Gutteridge, 1999).

β -carotene bleaching inhibition method was deliberate based on the ability of an antioxidant to slow down orange color reduction of β -carotene due to the oxidation occurred in linoleic acid/ β -carotene mixture (Kulisic *et al.*, 2004; Wang *et al.*, 2010). β -carotene is very responsive to free radical formed by linoleic acid oxidation (Fukumoto & Mazza, 2000; Apak *et al.*, 2007).

In case of *P. zeylanica*, we found that chloroform extract among all extracts showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 70% inhibition at 30 minutes which was reduced to 45% at the time of 120 minutes.

Hydroxyl radicals are well thought-out to be one of the rapid initiators of lipid peroxidation process, abstracting hydrogen atoms from polyunsaturated fatty acid, which brings about peroxidic reactions of membrane lipids (Kitada *et al.*, 1979) and also, from each of the carbon atom of the sugar moiety of DNA causing oxidative smash up to DNA. These effects have been concerned in mutagenesis, carcinogenesis and aging (Halliwell & Gutteridge, 1999). Ferric-EDTA incubated with H_2O_2 and ascorbic acid at pH 7.4, produces hydroxyl radicals and was detected by their aptitude to degrade 2 deoxyribose into fragments, on heating with TBA at low pH forming a pink chromogen (Aruoma *et al.*, 1989).

In case of *P. zeylanica*, Hydroxyl radical scavenging ability calculated as that chloroform, methanol, butanol and water extracts have IC_{50} values of 47 μ g/mL, 100 μ g/mL, 200 μ g/mL and 150 μ g/mL, respectively. These results implied that chloroform and methanol extracts have the highest OH radical scavenging abilities as compared to butanolic and water extracts.

DPPH is a free radical constant at room temperature, and produces a purple color solution in methanol. It is condensed in the presence of antioxidant molecule, giving rise to a yellowish methanol solution. In case of *P. zeylanica*, DPPH scavenging ability calculated as IC_{50} shows that chloroform extract has IC_{50} of 36 μ g/mL followed by ascorbic acid (3.2 μ g/mL), gallic acid (3.5 μ g/mL), methanol (40 μ g/mL), butanolic (47 μ g/mL) and water (45 μ g/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability.

In our study of *Plumbago zeylanica* Linn. Learning and memory are associated with escape latency and time spent in target quadrant. Decline of EL and augment of TSTQ by mice in Morris water maze indicates improvement of learning and memory and vice versa. Among all the extracts, chloroform extract showed a highly significant effect on EL and TSTQ. Chloroform extracts significantly decreased ($P < 0.001$) EL and significantly increased TSTQ as compared to vehicle treated control.

In our study, all the extracts in a dose of 200 & 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. Chloroform extract of both plants significantly decreased EL during training and it significantly increased TSTQ during retrieval, indicating improvement of learning and memory.

Acetylcholine is measured as the most important neurotransmitter involved in the regulation of cognitive functions (Hasselmo, 2006). Selective defeat of cholinergic neurons or decreased synthesis of acetylcholine was reported to be an attribute feature of neurodegenerative disorder. Drugs that increases the overall quantity of acetylcholine was

considered as memory enhancing drug (Deutsch & Rocklin, 1967).

CONCLUSION

The deterioration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of Alzheimer's disease (Bartus *et al.*, 1982; Coyle *et al.*, 1983). Thus, the drugs which enhance cholinergic function can be used for treatment of dementia intimately related to Alzheimer's disease.

REFERENCES

- Kriti, S.K., Kasture, S.B., Mengi, S.A., Efficacy Study of Prunus amygdalus (almond) nuts in Scopolamine Induced Amnesia in Rats, Indian Journal of Pharmacology, 2010; 42(3):168-173. <https://doi.org/10.4103/0253-7613.66841>
- Wang YC, Huang TL. High-performance liquid chromatography for quantification of plumbagin, an anti-Helicobacter pylori compound of Plumbago zeylanica L. J of Chromato A; 2005; 1094:99-104. <https://doi.org/10.1016/j.chroma.2005.07.092>
- Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, et al. Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. Molecules. 2007; 12(7):1496-547. <https://doi.org/10.3390/12071496>
- Aruoma O., Laughton M., Halliwell B.: Biochem. J. 1989; 264:863. <https://doi.org/10.1042/bj2640863>
- Sundari, A., & Jayakumararaj, R. Herbal Remedies Used to Treat Skin Disorders in Arasankulam Region of Thoothukudi District in Tamil Nadu, India. Journal of Drug Delivery and Therapeutics, 2020; 10(5):33-38. <https://doi.org/10.22270/jddt.v10i5.4277>
- Bartus RT, Dean RL, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982; 217:408-14. <https://doi.org/10.1126/science.7046051>
- Chance B., Sies H., Boveris A.: Physiol. Rev. 1979; 59:527. <https://doi.org/10.1152/physrev.1979.59.3.527>
- Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervations. Science 1983; 219:1184-90. <https://doi.org/10.1126/science.6338589>
- Deutsch JA, Rocklin KW. Amnesia induced by scopolamine and its temporal variations. Nature 1967; 216:89-90. <https://doi.org/10.1038/216089b0>
- Domange C, Schroeder H, Violle N, Peiffer J, Canlet C, Paris A, Priymenko N. Mining the brain metabolome to understand behavioural disruptions induced in mouse fed Hypochaeris radicata (L.), a neurotoxic plant for horse. Neurotoxicology 2013; 38:74-83. <https://doi.org/10.1016/j.neuro.2013.06.005>
- Sundari, A., & Jayakumararaj, R. Herbal Remedies Used to Treat Skin Disorders in Arasankulam Region of Thoothukudi District in Tamil Nadu, India. Journal of Drug Delivery and Therapeutics, 2020; 10(5):33-38. <https://doi.org/10.22270/jddt.v10i5.4277>
- Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochemical Pharmacology 1961; 7:88-95. [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- Fukumoto LR, Mazza G. Assessing antioxidant and pro-oxidant activities of phenolic compounds. J Agric Food Chem. 2000;48(8):3597-604. <https://doi.org/10.1021/jf000220w>
- Halliwell B., Gutteridge J., Aruoma O.: Anal. Biochem. 165, 215 (1987). [https://doi.org/10.1016/0003-2697\(87\)90222-3](https://doi.org/10.1016/0003-2697(87)90222-3)
- Hasselmo ME. The Role of Acetylcholine in Learning and Memory. Current Opinion in Neurobiology 2006; 16:710-15. <https://doi.org/10.1016/j.conb.2006.09.002>
- Joshi H, Parle M. Nootropic activity of Calyces of Hibiscus sabdariffa Linn. IJPT; 2006; 5(1):15-20.
- Soorya C, Balamurugan S, Ramya S, Neethirajan K, Kandeepan C, Jayakumararaj R. Physicochemical, ADMET and Druggable properties of Myricetin: A Key Flavonoid in Syzygium cumini that regulates metabolic inflammations. Journal of Drug Delivery and Therapeutics. 2021; 11(4):66-73. <https://doi.org/10.22270/jddt.v11i4.4890>
- Kikuchi A, Kitamura K. Simple and Rapid Carotene Bleaching Tests for the Detection of Lipoxygenase Isozymes in Soybean Seeds. Japan J Breed. 1987; 37(1):10-16. <https://doi.org/10.1270/jsbbs1951.37.10>
- Kitada M., Igarashi K., Hirose S., Kitagawa H.: Biochem. Biophys. Res. Commun. 87, 388(1979). [https://doi.org/10.1016/0006-291X\(79\)91808-4](https://doi.org/10.1016/0006-291X(79)91808-4)
- Kokate, C.K., 1996, Practical Pharmacognosy. Delhi, Vallabh Prakashan.
- Kshirsagar SN. Nootropic Activity of dried Seed Kernels of Caesalpinia crista Linn against Scopolamine induced Amnesia in Mice. Int J Pharm Tech Res; 2011; 3(1):104-09.
- Kulicic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry. 2004; 85(4):633-40. <https://doi.org/10.1016/j.foodchem.2003.07.024>
- Kumar, N., Singh, S., Patro, N., Patro, L., Evaluation of protective efficacy of Spirulina platensis against collagen-induced arthritis in rats. Inflammopharmacology, 2009; 17(3):181-190. <https://doi.org/10.1007/s10787-009-0004-1>