"Pharmacological Evaluation of *Solanum Nigrum* Leaves extract for its Memory enhancing activity"

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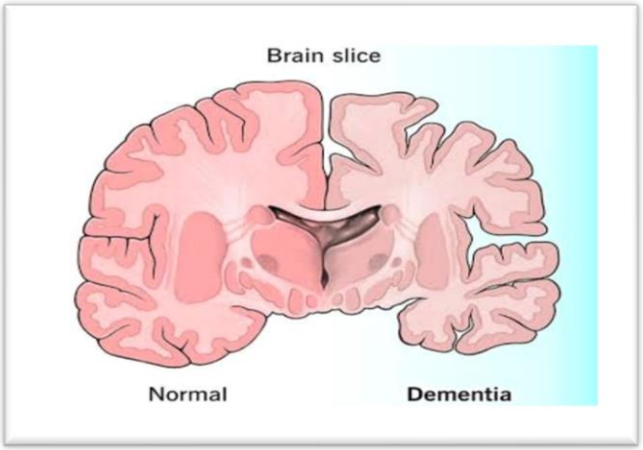
***Abstract*- In the traditional system of medicine, the Leaves and berries of *Solanum Nigrum* have been employed clinically for centuries for their anti-inflammatory, antiulcer, antimicrobial and anxiolytic activities. The present study was undertaken to investigate the effects of *Solanum Nigrum* (popularly known as Blackberry) on learning and memory in mice. Elevated plus-maze and Water Morris maze were employed to test learning and memory. Two doses (100 and 200mg/kg p.o.) of Methanolic extract of *Solanum Nigrum* Leaves were administered for 7 successive days in separate groups of animals. The dose of 200 mg/kg of the methanolic extract of Blackberry significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by Piracetam (200 mg/kg i.p.) and scopolamine (0.1 mg/kg i.p.). Anti-inflammatory and antioxidant properties of Blackberry may be contributing favourably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by Blackberry, it is possible that the beneficial effect on learning and memory was due to facilitation of cholinergic-transmission in mouse brain. However, further studies are necessitated to identify the exact mechanism of action. In the present investigation, *Solanum Nigrum* has shown promise as a memory enhancing agent in all the laboratory models employed.**

***Index Terms*: *Solanum Nigrum*, Scopolamine, Piracetam, Memory Enhancement, amnesia**

INTRODICTION:

Learning is the process of an acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as cognition. Without memory, which is a key mental function, we are powerless. It is a faculty by which sensations, impressions and ideas are stored and recalled. One of the areas of neuroscience that is most actively researched is learning and memory. Particularly in India, China, and Latin America, where dementia is quickly emerging as the primary public health issue, the ageing demographic shift is moving forward quickly. Approximately 10% of the adults older than 65 years and 50% of the adults older than 90 years have dementia [1]. Learning may be defined as the ability to alter the behaviour on the basis of experience and memory is the ability to recall past events at the conscious and unconscious level. A person may store more information and retain it for longer when his or her brain is alert. An emotional overload, which the modern language sometimes refers to as "tension," can cause memory loss [2]. Memory, mood, and behaviour are the three main areas that nootropic drugs like donepezil, piracetam, and pramiracetam are used to help. The resultant negative effects of these agents, however, have restricted their usage [3].



One of the largest and most diverse species groups in the genus is the Solanum section, which begins with the species Solanum nigrum L., sometimes known as the black, garden, or common nightshade. Although it is more frequently referred to as the section Morella (Maurella (Dun.) Dumort. or Morella (Dun.) Bitt.), the correct name for this section is Solanum [4] because S. nigrum is the generic type. From sea level to elevations above 3500 metres, this section's species are found in temperate and tropical climates. S. nigrum, which belongs to the lung and kidney meridians and which has a bitter taste, cold properties, and a little poisonous effect, can be utilised as medicine. In both traditional Chinese medicine (TCM) and Chinese folk medicine, S. nigrum has been used extensively in clinical settings. Since ancient times, the whole plant of Canker sores, skin dermatitis, urinary tract infections,

bacterial dysentery, prostate, chronic bronchitis, etc. have all been treated with S. nigrum. It also has beneficial effects on dispersing blood stasis and detumescence, clearing away heat, as well as detoxification [5].

MATERIALS AND METHODS

- **Processing of plant material:**

Shade drying of the leaves up to complete removal of moisture was done. (Took around 15 days) Dried leaves were powdered by hand crushing and sieved through sieve number 30 #.)

- **Pharmacological screening method for memory enhancing activity:**

1. Elevated Plus Maze

The procedure, technique, and end point for testing learning and memory were followed as per the parameters described earlier [8, 10, 11]. The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (i.e., 10th day of drug administration) for each animal. If the animal did not enter into one of the covered arms within 90 sec, it was gently pushed into one of the two covered arms and TL was assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examine24 h (11th day) after the first day trial.

Animal Grouping:

1. Control group
2. Scopolamine Induced
3. Scopolamine + Piracetam (Standard)
4. Scopolamine + Drug Extraction (100 mg/kg)
5. Scopolamine + Drug Extraction (200 mg/kg)

2. Morris Water Maze

Briefly, Morris water maze-(MWM) for mice consisted of a circular pool (60 cm in diameter, 25 cm in height) filled to a depth of 20 cm with water maintained at 25°C. The water was made opaque with nontoxic white coloured dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform was placed inside the target quadrants (Q4 in present study) of this pool 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days (starting from 6th day of drug administration to 9th day), during which they were allowed to escape on to the hidden platform and to remain there for 20 s. During the training session, the mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. If the mouse failed to find the platform within 120 s, it was guided gently on to the platform and allowed to remain there for 20 s. Escape latency (EL) is the time taken by the animal to move from the starting quadrant to find the hidden platform in the target quadrant. EL was recorded on the 6th day to 9th day for each animal. Each animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

Day1 Q1 Q2 Q3 Q4.
Day2 Q2 Q3 Q4 Q1.
Day3 Q3 Q4 Q1 Q2.
Day4 Q4 Q1 Q2 Q3.

On the fifth day (i.e., 10th day of drug administration), the platform was removed and mouse was placed in any of the three quadrants and allowed to explore the target quadrant for 300 s. Mean time spent in all the three quadrants that is, Q1, Q2, and Q3 was recorded. The mean time spent in the target quadrant in search of the missing platform was noted as index of retrieval or memory. The observer always stood at the same position. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory.

Animal Grouping:

1. Control group
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4. Scopolamine + Drug Extraction (100 mg/kg)
5. Scopolamine + Drug Extraction (200 mg/kg)

RESULT

Statistical analysis

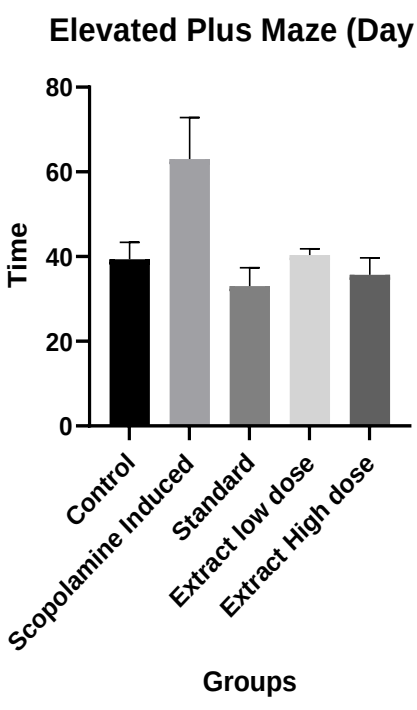
- Effect of Solanum Nigrum Leaves Extract and other drug employed on transfer latency (TL) of mice using elevated plus maze.

Groups	Day 1	Day 7
Control	39.33±2.3**	35.67±0.66##
Scopolamine Induced	63±5.6	46.67±2.4
Standard	33±2.5@@@	22±1.5$$$
Extract Low dose	40.33±0.88@@	32±1.5$
Extract High does	35.67±2.33@@@	29.67±1.4$$

Table No:1

(n=3); Values are in mean ±SEM; Statistical analysis done by one-way ANOVA which is followed by Tukey test

* Indicate Comparison of Scopolamine inducer with control on day 1
@ Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 1
# Indicate Comparison of Scopolamine inducer with control on day 7
$ Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 7
$ p<0.033
$$ p<0.0021
$$$p<0.0001
** p<0.0021
p<0.0021
@@ p<0.0021
@@@p<0.0001



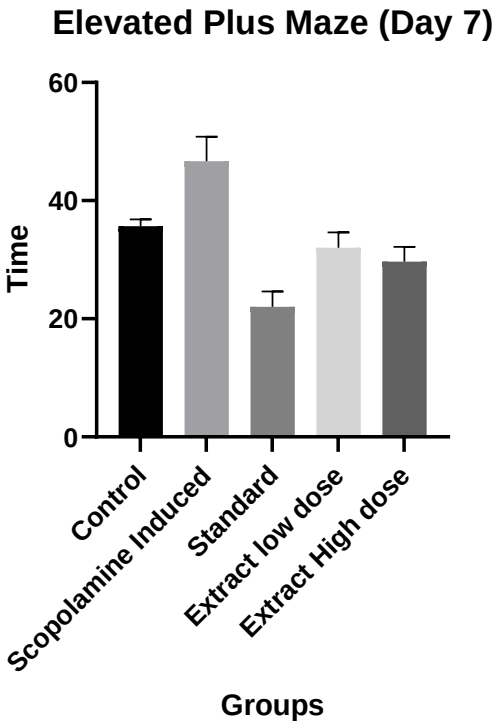


Fig No:1
Graphical representation of Elevated plus maze of Day 1

Fig No:2
Graphical representation of Elevated plus maze of Day 7

In elevated plus maze method, the study was done for the 7 days.
When the scopolamine induced group compared with STD, low dose and high dose of extract extract it possess the memory enhancing activity. STD and high dose of extract shows the significant value at (p<0.0001)

- Effect of Solanum Nigrum Leaves Extract and other drug employed on escape latency (EL) of mice using Water Morris maze.

Groups	Day 6	Day 7	Day 8	Day 9
Control	110.3±2.6*	105.3±2$^{@}$	87.67±0.88$^{\#}$	72.33±1.4$^{\$\$}$
Scopolamine Induced	118.3±0.88	112.7±1.7	97.33±1.4	83.33±0.88
Standard	101.7±0.88$^{\%\%\%}$	93.67±0.88$^{\wedge\wedge\wedge}$	74.33±0.6$^{\&\&\&}$	42.33±1.4$^{!!!}$
Extract Low Dose	110.7±1.2$^{\%}$	104.7±1.4$^{\wedge}$	83.67±0.88$^{\&\&}$	66±2$^{!!}$
Extract High Dose	108.3±0.33$^{\%\%}$	95.33±0.88$^{\wedge\wedge\wedge}$	76±3.05$^{\&\&\&}$	46.67±1.76$^{!!!}$

Table No:2

(n=3); Values are in mean ±SEM; Statistical analysis done by one-way ANOVA which is followed by Tukey test

* Indicate Comparison of Scopolamine inducer with control on day 6
% Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 6
@Indicate Comparison of Scopolamine inducer with control on day 7
^ Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 7
\# Indicate Comparison of Scopolamine inducer with control on day 8
& Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 8
$ Indicate Comparison of Scopolamine inducer with control on day 9
! Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 9

*p<0.033 #p<0.033 @p<0.033 %p<0.033
^p<0.033 %% p<0.0021 $$ p<0.0021 !! p<0.0021
&&p<0.0021 %%% p<0.0001 &&&p<0.0001 ^^^p<0.0001
!!!p<0.0001

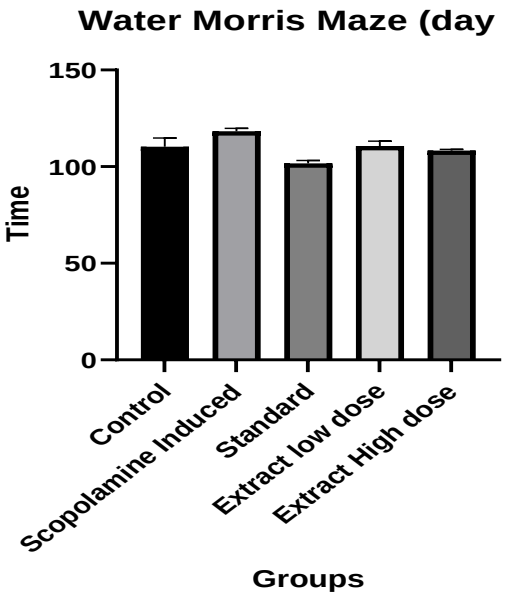


Fig No:3 Graphical representation of water morris maze of Day 6

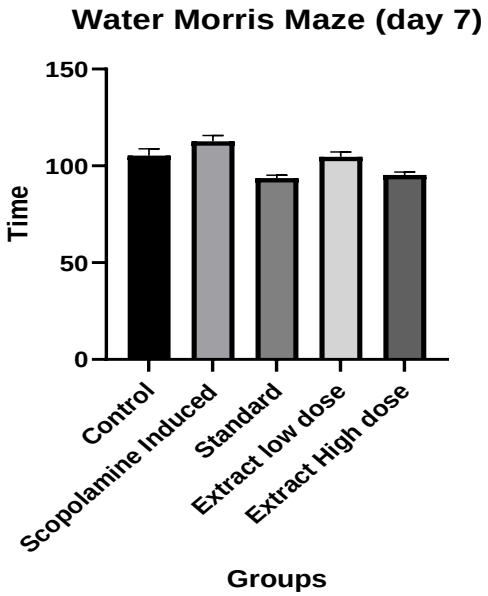


Fig No:4 Graphical representation of water morris maze of Day 7

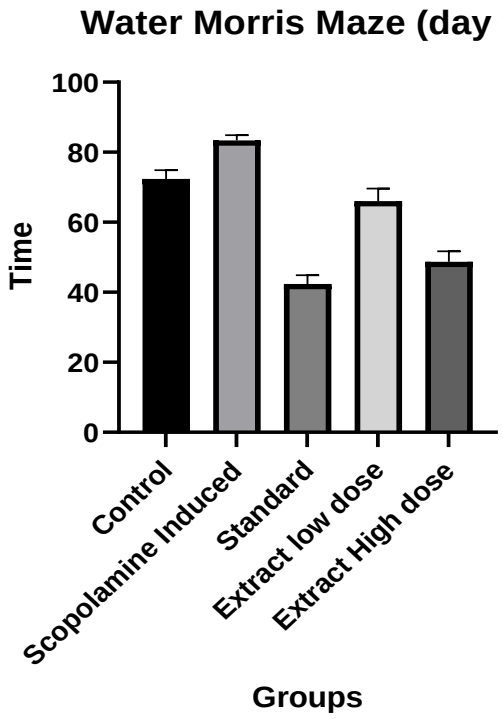


Fig No:5 Graphical representation of water morris maze of Day 8

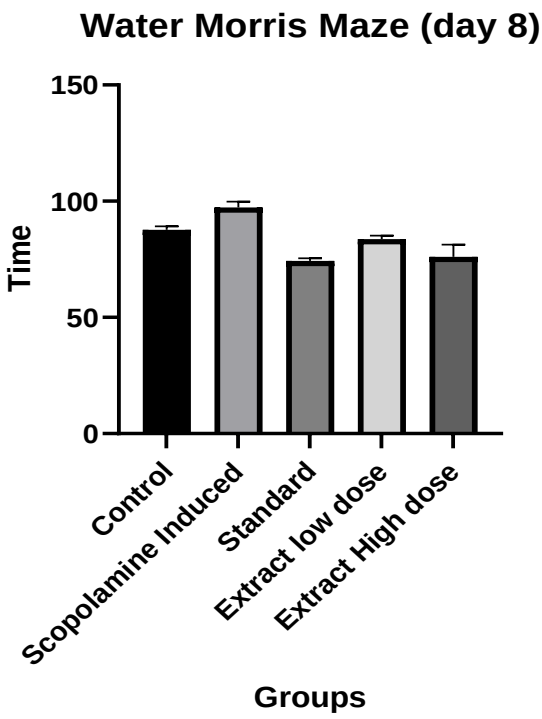


Fig No:6 Graphical representation of water morris maze of Day 9

- Effect of Solanum Nigrum Leaves Extract and other drug employed on time spent in target quadrant of mice using Water Morris maze

Groups	Day 10
Control	94.33±0.88@
Scopolamine Induced	106.7±1.45
Standard	56.33±1.85***
Extract Low dose	83.67±1.85**
Extract High Dose	68.33±3.75***

Table No:3

(n=3); Values are in mean ±SEM; Statistical analysis done by one-way ANOVA which is followed by Tukey test
@ Indicate Comparison of Scopolamine inducer with control on day 10
* Indicate Comparison of Scopolamine inducer with Treatment Standard, t_1 and t_2 on day 10
* p< 0.033
** p<0.002 1
*** p<0.0001
@p< 0.033

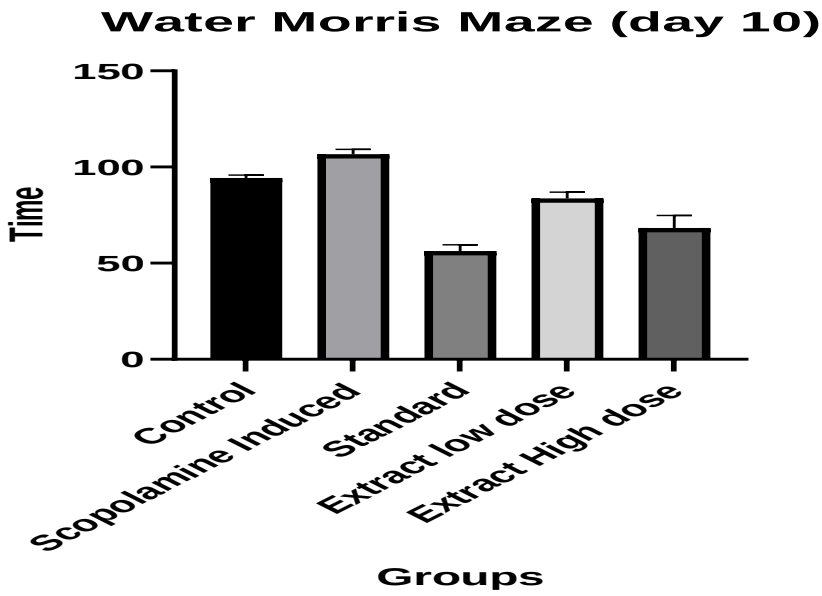


Fig No:7 Graphical representation of water morris maze of Day 10

In water Morris maze method, the study was done for the 10 days.
The escape latency of mice decreased on 9 day and increased on 10day as compared to control group, thus showed the significant improvement of learning and memory. When compared to the standard the low dose gives less significance as compared to high dose.

DISCUSSION

In recent years, much attention has been focused on the protective biochemical function of naturally present antioxidants in biological systems, and on the mechanisms of their action [6]. Our current study examines the memory enhancing activity of *Solanum Nigrum* Leaves extract in mice oral route was chosen to meet the way used by people in traditional medicine. The lethal dose LD_{50}Value of the *Solanum Nigrum* indicated that the methanolic extract was safe and nontoxic up to 2000 mg/kg [7]. Previous studies were reported cancer, inflammation, infectious, cardiopathy, diabetes, hepatotoxicityactivity and improving function of liver and kidney [8].
The mechanism of action by which amnesia was induced by scopolamine the methanolic plant extract was also investigated and compared with standard reference drug piracetam and control group [9].
Amnesia is enabled to remember event for a period of time often due to brain injury illness or effect of drug or alcohol. When the brain forms new short-term memories, it creates new neurons in a region of a hippocampus called the dentate gyrus. This process also clears outdated memories making room for new one. Commonly used memory enhancers are caffeine, Brahmi, etc. Amnesia in experimental animal can be produced by benzodiazepine.
In the present study the attempt has been focused to evaluate the memory enhancing activity of extract of plant *Solanum Nigrum* Leaves by using elevated plus maze method and water morris maze method.
Study of literature survey revealed that leaves are aromatic and contains tannins, carbohydrates, flavonoids, on the basis of literature survey [10] and solubility of chemical constituents, solvents are selected for extraction are as follows.
In the present study extraction was done in methanol and it gives good yield, extraction was done by Soxhlet apparatus. Methanolic extract contains flavonoids, glycosides, steroids, carbohydrates, tannins etc.
On the basis of observation methanolic extract was found to be 13.48 %.
In pharmacological study it observed that safe dose was found to be 200 mg /kg to check memory enhancing activity. Presence study includes elevated plus maze method and water morris maze method, in 1st technique of this method is consist of four arms, two arms are closed (16 cm×5 cm×15 cm) and two are open (16 cm×5cm) and one is central platform (5 cm ×5 cm). Second one contains circular pool and its diameter is 60 cm and height is 25 cm.
The memory enhancing effect of solanum nigrum are presented in current study. It was observed that administration of methanolic extract at the dose of 200 mg/kg resulted in a significant value.
When it compared with the scopolamine treated group, it has shown effect nearby similar to that of piracetam.

CONCLUSION

Solanum nigrum leaves contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like cancer, inflammation, infectious, cardiopathy, diabetes, hepatotoxicity and other infectious infections where its purported ability to improve memory is said to be helpful. The extracts of Solanum nigrum leaves tested for memory enhancing activity by researchers. There were no scientific studies on the Solanum nigrum leaves' ability to improve memory.

The present study aimed at evaluating the In-vivo memory enhancing activity of Solanum nigrum leaves extract in mice. methanolic extracts were prepared by the hot extraction process, i.e., by using Soxhlet apparatus. Preliminary phytochemical evaluation of methanolic extract was carried out for the determination of presence of phytoconstituents.

The result of acute oral toxicity studies of plant extracts as per standard references revealed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD50) could be greater than 2000 mg/kg body weight in rats. Accordingly safe experimental dose was calculated as ≤ 200mg/kg & was used accordingly for further screening of extracts. In- Vivo study has showed that methanolic extracts of Solanum nigrum does possess significant memory enhancing activity with 100 mg/kg and 200 mg/kg, but high doses of the methanolic extract 200 mg/kg being more superior and showed significant to highly significant percentage inhibition when compared with standard Piracetam. The finding of the present study reveals that Solanum nigrum leaves has potent memory enhancing activity. Further study is requiring evaluating the mode of action of memory enhancing effect of Solanum nigrum leaves extracts.

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