IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2020 Vol.:19, Issue:3

Phytochemical Investigation and Pharmacological Evaluation of *Solanum nigrum* L. Leaves Extracts for Its Memory Enhancing Activity

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Submission: 22 September 2020
Accepted: 28 September 2020
Published: 30 October 2020

Keywords: *Solanum nigrum*, ethyl acetate and Methanolic extract, phytochemical screening, Antioxidant effect, Memory enhancing activity

ABSTRACT

The present study reports physicochemical characterization, antioxidant and Memory enhancing activity of extracts from *Solanum nigrum* leaves collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using ethyl acetate and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phytochemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of ethyl acetate and methanol leaves extracts. Maximum percentage inhibition i.e. 80.97% was shown by ethyl acetate extract at concentration of 150 µg/ml and was compared with Ascorbic acid as reference standard. The *In-Vivo* memory enhancing activity of *Solanum nigrum* leaves was evaluated by radial arm maze model in rats using Piracetam as a standard. Both the extracts at 200mg/kg concentration showed significant to highly significant number of entries and time spent in P zone (from $P < 0.05$ to $P < 0.001$). The result suggests that *Solanum nigrum* leaves extracts possess memory enhancing activity and this might be due to flavonoids, Phenolic compound, steroid and proteins present in extract.

INTRODUCTION:

Learning is the process of acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as cognition. Memory is a fundamental mental process and without it we are capable of nothing. It is a faculty by which sensations, impressions and ideas are stored and recalled. Learning and memory is one of the most intensively studied subjects in the field of neuroscience. Dementia (loss of memory) is a syndrome caused by disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, and orientation, and comprehension, calculation, learning capacity, language and judgment. Aging demographic transition is proceeding rapidly especially in India, China and Latin America, where dementia is rapidly becoming the major public health problem. Approximately 10% of the adults older than 65 years and 50% of the adults older than 90 years have dementia. Nootropic agents such a Piracetam, Pramiracetam, Aniracetam and choline esterase inhibitors like donepezil are being primarily used to improve memory. However, the resulting adverse effects associated with these agents such as hepatotoxicity, nasal congestion, hypotension, gastrointestinal disturbances, rashes, constipation, tiredness, headache and drowsiness systemic side effects upon chronic use have limited their use. Learning may be defined as the ability to alter the behavior on the basis of experience and memory is the ability to recall past events at the conscious and unconscious level. These two are obviously closely related to each other and should be considered together. The brain and the computer working similar fashion to store day to day happenings, incidents and visuals. Alzheimer's disease is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes and ultimately death. The personality distortions interfere with the patient's professional life, social activities and relationships. The present attempt is review and compiles updated information on various aspects of *Solanum nigrum*, a plant used all over the world. The plant *Solanum nigrum* Linn belongs to family *Solanaceae* which is commonly known as Makoya or kakamachi. Makoya consist of dried whole plant shrub that grows wild and abundantly in open fields. *Solanum nigrum* is also called as black nightshade which is annual to short lived perennial plant has white or mauve flowers followed by berries that are first green but change to black as they ripen.

Solanum nigrum is a well known traditionally used medicinal plant. It is reported to possess hepatoprotective, anthelmentic, anti-inflammatory, antimicrobial, antihyperlipidemic, antitumour and neuropharmacological properties. The leaves of the plant reported to contain several phytoconstituents like quercetin, rutin, hyperoside, sitosterol, solamargine, salidroside, stigmasterol, cholesterol and solasodine.

The leaves of *Solanum nigrum* (Solanaceae) have been found to contain four flavonoids. one is a flavonoid aglycone quercetin, two are flavonoid glycosides Quercetin-3-O-D-glucopyranoside and quercetin-3-O-L- rhamnopyranoside and one is a flavonoid diglycoside- rutin. The occurrence of all these compounds Quercetin, Quercetin 3-O-b-D glucopyranoside, Quercetin 3-O-b-D rhamnopyranoside and Rutin are reported for the first time from this plant. (**Potawale *et al. 2008)***

MATERIAL AND METHODS:

1. Collection, identification and authentication of plant material

The fresh Leaf of *Solanum nigrum* L. was collected from local region of Nanded i.e. from local market and authenticated by **Dr. Shrirang S. Bodke,** Head, Department of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded.

2. Processing of crude drug:

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 #.

3. Preparation of Extracts:

Three extracts of *Solanum nigrum* leaf powder were prepared with solvents petroleum ether, ethyl acetate, methanol. The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\text{(\%) yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

a. Preparation of ethyl acetate extract

Ethyl acetate extract of powdered leaves of *Solanum nigrum* was prepared in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube. 180 gm of the powdered and 1000 ml Ethyl acetate was used for extraction. After completion of extraction, extract was cooled and dried. The extract was stored in airtight container till use. Percentage yield of extract was calculated.

b. Preparation of methanol extract

Methanol extract of powdered leaves of *Solanum nigrum* was prepared in Soxhlet extractor according to the standard method till colourless solution was observed in siphon tube. 178gm of the powdered husk and 1200 ml Methanol was used for extraction. After completion of extraction solvent was cooled and dried. The extract was stored in airtight container till use. Percentage yield of extract was calculated.

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

Phytochemical Evaluation:

1. Total Phenolic Content

Total Phenolic Content was determined by using the Folin-Ciocalteu assay. An aliquot (1m) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10 ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCo_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

2. Total Flavonoids Content

Total Flavonoid Content was measured by the aluminium trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five

minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

3. *In vitro* antioxidant activity

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

2,2 Diphenyl- 1 picryl-hydrazyl radical scavenging (DPPH) Activity-

Principle:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple colour is monitored at 517 nm. The free radical scavenging activity can be measured by using 1, 1- diphenyl-2-picryl-hydrazyl. ***Vani T, Rajani, M et al., (1997)***

Reagents Required:

1) DPPH

2) Pure Methanol

Preparation of samples and standard solutions:

Accurately weighed 10 mg of ethyl acetate and methanolic extracts and the standard ascorbic acid and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure:

The reaction mixture (3.0 ml) consists of 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was

vortexes and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

The percentage of inhibition can be calculated using the formula:

$$\%\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: Absorbance of control.

A_{test}: Absorbance of test.

Animal used:

For the study, Wistar rats of either sex, of weight 150-200gm were selected.

Test group:

For the study, six groups of animals were made. Each group having six rat.

Route of administration: Oral route administration.

Housing Condition:

Animals were housed six groups in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of three mice.

Methodology:

During the test, rats are placed at the end of their body weights maintained at 85% of their free feeding weight to motivate the rat to run the maze. The animals are trained on daily basis in the maze to collect the food pellets. The session is terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors. Animals were divided randomly into six treatment groups; each group consisting of six rats, each treatment group received orally the extracts of *Solanum nigrum* leaves in a dose of 100 mg/kg and 200mg/kg of both the extract as per the group. Extracts was given to rats, once

daily for period of 8 days and daily evaluation was done. The endpoint will be taken as per the number of entries in P zone and time spent in P zone. The mean of number of entries and time spent in P zone for each group is calculated.

Evaluation

Evaluation was done on the basis of time spend in C zone and selected arms P zone and number of entries in C zone and selected arms P zone.

The Number of errors (Entries to non-baited arms) will be counted during the session.

Memory Enhancing Activity:

In-vivo memory enhancing activity of *Solanum nigrum* leaves was carried out by using radial arm maze. In which piracetam was used as standard drug. Test drug ethyl acetate and methanolic extract was used, the apparatus is a fabric elevated maze eight-arm radial maze with the arms extending from a central platform 26 cm in diameter each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm. The maze was well illuminated and numerous cues were present. Food pellets (reward) were placed at the end of the arms.

During the test, rats were placed at the end of their body weights maintained at 85% of their free feeding weight to motivate the rat to run the maze. The animals were trained on daily basis in the maze to collect the food pellets. The session was terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors.

IAEC Approval

Wistar rats of either sex weighing 150 to 200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Memory enhancing model

(Radial Arm Maze)

Principle:

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. These two are obviously closely related to each other and should be considered together. The brain and the computer work in similar fashion to store day to day happenings, incidents and visuals. When a person's brain is fresh he is able to store more information and remember the matter for long. An emotional overload that in the modern language is sometimes called –'tension' which may leads to loss of memory. Alzheimer's disease is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behaviour, personality changes and ultimately death. Personality distortions interfere with the patient's professional life, social activities and relationships. Nootropic agents such as Piracitam, Pramiracetam, aniracetam and choline esterase inhibitors like Donepezil are being primarily used to improve memory, mood and behavior.

So the present study was therefore carried out for confirming veracity of aforementioned traditional claim of using two animal models, namely Radial arm maze.

***In vivo* Memory enhancing activity:**

Radial arm maze

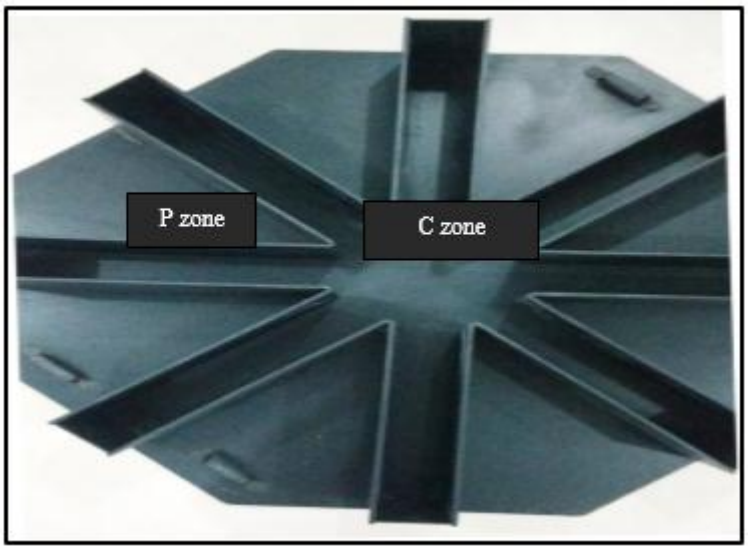


Figure No. 1: Radial arm maze model

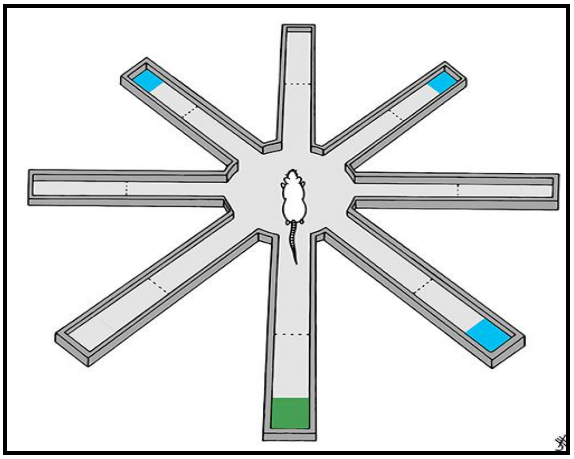


Figure No. 2: Radial arm maze model during experiment.

Procedure:

- Rats of either sex (150-200) gm were used for Memory enhancing study.
- The animals were grouped to different groups like control group (Vehicle treated), Test groups (Extract treated) and Standard drug treated group.
- The rats were subjected to central zone of radial arm maze which The apparatus is a fabric elevated maze eight-arm radial maze with the arms extending from a central platform 26 cm in diameter each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm.
- Each Rat maintain at 85% of its total diet weight was exposed to the maze with the food pellet in a fix arm followed by respective drug treatment for the period of 8 days.
- On each day the evaluation was done.
- Following evaluation parameter consider for the evaluation of memory i.e Time spent in C zone and selected arms P zone and number of entries in C zone and selected arms P zone.

Animal Grouping and drug administration:

Wistar rats of either sex weighing 150-200gm, obtained from animal house of college. The Animals were randomly divided into Six groups of six animals in each group namely.

1) Control group (Vehicle-treated)

2) Standard drug.(200 mg/kg Piracetam IP)

3) *Solanum nigrum* Ethyl acetate extract (100mg/kg orally)

4) *Solanum nigrum* Ethyl acetate extract (200mg/kg orally)

5) *Solanum nigrum* Methanolic extract (100mg/kg orally)

6) *Solanum nigrum* Methanolic extract (200mg/kg orally)

Statistical Analysis

The data were expressed as mean + standard of mean (SEM). Statistical analysis were performed by one way analysis of variance (ANOVA).

RESULTS:

1. Phytochemical Test of *Solanum nigrum* leaves extracts:

Table No. 1: Observations for Phytochemical qualitative analysis

Test	Pet. Ether	Ethanol	Water
Alkaloids			
a. Dragendroff's test	+	+	+
b. Mayer's test	-	+	-
c. Wagner's test	+	+	+
d. Hagers test	-	+	-
Flavonoids			
a. Shinoda test	+	+	+
b. Sulphuric acid test	-	+	+
c. Lead acetate test	+	+	+
Glycosides			
a. Keller Killani test	+	+	+
b. Legal test	-	+	-
c. Foam test	+	+	+
Steroids			
a. Salkowski test	+	+	+

b. Liberman Burchard	-	+	+
Protein			
a. Biuret test	+	+	+
b. Millions test	-	-	-
Carbohydrates			
a. Molish test	+	+	+
b. Fehlings test	+	+	+
c. Benedicts test	+	-	-
d. Barfoed's test	-	+	+
Tannin & Phenolic Compound			
a. Lead acetate	+	+	+
b. Dil. HNO3 test	-	+	+
c. Dil. Iodine solution	-	+	+

TLC Fingerprinting:

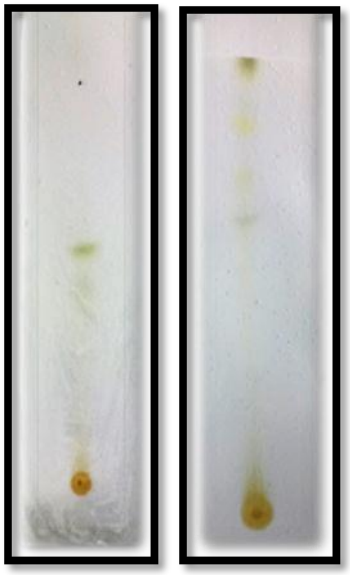


Figure No. 3: TLC of Ethyl acetate and Methanolic extracts

Table No. 2: Results of TLC profile of extracts:

Sr. No.	Extracts	Solvent systems	Proportions	Spraying Reagent	R_f
1.	Ethyl acetate extract	Chloroform: Acetic acid: Methanol	(5:1:1)	Sulphuric acid	0.57
2.	Methanolic extract	Chloroform: Acetic acid: Methanol	(5:1:1)	Sulphuric acid	0.68

Ethyl acetate extract of ***Solanum nigrum*** when subjected to TLC fingerprinting showed R_f value at 0.57 in the solvent system of Chloroform: Acetic acid: Methanol (5:1:1).

Total Phenolic Content:

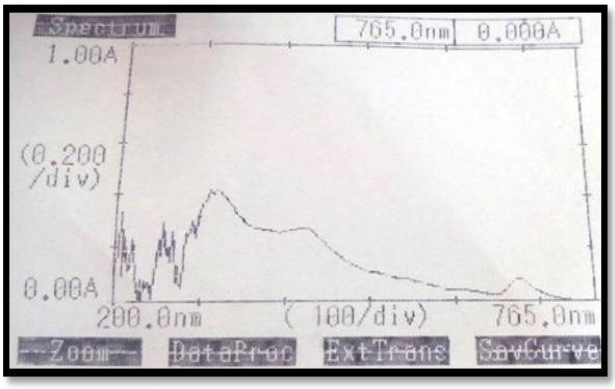
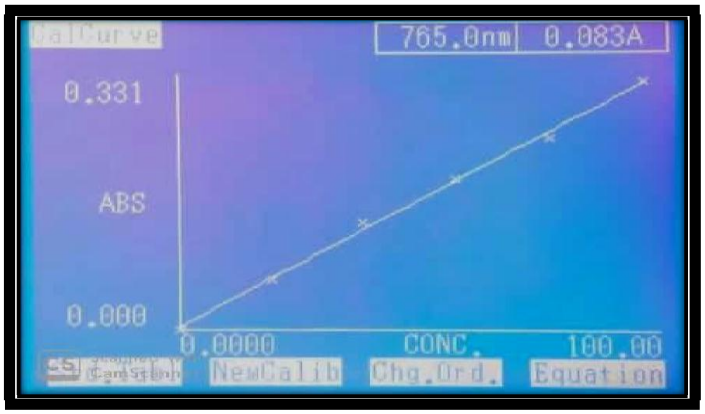


Figure No. 4: λmax Determination Gallic acid

Table No. 3: Total phenolic content of standard Gallic acid

Sr. No.	Conc. µg/ml	Absorbance
1	**20**	**0.067**
2	**40**	**0.144**
3	**60**	**0.201**
4	**80**	**0.256**
5	**100**	**0.331**



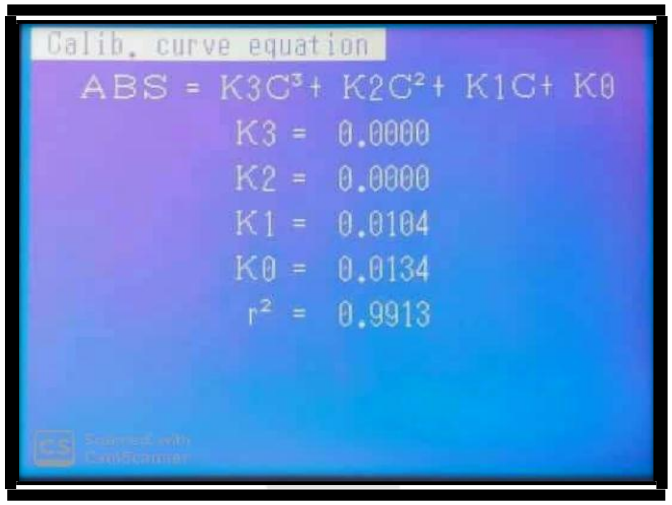


Figure No. 5: Calibration Curve of Gallic acid

Table No. 4: Total phenolic content of *Solanum nigrum* leaves extracts

Sr. No.	Conc. µg/ml	Extracts	Absorbance	Phenolic content (mg GAE/g DW)
1	100	Petroleum ether	0.090	**27.27 ± 0.16**
2	100	Ethyl acetate	0.107	**32.42 ± 0.27**
3	**100**	**Methanol**	**0.196**	**59.39 ± 0.19**

(N=3) Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Methanol have

Phenolic content as 27.27 (mg GAE/g DW), 32.42 (mg GAE/g DW), 59.42 (mg GAE/g DW) respectively. Methanol extract shows more phenolic content than Petroleum ether and Ethyl acetate as per comparative evaluation of phenolic content of extracts.

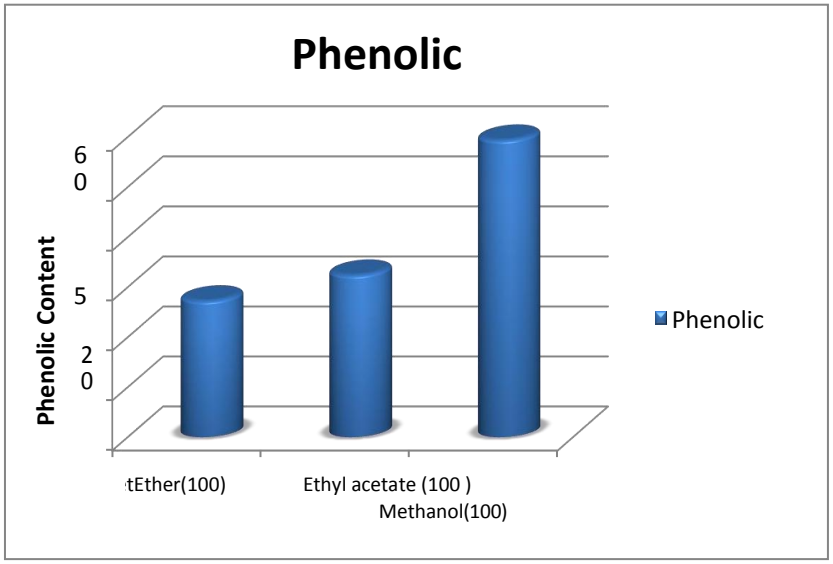


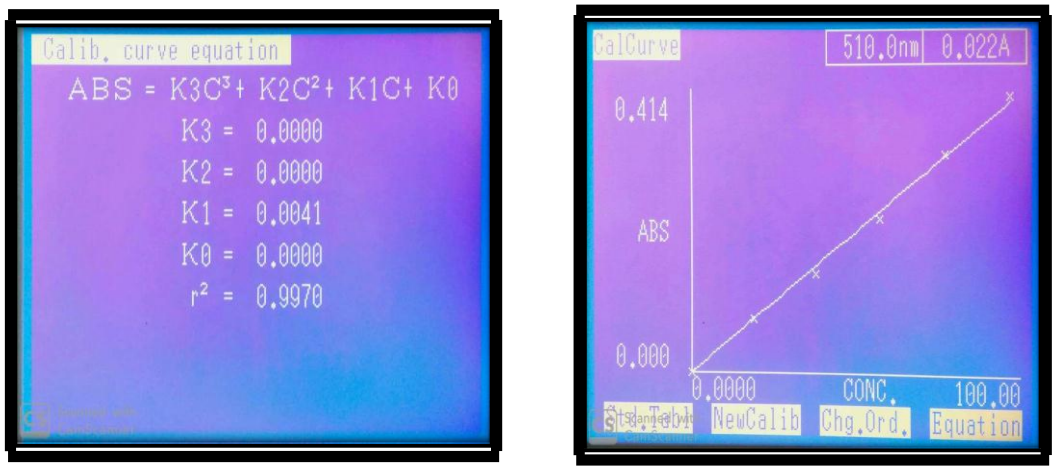
Chart 1. Effect of Phenolic content of extracts

The concentration absorbance calibration curve for sequentially and separately prepared stock solution of standards of Gallic acid solution was taken. The absorbance measured at 765 nm for 20, 40, 60, 80, 100 µg/ml concentration Gallic acid solution are in a range of 0.067 to 0.331 within the range of concentrations, the calibration curve of Gallic acid has clearly exhibited linearity. Above table indicate that the Methanolic extract contain more phenolic content (59.39 mg GAE/g DW) than Ethyl acetate and Petroleum ether extract (32.42 mg GAE/g DW, 27.27 mg GAE/g DW) respectively equivalent to Gallic acid.

Total Flavonoid Content

Table No. 5: Total flavonoid content of standard Rutin

Sr. No.	Conc. µg/ml	Absorbance
1	20	**0.080**
2	40	**0.150**
3	60	**0.232**
4	80	**0.328**
5	**100**	**0.414**



Graph 1. Calibration Curve of Rutin

Table No. 6: Total flavonoid content of *Solanum nigrum* leaves extracts

Sr. No.	Conc. µg/ml	Extracts	Flavonoid content (mg Ru/g DW)
1	100	Petroleum ether	40.00 ± 0.23
2	100	Ethyl acetate	52.19 ± 0.12
3	100	Methanol	62.43 ± 0.17

(N=3) Note: Ru/g DW denotes Rutin Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Methanol have Flavonoid content as 40.00 (mg Ru/g DW), 52.19 (mg Ru/g DW), 62.43 (mg Ru/g DW) respectively. Methanol extract shows more Flavonoid content than Petroleum ether and Ethyl acetate as per comparative evaluation of Flavonoid content of extracts.

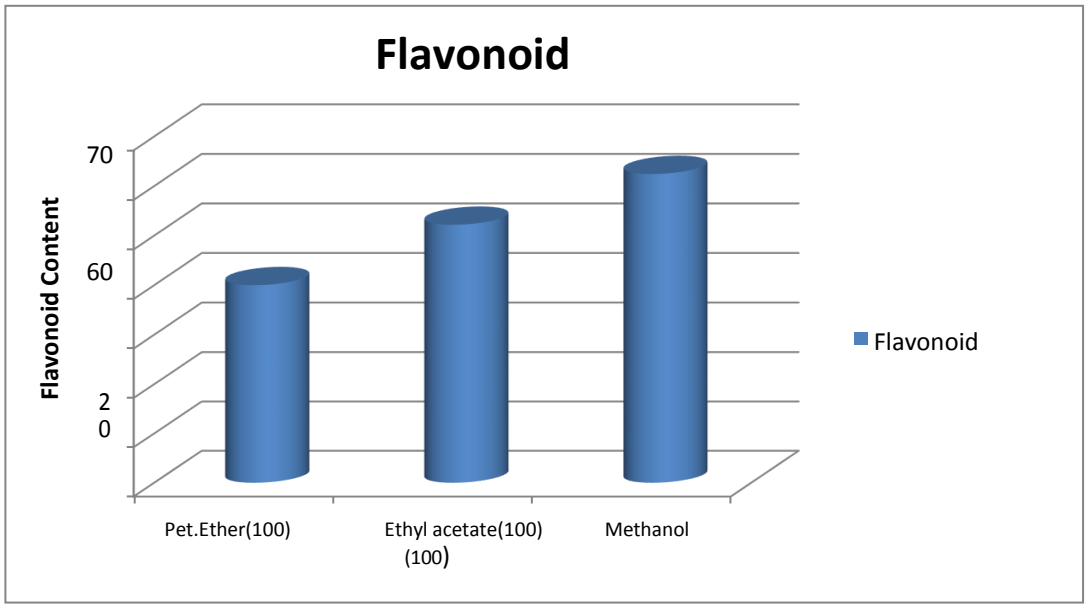


Chart 2. Effect of Flavonoid content of extracts

The calibration curve for sequentially and independently prepared stock solution of rutin that depicts the concentration of rutin against the absorbance. The absorbance values increased proportionally upon increasing the concentration of rutin from 20µg/ml to 100 µg/ml. Above table indicates that the Methanol extract contain more flavonoids (62.43 mg Ru/g DW) of extract than Ethyl acetate and Petroleum ether extract (52.19 mg Ru/g DW and 40.00 mg Ru/g DW) respectively equivalent to rutin.

Total Antioxidant Activity

Table No. 7: Total Antioxidant Content of Standard

Sr. No.	Conc. µg/ml	Absorbance of Ascorbic acid	Absorbance of Gallic acid	Absorbance of Rutin
1	25	0.182 ± 0.0008	0.287 ± 0.0013	0.285 ± 0.0013
2	50	0.129 ± 0.0012	0.224 ± 0.0004	0.236 ± 0.0011
3	75	0.088 ± 0.0019	0.116 ± 0.0009	0.124 ± 0.0013
4	100	0.059 ± 0.0015	0.093 ± 0.0006	0.102 ± 0.0003
5	125	0.024 ± 0.0009	0.043 ±0.0010	0.032 ± 0.0003

% inhibition of Standards:

Sr. No.	Conc. µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62 ± 0.23	41.06 ± 0.33	41.47 ± 0.19
2	50	73.51 ± 0.22	54.00 ± 0.26	51.54 ± 0.17
3	75	81.93 ± 0.21	76.18 ± 0.27	74.53 ± 0.25
4	100	87.88 ± 0.04	80.90± 0.27	79.05 ± 0.31
5	125	95.07 ± 0.25	91.17 ± 0.28	93.42 ± 0.31

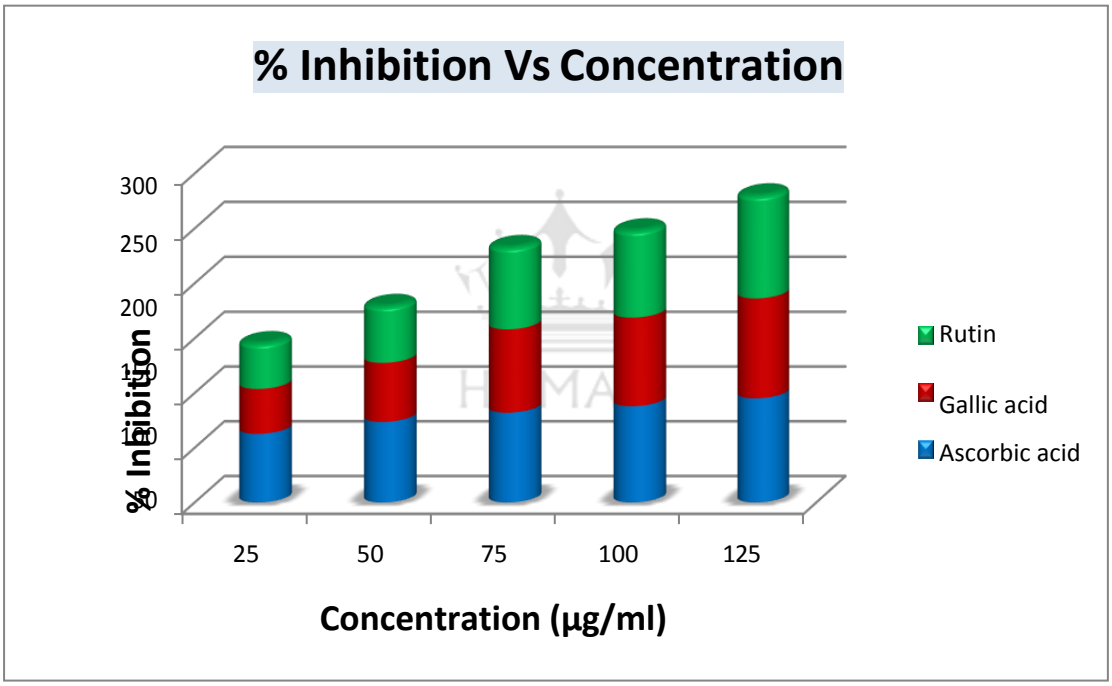
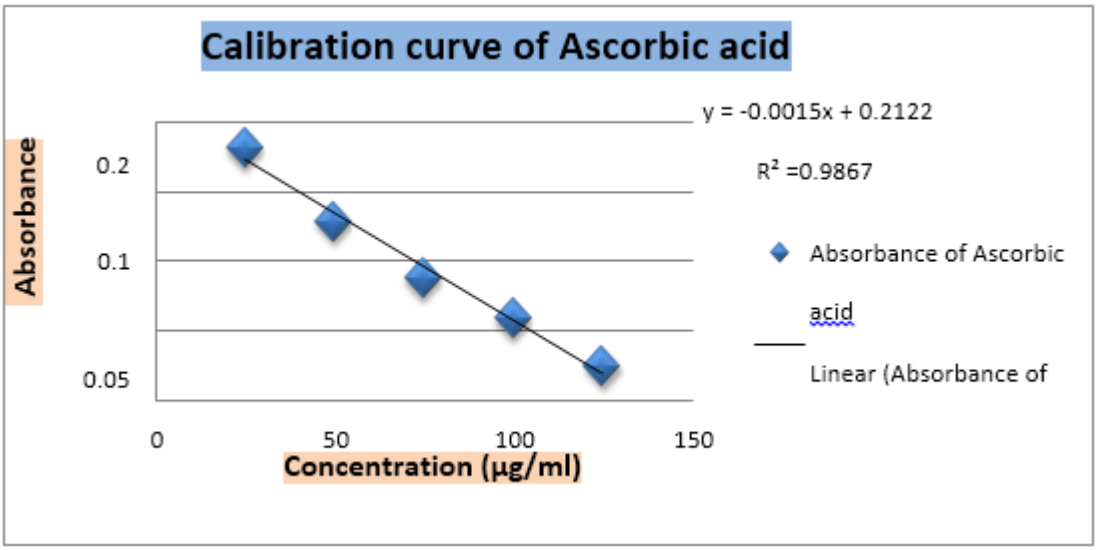


Chart 3. Effect of % Inhibitions of standard



Graph 2. Calibration curve of Ascorbic acid

Table No. 8: Total Antioxidant Content of *Solanum nigrum* leaves Extracts

Sr. No.	Conc. µg/ml	Absorbance of Pet. Ether Extract	Absorbance of Ethyl acetate Extract	Absorbance of Methanolic Extract
1	25	0.221 ± 0.0050	0.209 ± 0.0011	0.198 ± 0.0027
2	50	0.200 ± 0.0036	0.189 ± 0.0009	0.142 ± 0.0036
3	75	0.142 ± 0.0024	0.110 ± 0.0025	0.097 ± 0.0008
4	100	0.122 ± 0.0009	0.082 ± 0.0010	0.062 ± 0.0017
5	125	0.110 ± 0.0007	0.050 ± 0.0018	0.043 ± 0.0019

Sr. No.	Conc. µg/ml	% Inhibition of Pet. Ether Extract	% Inhibition of Ethyl acetate Extract	% Inhibition of Methanolic Extract
1	25	54.62 ± 0.18	57.08 ± 0.37	59.34 ± 0.35
2	50	58.93 ± 0.34	61.19 ± 0.30	70.84 ± 0.06
3	75	70.84 ± 0.29	77.41 ± 0.38	80.08 ± 0.25
4	100	75.00± 0.36	83.20± 0.34	87.26 ± 0.31
5	125	77.41 ± 0.27	89.80 ± 0.33	91.20 ± 0.31

Table No. 9: Comparative DPPH Scavenging assay method of *Solanum nigrum* leaves powder (Pet. Ether, Ethyl acetate and Methanolic) leaves extracts-

Sr. No.	Conc. µg/ml	Petroleum ether % inhibition	Ethyl acetate % inhibition	Methanol % inhibition	Ascorbic acid % inhibition
1	25	54.62 ± 0.18	57.08 ± 0.37	59.34 ± 0.35	62.62 ± 0.23
2	50	58.93 ± 0.34	61.19 ± 0.30	70.84 ± 0.06	73.51 ± 0.22
3	75	70.84 ± 0.29	77.41 ± 0.38	80.08 ± 0.25	81.93 ± 0.21
4	100	75.00± 0.36	83.20± 0.34	87.26 ± 0.31	87.88 ± 0.04
5	125	77.41 ± 0.27	89.80 ± 0.33	91.20 ± 0.31	95.07 ± 0.25

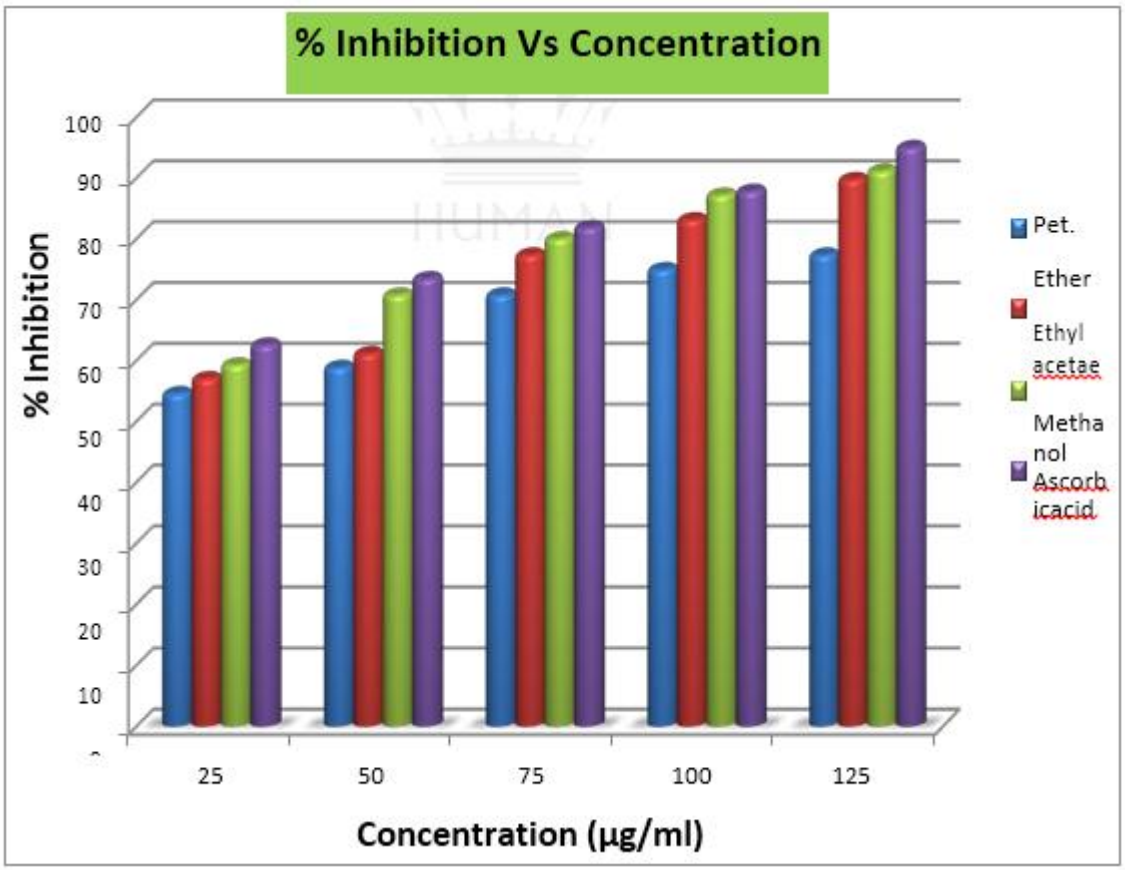


Chart 4. Effect of % Inhibition of Extracts

The calibration curve for sequentially and independently prepared stock solution of ascorbic acid that depicts the concentration of ascorbic acid against the absorbance. The absorbance values increased proportionally upon increasing the concentration of ascorbic acid from 25 µg/ml to 125 µg/ml. Above table indicates that the methanol extract contain more antioxidant content (59.34 µg/ml, 91.20 µg/ml) of extract than Ethyl acetate and Petroleum ether extract (57.08 µg/ml, 89.80 µg/ml and 54.62 µg/ml,77.41 µg/ml) respectively at concentration 25 and 125 µg/ml.

***In- vivo* memory enhancing activity:**

Table No. 10: Number of entries in C and P zone of rats for Memory enhancing activity of *Solanum nigrum* Ethyl acetate (SNEA) and methanolic extract (SNME) leaves extracts.

Sr. No	Group name	Number of Entries in C zone	
		Day 1	Day 8
1	Ctrl	25	23
2	Std	16	14
3	SNEA-100	19	18
4	SNEA-200	17	17
5	SNME-100	18	15
6	SNME-200	21	19

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

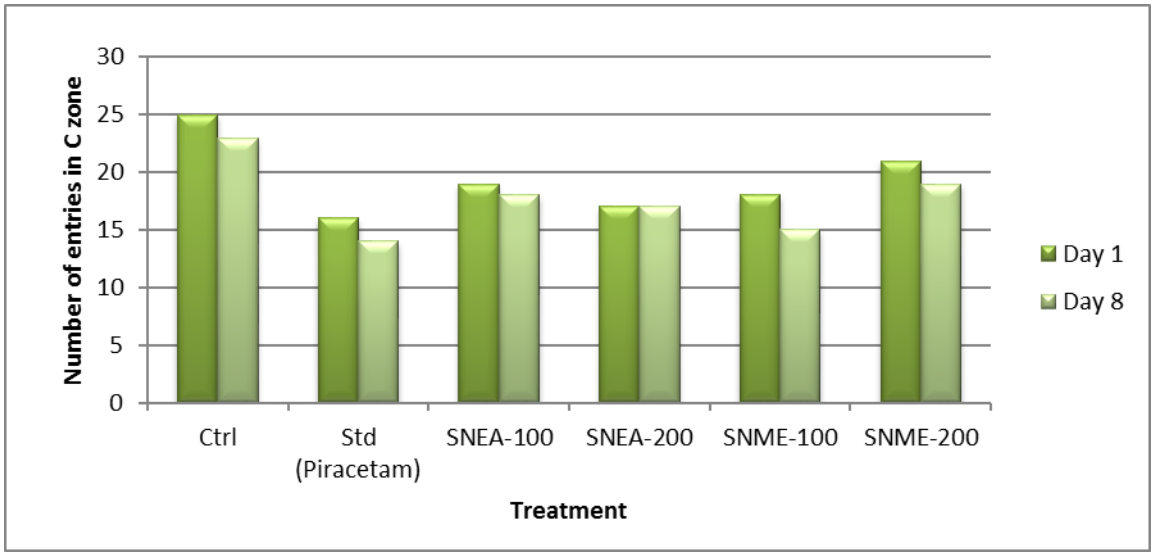


Chart 5: Number of entries in C zone of rats during experimental period

The number of entries in C zone on day 8 when compared with day 1 it was found that SNEA-100, SNEA-200 & SNME-100, SNME-200 shows no significant difference as compared to control but standard shows highly significant difference on day 8. All test doses shows no significant difference when compared with Standard.

Sr. No	Group name	Number of Entries in P zone	
		Day 1	Day 8
1	Ctrl	15.2	23.2
2	Std	23.4	38.0**
3	SNEA-100	27.4	35.8**#
4	SNEA-200	28.8	36.8**#
5	SNME-100	26.6	36.8**#
6	SNME-200	27.2	37.4**#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

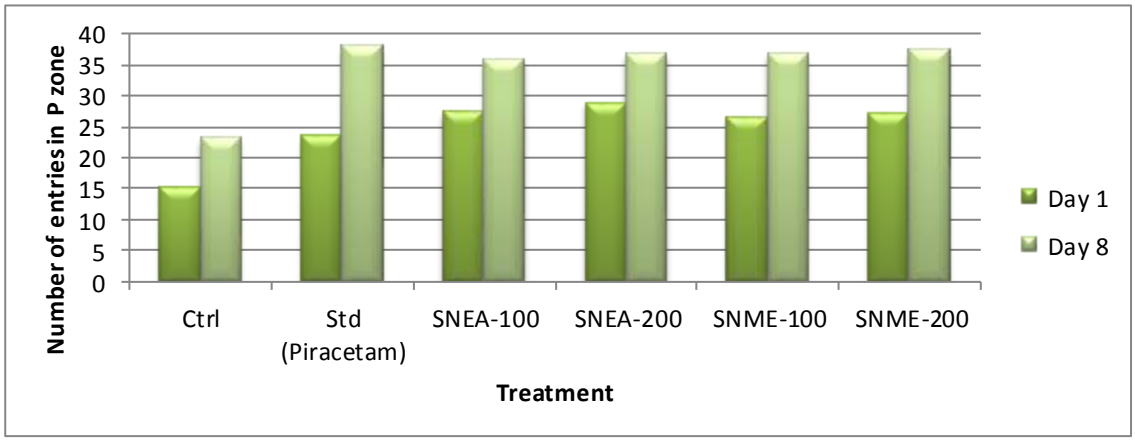


Chart 6: Number of entries in P zone of rats during experimental period

The number of entries in P zone on day 8 when compared with day 1 it was found SNEA-100, SNEA-200 & SNME-100, SNME-200 shows highly significant difference as compared to control (P<0.001). All test doses shows no significant difference when compared with Standard.

Table No. 11: Time spent in C and P zone of rats for Memory enhancing activity of *Solanum nigrum* (Ethyl acetate and Methanolic) leaves extracts

Sr. No	Group name	Time spent in C zone	
		Day 1	Day 8
1	Ctrl	74.6	73.4
2	Std	62.2	31.0**
3	SNEA-100	65.6	50.6**
4	SNEA-200	62.4	48.8**
5	SNME-100	60.8	45.4**
6	SNME-200	58.0	43.2**

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly Significant difference when compared to control, #-No Significant difference when

compared to Standard, Δ-Significant difference when compared to Standard but more activity.

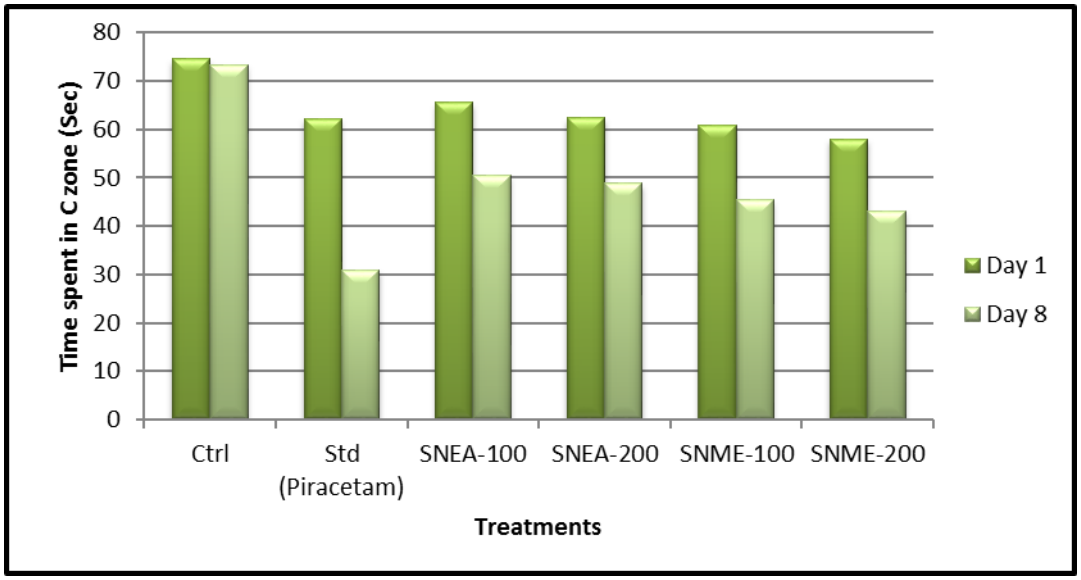


Chart 7: Time spent in C zone of rats during experimental period

The time spent in C zone on day 8 when compared with day 1 it was found that SNEA-100, SNEA-200 & SNME-100, SNME-200 shows highly significant difference as compared to control(P<0.001). All test doses shows no significant difference when compared with Standard.

Sr. No	Group name	Time spent in P zone	
		Day 1	Day 8
1	Ctrl	161.2	161.4
2	Std	145.8	220.2**
3	SNEA-100	129.6	172.4**#
4	SNEA-200	140.2	177.2**#
5	SNME-100	131.2	183.0*#
6	SNME-200	142.6	190.2#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One way ANOVA, followed by Tukey test. * P<0.05-Significant difference when compared to control, ** P<0.001- Highly

Significant difference when compared to control, #-No Significant difference when compared to Standard, Δ-Significant difference when compared to Standard but more activity.

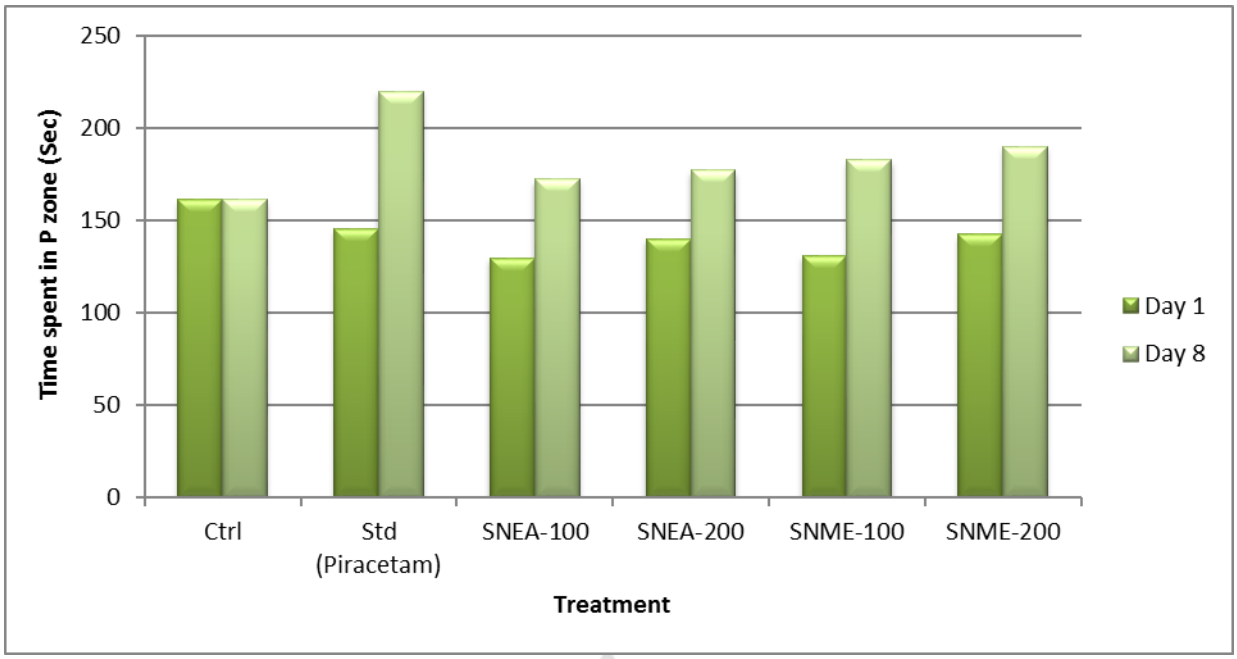


Chart 8: Time spent in P zone of rats during experimental period

The time spent in P zone on day 8 when compared with day 1 it was found that the SNEA-100 and SNEA-100 shows significant difference when compared to control($P<0.05$) but SNME-100 & SNME-200 shows highly significant difference ($P<0.001$). All test doses shows significant difference when compared with Standard.

DISCUSSION

We conclude from the literature study and experimental results analysis that *Solanum nigrum* is a traditional remedy for hepatitis, fever, ulcer, and various immunological applications in cancer and others. The plant is beneficial in preventing hepatotoxicity & cytotoxicity thus improving functions of liver and Kidney. It also finds in analgesic, anti inflammatory, antimicrobial, anti-diabetic, immune-stimulant, central nervous system and brain functioning. It can really contribute to medical and pharmaceutical practices.

Preliminary phytochemical evaluation of Ethyl acetate & methanolic extracts was carried out for the determination of presence of phytoconstituents along with TLC fingerprinting. Both extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids,

and saponins. The spots at R_f values (Ethyl acetate extract) 0.18, 0.50, and (methanolic extract) 0.52, 0.72, 0.88 represents the presence of Morin, Flavanone, 6- Hydroxyflavone, Galangin, Flavone in the extracts. Antioxidant property of *Solanum nigrum* leaves extracts was carried out by using DPPH radical scavenging assay technique. In this method percentage inhibition of test sample was calculated and compared with percentage inhibition of standard (ascorbic acid). By this method the percentage inhibition shown by the Ethyl acetate & methanolic extracts were 80.97% and 74.14% respectively. Whereas standard Ascorbic acid showed 96.22% percentage inhibition at 150 µg/ml. This provides evidence that ethyl acetate extract of *Solanum nigrum* leaves has potent antioxidant activity and it can be used as an antioxidant agent.

Acute toxicity studies (OECD 423: Acute Oral Toxicity-Class Method) of extracts of *Solanum nigrum* leaves conducted by researchers revealed that the graded doses administration of extracts (up to a dose of 2000 mg/kg) did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and in appearance of the animals. No death was recorded up to the dose of 2000 mg/kg body weight. The result of such studies showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD_{50}) 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 memory enhancing activity of *Solanum nigrum* leaves extracts was evaluated by using the Radial arm maze and by using Wistar rats as an animal model.

The memory enhancing activity of Ethyl acetate and methanolic extracts of *Solanum nigrum* leaves was evaluated in rats by daily exposing them to the radial arm maze with the food pellet in a fix arm of maze. Food pellets were placed in a variable arm for evaluation of working memory. It is characterized by increase in latency to find the food and time spent in selected arm. The results were drawn by evaluating time spent & number of entries in P zone.

The results showed that the highest dose (200mg/kg) of both the extracts, showed highly significant memory enhancing activity when given orally in daily single dose. The findings suggest effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through ability of the animals to cause a significant decrease in number of errors and increase in latency to find the food and time spent in selected zone as well.

At the end of the study, it was observed that group no. 6 i.e. ethyl acetate extract treated group at dose of 200mg/kg showed maximum number of entries at P zone **(37.4±0.42**#).** The same group showed maximum time spent at P zone **(190.2±12.4#)** as well. Whereas the other groups i.e. group no. 3 (ethyl acetate at dose of 100 mg/kg) group no. 4 (methanolic at dose of 200mg/kg) and group no. 5 (mehtanolic at dose of 100 mg/kg) represents **183±5.74*#, 177.2±9.77**#, 172.4±0.91**#**values respectively for time spent in P zone. The number of entries of these groups at P zone were found to be **36.8 ±0.30**#, 36.8±0.30**#, 35.8 ±0.65**#**respectively. All these values were compared with standard drug i.e. Piracetam at dose of 200mg/kg.

From the results it was revealed that both extract i.e. ethyl acetate and methanolic showed effective memory enhancing activity. Although methanolic extract at 200 mg/kg showed more superior and significant to highly significant (from $P < 0.05$ to $P < 0.001$) memory enhancing activity by using radial arm maze in rats.

SUMMARY AND 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 many microbial attacks where its memory enhancing potential is claimed to be useful. The extracts of *Solanum nigrum* leaves tested for memory enhancing activity by researchers. No methodical reports on memory enhancing activity of *Solanum nigrum* leaves were available. The present study aimed at evaluating the *In-vivo* memory enhancing activity of *Solanum nigrum* leaves extract in rats. Ethyl acetate and methanolic extracts were prepared by the hot extraction process, i.e. by using Soxhlet apparatus. Preliminary phytochemical evaluation of ethyl acetate and methanolic extract was carried out for the determination of presence of phytoconstituents.

Antioxidant property of *Solanum nigrum* leaves was carried out by using DPPH radical scavenging assay technique respectively. In DPPH assay all the extracts showed promising antioxidant activity, however Methanolic extract of *Solanum nigrum* leaves revealed significant antioxidant activity.

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 (LD_{50}) 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 ethyl acetate and 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 (from $P < 0.05$ to $P < 0.001$) 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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