INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD
ISSN(O): 2455-0620 [ Impact Factor: 7.581 ]
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value : 86.87
Volume - 9, Issue - 8, August - 2023 Publication Date: 05/08/2023

DOIs:10.2015/IJIRMF/202308001 --:-- Research Paper / Article / Review

# "TO EVALUATE THE DOSE DEPENDENT EFFECT OF BERBARINE ON SCOPOLAMINE INDUCED MEMORY DEFICIT IN RATS"

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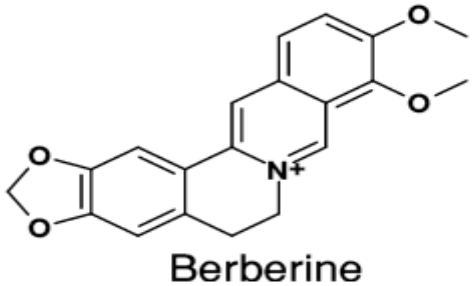
***Abstract:*** *The effect of Berberine (BER) on Scopolamine (SCOP)-induced amnesia was investigated in a step-through passive avoidance task in rats. It was observed that BER at the doses of 3mg/kg Scopolamine 3mg/kg and Berberine 100mg/kg after 7-day administration significantly improved SCOP-induced amnesia. The anti-amnesic effect of BER after 7-day administration on the SCOP-induced amnesia was significantly augmented by physostig mine or neostigmine, and completely reversed by scopolamine N-methylbromide. These results suggest that the antiamnesic effect of BER after 7-day administration may be related to the increase in theperipheral and central cholinergic neuronal system Activity.*

***Keywords :*** *Berberine, Scopolamine, cholinergic , peripheral, antiamnesic, neostigmine.*

## 1. INTRODUCTION :

Berberine is an isoquinoline alkaloid isolated from the coptidis rhizome, which has been used in Chinese medicine with a long history of clinical benefits. With evidence of safe and efficient application in human and animals, Berberine has used in clinical therapies for several types of dementia in china and Japan. Recent study also shows that beyond its main applications, Berberine can provide beneficial effects via the induction of autophagy in pulmonary fibrosis and atherosclerosis.

Berberine is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids. Berberine is a natural alkaloid found in a wide variety of traditional herbs, including golden seal, barberry, gold thread, Oregon grape, tree turmeric and phellodendron. Within these plants, the Berberine alkaloid can be found in the stem bark, roots and rhizomes (root like subterranean stems) of the plants. The chemical Berberine is found in a handful of plants widely used in botanical Medical practice including Goldenseal (Hydrastis Canadensis). Oregon grape (Berberis aquifolium), Barberry (Berberis vulgaris), and Chinese Goldthread (Coptischinensis).Berberine is a bitter-tasting, yellow compound found in plants. And Berberine is slowly soluble in water. It has a long history of medical use in Chinese and Ayurvedic medicine. Berberine is found in the roots and stem bark of many plants, including goldenseal, coptis or golden thread, Oregon grape, barberry, and tree turmeric. Berberine has also been used as a yellow dye.



Berberine

There is some evidence to support the use of Berberine for eye infections, bacterial diarrhea, and some parasite infections (leishmaniasis). Berberine may also protect against viruses, fungi, protozoans, worms, and chlamydia. More research is needed in these areas, as well as heart disease, skin disorders, and liver disorders.Antagonism against the effects of cholera and E coli heat-

stable enterotoxin. Inhibition of intestinal ion secretion and of smooth muscle contraction and Reduction of inflammation astimulation of bile secretion and bilirubin discharge. Berberine is generally thought safe. However, it may interact with many prescription medications. Berberine should not be used by pregnant or breastfeeding women, due to the risk of problems in the newborn.

Berberine has several pharmacological properties including neuroprotective effect against cerebral ischemia, mental depression, and anxiety. There are several protective mechanisms reported for Berberine in the literature like antioxidant activity, anti – apoptotic, anti – inflammatory activity. The neuroprotective effect different drugs have been reported to improve memory. Therefore in this study it was speculated that berberine could prevent memory impairment. Thus in this study effect of Berberine was evaluated in scopolamine induced memory deficit.

## 2. Materials and Method:

### 2.1 Animals

25 Sprague Dawley Male rats (5 rats per group; 170 -280 g) were housed in standard cages at 25±2°C degree. In this study five groups were taken and each group was containing 5 rats.Group 1 was Naive group and group 2 received scopolamine (3 mg/kg; i.p.), group 3 received scopolamine and berberine (25 mg/kg; p.o.), group 4 received scopolamine and berberine (50 mg/kg; p.o.) and group 5 received scopolamine and berberine (100 mg/kg; p.o.). The treatment schedule was followed up to 7 days.

| **Groups (5 rats / group)** | **Treatment** |
|---|---|
| Group 1 | Naïve |
| Group 2 | Scopolamine 3mg/kg |
| Group 3 | Scopolamine 3mg/kg + Berberine25mg/kg |
| Group 4 | Scopolamine 3mg/kg + Berberine 50mg/kg |
| Group 5 | Scopolamine 3mg/kg + Berberine 100mg/kg |

On day 6, effect on locomotor activity (acquisitions), passive shock avoidance test and T maze was evaluated. On day 7, animals were againlocomotor activity (retention), passive shock avoidance test and T maze was evaluated

After behavioral estimation, animals were sacrificed under deep anesthesia and blood was collected through retro orbital sinus. Different organs (heart/kidney/brain/liver) was isolated and stored at -20ºC.

### 2.2 Behavioral assessment

#### 2.2.1 Locomotor activity

Instrument used: Animal activity meter-opto-varimax-4 auto track with six boxes (Columbus instruments) Locomotor behavior of the exposed and treated animals was evaluated in the open- field test. The open-field behavior of rats was assessed in a box measuring 90 cm X 90 cm X 30cm. sub-divided into 19 equal squares by black lines. Immediately after a rat was placed in the center of the open field, the movements of the rat were scored. Variables which were recorded during a 5 minute session include: number of squares crossed with all paws (crossings), standing on legs (rearings), standing on hind legs and placing fore limbs on the wall (wall rearings) and placing nose against floor (sniffing) were counted in all sessions. Testing was carried out on two consecutive days in five minute session. Locomotor activity of the animal was recorded in following parameters Distance Travelled (DT), Resistance Time (RT), Ambulatory Time (AT), Stereotypic Time (ST), Burst of Stereotypic Movement (BSM), Horizontal Count (HC), and Ambulatory Count (AC).

#### 2.2.2 Learning and memory in passive shock avoidance paradigm

Instrument Used: Passive and active Avoidance Chamber System's (PACS-30) (Columbus Instruments) The learning and memory capacities (acquisition and processing of information, decision making and response initiating) are being assessed in Mn exposed and treated rats by passive avoidance in a shuttle box. It is a standard, sensitive psychopharmacological test of learning and memory in rats. The apparatus consisted of two identical chambers, one of which was well lit. Chamber without light is darkened using a black partition. The rats were placed one at a time, in the lighted compartment, following which the door separating the two chambers was lifted. As soon as the rat entered the dark compartment within 90 seconds the door was shut and was presented at randomly selected time intervals of 10-50s. Each tone lasted 5 s, and was followed by 30s exploration time, 0.3 mA grid intensity, 5 lux light intensity.

### 2.2.3. Spontaneous alternations in T maze

A rat has made its choice when it has placed all four of its feet onto one arm. Some rats will tentatively place a foot onto each arm before making a final decision. Return the rat to the starting platform for a set time (from 0 sec to many min; 5 sec is ideal to begin). Repeat this nine times and record the number of entries into each arm (Control rats should alternate their arm choices within each test session).Express results as the number of alternations divided by the total number of choices made per session. Present data either as a histogram showing the percent correct choices averaged across a given number of tests for controls, or drug-treated groups, or as a line drawing comparing percent alternations for each group versus each daily test session (Data from daily test sessions can also be averaged into 2-day blocks).

### 2.2 Hematological assays

After behavioral assessments rats were anesthetized and 1ml blood was collected through cardiac puncture.
Note: The blood was mixed with anti-coagulating agent (Tri Sodium Citrate)

### 2.3.1 Red Cell Count

For RBC Count Blood was sucked in RBC pipette to the mark 0.5.Pipetted blood was then diluted with RBC diluting fluid i.e. Hayem's solution up to the mark 101.This is ideal fluid for diluting the bloodasitisotonic and neither cause hemolysis nor crenation of red cells. It has a fixative to preserve the shape of RBCs and it also has the property to prevent blood from autolysis.After diluting blood discard few drops before filling the neubauer chamber. And a cover slip was placed on the neubauer chamber then the diluted blood was filled into the chamber. The excessive blood was removed. And the slide was observed under the microscope at 40 X magnification.

***Calculation of RBCs:***

Let x be the number of cells in 1/50 $mm^3$ of diluted blood.
Then, Cells in 1 $mm^3$ of diluted blood = x × 50
As, Dilution employed was 1 in 200
Therefore, the number of cells in 1 $mm^3$ of undiluted blood will be = x*50*200 = x*10000 Here dilution factor is 10000.

### 2.3.3 Platelet Count

For platelet count the blood was sucked in WBC pipette to the mark 0.5.Pipetted blood was then diluted with platelet diluting fluid up to the mark 11. And in this also few drops were discarded before filling the neubauer chamber. A cover slip was placed on the neubauer chamber and the diluted blood was filled into the chamber. The excessive blood was removed. And the slide was observed under the microscope at 40 X magnification.

*Calculation of Platelet:*

Let x be the number of cells in 1/50 $mm^3$ of diluted blood.
Then, Cells in 1 $mm^3$ of diluted blood = x × 50
As, Dilution employed was 1 in 200
Therefore, the number of cells in 1 $mm^3$ of undiluted blood will be = x*50*200 = x*10000 Here dilution factor is 10000.

### 2.3.4 Hemoglobin (Sahli-Adams Hemoglobinometer)

The Hb present in a measured amount of blood (20microlitre) is converted by adding 8-10 drops of dilute hydrochloric acid (0.1N) into acid hematin, which gives a golden brown color on mixing. More the intensity of colormore the concentration of acid hematintherefore, more is the concentration of Hb. After 6-8 minutes the color of the solution, is matched against the goldenbrown tinted glass rods by direct vision by diluting it with the help of water. And then readings were observed in g%.

### 2.3.5 Packed Cell Volume (PCV) Hematocrit (Hct)

In this, the Wintrobe's tube was filled with blood to the zero mark using Pasteur Pipette.Thetube was then centrifuged at 3000 rpm for 30 minutes at 4degree Celsius temperature.After centrifugation the reading was observed in mm.Even under optimum conditions red cells don't get completely pack together. And about 2% plasma remains trapped in between the red cells. This percentage is more (i.e., more plasma) if the red cells are abnormal in shape (e.g. spherocytosis, sickle cells). To compensate for the trapped plasma, the 'true' cell volume (true hematocrit) can be obtained by multiplying the observed Hct value with 0.98.

$$PCV = \frac{\text{Height of packed red cells (mm)} * 100}{\text{Height of packed RBCs and plasma (mm)}}$$

### 2.3.6 Mean Corpuscular Volume (MCV)

The MCV is the average or mean volume of a single red blood cell expressed in cubic micrometers (µm$^3$ or femtoliters). It can be calculated from the following two basic formulas:

MCV = PCV X10 or PCV per liter
RBC count in million/mm$^3$ RBC ($10^3$/mm)

### 2.3.7 Mean Corpuscular Hemoglobin (MCH)

The MCH is also determined indirectly. It is the average hemoglobin content (weight of Hb) in a single red blood cell expressed in picograms (micromicrogram, µµg). It is calculated from the following formula:

MCH = Hb in g% X 10
RBC count in million/) mm$^3$

### 2.3.8 Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC represents the relation between the red cell volume and its degree or percentage saturation with hemoglobin, that is, how many parts or volumes of a red cell are occupied by Hb. The MCHC does not take into consideration the RBC count, but represents the actual Hb concentration in red cells only, (i.e., not in whole blood)—expressed as saturation of these cells with Hb.MCHC is calculated from the following formula:

MCHC = Hb g% X 100
PCV%

## 3. Results :

### 3.1 Behavioural assessment:

During different treatments one animal from Berberine 100 & two animals from Berberine 25 died.

### 3.1.1. Effect of different treatment on Locomotor activity

**Locomotor activity in Naive group**

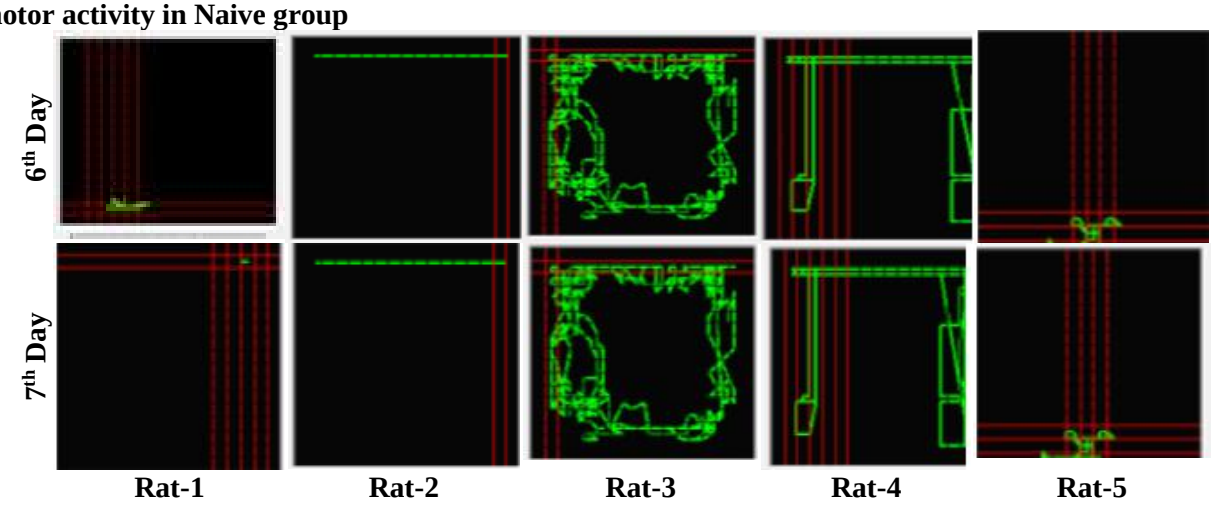



**Figure No. 1: The Locomotor activity Graph of Naive Group.**

**Table No. 1: The Locomotor activity of Naive group animals**

**6th day**

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 293 | 5 | 2 | 5 | 10 | 3 |
| 2 | 171 | 257 | 17 | 26 | 13 | 139 | 110 |
| 3 | 997 | 147 | 41 | 112 | 27 | 823 | 595 |
| 4 | 359 | 206 | 35 | 59 | 30 | 296 | 209 |
| 5 | 75 | 254 | 19 | 27 | 17 | 60 | 29 |
| **Mean** | **321.60** | **231.40** | **23.40** | **45.20** | **18.40** | **265.60** | **189.20** |
| SD | 357.95 | 50.45 | 12.98 | 37.99 | 9.15 | 295.02 | 215.20 |
| **SEM** | **160.08** | **22.56** | **5.80** | **16.99** | **4.09** | **131.94** | **96.26** |

**7th Day**

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 37 | 279 | 15 | 6 | 13 | 20 | 9 |
| 2 | 15 | 279 | 16 | 5 | 11 | 25 | 6 |
| 3 | 1622 | 73 | 53 | 174 | 35 | 1365 | 1086 |
| 4 | 382 | 176 | 46 | 78 | 39 | 303 | 221 |
| 5 | 31 | 284 | 8 | 8 | 6 | 33 | 15 |
| **Mean** | **417.40** | **218.20** | **27.60** | **54.20** | **20.80** | **349.20** | **267.40** |
| SD | 617.77 | 83.17 | 18.22 | 66.02 | 13.48 | 519.12 | 417.38 |
| **SEM** | **276.27** | **37.19** | **8.15** | **29.52** | **6.02** | **232.15** | **186.66** |

**Locomotor Activity in Scopolamine (control) treated group**

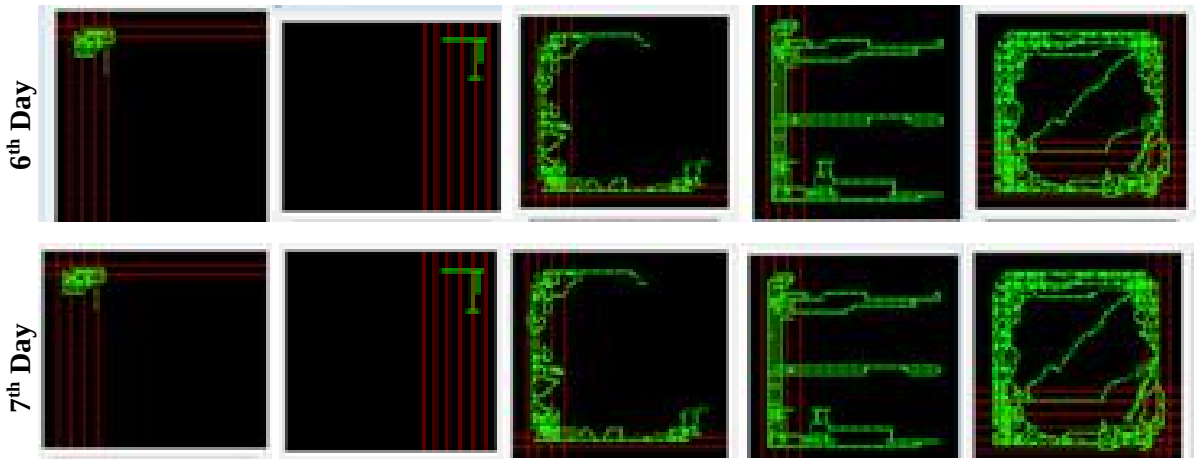



**Figure No. 2: The Locomotor activity graph of Scopolamine (control) treated group**

**Table No. 2: The Locomotor activity of Scopolamine (control) treated group**

**6th** day

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 135 | 237 | 30 | 33 | 16 | 132 | 70 |
| 2 | 15 | 278 | 17 | 5 | 14 | 36 | 17 |
| 3 | 720 | 103 | 81 | 116 | 56 | 616 | 388 |
| 4 | 406 | 197 | 37 | 66 | 32 | 363 | 276 |
| 5 | 1782 | 58 | 56 | 186 | 42 | 1314 | 1076 |
| **Mean** | **611.60** | **174.60** | **44.20** | **81.20** | **32.00** | **492.20** | **365.40** |
| SD | 633.55 | 82.23 | 22.30 | 64.11 | 15.84 | 457.21 | 380.02 |
| **SEM** | **283.33** | **36.77** | **9.97** | **28.67** | **7.08** | **204.47** | **169.95** |

**7th Day**

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 128 | 252 | 22 | 26 | 18 | 127 | 87 |
| 2 | 38 | 279 | 9 | 12 | 9 | 30 | 18 |
| 3 | 273 | 232 | 27 | 41 | 19 | 240 | 159 |
| 4 | 270 | 242 | 18 | 40 | 15 | 251 | 202 |
| 5 | 1022 | 169 | 38 | 93 | 29 | 750 | 595 |
| **Mean** | **346.20** | **234.80** | **22.80** | **42.40** | **18.00** | **279.60** | **212.20** |
| SD | 349.41 | 36.44 | 9.62 | 27.49 | 6.58 | 248.63 | 201.42 |
| **SEM** | **156.26** | **16.29** | **4.30** | **12.26** | **2.91** | **111.22** | **90.07** |

**Locomotor activity of scopolamine plus berberine (25 mg/kg) treated group**

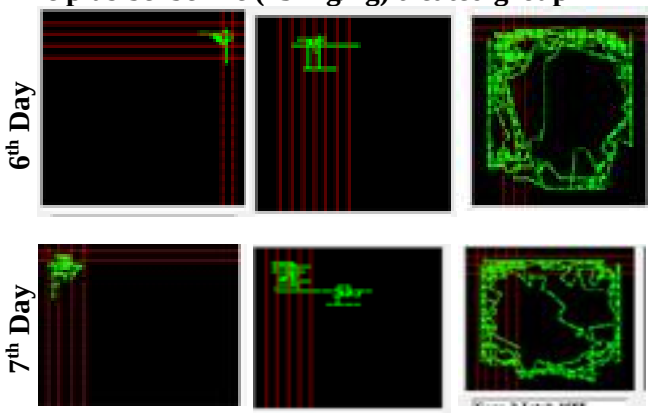



**Figure No. 3: The Locomotor activity graph scopolamine plus berberine treated group**

**Table No. 3: The Locomotor activity of scopolamine plus berberine (25 mg/kg) treated group**

**6th day**

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 70 | 215 | 65 | 20 | 44 | 135 | 34 |
| 2 | 133 | 205 | 59 | 36 | 33 | 187 | 92 |
| 3 | 1635 | 65 | 78 | 157 | 45 | 1799 | 1447 |
| **Mean** | **612.66** | **161.66** | **67.33** | **71.00** | **40.66** | **707** | **524.33** |
| SD | 723.35 | 68.47 | 7.93 | 61.16 | 5.43 | 772.45 | 652.85 |
| **SEM** | **417.62** | **39.53** | **4.57** | **35.31** | **3.13** | **445.97** | **376.92** |

**7th day**

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 101 | 212 | 62 | 26 | 42 | 136 | 56 |
| 2 | 434 | 180 | 49 | 71 | 35 | 290 | 146 |
| 3 | 1373 | 52 | 95 | 153 | 43 | 1651 | 1240 |
| **Mean** | **636.00** | **148.00** | **68.66** | **83.33** | **40.00** | **692.33** | **480.66** |
| SD | 538.57 | 69.12 | 19.362 | 52.57 | 3.55 | 680.78 | 538.18 |
| **SEM** | **310.94** | **39.91** | **11.17** | **30.35** | **2.05** | **393.05** | **310.72** |

**Locomotor activity of scopolamine plus berberine (50 mg/kg) treated group**

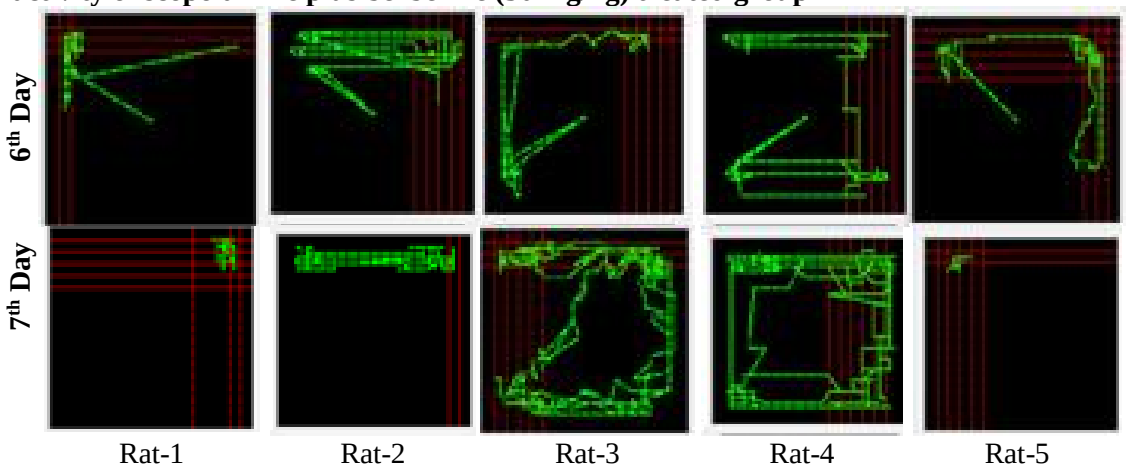



**Figure No. 4: The Locomotor activity graph of scopolamine plus Berberine (50 mg/kg) treated group**

**Table No. 4: The Locomotor activity of scopolamine plus Berberine (50 mg/kg) treated group**

$6^{th}$ day

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 176 | 182 | 77 | 41 | 57 | 226 | 136 |
| 2 | 508 | 172 | 55 | 73 | 36 | 438 | 313 |
| 3 | 1631 | 75 | 55 | 170 | 33 | 1513 | 1194 |
| 4 | 1560 | 91 | 45 | 164 | 32 | 1059 | 868 |
| 5 | 161 | 251 | 19 | 30 | 16 | 121 | 66 |
| **Mean** | **807.20** | **154.20** | **50.20** | **95.60** | **34.80** | **671.40** | **515.40** |
| SD | 655.87 | 64.38 | 18.78 | 60.01 | 13.10 | 531.84 | 440.83 |
| **SEM** | **293.31** | **28.79** | **8.40** | **26.83** | **5.86** | **237.85** | **197.14** |

$7^{th}$ day

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 194 | 173 | 88 | 39 | 42 | 1133 | 134 |
| 2 | 336 | 234 | 30 | 36 | 23 | 746 | 235 |
| 3 | 249 | 211 | 55 | 34 | 38 | 2809 | 125 |
| 4 | 468 | 178 | 63 | 59 | 26 | 572 | 294 |
| 5 | 159 | 239 | 34 | 27 | 23 | 235 | 78 |
| **Mean** | **281.20** | **207.00** | **54.00** | **39.00** | **30.40** | **1099.00** | **173.20** |
| SD | 110.87 | 27.44 | 21.04 | 10.75 | 8.01 | 902.67 | 79.16 |
| **SEM** | **49.58** | **12.27** | **9.41** | **4.808** | **3.58** | **403.68** | **35.40** |

**Locomotor activity of scopolamine plus berberine (100 mg/kg) treated group**

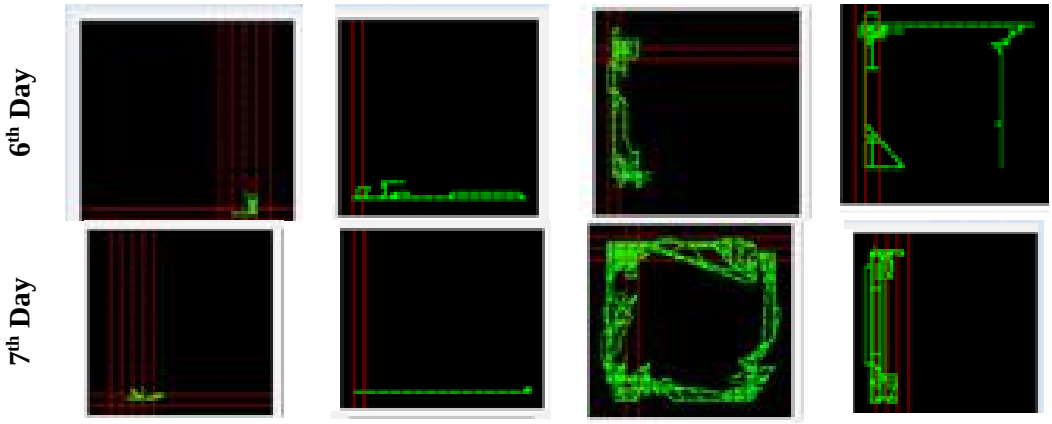



**Figure No. 5: The Locomotor activity graph of scopolamine plus berberine (100 mg/kg) treated group**

**Table No. 5: The Locomotor activity of scopolamine plus berberine (100 mg/kg) treated group**

$6^{th}$ day

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 221 | 229 | 32 | 39 | 26 | 151 | 72 |
| 2 | 581 | 167 | 51 | 82 | 38 | 471 | 340 |
| 3 | 1513 | 91 | 53 | 156 | 37 | 1350 | 1086 |
| 4 | 126 | 210 | 56 | 34 | 40 | 144 | 46 |
| **Mean** | **610.25** | **174.25** | **48.00** | **77.75** | **35.25** | **529.00** | **386.00** |
| SD | 548.13 | 53.05 | 9.40 | 48.87 | 5.44 | 492.06 | 420.21 |
| **SEM** | **274.06** | **26.52** | **4.70** | **24.43** | **2.72** | **246.03** | **210.10** |

7th day

| Animal No. | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| 1 | 24 | 277 | 14 | 9 | 13 | 30 | 13 |
| 2 | 575 | 168 | 57 | 75 | 38 | 529 | 390 |
| 3 | 493 | 192 | 36 | 72 | 26 | 420 | 292 |
| 4 | 227 | 208 | 48 | 44 | 37 | 179 | 108 |
| **Mean** | **329.75** | **211.25** | **38.75** | **50.00** | **28.50** | **289.50** | **200.75** |
| SD | 218.42 | 40.54 | 16.11 | 26.58 | 10.11 | 196.17 | 148.31 |
| **SEM** | **109.21** | **20.27** | **8.05** | **13.29** | **5.05** | **98.08** | **74.15** |

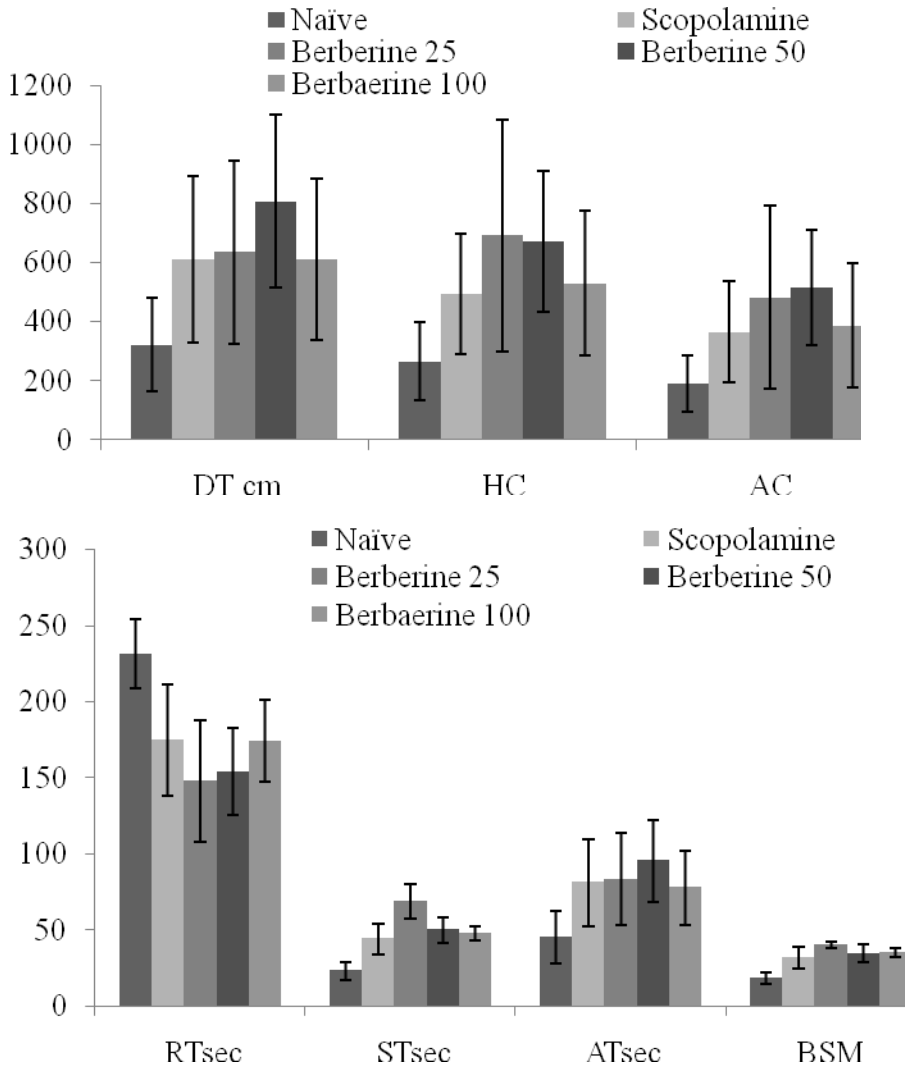



**Figure 6: Effect of different treatment on locomotor activity in rats on day 6**

All the data has been mentioned as Mean ± SEM. Statistical analysis was done using one way ANOVA followed by Tukey's test. Statistical significance was considered at $P < 0.05$.

**Table No. 6: Effect of different treatment on locomotor activity in rats**
**On 6th Day**

| Mean | DT (cm) | RT (s) | ST (s) | AT (s) | BSM | HC | AC |
|---|---|---|---|---|---|---|---|
| Naïve | 321.60±160.08 | 231.40±22.56 | 23.40±5.80 | 45.20±16.99 | 18.40±4.09 | 265.60±131.94 | 189.20±96.26 |
| Scopolamine | 611.60±283.33 | 174.60±36.77 | 44.20±9.97 | 81.20±28.67 | 32.00±7.08 | 492.20±204.47 | 365.40±169.95 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Berberine 25 | 612.66±417.62 | 161.66±39.91 | 67.33±4.57 | 71.00±35.31 | 40.66±3.13 | 707.00±445.97 | 524.33±376.92 |
| Berberine 50 | 807.20±293.31 | 154.20±28.79 | 50.20±8.40 | 95.60±26.83 | 34.80±5.86 | 671.40±237.85 | 515.40±197.14 |
| Berberine 100 | 610.25±274.06 | 174.25±26.52 | 48.00±4.70 | 77.75±24.43 | 35.25±2.72 | 529.00±246.03 | 386.00±210.10 |

**On 7th Day**

| **Mean** | **DT (cm)** | **RT (s)** | **ST (s)** | **AT (s)** | **BSM** | **HC** | **AC** |
|---|---|---|---|---|---|---|---|
| Naïve | 417.40±276.28 | 218.20±37.19 | 27.60±8.15 | 54.20±29.53 | 20.80±6.03 | 349.20±232.16 | 267.40±186.66 |
| Scopolamine | 346.20±156.26 | 234.80±16.29 | 22.80±4.30 | 42.40±12.27 | 18.00±2.91 | 279.60±111.22 | 212.20±90.08 |
| Berberine 25 | 636.00±310.94 | 148.00±39.91 | 68.67±11.17 | 83.33±30.35 | 40.00±2.05 | 692.33±393.05 | 480.67±310.72 |
| Berberine 50 | 281.20±49.58 | 207.00±12.27 | 54.00±9.41 | 39.00±4.81 | 30.40±3.58 | 1099.00±403.69 | 173.20±35.40 |
| Berberine 100 | 329.75±109.21 | 211.25±20.27 | 38.75±8.06 | 50.00±13.29 | 28.50±5.06 | 289.50±98.09 | 200.75±74.16 |

All the data has been mentioned as Mean ± SEM. Statistical analysis was done using one way ANOVA followed by Tukey's test. Statistical significance was considered at $P < 0.05$. * indicates significant change as compared to naive group; # indicates significant change as compared to scopolamine (Control) group.

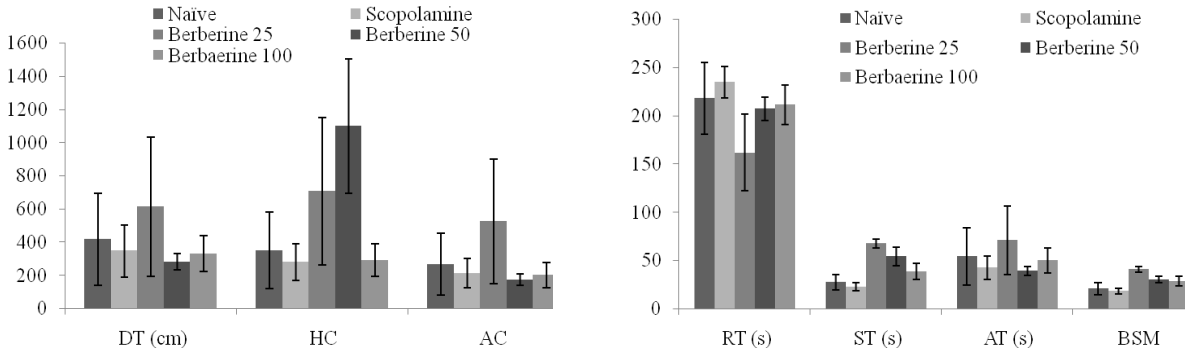



**Figure 7: Effect of different treatment on Locomotor activity in rats on day 7**
All the data has been mentioned as Mean ± SEM. Statistical analysis was done using one way ANOVA followed by Tukey's test. Statistical significance was considered at $P < 0.05$.

There was no significant change observed in different parameters of locomotor activity in different treatment groups. On day 6 and 7, there was no significant change observed in DT, RT, AT, ST, BSM, HC, and AC of the scopolamine treated rats as compared to naïve rats. Similarly, treatment with different doses of bereberine did not change the locomorotor activity as compared to scopolamine treated alone.

### 3.1.2. Effect of different treatment on transfer latency in Passive shock avoidance System

**Table No. 7: Effect on Transfer Latency in naive group**

| **Animal No.** | **Transfer latency time** | | **No. of trials** |
|---|---|---|---|
| | **6th Day** | **7th Day** | |
| 1 | 13.60 | 90.00 | 2 |
| 2 | 7.30 | 2.40 | 2 |
| 3 | 11.20 | 24.80 | 2 |
| 4 | 12.20 | 7.30 | 2 |
| 5 | 14.20 | 19.20 | 2 |
| **Mean** | **11.70** | **28.74** | **2** |
| SD | 2.43 | 31.66 | 0 |
| **SEM** | **5.23** | **12.85** | **0.89** |

**Table No 8: Effect on Transfer Latency in scopolamine (control) group**

| Animal No. | Transfer latency time | | No. of trials |
|---|---|---|---|
| | 6th Day | 7th Day | |
| 1 | 15.30 | 8.60 | 2 |
| 2 | 90.00 | 12.20 | 2 |
| 3 | 90.00 | 90.00 | 2 |
| 4 | 90.00 | 25.80 | 2 |
| 5 | 90.00 | 90.00 | 2 |
| **Mean** | **75.06** | **45.32** | **2** |
| SD | 29.88 | 36.92 | 0 |
| **SEM** | **33.56** | **20.26** | **0.89** |

**Table No 9: Effect on Transfer Latency in Scopolamine and Berberine (25mg/kg) treated group**

| Animal No. | Transfer latency time | | No. of trials |
|---|---|---|---|
| | 6th Day | 7th Day | |
| 1 | 90.00 | 10.20 | 2 |
| 2 | 90.00 | 5.40 | 2 |
| 3 | 12.70 | 7.70 | 2 |
| **Mean** | **64.23** | **7.76** | **2** |
| SD | 36.43 | 1.96 | 0 |
| **SEM** | **21.03** | **1.13** | **0** |

**Table No 10: Effect on Transfer Latency in Scopolamine and berberine (50mg/kg) treated group**

| Animal No. | Transfer latency time | | No. of trials |
|---|---|---|---|
| | 6th Day | 7th Day | |
| 1 | 12.80 | 51.00 | 2 |
| 2 | 5.20 | 90.00 | 2 |
| 3 | 90.00 | 90.00 | 2 |
| 4 | 90.00 | 90.00 | 2 |
| 5 | 19.2.0 | 90.00 | 2 |
| **Mean** | **43.44** | **82.20** | **2** |
| SD | 38.27 | 15.60 | 0 |
| **SEM** | **17.11** | **6.97** | **0** |

**Table No 11: Effect on Transfer Latency in Scopolamine and Berberine (100mg/kg) treated group**

| Animal No. | Transfer latency time | | No. of trials |
|---|---|---|---|
| | 6th Day | 7th Day | No. of trials |
| 1 | 25.40 | 90.00 | 2 |
| 2 | 20.50 | 38.50 | 2 |
| 3 | 5.10 | 33.50 | 2 |
| 4 | 4.70 | 90.00 | 2 |
| **Mean** | **13.95** | **63.00** | **2** |
| SD | 9.19 | 27.05 | 0 |
| **SEM** | **4.59** | **13.52** | **0** |

**Table No. 12. Effect of different treatment on Acquisition and retention phase**

| Treatments Groups | Acquisition phase (6th Day) | Retention phase (7th Day) |
|---|---|---|
| **Naïve** | 11.70±5.23 | 28.74±12.85 |
| **Scopolamine** | 75.06±33.57 | 45.32±20.27 |
| **Berberine 25** | 64.23±21.04 | 7.767±1.31 |
| **Berberine50** | 43.44±17.12 | 82.20±6.98 |
| **Berberine 100** | 13.93±4.60 | 63.00±13.53 |

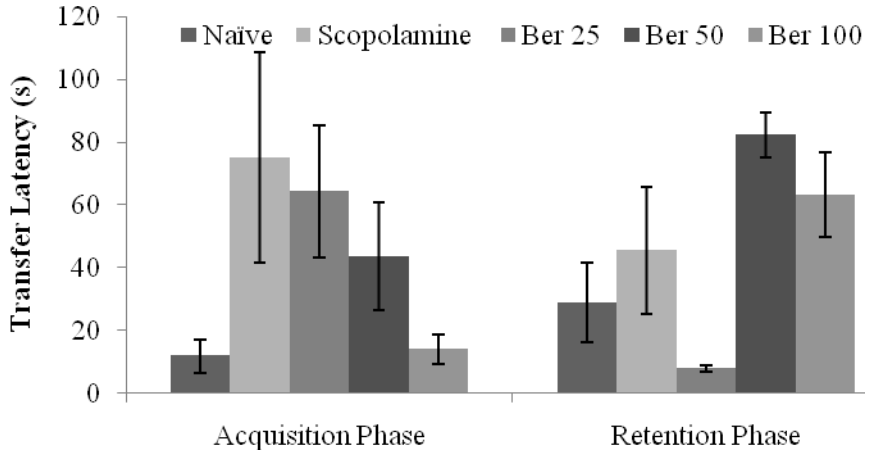



**Figure 8: Effect of different treatment on Acquisition and retention phase**

All the data has been mentioned as Mean ± SEM. Statistical analysis was done using one way ANOVA followed by Tukey's test. Statistical significance was considered at $P < 0.05$. * indicates significant change as compared to naive group; # indicates significant change as compared to scopolamine (Control) group.

### 3.1.3. Effect of different treatment on Spontaneous alternations in T-maze

**Table No 13: Effect of different treatment on Spontaneous alternations in T-maze**

| No. of trials | Group 1 | | | | | Group 2 | | | | | Group 3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rat-1 | Rat-2 | Rat - 3 | Rat-4 | Rat-5 | Rat-1 | Rat-2 | Rat - 3 | Rat-4 | Rat-5 | Rat-1 | Rat-2 | Rat - 3 |
| 1 | L | R | R | L | R | L | L | L | R | R | R | R | R |
| 2 | L | L | R | R | R | R | R | L | R | L | L | L | L |
| 3 | R | L | L | L | R | R | R | L | L | L | L | L | L |
| 4 | R | L | L | L | L | L | L | L | R | L | R | R | R |
| 5 | R | R | R | R | R | R | L | L | R | L | L | R | L |
| 6 | R | R | R | R | L | L | L | L | R | R | R | R | R |
| 7 | L | L | R | R | R | R | R | R | R | R | L | L | L |
| 8 | L | R | L | L | L | L | L | L | L | L | R | R | L |
| 9 | R | R | L | L | R | R | L | R | R | L | L | R | L |
| Total | 3 | 4 | 3 | 4 | 6 | 7 | 4 | 3 | 4 | 3 | 7 | 4 | 4 |
| Percentage | 33.33 | 44.44 | 33.33 | 44.44 | 66.67 | 77.78 | 44.44 | 33.33 | 44.44 | 33.33 | 77.78 | 44.44 | 44.44 |

| No. of trials | Group 4 | | | | | Group 5 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rat-1 | Rat-2 | Rat -3 | Rat-4 | Rat-5 | Rat-1 | Rat-2 | Rat -3 | Rat-4 |
| 1 | L | L | R | R | L | R | L | L | R |
| 2 | L | R | R | R | R | L | L | R | L |
| 3 | L | L | L | R | R | L | L | R | L |
| 4 | R | L | R | R | L | L | R | R | L |
| 5 | R | L | R | L | L | L | L | R | L |
| 6 | L | L | L | L | L | R | L | R | L |
| 7 | L | L | L | R | L | L | L | R | L |
| 8 | L | R | R | R | R | L | L | L | L |
| 9 | L | R | L | R | R | L | R | L | R |
| Total | 2 | 2 | 5 | 2 | 3 | 3 | 3 | 2 | 2 |
| Percentage | 22.22 | 22.22 | 55.56 | 22.22 | 33.33 | 33.33 | 33.33 | 22.22 | 22.22 |

| Percentage alternations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | **Mean** | SD | **SEM** |
| **Naïve** | 33.33 | 44.44 | 33.33 | 44.44 | 66.67 | **44.44** | 13.61 | **6.09** |
| **Scopolamine** | 77.78 | 44.44 | 33.33 | 44.44 | 33.33 | **46.66** | 18.26 | **8.17** |
| **Berberine 25** | 77.78 | 44.44 | 44.44 | | | **55.56** | 19.25 | **11.11** |
| **Berberine 50** | 22.22 | 22.22 | 55.56 | 22.22 | 33.33 | **31.11** | 14.49 | **6.48** |
| **Berberine 100** | 33.33 | 33.33 | 22.22 | 22.22 | | **27.78** | 6.42 | **3.21** |

**Table No 14: Effect of different treatments on percentage alternations in T Maze**

| | **Percentage Alternations** | |
|---|---|---|
| **Naive** | 44.44 | ± 6.09 |
| **Scopolamine** | 46.66 | ± 8.16 |
| **Berberine 25** | 55.56 | ± 11.11 |
| **Berberine 50** | 31.11 | ± 6.48 |
| **Berberine 100** | 27.78 | ± 3.21 |

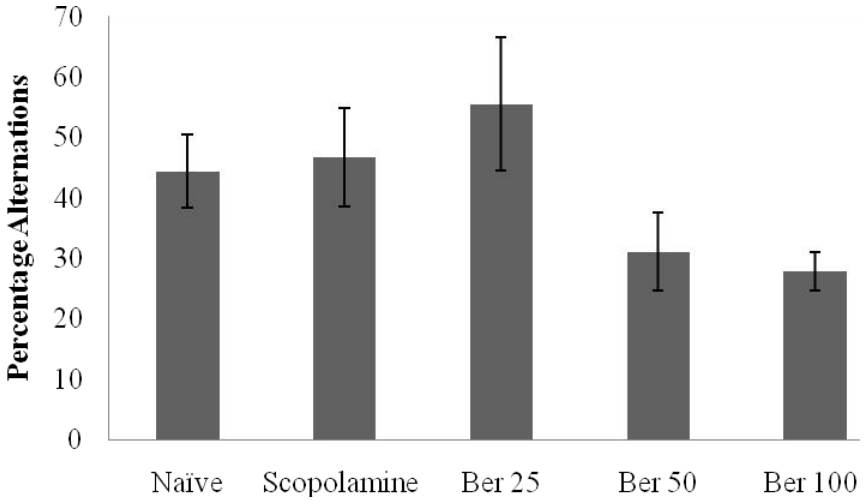



**Figure 9: Effect of different treatments on percentage alternations in T Maze**

| Table 15. Effect of Different Treatments on Hematological Variables | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Naive** | **RBC Count ($10^4$ cells/mm$^3$)** | **Platelet Count ($10^4$ cells/mm$^3$)** | **Hb (g%)** | **PCV** | **MCV (µm$^3$)** | **MCH (µm$^3$)** | **MCH C** |
| 1 | 421.01 | 487.03 | 12.20 | 2.40 | 9.86 | 32.75 | 0.48 |
| 2 | 635.06 | 456.09 | 13.80 | 3.80 | 7.82 | 27.65 | 0.36 |
| 3 | 445.02 | 365.07 | 11.00 | 2.50 | 8.65 | 35.75 | 0.39 |
| 4 | 386.04 | 378.03 | 9.00 | 1.50 | 6.35 | 26.45 | 0.45 |
| MEAN | 471.78 | 421.55 | 11.50 | 2.55 | 8.17 | 30.65 | 0.42 |
| SD | 111.51 | 51.39 | 1.75 | 0.82 | 1.27 | 3.77 | 0.05 |
| SEM | 49.87 | 22.98 | 0.78 | 0.36 | 0.57 | 1.68 | 0.03 |

| Scopolamine | RBC Count ($10^4$ cells/mm$^3$) | Platelet Count ($10^4$ cells/mm$^3$) | Hb (g %) | PCV | MCV (µm$^3$) | MCH (µm$^3$) | MCHC |
|---|---|---|---|---|---|---|---|
| 1 | 389.09 | 345.09 | 9.00 | 2.30 | 8.32 | 21.56 | 0.58 |
| 2 | 569.34 | 451.56 | 13.00 | 2.20 | 6.35 | 20.54 | 0.37 |
| 3 | 456.21 | 423.09 | 7.00 | 2.30 | 9.52 | 27.89 | 0.56 |
| 4 | 540.38 | 315.34 | 9.80 | 2.10 | 7.46 | 25.78 | 0.65 |
| MEAN | 488.75 | 383.77 | 9.70 | 2.22 | 7.91 | 23.94 | 0.54 |
| SD | 81.95 | 64.08 | 2.49 | 0.09 | 1.34 | 3.47 | 0.13 |
| SEM | 36.65 | 28.65 | 1.12 | 0.04 | 0.59 | 1.55 | 0.05 |

| Ber25 | RBC Count ($10^4$ cells/mm$^3$) | Platelet Count ($10^4$ cells/mm$^3$) | Hb (g %) | PCV | MCV (µm$^3$) | MCH (µm$^3$) | MCHC |
|---|---|---|---|---|---|---|---|
| 1 | 485.06 | 380.08 | 9.00 | 3.30 | 9.56 | 34.56 | 0.76 |
| 2 | 407.56 | 402.07 | 7.00 | 3.40 | 6.78 | 35.67 | 4.67 |
| 3 | 398.09 | 325.03 | 8.00 | 3.20 | 7.34 | 37.42 | 3.65 |
| MEAN | 430.23 | 369.06 | 8.00 | 3.30 | 7.89 | 35.88 | 3.02 |
| SD | 47.71 | 39.68 | 1.00 | 0.10 | 1.47 | 1.44 | 2.02 |
| SEM | 27.54 | 22.91 | 0.57 | 0.05 | 0.84 | 0.83 | 1.17 |

| Ber50 | RBC Count ($10^4$ cells/mm$^3$) | Platelet Count ($10^4$ cells/mm$^3$) | Hb (g %) | PCV | MCV (µm$^3$) | MCH (µm$^3$) | MCHC |
|---|---|---|---|---|---|---|---|
| 1 | 467.89 | 468.5 | 9.60 | 3.2 | 4.89 | 27.78 | 0.45 |
| 2 | 237.56 | 389.07 | 9.00 | 2.6 | 6.78 | 25.98 | 0.59 |
| 3 | 432.87 | 345.87 | 6.60 | 2 | 5.65 | 31.67 | 0.69 |
| 4 | 278.78 | 459.43 | 6.70 | 3.2 | 6.98 | 32.89 | 0.78 |
| 5 | 345.98 | 345.78 | 6.00 | 1.2 | 8.21 | 30.45 | 0.81 |
| MEAN | 352.61 | 401.73 | 7.58 | 2.44 | 6.50 | 29.75 | 0.66 |
| SD | 98.05 | 59.57 | 1.60 | 0.85 | 1.27 | 2.83 | 0.14 |
| SEM | 43.85 | 26.64 | 0.71 | 0.38 | 0.57 | 1.26 | 0.06 |

| Ber100 | RBC Count ($10^4$ cells/mm$^3$) | Platelet Count ($10^4$ cells/mm$^3$) | Hb (g %) | PCV | MCV (µm$^3$) | MCH (µm$^3$) | MCHC |
|---|---|---|---|---|---|---|---|
| 1 | 302.98 | 287.89 | 6.00 | 3.00 | 5.67 | 23.45 | 0.56 |
| 2 | 341.67 | 209.34 | 8.00 | 2.50 | 6.78 | 20.78 | 0.67 |
| 3 | 278.89 | 230.13 | 6.40 | 3.10 | 4.89 | 29.81 | 0.59 |
| 4 | 401.45 | 265.12 | 11.00 | 3.50 | 5.78 | 27.56 | 0.45 |
| MEAN | 331.24 | 248.10 | 7.85 | 3.02 | 5.78 | 25.40 | 0.56 |
| SD | 53.47 | 35.11 | 2.27 | 0.41 | 0.77 | 4.05 | 0.09 |
| SEM | 26.73 | 17.55 | 1.13 | 0.20 | 0.38 | 2.02 | 0.05 |

| Mean | RBC Count ($10^4$ cells/mm$^3$) | Platelet Count ($10^4$ cells/mm$^3$) | Hb (g %) | PCV | MCV (µm$^3$) | MCH (µm$^3$) | MCHC |
|---|---|---|---|---|---|---|---|
| **Naïve** | 471.78±49.87 | 421.55±22.98 | 11.50±0.78 | 2.55±0.37 | 8.17±0.57 | 30.65±1.69 | 0.42±0.02 |
| **Scopolamine** | 488.76±36.65 | 383.77±28.66 | 9.70±1.12 | 2.23±0.04 | 7.91±0.60 | 23.94±1.55 | 0.54±0.05 |
| **Berberine 25** | 430.24±27.55 | 369.06±22.91 | 8.00±0.58 | 3.30±0.06 | 7.89±0.85 | 35.88±0.83 | 3.03±1.17 |
| **Berberine 50** | 352.62±43.85 | 401.73±26.64 | 7.58±0.72 | 2.44±0.38 | 6.50±0.57 | 29.75±1.27 | 0.66±0.07 |
| **Berbaerine 100** | 331.25±26.74 | 248.12±17.56 | 7.85±1.14 | 3.03±0.21 | 5.78±0.39 | 25.40±2.03 | 0.57±0.05 |

**4. Conclusion:**

The treatment with berberine in scopolamine treated rats did not affect the locomotor activity, which indicates berberine does not interfere with the motor performance. Fear conditioning memory is evaluated in passive shock avoidance apparatus. While evaluating fear conditioning memory in passive shock avoidance paradigm, berberine improved fear conditioning memory in scopolamine treated rats. Spatial memory was evaluated by recording spontaneous alternations in T Maze. The treatment of berberine did not change the percentage alternations in T maze. While haematological analysis, there was not significant change observed on different hematological variables. Therefore, it can be inferred that berberine did not altered the spatial memory in rats.

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