Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(4):1191-1199

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of Nootropic Effect of Ethanolic Extract of *Beta Vulgaris* L. Roots in High fat diet induced amnesia in Swiss albino mice

Ahuja D.

Research Scholar, Faculty of Pharmacy, Rajasthan University of Health Science, Jaipur, Rajasthan

---

ABSTRACT

Learning refers to the process by which brain acquires new information about the events occurring in the surroundings and recalling of information is referred to as memory. Memory comprises of registration, consolidation and retrieval. Now a days a prevalence of neurodegenerative disorders is increasing day by days. Beta vulgaris l. (beet) is the abundantly available plant in indian subcontinent, the antioxidant, anti-inflammatory & hepatoprotective potential of Beta vulgaris l. Has been established but nootropic potential of the plant has not been explored the present study is designed to evaluate the nootropic potential of Beta vulgaris l. Roots extract. In this study hydroalcoholic extract of Beta vulgaris l. Roots was evaluated for nootropic potential by using morris water maze model. The present study has been designed to investigate the effect of hydroalcoholic extract of Beta vulgaris l. Roots in high fat diet induced experimental amnesia in mice. The mice were divided into groups i.e control group, normal saline treated group, high fat diet treated group, high fat diet + metrifonate treated group, high fat diet + piracetam treated group & high fat diet + beat root 250,500,1000 mg/kg treated groups. On analysis of results it was found that the extract treated animals showed increased escape latency time spent in search of missing platform in day 5 in morris water maze. This suggests that hydroalcoholic extract of beta vulgaris l. Roots shows significant nootropic activity.

Key words: *Beta vulgaris* L., Nootropic, Morris water maze, Escape latency time.

INTRODUCTION

In resent era amnesia has become a serious problem in front of world population and prevalence of diseases having amnesia as major symptom is increasing day by day [20].

The agents already available for treating amnesic disorders in modern system of medicine has various side effects so there is a need of a natural & safe alternative to treat amnesia.

Beta Vulgaris L. roots has significant antioxidant anti-inflammatory, haemopoietic, cholesterol reducing and immunomodulatory actions due to it's specific active constituents, which can be stated by following evidances.

Evidances of antioxidant activity of *Beta vulgaris* L. roots extract's and active constituents responsible for it:
- Frank *et al.*, in 2005 reported that the ingestion of a single dose of red beet juice resultes in an increase of antioxidant compounds including betalains in urinary excretion. Kujala *et al*., in 2001 reported that Beta vulgaris L. possess antioxidants properties because of the presence of nitrogenous pigments called betalains, mainly comprise of red–violet-colored betacyanins (betanin, isobetanin, probetanin and neobetanin) and yellow–orange-colored

betaxanthyns. Escribano *et al.,* in 1998 reported that Betacyanins are a group of compounds exhibiting antioxidant and radical-scavenging activities.

Evidances of cholestrol reducing action of *Beta vulgaris* L. roots extract's and active constituents responsible for it:

- Khalili *et al.* in 2004 has evaluated it's cholestrol reducing action as the augmented triglyceride and cholesterol due to diabetes were significantly decreased by the extract. Tesoriere et al. in 2004 said that betalains enrich human low density lipoproteins.

Evidances of anti-inflammatory and antimicrobial action of *Beta vulgaris* L. roots extract's and active constituents responsible for it:

- According to Jain *et al.,* 2011 *Beta vulgaris* L. roots has significant anti-inflammatory action as it's aqueous extract showed anti-inflammatory activity in the carrageenan-induced rat paw oedema. Strack *et al.,* in 2003 reported that it has anti-inflammatory, antimicrobial and antiviral effects effects due to betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) [6,10,22].

Evidances of cancer prevenive and immunomodulator action of *Beta vulgaris* L. roots extract's and active constituents responsible for it:

- Reddy *et al. in* 2005 stated that it can also inhibit the cell proliferation of human tumor cells. Kapadia *et al.,* in 1996 stated that beetroot ingestion can be considered a factor in cancer prevention because Beetroots (Beta vulgaris) are rich in valuable, active compounds such as carotenoids [7], glycine betain [5], saponins [3], folates [13].

By above it is clear that Beta Vulgaris L. roots has significant antioxidant anti-inflammatory, cholesterol reducing and immunomodulatory action and because major causes of amnesia are oxidative damage [11], inflammation[18], high cholesterol level [2], and exaggarated auto immune response [1] so study is undertaken to evaluate *Beta vulgaris* L. roots extract's amnesia relieving action as it has still also not been scientifically evaluated.

EXPERIMENTAL SECTION

ANIMALS

All the experiments were carried out using swiss albino mice of either sex produced from ivri bareilly, u.p. India. Young mice (3-4 month old) weighing around 20 g and aged mice (12-18 month old) around 35 g were used in the present study. The animals were housed, 12 hr. Light and 12 hr. Dark cycle in the departmental animal house with free access to water and standard diet. The animals were acclimatized to the laboratory condition five days prior to behavioral study.

All experiments were performed as per the norms of the institutional animal ethical committee of jaipur college of pharmacy (931/ac/06/CPCSEA) and the studies were approved and clearance obtained by the 'institutional review board'.

EXTRACTION OF BETA VULGARIS

Procurement and Authentification of crude drug

the plant material was procured from market of jaipur & it was authentified at jayoti vidyapeeth women's university, jaipur by botanist "Shazia Khan" and herbarium sheet was deposited with voucher specimen number JVWU/PCG/H/2010/99.

Extraction

The fresh roots of *Beta vulgaris L.* (1 kg, cut into small pieces) were exhaustively extracted in 95% (1.5 L) ethanol by continuous heat extraction in soxhlet apparatus. The obtained alcoholic extract was then evaporated on water bath till complete drying. Percent yield was calculated for each extract after drying [4] .

Determination of percentage yield

The percentage yield of extract was calculated by using following formula: Percentage yield = (Weight of extract / Weight of raw material taken) X 100

Acute toxicity study

The acute toxicity study was carried out in adult female wistar rats by "fixe dose" method of OECD (organization for economic co-operation and development) guideline no.420. Fixed dose method as in annex 2d: test procedure with a starting dose of 2000 mg/kg body weight was adopted. The animals were fasted overnight and next day extracts were administered orally at dose level 2000 mg/kg. Then the animals were observed continuously for three

hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days. The observations were tabulated according to 'irwin's table' (OECD guidelines-420).

Selection of doses

For the assessment of nootropic activity, dose level was chosen in such a way that, dose was approximately one tenth to one half of the maximum dose taken during acute toxicity studies (200 to 1000 mg/kg).

MORRIS WATER MAZE

Morris water maze [26], was employed to evaluate learning and memory. It consisted of a circular water tank (diameter 122 cm. and height 51 cm.) and was filled with water up to 30 cm. (at 25 C). 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 platform (10 cm") of 29 cm. height was located in the centre of one of these four quadrants. The position of the platform and clues were kept constant throughout the training session. In the present study, the target quadrant was Q4. Each animal was subjected to four consecutive trials on each day with an interval of 5 min, during which they were allowed to remain on the platform for 20 sec. In case the animal was unable to locate the hidden platform with in 120 sec. It was gently guided by hand to the platform and was allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition. Rats were subjected to acquisition trial for four consecutive days. On the 5th day. The platform was removed and time spent by animal in each quadrant was noted. The time spent by the animal in target quadrant and (Q4) in search of missing platform was noted as an index of retrieval.

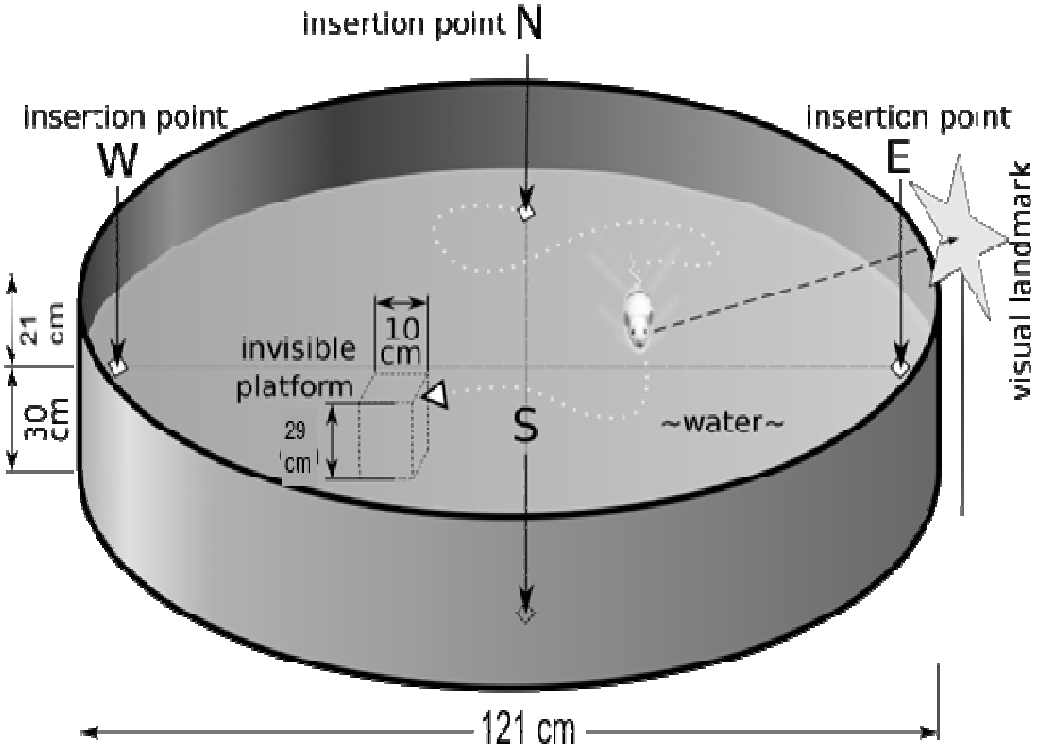

Fig.No-1: Diagrammatic Presentation of Morris Water Maze

a. Acquisition trial

Each mouse was subjected to four trials on each day (after 16 day of drug treatment). A rest interval of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four-acquisition trial was changed as described below and Q4 was maintained as target quadrant in all acquisition trials.

Day 1	Ql	Q2	Q3	Q4
Day II	Q2	Q3	Q4	Ql
Day III	Q3	Q4	Ql	Q2
Day IV	Q4	Ql	Q2	Q3

Mean escape latency time (ELT) calculated each day during acquisition trial was used as an index of acquisition.

b. Retrieval trial

On day 5th. the platform was removed. Each mouse was placed in water maze and allowed to explore the maze for 120 sec. Each rat was subjected to four such trials and each trial was started from different quadrant. Mean time spent in target quadrant i.e. Q4 in search of missing platform provided an index of retrieval. Care was taken that relative location of water maze with respect to other subject in laboratory serving as visual clues were not disturbed during the total duration of the study.

EXPERIMENTAL DESIGN

Animals were divided into groups and each group comprised of 6 mice.

A. Design for Morris water maze

Group I Control Group [26] **:** Control, mice were administered distilled water (10 ml/kg, i.p.) 45 min before conducting the acquisition trials (day 1 to day 4) and retrieval trial (day 5).

Group II Normal Saline Treated Group [26]: Vehicle treated, mice were administered normal saline (10 ml/kg, i.p.) 45 min before conducting the acquisition trials (day 1 to day 4) and retrieval trial (day 5).

High fat diet (HFD) induced amnesia

Group III High fat diet (HFD) induced amnesia [26]**:** Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were exposed to the water maze for four consecutive days during acquisition trials (day l to day 4) and retrieval trial was carried out on day 5.

Group IV High fat diet (HFD) + metrifonate [26]:Mice were administered metrifonate (50 mg/kg, i.p.) daily for 4 days and again for fifth consecutive day (day l to day 5, 45 min before) during acquisition and retrieval trials.

Group V High fat diet (HFD) + piracetam [26]: mice were administered piracetam (400 mg/'kg, i.p.) daily for 4 days and again for fifth consecutive day (day l to day 5, 45 min before) during acquisition and retrieval trials.

Group VI, VII, VIII High fat diet (HFD) + beat root [8]**:** HFD mice were administered *Beta vulgaris* L. (hydroalcoholic extract 250, 500 and 1000 mg/kg body weight respectively for 70 days) and again for five consecutive days (day l to day 5) during acquisition and retrieval trials.

Statistical analysis

The data of activity were analyzed by one way analysis of variance (ANOVA) followed by "Dunnet's test" by using graph pad prism version 4 software.

RESULTS

Extractive yield : The percentage yield obtained for Hydroalcoholic Extract of *Beta Vulgaris* L. Roots was 57% w/w.

Pharmacological studies

Acute Toxicity studies

Acute Toxicity studies on female mice showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days. The Behavioral, Neurological, Autonomic responses were studied for a time period of 6 hrs of toxicity study. During the study no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

Mortality in oral toxicity study (Roots Extract)

S.No.	Treatment (Hydroalcholic extract) 50:50	Dose (mg/kg)	Number of animals	Mortality			Toxicity profile
				After 24 Hrs.	After 7 days	After 14 days	
1.	Dried leaves Extract orally	2000	5	0	0	0	Safe

Escape latency time (ELT) - time spent in target quadrant

Mice, were administered distilled water (10 ml/kg, i.p.) 45 min before acquisition trials conducted on day 1 to day 4. Escape latency time was significantly decreased as compared to its value noted on day 1 with successive acquisition trials conducted on day 2 to day 4.

Mice, administered distilled water (10 ml/kg i.p.) 45 min before retrieval trial (day 5) spent significantly more time in the target quadrant (Q4) in search of missing platform as compared to time spent in other quadrants (Q1, Q2, Q3) during retrieval trial.

Table: 1

Treatment with Distilled Water (10 ml/kg, i.p.)	Mean Avg. Time Spent in Target in seconds (Quadrant 1)	Mean Avg. Time Spent in Target in seconds (Quadrant 2)	Mean Avg. Time Spent in Target in seconds (Quadrant 3)	Mean Avg. Time Spent in Target in seconds (Quadrant 4)
Day 1 (Acquisition Trial)	20±0.577	21±0.365	20±0.365	20±0.000 Sec (Constant)
Day 2 (Acquisition Trial)	18±0.577	19±0.365	18±0.577	20±0.000 Sec (Constant)
Day 3 (Acquisition Trial)	17±0.447	19±0.365	17±0.447	20±0.000 Sec (Constant)
Day 4 (Acquisition Trial)	17±0.447	18±0.577	17±0.447	20 ±0.000 Sec (Constant)
Day 5 (Retrival Trial)	24 ±0.577	26 ±0.516	24 ±0.577	80 ±0.577

Effect of Normal Saline on acquisition and retrieval of memory

Administration of normal saline (10 ml/kg, i.p.) daily for 4 days 45 min before acquisition trials conducted on day 1 to day 4 did not produce any significant effect on decrease in ELT when compared with control group. Normal saline (10 ml/kg, i.p.) did not produce any marked effect on time spent in target quadrant (Q4) in search of missing platform during retrieval trial condiicted on day 5 when compared with control group.

Table: 2

Treatment with Normal Saline (10 ml/kg, i.p.)	Mean Avg. Time Spent in Target in seconds (Quadrant 1)	Mean Avg. Time Spent in Target in seconds (Quadrant 2)	Mean Avg. Time Spent in Target in seconds (Quadrant 3)	Mean Avg. Time Spent in Target in seconds (Quadrant 4)
Day 1 (Acquisition Trial)	19 ±0.365	20 ±0.577	19 ±0.365	20 ±0.000 Sec (Constant)
Day 2 (Acquisition Trial)	17 ±0.447	18 ±0.577	17 ±0.447	20 ±0.000 Sec (Constant)
Day 3 (Acquisition Trial)	17 ±0.447	17 ±0.447	17 ±0.447	20 ±0.000 Sec (Constant)
Day 4 (Acquisition Trial)	16 ±0.258	17 ±0.447	16 ±0.577	20 ±0.000 Sec (Constant)
Day 5 (Retrival Trial)	23 ±0.577	25 ±0.447	23 ±0.365	79 ±0.577

Effect of high fat diet (HFD) on acquisition and retrieval of memory

HFD fed mice significantly showed increase in ELT during acquisition trials conducted on day 1 to day 4, as compared to control group. HFD also decreases time spent in target quadrant (Q4) in search of missing platform during retrieval trial conducted on day 5. It may be noted that impairment of acquisition due to HFD may lead to failure of retrieval.

Table No:3

Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were exposed to the water maze	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	23 ±0.365	24 ±0.577	23 ±0.365	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	21 ±0.365	22 ±0.365	21 ±0.365	20 ±0.000 Sec (Constant)
Day 3 (Acquisition trial)	21 ±0.333	21 ±0.365	21 ±0.365	20 ±0.000 Sec (Constant)
Day 4 (Acquisition trial)	20 ±0.577	21 ±0.365	20 ±0.601	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	27 ±0.577	29 ±0.258	27 ±0.577	70 ±0.447

Each column represents mean ±standard error of the mean (N=6)

Effect of metrifonate on high fat diet (HFD) induced amnesia:

Metrifonate prevented high fat diet (HFD) induced decrease in time spent in target quadrant (Q4) in search of missing platform during retrieval trial conducted on day 5.

Table No:4

Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were were administered metrifonate (50 mg/kg, i.p.) daily for 7 days and again for five consecutive days 45 minutes before conducting the trials	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	21 ±0.365	22 ±0.365	21 ±0.365	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	19 ±0.365	20 ±0.577	19 ±0.365	20 ±0.000 Sec (Constant)
Day 3 (Acquisition trial)	18 ±0.577	20 ±0.577	18 ±0.577	20 ±0.000 Sec (Constant)
Day 4 (Acquisition trial)	18 ±0.577	19 ±0.365	18 ±0.577	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	25 ±0.447	27 ±0.577	25 ±0.447	77 ±0.577

Each column represents mean ±standard error of the mean (N=6)

Effect of piracetam on high fat diet (HFD) induced amnesia:

Piracetam prevented high fat diet (HFD) induced decrease in time spent in target quadrant (Q4) in search of missing platform during retrieval trial conducted on day 5.

Table No:5

Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were were administered piracetam (400 mg/'kg, i.p.) daily for 7 days and again for five consecutive days 45 minutes before conducting the trials	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	20 ±0.577	21 ±0.365	20 ±0.577	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	18 ±0.577	19 ±0.365	18 ±0.577	20 ±0.000 Sec (Constant)
Day 3 (Acquisition trial)	17 ±0.447	18 ±0.577	18 ±0.577	20 ±0.000 Sec (Constant)
Day 4 (Acquisition trial)	17 ±0.447	18 ±0.577	17 ±0.447	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	24 ±0.577	26 ±0.516	24 ±0.577	78 ±0.365

Each column represents mean ±standard error of the mean (N=6)

High fat diet (HFD) + Beat Root

Group XXX, XXXI, XXXII: HFD + Beta vulgaris L., HFD mice were administered Beta vulgaris L. (hydroalcoholic extract 250, 500 and 1000 Mg/Kg body weight respectively for 70 days) and again for five consecutive days (day l to day 5) during acquisition and retrieval trials.

Table No:6

Mice were fed high fat diet (hfd) for 3 months. hfd fed mice were administered *Beta vulgaris* L.(hydroalcoholic extract 250 mg/kg body weight respectively for 70 days) 45 min before conducting the trials.	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	22 ±0.365	23 ±0.577	71 ±0.577	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	19 ±0.365	20 ±0.577	20 ±0.577	20 ±0.0000 Sec (Constant)
Day 3 (Acquisition trial)	19 ±0.365	19 ±0.365	20 ±0.577	20 Sec (Constant)
Day 4 (Acquisition trial)	20 ±0.577	19 ±0.365	68 ±0.516	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	27 ±0.577	27 ±0.577	26 ±0.516	75 ±0.258

Each column represents mean ±standard error of the mean (N=6)

Table No:7

Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were administered *beta vulgaris* L. (hydroalcoholic extract 500 mg/kg body weight respectively for 70 days) 45 min before conducting the trials.	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	21 ±0.365	22 ±0.365	20 ±0.577	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	18 ±0.577	19 ±0.365	19 ±0.365	20 ±0.000 Sec (Constant)
Day 3 (Acquisition trial)	18 ±0.577	18 ±0.577	19 ±0.365	20 ±0.000 Sec (Constant)
Day 4 (Acquisition trial)	19 ±0.365	18 ±0.577	17 ±0.447	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	26 ±0.516	26 ±0.516	25 ±0.447	76 ±0.258

Each column represents mean ±standard error of the mean (N=6)

Table No:8

Mice were fed high fat diet (HFD) for 3 months. HFD fed mice were administered *Beta vulgaris* L.(hydroalcoholic extract 1000 mg/kg body weight respectively for 70 days) 45 min before conducting the trials.	Mean avg. time spent in target quadrant in seconds (Quadrant 1)	Mean avg. time spent in target quadrant in seconds (Quadrant 2)	Mean avg. time spent in target quadrant in seconds (Quadrant 3)	Mean avg. time spent in target quadrant in seconds (Quadrant 4)
Day 1 (Acquisition trial)	21 ±0.365	21 ±0.365	70 ±0.447	20 ±0.000 Sec (Constant)
Day 2 (Acquisition trial)	18 ±0.577	69 ±0.365	18 ±0.577	20 ±0.000 Sec (Constant)
Day 3 (Acquisition trial)	18 ±0.577	69 ±0.365	67 ±0.365	20 ±0.000 Sec (Constant)
Day 4 (Acquisition trial)	18 ±0.577	18 ±0.577	67 ±0.365	20 ±0.000 Sec (Constant)
Day 5 (Retrival trial)	25 ±0.447	76 ±0.258	24 ±0.577	77 ±0.258

Each column represents mean ±standard error of the mean (N=6)

Table No:9 Final analysis of effect on escape latency in high fat diet induced amnesia

Group	Control	Normal saline treated animals	High fat diet treated animals	High fat diet + metrifonate treated animals	High fat diet + piracetam treated animals	High fat diet (HFD) + beat root roots extract treated animals		
						250 Mg/Kg	500 Mg/Kg	1000 Mg/Kg
Time spent in quadrant 4 on day 5 (retrival trial).	80 ±0.577	79 ±0.577	70 ±0.447	77 ±0.577	78 ±0.365	75 ±0.258	76 ±0.258	77 ±0.258

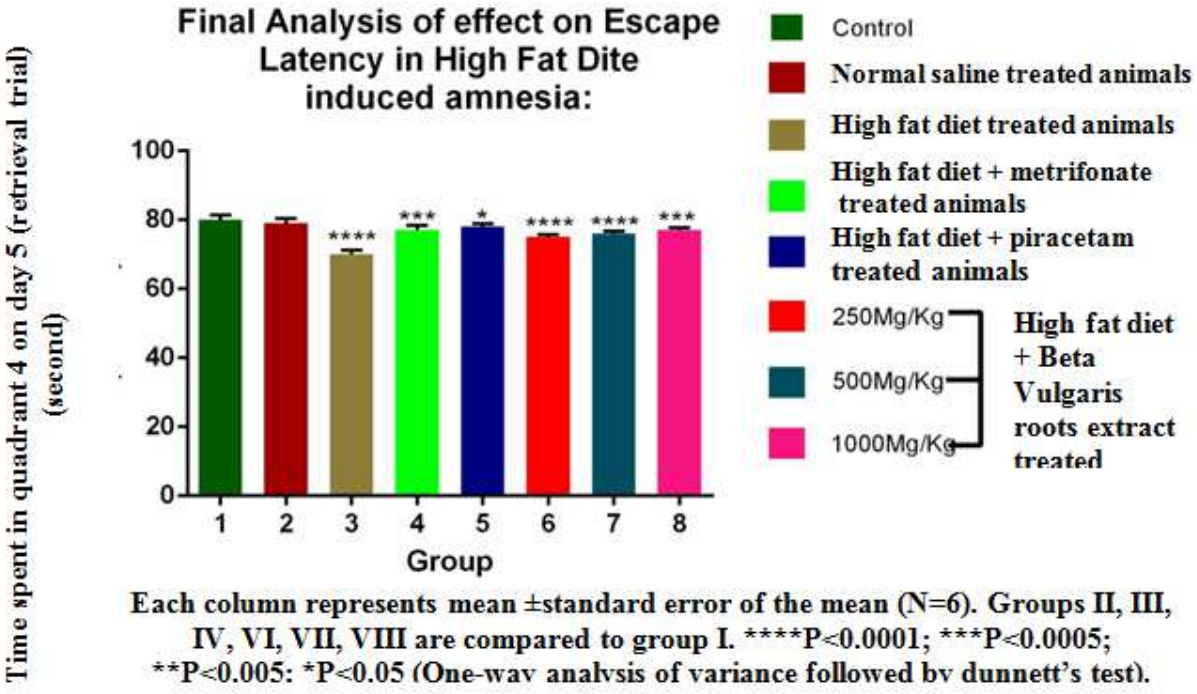

Fig.No.6 - Final analysis of effect on escape latency in high fat diet induced amnesia

The extract treated animal showed significant increase in Escape Latency Time spent in search of missing platform in day 5. This suggests that Beta Vulgaris Linn. Roots Hydroalcoholic Extract shows significant Nootropic potential.

DISCUSSION

The Memory enhancing effect of beet root extract in high fat diet induced amnesia may be due to it's significant fall in total serum cholesterol level shown by *Beta vulgaris* L.[8]. which may be due to phenolic compounds [17] and betalains which enrich human low density lipoproteins [24,25] which was found present in roots extract.

REFERENCES

[1] Ahmad A, Ramakrishna S, Meara J, Doran M., *J R Coll Physicians Edinb.*, Jun. **2010**;40(2):123-5.
[2] Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL., *Neurobiol. Aging*.,**2000**; 21: 383–421.
[3] Atamanova, A., Brezhneva, T.A., Slivkin, A.I., Nikolaevskii, V.A., Selemenev, V.F., Mironenko, N.V., "*Pharmaceutical Chemistry Journal*., **2005**; 39 (12):650–652.
[4] Chakole RD, Zade S, Charde MS., *Int. Jour. of Biomed and Adv Res*.,**2001**; 02(4): 124-127.
[5] De Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA, Chambers ST., *Food Chemistry.*, **2003**; 83: 197–204
[6] Delgado-Vargas F, Jiménez AR, Paredes-López O., *Crit Rev Food Sci Nutr.*, May **2000**;40(3):173-289.
[7] Dias MG, Camoes MFGFC, Oliveira L., *Food Chemistry.*, **2009**; 113: 808–815.
[8] Dosari Mohd Al, Saleh A, Majid A, Mohd Al Yahya., *Farmacia*.,**2011**; 59(5): 669-672.

[9] Escribano Josefa, Maria Angeles Pedreño, Francisco García-Carmona, Romualdo Muñoz., Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots., June **1998**; 9(3): 124–127.
[10] Frank T, Stintzing FC, Carle R, Bitsch I, Quaas D, Strass G, Bitsch R, Netzel M., *Pharmacol Res.*, Oct. **2005**;52(4):290-7
[11] Haddadi M, Jahromi SR, Sagar BK, Patil RK, Shivanandappa T, Ramesh SR., *Behav Brain Res.,* Feb. **2014** ;259:60-9.
[12] Jain Swati, Vipin Kumar Garg, Pramod Kumar Sharma., *Journal of Basic and Clinical Pharmacy.*, May **2011**; 2(2): 83–86.
[13] Jastrebova, J., Witthoft, C., Grahn, A., Svensson, U., Jagerstad, M., *Food Chemistry.*, **2003**; 80:579–588.
[14] Kapadia GJ, Tokuda H, Konoshima T, Nishino H., *Cancer Letters.*, **1996**;100: 211–214.
[15] Khalili M, Vaez Mahdavi MR., *Iranian Journal of Pharmaceutical Research*., Jun. **2004**: 2: 55-55.
[16] Kujala Tytti, Loponen Jyrki, Pihlaja Kalevi ., *Extraction and Characterisation Naturforsch*., 20**0**1; 56 c: 343-348.
[17] Lee Lan-Sook, Chang-Won Cho, Hee-Do Hong, Young-Chul Lee, Ung-Kyu Choi, Young-Chan Kim., *Molecules*., **2013**; 18(10): 12548-12560.
[18] Maes M., N*euro Endocrinol Lett*., June **2015**;36(1):1-6.
[19] OECD guidelines for testing of chemicals, acute dermal toxicity fixed dose procedure., **2004**; 434:1-13.
[20] Owen D , Paranandi B , Sivakumar R , Seevaratnam M ., *Postgrad Med J*., Apr. **2007**; 83(978): 236–239.
[21] Reddy KM, Ruby L, Lindo A, Nair GM., *Journal of Agricultural and Food Chemistry.*, **2005**;53: 9268–9273.
[22] Stintzing FC, Carle R., *Trends in Food Science and Technolgy.*, **2004**; 15:19–38.
[23] Strack D, Vogt T, Schliemann W., *Phytochemistry.*, **2003**; 62: 247–2.
[24] Tesoriere L, Allegra M, Butera D, Livrea MA., *Am J Clin Nutr*., Oct. **2004**;80(4):941-5.
[25] Tesoriere L, Allegra M, Butera D, Livrea MA., *Am J Clin Nutr*., Oct. **2004**;80(4):941-5.
[26] Vhores V Charles, Williams T Michale., Morris Water Maze Procedures for assessing memory Nat., Protoc.,**2006**;1(2):848-858.