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Effect Of Embellica Officinalis On Scopolamine And High Fat Diet Induced Memory Loss

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Abstract: Objective: To study the effect of Amla on High fat Diet induced memory loss as well as the Scopolamine induced memory loss on animals and checked the effect of Amla in case of disruption in the cholinergic transmission and Piracetam used as a standard substance for inducing memory power. **Materials and Methods:** This prospective study was carried out the Morris Water Maze and Elevated Plus Maze technique and different types of experimental protocol was generated based upon the High Fat Diet induced memory loss. Also checked the blood glucose and blood cholesterol level which was the most important parameter in case of obese patients.
Results: In this study, scopolamine and high fat diet induced animals group showed an increase in ELT during the acquisition trials conducted from day 1 to day 4 where as groups treated with Amla showed a decrease in the ELT during the acquisition trials. High fat diet and scopolamine treated groups reduced the time spent in target quadrant (Q4) where as Amla treated groups showed an increase in the time spent in target quadrant (Q4). High fat diet was feed to animals for 3 months and that showed an increase in the cholesterol levels whereas the groups treated with Amla showed a significant decrease in the cholesterol level which ultimately stops the neuronal damage and neurotransmission is increased.
Keywords: Embellica Officinalis ,Scopolamine ,High Fat Diet Induced Memory Loss.

Introduction

Hypercholesterolemia is a basic risk factor for inducing Alzeihmer's disease due to the deposition of amyloidal plaques in the cerebral cortex, mainly the amyloid-β peptide (Aβ1–40). These peptides are mainly inhibiting the neurotransmission of cholinergic system[1,2]. While growing evidence has shown that synaptic and cognitive dysfunction in AD is associated with intraneuronal accumulation of Aβ, the relationships between hypercholesterolemia[3] memory impairment, and intraneuronal Aβ remains unclear[4]. *Embellica officianlis* is reported to reduce the cholesterol deposition in rats as well as initiates the suppression of cholinergic system by decreasing the formation of neurofibrillary tangles and brain cholinesterase activity[5]. Excess cholesterol has

many consequences including peripheral pathology that can signal brain via cholesterol metabolites, pro-inflammatory mediators and antioxidant processes.

The present study has been designed to investigate the effect of *Embelica officinalis* on scopolamine and high fat diet induced memory loss and the effect of blood cholesterol level was examined.

Materials and Methods

Drug collection

The fresh fruits of *Embelica officinalis* were collected from the local market as the fruits are easily available. After authentication the fruits were purchased from Himgiri Traders, Dehradun in bulk, from local market they were than dried in sun and turned in to coarsed powder by grinding with the help of a mechanical grinder.

Extraction of fruits

300 gm of powdered fruit was extracted with methanol in soxlet apparatus. After the complete extraction the extract was concentrated on a water bath and finally solvent was removed under pressure. The weight of extract was recorded.

Animals

Wister rats of either sex weighing 40-60 gm considered as young were procured from Indian Veterinary Research Institute, Bareilly (U.P).

Animals were fed standard pallet diet supplied by Aashirwad industries, Punjab. The animals were housed, 12hours light and 12 hours dark in the department animal house.

Morris water maze process

Morris water maze was employed to evaluate learning and memory. It consisted of a circular water tank (diameter 150 cm and height 45 cm) and was filled with water up to 30 cm (at 25^{o}c). The tank was divided in to four quadrants with the help of two threads, fixed at right angles to each other on the rim of the pool. A platform ($10cm^2$) of 29cm,height was located in the center of one of these four quadrants. The position of the platform and clues were kept constant through the training session. In the present study the target quadrant was Q4.Each animal was subjected to four trials each day with an interval of 5 mints, during which they were allowed to stand on the platform for 20 seconds. In case if the animal was unable to locate the hidden platform in 120 seconds, the animal was gently guided with hands to platform. Escape latency time 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 5^{th} day the platform was removed and the time spent by each animal in searching for the platform in each quadrant and Q4 was noted. This time spent by the animal in target quadrant and Q4 in search of missing platform was noted as an index of retrieval [6].

Acquisition trial

Each mouse was subjected to four consecutive trials each day (after 16 days 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 for each day to conduct four-acquisition trials was changed as followed and Q4 was maintained as target quadrant in all acquisition trials.

Day 1 Q_1 Q_2 Q_3 Q_4

Day2 Q_2 Q_4 Q_3 Q_1

Day3 Q_4 Q_3 Q_1 Q_2

Day4 Q_3 Q_1 Q_2 Q_4

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

Retrieval trial

On 5th day the platform was removed. Each mouse was placed in the water maze and allowed to explore the maze for 120 seconds. Each mouse was subjected to four such trials each day starting from different quadrants. Mean time spent in target quadrant Q_4 was considered as the Index of retrieval, care was taken that the relative location of the water maze was not disturbed by other factors like changing of the background or any other changing visual clues during the total duration of study.

Elevated plus maze

The plus maze consisted of two open (50 × 10 cm) and two enclosed (50 × 10 × 40 cm)arms, connected by central platform(5× 5cm).The apparatus was subjected to a height if 25 cm above the floor. A fine line was drawn on the middle of the floor of each enclosed arms. On the first day (16th) day of the drug treatment each mice was placed at the end of the open arm, facing away from the end of the central platform. Transfer latency time was noted first day (training session). The mouse was allowed to explore the maze for 2 mints and returned to home case. Retention of this learned task was examined 24 hrs after the first task [7].

Estimation of blood glucose

Blood sample was collected from retero orbital plexus then, blood glucose was determined by the glucose oxidase peroxidase (GOD-POD). This diagnostic reagent kit (AGAPPE Diagnostics Kerala, India) is intended for simpler determination of glucose in serum/plasma/urinate samples. Glucose oxidizes the hydrogen peroxide thus produced reacts with enzyme peroxiase present in the system to liberate oxygen. This liberated oxygen reacts with the chromate system consisting phenolic compound and 4-amino antipyrene to produce quinonimine, the pink colored complex. The intensity of the color is directly proportional to the amount of glucose present in the sample and it is measured spectrophotometricallys at 530nm [8].

$$\text{Glucose} + O_2 \longrightarrow \text{Glucose acid} + H_2O_2$$

$$4H_20_2 + \text{Phenol} + 4\text{Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Red quinine} + 4H_2O$$

Estimation of Serum Cholesterol

The estimation is done on the basis of CHOD-PAP methodology [9].

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} 4\text{Cholesten-3-one} + H_2O$$

$$2H_2O_2 + \text{Phenol} + 4\text{ Aminoantipyrine} \xrightarrow{\text{POD}} \text{Red quinine} + 4H_2O_2$$

	Blank	**Standard**	**Sample**
Working reagents Standard Sample	1000µl --------- ---------	1000µl 10µl --------	1000µl --------- 10 µl

Mix and incubate for 5 minutes at 37^0C, measure the absorbance of sample and standard against the reagent blank.

Experimental protocol

Group1- Control (Animals were kept on free access to standard food pallets chow diet and water and normal saline was administered 30 mints before trials).

Group2- Scopolamine retrograde (Animals were kept on free access to standard pallets chow diet and water, normal saline was administered for 4 days 30 mints before trials and scopolamine was given on the 5th day).

Group 3- Scopolamine anterograde (Animals were kept on free access to standard food pallet chow diet and water , scopolamine was given for 4 consecutive days 30 minutes before the trial, and on 5th day it was replaced by normal saline).

Group 4- Scopolamine Retrograde +Standard (Animals were given standard food pallets chow diet and water, normal saline was administered firstly through i.p route followed by Piracetam after 15 mints for 4 consecutive days, and on 5th day saline was exchanged with Scopolamine, and trials were performed thereby15 mints .

Group5- Scopolamine anterograde + Standard (Animals were kept on free access to standard food pallet chow diet and water, Scopolamine was administered firstly through i.p route followed by Piracetam after 15 mints for 4 consecutive days and on 5th day Scopolamine was exchanged with normal saline and then trials were performed 15 mints later).

Group6- Scopolamine retrograde + Amla (Animals were given standard food pallets chow diet and water, normal saline was administered firstly through i.p route followed by Amla through oral route after 15 mints for 4 concecutive days and on 5th day saline was exchanged with scopolamine, and then trials were performed 30 mints later).

Group7- Scopolamine anterograde + Amla (Animals were given standard food pallets chow diet and water, Scopolamine was administered firstly through i.p route followed by Amla through oral route after 15 mints for 4 consecutive days and on 5th day Scopolamine was exchanged with normal saline and then trials were performed 30 mints later).

Group8- High Fat diet (animals were fed on high fat diet for three months with fee access to water and normal saline was administered through intra peritonial route 30 mints before the trials).

Group9- High fat diet + Amla (Animals were fed on high fat diet for three months with fee access to water and Amla was administered through intra peritonial route 30 mints before the trials through oral route).

Group10- High fat diet + Standard (Animals were fed on high fat diet for three months with fee access to water and Piracetam was administered through intra peritonial route 30 mints before the trials through intra peritonial route).

Stastistical analysis

All the results were expressed as mean and Standard error mean, data was analyzed by using one way ANOVA.

Results

In this study, scopolamine and high fat diet induced animals group showed an increase in ELT during the acquisition trials conducted from day 1 to day 4 where as groups treated with Amla showed a decrease in the ELT during the acquisition trials. High fat diet and scopolamine treated groups reduced the time spent in target quadrant (Q4) where as Amla treated groups showed an increase in the time spent in target quadrant (Q4). High fat diet was feed to animals for 3 months and that showed an increase in the cholesterol levels whereas the groups treated with Amla showed a significant decrease in the cholesterol level which ultimately stops the neuronal damage and neurotransmission is increased.

Discussion

Effect of Scopolamine on Escape Latency Time (E.L.T)

Escape Latency Time is the time taken by the animal to escape from the target quadrant. As per the Graph 1, we can interpret that the tendency to escape from the target quadrant is decreased from day1 to day 4 in the control group, which indicates that the animal has a good memory and as the trials conducted from day 1-day4. Animals start identifying the target quadrant that's why the tendency to escape from the target quadrant is decreased whereas in the case of Scopolamine and High Fat Diet treated groups the ELT is increases because the memory is abolished thus they are unable to remember the target quadrant even if the trials are conducted on all four days [Figure 1].

Effect of Scopolamine on Time spent in Target Quadrant

We can conclude that the control spent maximum time in the target quadrant (Q4) in comparison to High Fat Diet and Scopolamine , this is due to abolition in the memory of HFD treated group and Scopolamine[Figure 2].

Effect of Amla on HFD Induced decrease in Escape Latency Time (E.L.T)

Escape latency time induced by High fat diet is maximum as compared with other groups. High Fat Diet with Amla 8% treated group showed second highest tendency to leave the target quadrant while control shows minimum intent of leaving the target quadrant, which indicates that HFD causes memory losses due to which animals are unable to remembers the target quadrant which increases the ELT. But the groups treated with Amla improved in memory state and dcreased in ELT [Figure 3].

Effect of Amla on Time Spent in Target Quadrant with reference Piracetam

Here the control and HFD with Piracetam treated animals spent the maximum time in Q4 quadrant, while groups having HFD treatment spent minimum time in Q4, which is due to memory abolishment. Groups treated with HFD and Amla showed an improved memory as compared with HFD treated animals group [Figure 4].

Effect of Amla on Scopolamine induced decrease in E.L.T and time spent in Target Quadrant

Here the time spent by animals treated with scopolamine in target quadrant is minimum which indicates memory abolition due to anti cholinergic action of scopolamine groups treated with SCOP-Amla showed a gradual increase in time-spent in target quadrant (Q4) [Figure 5].

Effect of Amla on Blood Glucose Level

Here the graphs are drawn between various groups indicates that groups treated with HFD had maximum level of glucose and groups with Amla have gradual decrease in glucose level on day 1 and day 15 [Figure 6].

Effect of Amla on Blood Cholesterol Level

Here the graphs are drawn between various groups indicates that groups treated with HFD had maximum level of cholesterol and groups with Amla have gradual decrease in cholesterol level on day 1 and day 15 [Figure 7].

Table 1: Effect of Scopolamine on Time spent in Target Quadrant

SL NO	Factors	Day1	Day2	Day3	Day4
1	Control	80	58	35	15
2	High Fat Diet Induced	85	78	70	60
3	Scopolamine	80	83	87	90

Table 2: Effect of Scopolamine on Time spent in Target Quadrant

SL NO	Factors	Control	High Fat Diet induced	Scopolamine
1	q1			
2	q2	23.33	22.5	37
3	q3	28.33	28.76	27
4	q4	26.66	37.5	30
		41.67	31.25	22.5

Table 3: Effect of Amla on Time spent in Target Quadrant

SL NO	Factors	ql	q2	q3	q4
1	Control	23.33	28.33	26.66	41.67
2	HFD	22.5	28.75	37.5	31.25
3	HFD-Piracetam	23.75	26.25	23.75	46.25
4	HFD-Amla2%	21.25	21.25	37.5	40
5	HFD-Amla 4%	20.75	16.25	34.5	49
6	HFD-Amla 8%	15.5	19.5	32	53

Table 4: Effect of Amla on High Fat Diet induced decrease in the E.L.T

SL NO	Factors	Control	High Fat Diet induced (HFD)	HFD-Piracetam	HFD-Amla 2%	HFD-Amla 4%	HFD-Amla 8%
1	Day1	80	89	85	90	81	82
2	Day2	52	80	68	78	60	72
3	Day3	28	70	58	55	45	68
4	Day4	11	60	35	32	28	45

Table 5: Effect of Amla on Scopolamine induced decrease in E.L.T and time spent in Target Quadrant

SL NO	Factors	Control	Scopolamine (SCOP)	SCOP-Piracetam	SCOP-Amla 2%	SCOP-Amla 4%	SCOP-Amla 8%
1	Day1	78	80	80	80	82	83
2	Day2	58	85	40	78	64	68
3	Day3	38	92	32	58	54	58
4	Day4	18	98	20	20	24	38

Table 6: Effect of Amla on Blood Glucose Level

SL NO	Factors	Day 1	Day 15
1	Control	114.41	120.12
2	Scoplamine (SCOP)	101.665	109.33
3	SCOP –Amla	102.8	106.33
4	HFD-Amla2 %	101.483	100.21
5	HFD	102.35	106
6	SCOP -Piracetam	92.41	95.33

Table 7: Effect of Amla on Blood Cholesterol Level

SL NO	Factors	Day 1	Day 15
1	Control	110.97	110
2	SCOP	93.44	98
3	Control	92.44	82.56
4	HFD	118	125
5	HFD-Amla	120	88.78
6	HFD-Piracetam	119.45	107

Figure 1: Effect of Scopolamine on Time spent in Target Quadrant

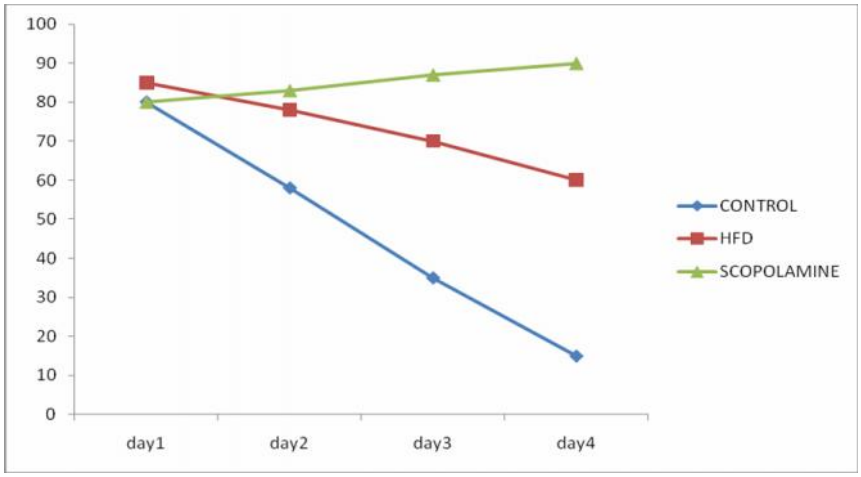


Figure 2: Effect of Scopolamine on Time spent in Target Quadrant

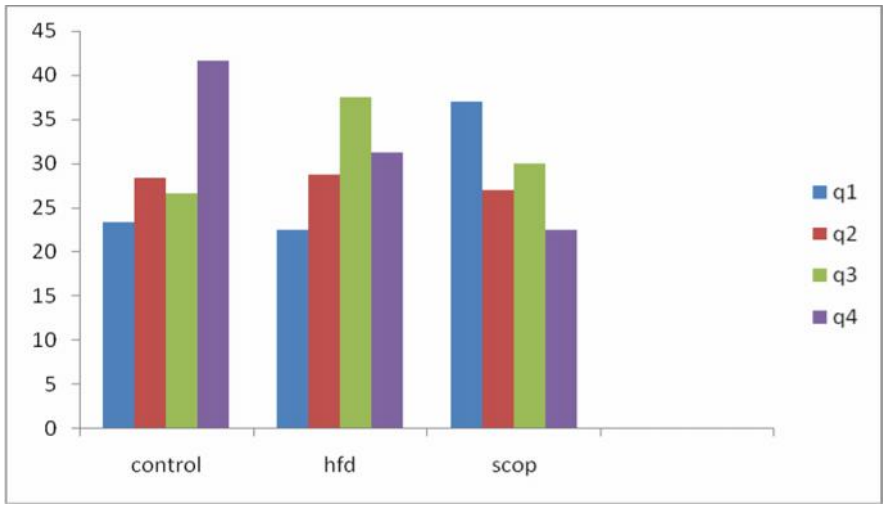


Figure 3: Effect of Amla on Time spent in Target Quadrant

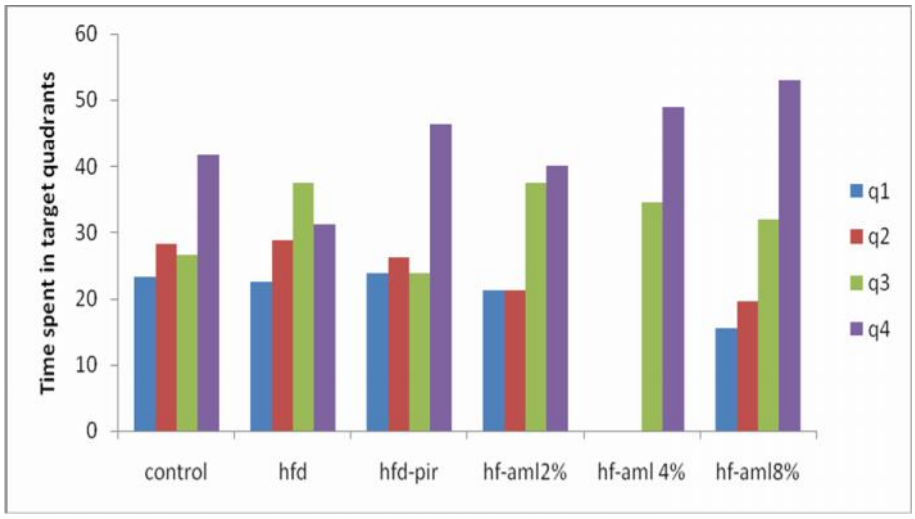


Figure 4: Effect of Amla on High Fat Diet induced decrease in the E.L.T

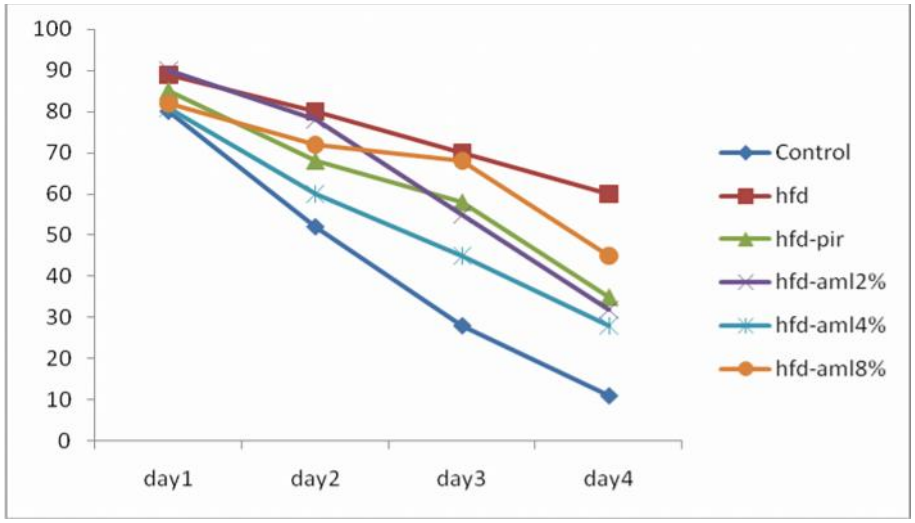


Figure 5: Effect of Amla on Scopolamine induced decrease in E.L.T and time spent in Target Quadrant

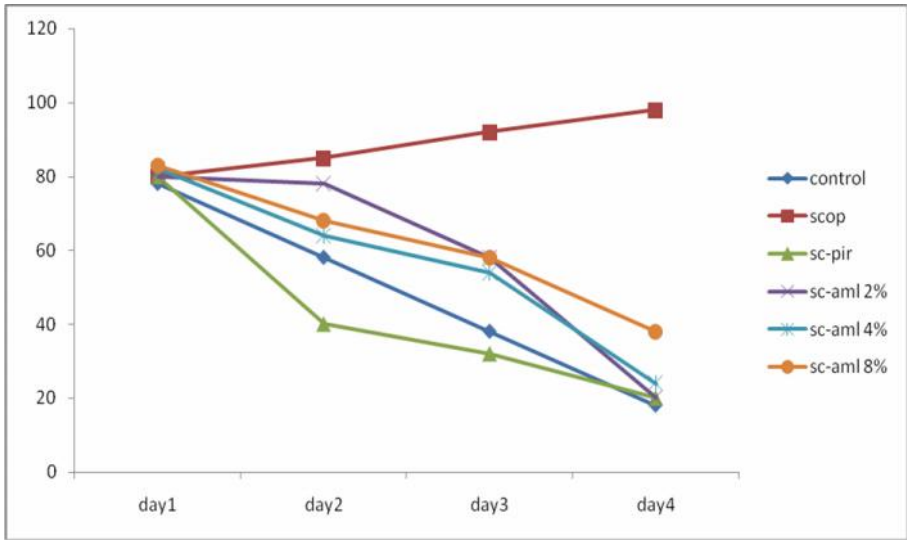


Figure 6: Effect of Amla on Blood Glucose Level

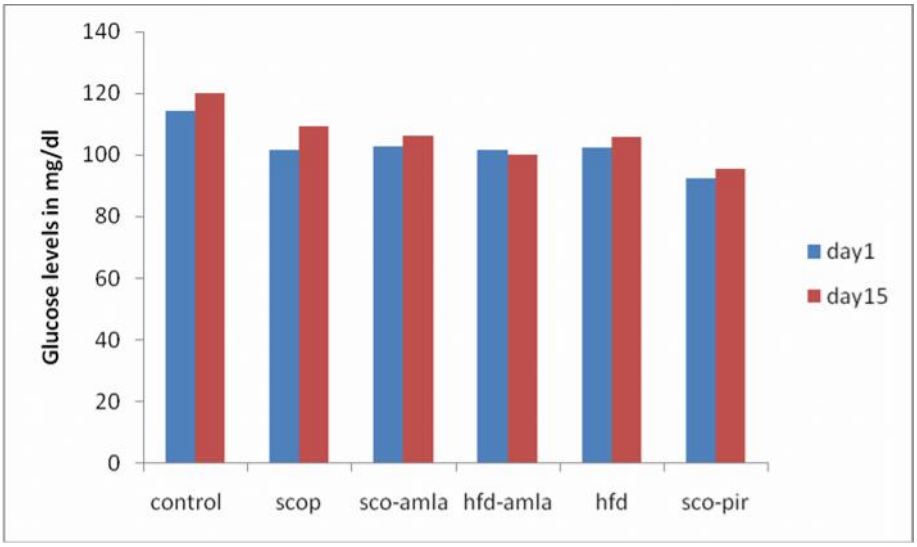
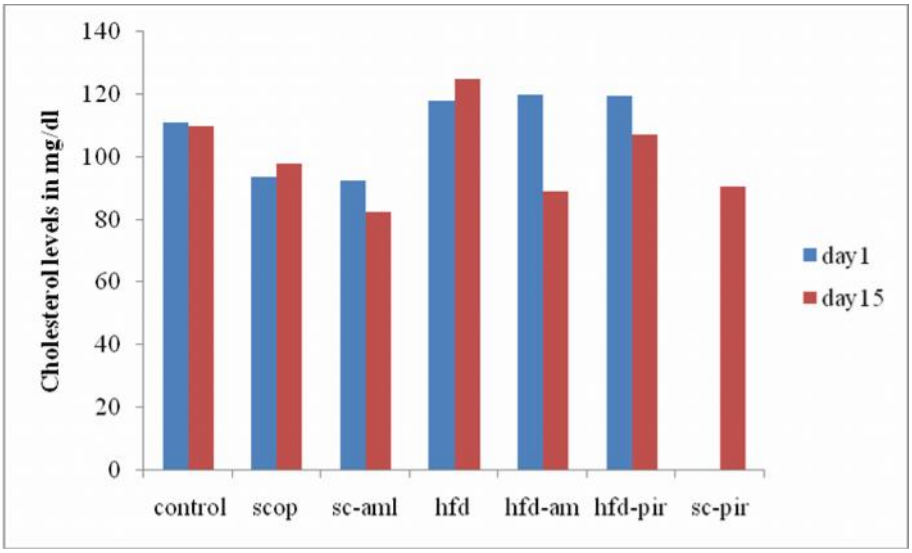


Figure 7: Effect of Amla on Blood Cholesterol Level



Conclusion

We conclude that Amla is very effective against memory loss due to the fatalness of cholinergic transmission and this is the most prevalent plant material and cost effective also. In case of highly obese patients those who are suffering from the memory impairment are also helpful by this natural boon.

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