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Article

Restoration of Memory Potential by *Piper betel* Leaves Extract in High-Fat Diet (HFD)-Induced Dementia in Mice

Pooja Sharma*, Ritu Rani, Ajeet Pal Singh, Amar Pal Singh

Department of Pharmacology, St. Soldier institute of pharmacy, Lidhran Campus, Behind NIT (R.E.C.), Jalandhar –Amritsar by pass, NH-1, Jalandhar -144011, Punjab, India.

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Abstract

The present study has been designed to evaluate the effect of ethanolic extract of leaves of Piper betel in high fat diet (HFD) induced dementia and memory restoration effect in Albino mice by various parameters like Morris water maze (MWM) test, Elevated Plus Maze etc. and Estimation of serum total cholesterol using commercially available kit. Dementia is a syndrome in which the deterioration of cognitive functions occurs.

The results of this study indicate that the administration of a high-fat diet to the Swiss mice produces a severe deterioration of spatial memory as determined by the Morris water maze (MWM) test, Elevated Plus Maze, T Maze delayed alteration task performed. Administration of ethanolic extract of *Piper betel* (250 and 500 mg/kg, p.o.) for 14 days significantly attenuated high-fat diet-induced memory deficits. Moringa Oleifera treatment to HFD induced mice improves cognition by virtue of its results support that the protective effect obtained with *Piper betel* may be through the activation of pregnane x receptors.

ethanolic extract of *Piper betel* treatment to HFD induced mice improves cognition by virtue of its results, supports that the protective effect obtained with *Piper betel* may be through the activation of pregnane x receptors.

Keywords: Dementia, *Piper betel*, high fat diet, Morris water maze, Elevated plus Maze, T Maze and Piracetam.

*Address for Correspondence: Pooja Sharma, Department of Pharmacology, St. Soldier institute of pharmacy, Lidhran Campus, Behind NIT (R.E.C.), Jalandhar –Amritsar by pass, NH-1, Jalandhar -144011, Punjab, India.

INTRODUCTION

Overweight and obesity is a global epidemic among children of all ages. Medically, obesity is a condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Overweight is defined as a body mass index (BMI) in the 25 to 29 kg/m^2 range, whereas obesity is a BMI in excess of 30 kg/m^2.[1] Dementia is a progressive neurodegenerative disorder characterized by multiple cognitive deficits which results in the loss of brain function and neuronal cell death.[2] Dementia may occur as a result of decrease in acetylcholine, nor-epinephrine and serotonin levels in brain.[3] The prevalence rate of dementia increases with age.[4]

The betel leaf is an evergreen and perennial, creeper and white catkin. Betel leaf (*Piper betle L.*) having family Piperaceae is a heart-shaped deep green leaf that grows on a root-climbing vine[5] It is a perennial plant belonging to the family Piperaceae, including black pepper.

Betel leaf is a versatile plant with a rich culture and medicinal history. these leaves have been reported to exhibit antioxidant, anti-inflammatory, immune-modulatory, and antitumor activity.[6] The proximate analysis of the leaves of *Piper betel* showed that it contained macro and micro nutrients as well as phytochemical. The plants of genus Piper are also used for many other purposes such as foods and spices, fish bait, fish poison, hallucinogens, insecticides, oils, ornaments, perfumes, *etc.*

Lee et al. (2022) examined the mechanisms of anti-cancer effects of the *Piper betle (P. betle) stem*.

Yoonus et al. (2021b) used *Piper betel* leaves extract to synthesize iron oxide nanoparticle (α-Fe2O3) from anhydrous ferric chloride. The material was investigated for its anticancer potential against A549 (Lung Cancer) cells by MTT assay.

Figure 1: Leaves of *Piper betel* Pant

Aims and Objectives

The present study has been designed to evaluate the effect of *Piper betel* in high fat diet (HFD) -induced dementia in Mice. It also aims to explore the involvement of pregnane x receptors in the Pathophysiology of dementia. The present work is aimed to evaluate the memory restoration effect of ethanolic extract of *Piper betel* plant leaves in experimental animals.

MATERIALS AND METHODS

Chemical:

All chemicals of analytical grade were procured from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

Collection and Preparation of plant material:

The ethanolic extract of leaves part of plant *Piper betel* was procured from Shreedha Phyto Extract, Jaipur.

Experimental Animals

Animals: Healthy, adult Swiss albino mice of either sex weighing (25-40 g), maintained under standard laboratory conditions, at temperature 25 ± 2°C and a 12 hr. light-12 hr. dark period was employed for the experimentation. Food and water were provided ad libitum.

METHODS

Morris Water Maze, Elevated Plus Maze, T Maze delayed alteration task employed in the present study to evaluate learning and memory in experimental Mice. The serum total cholesterol was estimated by cholesterol oxidase peroxidase (CHOD-PAP) method.

RESULTS

Chemical test

Table 1: Chemical Test of Herbal Betel Leaf

Sl.no.	Chemical test	**Observation**	Results
1	Tannins	Brownish green Color	Positive
2	Anthraquinones	Rose pink color	Positive
3	Flavonoids	Yellowish green color	Positive
4	Alkaloids	Brownish precipitate	Positive
5	Terpenoids	Reddish brown color	Positive
6	Saponins	Blue black precipitate	Positive
7	Cardiac glycosides	Reddish brown color	Positive

Phytochemical screening

Table 2: Preliminary Tests for *Piper betel* Linn.

Test	Observation
Alkaloids	
Mayer's reagent	-
Dragendorff's reagent	-
Hager's reagent	-
Wagner's reagent	-
Test for purine group (murexide test)	-
Carbohydrates	
Molisch's test	+
Fehling's test	+
Benedict's test	+
Glycosides	
Anthraquinone glycosides	-
Borntrager's test	-
Modified Borntrager's test	-
Cardiac glycosides	
Keller Killiani test	-
Raymond test	-
Legal test	-
Cyanogenic glycosides	-
Coumarin glycosides	-
Sterols	
Salkowski test	+
Lieberman–Burchard's test	+
Saponins	+
Tannins	
Ferric chloride	+
Gold Beater's skin test	+
Proteins and free amino acids	
Millon's test	+
Biuret test	+
Ninhydrin test	+
Mucilage	+
Terpenoids	+
Flavonoids	
Shinoda test	+
Alkali test	+
Acid test	+
Zn/Hcl test	+
Volatile oil	+

Effect of *Piper betel* extract on body weight (g) of mice

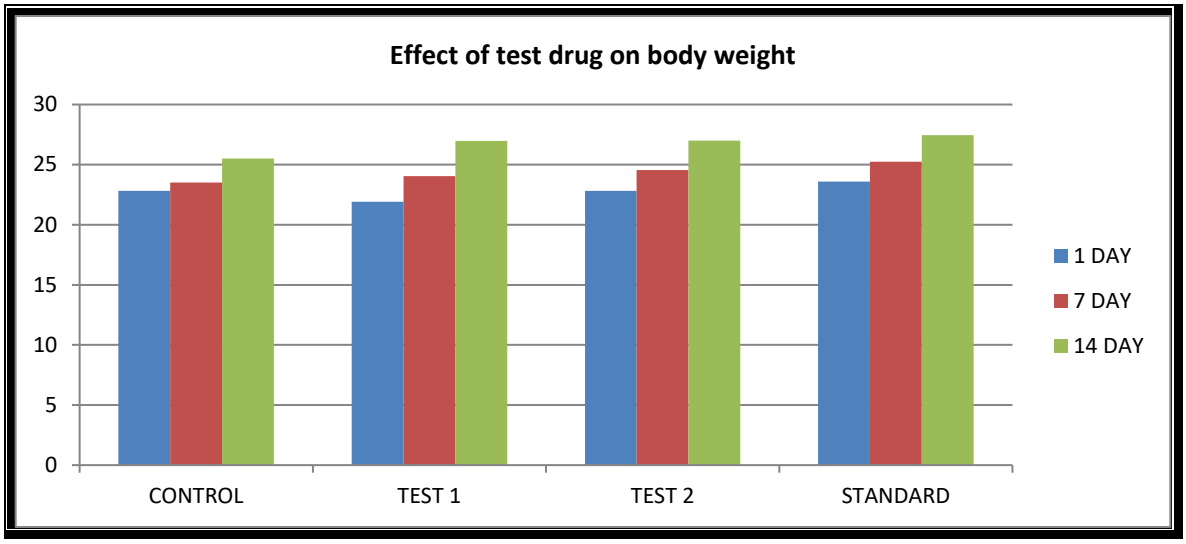

Figure 2: Graph Showing Effect of *Piper betel* Extracton Body Weight (G) of Mice

- **Effect of *Piper betel* extract on Feed intake (g) of mice.**

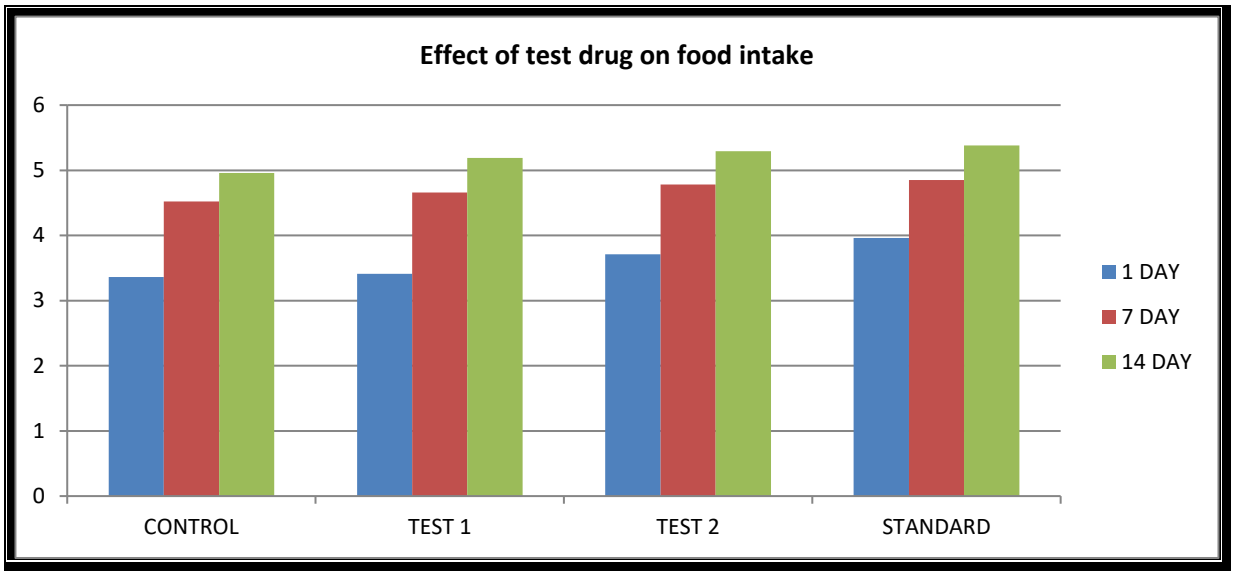

Figure 3: Graph Showing Effect of *Piper betel* Extract on Water Intake (Ml) of Mice.

Effect of *Piper betel* extract on Water intake (ml) of mice.

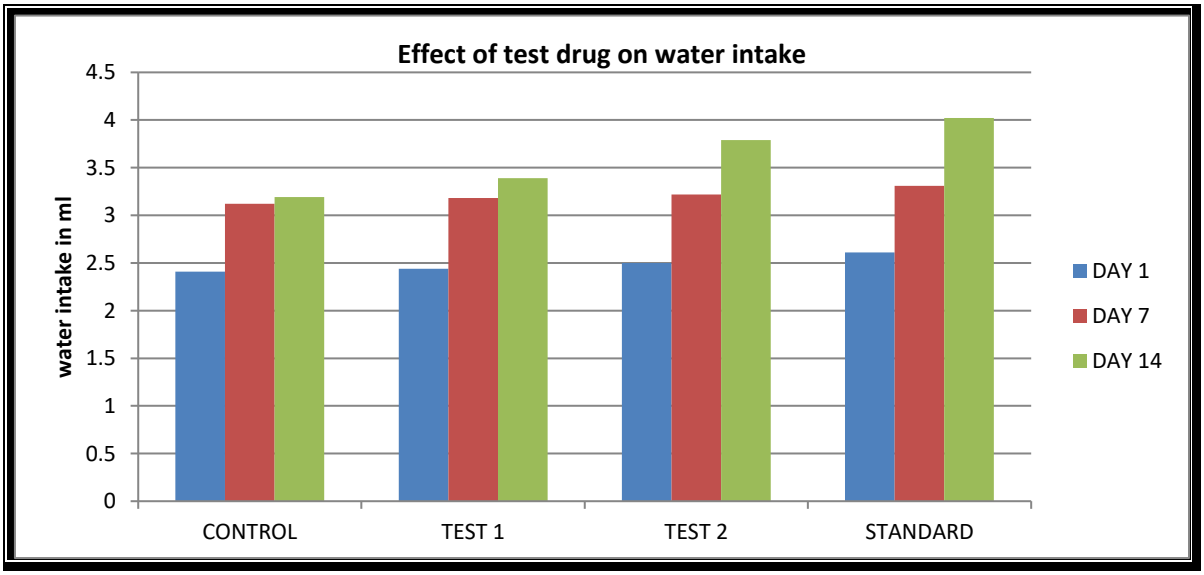

Figure 4: Effect of *Piper betel* Extract on Water Intake (Ml) of Mice.

Morris water maze test

HFD = High fat diet,

P.B. (LD) = *Piper betel* low dose (250 mg/kg; p.o.),

P.B. (HD) = *Piper betel* high dose (500 mg/kg; p.o.)

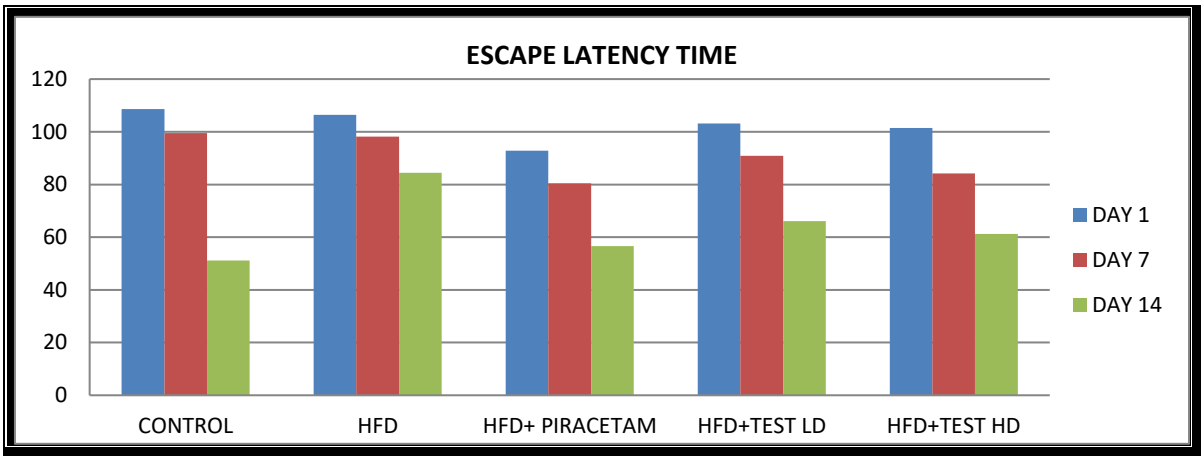

Figure 5: Graph Showing Effect of Pharmacological Interventions of *Piper betel* and Other Drugs on Escape Latency Time (ELT) on Day 1st, 7th and 14th (Time in Seconds) using Morris Water Maze Test in HFD Induced Dementia.

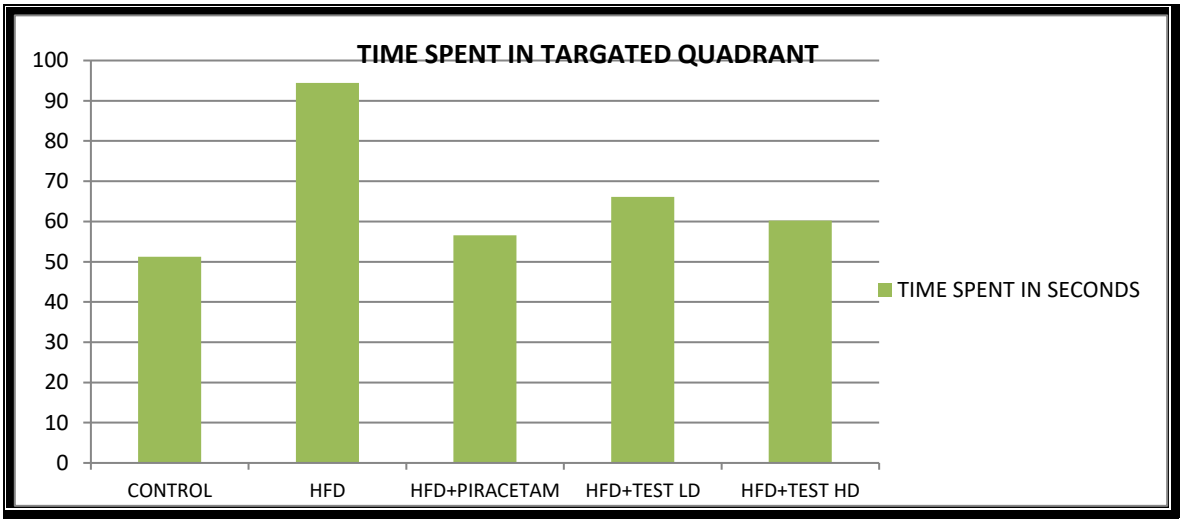

Figure 6: Graph Showing Effect of *Piper betel* and other Drugs on Various Pharmacological Interventions on Mean Time Spent in the Target Quadrant (TSTQ) Using Morris Water Maze in HFD Induced Dementia.

Elevated Plus Maze

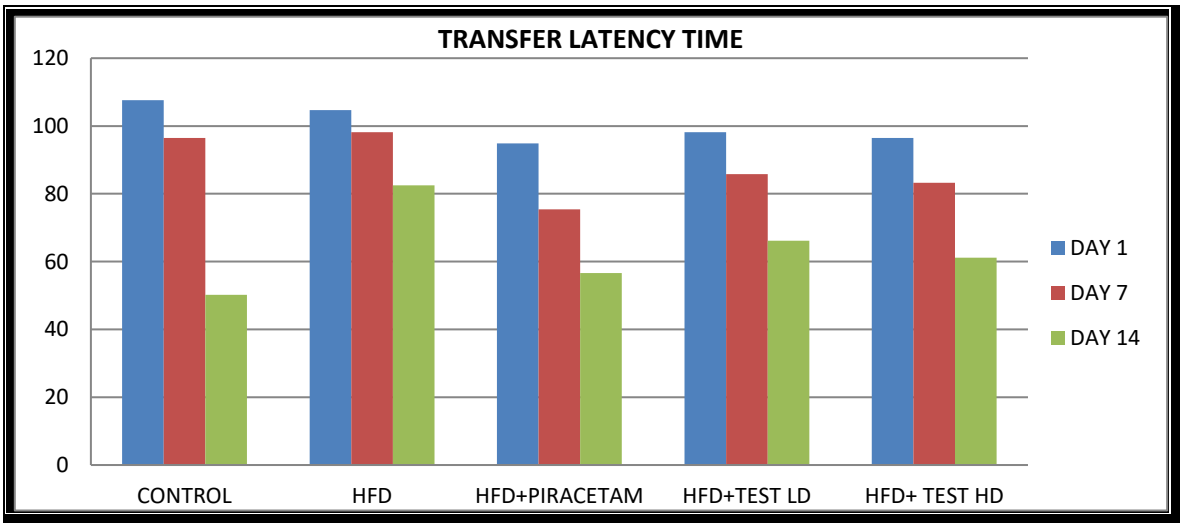

Figure 7: Graph Showing Effect of Pharmacological Interventions of *Piper betel* and Other Drugs on Transfer Latency Time (TLT) Of Mice Using Elevated Plus Maze (Time in Seconds) HFD Induced Dementia.

T maze

Graphs showing Effect of Pharmacological interventions of *Piper betel* and other drugs on Forced alteration task **(FAT)**, Left Right discrimination **(LRD)** latency time **(LT)** and distance travelled **(DT)** of mice using **T maze**, HFD induced dementia.

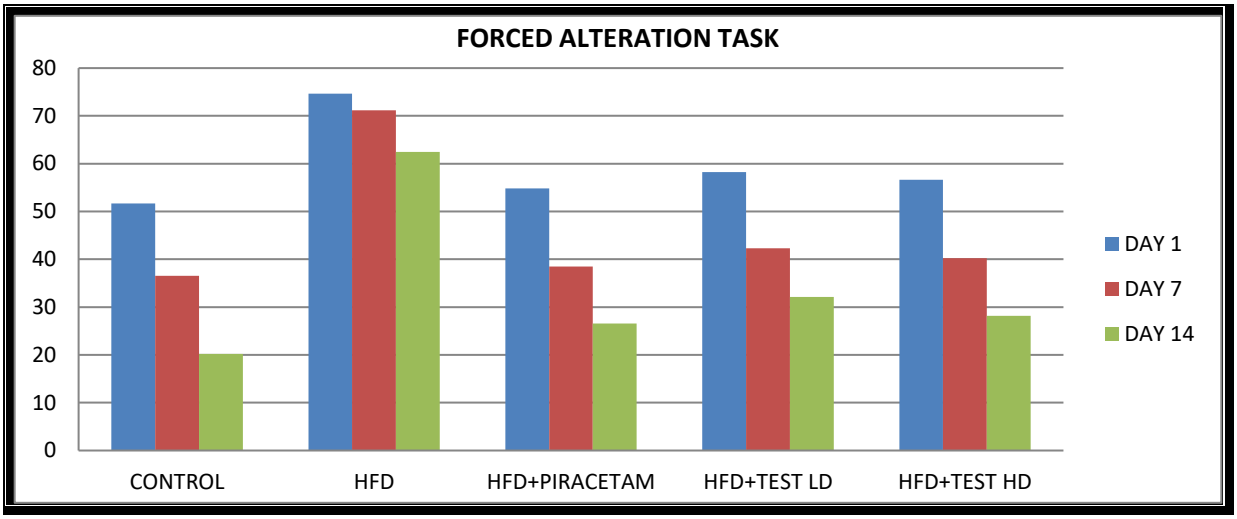

Figure 8: Graphs Showing Effect of Pharmacological Interventions of *Piper betel* and other Drugs on Forced Alteration Task (FAT)

- **Left Right discrimination (LRD)**

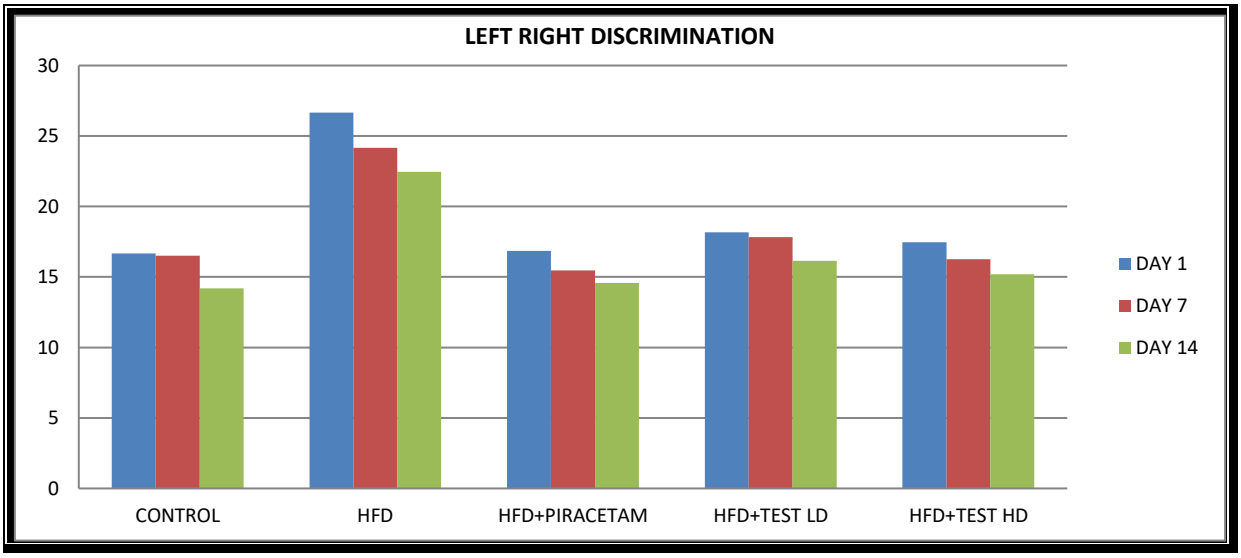

Figure 9: Left Right Discrimination (LRD)

Latency time (LT)

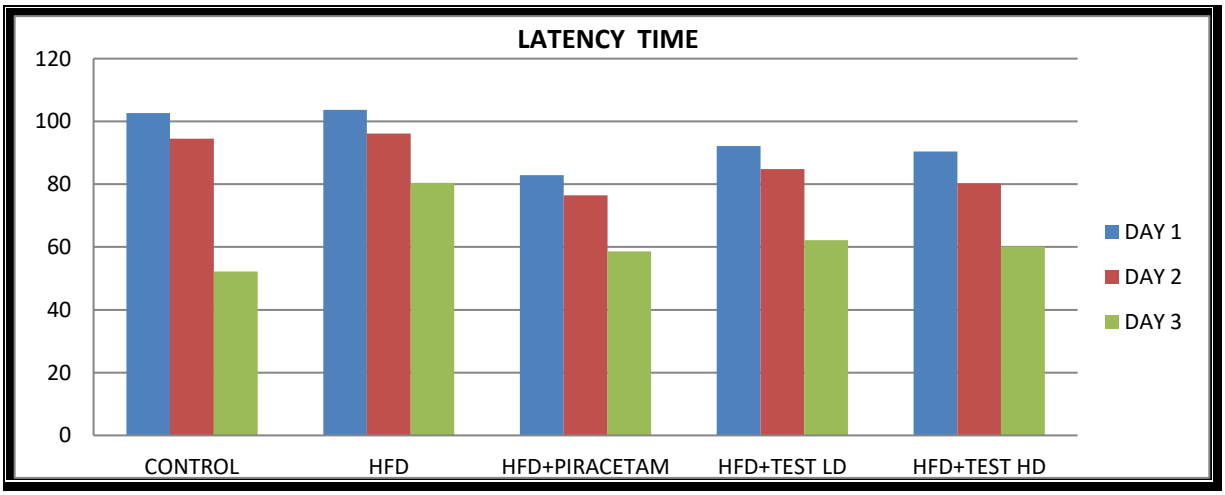

Figure 10: Latency Time (LT)

Distance travelled (DT)

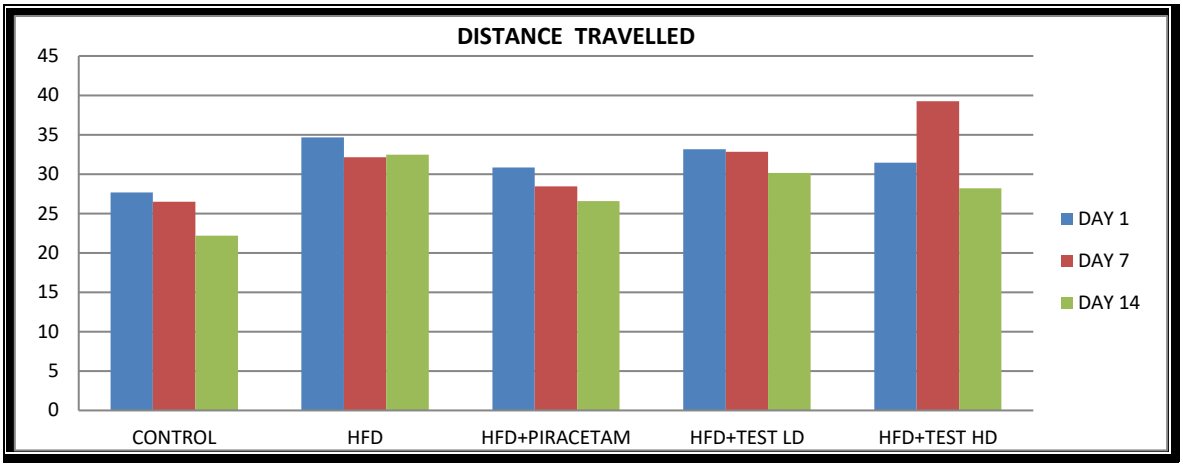

Figure 11: Distance Travelled (DT)

Total Cholesterol Levels

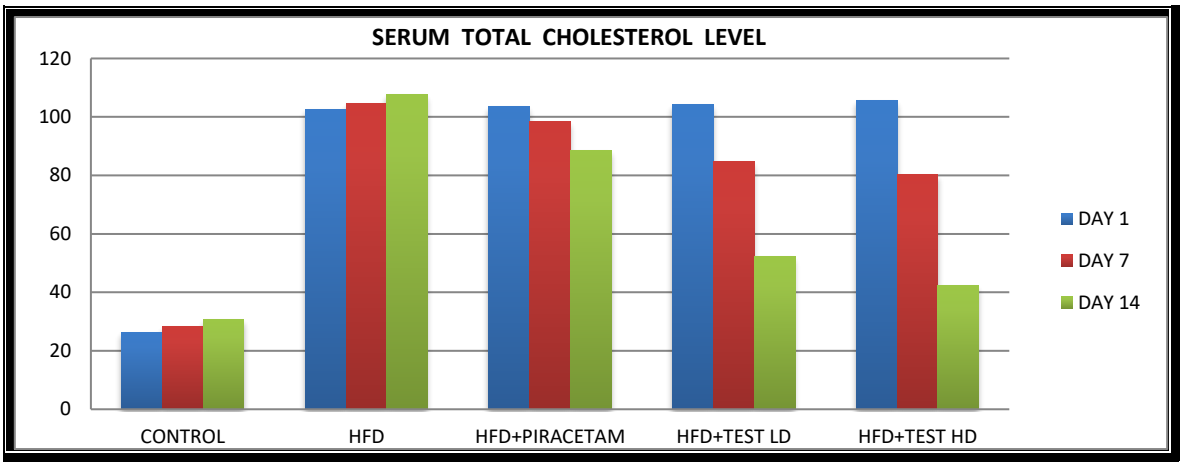

Figure 12: Graphs Showing Effect of Pharmacological Interventions of Test and Standard Drugs on Serum Total Cholesterol Levels of Mice.

DISCUSSION

Forced alternation and left-right discrimination tasks using the T-maze are used extensively to assess working and reference memory, respectively, in rodents.

Alzheimer's disease (AD), the most common neurodegenerative disorder associated with dementia (60-80%), is typified by the pathological accumulation of amyloid Aβ peptides, neurofibrillary tangles (NFT) and hyperphosphorylation of tau protein within the brain.[7,8,9]

In the present study, we provide evidences for the involvement of Pregnane X receptors in neuropathology of dementia. Pregnane X receptors (PXR, *NR1I2*) has been characterized as one of the central components in coordinated responses to xenobiotic stimulation by controlling the transcription of numerous transporter genes associated with xenobiotic metabolism.[10,11,12]

Some of the first studies examining pregnane x receptor's role in brain were those focused on blood-brain barrier, which was similar to other excretory organs in the body.[13]

In T-maze tasks, Orientation of the maze in a room and its stability, absence or presence of polarizing cues in the room, and ability of rodents to see cues in the room may affect strategies. Thus, researchers need to consider configuration and orientation of apparatus and cues in a room in conducting an experiment and an interpretation of behavioral data. In our laboratory, we place two apparatus facing in the same direction toward a wall in a soundproof room and set objects, such as a door of the room, fluorescent lights on the ceiling, walls of the room, CCD cameras of the apparatuses, and racks to accommodate mouse cages, that may serve as extra-maze spatial cues for mice.

As shown in the representative results, the percent correct responses of the control mice gradually increased across sessions in both tasks. The findings confirm that *Piper betel* treated mice can learn to make correct choices in the modified automatic T-maze.

In this study, the mice stayed at around 80% correct choices and not more even after extensive training. Considering that they keep showing some omission errors throughout the trainings, their motivation may not be so high for the mice as to reach higher level of performance. In the forced alternation task, mice

showed a higher percentage of correct responses than control mice.

The mice also displayed more omission errors than control mice during the reversal learning sessions. The increase in the number of omission errors could reduce the opportunity to learn which arm is associated with the reward. Therefore, the delayed learning acquisition could be due to the increase in the number of omission errors during the initial sessions, but not to impaired reversal learning. Another possibility is that the mutants could be confused by the change in rules, which might induce errors of omission and interfere with executive function. Thus, to draw a reasonable conclusion, omission errors should be examined as well as correct choice percentage.

The Image TM program generates the additional results for the latency and distance traveled to complete a session as well as the percentage of correct response and the number of omission error. The differences in the latency and distance traveled to complete a session may be interpreted as a difference in locomotor activity level, impulsive tendency to choice the arms, motivation to perform the task, habituation level to the task, different learning strategy and etc.

Regarding the representative results, mice showed shorter latency and shorter distance traveled than those of the controls. In fact, mice showed a hyperlocomotor activity compared to the control mice and this phenotype could underlie the differences in the indices.

The improved T-maze apparatus leads to the automation of test procedures, which can contribute to the standardization of protocols used across laboratories.

Taken together, T-maze forced alternation and left-right discrimination tasks using the modified automatic apparatus are useful for assessing working and reference memory and behavioral flexibility in mice. In Acute oral toxicity study, it has been observed that no change in behavioral responses and observation shows any acute oral toxicity.

Assessment of Dementia

Morris water maze (MWM) test elevated plus maze (EPM) and T maze delayed alternation task. The treatment was continued for 14 days after feeding HFD for 60 days duration, during acquisition trials, *i.e.* from day 1 to day 14 (61th TO 74th).

Effect of high fat diet (HFD) on escape latency time (ELT) and mean time spent in target quadrant (TSTQ) using Morris water maze (MWM) test. Animals fed with cholesterol-rich high fat diet for 60 days showed a significant increase in escape latency time (ELT) in comparison with the normal control group animals and decrease in mean time spent in target quadrant (TSTQ) indicating the impairment of learning and memory respectively. Administration of *Piper betel* (250 mg/kg/day and 500 mg/kg/day; *p.o.* for 14 days) to the mice fed with a cholesterol-rich high fat diet, showed a significant fall in escape latency time (ELT) when compared with the high-fat diet control group animals. Furthermore, a significant rise in the TSTQ was observed indicating the reversal of learning and memory impairment.

Effect of Pharmacological interventions of *Piper betel* and other drugs on transfer latency time (TLT) of mice using elevated plus maze (Time in seconds) HFD induced dementia shows, Effect of Pharmacological interventions of *Piper betel* and other drugs on Forced alteration task (FAT) less time, Left Right discrimination (LRD) 80% corrected response, latency time (LT) shorter and distance travelled (DT) shorter of mice using T maze, HFD induced dementia.

Assessment of Lipid profile

Pharmacological interventions of Kavalactone on total serum cholesterol level decreased on 7th and 14th days respectively. Piracetam didn't have any effect on raised cholesterol level of mice.

Elevated plus maze, these models are widely employed for evaluating the effect of drugs on learning and memory. In elevated plus maze, decrease in transfer latency on 2nd day (i.e., 24 h after the first trial) indicated improvement of memory and vice-versa. In Morris water maze, a decrease in escape latency during training and increase in time spent in target quadrant during retrieval indicated improvement of learning and memory respectively; and *vice versa*.

Piper betel did not show any significant change in locomotors functions of mice as compared to the vehicle treated control, so this did not produce any motor effects. Thus, memory enhancing effect of *Piper betel* is specific and not false positive. Out of the two effective doses of *Piper betel* produced better memory enhancing effect in mice as compared to the lower dose in both the behavioural models employed, hence the higher dose was employed for elucidating the probable mechanisms of memory enhancing activity.

CONCLUSION

Hence, it may be concluded that HFD administration induces memory deficits mice. *Piper betel* treatment to HFD induced mice improves cognition by virtue of its antioxidant, anti-cholinesterase, anti-inflammatory, hypolipidemic and amyloid-lowering potential and these results supports that the protective effect obtained with *Piper betel* may be through the activation of pregnane x receptors.

Properties of *Piper betel* are multidimensional and thus, have varied economic applications. It's easy cultivation within unfavorable environmental condition and wide availability makes it an excellent potential for growth in economy and health & nutrition sector in a developing country like India. Maximum yield of its various parts and constituents could be achieved to derive supplements and therapeutics of multifarious nature for human consumption. So far numerous studies have been conducted on different parts of *Piper betel* and the chemical constituents, but there is a need to isolate

and identify newer compounds from different parts of the tree. Further, more rigorous studies focusing on identification, characterization and commercialization of bioactive compounds of *PB* can lead to the development of remedies and prevention of several ailments.

Nowadays, with continuously changing socio-economic status, people have become more concerned about their health. Utilization of natural products of plant origin having lesser side effects has gained popularity over the years. There is immense scope for foods that can impart health benefits beyond traditional nutrients. Various researches have concluded that *PB* should be used as functional ingredient in food products.

Acetylcholine is considered to be one of the important neurotransmitter involved in the regulation of cognitive functions. Cognitive dysfunction has been shown to be associated with impaired cholinergic transmission and the facilitation of central cholinergic transmission resulting in improved memory. Moreover, selective loss of cholinergic neurons in certain brain parts appeared to be a characteristic feature of senile dementia. The degeneration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of AD. Thus, the drugs which enhance cholinergic function can be used for treatment of dementia closely related to AD.

The memory enhancing activity is also supported by its beta-site amyloid precursor protein-cleaving enzyme 1 (BACE 1) inhibiting property. BACE1 is the major beta-secretase to cleave the beta-amyloid precursor protein to generate beta-amyloid. Oxidative stress has been shown to affect amyloid-beta generation in the AD pathogenesis. Upregulation of BACE 1 gene transcription by oxidative stress may contribute to the pathogenesis of AD. *Piper betel* has also been reported to possess antioxidant activity. Thus, *Piper betel* produced significant memory enhancing effect in mice probably due to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improvement of neuronal function.

In conclusion, *Piper betel* showed memory enhancing activity in mice probably by inhibiting brain acetylcholinesterase activity, through involvement of GABA-benzodiazepine pathway and due to its antioxidant activity.

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Conflicts of Interests: There are no conflicts of interest.

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Authors Contributions: All the authors have contributed equally.

Ethics approval: Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2022/PR-027). (Reg. No. 2011/PO/Re/S/ 18/CPCSEA and date of registration is 1/5/2018)

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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