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## Comparative assessment of nootropic activity of Yashtimadhu Choorna and Yashtimadhu Kshirapaka in young and aged mice

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### Abstract

Yashtimadhu is one of the “Medhya Rasayan” drugs advocated to achieve desired effect on cognitive functions especially when given with Cow milk. In present study two dosage forms of Yashtimadhu i.e. Choorna [Powder] and Kshirapaka [Medicated Milk] were prepared as per standard guidelines given in ayurvedic classical text and were analysed with API parameters. Therefore, it can be said that a preparation method of both formulations is developed and validated with physic-chemical analysis. In extension, comparative assessment of Nootropic activity of both formulations was done by using two animal models, viz. Diazepam induced amnesia using elevated plus maze [EPM] in young mice and spatial learning and memory in radial arm maze [RAM] in aged mice. In EPM test and RAM test drug administration was done daily for 15 and 7 days respectively. Study finding suggests that Yashtimadhu Kshirapaka at therapeutic dose exhibited anti-amnesic effect and learning-memory enhancement action in EPM test and RAM test ( $P < 0.001$ ). The result also specified comparative significant effect of Yashtimadhu Choorna with cow milk on animal’s cognition than Yashtimadhu Choorna with water. Thus it can be concluded that addition of cow milk as Anupana [Vehicle] to Yashtimadhu or processing of Yashtimadhu Choorna with cow milk in “Kshirapaka formulation” has potential to maintain healthy cognitive process in animals’.

Hence both potent formulations can be employed as nootropic drugs to maintain human cognition in balanced state or can be utilized as adjuvant to conventional treatment.

**Keywords:** Yashtimadhu, Medhya Rasayan, Choorna, Kshirapaka, EPM, RAM

### Introduction

Learning, memory and cognitive skills are considered under the concept of Medha. Learning and memory work together in process of acquisition of knowledge. Proper and systemic learning will enhance memory. According to Ayurved acquisition of knowledge is result of complex interaction and co-ordination of Atma, Mana, Indriya, Indriyarth and Indriya Buddhi [1]. When any of these components are not in normalcy or not functioning properly, either individually or in co-ordination with one another, then learning problems can be seen [2].

Know a day, due to poor nutrition, old age, long term mental illnesses, peer pressures and stressful life occurrence of cognitive dysfunction is seen. Cognitive disorders that primarily affect learning, memory, perception and problem solving, may lead to amnesia, dementia, and delirium [3]. Senile dementia might be leading to Alzheimer disease which is worsening the daily activity of person. In Ayurved also it is explained that after fourth decade i.e. from age of forty functions of “Medha” starts to get decline [4].

According to WHO report an estimated 35.6 billion people were living with dementia in 2010. The numbers of people living with dementia will almost double every 20 years to 42.3 million in 2020. The rate of growth will be the highest in India [5].

Thus it is always advantageous to treat early symptoms of memory impairment which slowdowns occurrence of symptoms of Mild cognitive impairment (MCI), Dementia etc. [4] Symptomatic drugs used up till now in conventional science include Donepezil, Rivastigmine and Piracetam. These medicines demonstrate positive effects but have certain limitations [6]. Therefore “Medhya Rasayana” drugs prescribed in ayurvedic science might be helpful when administered with standard guidelines.

Yashtimadhu is one of the “Medhya Rasayan” drugs advocated to achieve desired effect on cognitive functions especially when given with Cow milk [7]. Ayurvedic literature revealed that Yashtimadhu has Madhur Rasa, Sheeta, Guru, Snigdha properties and Balya [strengthening of bodily metabolites and mind attributes] activity [8]. Thus in present study extraction of fat soluble active components of Yashtimadhu will be done in cow milk by preparing ayurvedic unique dosage form Kshirapaka, which might show significant effect than plain Choorna form of Yashtimadhu. Hence assessment of effect of Yashtimadhu Choorna administered with cow milk and plain water will be compared with Yashtimadhu Kshirapaka on learning and memory of young and aged mice using Elevated plus maze [EPM] and eight arm radial maze [RAM].

### Aim and Objectives

Comparative assessment of Nootropic activity of Yashtimadhu Choorna and Yashtimadhu Kshirapaka in Young and Aged mice using current pharmacological models.

### Materials and Methods

**Materials:** In this study three test drugs were used

1. Yashtimadhu Choorna with milk (YC-CM)
2. Yashtimadhu Choorna with water (YC- W)
3. Yashtimadhu Kshirapaka (YK)

**Methodology:** The study will be conducted in two phases

- a) Pharmaceutics.
- b) Experimental study.

#### a) Pharmaceutics

Identification and Authentication: Consensus method was applied for identification and selection of ideal sample of

Yashtimadhu wherein opinions of Ayurvedic experts were taken. Authentication of raw drug was carried out in authenticated by authorized laboratory as per Ayurvedic Pharmacopoeia of India parameters [9]. For preparation of medicated milk of Yashtimadhu, Fresh Cow milk was required. Thus Cow milk was collected from renowned milk product dairy unit situated in Pune. Two formulations were prepared i.e. Yashtimadhu Choorna and Yashtimadhu Kshirapaka prepared as per the standard guidelines given in ayurvedic classical texts.

Standard operating procedures were developed and followed throughout the manufacturing process.

Preparation of Yashtimadhu Choorna and Yashtimadhu Kshirapaka:

- **Yashtimadhu Choorna** [10]: The selected sample of Yashtimadhu (roots) was taken in 200 g in quantity. Coarse powder of Yashtimadhu was formed with the help of iron mortar and pestle where pounding was carried out. This coarse powder of Yashtimadhu was further grinded in grinder, to form fine particle size of Yashtimadhu [Choorna form]. Further it was sieved through 60 mesh size sieve to obtain uniform form of fine particle size of drug. Then prepared final product, Yashtimadhu Choorna 180 gm. in quantity was stored in food grade plastic bottle.
- **Yashtimadhu Kshirapaka** [11]: 5gm of Yashtimadhu coarse powder was mixed with 40 ml of cow milk, after that 160 ml of potable water was added to it; the mixture was then stirred well to formulate homogenous mixture. Heat was applied and mixture was boiled on low flame till total water content gets evaporated and extracted form of medicated milk remains as final product. Afterwards heat was cut off and the product was filtered through filter aid [cotton cloth], was stored in food grade plastic bottle.

### Analysis of Yashtimadhu Choorna and Yashtimadhu Kshirapaka

**Table 1:** Organoleptic tests of Yashtimadhu Choorna and Yashtimadhu Kshirapaka

Sample name	Sparsha	Roopa Yellowish	Rasa	Gandha Faint and Characteristic
Yashtimadhu Choorna	Soft	Brown	Sweet	(Sweetish Odour)
Yashtimadhu Kshirapaka	Watery Sweet	Cream colour		Characteristic

**Table 2:** Physico-chemical analysis of Yashtimadhu Choorna

Parameters	Yashtimadhu Choorna	
	(Standard values API) [9]	(Obtained values)
Water soluble extractive	Not less than 20%	21.07%
Alcohol soluble extractive	Not less than 10%	11.60%
Total Ash	Not more than 10%	8.89%

Obtained values of Yashtimadhu Choorna are comparable with standard established values given in API

**Table 3:** Physico-chemical analysis of Yashtimadhu Kshirapaka

Parameters	Yashtimadhu Kshirapaka
pH	7.06
Specific gravity	1.015
Total solid contain	5.84

Analysis of Yashtimadhu Kshirapaka was not mention in standard analytical books, through this study analytical values for Yashtimadhu Kshirapaka were established.

#### b) Experimental study

Experimental study: To initiate experimental study, permission of institutional animal ethical committee (IAEC)

was taken. The study was conducted at CPCSEA approved, central animal house, BVDU Medical College, Pune, 411043. Standard housing conditions were maintained throughout the study. All animals were housed in cages. 12 hour day and night cycle was maintained. Rodent pellet diet was provided and aqua guard water was supplied ad libitum to animals. Thirty-six, young Swiss albino mice of either

sex weight between 18-20gm were taken. Along with this thirty, aged Swiss albino mice of either sex weight between 30-35gm were taken. They were screen and divided in groups, each group consisting six animals.

**Model 1:** Diazepam induced amnesia in mice [12];

Drug dose administration GROUPS:

**Group 1-** Normal Control [Normal saline] - 1ml/kg- Orally

**Group 2-** Standard drug [Piracetam]- 500mg/kg – Orally

**Group 3-** Induction drug [Diazepam]- 1mg/kg- i. p

**Group 4-** Test drug 1[Yashtimadhu Choorna with milk] - 10gm/70 kg- Orally Group5- Test drug 2[Yashtimadhu Choorna with water] -10gm/70 kg- Orally Group6-Test drug 3[Yashtimadhu Kshirapaka]- 80ml/70kg- Orally All groups were treated with respective drugs with extrapolated drug dose for mice with extrapolation factor 0. 0026mg/20gm, for 15 days daily.

Assessment of Learning and Memory:

1. On the eight day, after 45 mints of last dose of drug administration, diazepam (1mg/kg) was given intra peritoneal [i. p] to each animal. After 45 mints of administration of induction drug diazepam, Transfer

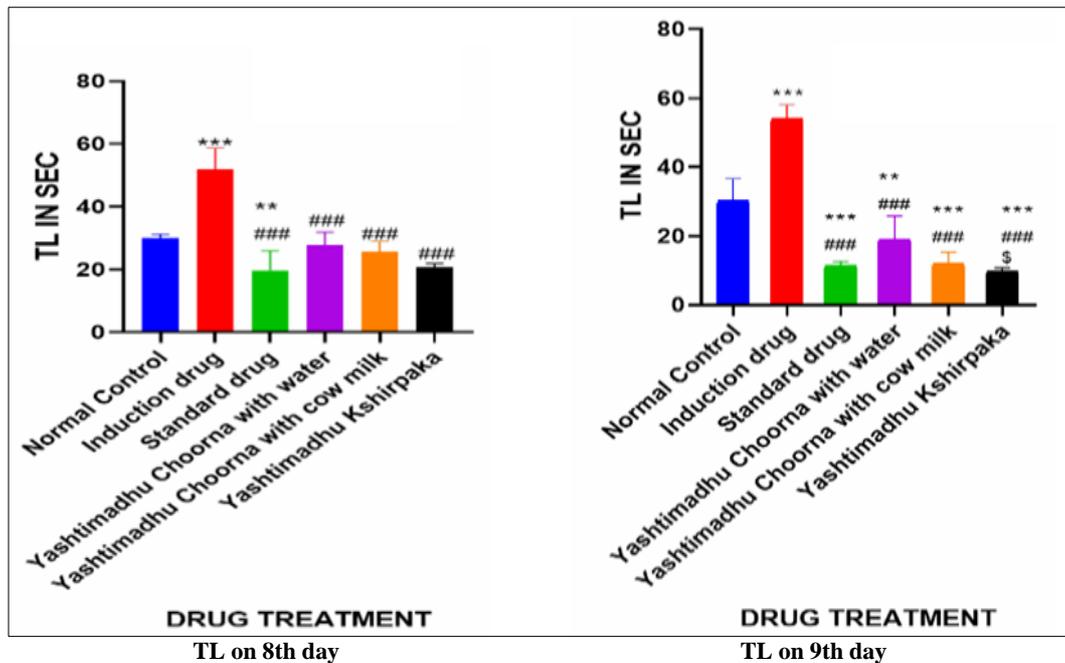
Latency [TL] of each animal was recorded to asses learning of animals.

2. After 24 hours of last drug dose administration i.e. on 9th day, TL was again noted to assess retrieval of learning i.e. memory.
3. On the same day after completion of recording of TL, all respective drugs were administered to concerned group animals and drug administration was continued for further seven days i.e. up to fifteen day.
4. On day 15th and 16th same procedure was carried out as on 8th and 9th day.

**Assessment Criterion:** Transfer latency (TL), The time taken (in seconds) by animal to move from open arm to one of the covered arm with all its four legs. Significant reduction in Transfer Latency (TL) value indicates improvement in acquisition and memory in animals.

**Statistical Analysis:** Data collected from EPM test was statistically analysed by One way ANOVA followed by Tukey’s test using Graph pad prism 7 version.

**Observations and results**



n=6/group, all values represent mean ± SD, one way ANOVA followed by Tukey’s test.  
 \*P<0. 05, \*\*P<0. 01, \*\*\*P<0. 001 as compared to Normal Control,  
 #P<0. 05, ##P<0. 01, ###P<0. 001 as compared to Induction drug,

**Fig 1:** Transfer latency of all drug treated groups on eight and ninth day

**Statistical analysis of eighth and ninth day**

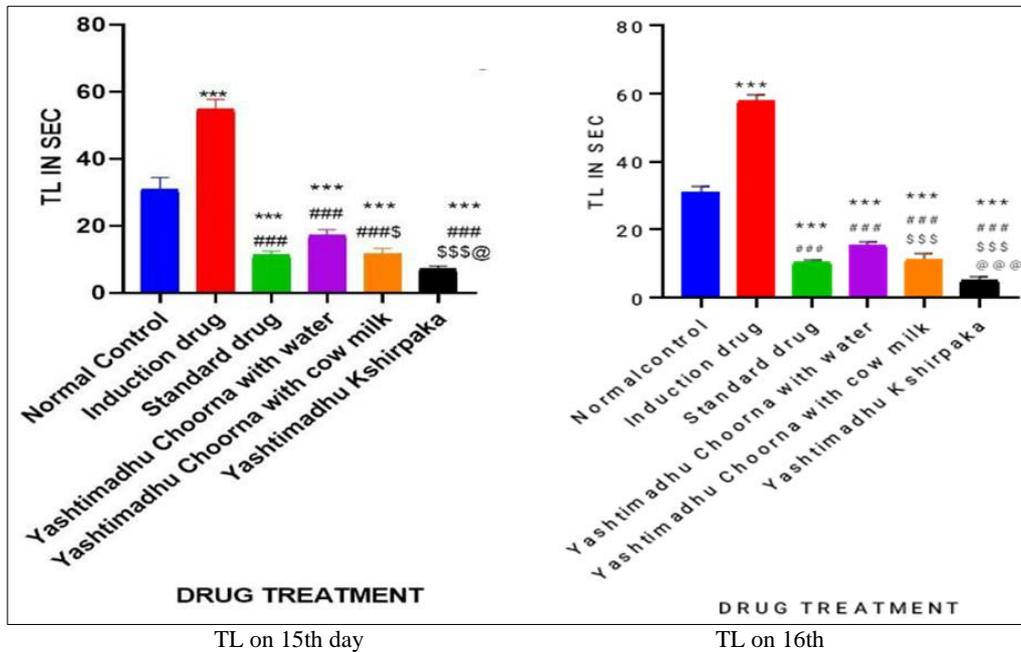
Induction drug group [diazepam] showed increase in TL as compared with normal control group. It indicates that amnesia was developed in animals (P< 0. 001).

Treatment with Standard drug (Piracetam) and three groups of Yashtimadhu showed decreased TL in comparison to Induction drug [diazepam] (P<0. 001).

The data of eight day showed no significant difference in TL

in all three groups of Yashtimadhu when compared with each other.

Data of TL of ninth day, showed significant decrease in Yashtimadhu Kshirapaka group when it was compared with Yashtimadhu Choorna with water group (P<0. 05). However no significant change in TL of Yashtimadhu Kshirapaka and Yashtimadhu Choorna with cow milk was observed.



n =6/group, All values represent mean ± SD, One way ANOVA followed by Tukey's test.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to Normal control,

# $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  as compared to Induction,

\$ $P < 0.05$ , \$\$ $P < 0.01$ , \$\$\$ $P < 0.001$  as compared to Yashtimadhu Choorna with water,

@ $P < 0.05$ , @@ $P < 0.01$ , @@@ $P < 0.001$  as compared to Yashtimadhu Choorna with milk.

**Fig 2:** Transfer latency of all drug treated groups on fifteen and sixteen day

**Statistical analysis of fifteenth and sixteenth day**

Induction drug group (Diazepam) showed increase in TL as compared to Normal Control group on day 15 and 16. It indicates that amnesia was developed in animals ( $P < 0.001$ ). Standard drug group (Piracetam) and three groups of Yashtimadhu have shown significant decrease in TL as compared to Induction drug group ( $P < 0.001$ ).

On day 15, Yashtimadhu Choorna with cow milk group showed significant decrease in TL when compared with Yashtimadhu Choorna with water group. ( $P < 0.05$ ).

Similarly, Yashtimadhu Kshirapaka group showed significant decrease in TL in comparison to Yashtimadhu Choorna with water ( $P < 0.001$ ) and Yashtimadhu Choorna with milk groups ( $P < 0.05$ ).

On day 16, Yashtimadhu Choorna with cow milk showed significant decrease in TL, when compared with Yashtimadhu Choorna with water group ( $P < 0.001$ ).

Wherein on the same day, Yashtimadhu Kshirapaka has shown decreased TL in comparison to Yashtimadhu Choorna with water and Yashtimadhu Choorna with cow milk groups. ( $P < 0.001$ ).

**Interpretation-** All together EPM test result confirmed the positive effect of Yashtimadhu Choorna with cow milk on acquisition and retention of learning in mice. Moreover; it is also proved that Yashtimadhu Kshirapaka formulation has encouraging effects on acquisition as well as retention of learning in comparison to both other groups of Yashtimadhu choorna. Thus the result of this test suggests that either Yashtimadhu choorna should be administered with cow milk or in the form Yashtimadhu Kshirapaka to attain desired effect on acquisition and memory.

**Model II:** Spatial learning and memory in the Radial Arm Maze <sup>[13]</sup>

Drug dose administration GROUPS:

**Group 1-** Normal Control [Normal saline] - 1ml/kg- Orally

**Group 2-** Standard drug [Piracetam]- 500mg/kg –Orally

**Group 3-** Test drug 1[Yashtimadhu Choorna with milk] - 10gm/70 kg- Orally

**Group 4-** Test drug 2[Yashtimadhu Choorna with water] - 10gm/70 kg- Orally

**Group 5-** Test drug 3[Yashtimadhu Kshirapaka]- 80ml/70kg- Orally

All groups were treated with respective drugs with extrapolated drug dose for mice with extrapolation factor 0.0026mg/20gm, for 8 days daily.

Study procedure:

**Learning trial:** Each animal from every group was exposed daily to RAM by keeping food in fixed arm as learning trial to them, for seven days. Afterward's, respective drugs were administered daily and it was continued for next seven days. After every trial, maze arms were cleaned to avoid olfactory stimulus.

**On seventh day:** Evaluation of reference memory was done i.e. after last dose of drug administration where food was kept in fixed arms.

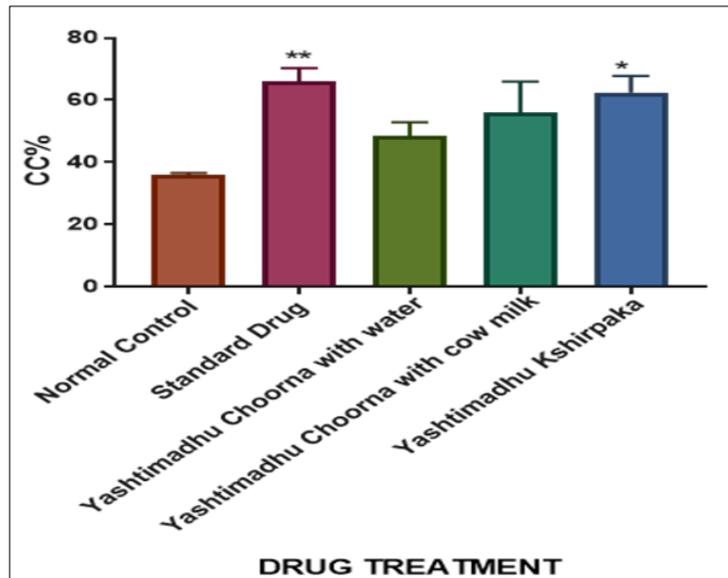
On day eighth day i.e. after 24 hrs. : of drug treatments, each animal was exposed to RAM by keeping food in variable arms to assess the effect of drug treatment on working memory. Latency to find out the food in 5 minutes and one re-entry in the variable arm was recorded. Data related TL and number of re-entries of all groups was compared and analysed statistically.

**Assessment parameters**

i) Percentage of correct choices (CC %) - Total entries in baited arm divided by total entries in unbaited arm into hundred.

ii) Working memory errors (WMEs) - Total repeat entries to baited arms.

iii) Reference memory errors (RMEs) - Total initial entries to unbaited arms Observations and results:

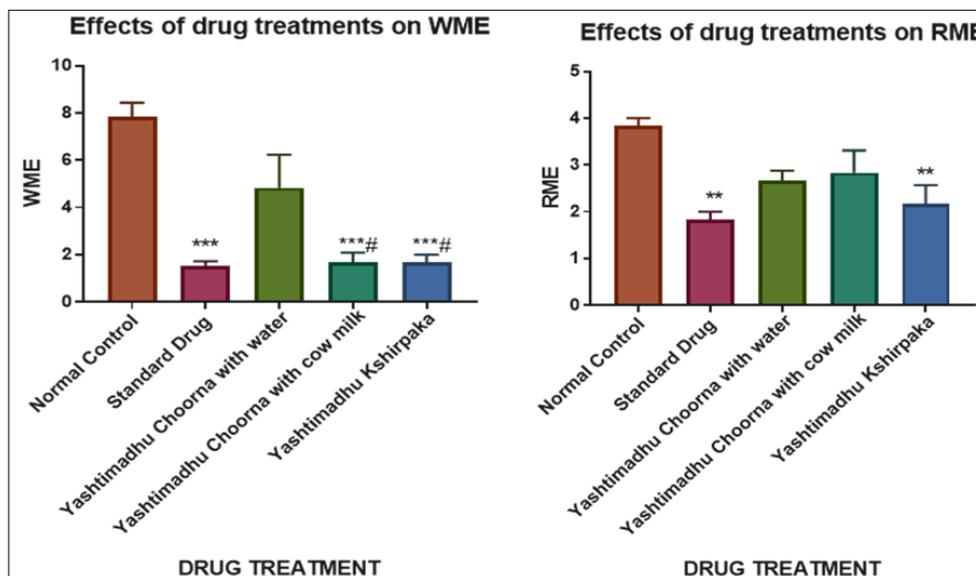


n=6/group, All values represent mean ± SEM, One way ANOVA followed by Tukey's test.  
\* $P < 0.05$ , \*\* $P < 0.01$  as compared to Normal control.

**Fig 3:** Percentage of Correct Choices of all drug treated group

On day seven, Yashtimadhu Choorna with water and Yashtimadhu Choorna with cow milk showed markedly decrease number of correct choices when it was compared with Normal Control group. Animals treated with standard drug (Piracetam) and Yashtimadhu Kshirapaka group showed distinctly increase number of correct choices, when

compared with normal control group ( $P < 0.01$  and  $P < 0.05$ ). This result suggests that Yashtimadhu Kshirapaka has significant effect on increase in reference memory in comparison to other two groups of Yashtimadhu i.e. Choorna with water and Choorna with cow milk.



n=6/group, All values represent mean ± SEM, One way ANOVA followed by Tukey's test.  
\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared to Normal control,  
#  $P < 0.05$  as compared to Yashtimadhu Choorna with water

**Fig 3:** WMEs and RMEs of all drug treated groups:

On day eighth, standard drug (Piracetam) and Yashtimadhu Kshirapaka have shown significant reduction in WMEs ( $P < 0.001$ ) and RMEs ( $P < 0.01$ ) when compared with Normal Control group. Eight days of treatment of Yashtimadhu Choorna with water group showed elevated

WMEs and RMEs when compared with Normal Control group. However, statistical reduced WMEs ( $P < 0.001$ ) and elevated RMEs ( $P < 0.05$ ) were demonstrated in Yashtimadhu Choorna with cow milk group, when it was

compared with Normal Control group. It is also resulted that YC with cow milk had significant effect on reduction in merely on WMEs not in RMEs, when it is compared with Yashtimadhu Choorna with water.

**Interpretation:** The result of RAM test implies the positive effect of Yashtimadhu Choorna with cow milk on working memory of aged mice. Piracetam, standard conventional drug has also shown affirmative effect on working and reference memory of aged mice, as expected. Similarly, therapeutic potential and positive effect of Yashtimadhu Kshirapaka formulation on spatial [reference and working memory] learning of aged mice is confirmed in RAM test as against other two groups of Yashtimadhu groups.

### Discussion

Human being is known to be the most intelligent animal on the earth. In normal human, intelligence is the result of normal functioning of Tridosh and Triguna <sup>[14]</sup>.

Optimum functioning of brain is the most important aspect of intelligence. While during the process of aging, healthy process of memory gets decline along with the disorientation of navigation. Know a day, due to poor nutrition, old age, peer pressures and stressful life occurrence of cognitive dysfunction is seen. These factors are prone to develop cognitive disorders that primarily affect learning and memory, lead to develop memory loss, mild cognitive impairment (MCI), amnesia, and dementia and further into Alzheimer disease.

In conventional science, medicines used for cognitive disabilities or maintenance of memory functions demonstrate positive effects but have certain limitations. Therefore “Medhya Rasayana” drugs prescribed in ayurvedic science might be helpful when administered with standard guidelines. Medhya word indicates improvement of functions of Medha and Smriti. Yashtimadhu is one of the “Medhya Rasayan” drugs advocated to achieve positive effect on cognition especially when given with Cow milk. <sup>7</sup> To create evidence and find out the therapeutic potential on short term, working and reference memory above said forms of Yashtimadhu were evaluated preclinically by most widely used animal models viz. Elevated plus maze [EPM] test and Eight arm radial maze [RAM] test.

**In EPM test:** Diazepam is used in EPM test, to develop amnesia in animals as diazepam works on multiple areas of cognition when used in short term. Administration of diazepam induces short term memory loss and acquisition (learning) is also diminished. Especially young mice were used in this test as due to induction of diazepam, amnesia is developed which is confirmed in “diazepam with Normal saline” group. It is said that due to certain stress factors, in human mild impairment in cognition or loss of short term is pertained. “Yashtimadhu Choorna plus cow milk” group proved to reduce the amnesia effect of diazepam in animals. Additionally, different dosage form of Yashtimadhu i.e. Kshirapaka pertained its effect as anti- amnesic as against “Yashtimadhu Choorna plus water” group. It is evident in this study that Yashtimadhu Choorna administered with cow milk and processed with cow milk has potential and positive effect on learning and memory.

**In RAM test:** The present study investigated age related cognitive impairment in mice. It is well quoted in ayurvedic

classical texts that after the 4th decade of life span “Medha” starts to get decline. Thus the functions of components of Medha (Dhi, Dhriti and Smriti) might be deteriorated and memory loss or further deficits might be occurred. It is well stated that in aging changes in the parts brain (hippocampus) takes place. Thus considering these facts, instead of young mice, aged mice were utilized in RAM test. In the process of brain aging, spatial learning in relation to navigation is hampered, thus widely used RAM instrument was used which is widely used to study spatial learning and memory in rodents. Seven days treatment of Yashtimadhu Choorna with cow milk and Yashtimadhu Kshirapaka demonstrated significant effect on WMEs. While Yashtimadhu Kshirapaka showed positive effects on reduction of RMEs, and elevation of percentage of correct choices. All together the result of both tests suggest a positive effect of Yashtimadhu Kshirapaka on spatial learning, reduction in short term and long term memory deficits. Yashtimadhu has some phytochemicals which has effect on cognition like glabridin, it is only fat soluble phytochemical. When Yashtimadhu was process with cow milk to form Kshirapaka fat soluble phytochemicals like glabridin in Yashtimadhu might be percolated in Kshirapaka. Due to this Yashtimadhu Kshirapaka may act on brain parts like hippocampus and show positive effect on learning and memory.

EPM test and RAM test results indicate that Yashtimadhu Choorna with cow milk and Yashtimadhu Kshirapaka play an important role in maintain the healthy functioning of cognition. However results indicates that addition of cow milk as Anupana or processing drug Yashtimadhu Choorna with cow milk plays an important role.

In progressive dementia shrinkage of brain parts are seen which may lead to loss of learning and memory process this condition of brain can be correlated with the condition of Kshay of Majja Dhatu stated in ayurvedic science. Study drugs Yashtimadhu and Cow milk both possesses sweet taste, cold potency and main action on like Balya (immune booster), Bhruhaninya (promotes physical strength) and Medhya (memory enhancing activity). May be, the synergistic action of their two drugs might be helpful in the nourishment of brain tissue and regularizing the functions of learning and memory, which is demonstrated in Yashtimadhu Choorna plus cow milk group and Yashtimadhu Kshirapaka group. Thus it can be said that to obtain desired effect on learning and memory, Yashtimadhu Choorna should be given with cow milk or Yashtimadhu Choorna should be converted into Yashtimadhu Kshirapaka form. These two formulations would be the better choice of drug to maintain cognition in balance states or can be used to treat as adjuvants.

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