Evaluation of *YashtimadhuKalpa* formulation as Neuro-nutrient for Learning and Memory improvement in experimental animals

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**ABSTRACT**

The unique functions of the brain are learning, memory, and ability to find objects, recollecting them, and cognition. The foremost intention of the study is to appraise the memory-enhancing potential of “YashtimadhuKalpa” using Wistar rats. The formulation *YashtimadhuKalpa* was prepared using various ingredients and was evaluated for memory-enhancing activity by using Morris water test and elevated plus maze apparatus for the parameter like transfer latency. The formulation - *YashtimadhuKalpa* (75, 150, and 300 mg/kg, p.o.) used for consecutive four weeks significantly reduced TL at the 29th day as correlated to the respective control sets, show enhancement in memory. All doses of formulation notably decreased EL of rats when checked for Day 1, day 15 and on day 30th as related to the control and showed noteworthy enhancement of learning and memory. Among all the doses, the higher dose at 300 mg/kg, p.o. Presents a highly important effect on TL as related to the vehicle-treated control set. The decline of transfer latency dose-dependently proved its potency against Neurodegeneration and confirmed its nootropic activity.

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INTRODUCTION

The progressive neurodegenerative disorder was characterized by the steady onset of dementia which is distinguished by step by step progressive rebuff in cognitive function, with the shortfall, especially in memory reollections ([Uplanchiwar et al., 2018](https://doi.org/10.26452/ijrps.v11iSPL4.4513)). The term cognition concern with people’s mental potential that are part of nearly every human action while conscious. The crucial basis behind neurodegenerative disorder is the involvement of cholinergic commotions ([Uplanchiwar, 2018](https://doi.org/10.26452/ijrps.v11iSPL4.4513)).

Nootropic specialists utilized in allopathic medicine and cholinesterase inhibitors such as donepezil are utilized widely for enhancing memory, disposition, and conduct. In contrast to the present, the result related to adverse effects of those agents have restricted their utilization ([Cumin et al., 1982](https://doi.org/10.26452/ijrps.v11iSPL4.4513)), and it’s sensible to get the efficacy of traditional medicines within the management of diverse cogni-
While dealing with Ancient Indian medicinal system, i.e. Ayurveda, which includes MedhaRasayana, which proved its potency in the field of mental disorders for geriatric to pediatric illness. These drugs act by improving intellect (Dhi), retention power (Dhriti) and memory (Smriti). If this is true, then this category can be considered as neuro-nutrients improving cerebral metabolism by improving intellectual ability, intelligence, memory and cognitive functions (Gómez-Pinilla, 2008). These categories of drugs generally shrink stress, tranquil the mind and improve skills of rational thinking and reasoning (Kabir and Muhammad, 2017). Herbal nootropics comprising a wide range of plants with claimed potential, but the overall mechanisms of action have not been well elucidated in the modern era of science. There is a lack of satisfactory drugs in the allopathic system of medicine with fewer side effects. So, by considering all these issues, an attempt was carried to appraise the prospective of a novel formulation YashtimadhuKalpa - a milk dissolvable, with better shelf life and palatable form for its nootropic potential.

**MATERIALS AND METHODS**

The experimental protocol was carried out at the Department of Central preclinical (Animal) facility lab, Sawangi (Meghe), Wardha, (M.H.)– 442004 India. The ethical clearance was acquired from the Institutional Animal Ethics Committee (letter ref. no. DMIMS (DU) / IAEC / 2018-19-08 dated 14th August 2018). The study protocol was followed by OECD Guidelines for testing of chemical (No.408, Section 4: Health Effects) on the conduct of “Effects of Glycyrrizaglabra Root Extract on Learning and Memory in Wistar Albino Rats” (Adopted: 21st September 1998).

**Drugs and chemicals**

The formulation, YashtimadhuKalpa contains coarse YashtimadhuChurna (powder of Glycyrrhiza glabra Linn.) and Sita (candy sugar) as main ingredients. It was prepared on the basis of GhanaKalpana (solid preparations of herbal extract) by adopting the reference of VriddhaVaidyaParampara. Authentication, standardization, validation and microbial analysis of finished product have been done.

**Animals**

Disease-free male Wistar rats were procured from Bharat serum and vaccines under the supervision of a veterinarian. Animals were housed independently in polycarbonate cage in sets of 6 to 8 per cage under correct laboratory situations with changing light and dark cycle of 12 hours each. The experimental protocol became accepted with the aid of using the Institutional Animal Ethics Committee and animal care was taken as per the rules of CPCSEA, Ministry of Environment and Forests, Government of India. (Registration no. 571/PO/ReRcBiBt/S/02/CPCSEA dated ———).

**Preparation of test Solution**

All the solutions were prepared freshly before administration. The different doses in different concentrations were prepared and a uniform volume of administration was maintained to avoid any bias. The oral route was considered as a route of administration to experimental animals. All the vehicles used were of analytical grade.

**Administration of drug**

The test items were administered by oral gavage to each rat using an incubation needle (16G) fitted into a disposable syringe of appropriate size. The amount to be dosed to an individual animal adjusted according to its weekly body weight. The dose-volume did not exceed 1 ml per body weight.

**Acute Toxicity study**

Acute oral toxicity has a look at ”YashtimadhuKalpa” changed into carried out in step with the OECD guidelines-425 (OECD, 2006). Female rats (Charles Foster; n = 5) fasted nightlong were utilized in the test and also the test drug administered orally following the up and down method, and also the Wistar rats were determined singly for continuous forty-eight hours for any neurological and behaviour changes alongside any sign and symptoms of mortality or toxic effect (Deshmukh et al., 2020).

**Preliminary phytochemical Analysis**

The formulation YashtimadhuKalpa was screened to detect alkaloids, terpenoids, fatty acids, steroids, flavonoids, and glycosides using a pharmacognostic approach (Kokate, 1996).

**Experimental Design**

Male Wistar rats weighing between 150-200g were separated into six sets with six animals per group. The experimental setup for both models was used as

- **Group 1- Control group**: Vehicle alone (Distilled water)
- **Group 2- Toxicant group**: Diazepam (7mg/kg/i.p.) alone
- **Group 3- Low dose of YashtimadhuKalpa**: (75 mg/kg/p.o)
Group 4- Medium dose of YashtimadhuKalpa (150 mg/kg/p.o)
Group 5- High dose of YashtimadhuKalpa (300 mg/kg/p.o)
Group 6- Medium dose of YashtimadhuKalpa (150 mg/kg/p.o) + Diazepam (7mg/kg/i.p)

Elevated plus maze (EPM)
The EPM is considered a simple method in evaluating learning and memory by using transfer latency (TL) as an important parameter (Itoh et al., 1990). A number of plants were assessed by using EPM for nootropic potential. The fabricated EPM comprises of two opposite open arms, 50X10 cm, crossed with two enclosed arms, of equal dimensions with forty cm excessive walls (Kaikade et al., 2020). The arms have been linked with an imperative square (10X10 cm) to present the equipment a plus sign appearance. The maze was unbroken during a dimly lit area elevated fifty cm higher than floor level (Ojha et al., 2010).

On the primary day (i.e., ninety minutes after the closing dose), every rat became located on the quit of the open arm, dealing with far from the primary platform. Transfer latency (TL) became described because of the time (second) taken with the aid of using the animal to budge from the open arm into one of the closed arms with its all four legs. The TL was noted on the primary day considered as a training session for every animal. Retention of this learned-task (reminiscence) began tested 24 hours after the primary day trial (i.e., the twenty-ninth day, 24 hours after the closing dose of four weeks duration). All experimental animals were tested for abstraction memory the usage of EPM, ninety minutes after the last dose. Considerable diminution in TL value of retention signifies enhancement in memory (Domange et al., 2013).

Morris water maze (MWM)
The Morris water maze task was considered widely to explore spatial learning and memory in rodents. The method and parameters for learning and memory of mice considering the Morris water maze were followed, as reported earlier (Morris, 1984). The test substance “YashtimadhuKalpa” in the powder forms, considered as a single ingredient, was experimented with using male Wistar rats (Parle and Singh, 2007).

This methodology is employed to evaluate studying memory in test four week old male Wistar rats. Each rodent become exposed to 4 procurement preliminaries in accordance with the day for four successive days (i.e., after the end portion of about a month term) and their memory becomes analyzed at the fifth day, all through which the stage become taken out (test preliminary). A plastic round pool (58.5 cm in radius, 60 cm high) was packed to a profundity of twenty-five cm with water. 200 millilitres of milk were esteem added to frame the water hazy and spare the perception of the stage. Four variables at the edge of the pool were chosen as north (N), south (S), east (E), and west (W), in this manner separating the pool into four quadrants (NW, NE, SE, and SW). An 8 × 8 cm Plexiglass stage, onto that the rodent may getaway, was situated inside the centre of one of the quadrants, 1 cm underneath the water surface. One day sooner than the test, each rodent becomes situated inside the pool for 60 seconds without the stage presence; this free swim empowered the rodent to come to be adjusted to the preparation air. The dormancy (TL) from inunction into the pool to escape onto the stage gets noted for each preliminary. On the off chance that the rodent neglected to see the platform in a hundred and twenty seconds, it turns out to be physically situated at the stage for a 30-second rest. On the third day (i.e., 30th day, 96 hours after the end portion of about a month span), the stage was taken out and for sixty seconds, the rodent was considered with the expectation of complimentary swimming. The changes in parameter like Transfer latency (TL) was noted down for assessment.

Statistical Analysis
Information was recorded by investigation of change followed by Tukey’s post hoc test in Graph Pad crystal. The p<0.05 was considered as measurably huge. All the outcomes are communicated as a mean ± standard mistake of the mean.

RESULTS AND DISCUSSION
Phyto-chemical testing of formulation YashtimadhuKalpa
The preliminary phyto-analysis exposed the presence of various lively constituents of such as amino acids, fat, carbohydrates, proteins oils, glycosides, alkaloids, steroids, terpenoids, tannins and other Phenolic compounds.

Effect of YashtimadhuKalpa using EPM
The TL is described as the time taken by the rodent to go from the open into covered arms with all its four legs. The comparatively decrease in TL esteem showed the enhancement of memory. The formulation - YashtimadhuKalpa (75, 150, and 300 mg/kg, p.o.) used for consecutive four weeks significantly reduced TL at the 29th day as correlated to the respective control sets, show enhancement in memory. The YashtimadhuKalpa, along with Diazepam in
Table 1: Potency of Formulation

| Treated Groups | TL after 24 Hour | TL after 28 Days |
|----------------|------------------|------------------|
| Group I (Control group) | 48.55 ± 2.23 | 52.08 ± 2.85 |
| Group II (Diazepam control) | 72.64 ± 1.62*** | 61.09 ± 1.16*** |
| Group III (75 mg/kg of YashtimadhuKalpa) | 44.19 ± 1.28*** | 43.30 ± 0.80*** |
| Group IV (150 mg/kg of YashtimadhuKalpa) | 19.55 ± 0.84*** | 12.76 ± 0.9*** |
| Group V (300 mg/kg of YashtimadhuKalpa) | 39.56 ± 1.18*** | 33.08 ± 2.73*** |
| Group VI (150 mg/kg of YashtimadhuKalpa + Diazepam 7 mg/kg) | 19.74 ± 0.90*** | 15.03 ± 1.24*** |

Values are expressed as mean ± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control.

Table 2: Effect of YashtimadhuKalpa using MWM

| Groups | TL - Day 1 | TL - Day 15 | TL - Day 30 |
|--------|------------|-------------|-------------|
| Group I (Control group) | 65.51 ± 2.81 | 26.23 ± 0.58 | 24.07 ± 0.58 |
| Group II (Diazepam control) | 74.01 ± 2.81*** | 19.32 ± 2.24** | 17.23 ± 0.96*** |
| Group III (75 mg/kg of YashtimadhuKalpa) | 50.41 ± 2.36*** | 24.42 ± 1.23 | 22.07 ± 2.55 |
| Group IV (150 mg/kg of YashtimadhuKalpa) | 29.50 ± 1.63*** | 22.14 ± 0.97 | 20.65 ± 1.93* |
| Group V (300 mg/kg of YashtimadhuKalpa) | 39.55 ± 2.77*** | 20.22 ± 1.88** | 18.08 ± 0.63*** |
| Group VI (150 mg/kg of YashtimadhuKalpa + Diazepam 7 mg/kg) | 26.70 ± 1.16*** | 20.90 ± 3.23* | 20.04 ± 2.83* |

Values are expressed as mean ± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control.

group VI, also significantly improved the TL of the 29th day, indicating the potency of formulation as shown in Table 1.

Effect of YashtimadhuKalpa using MWM

As per the results obtained, which were mentioned in Table 2, Learning and memory are associated with transfer latency. The decline of TL by rats in MWM indicates the enhancement of learning and memory. Different doses of YashtimadhuKalpa along with diazepam (7 mg/ kg, i.p.) used for 4 weeks successively, notably decreased EL of rats when checked for Day 1, day 15 and on day 30th as related to the control, present noteworthy enhancement of learning and memory. Among all the doses, the higher dose at 300 mg/kg/p.o. Present a highly important effect on TL as related to the vehicle-treated control set.

The current research reveals the memory-enhancing potential of various doses of “YashtimadhuKalpa” in suitable Exteroceptive behavioral animal models. The formulation was administered to all experimental animals at various dose levels and the effects were assessed on the basis of transfer latency. Memory improving effects of the formulation was comparable to amnestic drug diazepam. The MWM and EPM were involved as a behavioral model for the assessment of learning and memory. The formulation significantly decreased TL during training and experimentation, indicating the improvement of learning and memory.

The formulation was based on the presence of Glycyrrhiza glabra, having a chief constituent as glycyrrhizin which proved its potency in oxidative stress (Ju et al., 1989) as well as in inflammation (Yokota et al., 1998). The immunohistochemical investigation uncovered the presence of endless irritation in specific districts of the mind in Alzheimer’s infection patients. As irritation can be harmful to have a tissue, it was guessed that calming medications may be repressing both the beginning and the movement of Alzheimer’s sickness (Mcgeer and Mcgeer, 1999). The Anti-inflammatory action of “YashtimadhuKalpa” might play an imperative position in Neuroprotection.
The phytochemical investigation conforms to the existence of flavonoids, phenols and triterpenoids, as well as the major constituent present in the formulation - glycyrrhizin altogether contributed as defensive factors in the management of oxidative stress as damage of neurons results in neurodegeneration and memory loss.

Cognitive dysfunction can be interconnected with impeded cholinergic transmission and the help of focal cholinergic transmission bringing about reduced memory. The disintegration and brokenness of cortical cholinergic neurons are firmly associated with intellectual deficiencies of Alzheimer’s sickness (Ferreira-Vieira et al., 2016). Henceforth, the specialist who increments cholinergic capacity can be used for the treatment of dementia personally identified with Alzheimer’s illness.

Acetylcholine is the most valuable synapse liable for the guideline of psychological capacities (Jensen et al., 1987). The loss of cholinergic neurons or diminished combination of acetylcholine was noted and answered to be a trademark highlight of demen-tia of Alzheimer’s infection type (Hasselmo, 2006). The chief phytoconstituents of formulation, i.e. glycyrrhizin which categorized as triterpene, was evaluated for its AchE potential confirmed its potency against neurodegeneration (Bar et al., 2019).

So, from all these parameters and results obtained, we can claim as a nootropic and as it is consumed largely without any potential adverse effect, the formulation “YashtimadhuKalpa” can be considered as neuro-nutrient.

CONCLUSIONS

The present study concluded that the formulation “YashtimadhuKalpa” claim for memory-enhancing effect according to Ayurveda and other systems of medicine. It importantly enhances learning and memory of rats doubtless through transfer latency with the involvement of the GABA-benzodiazepine pathway, AchE inhibition and through anti-inflammation. A further detailed study is required to explore the other possible mechanisms for the management of cognitive disorders with its worth in the management of cognitive and neurodegenerative disorders. Moreover, this formulation can be used as nutraceuticals in the control of Alzheimer’s illness.

Conflict Of Interest

The authors declare that they have no conflict of interest for this study.

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