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Experimental evaluation of *Yashtimadhu* (liquorice) Nootropic action

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Abstract
Human beings are the intelligent organisms present in this universe. The only thing that separates human beings from all the other creatures is the brain and its complexity. The unique functions of brain are learning, memory and ability to find objects, recollecting them and cognition. Even though certain nootropic drugs of other systems of medicine are available, their effectiveness and safety is only limited. Here Ayurveda provides fruitful solutions for all these problems. As per Ayurveda the one that benefits intellect *(Medha)* is said to be *Medhya*. *Yashtimadhu* (*Glycyrrhiza glabra* Linn.) is one among the four *Medhya Rasayanas* (memory promotive drugs) mentioned by *Acharya Charaka*. The present study is intended to find out the efficacy of *Yashtimadhu* root with Cow's milk and milk powder as Medhya drugs on the behavior of Wistar Albino Rats in certain selected Pharmacological animal models.

Keywords: Learning; Memory; Medhya; Behaviour; Pharmacological Animal Model

Introduction
Right from the Vedic period, the interest of the sages has been in understanding and in controlling the psychological conditions in human beings. The holy Gayatri mantra is a prayer offered to the sun, the illuminator of the Universe, to stimulate and enlighten the mind [1]. In one or the other way man has always tried to achieve great abilities in terms of physical and psychological excellences. The changes, improvements and evolutions that have occurred from civilization to civilization, generation to generation are the proofs of developing skills of mankind. Man has been to conquer the peak in all walks of life because of his unlimited thoughts as well as ambitions. For the successful survival of man in this competitive world there is a need for promotion of psychological health and management of various psychological and psycho-somatic problems.

Brain is the organ that is responsible for what we call the mind. It is the basis for thinking, feeling, wanting, perceiving, learning and memory, curiosity and behaviour. Memory is a fundamental mental process and without memory we are capable of nothing but simple reflexes and stereo type behaviours [2]. Thus, learning and memory are one of the most intensively studied subjects in the field of neuroscience, which are complex phenomena requiring the coordinated interaction of multiple brain structures. Memory is a faculty by which sensations, impressions and ideas are stored and recalled, where learning is a process by which brain acquires new information about the events occurring in the given surroundings [3].

The scholars of Ayurveda gave due emphasis on the maintenance of health as well as cure of diseases which enabled us to enquire in depth about the mind and intellect. The analysis of the mind and intellect from different angles and their explanations are abundantly available in the Ayurvedic literature which provide evidence of the quantum of thoughts, clarity of vision as well as expertise of the ancient scholars. Ayurveda has laid down certain principles which can prove a great solution for many of the problems concerned with mind and body. The description regarding the concept of *Medhya Rasayana* (memory promotive rejuvenation therapy) is one among them. It requires reconsideration and application in present scenario as

it can improve the intellect in healthy individuals and also answer many of the psycho-somatic problems.

Baffling situations on the contemporary society are hampering memory faculties. As per *Acharya Charaka* many of the diseases like *Unmada* (psychosis), *Apasmara* (epilepsy), *Atatvabhinivesha* (derrangement of intellect) etc and other *manovikaras* (psychological disorders) result in which *smriti nasha* (loss of memory), *buddhi nasha* (loss of intellect) and *buddhi vibrama* (blasphemy of intellect) take place. That's why promotion of *Medha* (intellect) is desired and is the necessity of everyone. There are many Ayurvedic formulations which are mentioned as *Medhya Rasayanas*. *Yashtimadhu* (Liquorice) is one among the four *Medhya Rasayanas* mentioned by *Acharya Charaka* for promoting *Medha* [4]. It is necessary to analyze the effect of these *Medhya* (memory promotive) drugs in improving intellectual capacity. Nootropics of other systems of medicine are smart drugs or cognitive enhancers which are drugs, supplements, or other substances that improve cognitive function, particularly executive functions, memory, creativity, or motivation. The use of cognition-enhancing drugs by healthy individuals in the absence of a medical indication is one of the most debated topics among neuroscientists, psychiatrists, and physicians which spans a number of issues, including the ethics and fairness of their use, concerns over adverse effects, and the diversion of prescription drugs for non-medical uses, among others [5]. Their efficacy is also limited. Hence *Yashtimadhu* has been used for this study as it can be given even for healthy individuals without any interactions.

Evidence based health sciences are order of the day today. Any small evidence shown logically creates confidence and promotes practice. *Ayurveda* means to search ornamentation of evidences to become popular. Animal experimentation provides evidences provided properly designed. Hence the present study has been taken to study the efficacy of *Glycyrrhiza glabra* root powder with milk and *Glycyrrhiza glabra* root powder with milk powder formulations in ethanol induced amnestic rats in comparision to the normal control and negative control groups. The study here is designed in accordance with *Ayurvedic* methods of application. No synthetic drug is used to create a pathological condition in the animal. The study has provided interesting results.

Aims and Objectives

- To evaluate the efficacy of *Yashtimadhu medhya karma* (memory promoting activity) along with Cow's milk and milk powder on Wistar Albino Rats using current pharmacological Models.

Materials and Methods

a. Drug Material: **Fig. 1**

The test drugs *Glycyrhiza glabra* Linn. root powder with Cow's milk (GGM) and *Glycyrhiza glabra* Linn. root powder with milk powder (GGMP) are used for experimental purpose and administered to the experimental animals according to the calculated dose. As the availability of Cow's milk is a difficult task now-a-days, hence the efficacy of milk powder is also tested.

b. Animals

Wistar Albino Rats obtained from the animal house attached to Sree Vidyanikethan College of Pharmacy, Tirupati were divided into four groups consisting of 6 animals per group. The animals were maintained with Rat pellets feed and tap water. The laboratory animals were maintained under normal ambient conditions which are favorable for their survival.

c. Instruments Used

1. Rat Feeding Needles (No.18&20)
2. Syringe (Tuberculin)
3. Flask
4. Pipettes

d. Chemicals

1. Ethanol (0.5 ml/100gm body wt. orally)
2. 2% v/v tween 80 (as a control vehicle)

Fig. 1: Showing Yasthimadhu plant and its roots

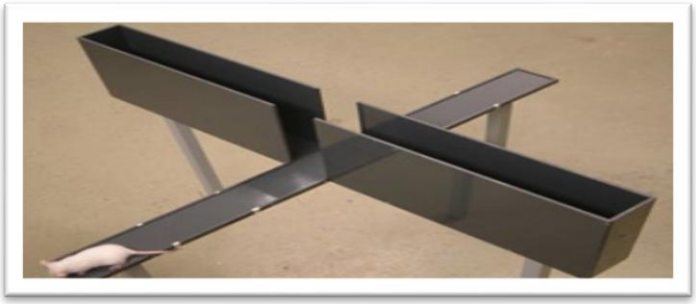

Fig. 2: Showing the model of the Elevated plus maze test used for the study

e. Methods

Behavioral paradigms to assess learning and memory

Passive Avoidance - step down model

This is a classical model for the assessment of cognitive performance after inducing brain lesions. The term "passive avoidance" is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behavior. The step-down type of

passive avoidance task is used to examine the long-term memory based on negative reinforcement.

The apparatus consists of a transparent acrylic cage (30cm*30cm*40cm) with a grid floor, inserted in a semi sound proof outer box (35cm*35cm*90cm). The cage is illuminated with a 15 W lamp during the experimental period. A wooden platform (4cm*4cm*4cm) is fixed in the centre of the grid floor (35cm above the floor) and electric shocks (1 Hz, 500 m sec, 40 V DC) are delivered using an isolated pulse stimulator.

Fig. 3: Showing the model of the passive avoidance paradigm used in the study

The training is carried out in two sessions. The animal is placed on the elevated platform. Due to its innate exploratory behavior, the animal steps down on to the grid. The step-down latency (SDL) is noted. As soon as the animal steps on the grid it is given an electric shock (for duration of 15 sec). SDL, number of flinching reactions and vocalizations are measured. Animals showing a SDL of 3 to 30s during the first training session are selected for second (60 to 90 min after first) and retention trials. Animals staying 60s on the platform are considered as remembering the task and do not receive electric shocks any more. The retention test is carried out 24 hr after training, in a similar manner, except that the electric shocks are not applied to the grid floor. Each animal is again placed on the platform and SDL is recorded with an upper cut-off time of 300s. Both saline-treated and amnestic drug treated animals show the same SDL during the first training session. However, amnestic drugs are expected to increase the SDL in the subsequent trials indicating the impairment of learning and retention tasks. The failure should be ameliorated by test drug.

Maze

Mazes are traditional tools for assessing learning and memory performance in laboratory animals. Conventionally, maze consists of open and enclosed arms. The rodents have a natural inclination towards enclosed area and spend more time there in comparison to open area. On the basis of this, the transfer latency of the animal is recorded. The animal learns to avoid open arms and shortens transfer latency to enclosed area. Nootropic agents are effectively screened using this paradigm in scopolamine-induced dementia. Elevated plus maze, radial maze, Y maze and figure 8 maze are based on this phenomenon.

The plus maze consists of two opposite open arms (50cm*10cm), crossed with two closed arms of the same dimensions with 40cm high walls. The arms were connected with a central square (10cm*10cm). Rats are placed individually at one end of an open arm, facing away from the central square. Time taken for the rat to move from the open arm and enter into one of the closed arms is recorded and termed as "initial transfer latency" (ITL). Animals are allowed to explore the maze for 30s after recording transfer latency. Retention transfer latency (RTL) is recorded by placing the rats similarly on the open arm at specified intervals [6].

Groups

1. Group I - Normal control (2 % v/v tween 80) – 2 % v/v Tween 80 (Polysorbate 80) is a hydrophilic non-ionic surfactant commonly used as an ingredient in dosing vehicles for pre-clinical in vivo studies.
2. Group II - Negative control (ethanol 0.5 ml/100gm body wt. orally)
3. Group III - *Glycyrhiza glabra* Linn. with Cow's milk (GGM)
4. Group IV - *Glycyrhiza glabra* Linn. with milk powder formulation (GGMP)

Methods to induce memory loss

- Using madya i.e. ethyl alcohol (ethanol)

"buddhim lumpathi yat dravyam madakari taduchyate
tamo guna pradhanam cha yatha madyam suraadhikam" [7]

As madya (alcoholic preparation) is described in Ayurveda to overshadow buddhi (intellect) by virtue of its tamo guna (drowsy nature), that is why it is chosen to create buddhi nasha (loss of intellect) prior to the administration of the test drugs. Inorder to induce memory loss oral route has been chosen as it is the natural way, in converse to the usually followed intra peritoneal route.

Route of Administration

Administration of compound plays a large part in experimental design using animals. Before administering any substance (therapeutic or experimental) to an animal subject, appropriate decision on the dose to be administered, frequency of administered volume, solvent (if necessary) and route of administration should be made.

Drugs are to be administered orally in Ayurveda because the drugs here follow the food pathway in the body. The oral administration of solution of drugs or test substances to experimental rats is often necessary in various pharmacological, toxicological and other biomedical researches. Oral ingestion is the most common method of drug administration to humans. It is also the safest, most convenient and most economical route in the animals.

It is scientifically sound and preferable to administer test substances to experimental animals by the same route(s) by

which it is taken or meant to be taken by humans. As systemic bioavailability, pharmaco-kinetics and toxicological parameters obtained for the substance will depend markedly on the route used to administer it. So in the present animal experimentation oral route is elected for drug administration. The test drugs were administered in suitable doses by oral route with the help of rat feeding needle daily for fourteen consecutive days.

Method of administration

1) The rat feeding needle is attached to a 3ml syringe containing the solution of test drug (GGM and GGMP of groups III and IV respectively) and held with the right (dominant) hand.
2) It is introduced into the Rat's oral cavity to the left side of the animal's incisor teeth in the midline and maintained in this position throughout the procedure to avoid damage to it from the animal's bite.
3) Carefully advanced down the oral cavity between the tongue and roof of the mouth, occasionally it passes easily straight on into the esophagus at other times a resistance is encountered. This resistance can be relieved by the maintenance of a gentle inward push on the needle while rotating the tip from side to side.
4) This soon stimulates a swallow reflex, which transmits the needle into the esophagus. Occasionally the animal may use any of its strong hands to attempt to push the needle out of the mouth at this stage or during the ingestion.
5) Working alone in this situation, instead of focusing on restraining the tail as explained above it greatly helps to use any of the other free fingers of left hand to hold down the dominant forelimb of the animal preventing any grip on the needle already situated within the esophagus. Once needle in the esophagus the plunger is pushed down the syringe, emptying its content into the esophagus en-route to the stomach.

Dose of the formulations

The dose is decided basing on the standard dose of the drug -

- For a 10-15yr (40kg=40,000gm) old boy = 1gm *Yasthi* is the dose
- i.e. for 150gm lab animal = *3.75mg GGM* is the dose
  (Animal Dose = AW x HD) (AW=Animal weight;
  40x1000 HD = Human dose)
- (Likewise, for a 10-15 yr old boy (40kg=40,000gm) = 1gm Yasthi + 2gm Milk powder = 3gm mixture is the dose
- i.e. for 150gm lab animal = *11.25mg GGMP* is the dose
- Formulation 1 GGM dose = 3.75 mg
- Formulation 2 GGMP dose = 11.25mg

Procedures done: Fig. 2

Elevated plus maze test

Elevated plus maze was described as a tool for testing memory by the investigator working in the field of psycho pharmacology [8]. Elevated plus maze served as exteroceptive behavioral model to evaluate learning and memory in rats [9]. The elevated plus maze consists of two open arms and two closed arms (50cm * 10cm * 40cm) with an open roof arranged so that the two arms are opposite to each other. The maze was elevated to a height of 50 cm. All the animals of groups 3 and 4 were given ethanol (0.5 ml/100gm body wt.) orally for 14 days and after 2nd hr of ethanol administration on every day the formulations GGM and GGMP were given to the respective groups 3 and 4. Prior to this training was given to the animals. First group served as a vehicle control. On the 14th day respectively each rat was placed at the end of the open arm, facing away from the central platform.

Transfer latency was time taken by the rats to move into covered arm with all its four paws, transfer latency was recorded. If the animals did not enter into one of the covered arms within 90s, it was gently pushed into one of the two covered arms and transfer latency was assigned as 90s. The rat was allowed to explore the maze for 10s and returned to the home cage. Twenty four hours later i.e. on 15th day transfer latency was recorded again. The measurement of transfer latency on the day 14 served as a parameter for acquisition and those on day 15 served as a parameter for retention of memory.

Passive avoidance paradigm Fig. 3

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory [10]. The apparatus consists of a box (27cm * 27cm * 27cm) having three walls of wood and one wall of Plexiglass, featuring a grid floor (made up of 3mm stainless-steel rods set 8mm apart), with a wooden platform (10cm*7cm*1.7cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock was delivered to the grid floor. The rats were initially trained and were gently placed on the wooden platform set in the center of the grid floor. When the rat stepped down and placed all its paws on the grid floor, shocks (50hz; 1.5mA; 1s) were delivered for 15 seconds and the step down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the rat to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 seconds during the training session were used for the acquisition and the retention test. The acquisition task was carried out 90 min after the training session. During the acquisition test, animals were removed from the shock free zone if they did not step down for a period of 60 seconds. Retention was tested after 24hr in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 s [11]. Significant increase in SDL value indicated improvement in the memory.

All the animals of groups 3 and 4 were given ethanol (0.5 ml/100gm body wt.) orally for 14 days and after 2nd hr of ethanol administration on every day the formulations GGM and GGMP were given to the respective groups 3 and 4.

Observation of the Elevated plus maze test

Table 1: showing the effect of formulations GGM and GGMP on transfer latency (elevated plus maze test) in ethanol induced amnesia in Wistar Albino Rats

Treatment groups	Transfer latency in sec.	
	Acquisition day 14	Retention day 15
Control	32.96±2.06 s	28.96±0.98s
Ethanol	127.56±1.28s	131.26±1.58s
Formulation 1 (GGM) (Dose:3.75mg)	26.39±4.55s*	9.36±0.63s*
Formulation 2 (GGMP) (Dose:11.25mg)	19.63±3.69s*	7.63±2.55s*

(Statistical significance test was done by ANOVA followed by Dunnet's t test (n=6), Values are mean ± SEM of 6 animals per group.*P < 0. 001 vs. Ethanol- treated group)

Observation of the Passive avoidance paradigm test

Table 2: showing the effect of the formulations GGM and GGMP on step down latency (passive avoidance paradigm) in ethanol induced amnesia in Wistar Albino Rats

Treatment groups	Step down latency in sec.	
	Acquisition day 14	Retention day 15
Control	3.69±0.69s	3.89±0.57s
Ethanol	1.06±0.28s	1.03±0.18s
Formulation 1 (GGM) (Dose:3.75mg)	13.39±1.25*s	17.89±0.51*s
Formulation 2 (GGMP) (Dose:11.25mg)	14.08±0.09*	21.05±1.58*s

(Statistical significance test was done by ANOVA followed by Dunnet's t test (n=6), Values are mean ± SEM of 6 animals per group.*P < 0. 001 vs. Ethanol- treated group)

Results

From the above tables it is clearly evident that the formulations 1 (GGM) and 2 (GGMP) of groups III and IV showed remarkable reduction in the transfer latency time (in elevated plus maze test) from the acquisition day to the retention day. Thus it can be ascertained that GGM (*Glycyrrhiza glabra* with Cow's milk formulation) and GGMP (*Glycyrrhiza glabra* with milk powder formulation) are statistically significant in comparison to the normal control and negative control groups.

From the above tables it is clearly evident that the formulations 1 (GGM) and 2 (GGMP) of groups III and IV showed remarkable increase in the step down latency time (in passive avoidance paradigm) from the acquisition day to the retention day. Thus it can be said that GGM (*Glycyrrhiza glabra* with Cow's milk formulation) and GGMP (*Glycyrrhiza glabra* with milk powder formulation) are statistically significant in comparison to the normal control and negative control groups.

Discussion

Even though nootropics are available in other systems of medicine, their utility is only limited as they pose many adverse effects with little efficacy. So, they can be used in only certain psychological disorders related to memory. Further they can't be used in healthy individuals to improve the memory faculties. In converse *Medhya rasayana* (memory promotive) drugs like *Yashtimadhu* prove to be efficacious not only to the psychologically ailed but also healthy individuals who want their memory faculties to be increased. Hence the present study has been carried out pre-clinically in Wistar Albino Rats.

Groups GGM and GGMP showed remarkable reduction in the transfer latency time (in Elevated plus maze test) which explains that they are significant. Groups GGM and GGMP showed outstanding increase in the step down latency time (in Passive avoidance paradigm) which explains that they are significant. From the above observations it can be said that *Yashtimadhu* administration along with either Cow's milk or milk powder is an equally effective remedial measure in memory related disorders which is a re-establishment of old fact as propounded by *Acharya Charaka.*

Conclusion

The use of cognition-enhancing nootropic drugs of other systems of medicine by healthy individuals in the absence of a medical indication is one of the most debated topics among neuroscientists, psychiatrists, and physicians which spans a number of issues, including the ethics and fairness of their use, concerns over adverse effects, and the diversion of prescription drugs for non-medical uses, among others. From the above observations of the study it can be concluded that *Yashtimadhu* with Cow's milk and milk powder have shown remarkable effect on the learning and memory capacity in Wistar Albino Rats in both the Pharmacological Models i.e. Elevated plus maze test and Passive avoidance paradigm. Hence as propounded by *Acharya Charaka Yashtimadhu* can be used as a memory promoting drug in both healthy as well as psychologically ailed individuals of all ages without any adverse effects. Findings also prove to be fruitful paving the way for future expansive clinical experiments for the betterment of learning and memory in human beings.

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