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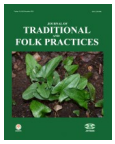

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Memory enhancing effect of *Kuvalaya* (*Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth) in Wistar albino rats

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Abstract

According to World Health Organization (WHO), dementia is one of the major causes of disability and dependency among older people for which piracetam and anticholinesterases are the only available treatment, but these are inadequate to provide optimum treatment. Ashtangahrdaya describes a formulation called *chathushkuvalaya rasayana* which is glorified as a potent memory enhancer, where *Kuvalaya* is the only herbal ingredient. In Kerala, *Kuvalaya* is botanically identified as *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth., belonging to Pontederiaceae family. The present study intended to assess the memory enhancing effect of *Kuvalaya Ghrta* (KG), *Kuvalaya swarasa* (KS), ethanolic extract of *Kuvalaya* (KEE) using T-Maze test. The acute toxicity of the ethanolic extract of *Kuvalaya* (*M. vaginalis*) was also assessed according to OECD guidelines 425. The T-Maze experiment was carried out in six groups having six rats each (36 rats). Initially the baseline memory was established in each group. The T maze test was done on the 15th day to assess the improvement in memory in different groups. The memory enhancing effect was assessed by comparing different groups before and after treatment and between different groups. It was observed that GIII Pira (Standard group-piracetam dose-500 mg/kg) and GIV-KG (*Kuvalaya Ghrta* 4.32ml/kg) showed an improvement in memory after chronic dosing of the medicines for 14 days. Multiple Comparison Post-Hoc analysis revealed GIV-KG provided considerable improvement in memory when comparing with all other groups which was statistically significant but did not have statistically significant memory enhancement when comparing with the standard drug (GIII-Pira). No acute toxicity was observed for the ethanolic extract up to 2000 mg/kg.

Keywords: Acute toxicity, Ashtangahrdaya, *Chatushkuvalaya rasayana,* Dementia, T-Maze

1. Introduction

Ayurveda is one among the most ancient systems of medicine in the world. The classical lexicons of Ayurveda describes that the main aim of this science is to provide physical, mental, social and spiritual well-being. For this, there is the need of developing diagnostic, therapeutic and prognostic approaches on scientific lines. Although Ayurveda has its own time tested validation of principles and treatments there is an emerging need for the development of standardization protocols and more research insights so that the science gets global acceptance. Thus, the present era is witnessing cutting edge researches and scientific revalidation of concepts.

India has a rich treasure of biodiversity which is serving as the backbone of ayurvedic treatment with its wide variety of climatic and soil conditions India has ample scope of gaining a foothold in the global plant based pharmaceutical market. Out of more than 25000 plants of medicinal value, only 10 % are used for their medicinal value. Around 1800 species are systematically documented in the codified Indian systems of medicine. These herbal products are preventive, protective, nutritive and curative (Mahalakshmi *et al.,* 2022). There is a rising need for exploring this rich biodiversity for development of new novel medicines.

Poor memory, lower retention and slow recall are some of the common problems faced by today's stressful and competitive world (Parle and Vasudevan, 2007). The acquisition of any new information and skill is termed as learning and its subsequent retention is known as memory, both are fundamental properties of Central Nervous System (CNS) and play a critical role in the 'process of thought' which is known as cognition (Bruel *et al*., 2007). Memory loss may be associated with diseases especially related to ageing though it can be found otherwise also. This can affect the quality of life of people to a greater extent (Silva and Martínez, 2022). Memory may be broadly classified into short term memory and long-term memory. As the name itself suggests, short term memory stores the information for a shorter period whereas long term memory stores information for a much longer period. Memory loss may also be associated with diseases like Parkinson's disease, Ischaemic brain damage, head injuries, etc. (Bird and Miller, 2005).

Contemporary medical research mainly focuses on understanding the neurobiology of learning and memory and explores nootropic agents that can prevent progressive memory loss or enhance memory capacity (Malve, 2015). Research on memory deficit and age-related memory loss has brought forward new treatment techniques to improve memory including diet, exercise, stress management, cognitive therapy and pharmaceutical medications.

Classical ayurvedic texts describe a set of rejuvenative measures to impart biological sustenance to body tissues known as '*rasayana*' and those specific to brain tissue are called as '*medhya rasayana*'. They help in slowing down brain ageing and regeneration of neural tissues besides producing antistress, adaptogenic and memory enhancing effects. Thus, they can be effectively utilised for delaying the loss of memory and can also be used for the preventive aspect of the same (Singh *et al.,* 2008). Ashtangahrdaya, a classical ayurvedic text presents a preparation namely *chathushkuvalaya rasayana* (Vaghbhata, 1995) while describing about *medhya* and *rasayana* preparations in *uttarasthana* and emphasizes its applicability as a potent memory enhancer. The only two ingredients present in it are *Kuvalaya* and *swarnapatra,* among which *Kuvalaya* is the only herbal ingredient and it is in the *Ghrta* dosage form.

The drug *Kuvalaya* is found widely throughout the *brhatrayee* in various contexts where *neelotpala* and *indeevara* are used as its most common synonyms. In Susruta Samhitha it has been mentioned as an ingredient of *Suvarna* yoga in medhyayushkameeya adhyaya (Susruta, 2014). While glancing through the traditional books of Kerala like Sahasrayogam (Vaidyan, 2011) and Ashtangahrdyam Malayalam commentary by Chepat Achyutha Varier., the *neelotpalam* has been taken as a plant locally known as *karimkuvalam* (Achyutha, 2014).

In Indian medicinal plants the plant with local name '*karimkoovalam*' with botanical identity *Monochoria vaginalis* (Burm.f.) C.Presl ex Kunth of Pontederiaceae has been identified as *Kuvalaya* (Anonymous, 2007). Hortus Malabaricus also accepts the botanical identity of '*karimkuvalam*' as *M. vaginalis* (Manilal, 2003). There is scarce data regarding its memory enhancing effect of this drug. Hence the present study was aimed to assess the preliminary memory enhancing effect of *Kuvalaya* in rats using T-Maze test (Ramakrishna *et al.*, 1998) and also the acute toxicity of the ethanolic extract of *Kuvalaya via* OECD guidelines 425.

2. Materials and methods

2.1. Plant materials and authentication

For the purpose of the study genuine samples of the drug *M. vaginalis* was collected from the natural habitat. The drug is commonly found along marshy regions and alongside paddy fields throughout the state especially during rainy season (Fig. 1). The drug was authenticated at the concerned authorities and a dried sample was preserved as Herbarium for further use (DGAVC195/18).

2.2. Preparation of *Kuvalaya Ghrta, swarasa* and ethanolic extract

2.2.1. Preparation of *Kuvalaya Ghrta*: *Ghrta* was prepared with *Kuvalaya* (Fig. 2) according to the standard procedure of preparation of *Ghrta* mentioned in Sharangadhara Samhitha Madhyama Khanda. *Paakalakshana* was taken as per Sharangadhara Samhitha Madhyamakhanda. The formulation composition is mentioned in Table 1 and the final yield of the product was 216.4 ml.

2.2.2. Preparation of *swarasa*: *Swarasa* (fresh juice) of the fresh drug was prepared according to the standard

Fig. 1. *Monochoria vaginalis* (Burm.f.) C. Presl ex Kunth

Fig. 2. Preparation of *Kuvalaya Ghrta*

procedure of preparation of *swarasa* mentioned in Sharangadhara Samhitha Madhyamakhanda. Fresh drug was collected from the natural surroundings. It was washed and cut into small pieces and the juice was extracted to get the required amount of *swarasa* (fresh juice).

2.2.3. **Preparation of ethanolic extract of *Kuvalaya***: Around 200 g of the powdered drug was taken and refluxed with ethanol in Libig's condenser. After 2 hrs of refluxing the mixture was filtered and then it was distilled. After distillation ethanol was collected in a conical flask. The extract was evaporated to dryness and stored for further use.

2.3. Experimental study

2.3.1 Acute toxicity of *M. vaginalis* according to OECD Guideline 425: The dose of 2000 mg/kg was used as the test dose as per OECD Guideline 425; Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure (Anonymous, 2022). Five female rats were taken for the study as the literature survey revealed that the female rats are more sensitive. The dose for each rat was calculated according to their body weight. The dose of the rat having body weight 120 gm (2000x120/1000) was 0.24 g/ml. The treated animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 14 days (Table 2).

2.3.2. Memory enhancing activity

(a) *Experimental animals*: Thirty-six adult healthy Wistar albino rats, female, 2-3 months of age were obtained from Sree Chitra Tirunal Institute of Medical Sciences and Technology, Poojappura, Kerala. The experiment protocols were approved by the Institutional ethical committee (order no IEC255/25.05.2017) and a written permission from Institutional Animal Ethics Committee, Govt. Ayurveda College, Thiruvananthapuram (No 06/01/2018/GAVC dated 09/07/2018) (CPCSEA Reg No. 2015/GO/Re/S/18/ CPCSEA) were followed for the maintenance and experimental animals were handled as per CPCSEA guidelines.

Table 2. Table showing the weight of the animals used and the dose of medicine administered

Animal	Body wt (g)	Dose(g/ml)
A	120	0.24 g/ml
B	150	0.3 g/ml
C	160	0.32 g/ml
D	140	0.28 g/ml
E	140	0.28 g/ml

Table 1. Table showing the ingredients and the formulation composition

Sl. No.	Material used	Parts	Quantity
1.	*Kuvalaya kalka* (fine paste of the drug)	1/8th Part	64.32 g
2.	*Ghrta* (Ghee)	1 part	514.5 ml
3.	*Kuvalaya swarasa* (Fresh juice)	4 Parts	2058.2 ml

(b) *Grouping of animals and housing conditions*: All the animals were maintained in appropriate environmental and nutritional circumstances throughout the experiment. The rats were maintained under standard laboratory conditions with conditions with natural dark and light cycle. They were provided with free supply of standard dry rat diet and water. Animals were acclimatized for 14 days prior to experimentation. They were given preliminary trials to reach the reward in T-Maze during last 5 days of acclimatization and the time was noted to calculate baseline memory of the rats in each group.

Three animals were housed in each cage made of polypropylene with stainless steel top grill. The bedding was changed on alternate days. Bedding provided in rat cages was in sufficient quantity to cover the whole floor.

The animals were weighed and randomly divided into 6 groups having 6 rats each. The random selection was unbiased distribution of animal with regard to weight, age, etc. in each group. The animals were marked for proper identification and kept in separately labelled cages. The dose of each animal was calculated according to the body weight and was put in tables for further reference. The study was conducted by grouping the healthy albino rats into 6 groups with each group containing 6 rats and the groups were as follows (Table 3).

24 hrs prior to testing, food was withdrawn from the cages. 6 groups were given the already described medicines for 14 days. On the 15th day the rats in each group were again tested on T-Maze to find out the change in the time taken for reaching the reward. 10 trials were given in a short duration and the average value of positive scores were noted. The memory enhancement was calculated by comparing the improvement in each group before and after the study and also comparing between the different drug treated groups to obtain an authentic result.

2.4. Statistical analysis

Descriptive statistics such as mean and standard deviation was calculated and the observed mean difference in time taken to reach the food reward was statistically tested using one-way ANOVA. Then Post-hoc multiple comparisons were done for mutual comparison of groups. A calculated 'p value of <0.05 was considered to be statistically significant. Paired t-test was done to analyse the difference in groups before and after treatment.

3. Results and discussion

3.1. Acute toxicity study

Throughout the observation period neither incidence of mortality nor animals found in a moribund condition. Body weight gain and animal behaviour were normal for 14 days. Thus, ethanolic extract of *M. vaginalis* is safe up to the dose of 2000 mg/kg, in tested rats.

3.2. Memory enhancing activity

The data on the time taken by each rat to reach the food reward was measured using a stop watch. The initial data to establish that each rat had normal baseline memory was carried out for 5 days and the data was recorded. After administering the medicine for 14 days, the rats were again tested for the time to reach the food reward *via* T-Maze and the results were recorded. Descriptive statistics such as mean and standard deviation was calculated and the observed mean difference in time taken to reach the food reward was statistically tested using One-way ANOVA (Table 4). The p value 0.000 ($p<0.05$) shows that there exists a significant difference between at least one pair of groups.

The p value 0.000 ($p<0.05$) shows that there exists a significant difference between at least one pair of groups. To find out which is the differing group we perform post-hoc analysis the results of which are tabulated.

Table 3. Table showing the grouping of animals and the medicine administered

Sl. No.	Groups	Medicine administered
1	Group-I	Distilled water (GI-NC)
2	Group II	Normal dose of Plain *Ghrta* (GII-G) (0.864 ml/200 g rat)
3	Group III	Standard drug piracetam in the dose of 500 mg/kg (GIII-Pira.)
4	Group IV	Normal dose of *Ghrta* prepared with *Monochoria vaginali*s Presl. (GIV-KG) (0.864 ml/200 g rat)
5	Group V	Normal dose of *swarasa* prepared with *Monochoria vaginali*s Presl. (GV-KS) (0.432 ml/200 g rat)
6	Group VI	Normal dose of ethanolic extract prepared with *Monochoria vaginali*s Presl. (GVI-KEE)

3.2.1. Multiple comparisons Post hoc Analysis (Table 5; Fig. 3)

Dependent variable: Difference

3.2.2. Paired t-test to analyse the difference before and after treatment in each group (Table 6): In the present study we have tried to explore the memory enhancing potential of the drug *M. vaginalis*. Achinthya Veerya has been explained to be the mode of action of *medhya* drugs by Acharya Nagarjuna. *Medhya rasayanas* may act at multiple levels including *rasa, agni* and *srotas*. These drugs help in improving *agni* by stimulating the function of *agni*, they open up and clean the microchannels which ultimately results in the improvement of *medha* (Ray and Ray, 2015)

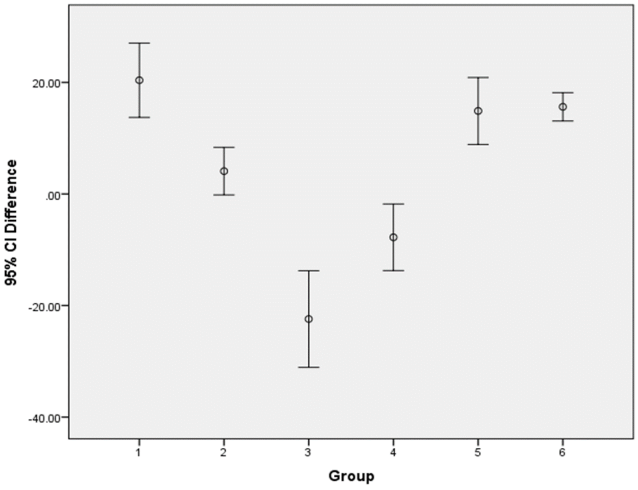

Fig. 3. Graph showing group wise comparisons *(1-GI-NC, 2-GII-G, 3-GIII-Pira., 4-GIV-KG, 5-GV-KS, 6-GVI-KEE.)*

Table 4. Table showing results of ANOVA

	Sum of Squares	**Df***	**Mean Square**	**F***	**Sig.**
Between Groups	8151.159	5	1630.232	49.923	.000
Within Groups	979.648	30	32.655	-	-
Total	9130.807	35	-	-	-

**Df – Difference, F value - variation between sample means/variation within the samples, Sig. - significance*

Table 5. Table showing Multiple comparisons between groups

Group comparison		**Mean difference**	**Standard error**	**Significance**	**95% confidence interval**	
					Lower bound	**Upper bound**
GI-NC	GII-G	16.30139*	3.29924	0.000	6.2664	26.3363
	GIII-Pira	42.81350*	3.29924	0.000	32.7786	52.8484
	GIV-KG	28.16073*	3.29924	0.000	18.1258	38.1957
	GV-KS	5.51114	3.29924	0.561	-4.5238	15.5461
	GVI-KEE	4.77595	3.29924	0.699	-5.2590	14.8109
GII-G	GIII-Pira	26.51211*	3.29924	0.000	16.4772	36.5471
	GIV-KG	11.85934*	3.29924	0.013	1.8244	21.8943
	GV-KS	-10.79025*	3.29924	0.029	-20.8252	-0.7553
	GVI-KEE	-11.52544*	3.29924	0.017	-21.5604	-1.4905
GIII-Pira.	GIV-KG	-14.65277*	3.29924	0.001	-24.6877	-4.6178
	GV-KS	-37.30236*	3.29924	0.000	-47.3373	-27.2674
	GVI-KEE	-38.03755*	3.29924	0.000	-48.0725	-28.0026
GIV-KG	GV-KS	-22.64959*	3.29924	0.000	-32.6845	-12.6146
	GVI-KEE	-23.38478*	3.29924	0.000	-33.4197	-13.3498
GV-KS	GVI-KEE	-0.73519	3.29924	1.000	-10.7701	9.2998

*The *mean difference is significant at the 0.05 level*

Table 6. Paired t test to analyse the difference before and after treatment in each group

Paired samples test									
Groups		Paired differences					T*	Df*	Sig. (2-tailed)*
		Mean	Std. deviation	Std. error Mean	95% confidence interval of the difference				
					Lower	Upper			
Pair 1	GI-NC Bef GI-NC After	-20.39581	6.34775	2.59146	-27.05736	-13.73426	-7.870	5	0.001
Pair 2	GII-G Bef GII-G After	-4.09442	4.05876	1.65698	-8.35383	0.16499	-2.471	5	0.056
Pair 3	GIII–Pira.Bef GIII–Pira.After	22.41769	8.25466	3.36995	13.75496	31.08043	6.652	5	0.001
Pair 4	GIV–KG Before GIV-KG After	7.76492	5.69440	2.32473	1.78901	13.74083	3.340	5	0.021
Pair 5	GV–KS Bef GV-KS After	-14.88467	5.71908	2.33480	-20.88648	-8.88286	-6.375	5	0.001
Pair 6	GVI-KEE.Bef GVI-KEE After	-15.61986	2.42665	0.99068	-18.16647	-13.07324	-15.767	5	0.000

**Df – Difference, Sig. – significance, T value - to test the difference between each pair of categories*

In the present study it was observed that GIII-Pira. (piracetam) and GIV-KG (*Kuvalaya Ghrta*) showed an improvement in memory after chronic dosing of the medicines for 14 days. Multiple Comparison Post-hoc analysis revealed that GIII-Pira. showed significant improvement in memory ($p<0.05$) when compared with other groups. GIV-KG has got a decrease which is larger than GIII-Pira but smaller than the remaining ($p<0.05$). GII-G is the next smaller group than GI-NC. There was not sufficient evidence to prove a significant difference between GI-NC, GV-KS and GVI-KEE (Table 5; Fig. 3) after treatment of 14 days which was suggestive of the memory enhancing effect of the study drug *Kuvalaya Ghrta*. GIII-Pira. mean is smaller in after treatment which implied that there is an improvement of memory in this group. GIV-KG mean is smaller in after treatment which implied that there is an improvement in memory after treatment which is the reason why they have a smaller mean after treatment. There is no significant difference in the other groups (Table 6).

The probable explanation of the results may be that water-soluble drug is usually distributed in the extracellular spaces and it may not readily diffuse in to cerebrospinal fluid (CSF) and other body cavities, whereas the lipid soluble drugs are rapidly distributed throughout the intra and extracellular spaces. The drugs that are rapidly absorbed from the gut due to their lipid solubility are known to readily diffuse into the CSF and the brain. The drugs given in the form of Ghee (*Ghṛta*), a form of lipid, is likely to be rapidly absorbed and distributed in the target areas of the body such as the nervous system in this case. The membrane separating the CNS tissue and the circulating blood is lipophilic in nature. Thus, it selectively allows the passage of lipids and lipid soluble drugs across it. Therefore, any drug given in the form of *ghee* will not only be digested and absorbed fast but will also be able to reach some of the most distant areas of the body such as the CNS. Thus, proving that *Ghrita Kalpanas* are one of the most effective drug dosage forms used in ayurvedic medicine.

The drug *Kuvalaya* possesses *madhura, tikta, kashaya* rasa, *laghu, snigdha, pichila* guna, *sheetha* veerya and *madhura vipaka*. *Ghrta* possesses *madhura rasa*, *guru*, *snigdha*, *mrdu* guna, *sheetha veerya* and *madhura vipaka* (Sharma, 2005). Both *Kuvalaya* and *Ghrta* possess *madhura rasa* and *madhura vipaka*. *Madhura rasa* mitigates *vata* and *pitta dosha*. It endows maximum strength to the tissue (*dhathunam prabalam balam*) and is good for the sense organs and pleasing to the mind (*shad indriya prasadaka*). *Madhura rasa* gives unctuousness (*snehana*) and helps in mitigating *vata*, nourishes the body (*tarpayati*) and plays a major role in *jeevana*. In addition to *Madhura rasa*, the drug also possesses *tikta rasa* which is most effective in mitigating *pitta* and *kapha dosa*. *Tikta rasa* in turn promotes memory and intellect as it has been highlighted in classics as *medhya* (Sreekumar, 2008). *Snigdha guna* present in *Kuvalaya* and *Ghrta* helps in mitigating *vata* and in turn is *dhatuvardhaka* (promotes all bodily tissues). *Mastishka* is made by *majja* and thus the *snigdha guna* definitely may have a positive impact on *masthishka*.

Ghrta has been glorified by Acharyas as said to have rejuvenating *(rasayana)* and intellect (*medhya*) properties. *Medha* have been defined by Dalhana as *grandha aavdharana shakthi* or that which helps in increasing memory (Susruta, 2014). So, the use of *Ghrta* as the base for preparing the medicine may have add on benefits in improving the *medha* of the subjects and the above maybe the reasons why *Kuvlaya Ghrta* showed positive memory enhancement effect *via* T-Maze.

4. Conclusion

It was observed that GIII-Pira. and GIV-KG showed an improvement in memory after chronic dosing of the medicines for 14 days. The acute toxicity study revealed that the ethanolic extract of the drug *Kuvalaya* is safe in tested rats upto 2000 mg/kg. It is the need of the hour to find out regionally used source plants which may be useful to reduce the stress on the existing plants and can also lead to the understanding of broader therapeutic uses of less explored but commonly available regional plants.

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