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RESEARCH ARTICLE

STANDARDIZATION AND EVALUATION OF *DIVYA MEDHA VATI*, AN AYURVEDIC FORMULATION FOR ITS ANTI-AMNESIC POTENCY

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ABSTRACT

Aim: To Investigate the Ayurvedic formulation for any sign of toxicity and biological evaluation. **Materials and methods:** The whole study was designed to carry out the research work on amnesia model in mice taking into consideration various parameters that were helpful analysed the potency of the test drug. The toxicity studies were also be performed on rodents prior to conduct the experimentation process in Morris Water Maze model. The study also includes the standardization of selected herbal formulation with various parameters like Ash value, extractive value, moisture content, pH and phytochemical investigation. Acute toxicity studies (50mg/kg, 300mg/kg and 2000 mg/kg orally of test sample)was conducted to determine the safe dose as per OECD-423 guidelines. Three Groups of animals (n=5) were used in this study. Group I was disease control group, Group II received Standard drug (Piracetam) and Group III received Test drug (Divya Medha Vati). **Result**: No sign of toxicity was observed. The test sample increases the latency time to find the hidden platform in M.W.M. Data of this method was analysed by annova and dunnetts multiple comparison at $p<0.01$. **Conclusion**: Results obtained in the present study suggest that the herbal formulation shows significant anti-amnesic activity when divya medha vati and piracetam were used separately.

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INTRODUCTION

Amnesia refers to the loss of memory. Memory loss may result from two-sided (bilateral) damage to parts of brain vital for memory storage, processing or recall (the limbic system, including the hippocampus in the medial temporal lobe).Some people with amnesia have difficulty forming new memories. Others can't recall facts or past experiences. People with amnesia usually retain knowledge of their own identity, as well as motor skills. (healthline.com)Permanent amnesia can be differentiated from more common forms of memory loss, such as memory loss associated with aging or depression. Amnesia can be a symptom of several neurodegenerative disease; however, people whose primary symptoms is memory loss (amnesiacs), typically remain lucid and retain their sense of self. They may even be aware that they suffer from a memory disorder. Memory of new experience, motor skills, past events and previous conditioning were grouped together in one system that relied on a specific area of the brain. Such research demonstrates that the brain has multiple systems for processing, storing and drawing on memory.

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(Amnesia. Blue Print for Health) The pathophysiology of amnesia varies with the extent of damage and the regions of the brain that were damaged. The most well described regions indicated in amnesia are the medial temporal lobe (MTL), basal forebrain and forming. Herbal drugs are naturally occurring, plant-derived substances that are used to treat diseases within local or regional healing practices. These products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant.(draxe.com) Chemical substances and plant extracts that are known to restores memory work in different ways. Brahmi is useful in amnesia, botanically known as Bacopa moniera. The bacosides are the memory chemicals in Brahmi. The bacosides help to repair damaged neurons and replace the old ones. Shankhapushpi is indicated as Medhya (brain tonic). It is one of the best and prominent medicine that help in improving memory. Piracetam was the first nootropic to be synthesized. It is a cognitive enhancer supplement that enhances cognitive abilities. It is considered to be the safest nootropic.

MATARIALS AND METHODS

Drugs and chemicals: Drugs- Divya Medha Vati (DMV) and Piracetam. Chemicals- Methanol, iodine, potassium iodide, conc^{h} Sulphuric acid, chloroform, HCL, acetic acid, NaOH,

ammonia, NaCl, ethanol, Molisch's reagent Hager's reagent, Wagner's reagent, Mayer's reagent, Dragendroff's reagent, gelatin, ferric chloride, sodium bicarbonate, 1% acasia solution, normal saline. All drugs and chemicals were of analytical grade.

Animals: Swice albino mice (28-40gm) were used in the present study. The animals were procured from disease free small animal house. They were provided normal diet and tap water and were exposed to 12hr. Light and 12hr.dark cycle. The animal was acclimatized to the laboratory condition before experiments. Experimental protocol was approved by Institution (IPS-College of pharmacy) animal ethics committee. Care of animal was taken as per guideline of the committee for the purpose of control and supervision of experiments on animal (CPCSEA), ministry of environment and forest government of India. Experimental protocol was approved by Institution animal ethic committee. The animals were kept in polypropylene cages under standard laboratory condition. The animal house was maintained at 27°c ±2°c temperature and 50 to 60% humidity. The animals were obtained from animal house of institute (IPS- College of pharmacy, Gwalior)

Dose

Piracetam: - 50mg/kg
Divya medha vati:- 74mg/kg

Dose of DMV and Piracetam were calculated for mice from the available therapeutic dose for the human use. The therapeutic dose was calculated by using dose conversion table (Paget and Barnes, 1964).

Extraction

Powder was taken and weighed for the further evaluation. The powder form for extraction with suitable solvent system after examining the solubility property of the drugs. Aqueous solvent system was the preferable solvent system for the study as per the available literature. Simple maceration technique of extraction was followed to get the purified drug for further investigations. (Kokate C.K, 2005).

Preliminary Phytochemical Study

Determination of Ash value, loss on drying, determination of extractive value and determination of pH value.

Phytochemical Screening

Test for starch, flavonoids, alkaloids, tannins, sapponins, carbohydrates, phenol and steroids.

Quantitative Study

Thin Layer Chromatography (TLC) (Kushwaha Richa-2011): TLC is the sepration of mixture into individual components using stationary phase and mobile phase. Mobile phase prepared by taking chloroform + methanol + toluene in the ratio 7:2:1. Sample A (Divya medha vati powder) and Sample B (Divya medha vati extract) were dissolved in methanol and the sample were spotted on the chromatographic plate.

Retardarion factor (Rf) defined as:

$$\text{Rf Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

High Performance Liquid Chromatography (HPLC)

HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that move the mobile phase(s) through the column, and a detector that shows the retention time of the molecules.

HPLC of Divya medha vati

- Preparation of mobile phase = methanol : water : acetonitrile (40:45:15)
- Wave length = 254nm
- Flow rate = 1ml/min.

Preparation of Standard solution/Sample solution:- Took100mg(std)drug/Sample drug dissolved in 100ml of mobile phase, prepared the concentration of solution 1000µg/ml. Took 1ml of 1000 µg/ml of solution then volume made up to 10 ml, prepared the concentration of solution100 µg/ml. Took 1ml of 100 µg/ml of solution then volume made up to 10ml,prepared the concentration of solution 10µg/ml.

Pharmacological Investigtion

Acute oral toxicity study: Acute toxicity study was carried out to determine the safe dose by acute toxic class method of oral toxicity as per Organization for Economic Co-operation and Development (OECD) 423 guideline(Anonymous-2001).

In vivo study: The animals were divided into 3 groups, and each group contains five animals. The cages were labeled properly with number, age and sex and weight of animals. The animals were marked with permanent marker on tail for identification. Oral route of administration was used for dosing. The groups were as follows

Groups of Animal

Group I: Diseased control group.
Group II: Piracetam (standard drug) treated group
Group III: Divya Medha Vati (test drug) treated group.

Morris water maze apparatus

The Morris water maze (MWM) apparatus was developed by Richard Morris in 1994. In its most basic form, it assesses spatial learning and memory along with nonspatial discrimination learning. The test apparatus consists of a circular fiberglass tank (130 cm in diameter, 50 cm in depth). The pool was filled to a height of 30 cm with water at room temperature, 21-22 ^{0}C. The pool was divided into four quadrants (Q1, Q2, Q3 and Q4) of equal surface area. A transparent escape platform (10 cm in diameter, 29 cm in height) is placed in a fixed location in the tank in the centre of one of the quadrants, 1 cm below the water surface. So, that platform is not visible. Several clues around the maze were available for the mice to used in locating the escape platform.

Method

The platform remains in a constant location in the centre of one quadrant, equidistant from the centre and the edge of the pool. Each training trial involves placing the animal into the pool facing the wall at one of the four quadrants. A different starting point was randomly used on each trial. The mice were allowed to swim freely until they find the escape platform. The latency to find the hidden platform is recorded and used as a measure of acquisition of the task. If a mice failed to locate the hidden platform within 60 sec, it is then manually guided to escape platform by the experimenter. The mice remains on the platform for at least 20 sec to orient itself to the visual clues. Mice were then turned to their home cage for a fixed interval (3-10min), until the series of trials were completed. Weighed the animals and marked properly. Animals were divided into three groups (n=5 in each group). Group 1: Disease Control group (n=5); mice were given normal saline at the equivalent dose of drug. Group 2: Standard group (n=5); mice were given Piracetam and observed the animals for 90sec/min(cutoff time). Group 3: Test group (n=5); mice were given DMV. Time to reach at the platform were recorded and the movement in the quadrant of the pool. (Bikas Medhi and Ajay Paraksh, 2008)

RESULTS

Standardization of Divya Medha Vati

Table 1. Showing the values for various parameters for Standardization of DMV

S. No.	Standardisation Parameter	Value
1.	Ash Value Analysis	
	• Ash content (Total ash)	15.4%
	• Acid insoluble ash	5.2%
	• Water soluble ash	1.2%
2.	Extractive value (Maceration process)	
	• Ethanol soluble	11.25%
	• Water soluble	17.75%
3.	Moisture content (Loss on During)	7.75%
4.	pH (1% aqueous solution)	5.58

Table 2. Phytochemical Screening of DMV

S. No.	Phyto constituents	Phytochemical Test	Result
1.	Starch	Iodine test	-
2.	Steroids	Salkowski test	+
3.	Flavonoids	Ferric chloride test	+
4.	Alkaloids	• Hager's test	+
		• Wagner's test	+
		• Mayer's test	++
		• Dragendroff's test	++
5.	Tannins	• Ferric chloride test	+
		• Gelatine test	-
6.	Saponins	• Froth test	+
		• Foam test	+
7.	Carbohydrates	Molisch's test	-
8.	Terpenoids	Salkowski's test	+
9.	Phenol	Ferric chloride test	+

(+) Positive, (++) Moderated Positive, (-) Negative

Quantitative Evaluation

(a)T.L.C (Thin layer chromatography)

A (Sample) = DMV extract
B (Standard) = DMV Powder

Rf value = Distance travelled by solute

Distance travelled by solvent

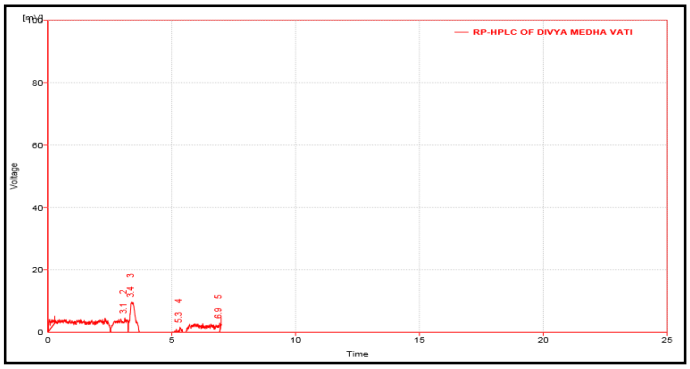
Table 3. Rf value of Sample and standard

S. No	Samples	Rfvalue
1.	A	0.79
2.	B	0.82

HPLC of Divya medha vati

Sample = DMV extract

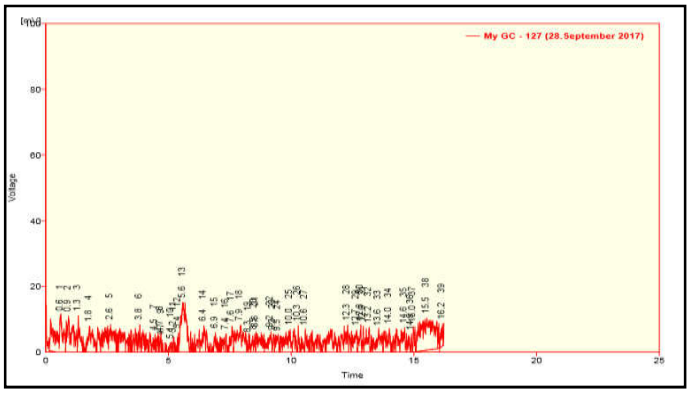
Standard = DMVpowder



Graph 1. Showing the chromatogram of sample (10µg/ml)

Table 4. Result analysis of sample (10µg/ml) by HPLC

S. No.	Reten. Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1.	0.063	2.705	0.340	0.3	1.8	0.06
2.	3.107	124.924	4.719	12.2	24.8	0.72
3.	3.390	246.730	7.361	24.0	38.7	0.52
4.	5.330	401.041	6.356	39.1	33.4	0.88
5.	6.940	250.905	0.263	24.4	1.4	0.10
	Total	1026.304	19.039	100.0	100.0	



Graph 2. Showing chromatogram of standard (10µg/ml)

Table 5. Result analysis of standard (10µg/ml) by HPLC

S. No.	Reten. Time (min)	Area (mV.s)	Height (mV)	Area (%)	Height (%)	W05 (min)
1.	0.613	22.856	1.147	3.6	2.8	0.09
2.	0.930	19.773	1.186	3.1	2.9	0.07
3.	1.327	9.857	1.270	1.6	3.1	0.04
4.	1.793	26.537	1.087	4.2	2.6	0.13
5.	2.643	64.712	1.270	10.2	3.1	0.31
	Total	143.735	5.96	22.7	14.5	

Acute oral toxicity study

Table 6. Acute oral toxicity study of DMV in mice (male) at various dosed

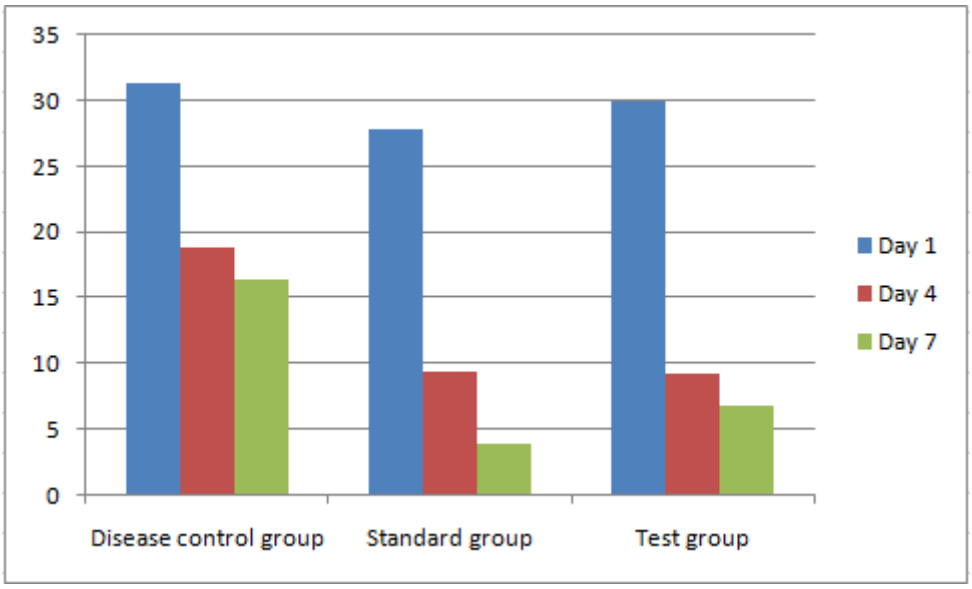
S. no.	Dose	Observation period (24 hr)	observation period 14 days
1.	50mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.
2.	300mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.
3.	2000mg/kg	All animals survived	No sign of toxicity, normal diet and feeding.

Table 7. Result of MWM showing Escape Latency Time (sec) in target quadrant

S. No.	Group/Dose	Day 1 ELT	Day 4 ELT	Day 7 ELT
1.	Disease control/Normal saline	31.42 ± 0.48	18.91± 0.55	16.46 ± 0.69
2.	Standard Group(50mg/kg)	27.79 ± 0.98**	9.46 ±1.01**	3.97 ± 0.19**
3.	Test Group(74mg/kg)	30.05 ± 0.46*	13.28 ± 0.28**	6.80 ± 0.66**

P Value is < 0.0001 considered Extremely Significant
* P> 0.05 - Non Significant
**P<0.01 Compared to control

Graph 3. Showing the graph of M.W.M model



DISCUSSION

- The physiochemical parameters viz ash content , extractive value moisture content pH indicated that the formulation. DMV intended for study was of inquisite pharmacopoeial standard. Phytochemical analysis is very important in the evaluation of active biological component of plant. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance in nootropic activity. Quantitative estimation of DMV T.L.C spot of sample very nearest to standard sample spot .and HPLC analysis of herbal formulation sample value almost similer to standard sample value.
- Acute oral toxicity study of the DMV (50mg/kg, 300mg/kg and 2000 mg/kg orally) invealed that there was no toxicity of any nature, all animals were survived during observation period.
- The study was undertaken in view of the therapeutic use of DMV ayurveda system of medicines for treatment of various disease like – insomnia, treatment of anxiety, increase memory, brain power, epilapsy, antioxidant, depression, neuralgia, migraine etc.

The nootropic activity of ayurvedic polyherbal formulation was investigated in present study.

- Memory enhancing drugs are thought to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes and hormones), improving brain's oxygen supply or by stimulating nerve growth. Nootropics agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used for improving memory, mood and behavior but not used generally because of more side effects associated with these agents have made their applicability limited. In the present study, we have focused upon exploring the potential of Ayurvedic poly-herbal formulation, DMV for its efficacy in reversing the memory deficits, improving acquisition and memory retention in experimental animals using morris water maze and elevate plus maze model. In the present study, DMV administered orally improved learning and memory of mice significantly in both models. Furthermore, pre-treatment with DMV (74mg/kg) protected the animals from learning and memory impairment produced naturally. These findings suggested the possible neuroprotective role for DMV.

Conclusion

There is growing interest in development of herbal medicine as alternative therapeutic options. Indian plants and their formulations are constantly being evaluated for possible anti-amnesic activity. Divya Medha Vati is an important Ayurvedic formulation used in the management of amnesia. A study on DMV for its anti-amnesic effect was performed. The dose of DMV was taken as 74mg/kg that was proved as safe and effective dose required for management of the activity. The data regarding model and activity has already been discussed in result. It can be concluded that the anti-amnesic activity is probably due to the presence of bioactive compound like alkaloids, phenols and tannins are responsible for management of amnesia.

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