

EVALUATION OF NEURO-PROTECTIVE ACTIVITY OF ASHWAGANDHA AND VACHA IN COMBINATION AS AYURVEDIC MEDHYA RASAYANA IN -VIVOChandreyee Ray¹ Mradu Gupta² Arijit Ghosh³¹Senior Research Fellow (Ayurveda), National Research Institute of Ayurvedic Drug Development, Kolkata, West Bengal, India²Professor & Head; ^{1,2}Department of Dravyaguna Vijnana, Institute of Post Graduate Ayurvedic Education & Research at S.V.S.P, Kolkata, West Bengal, India³Assistant Professor, Department of Pharmacology, Malda Medical College & Hospital, Malda, West Bengal, India**ABSTRACT**

Neuroprotection aims to prevent or slow disease progression and secondary injuries by halting or at least slowing the loss of neurons. *Medhya Rasayana* drugs are used for prevention and treatment of mental disorders of all the age groups. These drugs play an essential role in the Neuro-protection also. Mode of this therapy involves the individual to attain sedation, calmness, tranquility or stimulation of activities of brain. These drugs promote the Intellect (*Dhi*), Retention power (*Dhriti*), memory (*Smriti*). The present research drug is a unique combination of Ayurvedic *Medhya* herbs like *Vacha* (*Acrous Calamus Linn.*) and *Aswagandha* (*Withania Somnifera Dunal.*). Both have been mentioned in the *Brhatrayee* and *Laghutrayee* for *Medhya* as well as *Rasayana* purpose but have not been evaluated till date. The aqueous extract of formulation was prepared by mixing 2 part of root of *Aswagandha* and 1 part of rhizome of *Vacha* in the *Dravyaguna* Department of I.P.G.A.E & R, Kolkata after proper identification and authentication. The experimental study was carried out in the animal house of the same institute on Wister rats & Albino mice. The aqueous extract of the formulation was administered in two divided dose viz. 500mg/kg & 700mg/kg body weight in an oral dose after getting In the present study, dose 700mg/kg showed significant increase in the retention of conditioned avoidance response as compared to control group (80% vs 90%, $p<0.001$). ECS+ DOSE 700mg/kg, also significantly prevented the ECS induced memory loss, regarding retention of conditioned avoidance response, compared to only ECS group (25% VS 75% $p<0.001$). This paper is an attempt to evaluate the role of *Medhya* herbs in combination specially *Aswagandha* and *Vacha* in modern day experimental parameters with respect to memory and mind health in prevention and care for cognitive dysfunction as well as for neuro-protection.

Keywords: *Medhya Rasayan*, Neuro protection, *Aswagandha*, *Vacha*,

INTRODUCTION

The central nervous system (CNS) consists of interconnected neural networks, which are believed to form on the basis of neuronal function, including thinking, learning & emotionality. These networks are laid down under genetic constructions, but are fine tuned during development in a process that requires interaction with the environment.¹ Neuro protection is the active suppression of damaging neuro toxic conditions in the nervous system in an aim to inhibit apoptosis, neuronal dysfunction and neuro degeneration of nerve cells. This may be either achieved by endogenous neuro protectants or by exogenously added pharmacological agents/neuro protective therapies.² The goal of neuro protection is to limit neuronal dysfunction after injury and attempt to maintain the possible integrity of cellular interactions in the brain resulting in undisturbed neural function. There is a wide range of neuro protection products available or undergoing research and some products can potentially be used in more than one disorder, as many of the mechanism underlying is similar. These products may be of various kinds and can be classified as free radical scavengers, anti-excitotoxic agents, apoptosis inhibitors, neuro trophic factors etc.³

Ayurveda, an ancient system of Indian medicine, has defined a number of plants with therapeutic benefits for the treatment of neurodegenerative disease, having antioxidant activities.⁴ Recently there is a tremendous urge to explore medicinal plants globally for improving cognitive function owing to their less adverse effects. Ayurveda provides a list of herbs known for nootropic activity as well as their multi-dimensional utility in various conditions.⁵ Medhya Rasayana is a

group of medicinal plants that can be used singly or in combinations. Mode of this therapy involves the individual to attain sedation, calmness, tranquility or stimulation of activities of brain.⁶ Such Rasayanas retard brain aging and help in regeneration of neural tissues besides producing antistress, adaptogenic and memory enhancing effects.⁷ In Ayurveda, Ashwagandha (*Withania somnifera* Dunal) of Solanaceae family, commonly found as Winter cherry used for medicinal purposes is classified as a Rasayana (rejuvenation) and expected to promote physical and mental health, rejuvenate the body in debilitated conditions and increase longevity. The available scientific data support the conclusion that Ashwagandha is a real potent regenerative tonic (Rasayana of Ayurveda), due to its multiple pharmacological actions like anti-stress, neuroprotective, anti-tumor, anti-arthritis, analgesic and anti-inflammatory etc.⁸ Ashwagandha has been in use for more than 2500 years for diverse clinical condition especially for improvement of memory and cognition enhancement.⁹

Another drug of the combination, Vacha (*Acorus calamus* Linn.), of Araceae family is one of the important plants in the group 'Medhya Rasayana'. They are widely claimed as restorative, nervine and mental tonics. They have got prominent action on Central Nervous System where they improve grasping power, memory, intellect and speech, and correct aberrations of emotions, mood and personality of an individual. Vacha stimulates the power of self expression and intelligence. Rhizome contains calamediol, essential oil, tanning substances and vitamin - C. These constituents are val-

uable in a vast range of diseases.¹⁰ It has a special potency as a nervine tonic. It is a very vigorous brain tonic, because it shows results in a very short time. It increases the overall memory of the person and strengthens the nervous system. *Vacha* is prescribed to people who have amnesia. Improving the memory is a quest on which human beings have embarked centuries ago.¹¹ In addition to the long tradition of textual and experience-based evidence for their efficacy, certain recent studies are being conducted on neuroprotective herbs with scientific parameters have shown promising results in ensuring healthy brain ageing.

MATERIALS AND METHODS

Plant materials

The roots of *Aswagandha* (*Withania Somnifera* Dunal) & rhizome of *Vacha* (*Acorus Calamus* Linn.) were collected from a reputed plant materials supplier & authenticated by the Dravyaguna Department of I.P.G.A.E.&R, Kolkata.

Method of Extraction Of Research Drug:¹²

Continuous hot percolation (successive solvent extraction) process by using soxhlet apparatus and cold maceration

Extractive values of crude drugs are useful for their evaluation especially when constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the constituents present in a crude drug. In the soxhlet apparatus 6 solvents was used starting from –

- Petroleum ether
- Ethyle acetate
- Acetone
- Chloroform
- Ethyle-alcohol
- Water finally

Extraction Procedure¹³

Petroleum ether extract

The shade dried coarsely powdered whole research drug (320gm.) was extracted with petroleum ether (60-80°C) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. Dark green colour residue was obtained. The residue was then stored in desiccator.

Chloroform extract

The marc left after petroleum ether extraction was dried and then extracted with chloroform (55-56°C), until the extraction was completed. Dark greenish yellow colour residue was obtained. The residue was then stored in desiccator.

Acetone extract

The marc left after chloroform extraction was dried and then extracted with acetone (55-56°C), until the extraction was completed. Dark brownish green colour residue obtained, was stored in desiccator.

Ethanol extract

The marc left after acetone extraction was dried and then extracted with ethanol 95% v/v (75-78°C), until the extraction was completed. Dark brown colour residue was obtained. The residue was then stored in desiccator.

Aqueous extract

The marc left after ethanol extraction was dried and then extracted with chloroform water by cold maceration process for 7 days. At the end of 7th day, it was filtered through muslin cloth and the filtrate was concentrated. The remaining solution was evaporated by heating on a water bath. The brown colour residue was obtained. The residue was then stored in desiccator, which was to be administered as 500mg/kg body wt &

700 mg /kg body wt in two divided dose to the experimental animals.

Animal materials:

Experimental protocol was submitted and approved by Institutional Ethical Committee after procuring lawfully from an authorized licensed breeder as per the CPCSEA guidelines. Wister albino rats (150-200 gm) of approximate same age of either sex for CAR test and Swiss albino mice (15-20 gm) of either sex for Hot Plate study were employed in this investigation. Housing was maintained with provision of 12 hours, day and night cycle. Animals were fed with standard pellet diet and *ad libitum* and housed under standard conditions of temperature 22^o C (\pm 3^o C) humidity 35 % to 60 %, and light (12:12 hr light/dark cycle) in polypropylene mice cage. The animals received the drug treatments by oral gavages tube.

METHODS:

In Vivo for this study was a) Conditioned Avoidance Response test Apparatus, b) Eddy's Hot Plate Analgesiometer, c) Electro Convulsimeter. Analgesic & CAR test was carried out in the animal house (Regn no.1180/AC/08/CPCSEA Dated 27.03.08) under Dravyaguna Vigyan Department in I.P.G.A.E. & R at S.V.S.P. according to the guidelines of CPCSEA after the approval of IAEC.

Conditioned Avoidance response test:

Animals were divided into six groups (n=6 in each groups). Initially all 36 rats were trained in the conditioned avoidance response test apparatus, for 10 days. Training was supposed to be completed when they avoid shock on the floor grid only on light buzzer, not after foot shock. Group I served as control (received normal saline for 8 days), Group II (ECS+drug

500mg/kg)[where amnesia through ECS was given after 30 min of oral administration of dose 500mg/kg], Group III (ECS+drug 700 mg/kg) body wt, [where amnesia through ECS given after 30 min of the oral administration of dose 700mg/kg], Group IV & V administered orally with dose 500mg & 700 mg respectively, Group VI (ECS only) received MES for 8 days. On the 9th day, all rats were again placed into the CAR apparatus for the evaluation of retention of learning and memory.

Electro convulsimeter (for amnesia production): The MES pattern was induced in animals where 150V, 50 Hz sinusoidal with intensity of 180 mA for 0.3 sec through crocodile clip ear electrodes as a single daily dose for 8 days in group 2,3,& 6.

Hot Plate Method :(To evaluate Analgesic Property of PD-2): The animals divided into four groups (n=6 animals in each). Group I served as control & given normal saline, Group II served as standard and were injected Pentazocine (Fortwin) (0.5mg/kg, ip). Group III and IV were treated orally with test drug 500 and 700 mg/kg body weight, respectively. The animals were individually placed on the hot plate maintained at 55^oC, half hour after their respective treatment. Response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds. The reaction time is recorded by a stop watch. Repeated reading is taken at 30, 60,90minutes after drug administration.¹⁴

RESULT:

The standard test drug dose was taken as 700 mg/kg body weight. The retention of CART after drug administration and ECS

was calculated on 9th day. The rats in test drug group showed significant increase in retention of CAR as compared to Control (normal saline) ($p < 0.001$). [Table-no 6] ECS+ test drug significantly prevented the ECS induced attenuation of CART ($p < 0.001$). [Table-no 6]. As illustrated in Table no.6, varied significantly among the various groups of mice $F(3,20) = 48.76$; $p < 0.001$. The central analgesic effect of the formulation at the 700 mg/ kg in Hot Plate analgesiometer model showed significant increase in latency period compared to standard drug (Fortwin 0.5 mg/kg i.p) observed at 90 min pointing out the analgesic effect being sustained. In hot plate method the calculated percentage increase in latency period was 67.26%, 77.85%, 95.40%, in Group standard, 500mg/kg drug dose, & 700mg/kg drug dose, respectively at the 90min time interval also showed the sustainability of the drug as analgesic.

DISCUSSION:

The research drug has been selected from the mentioned medhya rasayan plants in the ancient Ayurvedic text Susruta Samhita (Medha Ayuskamiya Adhayaya)¹⁵ and prepared by taking two part of the powder of root of Aswagandha (*Withania Somnifera* Dunal) and one part of the rhizome of the vacha (*Acorus Calamus* Linn.) to find out its pharmacological action on memory, learning & cognitive function related to neuroprotection by experimental study on animals after pharmacognostical and phytochemical standardization of the research drug.

The central analgesic effect of the research drug at the 700 mg/ kg body wt and 500 mg/ kg body wt both were highly significant ($p < 0.001$) in comparison to the control group at 60 & 90 mins interval which indi-

cates the analgesic effect was sustained in the higher dose of the drug for long time.

Studies conducted on rats' brains showed that *Ashwagandha* produced an increase in the levels of three natural antioxidants superoxide dismutase, catalase and glutathione peroxidase.¹⁶ Active principles of *Ashwagandha*, for instance the sitoindosides VII-X and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress.¹⁷ *Vacha* has been proved for its analgesic and anticonvulsant activity. An ethanolic extract of *acorus calamus* rhizome is able to prevent 15.16% and 54.51% of the acetic acid induced writhing response with 250mg/kg and 500mg/kg of the extract; a potency comparable to *kigella pinata* and 500mg/kg being as potent as 25mg/kg diclofenac.¹⁸ 12.5-50mg/kg -asarone oral ingestion for 28 days (starting three days after -amyloid toxicity) is able to preserve cognition in rats with a potency comparable to 0.33mg/kg donepezil hydrochloride which was associated with a reduction in hippocampal apoptosis rates (of which donepezil was ineffective).¹⁹ An important active principle of *Withania somnifera* is Withaferin-A, have been shown to possess a remarkable range of therapeutic properties i.e. antistress, antioxidant, immunomodulatory.²⁰ It also contains flavonoids & many active ingredients of the withanolide class. Numerous studies over the past two decades indicate that it has antistress & rejuvenating properties.²¹

The aim of the experimental study was to evaluate the combine *Medhya* activity of *Ashwagandha and Vacha*. Flavonoids assist hard-working glial cells (oligodendrocytes) in getting rid of free radicals and other brain-robbers that play the biggest role in

memory decline, slowing of body movements and mental fatigue²². There is evidence that indigenous antioxidants may be useful in preventing oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in *Medhya Rasayana* herbs. Overall the formulation has been significantly evaluated, validated its literature information mentioned by *Acharya Susruta* in *Susruta Samhita* for the *Medhya* perpus and also proved its Neuroprotective activity following the standard protocols of the experimental pharmacology. This study has shown promising results in ensuring cognitive function in learning & memory and neuro-protection in experimental model. Randomized Clinical Trials should be done in the future to be more conclusive about the efficacy of the *medhya* formulation.

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Tables

Table 1: Ellustration of latent period For Paw Licking or Jumping for the Standard Drug (Fortwin) Group

Grouping	0min	30min	60min	90min
Rat-1	6.00 sec	4.70sec	6.15 sec	6.00sec
Rat-2	6.25 sec	4.90 sec	6.05 sec	5.95sec
Rat-3	6.85 sec	4.60 sec	7.00 sec	6.85sec
Rat-4	5.05 sec	4.00 sec	7.15	6.00 sec
Rat-5	7.00 sec	3.15	7.05 sec	5.75 sec
Rat-6	6.95 sec	2.90 sec	5.35 sec	5.65

Table 2: Ellustration of latent period For Paw Licking or Jumping for Control (Normal Saline)Group

Grouping	0min	30min	60min	90min
Rat-1	3sec	4sec	1.50sec	1.58sec
Rat-2	2.00sec	3.10sec	2.05sec	2.10sec
Rat-3	2.70sec	3.55sec	3.00sec	2.90sec
Rat-4	1.88sec	2.10 sec	1.20sec	1.70sec
Rat-5	1.55sec	3 sec	1.10sec	1.25 sec
Rat-6	2.30sec	3.05sec	2.70sec	2.05sec

Table 3: Ellustration of latent period for Paw Licking or Jumping for Drug at the dose of 500mg/kg b/wt:

Grouping	0 min	30 min	60 min	90 min
Rat-1	4.10 sec	5.05 sec	5.25 sec	6.25 sec
Rat-2	2.15 sec	4.20 sec	4.85 sec	5.05 sec
Rat-3	2.80 sec	4.05 sec	4.95 sec	5.12 sec
Rat-4	1.90 sec	4.70 sec	5.05 sec	6.32 sec
Rat-5	3.35 sec	5.10 sec	5.40 sec	4.48 sec
Rat-6	1.50 sec	5.00 sec	6.00 sec	5.18 sec

Table 4: Ellustration of latent period For Paw Licking or Jumping for Drug at the dose of 700mg/kg b/wt

Grouping	0min	30min	60min	90min
Rat-1	4.05sec	4.35sec	5.75 sec	5.85 sec
Rat-2	2.25 sec	4.38sec	5.68 sec	6.15 sec
Rat-3	3.35 sec	5.12sec	6.02sec	7.05sec
Rat-4	4.15 sec	4.95sec	5.33sec	7.00 sec
Rat-5	2.95sec	4.28sec	5.17 sec	7.15 sec
Rat-6	3.75 sec	6.02 sec	6.65sec	6.85 sec

Table 5` : Paw licking time for each group of rat-----Calculation of Mean & SEM :

Serial no.	Group	0 minute	30 minute	60 minute	90 minute
1.	Control (Normal saline)	2.238±0.22	3.133±0.259	1.925±0.324	1.93±0.3244
2.	Standard (Fort-win)	3.605±0.4065	6.35±0.3084	6.45±0.2950	6.03±0.1735
3.	Drug dose (500mg/kg body weight)	2.633±0.3978	4.683±0.1864	5.25±0.1707	5.4±0.2980
4.	Drug dose(700mg/kg body weight)	3.416±0.2962	4.85±0.2737	5.766±0.2159	6.675±0.2205

Table 6: ANOVA OF of Pain threshold activity by Hot plate Analgesiometer:

SOURCE	D _f	SUM OF SQUARE	MEAN SQUARE	F
BETWEEN GROUPS	3	80.739	26.913*	81.27***
ERROR	20	6.623	0.3311	
TOTAL	23	87.362		

Referring to F-ratio table for (3,20) degrees of freedom we get for F=48.76,*P<0.01 ***P<0.001,hence there is a significant diffrnce between groups.

{Between groups sum of squares =(X)²/c.f= 80.739,Error sum of squares=(total sum of squares)—(Between groups sum of squares)=6.623,Between groups (D.F)=No of groups-1=4-1=3,Total (D.F.)=Total ob-

servations of all groups-1=24-1=23,Error (D.F.)=Total (D.F.)-Between groups (D.F.)=23-3=20, Mean squares (between groups)=Between groups sum of squares÷ Degree of freedom = 80.739÷3=26.913,Mean squares (Error)=6.623÷20=0.3311,F =Between groups mean squares÷error mean squares=26.913/0.3311= 81.27

Table 7: Evaluation of CAR test on the basis of arbitrary scoring system:

Grouping	CAR TEST POSITIVE	CAR TEST NEGETIVE
CONTROL	80%	20%
ECS	10%	90%
PD-2 DOSE(500mg/kg)	80%	20%
PD-2 DOSE(700mg/kg)	90%	10%
ECS+ PD-2 DOSE (500mg/kg)	65%	35%
ECS+ PD-2 DOSE (700mg/kg)	75%	25%

Figures

Figure 1: Conditioned response test in Rat

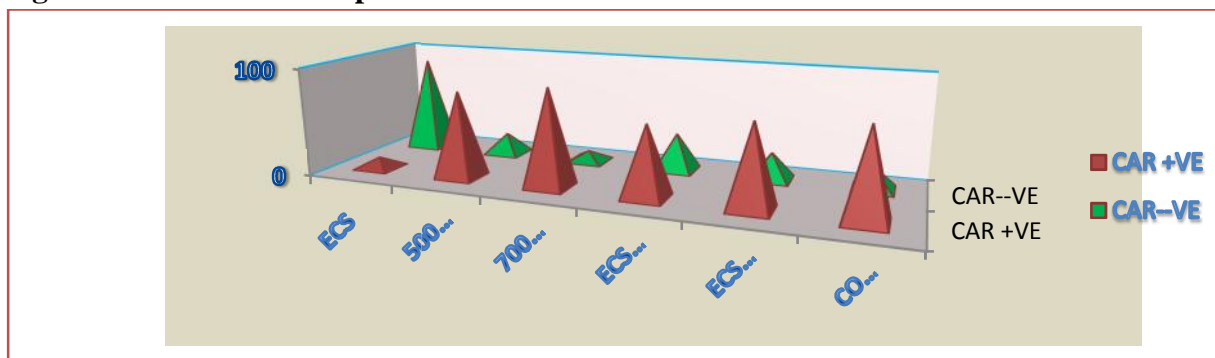
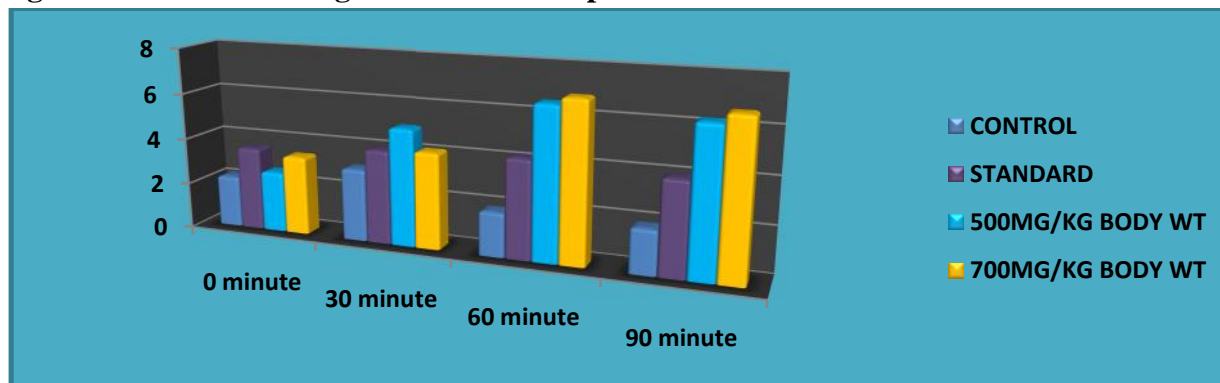


Figure 2: Hot Plate Analgesiometer test response



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