

## EXPERIMENTAL EVALUATION OF MANDOOKAPARNI SWARASA AS A MEDHYA RASAYANA IN ALUMINIUM CHLORIDE INDUCED NEUROTOXICITY - AN HISTOPATHOLOGICAL APPROACH

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### ABSTRACT

**Introduction:** Memory is one of the important higher mental functions of human nervous system. *Acharya Charaka* explained that by the intake of *Mandookaparni swarasa*, increases memory and individual becomes "Medhavi". For an experience to become part of memory, it must produce persistent structural and functional changes that represent the experience in brain. So with an intension to observe the structural changes in various parts of brain along with behavioural changes the albino rats were administered *Mandookaparni swarasa* and aluminium chloride. Later bio chemical analysis was also assessed. **Method:** 32 albino rats of either sex was randomly selected and grouped into 4 different groups. The dose of *Mandookaparni swarasa* and *Aluminium chloride* was fixed and were administered for 2 months daily. During the procedure of 2 months, the rats of all the 4 groups are simultaneously subjected spatial memory test and observed. At the end of 60th day all the animals were sacrificed by ether anesthesia and the parts of CNS were collected and processed for histological study and bio chemical study. **Result:** In the behavioural assessment *Mandookaparni swarasa* administered groups showed good memory power. Histology report showed neuroprotection in *Mandookaparni swarasa* against  $AlCl_3$  induced degenerative changes. In the bio chemical study, the group of rats which were administered *Mandookaparni swarasa* showed high level of protein concentration compared to other groups.

**Keywords:** *Medhya, Mandookaparni swarasa, Aluminium chloride*

### INTRODUCTION

Judgment, intelligence and memory are the higher mental functions of human nervous system. It is highly developed at the cost of regeneration. The

activities of nervous system are linked with every aspect of our physical, psychological and intellectual levels.<sup>1</sup>Primary function of the Nervous system is

controlling and coordinating the systems of our body. It regulates both voluntary and in-voluntary functions and adjusts the body to the surroundings. The sensory part of the Nervous system collects the information from the surroundings and helps in gaining knowledge and experience, whereas the motor part is responsible for the responses. One of our major mental activities is Memory. The brain is capable of storing and receiving both short term and long term memories<sup>1</sup> in Hippocampus.<sup>2</sup>when the memory centers are triggered repeatedly there will be permanent changes in the synapse in a specific circuit of neurons.

**It may be due to two reasons:**

- Increase in the number of pre-synaptic axon terminals or increase in number of receptors in postsynaptic neurons.<sup>3</sup>
- Changes in the concentration of neurotransmitters or functions of astrocytes.

In Ayurvedic classics various *Medhya rasayanas* have been mentioned to increase memory. *Mandookaparni swarasa* is one among them mentioned by Charaka. There are several references of the *Mandooka parni swarasa* in *Ayurvedic* literatures mentioning its actions like *Balya, Brumhana, Rasayana, Medhya, Tridosahar, and Vyadhidhvamsaka*. *Mandookaparni* is easily available, economical and can be used in day to day diet<sup>4</sup>. For the storage of previous experience as a part of memory, structural

changes in brain are necessary. So an attempt is made here to study the CNS histologically and behavioral changes by administration of *Mandookaparni swarasa* in Aluminium chloride induced albino rats. Since, Aluminium is a known neurotoxin and occupational exposure to Aluminium has implicated in neurological disease like Alzheimer’s disease<sup>5</sup>, administration of *Mandookaparni swarasa* which is *Medhya* may substantiate the *Aptha vaakya* of Acharya *Charaka* is the main intension of the study.

**AIM AND OBJECTIVES**

To study the histological and bio chemical changes in various parts of brain and spinal cord of Wister albino rats and to perform spatial memory test after administration of *Mandookaparni swarasa* and aluminium chloride.

**METHODOLOGY**

**MATERIALS AND METHODS:** Healthy young Wistar albino rats of either sex weighing about 200-250g were selected and divided into 4 groups. The animals were obtained from the animal house attached to S.D.M center for research in Ayurveda and allied sciences. The selected animals were maintained under prevailing husbandry conditions. They were fed Sai with ‘*Amrut*’ brand rat feed and water. The experiments were undertaken after obtaining permission from the institute’s animal ethics committee and as per CPSEA guidelines.

**Table 1:** Animal grouping: Each group had 8 albino rats and were kept in separate cages.

Group	Drug Used	Method	Number Of Rats
G-1	Normal diet and water	Control group	8
G-2	Normal diet and water with Aluminium Chloride	Disease control group (AlCl3 control group)	8
G-3	Normal diet and water with <i>Mandookaparni Swarasa</i>	Trial group 1 (MDP group)	8
G-4	Normal diet and water with Aluminium Chloride and <i>Mandookaparni Swarasa</i>	Trial group 2 (MDP + AlCl3 group)	8

**(MDP-Mandookaparni)**

**DRUGS:** *Mandookaparni* collected and *Swarasa* was extracted; Aluminium chloride from THOMAS BAKER Company.

**Dose fixation:** Human Therapeutic dose of *Man-dookaparni Swarasa* is ½pala i.e. 24ml<sup>[6]</sup>.

**Rats Dose:** Therapeutic human dose x surface area ratio (convertibility factor)

- single dose *Mandookaparni Swarasa*  
= Therapeutic human dose x 0.018x5/ kg body weight  
= 24x 0.018x5/kg body weight  
= 2.16/kg body weight
- dose of aluminium chloride-300mg/kg body weight

**Drug Preparation:**

- *Mandookaparni Swarasa* was extracted and administered according to the weight of the rat.
- 1.2mg aluminum chloride is weighed and mixed in 40ml of distilled water and administered.

**Drug Administration:**

- The drug was administered to disease control and trial group 1 and trial group2 for 60 days in the morning session between 9-10 AM orally.
- In trial group 2, aluminium chloride was administered after one hour of administration of *Man-dookaparni Swarasa*.

**EXPERIMENTAL PROTOCOL:**

The test formulation was administered orally once a day for 60 consecutive days. Assessment of behavioral changes was noted weekly.

Rats of all groups were subjected to Morris’s water maze test on scheduled days.

On 61<sup>st</sup> day, all animals were sacrificed under over dose of ether anesthesia. The head was opened through midline incision to record the autopsy changes followed by dissecting out the brain, spinal cord and weighed. The brain was transferred to bottles containing 10% formalin for the purpose of Histological study.

**OBSERVATION AND RESULT**

**Table 2:** Effect of *Mandookaparni* on retention memory recorded during Morris’s water test on 28th day

group	Latency(sec) MEAN ± SEM	No of crossing over MEAN ± SEM	Time spent in SW quadrant (sec) MEAN ± SEM
Control group	6.25±1.23	4.75±0.75	20.66±2.02
AlCl3 control@	6.2±0.91	4±0.44	27.6±1.83
MDP only#	5.80±1.06	4.4±0.44	32.8±2.97
MDP + AlCl3 ##	3.20±0.48	5±0.54	31.2±3.94

@-Compared with normal control, #-compared with normal control, ##-compared with @, \* indicates significance.

It was observed in the above data that, the latency of escape behavior, no of crossings and the duration spent in the target quadrant were not affected to significant extent in AlCl3 control group in comparison to the normal control group. In *Mandookaparni* alone group the latency and number of cross over

only marginally affected however, moderate prolongation in the duration spent in the target quadrant was observed but the observed change was non-significant in comparison to the control group. In *Mandookaparni* plus AlCl3 group moderate but non-significant decrease in latency of escape, increased cross over and time spent in the target quadrant was observed.

**Table 3:** Effect of *Mandookaparni* on retention memory recorded during Morris’s water test on 58th day

group	Latency (sec) MEAN ± SEM	No of crossing over MEAN ± SEM	Time spent in SW quadrant (sec) MEAN ± SEM
Control group	5.37±0.86	6.83±0.16	22±3.2
AlCl3 control@	4.6±0.24	6.60±0.24	24.2±0.37
MDP only#	3.8±0.37	4.80±0.58**	35.4±1.74**
MDP + AlCl3 ##	4±0.00	4.40±0.40**	25.4±1.03

@-Compared with normal control, #-compared with normal control, ##-compared with

@, \* indicates significance.

Table contains data related to the effect of different types of treatment on memory retention tested on 59<sup>th</sup> day of testing. It could be observed that AlCl3 treatment alone did not affect escape latency, number of cross over and duration spent in the target quadrant in significant manner in comparison to the control group. In *Mandookaparni* alone group non-

significant shortening of escape latency, significant increase in frequency of cross over and duration spent in the target quadrant was observed in comparison to the normal control group. In *Mandookaparni*-AlCl3 group non-significant marginal decrease in the escape latency, significant increase in cross over frequency and non-significant increase in the duration spent in target quadrant was observed in comparison to AlCl3 control group.

**Table 4:** Histological changes in hippocampus of AlCl3 group

GP-2					
Rat number	Dentate gyrus Mol Gran Pr layers	Mid brain	Blood vessels	CA (cornuammonis)	General remarks/ Pathological changes
Rat 1 ( R1)	NR	Extensive deg changes	NR	NR	R1- extensive deg changes and infarct formation R-2 oedematous changes and ventricular dilatation R3- oedematous changes in Dentate gyrus
R2	Oedematous changes	NR	--	NR	
R3	Oedematous changes	NR	NR	NR	
R4	NR	NR	--	NR	Ventricular dilatation

**Table 5:** Histological changes in hippocampus of MDP + AlCl3 group

GP-4					
Rat number	Dentate gyrus Mol Gran Pr layers	Mid brain	Blood ves- sels	CA ( cornuammonis)	General remarks/ Pathological changes
Rat 1 ( R1)	NR	NR	NR	NR	Normal cytoarchitecture
R2	NR	NR	NR	NR	
R3	NR	NR	NR	NR	
R4	Mild oedematous changes	Moderate degeneration			

In AlCl3 – control group oedematous changes, ventricular dilatation and degenerative changes were observed in dentate gyrus and nearby areas. In *Man-*

*dookaparni swarasa* group almost normal cytoarchitecture was observed. In AlCl3 + *Mandookaparni* combination group normal cytoarchitecture was ob-

served in ¾ rats in one rat moderate degenerative changes were observed. This clearly indicates neu-

roprotection in *Mandookaparni swarasa* against AlCl3 induced degenerative changes were observed.

**Table 6:** Effect of *Mandookaparni Swarasa* to assess protein concentration

Group	Protein concentration mg/g of wet tissue MEAN ± SEM
Control group	0.18±0.12
Alcl3 control@	0.15±0.002
MDP only#	0.23±0.01**
MDP + Alcl3 ##	0.17±0.02

@-Compared with normal control, #-compared with normal control, ##-compared with @, \* indicates significance.

The data shown in the above table shows that administration of AlCl3 lead to moderate but non-

significant decrease in tissue homogenate. Administration of *Mandookaparni swarasa* alone leads to significant increase in the protein content. The values of *Mandookaparni swarasa*-AlCl3 group did not differ significantly from AlCl3 control group.

**Drug:**

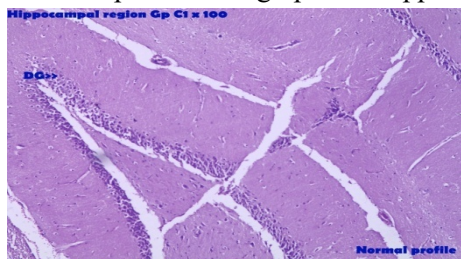
**Fig 1:** (*Mandooka parni*)



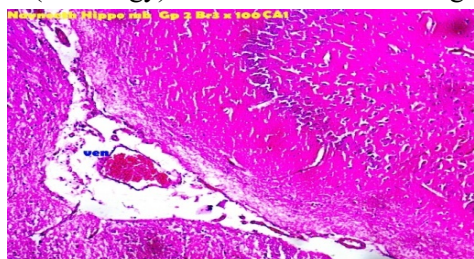
**Fig 2:** Dissected Brain and Spinal cord



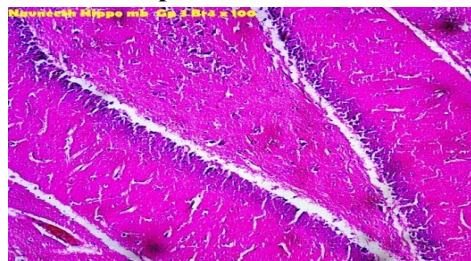
**Fig 3:** Shows photo micrographs of Hippocampus (Histology) sections from different groups.



**Group 1**



**Group 2**



**Group 3**



**Group 4**

## DISCUSSION

### Discussion on behavioral study:

#### Retention Memory

This was assessed by removing the hidden platform. Reduced escape latency and increased time spent in quadrant where platform is missing was considered to represent memory retention. Analysis of the data shows that administration of AlCl<sub>3</sub> did not influence memory retention when tested on 28<sup>th</sup> day of treatment schedule. *Mandookaparni* alone and its combination with AlCl<sub>3</sub> also did not produce any significant change in the parameters assessed. Analysis of the data related to 58<sup>th</sup> day testing shows that the toxicant did not impair memory retention even on chronic administration. *Mandookaparni swarasa* alone produced significant decrease in number of cross over and significant increase in the duration spent in the target quadrant- this clearly indicates presence of memory retention effect in it. It confirms presence of *Medhya* effect. In AlCl<sub>3</sub> + *Mandookaparni* combination group also significant decrease in the number of crossover was observed while the effect on duration spent in the target quadrant was moderate. This indicates preservation of memory retention benefit even in the presence of the toxicant. *Medhya* effect present in *Mandookaparni* is also effective in preventing the cognitive deficits as well as oxidative stress by increase in the catalase activity [7]. Their administration for memory effect in all aged seems to strongly justified.

#### Discussion on histology

Histological examination of brain and spinal cord revealed that administration of AlCl<sub>3</sub> did not lead to any change in the cytoarchitecture cerebellum. In cerebral part region hemorrhagic streaks were found in some sections of one rat. In AlCl<sub>3</sub> – control group oedematous changes, ventricular dilatation and degenerative changes were observed in dentate gyrus and nearby areas. In *Mandookaparni swarasa* group almost normal cytoarchitecture was observed. In AlCl<sub>3</sub> + *Mandookaparni* combination group normal cytoarchitecture was observed in ¾ rats in one rat moderate degenerative changes were observed.

This clearly indicates neuroprotection in *Mandookaparni swarasa* against AlCl<sub>3</sub> induced degenerative changes and should be considered as a significant finding.

Spinal cord – in one rat receiving AlCl<sub>3</sub> disturbance in the dorsal horn of the gray matter was observed- other features were normal. However, in AlCl<sub>3</sub> group thinning of the vertebral bone was observed – especially the compact bone of thoracic and lumbar vertebrae. These changes were found in one rat in *Mandookaparni* group also. These changes were not reversed significantly in AlCl<sub>3</sub> + *Mandookaparni* combination group.

## DISCUSSION ON ANTI OXIDANT STUDY

### Protein concentration

Protein concentration in tissue homogenate moderately decreased in AlCl<sub>3</sub> – control group in comparison to the normal control group. Significant moderate increase was observed in *Mandookaparni swarasa* alone treated group. This may be indicative of increased turnover of nitrogenous material. It may also be indicative of enhanced protein synthesis due to liver induction. In AlCl<sub>3</sub> + *Mandookaparni* combination group moderate non-significant increase was observed in comparison to AlCl<sub>3</sub> – control group.

## CONCLUSION

In the present study *Mandookaparni Swarasa* exhibited good learning and memory enhancing effect, which contribute to the Neuro-protective activity. This is an important finding since learning and memory are major issues now.

Observations based on the result obtained in this study, the test drug *Mandookaparni Swarasa* is having good potential in neurodegenerative disorders characterized by cognitive and memory deterioration and accelerates the growth of brain. It also boosts up utilization capacity and has the potential to modulate CNS activities as said by the *Acharyas*. Its administration for memory enhancing in all ages seems to be strongly justified. It can be concluded that *Man-*

*dookaparni swarasa* administered group has shown better results compared to normal control group. Mandookaparni swarasa along with the aluminium chloride administered group also showed better result compared to aluminium chloride administered group i.e. it suggests that damage caused by aluminium chloride had overcome by *Mandookaparni*. So this represents *Mandookaparni* has very good property of revitalizing the brain.

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