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Research paper

Phyto chemical and memory enhancer activity of the 2 polyherbal formulation: comparative activity on $AlCl_3$ induced albino wistar rats

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Abstract

Background: Epidemiological studies of Indian population reveal that dementia is largely a hidden problem in India. Ayurveda claims several plants are beneficial in cognitive disorders. Pharmaco-epidemiological studies reveal that herbal and allopathic learning and memory enhancing medicines are becoming very popular among Indian population.
Methodology: The present study was aimed at investing the effects of Dr. Brain Syrup and Capsule, Ayurvedic polyherbal formulation on memory enhancing activity in albino wistar rats. Drugs were administered through intraperitoneal at therapeutic dose for 28 days to different groups of the rats. Elevated Plus maze (EPM) and Radial Arm maze apparatus were served as the evaluating tool to identify the Transfer Latency (TL) in EPM and Average time taken to reach right arm in Radial Arm Maze models. Cognitive impairment was induced by administering the AlCl3 at dose of 4.2mg/kg i.p.
Result: At the end of 4 weeks, Transfer Latency in model control group increased to 90±0.00 sec as compared to47.66±7.39 sec ($p<0.01$) in Normal control rats. Dr. Brain Syrup and Capsule were significantly attenuated TL to 19.5±4.27 and 21.16±2.83 sec respectively as compared to Model Control Group after 4 weeks. Moreover, Treatment with Dr. Brain Syrup and Capsule reduced the mean time to find the right arm to 20.83±2.08 and 27.16±1.17 sec ($p<0.01$) as compared to 129.66±4.60 sec in Model control group after 4 weeks.
Conclusion: Dr. Brain Syrup and Capsule showed significant activity in improvement in Memory by evaluating the TL and Average time period in 2 different instruments in 4 weeks of the drug treatment.

Keywords: *EPM; Transfer Latency; Polyherbal Formulations; Cognitive Impairment.*

1. Introduction

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention, and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, or to more ominous threats like schizophrenia and Alzheimer's disease.(Parle M et al 2007).
The nature provides a new opportunity to regain one's full mental capacity. A number of herbs traditionally employed in the Indian system of Medicine "Ayurveda" have yielded positive results.
In the year 2000, Around 2.88 million people over the age of 60, were affected with dementia which was continuously rising in upcoming years to 4.41 million people in 2015 and still this ratio is expected to escalate up to 14.32 million in 2050. The increased numbers of people with dementia will have a marked impact on states' infrastructures and healthcare systems, which are ill prepared in many regions and also on families and caregivers. The data on prevalence clearly identifies the importance of dementia in India, and the growing number of older patients in upcoming years. (Shaji KS et al 2010) .
Aluminium (Al) is a potent environmental neurotoxin, which is involved in the progression of neurodegenerative processes. Prolonged Al exposure induces oxidative stress and pathological alterations in diverse areas of brain of neonatal rats. (Shaik A et al 2015, Yuan CY et al 2012) AlCl3 is a non-redox active metal capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties.(Shaik A et al 2015, Nayak P et al 2010)
In present study, two different memory enhancer formulations are used to evaluate the memory enhancing property. The various herbal constituents of the formulations show significant activity in the treatment of the dementia or in improving the memory power.
Bacopa monnieri: Bacopa monnieri (BM) extract may be able to increase memory formation by the enzyme Tryptophan Hydroxylase (TPH) and increasing the expression of the serotonin transporter (SERT). B.monneri does appear to have some connections with the serotonin system, and may have downstream effects on the cholinergic system.(Pandey S et al 2013, Charles PD et al 2011) Ethanolic extract of B. monnieri afforded a neuroprotective role against aluminium induced toxicity and prevented oxidative stress induced by aluminium in the hippocampus of rats.(Charles PD et al 2011, Jyoti A et al 2006).
Convolvulus pluricaulis: Sharma K et al suggested that the significant increase in AChE altered the cholinergic activity which directly helps in the improvement of the memory.(Sharma K et al 2010).
Centella asiatica: Aqueous extract of the herb showed significant effects on learning and memory and decreased the levels of nore-

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pinephrine, dopamine and 5-HT and their metabolites in the brain. (Nalini K et al 1992, Singh S et al 2010) It contains brahmicacid, isobrahmic acid, brahminoside and brahmoside. It has psychotropic, sedative and anticonvulsant properties. It is also useful in dementia, mental disorders and anxiety.
(Singh S et al 2010, Upadhyay SK et al 2002)
Celastrus paniculatus: Celastrus paniculatus showed significant improvement in cognitive ability in animals by reducing the level of the Norepinephrine, Dopamine and Serotonin which helps in depression and enhance the memory. (Bhanumathy M et al 2010)
Mukta sukti Bhasma: Mukta sukti Bhasma has supporting action in the treatment of the dementia. It also helps in enhancing the memory power in children.

2. Materials & methods

2.1. Chemicals & drugs

The toxic substance AlCl3 was obtained from the A.R. college of Pharmacy & G.H. Patel Institute of Pharmacy, Vidhyanagar. The drug products were manufactured at PETLAD MAHAL AROGYA MANDAL PHARMACY, Nadiad.

2.2. Animals

Albino Wistar rats of either sex weighing around 300-350 gm were used in the present study. Animals were acclimatized to the laboratory conditions for five days. Animals were maintained under standard housing conditions (Temp.24-27°C and humidity 60-65%) with 12:12 hour light dark cycle. The entire project was approved by the IAEC of the institute (No: - CPCSEA/IAEC/ARCP/2015-16/06) and experiments were carried out as per guidelines of CPCSEA.

2.3. Preparation of the active formulations

2.3.1. Dr. Brain capsule powder

Grind the individual ingredients of Dr. Brain capsule to obtain the "Kwatha" in the disintegrator with the help of 4# sieve. Follow the common procedure to obtain the extracts of all ingredients separately.
The individual "Kwathas" were soaked in water in 1:8 ratios and keep it for 8 hours. After 8 hours, Boil the liquid until approximately quarter part of water remain. This decocted matter was filtered with the help of sparkler filter. This filtered liquid was concentrated with constant heat in S.S. jacketed vessel to prepare thick paste. Once the paste was formed, Prepare the small lumps of it and dry in tray dryer at <60° C. Pass these dries lumps through 8 mm multimill and sieve through 16# screen to make fine powder. Finally, mix these fine powder of all the ingredients vigorously as per the label claim and prepare the final powder mixture.

2.3.2. Dr. Brain syrup

All the plant materials were carefully segregated, washed and dried in shade. Other side prepare the Sugar Syrup in S.S. vessel and add sufficient amount of 'Nimbus Satva" and use stirrer for proper mixing. All the active ingredients, previously mixed and crushed in mass mixer and passed through sifter 100# sieve were mixed with Sugar syrup and stirred it continuously, filtered the liquid with help of sparkler filter machine.

2.4. Induction of cognitive impairment

Cognitive impairment was induced in Albino wistar rats using AlCl3 [4.2 mg/kg i.p.]. AlCl3 was dissolved in distilled water and injected to rats through intra-peritoneal route at a dose of 4.2 mg/kg body weight.AlCl3 was given for 28 days and neurological damage was confirmed by measuring various cognitive activities weekly up to 28 days.(Murthy KS et al 2011).

Table 1: Constituents of 2 Polyherbal Formulation for Memory Enhancer

Formulation Name	Constituents	Quantity
Dr. Brain Capsule	Ext. Bacopa monnieri	150mg
	Ext. Convolvulus pluricaulis	150mg
	Ext. Centella asiatica	100mg
	Ext. Celastrus paniculatus	75mg
	Mukta shukti Bhasma	25mg
Dr. Brain Syrup	Ext. Bacopa monnieri	250mg
	Ext. Convolvulus pluricaulis	150mg
	Ext. Centella asiatica	100mg

2.5. Evaluation of the cognitive impairment

2.5.1. Elevated plus maze apparatus [EPM]

EPM was use to evaluate the retention of memory and Learning in rats. The plus-maze consists of the two opposite open arms (50×10 cm), crossed with two enclosed arms of the same dimensions with 40 cm high walls. The arms are connected with a central square (10×10 cm) to give the apparatus a plus sign appearance. The maze was kept in a dimly lit room elevated 50cm above floor level. On 1st day, rats were placed on the end of one of the open arms, facing away from the centre, and the time taken by the animals to enter one of the closed arms Transfer Latency (TL) was recorded with the help of stop watch. On 2nd day, the same. procedure was repeated and Transfer Latency was recorded. Similarly, at weekly interval up to period of 21daysTL was recorded. (Kulkarni SK 2012).

2.5.2. Radial arm maze apparatus

The radial arm plus maze was used to evaluate the working memory in animals. Each arm (50 × 12 cm) of the eight arm radial maze extends from an octagonal shaped central hub of 30cm diameter. The platform was elevated 40cm above the floor. Small black plastic cups (3 cm in diameter and 1 cm deep) mounted at the end of each arm as receptors for food. At the beginning of the trial, food pallets were placed in right arm and animal was placed at the centre and allowed to find right arm. Average time taken by rats of each group to reach the right arm was recorded. (Kulkarni SK 2012)**Error! Bookmark not defined.**

2.6. Statistical analysis

All values are expressed as Mean±SEM. All data were analysed statistically by performing one-way ANOVA followed by Dunnet's Test using Graphpad Instant 3.0 software. $p<0.05$ was considered as statistically significant.

2.7. Experimental design

All the animals were divided in to four different groups.

- **Group 1**: Normal Control: All the animals were treated with 0.9% NaCl p.o.
- **Group 2**: Model Control: All the Animals were treated with AlCl3 4.2 mg/kg i.p.
- **Group 3**: Dr. Brain Syrup: Animals were treated with 176 mg/kg p.o. in addition with AlCl3 (4.2 mg/kg i.p.)
- **Group 4**: Dr. Brain Capsule: Animals were treated with 132 mg/kg p.o. in addition with AlCl3 (4.2 mg/kg i.p.)

3. Result

The organoleptic Properties and Quality test for finished products have been examined before conducting the efficacy preclinical trial. The Results of the Phyto chemical evaluation is shown in Table II and Table III.

The non-toxic nature of both formulations reveals no lethality or toxic reactions on the treated animals at any doses till the end of the study period.

The average body weights of the different groups have been increased gradually due to proper intake of the food and Water for entire study period.

Table 2: Organoleptic Properties

No.	Product Name	Colour	Odour	Taste
1	Dr.Brain Syrup	Dark Brown colour Liquid	Aromatic	Sweetish Bitter
2	Dr.Brain Capsule	Light Brown Colour Powder	Faint	Slightly Bitter

Table 3: Quality Test for the Finished Product and Plant Extract.

Product Name	Quality Test	Specification	Result
Dr. Brain Syrup	Specific Gravity	1.000-1.4000g/ml	1.2414 gm/ml
	pH	4.0-8.0	4.52
	Gross amount of Dry Extract	55-65% from specified amount	58.10%
	Assay of Bitter	NLT 0.1% w/w	0.15%
	Assay of Saponin	NLT 1.0% w/w	1.50%
	Identification by TLC	As per Specification	Complies
Dr.Brain Capsule	Average weight of Capsule Content	500.0mg	507.41mg
	Disintegration Time	NMT 15 min	10.20 min
	LOD at 110˚C	NMT 7% w/w	4.15%
	Ash Content	NMT 18%w/w	12.65%
	Acid Insoluble Ash	NMT 3% w/w	1.90%
	Alcohol Soluble Extract	NLT 5% w/w	6.54%
	Water Soluble Extract	NLT 40% w/w	65.20%
	Identification by TLC	As per Specification	Complies
	Assay of Saponin	NLT 5.00% w/w	6.30%
	Assay of Calcium	NLT 1.00% w/w	1.25%

Table 4: Effect of Dr. Brain Syrup & Capsule on Transfer Latency (TL) of Rats using Elevated plus Maze

Treatment Group	Transfer Latency Time (Sec)				
	Basal Value (Day 0)	At end of 1st week	At end of 2nd week	At end of 3rd week	At end of 4th week
Normal Control Saline (0.9 % NaCl p.o.)	23.00±8.63	43.83±8.97	39.16±8.98	47.5±6.33	47.66±7.39
Model Control AlCl3 (4.2 mg/kg i.p.)	41.60±5.78	76.50±13.55	90.00±0.00	90.00±0.00	90.00±0.00##
Dr.Brain Syrup (176 mg/kg p.o.)	26.00±3.81	16.16±2.20	17.50±3.57	18.16±1.92	19.50±4.27**
Dr. Brain Capsule (132 mg/kg p.o.)	28.80±16.36	20.66±2.39	23.33±1.9	17.00±1.97	21.16±2.83**

Data are Expressed as Mean±SEM (n=6),
p<0.01, When Compared to Normal Control Rats;
**p<0.01, When compared to Model Control rats, using one way- ANOVA followed by Dunnet's Test

Table 5: Effect of Dr. Brain Syrup & Capsule on Time Taken by Rats to Find Right Arm Using Radial Arm Maze Apparatus

Treatment Group	Average Time Periods (Sec)				
	Basal Value (Day 0)	At end of 1st week	At end of 2nd week	At end of 3rd week	At end of 4th week
Normal Control Saline (0.9 % NaCl p.o.)	70.5±17.74	55.83±11.06	61.00±9.02	60.00±8.34	64.50±7.89
Model Control AlCl3 (4.2mg/kg i.p.)	61.00±10.62	75.66±8.12	97.50±4.78	115.66±14.12	129.66±4.60##
Dr. Brain Syrup (176mg/kg p.o.)	66.33±24.57	23.66±2.31	19.50±2.65	19.5±2.18	20.83±2.08**
Dr. Brain Capsule treated (132mg/kg p.o.)	74.50±5.99	45.83±3.01	38.5±1.48	33.0±2.07	27.16±1.17**

Data are Expressed as Mean±SEM (n=6),
p<0.01, When Compared to Normal Control Rats;
**p<0.01, When compared to Model Control rats, using one way- ANOVA followed by Dunnet's Test

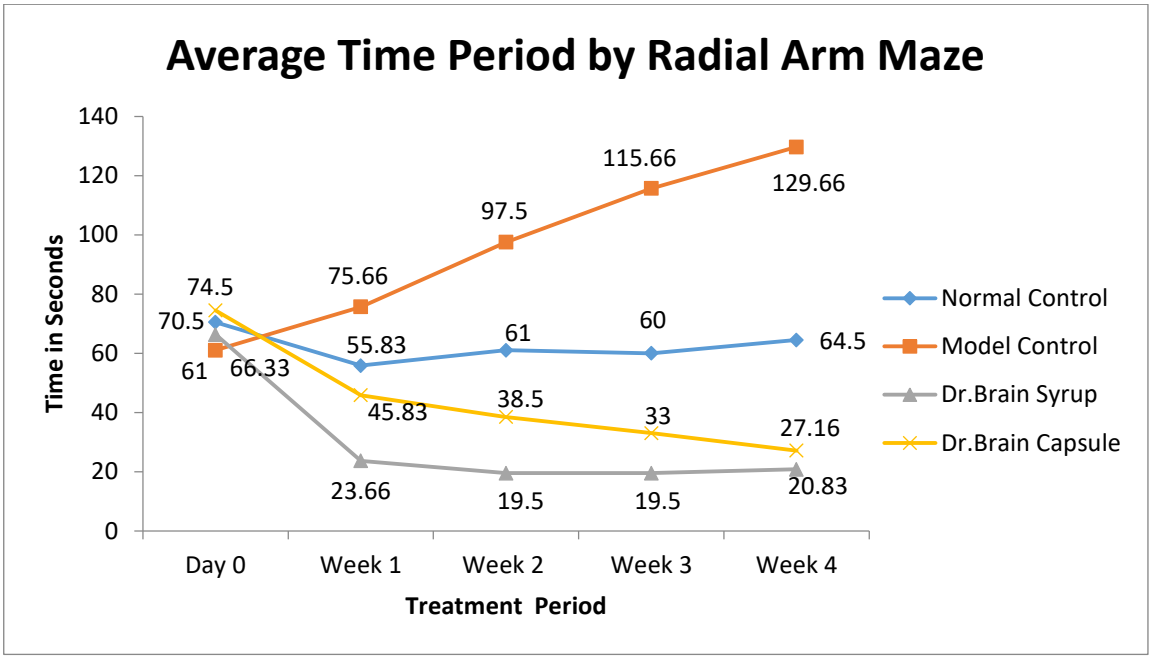


Fig. 1: Average Time Period Taken by Radial Arm Maze for 2 Formulations.

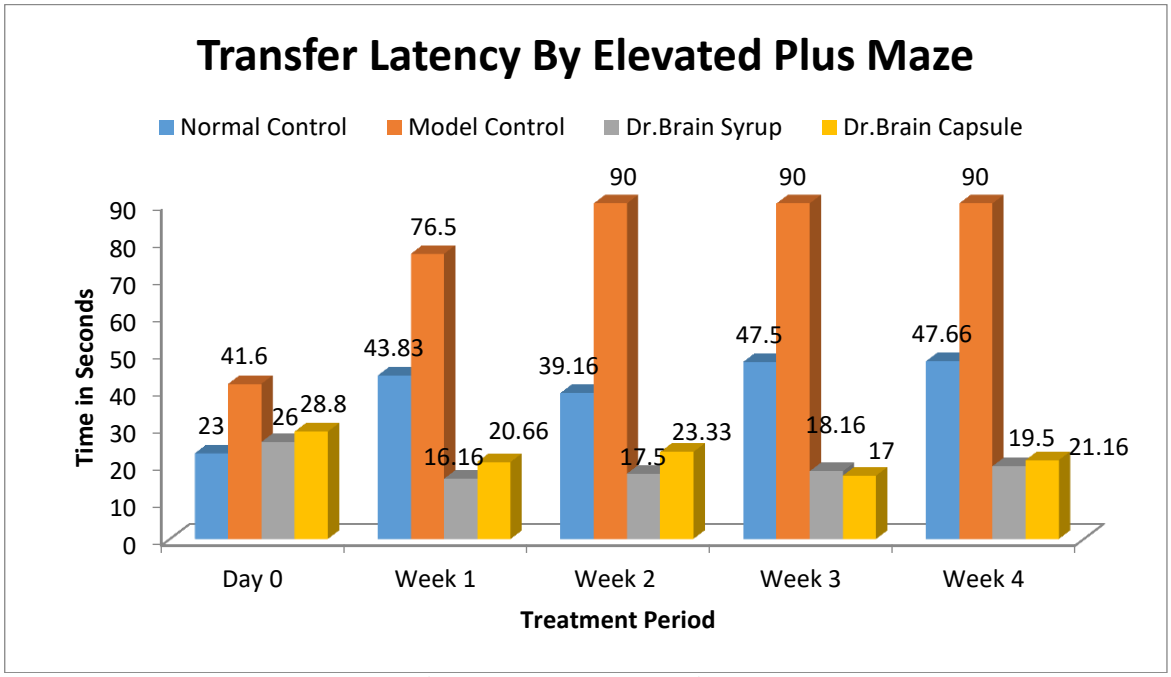


Fig. 2: Transfer Latency Evaluated by EPM for 2 Formulations.

Here, it is clearly indicating that, both the Polyherbal formulations shows significant reduction at the end of the treatment period (Week 4). In case of the Radial arm maze, Dr. Brain Capsule and Syrup showed gradually decrement in the Transfer Latency from 28.8 and 26 at Day 0 to 21.16 and 19.5 at week 4 respectively. Moreover, it is also noted that Dr. Brain Capsule and Syrup are also reduced the average time period in Radial arm maze apparatus. Hence, it is clearly shown that both the formulations are significantly enhancing the memory in to the animals at period of time.

4. Discussion

Dementia is generally defined as the "Loss of intellectual abilities, in dementia, memory capacity to solve problems of day-to-day living, performance of learned motor, social skills and control of emotions are primarily affected. Alzheimer's disease is a progressive and fatal neurodegenerative disorder manifested b cognitive and memory deterioration, Progressive impairment of routine activity of living, and a variety of neuropsychiatric symptoms and behavioural disturbances. (Ladde S et al 2011).

The clinical features of Alzheimer's disease are an amnesic type of memory impairment, deterioration of Language and visuospatial deficits. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide some satisfactory medications. (Wood AAJ 2004, Parle M et al 2007) Therefore, we were motivated to explore the new approach in Indian traditional system to manage this disorder through Ayurveda. In the present study, we have focused upon exploring the potential of an Ayurvedic poly herbal formulations "Dr. Brain Syrup" and "Dr. Brain Capsule" in reversing the memory deficits.

In the present study by using Elevated Plus Maze and Radial Arm Maze, the effect of AlCl3, Dr. Brain Syrup and Capsule exposure on Transfer Latency and the Average time spent were investigated. In present study, AlCl3 significantly ($p<0.01$) impaired learning and memory by increase in the Transfer Latency time in rats of Model Control group on Elevated Plus Maze up to 90.00±0.00 at the end of the 4th week, which was found only 47.66±7.39 in

Normal Control Group at the end of study. Dr. Brain Syrup and Capsule drastically reduced this time to 19.50±4.27 and 21.16±2.83 ($p<0.01$) respectively when compared to model and Normal Control group.

Similarly, in Radial Arm maze, AlCl3 increased time to find the correct arm suggesting loss of memory and impairment of cognition. Time to find right arm was significantly reduced up to 20.83±2.08 and 27.16±1.17 ($p<0.01$) by administration of the Dr. Brain syrup and capsule respectively, suggesting the memory enhancing ability.

Moreover, from the Fig:1 & 2, It can be easily seen that both Dr. Brain Syrup and Capsule gradually reduced the Time taken for Transfer latency in EPM and find the right arm in radial arm maze in the 4-week treatment period, which clearly indicates the potency of the drug to improve the memory in Rats.

5. Conclusion

In the light of above, it may be worthwhile to explore the potential of these formulations exhibited nootropic activity and useful in the management of enhancing memory. It can enhance the Therapy and treatment in to the patients suffering from the memory Loss, Anxiety and Depression. These formulation produce none side effects in animals shows rising supplement for the children as a memory tonic. Further study of these products with comparative allopathic drugs is advisable.

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