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NEUROPROTECTIVE EFFECTS OF POLYHERBAL FORMULATION ON ALUMINIUM CHLORIDE INDUCED ALZHEIMER'S DISEASE IN RATS

N. Tulasi Uma Rani*, S. K. Naina Sulthana and S. K. Meeravali

HINDU College of Pharmacy, Guntur - 522002, Andhra Pradesh, India.

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***Corresponding Author**
N. Tulasi Uma Rani
HINDU College of Pharmacy, Guntur - 522002, Andhra Pradesh, India.

ABSTRACT

Objective: The present study was designed to evaluate the Nootropic activities of Poly herbal formulation against aluminum Chloride induced Alzheimer's disease (AD) in rats. **Methodology:** The current study was designed to investigate the neuroprotective effects of polyherbal formulation (PHF), 200mg, 300mg/kg, for 28days, Albinowistar rats of group 2 were treated with Aluminium chloride for 28 days. Behavioural studies are done by using Y-MAZE and ELEVATED PLUS MAZE. Other parameters such as RBC-Cholineesterase, Blood glucose, Hemoglobin, Body weights, food habits were observed. **Results:** This Study shows that the Aluminium chloride 4.2mg/kg does neuronal damage and memory impairment was observed. With behavioral changes, high RBC Cholinesterase levels, low hemoglobin levels, high blood glucose levels, gradually decrease in body weight and food habits. Treatment of PHF 300mg/kg shown effective compared with other groups. **Conclusion:** The present study revealed that poly herbal formulation of 300mg/kg significantly reduces RBC-Cholinesterase levels, Blood Glucose levels, Increased Hemoglobin levels, Body weight, Food habits are normal. And increased neuroprotection.

KEYWORDS: alzheimer 's disease, Nootropic activity, Neuroprotection, Aluminium chloride, Donepezil, RBC-Cholineesterase, Behavioural changes, Blood glucose, Hemoglobin.

1. INTRODUCTION

AD, a Neuro degenerative disorder affecting neurons in the brain, leads to dementia and memoryloss.[1,2] Current studies suggest that approximately 50 million people globally are

affected by AD, with the numbers expected to double every 5 years, reaching 152 million by 2050.[3] Memory is a complicated process composed of three stages: encoding (the process of choosing which information is significant), storing, and recalling. Various parts of the brain are responsible for different types of memory. For an event to be stored in long-term memory, the brain must allocate attention and repeat information, moving it from short-term memory to long-term memory; this is known as encoding.

Short-term memory, alternatively known as working memory, takes place in the prefrontal cortex. It retains information for roughly one minute and has a restricted capacity of approximately 7 objects. As an illustration, it empowers you to input a phone number that someone recently shared with you. In addition, it plays a role in reading by committing the sentence you just read to memory, allowing the subsequent one to be comprehensible.[4] A permanent solution for AD is not available, but there is a treatment option that can reduce the symptoms of AD. AD is characterized by several risk factors, including increasing age, genetic factors, head injuries, vascular diseases, infections, and environmental factors. Currently, numerous studies are being conducted to comprehend the pathophysiology of AD. These studies target various mechanisms such as abnormal tau protein metabolism, ß-amyloid, inflammatory response, cholinergic activities, and free radical damage.

2. METHODOLOGY

2.1 MATERIALS USED

Donepezil 10mg, Aluminium chloride, Alcohol, Plant powders of Centella asiatica, Clitoria ternatea, Glycerhiza glabra, Nardosthys jatamansi from local pachari shop.

2.2 EXPERIMENTAL ANIMALS

Albino wistar rats of 150-200mg, of 8 weeks are used in the study. They are maintained under proper laboratory conditions and giving Standard pelletisest food as diet and maintain 12hrs day and 12hrs night cycles, and providing water ad libitum. The all the animals were acclimatized to the laboratory environment 5 days prior to experiment. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee of Hindu College Of Pharmacy. The study was conducted after obtaining ethical committee clearance from the Institutiona Animal Ethics committee No: **IAEC-HCOP/2023/05.**

2.3 ALZHEIMER'S INDUCTION AND GROUPING OF ANIMALS

The aluminum chloride was used to induce Alzheimer's. The aluminum chloride was dissolved in normal saline at a dose of 4.2 mg/kg body weight and injected through intra-peritoneal (i.p.) Route for 28 days. The induction of Alzheimer's was confirmed by measuring the nootropic activity at alternate days till the end of the study.

The animals are divided into 5 groups and 6 animals each group.

Group I (normal control group): receive distilled water *ad libitum*.

Group II (Negative control group): Receives Aluminiumchloride (4.2mg/kg)

Group III Aluminium Chloride (4.2mg/Kg) + Test drug (200mg/Kg)

Group IV Aluminium Chloride (4.2mg/Kg) + Test drug (300mg/Kg)

Group V Aluminium Chloride (4.2mg/Kg) + Standard Drug (10mg/Kg).

The (group 2) is a Negative group will be treated with Aluminium Chloride. The animal groups (3, 4) are treated with Aluminium chloride (4.2mg/kg) + the test drug (200,300mg/kg) and the group 5 is treated with Aluminium chloride (4.2mg/kg) + standard drug Donepezil (10mg/kg) for 28 days and on 28th day the animals will be projected to Y-Maze and Elevated Plus maze. And other biochemical parameters were observed.

3. INVIVO STUDIES

3.1 BEHAVIOURAL STUDIES

3.1.1 Y-MAZE

Spatial working memory was assessed using the Y-maze test with some modifications (Sierksma et al., 2014). The Y-maze consisted of three identical arms arranged at a 120-degree angle and a triangular central area as represented in the. Each animal was placed in the center of the maze and allowed to explore for 8 minutes. Rats typically prefer to explore the arm they haven't visited recently, resulting in alternation between the three arms. Efficient alternation requires the use of working memory, as rats must keep track of their most recent arm visits and update this information continuously (Wietrzych et al., 2005). An arm entry was considered when all four paws were inside an arm. The following measures were recorded: total number of arm entries, triads (consecutive visits to three different arms), and the percentage of alternation. Alternation was defined as entering three different arms consecutively. The percentage of alternation was calculated as

$$\frac{\text{No. of alterations}}{\text{Number of possible traids}} * 100$$

The maximum possible alternations were based on the total number of arm entries minus two. A low percentage of alternation indicates impaired spatial working memory, as the rat struggles to remember which arm it visited previously and therefore exhibits reduced spontaneous alternation.

3.1.2 ELEVATED PLUS MAZE

The EPM test evaluates behaviors that resemble anxiety (Pellow et al., 1985). The setup consisted of a black melamine central square platform (11×11 cm), with four black melamine arms (50×11cm) radiating from it, separated by 90 degrees. Two of the arms, known as "closed arms," were enclosed by walls (40 cm in height), except at the entrance, while the other two arms, known as "open arms," had no walls but had raised edges (0.25 cm) around them. The maze was elevated one meter above the ground by five legs. The light intensity in the open arms was 80–90 lux. Each rat was placed on the central platform, facing an open arm, and was allowed to freely explore the maze for 5 minutes. An entry into an arm was counted when a rat introduced all four paws into that arm. The following measurements were recorded: the total distance moved, the number of entries into closed arms, the percentage of entries into open arms (number of open arm entries divided by total entries, multiplied by 100), and the percentage of time spent in open arms (time spent in open arms divided by 300 seconds, multiplied by 100).

(No. of open arm entries /Total entries) × 100

Percentage of open arm entries

(No. of open arm entries / Total entries) × 100 and

Percentage of time spent in open arms

(Time spent in open arms / 300sec) × 100.[5].

4. STATISTICAL ANALYSIS

All data presented as mean ± S.E.M. The significance of difference among the groups were assessed by using one sample t-test.and $p<0.1$ was considered significant.

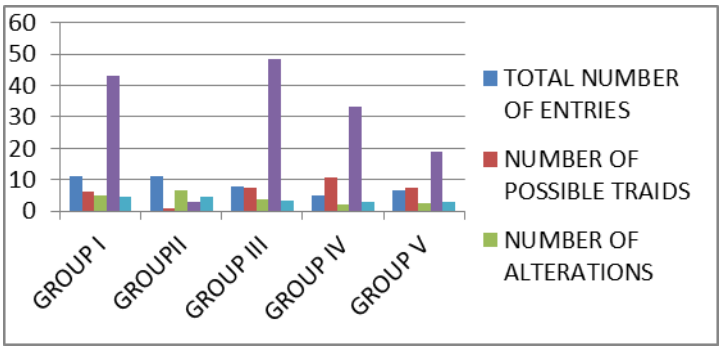
5. RESULTS

5.1 BEHAVIOURAL PARAMETERS

5.1.1 Y - MAZE

Table 1: Represents the values of Y-maze, The data obtained on the effect of treatment to various groups (Group I-control, Group II – Negative group, Group III – test 200+Alcl3 4.2mg/kg, Group IV – Test 300+Alcl3 4.2mg/kg ,Group V – Standard Donepezil 10mg/kg. Effect of various doses of PHF (200 mg/kg, 300 mg/kg) and standard (Donepezil10mg/kg) on learning and special memory on Y MAZE. Each value represents the mean ± SEM (n=6). * p<0.1, **p<0.01, *<0.001 in comparison with Standard (Donepezil 10mg) treated group.**

S.No	Group	Total no. of arm entries	No. of possible triads	No. of alterations	%Alterations
1	Group I	**11**±0.51**	**6.16**±0.0.4279**	**4.83**±0.75***	**42.95**±17.53***
2	Group II	**11**±0.36***	**1**±0.36***	**6.50**±1.04****	**3** ±112****
3	Group III	**8**±0.57**	**7.5**±0.42**	**3.66**±0.81**	**48.5**±2**
4	Group IV	**5**±0.57*	**10.8**±0.60*	**2**±0.63*	**33.4**±2.58*
5	Group V	**6**.50±0.57	**7.50**±0.42	**2.50**±0.54	**18.72**±2.77



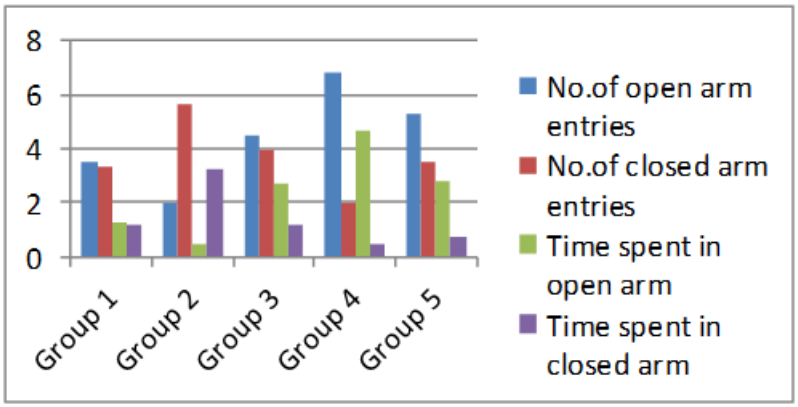
GRAPH 1: Represents the values of various doses of PHF (200 mg/kg, 300 mg/kg) and standard (Donepezil10mg/kg) on learning and special memory on Y MAZE. Each value represents the mean ± SEM (n=6). * p<0.1, **p<0.01, *<0.001 in comparison with Standard (Donepezil 10mg) treated group.**

5.1.2 ELEVATED PLUS MAZE

Table 4: Represents the values EPM (Elevated plus maze) are calculated the results are calculated based on the number of open and closed arm entries and Time spent in open and closed arms. the (Group I-control, Group II – Negative group, Group III – test 200+Alcl3 4.2mg/kg, Group IV – Test 300+Alcl3 4.2mg/kg ,Group V – Standard (Donepezil 10mg/kg)Each value represents the mean ± SEM (n=6). * p<0.1, **p<0.01, *<0.001 in comparison with Standard (Donepezil 10 mg).**

S.No	Groups	No. of entries in open arms	No. of entries in Closed arms	Time spent in open arms(min)	Time spent in closed arms(min)
1	Group I	3.5±0.76***	3.33±0.66***	1.28±0.03***	1.15±**0.51***
2	Group II	2±0.36****	5.66±0.61****	0.44±0.63****	3.21±**0.78****
3	Group III	4.5±0.42**	4±0.36**	2.68±0.11**	1.23±**0.80**

4	Group IV	6.8±0.60*	2±0.36*	4.72±0.18*	0.45±**0.53***
5	Group V	5.3±0.55	3.50±0.61	2.82±0.13	0.76 ±0.12

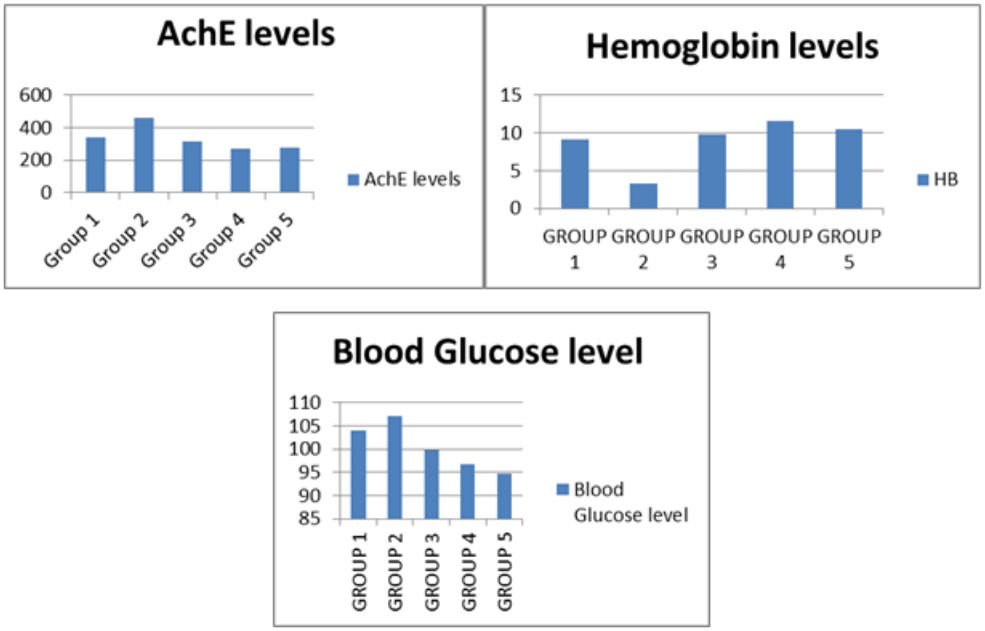


GRAPH 2: Represents the values of various doses of PHF (200 mg/kg, 300 mg/kg) and standard (Donepezil10mg/kg) on learning and special memory on ELEVATED PLUS MAZE. Each value represents the mean ± SEM (n=6). * p<0.1, **p<0.01, *<0.001 in comparison with Standard (Donepezil 10mg) treated group.**

5.2 OTHER PARAMETERS

Table 5: Represents the values of (Group I-control, Group II – Negative group, Group III – test 200+Alcl3 4.2mg/kg, Group IV – Test 300+Alcl3 4.2mg/kg ,Group V – Standard (Donepezil 10mg/kg). Effect of various doses of PHF (200 mg/kg, 300 mg/kg) and standard (Donepezil10mg/kg) on Acetylcholine esterase levels, Hemoglobin, Blood glucose levels. Each value represents the mean ± SEM (n=6). * p<0.1, **p<0.01, *<0.001 in comparison with Standard (Donepezil 10mg) treated group. Here the Group II shows the elevated RBC Choline esterase levels, increased blood glucose levels and decreased hemoglobin levels compared to other groups. And the group IV of test 300mg/kg shows more effective here there are decreased AchE levels, increased hemoglobin levels, and normal blood glucose levels in comparison with negative and standard groups.**

S.No	Group	ChE Levels	HB	GLUCOSE
1	Group I	339.16±7.12**	9.16±0.47**	104±0.42**
2	Group II	458.33±0.66***	3.3±0.26***	107±0.66***
3	Group III	313.33±2.06**	9.83±0.60**	99.83±0.47**
4	Group IV	269.16±14.72*	11.50±0.76*	96.83±1.85*
5	Group V	276.50±10.93	10.5±0.76	94.66±1.66



GRAPH 3: (a,b,c) Represents the parameters of Ache (in RBC) levels, HB, Glucose levels. Each value represents mean ±S.E.M (n=6)*p<0.1, **p<0.01, ***p<0.001 in comparison with Standard (Donepezil) treated group.

6. DISCUSSION

The aim of this research is to assess the Neuro protective effects of a Poly herbal Formulation on animals exposed to Aluminium chloride.

To achieve this, various tests such as Y-maze and Elevated Plus maze are conducted, as well as measurements of RBC-Cholinesterase levels, Blood Glucose levels, Hemoglobin levels, behavior changes, food consumption, and weight in the animals are observed. Cognitive dysfunction is a significant health issue in the 21st century, and a variety of neuropsychiatric and neurodegenerative disorders, such as schizophrenia, depression, Alzheimer's Disease dementia, cerebro vascular impairment, seizure disorders, head injury, and Parkinsonism, can be tremendously disabling in their effects. Over time, scientists have discovered various neurotransmitters and signaling molecules that have been recognized as potential targets for therapy.

Both conventional and newer substances have been tested against these targets. The phyto chemicals found in medicinal plants play a crucial role in maintaining the brain's chemical balance by influencing the function of receptors for major inhibitory neurotransmitters.

Traditional medicine has long reported the use of several plants to treat cognitive disorders. In this analysis, we aim to shed light on the use of medicinal herbs for cognitive disorders. Specifically, we provide a brief overview of certain medicinal herbs and their Neuro protective phyto chemical substances, such as fatty acids, phenols, alkaloids, Flavanoids, Saponines, and terpenes. Additionally, we discuss the activation of specific signal transduction pathways and transcription factors that allow neurons to resist various stressors.[6]

Some plants that are used in the treatment of AD are Bellis perennis[12,13], Calendula officinalis[14], Carthamus tinctorius[15], Cassia occidentalis[16,17], Coriandrum sativum[18,19], Crocus sativus[20,21], Cyperus rotundus[22], Dalbergia sissoo[23], Geum urbanum[24], Hyoscymusniger[25], Juglans regia[26,27,28], Lagerstroemia speciosa, Lithospermum officinale, Lycium barbarum, Mangifera indica, Matricaria chamomilla, Medicago sativa, Melilotus officinalis, Melissa officinalis, Mentha longifolia.[7]

Ayurveda frequently employs medicinal plants and concoctions to treat neurodegenerative illnesses, particularly Alzheimer's disease and its related indications. The current Study investigates the potential of Centella asiatica (leaves), Clitoria ternatea (flower and leaves), Glycerhiza glabra (stem), and Nardostachys jatamansi (roots) as a promising remedy for AD. These plants have already demonstrated beneficial qualities such as scavenging free radicals, neuroprotection, and anti-inflammatory activity. The study aims to explore their ability to modify Ach levels and enhance neuroprotection.[8-11]

Based on the protocol of 28 day study followed by single dose of Aluminium chloride (4.2mg/kg), Poly herbal formulation 200mg/kg + aluminium chloride 4.2mg/kg, Poly herbal formulation 300mg/kg + Aluminium Chloride 4.2mg/kg, Donepezil(standard)10mg/kg+ Aluminium chloride 4.2mg/kg.

The cognitive impairment was confirmed by various behavioral studies i.e. Y-maze, Elevated Plus maze Y-maze As it is one of the standard apparatus to do behavioral studies here in y-maze, Number of left arm entries, Number of right arm entries, Total number of entries, number of alterations,% of alterations, Number of possible traids are noted and calculated.

The Total number of arm entries of all groups are as follows, Control (11±0.51), Aluminium chloride treated group (11±0.36), Test 200+Aluminium chloride (8±0.57),Test

300+Aluminium chloride (5±0.57), Standard (Donepezil 10mg) + Aluminium chloride (6.50±0.57) here the number of arm entries are increased in Aluminium chloride treated group than other groups. The test 300mg/kg shown equally effective than the standard drug group.

As the Number of possible traids, number of alterations and % of alterations are low in negative group compared to other groups i.e., 1±0.36, 6.50±1.04, and 3 ±112.Where the test 300mg shown more effective than the standard group. Test 300mg/kg 10.8±0.60, 2±0.63, and 33.4±2.58 where the standard group shows 7.50±0.42**,** 2.50±0.54**,** and 18.72±2.77.

While in Elevated plus maze the parameters of number of open and closed arm entries and time spent in open and closed arm entries are calculated. The aluminium chloride treated group shown less open arm entries and more closed arm entries i.e., 2±0.36, 5.66±0.61 compared to other groups and spent less time in open arms and more time in closed arms i.e., 0.44±0.63, 3.21±0.78. where as control group shows 3.5±0.76, 3.33±0.66 and 1.28±0.03, 1.15±0.51. And coming to test 300mg/kg and standard the test 300mg/kg shown more effective the values include open and closed arm entries and time spent in open and closed arms i.e., 6.8±0.60, 2±0.36 and 4.72±0.18,0.45±0.53 and Standard group shows 5.3±0.55, 3.50±0.61 and 2.82±0.13, 0.76±0.12.

Coming to the parameters of RBC-Cholinesterase levels, Hemoglobin and Blood glucose levels. The Aluminium chloride group shows high choline esterase levels, low hemoglobin levels and high blood glucose levels .i.e., 458.33±0.66, 3.3±0.26, and 107±0.66 and control group shows 339.16±7.12, 9.16±0.47, and 104±0.42. Where the Test 300mg/kg and standard shows 269.16±14.72, 11.50±0.76 and 96.83±1.85, and standard group shows 276.50±10.93, 10.5±0.76, and 94.66±1.66.

CONCLUSION

From the present study, it can be considered that the Poly herbal formulation exhibited Neuroprotective activity in Aluminium chloride induced Alzheimer's rat model. Among All The Formulations the Polyherbal formulation of 300mg/kg treated group have shown better resultswhen compared with Standard (Donepezil 10mg/kg). The Neuro protection is may be due to thepresence of phyto chemicals but Further studies are to be conducted to know the exactmechanisms of Neuro protection.

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Conflict consent

Not applicable.

Ethical statement

The study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics committee No: **IAEC-HCOP/2023/05.**

Author Contribution

All authors contributed equally.

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