

How to Cite:

Himabindu, K., & Vijayalakshmi, A. (2022). Neuroprotective effects of *Ficus dalhousie* and *Melissa parviflora* benth against aluminium chloride induced Alzheimer's disease in rat model. *International Journal of Health Sciences*, 6(S5), 1129–1137. <https://doi.org/10.53730/ijhs.v6nS5.9097>

Neuroprotective effects of *Ficus dalhousie* and *Melissa parviflora* benth against aluminium chloride induced Alzheimer's disease in rat model

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Abstract---In present study, the protective effects of *Melissa parviflora* and *Ficus dalhousie* were evaluated against $AlCl_3$ induced Alzheimer's infection in albino Wistar rodents. 42 wistar rodents divided in seven groups, six in each, and treated for 21 days. Toward the finish of the investigation, different social, biochemical and histopathological assessments were done. The outcomes showed an abatement in muscle strength as estimated by the rotarod. Muscle in coordination and decline in locomotor action saw in the negative control group, while in the groups treated with plants, they show an improvement in muscle strength and good locomotor development. The biochemical review uncovered that persistent organization of $AlCl_3$ altogether diminished lipid peroxidation and diminished degrees of acetylcholinesterase, catalase, decreased glutathione and glutathione reductase, a record of oxidative pressure process. A marked changes at the subcellular level observed in histopathology studies of control group and better improvement in these parameters was seen in Rivastigmine treated group.

Keywords--- $AlCl_3$, *Melissa parviflora*, *Ficus dalhousie*, Actophotometer

Introduction

Alzheimer's is the most widely recognized type of dementia in the old populace. The significant downside with Alzheimer's is that we don't have a total and long-lasting remedy (Lakshmi, BV et al., 2015). Preclinical studies on animal models always play a crucial role in drug development with a meaningful full contribution to the development of a successful therapeutic agent (A, Abyad et al., 2015). In the last two decades, work on transgenic animal models played a crucial role in a basic understanding of the molecular mechanisms involved in the onset and progression of the disease (H. Aebi et al., 1984).

Materials and methods

Plant Materials

Leaves of *Melissa parviflora* and Fruits of *Ficus dalhousie* were collected from various parts of Tirupati, Andhra Pradesh, India during the month of March 2020. The Collected Plant materials were identified and authenticated by a renowned Botanist Dr. Madhav Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Andhra Pradesh. The plants were authenticated and the specimen kept in herbarium as *Melissa parviflora* Benth voucher No: 0046 and *Ficus dalhousiae*, voucher No: 0703 for future references.

Extraction procedure

The leaves of *Melissa parviflora* and fruits of *Ficus dalhousie* were thoroughly washed, shade-dried at room temperature and coarsely powdered. The Fine powder of crude drug used for extraction in different solvents by Continuous Hot Extraction (Ingle, KP et al., 2017). The solvent used in extraction is arranged according to their polarity from least polar to the most polar like Petroleum Ether, Ethylacetate and Hydro alcoholic extract.

The marc left after Ethylacetate extraction was further used for Hydroalcoholic extraction. The extraction was completed with ethanol in addition to water in the proportion 70:30 v/v, then at that point, the blend was mixed at room temperature (25°C) for 24 hours. In this manner, the blend was sifted under vacuum, holding the filtrate (Pandey et al., 2014). 10 ml of ethanol were added to the strong buildup, centrifuged at 3000 rpm for 5 minutes and re-filtered utilizing the vacuum siphon. The last extract was then stored in dark at 4° for further experiments (Sasidharan, S et al., 2011).

Experimental setup

Forty-two albino wistar rats weighing ranges 100–150g were utilized in this investigation. All animals were divided into seven groups, each group contains six animals. All the experimental procedures conducted according to animal ethical guidelines of CPCSEA.

Aluminium chloride hexahydrate stock solution was prepared in (0.9%) saline. Group II-VII animals received AlCl₃ at a dose of 300 mg/kg, P.O., whereas control group received an equivalent amount of saline for 7 days. Behavioural studies were carried out with regular interval and animals were sacrificed by decapitation.

The brains were removed and rinsed with ice-cold saline immediately after decapitation and store at -70°C for further analysis. The cognitive abilities of rats were assessed by the Ymaze and actophotometer was used for the assessment of rats locomotive activity.

The rats were treated for 20th days and behavioural parameters were determined on 0, 6th, 12th and 20th day(S.,Bhattacharjee et al., 2014; E.,Cheraghi et al., 2017).

Biochemical estimations

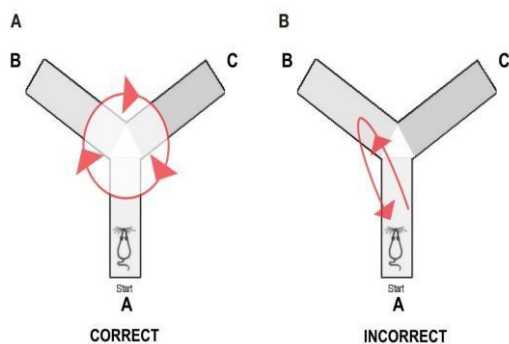
Oxidative parameters

The cerebrum tests were taken out from the skull rinse di-isotonic saline and weighed. The tissue homogenate was plated 10% (w/v) with 0.1 M phosphate support pH 7. The mind homogenates were then centrifuged at 12,000 xg for 20 minutes at 4°C , the supernatant was gathered and used to assess different oxidative boundaries like MDA, SOD and GSH(L.,Flohe et al., 1984; M.B., Ćoloviċ et al., 2013).

Estimation of Acetyl cholinesterase activity using colorimetric assay

The brain part like cerebrum, hippocampus and septum of sacrificed rats taken out on a Petridish chilled on squashed ice. The homogenized tissue was first treated with 5, 50-dithionitrobenzoic acid (DTNB), and the optical density (OD) of the yellow colour compound formed during the reaction at 412 nm every minute for a period of 3 min was measured using Folin's method(G.L.,Ellman et al., 1961; V.P.,Bingman et al., 2014).

Assessment of the recognition memory activity using Ymazes patial learning apparatus



The Ymaze was the better way to understand their cognition memory in experimental rats. The test represents the damage to the hippocampal region. The animal's memory function was tested with Y-maze and observed the returned and spend time tendency in the arms(M.E.,Hasselmo et al., 2006).

Results

The biochemical parameters of the levels of SOD, MDA and GLUTATHIONE are given in below table

Table no 1: Estimation of biochemical parameters in drug treated rat brain

Treatment Groups	SOD (U/g of brain tissue)	MDA (nM/mg of tissue)	Glutathione(μ g/g of tissue)
Normal Control	7.18 \pm 1.21	5.16 \pm 0.55	15.17 \pm 4.75
Test Control	11.10 \pm 2.10	14.06 \pm 4.28	5.67 \pm 2.31
Standard	8.73 \pm 2.23	4.99 \pm 0.72	14.7 \pm 4.71
<i>Melissa Parviflora</i> (200mg/kgp.o)	10.80 \pm 3.22	10.5 \pm 2.47*	7.92 \pm 3.26
<i>Melissa Parviflora</i> (400mg/kgp.o)	8.48 \pm 2.81*	6.98 \pm 2.05*	12.09 \pm 4.1*
<i>FicusDalhousie</i> (250mg/kgp.o)	10.90 \pm 3.09	11.06 \pm 1.5	8.20 \pm 3.09
<i>FicusDalhousie</i> (500mg/kgp.o)	8.82 \pm 3.86*	7.09 \pm 1.20*	11.56 \pm 4.24*

Values are expressed in mean \pm SEM at n=6 , Statistical analysis was carried out by one- way ANOVA followed by Dunnett's test in compare to control animals.

The biochemical parameters of Acetylcholinesterase, Noradrenaline and Dopamine are given in the below table

Table no 2: Estimation of biochemical parameters in drug treated rat brain

TreatmentGroups	Acetylcholinesterase(μ mol/L/min/gti ssue)	Noradrenaline(ng/gbraintissue)	Dopamine (ng/gbraintissue)
NormalControl	633 \pm 12.49	2.26 \pm 0.18	232.02 \pm 6.63
TestControl	943.75 \pm 26.88	5.01 \pm 0.25	180.03 \pm 4.19
Standard	566.68 \pm 19.11	2.31 \pm 0.21	293.29 \pm 5.93

<i>Melissa Parviflora</i> (200mg/kgp.o)	786.93 ±12.45	3.86±0.23	259.33±5.10*
<i>Melissa Parviflora</i> (400mg/kgp.o)	666.87±11.16*	2.79±0.18*	290.87± 5.86*
<i>FicusDalhousie</i> (250mg/kgp.o)	845.21 ±17.11	4.06±0.13	231.33±3.14
<i>FicusDalhousie</i> (500mg/kgp.o)	710.11±12.09*	3.61±0.22*	265.33±4.11*

Values are expressed in mean ± SEM at n=6 , Statistical analysis was carried out by one- way ANOVA followed by Dunnett's test in compare to control animals. The spontaneous locomotor activity is estimated and given in the table below

Table no 3: Estimation of spontaneous locomotor activity in experimental rats

TreatmentGroups	Locomotor activity score			
	0day	6day	12day	20day
Normal Control	151.00± 8.40	154.00± 8.46	148.00± 8.10	136.00± 7.08
Test Control	149.33± 9.02	141±9.28	101.12 ±7.98	68.58±4.95
Standard	150.31 ±9.72	148.30 ± 8.72	134.01 ±7.72	128.41 ±8.01
<i>Melissa Parviflora</i> (200mg/kgp.o)	148.83± 9.12	132.03±8.12	113.24 ±7.12	103.62 ±6.74
<i>Melissa Parviflora</i> (400mg/kgp.o)	149.17±8.03	141.36 ±8.93	128±6.98	121.07±9.30
<i>Ficus Dalhousie</i> (250mg/kgp.o)	158.38 ±8.21	155±8.05	124.4±6.82	103.82 ±6.44
<i>Ficus Dalhousie</i> (500mg/kgp.o)	154.21 ±8.33	143.63 ±8.03	132±7.82	128.9 ±9.01

Values are expressed in mean ± SEM at n=6. Statistical analysis was carried out by one- way ANOVA followed by Dunnett's test in compare to control animals. The spatial learning activity is estimated and given in the table below

Table no. 4: Estimation of learning activity in experimental rats

Treatment Groups	Time to reach pre-visited arm in sec.			
	0day	6day	12day	20day
Normal Control	27±1.58	32.06± 2.07	28±1.37	30.2± 1.47
Test Control	34±1.58	36±1.57	42.23± 2.09	58.8± 3.11

Standard	27.4±1.14	31.02± 1.12	32.04± 1.55	38±1.77
Melissa Parviflora(200mg/kgp.o)	32.2±2.49	33.45± 1.47	38.5±1.74	42.7± 2.41
Melissa Parviflora(400mg/kgp.o)	33±1.58	31.88± 1.80	36.4±1.67	40±1.98
Ficus Dalhousie(250mg/kgp.o)	34.1±1.29	36.5±2.07	41.9±2.14	46.7± 2.61
Ficus Dalhousie(500mg/kgp.o)	32.8±1.71	34.18± 2.0	35.9±1.76	38±2.01

Values are expressed in mean ± SEM at n=6. Statistical analysis was carried out by one- way ANOVA followed by Dunnett's test in compare to control animals. The motor coordination activity is estimated and given in the table below

Table no.5: Estimation of motor coordination activity in experimental rats

Treatment Groups	Number of animals completing 100 % duration on rotating rod at various treatment days intervals			
	Oday	6day	12day	20day
NormalControl	6/6	6/6	5/6	6/6
TestControl	6/6	4/6	3/6	2/6
Standard	6/6	5/6	4/6	4/6
Melissa Parviflora(200mg/kgp.o)	5/6	4/6	3/6	3/6
Melissa Parviflora(400mg/kgp.o)	6/6	5/6	4/6	4/6
FicusDalhousie(250mg/kgp.o)	6/6	5/6	4/6	3/6
FicusDalhousie(500mg/kgp.o)	5/6	4/6	4/6	3/6

Values are expressed in mean ± SEM at n=6. Statistical analysis was carried out by one- way ANOVA followed by Dunnett's test in compare to control animals.

Histopathology of brain:

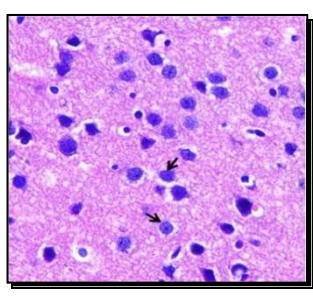


Figure 1: Normal control

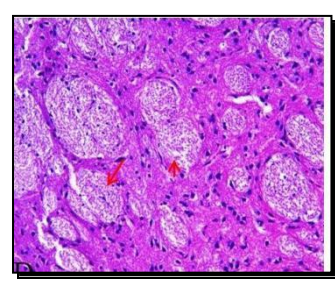
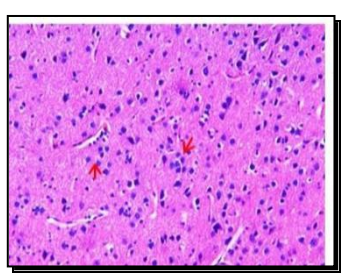


Figure 2: Test control



**Figure 3: Standard group
Melissaparviflora(200mg/kgp)**

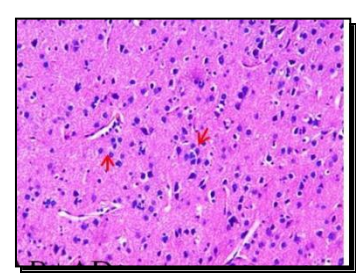
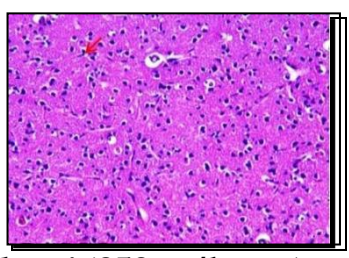


Figure 5: Melissaparviflora (400mg/kg p.o)

**Figure 4:
Figure 6: Ficus**



dalhousie(250mg/kg p.o)

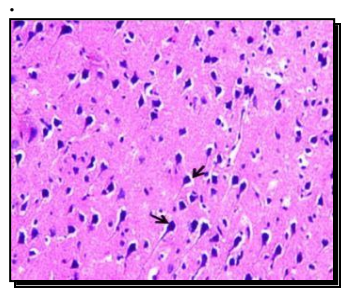
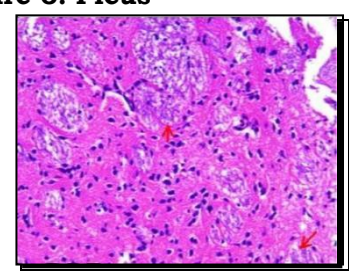


Figure 7: Ficus dalhousie(500mg/kg p.o)

Summary and Discussion

Alzheimer's infection (AD) is a dynamic neurodegenerative illness portrayed by obliterating loss of memory and mental capacity. Oxidative pressure assumes a vital part in the aetiology of AD. Oxidative pressure for the most part happens because of the over production of oxygen-free extremists by the mitochondria. Responsive oxygen species (ROS) superoxide is delivered by edifices I and III of the mitochondrial respiratory chain. SOD level in the test control group increased in comparison to the standard group.

The other evidence supporting our results are the MDA level. Usually, MDA levels will increase in Alzheimer's disease. The results representing that the MDA level increased significantly in Test Control in comparison to the standard group. Extracts of both plants exhibited a significant protective effect and reduce MDA levels.

Low glutathione levels in the test group represent the proximity of Alzheimer's disease and increase the chances of AD. As the data representing Glutathione level increased in the extract-treated group in comparison to test control show the protective effects of plant extracts against Alzheimer's disease. Ethyl Acetate Extract of *Melissa parviflora* at the doses of 200 and 400 mg/kg showed a critical defensive impact against acetylcholine corruption contrasted with the benchmark group. There was a critical increase in acetylcholinesterase movement in the standard group of rodents.

On oral administration, *Melissa parviflora* at the doses of 400mg/kg and *Ficus dalhousie*(500mg/kg p.o) exhibited marginal in the noradrenaline levels compared with the vehicle control group. On oral administration, *Melissa parviflora* at the doses of 200 and 400mg/kg and *Ficus dalhousie*(500mg/kg p.o) exhibited a significant($P < 0.01$) increase in the DOPA levels in compare with the test control group.

Both plant extracts demonstrated a significant dose-dependant decrease in time to reach the pre-visited arm. Whereas time significantly increased in the test control group. Whereas time was significantly increased in test control in comparison to the normal control group. Treatment with the leaf extract of *Melissa parviflora* at the portion of 200 and 400 mg/kg and of *Ficus dalhousie* at the portion of 250 and 500 mg/kg po showed no adverse consequence on the length of stay on the turning bar. All the treated albino wistar rats were observed on a rotating rod for 90sec.

The results revealed that 4 animals out of 6, completed 100% duration in *Melissa parviflora*(400mg/kg p.o) treated animals whereas in test control-treated only 2 animals completed 100% duration on rotating rod. The results revealed that *Melissa parviflora* (400mg/kg p.o) and *Ficus dalhousie* (500mg/kg p.o) treated animals have good nerve coordination in comparison to Test control.

On the other side, *Melissa parviflora*(400mg/kg p.o) treated the animal reflected Normal neuronal morphology with less or minimal cytoplasmic vacuolation and perinuclear space. It clearly indicates that the *Melissa parviflora*(400mg/kg p.o)

treated animals have less neuro-degeneration compare to test control and another group, which prove its effectiveness in the treatment of Alzheimer Disease. These animals also showing the normal morphological appearance of neurons with less cytoplasmic vacuolation. The histopathology clearly indicates the effectiveness of this plant extract against Aluminiumchloride-induced Alzheimer disease.

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