Acta Pharm. Sci. Vol 58 No: 3. 2020
DOI: 10.23893/1307-2080.APS.05816

Effect of *Salvia Officinalis* on Neuromodulating and Oxidative Stress Status in Brain of Male Albino Wistar Rats Intoxicated with Aluminium

Nishat Fatima[1*], Afreen Tabassum[1]

1 Department of Pharmacology, Shadan Women's College of Pharmacy, Khairtabad, Hyderabad

ABSTRACT

The present study reflects the effect of plant extracts of *Salvia officinalis*on neuromodulating and oxidative stress status of Male Albino Wistar rats intoxicated with Aluminium chloride.

Rats were divided into7 groups of 6 in each. Apart from normal control, toxicant and standard, rats also received 250mg/kg and 500mg/kg doses of aqueous and ethanolic extracts of *Salvia officinalis* for20 days. Behavioral parameters, along with acetylcholinesterase enzyme levels, antioxidant markers and histopathology of brain tissues were determined.

Salvia officinalis improved behavioral parameters and reversed the reduced Acetylcholinesterase content thereby increased SOD and decreased MDA and NO when compared to $AlCl_3$ induced rats.

The study demonstrated the beneficial effects of *Salvia officinalis* in Alzheimer's disease by showing antioxidant, AchE inhibiting activity and by improving memory and cognitive functions.

Keywords: Alzheimer's disease, Aluminium chloride, Acetylcholinesterase, *Salvia officinalis*.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative, multifactorial, complex mental illness, and a form of dementia causing memory loss and neuronal death throughout the brain. It causes progressive behavioral (i.e., depression, agitation and psychosis), and neurological changes involving functional impairment,

*Corresponding author: Nishat Fatima, e-mail: nishat_fatima50004@yahoo.com
Nishat Fatima ORCID Number: 0000-0003-4906-1798
Afreen Tabassum ORCID Number: 0000-0003-0023-4715
(Received 22 October 2019, accepted 28 November 2019)

loss of independency, frustration, forgetting names, mood swings irritability and hostility, emotional problems, characterized by worsening of cognition and memory. [1] AD is mostly diagnosed in individuals above 65 years of age. Currently, it affects nearly 5% population of 65-year old, rising to 20% of those over 80 years and over 30% of 85-year old. Globally more than 27 million people are suffering with AD in the world and mostly in developed nations. [2]

Multiple pathogenic factors causing AD include aggregated extracellular ß-amyloid plaques, the formation of neurofibrillary tangles (NFTs) (highly phosphorylated tau proteins), cholinergic dysfunction and oxidative stress. [3] Oxidative cell damage occurs with an increase in level of free radicals which are usually held in balance by the body antioxidant system. Accumulation of intracellular reactive oxygen species (ROS) leads to oxidation of protein, lipids and DNA causing cellular damage. Elevated ROS levelsare also associated with amyloid-ß deposition which is an early feature of AD. [4]

Aluminum is a well-known neurotoxin. It causes neurodegeneration resulting in neurological changes in the hippocampus, cerebrum and also promotes biochemical changes. Literature shows that Aluminium induces neurotoxicity through production of free radicals resulting oxidative stress. It has a greater affinity to bio-membrane promoting the formation and aggregation of insoluble ß-amyloidal plaques which is vital characteristic of Alzheimer's disease. [5]

Medicinal plants have been used since ancient time to cure diseases, their progression and development. Medicinal plants with antioxidant properties have been used in the treatment of human diseases like cardiovascular disorders, cancer and neurological diseases such as AD.

So drugs for complete cure of Alzheimer's disease are not available clinically and greatly needed. Pharmacological activity and antioxidant property of phytoconstituents obtained from crude extract of medicinal plants are found its importance in various degenerative disorders.[6]

Salvia officinalis belongs to the family Lamiaceae is a native plant of East Mediterranean region which has been used as a traditional medicine by Middle Eastern and Asian countries to treat many disorders. *Salvia officinalis* (sage) has dual cholinergic activity. It is active against both Acetylcholine esterase and butyrylcholine esterase enzymes. Besides the cholinergic activity, it also have potent activity for CNS disorders, antioxidant activity, anti-inflammatory properties, nicotinic activity, glutamergic activities, and memory-enhancing effect. Its high antioxidant potential is due to its high phenolic contents isolated from this herb such as hydroxyl benzoicacid derivatives, ferulic acid, flavonoid de-

rivatives; luteolin and quercetin, caffeic acid derivatives (e.g., rosmarinic acid).[7] Hence in the present investigation we have attempted to demonstrate the anti-Alzheimer's property of *Salvia officinalis*.

METHODOLOGY

Collection of drugs

The whole plant of *Salvia officinalis* belonging to family Lamiaceae was collected, identified and authenticated by the botanist Dr. K. Madhavachetty, HOD, department of Botany in Sri Venkateshwara University, Tirupati, A.P. India.A voucher specimen (Voucher number: 1279) has been deposited in the department.

Chemicals:

a. Aluminium chloride - 300mg/kg b.w

Aluminium chloride anhydrous LR (granular)- SD fine-chem limited, industrial estate, 248, Worli road,

Mumbai-30. Batch No: A17A/0216/3108/13

MFD JAN 2017, Expiry Date DEC 2021

b. Donepezil – 0.75mg/kg b.w.

donepezil hydrochloride syrup – Donep syrup 5mg, Alkem laboratories Ltd, Thana Baddi, Himachal Pradesh-173205, India. Batch No: DNS 6002GB

MFD OCT 2016, Expiry date SEP 2018.

Preparation of plant extract:

The dried grounded powder of whole plant was subjected to ethanolic extraction using soxhalation technique[8] and aqueous extraction by decoction method.

Toxicity Studies

Extracts were tested for acute toxicity studies using 3 healthy male Albino Wistar rats weighing 150-180gms. Animals were fasted overnight priorto the experiment. Fixed dose acute toxicity studies were carried out according to the OECD guideline no.423.[9] The animals were given a dose of 2000mg/kg body weight of *Salvia officinalis* extracts and observed for any signs of mortality at 30minutes, 4hrs and thereby for next 24-hour post treatment. The animals were alsoexamined visuallyfor changes in behavior, skin color, and furfor 14 days. Dose was selected for the main study as per the oral acute toxicity results.

Experimental Design

The experiments were conducted with guidelines of Institutional animal ethical committee (IAEC), having approval number IAEC -01/SES/2018/101, governed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.Male Albino Wistar rats weighing between 180-200g were obtained from the animal house of Sainath agencies, Uppal, Hyderabad (282/PO/Bt/S/2000 CPCSEA).Rats were divided into 7 groups of six each **(Table 1)** and maintained at conditions of temperature (22 ±2°), humidity (50±5%) and 12-12 hour's light-dark cycles. All the animals were acclimatized for 7days before the study and provided with free access to food and water *ad libitum*.[10] During the experimental study rats were fed with pellets obtained from (Pranav Agro Industries limited, rat feed, India). The rats received humane care according to the criteria outlined in CPCSEA guidelines 2003, Government of India.

Table 1. Grouping of Animals with doses

SL.NO	GROUPS	AGE OF ANIMALS	TREARMENT
1.	Normal control	12 weeks	Normal saline (0.5ml p.o)
2.	Toxicant control	12 weeks	Aluminium chloride (300mg/kg, p.o)
3.	Standard control	12 weeks	$AlCl_3$ (300mg/kg, p.o) + Donepezil (0.75mg/kg, i.p).
4.	Aqueous extract (low dose)	12 weeks	$AlCl_3$ (300mg/kg, p.o) + aq extract (250mg/kg p.o)
5.	Ethanolic extract (low dose)	12 weeks	$AlCl_3$ (300mg/kg, p.o) + ethanolic extract (250mg/kg p.o)
6.	Aqueous extract (high dose)	12 weeks	$AlCl_3$ (300mg/kg, p.o) + aqueous extract (500mg/kg p.o)
7.	Ethanolic extract (high dose)	12 weeks	$AlCl_3$ (300mg/kg, p.o) + ethanolic extract of plant (500mg/kg p.o)

The above dosing schedule was continued for 20 days and behavioural parameters like locomotor activity, Conditioned avoidance response test, spatial long-term memory assessment, and motor coordination were determined.

Behavioral Study

Locomotor activity

It is an index of wakefulness or mental alertness. Locomotor activity of animals was determined using digital photoactometer. When the light beam that falls on the photocells is cut off by the animal, account is recorded. The movements of animal were recorded for 5 min which can be expressed as counts for 5 min per animal. Assessment was done in control and experimental groups.[11]

Motor coordination

It was assessed by using Rota-rod apparatus. Animals were initially trained to hold the rotating rod at a certain slow speed for 3 min. Then afterwards the speed of rod was increased to 50 rpm. Motor function and coordination of the animal was assessed by the time latency or fall off time from the placement of rat on the rod until it falls off onto the plate below. Assessment was done in control and experimental groups. [12]

Conditioned avoidance response test

This is done by using Pole climbing apparatus to evaluate memory and cognitive function. Animals were placed in the chamber individually and allowed to move for 1 min. After that, a warning sound was introduced for 3 seconds followed by an electric shock. If the rat did not climb the pole to escape from electric shock, it was noted as none. If the rat escaped the shock by climbing the pole, this was noted as escape, and if the rat avoided the shock by climbing the pole within the warning period, before warning sound is ceased, then it is termed as avoid response.[13]

Spatial long-term memory assessment

Spatial long-term memory assessment was performed by using elevated plus maze. The parameters used for the assessment were frequency of entries of animal into the open and closed arms and transfer latency (TL). Transfer latency is defined as the time taken by an animal to move from open arm to closed arm. Assessment was done in control and experimental groups.[14]

Blood Sampling and Brain Isolation

At the end of the experiment (after 20 days), animals were kept fasted overnight. After overnight fasting, animals were kept in desiccation chamber for the inhalation of carbon dioxide. Blood samples were collected through retro-orbital puncture by using capillary tubes. Blood samples of all animals were subjected to centrifugation at 1000rpm for 15 minutes to obtain serum. After taking blood samples, the animals were sacrificed. The whole brain of each animal was rapidly dissected by opening the skull carefully, and washed thoroughly with saline, dried and weighed. Each brain sample was fixed in 10% formalin solution for histopathological investigations. [15]

Biochemical Analysis

Blood samples were collected, and serum was separated and analyzed for biochemical parameters. Acetylcholine esterase (AchE) activity was determined in serum colorimetrically by referring *Dietz et al.* [16] Antioxidant parameters like

superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) were estimated in serum. Superoxide dismutase (SOD) levels in serum were measured by using the method Kono et al., 1978.[17] MDA was determined by the method Okhawa et al., 1979.[18] Nitric oxide (NO) levels were estimated in serum by the method described by Berkel et al., 2004.[19]

Histopathological Study

The isolated brains from the sacrificed animals were kept immediately in 10% formalin solution for a period of 24 hours. Washed with distilled water and dehydrated using serial dilutions of alcohol (methyl, ethyl and absolute ethyl). Xylene was used to clean the specimens and then embedded in paraffin at 56^{o}C in hot air oven and kept for 24 hours. Paraffin bees wax Tissue blocks were prepared by sectioning at 4 microns by microtome. The resulting tissue sections were kept on glass slides and subjected to removal of paraffin (deparaffinized). Hematoxylin and eosin stains were used for staining of tissue for histopathological examination using the light microscope. [20]

Statistical Analysis

The outcomes were expressed as the Mean ± SEM. Statistical evaluation (data) was carried out by one-way analysis of variance (ANOVA), followed by Dunnet 't' test using Graphpad Prism 5 software, version 5.3 La Jolla, San Diego, California, USA to compare significance between groups. $p<0.05$ was considered to be significant. [21]

RESULTS AND DISCUSSION

Results of Acute Toxicity Study

Both extracts were administeredup to a dose 5gm/kg body weight and it was found that none of the two extracts produced any mortality thus indicating their practically nontoxic nature. The dose was calculated as1/8thand 1/10th dose of maximum tested (5gm/kg) of both extracts and selected for the main experiment.

Aqueous Extract - 250 mg/kg, b.w and 500mg/kg, b.w

Ethanolic Extract - 250 mg/kg, b.w and 500mg/kg, b.w

Results of Behavioral Study

Locomotor Activity

From the **Table 2,** it is observed that locomotor activity of rats treated with $Alcl_3$ is reduced compared to the control group. Treatment with low and high doses of extracts of *Salvia officinalis* found to be efficient in improving the locomotor activity in group 4-7 with maximum improvement in high dose (500mg/kg) of ethanolic extract.

Table 2. No. of counts/5 min in Actophotometer

Group	Treatment	Locomotor activity (No. of counts/5 min)
Group 1	**Normal control** Saline, 0.5ml, p.o	583.8 ± 38.18
Group 2	**Toxicant control** $Alcl_3$ (300mg/kg, p.o)	214.7 ± 15.71@
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	466.3 ± 17.06#
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	354.3 ± 14.16@
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	359.3 ± 17.19@
Group 6	**Aqueous extract (high dose)** $Alcl_3$ +aqueous extract (500mg/kg p.o)	422.7 ± 15.09@
Group 7	**Ethanolic extract (high dose)** $Alcl_3$ + ethanolic extract (500mg/kg p.o)	495.8 ± 12.17$

All values are expressed as mean± SEM. @-p<0.001 compared to normal control, # p<0.01 compared to normal control, $- p<0.05 compared to normal control

Motor coordination

From the **Table 3,** it is inferred that animals treated with $Alcl_3$ show significant decrease in the fall off time and decreased motor coordination compared to the control group while, rats treated with donepezil, aqueous and ethanolic extracts proved to be enhancing the motor coordination in extract and standard treated groups compared with toxicant group.

Table 3. Fall off time in seconds using Rota rod test for motor coordination

Group	Treatment	Rota rod test (fall off time in sec)
Group 1	**Normal control** Saline, 0.5ml, p.o	68 ± 9.73
Group 2	**Toxicant control** $AlCl_3$ (300mg/kg, p.o)	25.17 ± 2.04$^{\#}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	59 ± 5.41^{ns}
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	45.17 ± 1.81$^{@}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	45.83 ± 2.52$^{\$}$
Group 6	**Aqueous extract (high dose)** $AlCl_3$ +aqueous extract (500mg/kg p.o)	58.67 ± 3.38^{ns}
Group 7	**Ethanolic extract (high dose)** $AlCl_3$ + ethanolic extract (500mg/kg p.o)	63 ± 3.44$^{@}$

All values are expressed as mean± SEM. #- p<0.0001 compared to normal group, ns- nonsignificant compared to normal, @- p<0.01 compared to normal, $- p<0.05 compared to normal

Conditioned Avoidance response test

The **table 4** showed reduction in time taken to climb the pole in standard and extract treated groups when compared to the toxicant group. Animals treated with high dose (500mg/kg) of aqueous and ethanolic extracts show "Avoidance" response which means that they avoided the shock by climbing the pole within the warning sound period. The animals treated with low dose of extracts show "Escape" as they climb the pole after the warning sound by escaping the shock. No response is taken as "none".

Table 4. Time taken to climb pole in seconds using pole climbing apparatus

Group	Treatment	Time taken to climb pole in sec
Group 1	**Normal control** Saline, 0.5ml, p.o	0
Group 2	**Toxicant control** $Alcl_3$ (300mg/kg, p.o)	157.3 ± 4.45@
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	112.8 ± 4.9@
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	125.7 ± 2.5@
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	126.2 ± 2.99@
Group 6	**Aqueous extract (high dose)** $Alcl_3$ +aqueous extract (500mg/kg p.o)	118.2 ± 4.96@
Group 7	**Ethanolic extract (high dose)** $Alcl_3$ + ethanolic extract (500mg/kg p.o)	116.2 ± 4.13@

All values are expressed as mean± SEM. @- p<0.0001 compared to control group.

Spatial long-term memory assessment

There is an improvement in memory in the extract treated group with maximum effect in high dose (500mg/kg) aqueous extract treated group. Transfer of latency is reduced in the extract treated groups compared to the $Alcl_3$ treated group and the frequency of entries in the closed arm and the open arms is increased in standard and extract treated group compared to toxicant as shown in **table 5.**

Table 5. Transfer of latency in seconds

Group	Treatment	Transfer of latency in sec
Group 1	**Normal control** Saline, 0.5ml, p.o	22.83 ± 1.54
Group 2	**Toxicant control** $Alcl_3$ (300mg/kg, p.o)	50.17 ±2.61$^{!}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	27.33 ± 1.08^{ns}
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	32.17 ± 1.39$^{@}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	31 ± 1.72$^{@}$
Group 6	**Aqueous extract (high dose)** $Alcl_3$ +aqueous extract (500mg/kg p.o)	28.33 ± 0.94$^{@}$
Group 7	**Ethanolic extract (high dose)** $Alcl_3$ + ethanolic extract (500mg/kg p.o)	26.67 ± 1.83^{ns}

All values are expressed as mean± SEM. !- p<0.0001 compared to normal, ns- nonsignificant compared to normal, @-p< 0.01 compared to normal group.

Results Acetylcholinesterase activities on AD-induced and treated groups:

AchE levels in plasma were determined in control, toxicant, standard, low and high doses of aqueous and ethanol extract treated groups.

The result in the **table 6** showed significant increases in AchE in $Alcl_3$ treated group when compared to normal control, standard and extracts treated group. This indicates cholinergic reduction in AD-induced rats. Treatment of AD-induced rats with donepezil showed significant reduction in AchE enzyme levels and treatment with extracts of *Salvia* showed reduction in AchE level compared to the $Alcl_3$ treated group as show in the **figure 1**. Effect of high doses both extract of *Salvia* showed almost similar effects in AD-induced rats by facilitating elevation of Ach level, by significantly reducing AchE enzyme levels. AchE enzyme levels were measure in units/liter.

Table 6. Effect of treatment on AchE levels in plasma

Group	Treatment	AchE (U/L)
Group 1	**Normal control** Saline, 0.5ml, p.o	268.9 ± 28.58
Group 2	**Toxicant control** $AlCl_3$ (300mg/kg, p.o)	604.5 ± 15.63$^{\#}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	257.1 ± 17.29^{ns}
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	492.4±8.58$^{\#,\$}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	481.3±12.89$^{\#,@}$
Group 6	**Aqueous extract (high dose)** $AlCl_3$ +aqueous extract (500mg/kg p.o)	417.1±7.28$^{\#,@}$
Group 7	**Ethanolic extract (high dose)** $AlCl_3$ + ethanolic extract (500mg/kg p.o)	427.3±38.22$^{\#,@}$

All values are expressed as mean± SEM. #-p<0.001 compared to normal control, ns-nonsignificant compared to normal control, $-p<0.01 compared to toxicant control, @-p<0.001 compared to toxicant control

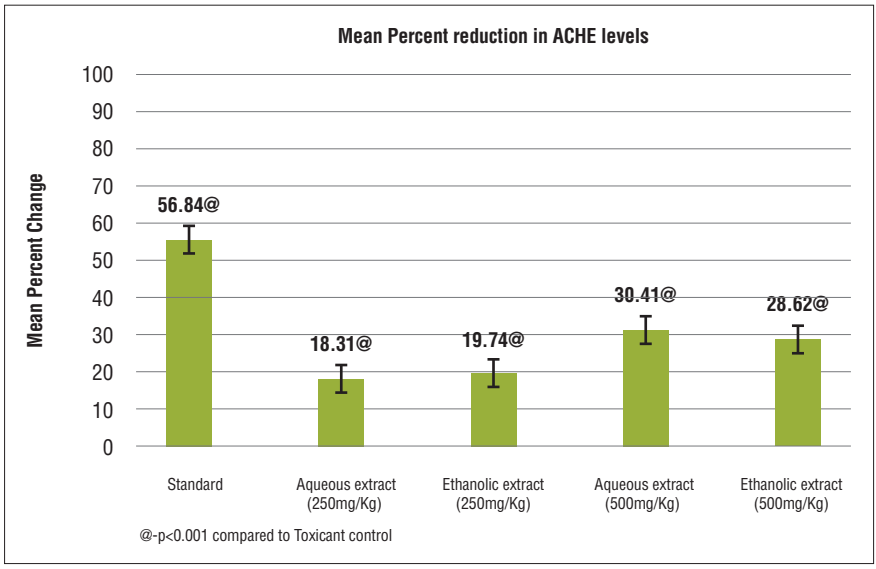


Figure 1. Effect of treatment on AchE levels

Results of Histopathology

Group 1-Treated with normal saline: Group 1 animals were given only saline. They show normal cerebral cortex and hippocampus and no enlarged ventricles are seen in **figure 2.**

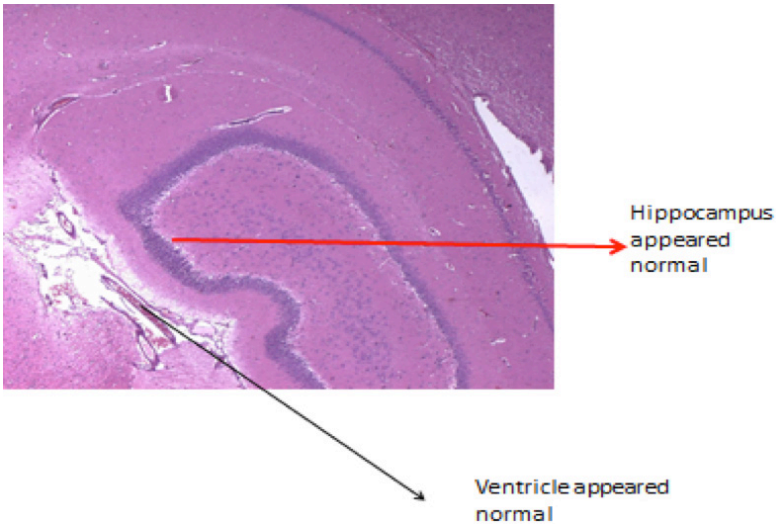


Figure 2. Brain section of control group rats showing normal structure of hippocampus.

Group 2-Treated with Aluminium chloride: Microscopic investigation of brain of $Alcl_3$ treated rats show neurodegeneration, enlarged ventricles and amyloidal plaques in hippocampus and brain atrophy when compared with the histological structure of brain of control group **(Figure 3).**

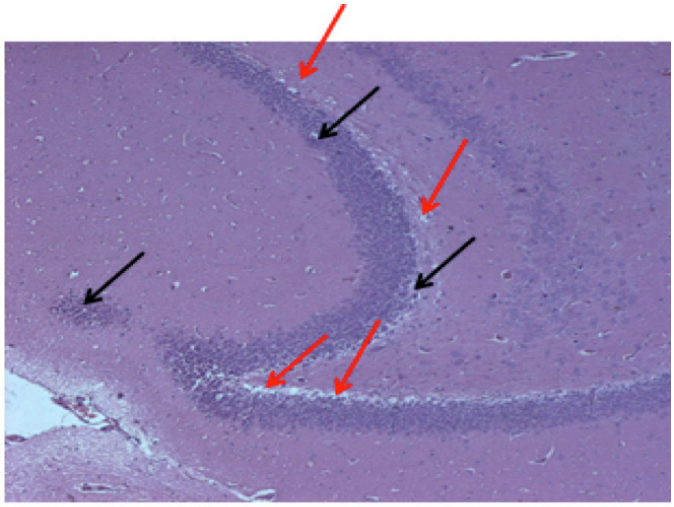


Figure 3. Micrograph of brain section of Alcl3 treated rats showing apoptosis of neurons and amyloidal plaques in hippocampus.

Group 3-Treated with Donepezil:

Group3 treated with donepezil revealed the disappearance of amyloid plaques formed due to $Alcl_3$ and normal histological structure of cortex and hippocampus compared to $Alcl_3$ treated which can be observed in **figure 4**.

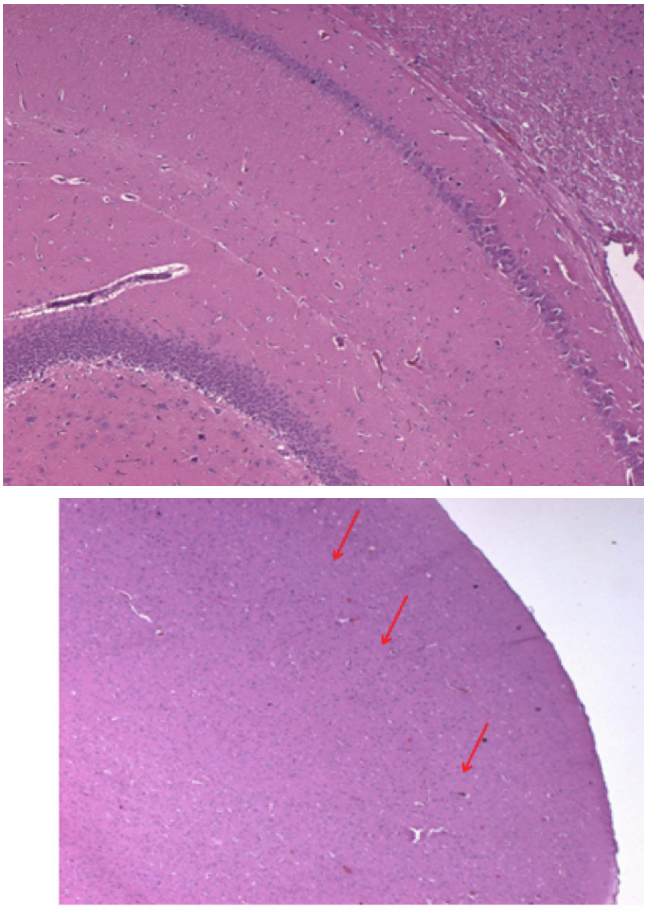


Figure 4. Brain section of AD-induced rats treated with donepezil showing normal cerebral cortex and hippocampus.

Group 4-Treated with aqueous extract (low dose) of *Salvia officinalis*:

Group 4 rats treated with low dose (250mg/kg, b.w) of aqueous extract shows mild neurodegeneration in the hippocampus region compared to $Alcl_3$ treated rats and disappearance of few amyloidal plaques formed due to treatment of $Alcl_3$ in **figure 5**.

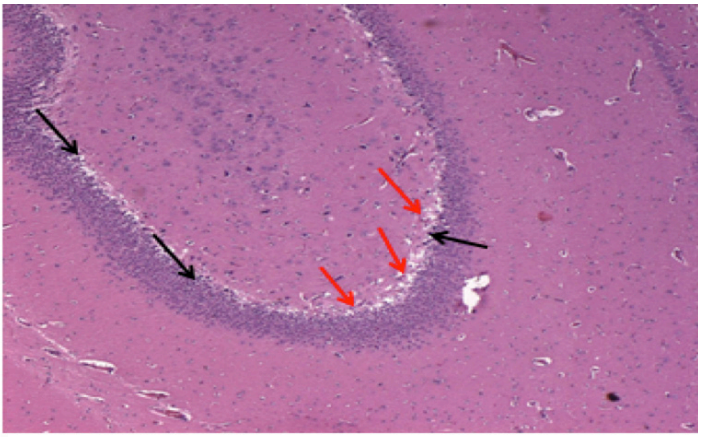


Figure 5. Histology of brain section of AD-induced rats treated with low dose of aqueous extract shows disappearance of amyloid plaques and mild neurodegeneration compared to Alcl3 treated rats.

Group 5- Treated with ethanolic extract (low dose) of *Salvia officinalis*:

Group 5 rats treated with low dose (250mg/kg, b.w) of ethanolic extract shows mild neurodegeneration in the hippocampus region compared to $Alcl_3$ treated rats and disappearance of few amyloidal plaques formed due to treatment of $Alcl_3$ in **figure 6.**

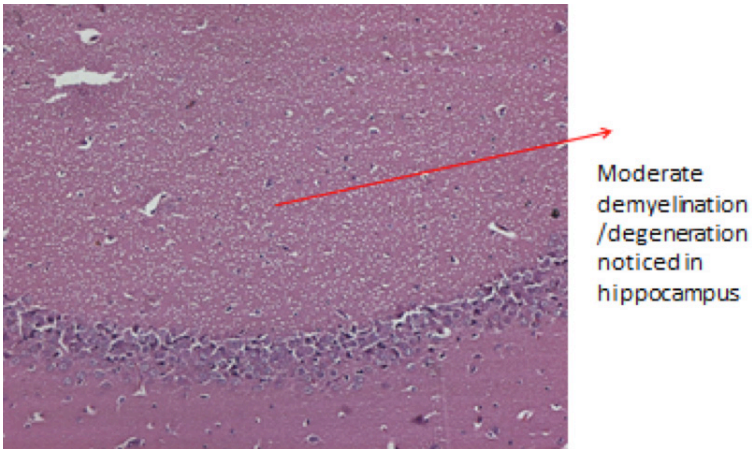
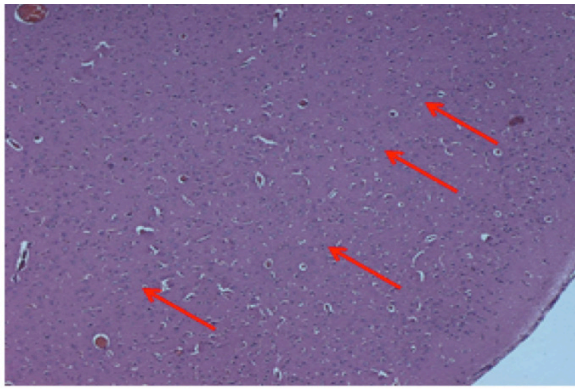


Figure 6. Micrograph of brain section of AD-induced rats treated with low dose of ethanolic extract shows disappearance of amyloid plaques and mild neurodegeneration compared to Alcl3 treated rats.

Group 6-Treated with high dose of aqueous extract of *Salvia officinalis*:

Group 6 rats treated with high dose (500mg/kg, b.w) of aqueous extract shows normal histological structure of hippocampus and cerebral cortex compared to $Alcl_3$ treated rats and disappearance of amyloidal plaques formed due to treatment of $Alcl_3$ **(figure 7).**It is inferred that high dose shows more potent effect with few dislocation of hippocampal cells.



Frontal cortex- cerebral hemisphere appeared normal–
Arrow. No necrosi s or inflammation noticed

Figure 7. Micrographic picture of brain section of AD-induced rats treated with high dose of aqueous extract shows normal histological structure of cerebral cortex and hippocampus with dislocation of few hippocampus cells and disappearance of amyloid plaques compared to Alcl3 treated rats.

Group 5 - Treated with high dose of ethanolic extract of *Salvia officinalis*:

Group 7 rats treated with high dose (500mg/kg, b.w) of ethanolic extract shows normal histological structure of hippocampus and cerebral cortex compared to $Alcl_3$ treated rats and disappearance of amyloidal plaques formed due to treatment of $Alcl_3$ **(figure 8).**

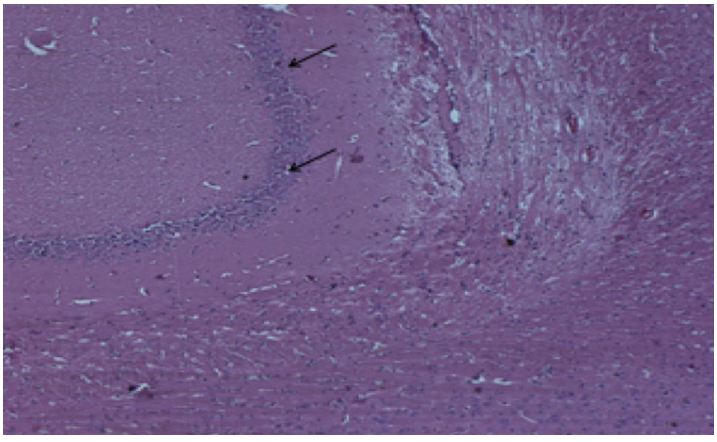


Figure 8. Brain section of AD-induced rats treated with high dose of ethanolic extract shows normal structure of cortex and hippocampus and disappearance of amyloid plaques compared to Alcl3 treated rats.

5. Results of Antioxidant Activity

Results of Malondialdehyde (MDA):

From **Table 7** it is inferred that toxicant control group treated with $Alcl_3$ shows elevated levels of MDA compared to normal due to oxidative stress. There is a reduction in the MDA level in groups treated with standard, drug extracts as per dose **(figure 9).**

Table 7. Effect of treatment on serum MDA levels.

Group	Treatment	MDA (nmol/mg protein)
Group 1	**Normal control** Saline, 0.5ml, p.o	6.58 ± 0.13
Group 2	**Toxicant control** $Alcl_3$ (300mg/kg, p.o)	$10.43 \pm 0.17^{@}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	6.97 ± 0.12^{ns}
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	$8.6 \pm 0.24^{@\#}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	$8.8 \pm 0.27^{@\#}$
Group 6	**Aqueous extract (high dose)** $Alcl_3$ +aqueous extract (500mg/kg p.o)	$7.9 \pm 0.13^{@\#}$
Group 7	**Ethanolic extract (high dose)** $Alcl_3$ + ethanolic extract (500mg/kg p.o)	$7.8 \pm 0.17^{@\#}$

All values are expressed as mean± SEM.@- $p<0.0001$ compared to normal group, ns-nonsignificant to normal, #-$p<0.0001$ compared to AlCl3 treated group.

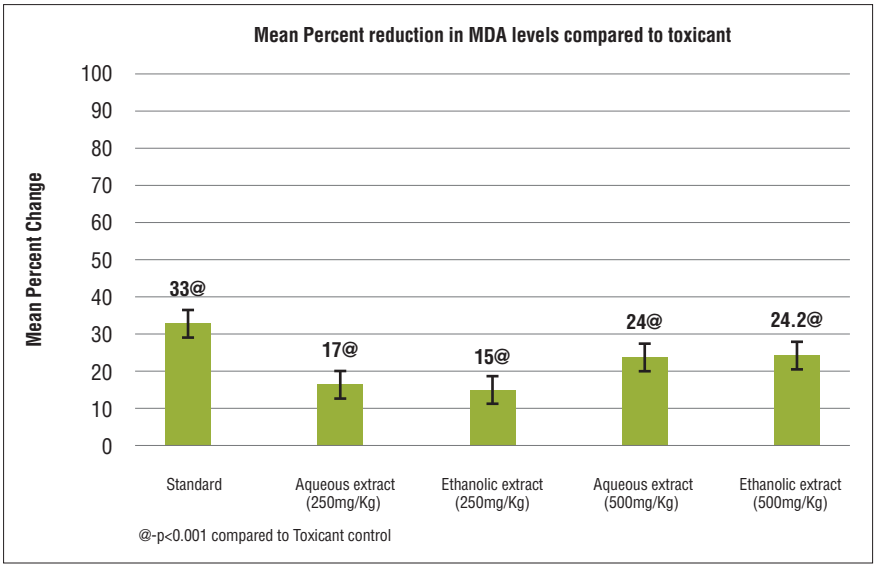


Figure 9. Mean percent reduction of MDA levels in serum

Results of Nitric oxide (NO):

Table 8 inferred that the elevated level of NO indicates oxidative stress in toxicant group treated with $Alcl_3$, whereas in groups treated with standard and extracts, the levels of NO is reduced compared to the toxicant group **(figure 10).**

Table 8. Effect of treatment on serum NO levels.

Group	Treatment	NO (µ/mg protein)
Group 1	**Normal control** Saline, 0.5ml, p.o	4.6 ±0.16
Group 2	**Toxicant control** $Alcl_3$ (300mg/kg, p.o)	10.46 ±0.12$^{@}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	6.11 ±0.23$^{@}$
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	8.12 ±0.1$^{@\#}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	8.09 ±0.11$^{@\#}$
Group 6	**Aqueous extract (high dose)** $Alcl_3$ +aqueous extract (500mg/kg p.o)	7.94 ±0.15$^{@\#}$
Group 7	**Ethanolic extract (high dose)** $Alcl_3$ + ethanolic extract (500mg/kg p.o)	7.68 ±0.12$^{@\#}$

All values are expressed as mean± SEM. @-p<0.0001 compared to normal control, #-p<0.0001 compared to AlCl3 treated group.

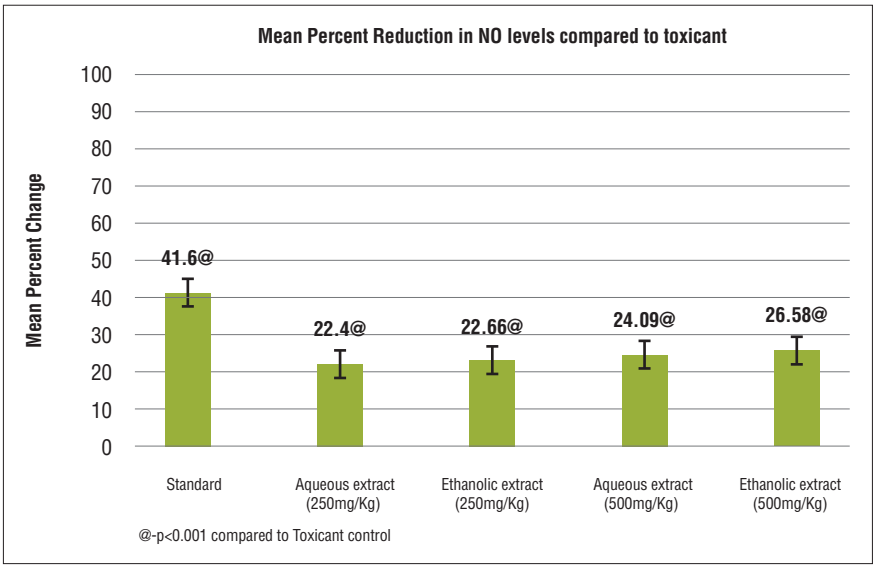


Figure 10. Mean percent reduction in NO levels in serum

Results of Superoxide Dismutase:

SOD level is less in animals treated with $Alcl_3$ when compared to normal group which indicates oxidative stress **(table 9).** SOD levels increased in group treated with standard, drug extracts when compared to the AD-induced rats treated with $AlCl_3$ **(figure 11).**

Table 9. Effect of treatment on serum SOD levels.

Group	Treatment	SOD (U/mg protein)
Group 1	**Normal control** Saline, 0.5ml, p.o	2.71 ±0.11
Group 2	**Toxicant control** $AlCl_3$ (300mg/kg, p.o)	1.21 ±0.09$^{@}$
Group 3	**Standard control** Alcl3 + Donepezil (0.75mg/kg, i.p).	2.43 ±0.13^{ns}
Group 4	**Aqueous extract (low dose)** Alcl3 + aqueous extract (250mg/kg p.o)	2.20 ±0.09$^{\#*}$
Group 5	**Ethanolic extract (low dose)** Alcl3 + ethanolic extract (250mg/kg p.o)	2.29 ± 0.08$^{\$*}$
Group 6	**Aqueous extract (high dose)** $AlCl_3$ +aqueous extract (500mg/kg p.o)	2.34 ±0.12$^{\#*}$
Group 7	**Ethanolic extract (high dose)** $AlCl_3$ + ethanolic extract (500mg/kg p.o)	2.36 ±0.10^{ns*}

All values are expressed as mean± SEM.@-p<0.0001 compared to control group, ns-nonsignificant to control group, #-p<0.01 compared to control group, $-p<0.05 compared to control group, *-p<0.0001 compared to AlCl3 group.

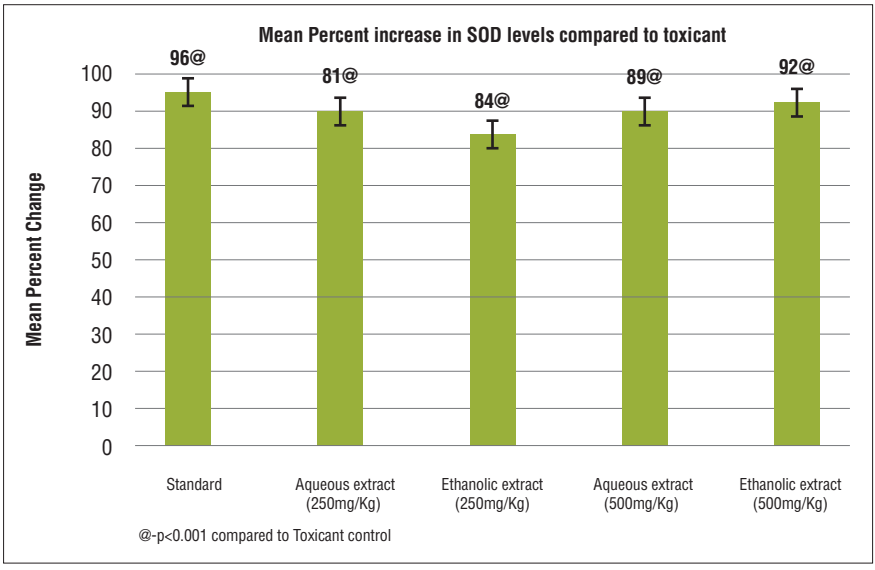


Figure 11. Mean percent increase in SOD levels in serum.

RESULTS AND DISCUSSION

Alzheimer's disease rises continually all over the world. It becomes a challenge for the modern health care to develop a treatment for the neurodegenerative diseases like Alzheimer. It's a complex, multifactor, progressive neurodegenerative disorder causing atrophy of brain. Pathogenic factors of AD include aggregated extracellular ß-amyloid plaques, the formation of neurofibrillary tangles (NFTs) (highly phosphorylated tau proteins), cholinergic dysfunction and oxidative stress. [22]

Aluminum is a well-known neurotoxin which causes neurodegeneration. Aluminium alters blood-brain barrier (BBB) and gets deposited in the cortex and hippocampus region causing brain toxicity. It promotes the formation and aggregation of insoluble ß-amyloidal plaques characteristics of Alzheimer's disease. It also cause disturbance in the enzyme activity of acetylcholinesterase involved in acetylcholine metabolism and leads to cognitive dysfunction.[23,24] Similarly in our study, we have observed that aluminum intoxicated rats (toxicant control group) showed significant elevation in AchE level compared to the normal, and this was supported by histopathological study, which showed the presence of amyloid plaques and neural damage in the brain tissues. Such results are in harmony with those obtained by Kaizeret al.[25]

From this study we found that the Acetylcholine esterase enzymes levels in the brain increases in Alzheimer induced rats due to aluminium chloride. This elevated level of AchE was found to be reduced with the treatment of extracts of *Salvia* and the standard drug. When compared to the toxicant control there is a reduction of 58% of AchE enzyme in group treated with donepezil, 28% reduction in high dose (500mg/kg) of ethanolic extract and 30% reduction in aqueous extract. Aqueous extract of *Salvia* showed potent effect in reduction of AchE enzyme level, thereby increasing Ach level. Hence the cholinergic activity in the extract treated groups was observed to be improved in the animals treated with ethanolic and aqueous extracts. These results are coincided with Perry et al, [26] who stated that the relevant component of *Salvia* can cross the blood-brain barrier and increase cholinergic transmission via inhibition of cholinesterase enzyme.

It has been well documented that Aluminium induces neurotoxicity through free radical production causing oxidative stress. According to Dickstein et al,[27] oxidative stress play an important role in the pathogenesis of AD. Accumulation of ROS takes place as a consequence of oxidative stress. If this ROS level exceeds the cellular protective mechanism, oxidative damage occur leading to cell death. However, the increased Al concentration deleteriously affects the

neurons, causing depletion of antioxidants which exhaust the SOD capacity to neutralize the free radical processes. This results in decreased activity of SOD, and increased activity of MDA and NO. Therefore, substances having antioxidant potential which can reduce oxidative stress are selected as the potential drug for treatment of AD. [28, 29]

The present study showed that oxidative stress was found in the group of animals treated with $Alcl_3$ which was analyzed by the high levels of MDA and NO which are the parameters of oxidative stress. And also, the low levels of SOD (antioxidant parameter) due to oxidative stress. These results are coincided with Gustaw-Rothenberg at al [30].This oxidative stress was recorded to be reduced in the groups treated with extracts of *Salvia* which was estimated by the reduced levels of MDA and NO and increased levels of SOD in the extract treated group.[31] The aqueous and ethanolic extracts higher doses i.e., 500mg/kg showed more potent anti-oxidant activity. This antioxidant activity of *Salvia* is due to its high phenolic content such as rosamarinic acid, caffeic acid, sage coumarin etc.

AchE inhibitors are the drugs approved by FDA for the treatment of AD. Acetylcholinesterase inhibitors (AchE-Is) prevent the metabolism of the Ach in the brain and found to improve cognition in patients with AD. AchE-Is are used currently for the symptomatic treatment of AD to improve and maintain central cholinergic function. Acetylcholine esterase inhibitors like Donepezil, rivastigmine, galantamineare currently used as a symptomatic treatment to improve and maintain central cholinergic function. In the present work, we used *Donepezil*as a standard drug for the comparison of drug extracts. This was done in accordance with Cutuli et al. [32]

Salvia officinalis (sage) is considered as a medicinal plant since ancient times. It has dual cholinergic activity. It has both Acetylcholine esterase and butyrylcholine esterase inhibiting activity. Besides the cholinergic activity, it has potent activity for CNS disorders, antioxidant activity, anti-inflammatory properties, nicotinic activity, glutamergic activities, and memory-enhancing effect. The plant is known to improve the mental functions according to Howes et al [33]. Sage extracts have been shown to possess antioxidant, anti-inflammatory, anticancer and antimicrobial activities. Its high antioxidant activity is due to its high phenolic contents isolated from this herb such as hyd-roxybenzoic acid derivatives, ferulic acid, flavonoid derivatives; luteolin and quercetin, caffeic acid derivatives (e.g., rosmarinic acid).[34] *Salvia* act as acetylcholinesterase inhibitor in comparison with standard drug Donepezil by inhibiting the enzyme activity and increasing Ach levels.

Behavioral study reveals the improvement of motor coordination, memory, functional ability, learning with the treatment of *Salvia* extracts when compared to the toxicant animals. High dose (500mg/kg) of aqueous and ethanolic extracts shows significant improvement in behavioral parameters. Such results are in harmony with those obtained by Somasekar et al, [1]who reported that *Salvia* plant extract having maximum antioxidant activity which may be due to the presence of high amount of flavonoids and phenols showed improvement of behavioral parameters like motor coordination, locomotor activity, functional ability and memory. Hasanein et al, in their study has explained that the protective effect of hydroalcoholic extract of *Salvia* against diabetes induced memory and learning deficit could be due to the presence of antioxidants such as rosimarinic acid as main flavonoid constituent. Mirrodi et al in their clinical investigation proved the beneficial effect of *Salvia* on cognitive functions in both healthy and patients with deficient cognition. Similar results were published by Moss et al, which indicated the aroma from essential oil of *Salvia* can improve learning and memory in healthy volunteers. Likewise, Scholey et al have demonstrated the preventive effect of ethanolic extract of *Salvia* on cognizance in healthy elderly subjects. [35]

Histological study of $Alcl_3$ treated group revealed neuronal damage, enlarged ventricles and amyloidal plaques in the brain. The amyloidal plaques formed due to induced AD were disappeared in the groups treated with low and high doses of *Salvia* extracts. Histological structure of brain of rats treated with low dose of extracts showed mild neurodegeneration whereas high dose showed more potent effect with normal histological structure of brain. Aqueous and ethanolic extracts showed overlapping results in treating the neurodegeneration with a lesser and higher effects as per low and high dose. High doses were proved to be more effective than the low doses of *Salvia* extracts. [36, 37]

The whole investigation concludes that the treatment of Alzheimer's disease with extracts of *Salvia officinalis*, and Donepezil (standard drug) were significantly reduced the oxidative stress and improves neurodegeneration of brain in Male Albino Wistar rats. Neurological and behavioral functions like memory, learning, physical activity, motor functions were enhanced with the treatment of Aqueous and ethanolic extracts. High dose (500mg/kg, b.w) of both the extracts of *Salvia* showed more potent effect on AD induced rats then the lower dose (250mg/kg, b.w). Biochemical Analysis revealed the improvement of cholinergic functions by the inhibition activity of AchE enzyme resulting in Ach elevations. Oxidative stress markers MDA and NO were decreased, and the antioxidant biomarker SOD was increased in the extract treated groups compared

to Alcl3 treated. Histopathological investigations proved that there was disappearance of amyloidal plaques, neuronal damage characteristic of AD with the extracts treatment compared to the AD-induced rats with Aluminium chloride. The results of the study give rise to the potent effect of *Salvia officinalis* extracts on the progressive disease of Alzheimer with improvement in oxidative stress. The possible mechanism by which the *Salvia* offcinalis extracts improve learning and memory functions could be due to its plausible involvement with cholinergic network. Further studies are warranted in clinical set up for elucidating the molecular mechanisms involved in *Salvia officinalis* which are responsible for producing favourable action in Alzeimers.

ACKNOWLEDGEMENTS

The authors thank Shadan Educational Society, Hyderabad, India, for providing necessary facilities for carrying out this research study. The authors are also thankful to Mr. M.A. Hafeeze Khan for assisting in study related procedures.

REFERENCES

1. Reddy, S. K.; Sudheer, A.; Arunamma, M.; LikithaSree, P.; Jyothirmayi, E. Protective effect of Picrorhizakurroa on Alzheimer's disease induced by aluminium chloride in rats. *Int. J. Basic Clin. Pharmacol.* **2017,** *6*, 602-607.

2. Imbimbo, B. P.; Lombard, J.; Pomara, N. Pathophysiology of Alzheimer's Disease. *Neuroimag Clin N Am*, **2005**, *15,* 727–753.

3. Eidi, A.; Sarkaki, A.; Mortazavi, P.; Hajipour, S.; Farbood, Y.; Valizadeh, Z. Effect of gallic acid on dementia type of alzheimer disease in rats: electrophysiological and histological studies. *Basic Clin. Neurosci.* **2016**, *7*, 97-106.

4. Nelson, V. M.; Dancik, C. M.; Pan, W.; Jiang, Z. G.; Lebowitz, M. S.; Ghanbari, H. A. PAN-811 inhibits oxidative stress-induced cell death of human Alzheimer's disease-derived and age-matched olfactory neuroepithelial cells via suppression of intracellular reactive oxygen species. *JAD*, **2009**, *17*, 611-619.

5. Shati, A. A.; Elsaid, F. G.; Hafez, E. E. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup, *Neurosci.* **2011**, *175*, 66-74.

6.Silva, C.; Herdeiro, R.; Mathias, C.; Panek, A. Evaluation of antioxidant activity of Brazilian plants. *Pharm. Res.* **2005**, *52*, 229-233.

7. Baricevic, D.; Bartol, T. The biological/pharmacological activity of the *Salvia* genus. In Kintzios S.E. (Ed.), SAGE – The genus Salvia, Harwood Academic Publishers, Amsterdam, The Netherlands: **2000**, 143-184.

8. Jensen, W. B. The origin of Soxhlet Extractor. *J. Chem. Educ.* **2007**, *84*, 1913-1914.

9. OECD Guideline for Testing of Chemicals. Acute Oral Toxicity-Fixed dose Procedure, 420 Adopted: 17th December; **2001**.

10. Nishat, F.; Hajera, S. Evaluation of protective effect of Terminalia bellerica against gentamicin induced nephrotoxicity in albino rats. *Pharm. Biol. Eval.* **2016**, *3*, 486-494.

11. Suba, V.; Murugesan, T.; Rao, R. B.; Pal, M.; Mandal, S. C.; Saha, B. P. Neuropharmacological profile of Barleria lupulina Lindl. Extract in animal models. *J. Ethnopharmacol.* **2002**, *81*, 251-255.

12. Reddy, D. S.; Kulkarni, S. K. Possible Role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpine-induced learning impairment. *Brain Res.* **1998**, *799*, 215-229.

13. Hirakawa, M.; Tamura, A.; Nagashima, H.; Nakayama, H.; Sano, K. Disturbance of retention of memory after focal cerebral ischemia in rats. *Stroke*, **1994**, *25*, 2471-2475.

14. Cutuli, D.; Foti, F.; Mandolesi, L.; De Bartolo, P.; Gelfo, F.; Federico, F.; Petrosini, L. Cognitive performances of cholinergically depleted rats following chronic donepezil administration. *J. Alzheimers Dis.* **2009**, *17*, 161–176.

15. Sargazi, M.; Shenkin, A.; Roberts, N. B. Aluminium-induced injury to kidney proximal tubular cells: Effects on markers of oxidative damage. *J. Trace Elem. Med. Biol.* **2006**, *19*, 267-273.

16. Diets, A. A.; Rubenstein, H. M.; Lubrano, T. Colorimetric determination of serum cholinesterase and its genetic variants by the propionylthiocholine-dithiobis(nitrobenzoic acid) procedure. *Clin Chem*, **1977**, *8*, 41-46.

17. Kono, Y. Generation of superoxide radical during auto oxidation of hydroxylamine and an

assay of superoxide dismutase. *Arch. Biochem. Biohys.* **1978**, *186*, 186-189.

18. Okhawa, H.; Ohisni, N. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Annal. Biochem.* **1979**, *95*, 351.

19. Berkels, R.; Purol-Schnabel, S.; Roesen, R. Measurement of nitric oxide by reconversion of nitrate/nitrite to NO. *Methods Mol Biol.* **2004**, *279*, 1-8

20. Banchroft, J. D.; Steven, A.; Turner, D. R. Theory and practice of histological technique. Fourth Ed. Churchil Livingstone, New york, London, San Francisco, Tokyo, **1996**.

21. Armitage, P.; Berry, G. Comparison of several groups. In: statistical method in medical research 2nd Ed. Blockwell Significant Publication, Oxford; **1987**, pp. 186-213.

22. Brookmeyer, R.; Johnson, E.; Ziegler-Graham, K.; Arrighi, H. M. Forecasting the global burden of Alzheimer's disease. *Alzheimer Dem.* **2007**, *3*, 186-191.

23. Moshtaghie, A. Aluminium administration on acetylcholinesterase activity of different regions of rat brain. *Med. J. Islamic Acad. Sci.* **1999**, *12*, 105–108.

24. Szutowiicz, A.; Bielarcczyk, H.; Kisielevski, Y.; Jankowska, A.; Madziar, B.; Tomaszewicz, M. Effects of aluminium and calcium on acetyl-CoA metabolism in rat brain mitochondria. *J. Neurochem.* **1999**, *71*, 2447–2453.

25.Kaizer, R. R.; Corrêa, M. C.; Spanevello, R. M.; Morsch, V. M.; Mazzanti, C. M.; Gonçalves, J. F.; Schetinger, M. R. C. Acetylcholinesterase activation and enhanced lipid peroxidation after long-term exposure to low levels of aluminum on different mouse brain regions. *J. Inorg. Biochem.* **2005**, *99*, 1865–1870.

26. Perry, N. S. L.; Bollen, C.; Perry, E. K.; Ballard, C. *Salvia* for dementia therapy: Review of pharmacological activity and pilot tolerability clinical trial. *Pharmacol. Biochem. Behav.* **2003**, *75*, 651-659.

27. Dickstein, D. L.; Walsh, J.; Brautigam, H.; Stockton, S. D.; Gandy, S.; Hof, P. R. Role of vascular risk factors and vascular dysfunction in Alzheimer's disease. *Mt. Sinai. J. Med.* **2010**, *77*, 82-102.

28. Nelson, V. M.; Dancik, C. M.; Pan, W.; Jiang, Z. G.; Lebowitz, M. S.; Ghanbari, H. A. PAN-811 inhibits oxidative stress-induced cell death of human Alzheimer's disease-derived and age-matched olfactory neuroepithelial cells via suppression of intracellular reactive oxygen species. *JAD*, **2009**, *17*, 611-619.

29. Prins, H. K.; Loose, J. A. Glutathione in biochemical methods in red cell genetics. Edited by Yunis, J. J. Academic Press, N. Y. D. London, **1969** 126-129.

30. Gustaw-Rothenberg, K.; Kowalczuk, K.; Stryjecka-Zimmer, M. Lipids peroxidation markers in Alzheimer's disease and vascular dementia. *Geriatr. Gerontol. Int.* **2010**, *10*, 161-166.

31. Keller, J. N.; Schmitt, F. A.; Scheff, S. W.; Ding, P.; Chen, Q.; Butterfield, D. A.; Markesbery, W. R. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* **2005**, *64*, 1152-1156.

32. Cutuli, D.; De Bartolo, P.; Caporali, P.; Tartaglione, A. M.; Oddi, D.; Nobili, A.; D'Amelioand, M.; Petrosini, L. Neuroprotective effects of donepezil against cholinergic depletion. *Alzheimers Res. Ther.* **2013**, *5*, 50.

33. Howes, M. R.; Perry, N. S. L.; Houghton, P. J. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother. Res.* **2003**, *17*, 1-18.

34. Durling, N. E.; Catchpole, O. J. Extraction of phenolics and essential oil from dried sage

(*Salvia officinalis*) usning ethanol water mixtures. *Food Chem*. **2007**, *101*, 1417-1424.

35.Ahmad, G.; Esmaeilizadeh, M. Pharmacological Proerties of *Salvia officinalis* and its components. *J. Tradit. Complement. Med*. **2017**, *7*, 433-440.

36. Proestos, C.; Sereli, D.; Komaitis, M. Determination of phenolic compounds in aromatic plants by RPHPLC and GC-MS. *Food Chem*. **2006**, *95*, 44-52.

37. Halliwell, B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging*, **2001**, *18*, 685-716.