RESEARCH PAPER

Neuroprotective Role Of Medicinal Plant Of Moringa Oleifera: Behavioral, Biochemical And Antioxidant Studies On Animal Model

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Abstract

Neuroprotection is a term used to allude to methodologies and relative components that shield the focal sensory system from neuronal wounds caused by incessant (e.g., alzheimer's and parkinson's ailments) or intense (e.g. stroke) neurodegenerative diseases (nds). Alzheimer'S disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have non-cognitive symptoms, such as depression, apathy, and psychosis that impair daily living, alzheimer'S disease can occur at any age, even as young as 40 years. It is mainly caused by beta-amyloid plaques, which are the dense deposit of protein and cellular material that accumulate outside and around nerve cells. Neurofibrillary tangles, which are twisted fiber that builds up inside the nerve cell. A depletion of short-term memory, failure to learn new knowledge, mood changes, difficulty recognizing terms, forgetting identities, and losing things are demonstrated by earlier illness. There is still considerable research into medications capable of preventing or at least effectively altering the trajectory of ad, referred to as 'disease-modifying' medicines. In the present study, the efficacy dried leaves of moringa oleifera tested for anti-alzheimer model in novel object recognition experiment which is employed in animal models of neurological disorders to assess memory, particularly memory recognition. The decision to explore the unfamiliar object represents the utilization of memory for learning and identification. M. Oleifera tested for antialzhiemer model in novel object recognition test showed dose-dependent increase in recognition index. From the behavioral, biochemical and dpph antioxidant test results, memo and moe showed statistically significant results, hence, memo and moh was further used for the estimation of free radical scavenging and mao levels in amnesia mice. In case of mao-a and b levels, in negative control group (31.88 ± 0.53 and 34.60±0.86 for mao-a and b, respectively) the levels are increased when compared with the control group (20.32 ± 0.82 and 21.33±0.69 for mao-a and b, respectively). In the treatment groups there was a significant reduction of mao-a ($p<0.001$) and b ($p<0.05$) in low dose (200 mg/kg) treated animals on comparing to the control group. The high dose treated group shown a significant decrease in both mao-a and b with $p<0.001$ on comparison with the neurotoxicity induced animals. In case of free radical scavenging activity, the levels of sod, catalase and vitamin c were significantly ($p<0.05$) increased and also showed dose dependent effect when compared to negative control group.

Keywords: Alzheimer'S Disease, Methanolic Extract, Moringa Oleifera, Neuroprotective Activity.

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I. INTRODUCTION

Neuroprotection is a term used to allude to methodologies and relative components that shield the focal sensory system from neuronal wounds caused by incessant (e.g., Alzheimer's and Parkinson's ailments) or intense (e.g. stroke) neurodegenerative diseases (NDs) (Iriti *et al*., 2010). These intense or unending ailments result from break down and crumbling of neurons of the central nervous system (CNS) and regularly result in the decay of the psychological and additionally the scholarly resources of the sufferers[1]. Neurodegenerative ailment is a term used to allude to

different conditions which happen because of neuronal cell demise, especially, those of the CNS[2]. This decay is frequently connected with continuous beginning of dynamic manifestations, a noteworthy side effect being loss of memory. The NDs incorporate Alzheimer's disease (AD), Parkinson's disease (PD), Lowy body dementia, numerous sclerosis, amyotrophic lateral sclerosis (ALS), and spongiform encephalopathy (Chen and Pan, 2014) of these NDs, AD is the most predominant, representing more than 60– 70% of all types of dementia (Francis *et al*., 1999). Protein conglomeration, irritation, excitotoxicity, oxidative anxiety, and neurotoxicity have been ensnared in the pathophysiology of NDs[3]. Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities. Patients also frequently have non-cognitive symptoms, such as depression, apathy, and psychosis that impair daily living, Alzheimer's disease can occur at any age, even as young as 40 years. It is mainly caused by beta-amyloid plaques, which are the dense deposit of protein and cellular material that accumulate outside and around nerve cells. Neurofibrillary tangles, which are twisted fiber that builds up inside the nerve cell. A depletion of short-term memory, failure to learn new knowledge, mood changes, difficulty recognizing terms, forgetting identities, and losing things are demonstrated by earlier illness. There is still considerable research into medications capable of preventing or at least effectively altering the trajectory of AD, referred to as 'disease-modifying' medicines[4]. Clinically, AD is described by a dynamic and irreversible memory deficiencies, psychological decay, and identity changes, with a mean span of around 8.5 years between beginning of clinical side effects and demise[5].

Plant Profile *Moringa oleifera*

Moringa oleifera is an edible plant with a wide variety of nutritional and medicinal virtues, which have been attributed to its roots, bark and leaves, flowers, fruits and seeds. *Moringa oleifera* is one of the vegetables of the Brassica order and belongs to the family Moringaceae. The Moringaceae is a single genus family with 13 known species. Moringa is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its leaves, pods and seeds. In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach. Moringa is rich in phytosterols like stigmasterol, sitosterol and campesterol which are precursors for hormones. These compounds increase the estrogen production, which in turn stimulates the proliferation of the mammary gland ducts to produce milk. It is used to treat malnutrition in children younger than 3 years [6-7].

Figure 1: Plant of *Moringa oleifera*

II. MATERIALS AND METHODS

2.1. Plant Material

The leaves of *Moringa oleifera was* collected from the Vangapally, Yadagirigutta, Yadadri-Bhongir Dist, Telangana, India, during month of March and authenticated by botanist Prof. Srinivas, Department of Botany, SLNS Degree and PG College, Bhongir, Telangana, India. The plant leaves were washed with tap water and shade dried at normal room temperature with the aid of circulating airflow using a fan. The stem was dried, and coarse powder was made of the Leaves in a mixture and it was stored in a container[8-10].

2.2. Preparation of Extract

The Methanolic extract of *Moringa oleifera* was obtained by the Soxhlet method. The fleshy leaves of about 3 kg were washed, cut into small pieces, shade dried, and crushed into powder. Two hundred and fifty grams of the powdered leaves were Soxhlet extracted with 95% methanol. The extract was evaporated in vacuum under reduced pressure, to make a syrupy consistency, and the final extract was stored in a glass container at room temperature[11-12].

2.3. Toxicity

The alcoholic extract's LD50 was estimated to be 3000 mg/kg. This plant has also known to be edible, making it safe and accordingly, 1/10th and 1/20th dose was selected for study, *i.e*., low dose 100 mg/kg, intermediate-dose 200 mg/kg, and high dose 300 mg/kg for the test groups with dose conversion[13-15].

2.4. Drug Treatment

The Methanolic extract was suspended in Normal saline in doses of 100, 200, 300 mg/kg p.o. for the pharmacological tests. The doses were selected based on the previous studies on Methanolic extract. *Moringa oleifera* leaves extract (MEMO) was administered to individual rats in group 4, 5, 6, 7. None of the rat was dead due to treatment till the end of the observation period.

The *Moringa oleifera* leaves extract (MEMO) caused no abnormality till the duration of treatment[16-17].

2.5. Equipments and Chemicals

Electronic balance, Morris water maze, Y-maze, novel object apparatus, syringes, and needles, ethanol, NS and scopolamine. Donepezil tablet, 5, 5'-dithiobis-(2- nitrobenzoic acid) (DTNB), acetylcholine, thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2), Griess reagent.

2.6. Animals

All the animals handling and experimentation were conducted in accordance with the prior approved guidelines from the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi and The Institutional Animal Ethical Committee (IAEC/01/ASPN/2018) of, Aspen Biopharma Labs, India has reviewed the experimental protocol to conducting Acute oral toxicity was performed as per the OECD 423 guidelines. Three female Albino mice weighing 22–25 g were used in the study. The animals were fasted over night before the treatment. Each animal was administered orally a dose level of 5 mg/kg body weight by gastric intubation. The animals were kept in a well-ventilated, air-conditioned animal house at a constant temperature of 24 ± 2 °C, with 12:12 hours dark: light cycle and a relative humidity of 55-60%. The animals were placed on bedding material in spacious polypropylene cages with a paddy husk. The animals were held on a normal diet with pellets and filtered water[18-20].

2.7. Acute Oral Toxicity Study

Acute Oral Toxicity Study has been performed previously on this plant as per OECD 423 guideline[21-23].

2.8. Experimental Design[24-26]

Grouping of Animals: Animals were divided into seven groups, each of six animals.

Group I: Control group oral administered by Distilled water (20 ml/kg).

Group II: Intraperitoneal injection by scopolamine hydrochloride (0.5 mg/kg) (Disease control).

Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with scopolamine (Standard).

Group IV: Animals oral administered by extract which is dissolved in NS (100 mg/kg) and Alzheimer's induced with scopolamine (Low dose).

Group V: Animals oral administered by extract which is dissolved in NS (200 mg/kg) and Alzheimer's induced with scopolamine (Intermediate dose).

Group VI: Animals oral administered by extract which is dissolved in NS (300 mg/kg) and Alzheimer's induced with scopolamine (High dose).

Group VII: Animals oral administered by extract which is dissolved in NS (300 mg/kg) (Extract only).

2.9. Anti-Alzheimer's Study

In behavioral studies, the methanolic extract of *M. oleifera* and its fractions exhibited improvement in cognitive function associated with changes of memory and behavior in amnesia induced mice. The results of different behavioral tests (jumping box, rectangular and Y- maze test)[27].

III. RESULTS AND DISCUSSION

3.1. Studies on methanolic extract and frations of *Moringa oleifera*

3.1.1. Physical nature and percentage yield of extracts

The percentage yield and physical status of the methanolic leaf extract (MOME) and its ethyl acetae (MOEA), n-Hexane (MOH), chloroform (MOC) and aqueous (MOA) fractions *Moringa oleifera* are showed in the Table 1. Yield of the extracts calculated with reference of the weight raw material used. For fractions, the yield was calculated with respect to corresponding methanolic extract.

Table 1: Percentage yield and physical status of the methanolic extract and fractions of *Moringa oleifera*

Extract/Fraction	Code	Yield (%)
Methanolic	MOME	**9.83**
Ethyl acetate	MOEA	**4.66**
Chloroform	MOC	**10.2**
n-hexane	MOH	**3.12**
Aqueous	MOA	**14.45**

3.1.2. Acute toxicity studies

The MOME and fractions were considered as nontoxic, because it did not show any toxic signs or symptoms and mortality in the oral dose of 2000 mg/kg in mice. According to OECD-423 guidelines, the LD_{50} of 2000 mg/kg and above is mentioned as unclassified. So further pharmacological screening is carried out. Hence, two doses ($1/10^{th}$ and $1/5^{th}$ of 2000 mg) methanolic extracts and their fractions were selected for the neuroprotective study.

3.1.3. *In-Vivo* pharmacological activity

The Stereotaxic apparatus used to identify the bregma point over the skull (approximately 1-3 mm rostral to the line drawn through anterior base of ears). After the identification, amnesia in mice was induced by Aβ by i.c.v. injection and the mice were grouping for the treatment after the induction. The animals were subjected to behavioral, biochemical and free radical scavenging activity.

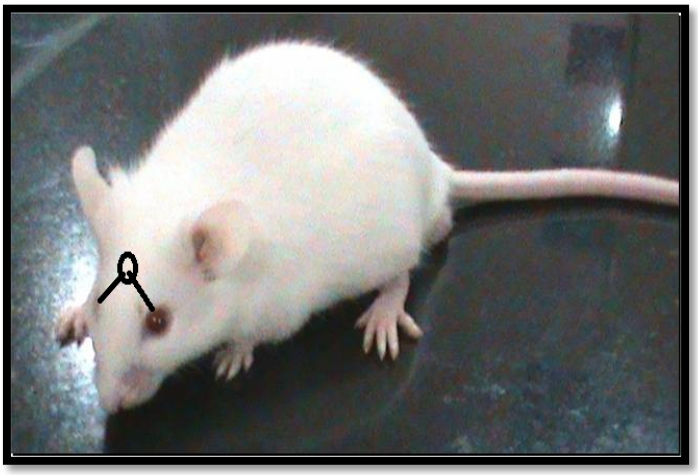

Figure 2: Identification of bregma point by Stereotaxic apparatus

3.1.4. Bhevarioual studies

In behavioral studies, the methanolic extract of *Moringa oleifera* and its fractions exhibited improvement in cognitive function associated with changes of memory and behavior in amnesia induced mice. The results of different behavioral tests (jumping box, rectangular and Y- maze test) are showed in Table 4.10 and 4.11.

3.1.4.1. Jumping box test

The jumping box test also considered as conditioned avoidance test (memory). The activity was expressed in latency periods with sec time in amnesia induced mice. In jumping box test, there was an in increase in latency period in negative control group (25.86 ± 1.73) when compared to vehicle control (10.87 ± 0.76) (Table 2 and Figure 3). The MOME treated group showed decrease in latency period in groups treated (19.1 ± 1.22and 15.7 ± 2.14for 200 and 400 mg/kg).

Table 2: Effect of MOME and its fractions on behavioral activity by Jumping box, rectangular maze and Y-maze test (Mean±SD, n=6)

Moringa oleifera extract			
Group	**Jumping box test (Sec)**	**Rectangular maze test (Sec)**	**Y-maze test (% alterations)**
I (PBS)	10.87 ± 0.76	27.61 ± 2.07	41.73 ± 2.75
II (Aβ)	25.86 ± 1.73	155.9 ± 1.85	26.25 ± 1.88
III (Aβ + DPZ)	13.4 ± 0.64***	68.81 ± 3.76**	39.88 ± 2.03***
IV (200mg/kg MOME+ Aβ)	19.1 ± 1.22*	96.9 ± 3.76*	32.54 ± 1.84**
V (400 mg/kg MOME + Aβ)	15.7 ± 2.14**	72.84 ± 1.86**	37.80 ± 2.04***

*p < 0.05, **P < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one way) followed by Bonferroni's test.

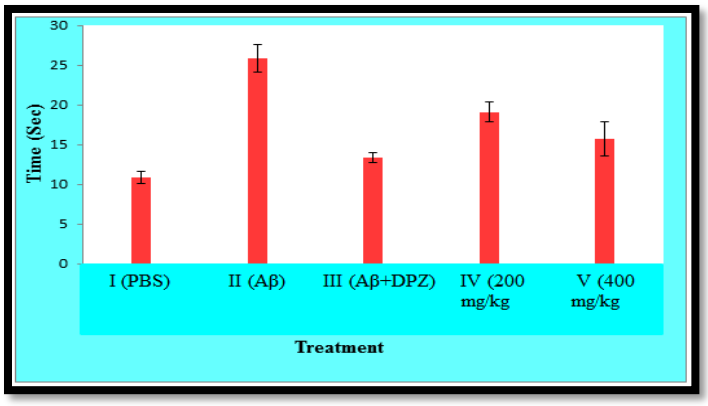

Figure 3: Effect of MOME on jumping box test in amnesia mice (mean±SD, n=6)

In case of fractions treated groups, there is a decrease in latency period in groups treated with MOEA (19.7 ± 1.23and 15.8 ± 1.06 for 200 and 400 mg/kg), MOC (22.2±0.83and 19.2±0.71 for 200 and 400 mg/kg), MOH (18.9 ± 0.88 and 14.2 ± 0.69for 200 and 400 mg/kg) and MOA (21.2±0.67and 18.2±0.54 for 200 and 400 mg/kg). From the results, the MOH fraction shows statistically significant more activity compared to MOEA, MOC and MOA fractions and also all the fractions exhibited dose dependent effect (Table 3 and Figure 4).

Table 3: Effect of different fractions on behavioral activity by Jumping box, rectangular maze and Y-maze test

Group	**Jumping box test (Sec)**	**Rectangular maze test (Sec)**	**Y-maze test (% alterations)**
I (PBS)	13.4±0.76	29.61±2.43	43.73±2.45
II (Aβ)	19.66±1.34	99.23±1.20	30.78±2.21
III (Aβ + DPZ)	14.35±2.46	74.89±2.67	36.54±2.87

IV(Aβ + 400	20.98±1.98	112.98±1.72	30.12±1.23
V(Aβ + 400	18..43±2.32	83.47±2.98	33.92±2.12
VI (Aβ + 200	18.23±1.45	95.13±1.78	32.43±2.23
VII (Aβ + 400 mg/kg MOEA)	13.10±1.23	70.25±2.43	40.23±3.23
VI II (Aβ + 200 mg/kg MOH)	20.12±2.32	112.34±2.12	31.12±2.67
IX (Aβ + 400 mg/kg MOH)	14.89±1.34	75.23±3.76	34.13±2.56
X (Aβ + 200 mg/kg MOA)	18.23±1.45	95.13±1.78	32.43±2.23
XI (Aβ + 400 mg/kg MOA)	13.10±1.23	70.25±2.43	40.23±3.23
XII (Aβ + 200 mg/kg MOC)	20.12±2.32	112.34±2.12	31.12±2.67
XIII (Aβ + 400 mg/kg MOC)	14.89±1.34	75.23±3.76	34.13±3.56

*p < 0.05, **P < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test.

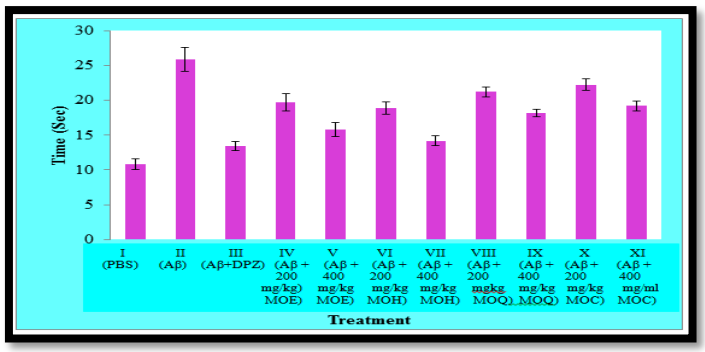

Figure 4: Effect of different fractions on jumping box test in amnesia mice (mean±SD, n=6)

3.1.4.2. Rectangular maze test:

The hippocampal learning of Aβ induced group (negative control) was declined and shown a significant (p<0.001) increase in escape latency while comparing the control group. The readings were showed in Table 4.10 and Table 4.11. From the results, in rectangular maze test there was an increase in maze traverse period in negative control group (155.9 ± 1.85) when compared to control (27.61 ± 2.07), and there is a decrease in traverse period in groups treated with methanol extract (MOME) (96.9 ± 3.76 and 72.84 ± 1.86 for 200 and 400 mg/kg). The fractions treated groups also showed a dose dependent decrease in traverse period in MOEA (90.55±2.84 and 84.72±3.91), MOC (93.1±3.14 and 86.65±2.74), MOH (80.22 ± 2.21 and 70.03 ± 3.17) and MOA (107.2±2.89 and 89.54±3.72) fractions. The results of test were represented in Figures 5 to 6 for MOME and fractions of MOF.

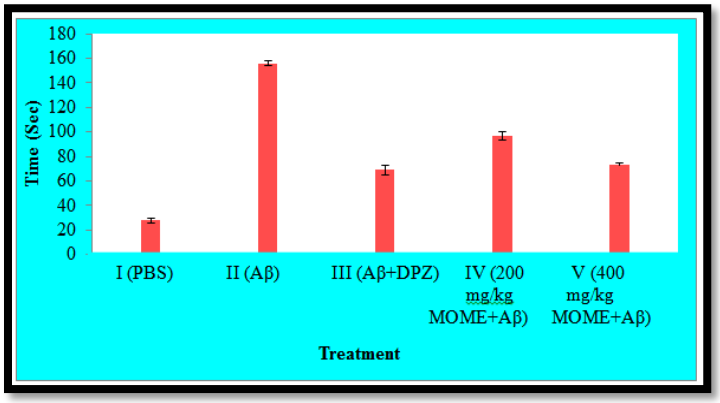

Figure 5: Effect of MOME on rectangular maze test in amnesia mice (mean±SD, n=6)

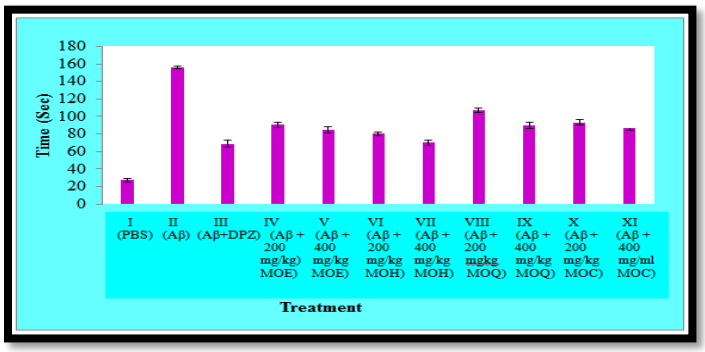

Figure 6: Effect of different fractions of MOF on rectangular maze test in amnesia mice (mean±SD, n=6)

3.1.4.3. Y-maze test

The Y-maze test was performed in amnesia mice and reported the outcomes in % alterations. The results showed in Table 4.2 and 4.3 and Figures 7 and 8. As the dose increased from 200 to 400 mg/kg, % alteration was increased. From the results, in negative control and vehicle groups the % alteration was found to be 26.25 ± 1.88 and 41.73 ± 2.75, respectively. MOME showed 32.54 ± 1.84 and 37.80 ± 2.04% alterations for 200 and 400 mg/kg respectively, which was statistically significant (p<0001) compared to negative control group. In case of MOEA, MOH, MOC and MOA the % alteration were found to be 32.73±1.86 and 37.88±2.17, 33.77±1.86 and 39.85±2.75, 27.91±0.82 and 31.27±1.53; 29.13±1.62 and 34.82±1.91 for 200 and 400 mg/kg, respectively and was significantly (p<0.001).

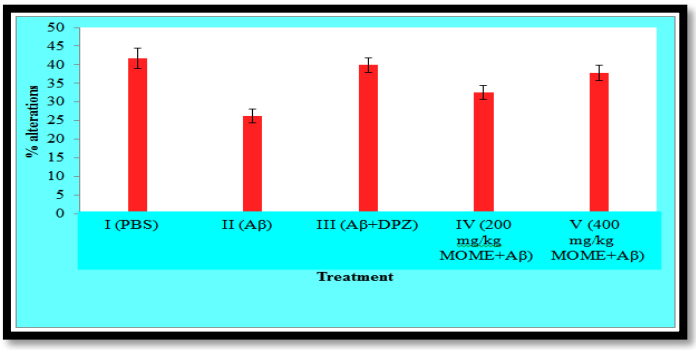

Figure 7: Effect of MOME on Y-maze test in amnesia mice (mean±SD, n=6)

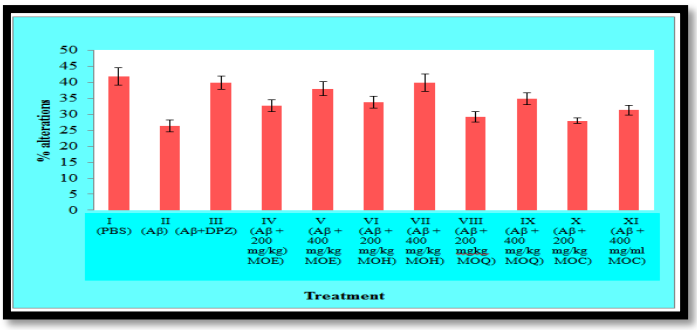

Figure 8: Effect of different fractions on Y-maze test in amnesia mice (mean±SD, n=6)

3.1.5. Biochemical estimations

The MOME and fractions (MOEA, MOH, MOC and MOA) were subjected to various biochemical parameters i.e., AChE, glutathione and MDA levels. The differences in biochemical parameters in treatment groups were summarized in the Table 4 and 5.

5.2.5.1. Acetylcholinesterase (AChE) enzyme:

The i.c.v injection of Aβ peptide in negative control animals showed an extremely significant (P<0.001) increase in brain AChE levels. The AChE enzyme levels were significantly decreased in the treatment groups and indicated a difference with p<0.01, p<0.05, p<0.001 and p<0.05, respectively when compared with the amnesia induced group. The levels of each animal expressed in µmole/min/mg. From the results, it was found that acetyl cholinesterase levels are increased in negative (24.77 ± 0.76) when compared to control (8.64 ± 1.12), and decreased levels are observed in methanol extract (16.58 ± 1.63and 12.91 ± 2.01 for 200 and 400 mg/kg, respectively) (Figure 9). The AChE enzyme levels were significantly decreased in the treatment groups of MOEA (21.20 ± 1.10 and 18.76 ± 1.21), MOC (19.36 ± 1.16 and 14.81 ± 1.05), MOH (15.34 ± 0.82 and 11.65 ± 0.73) and MOA (17.81 ± 1.26 and 13.64 ± 0.85) and indicated a difference with p<0.01, p<0.05, p<0.001 and p<0.05, respectively when compared with the amnesia induced group (Figure 8). The decreased levels of AChE were increased along with dose of the extract as well as fractions.

Table 4: Effect of MOME on biochemical parameters (mean±SD, n=6)

Group	AChE (µmole/min/g)	GSH (µmole/min/mg protein)	MDA (µg/gm wet tissue)
	Mean ± SD	Mean ± SD	Mean ± SD
I (PBS)	8.64 ± 1.12	23.69 ± 1.53	15.64 ± 0.53
II (Aβ)	24.77 ± 0.76	16.8 ± 0.97	52.85 ± 2.74
III (Aβ +DPZ)	10.72 ± 0.83***	21.37 ± 0.93***	20.91 ± 0.66**
IV(200mg/kg MOME + Aβ)	16.58 ± 1.63**	18.28 ± 1.66*	31.64 ± 0.74*
V (400 mg/kg MOME+ Aβ)	12.91 ± 2.01***	20.59± 1.54***	29.79 ± 0.93**

*p < 0.05, **p < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test

Table 5: Effect of different fractions of MOME on different biochemical parameters (mean±SD, n=6)

Group	AChE (µmole/min/g)	GSH (µmole/min/mg protein)	MDA (µg/gm wet tissue)
I (PBS)	8.64 ± 1.12	23.69 ± 1.53	15.64 ± 0.53
II (Aβ)	24.77 ± 0.76	16.8 ± 0.97	52.85 ± 2.74
III (Aβ + Donepezil)	10.72 ± 0.83**	21.37 ± 0.93***	20.91 ± 0.66**
IV (Aβ + 200 mg/kg MOEA)	21.20 ± 1.10*	18.13 ± 0.92*	41.60 ± 1.72*

V (Aβ + MOEA 400 mg/kg)	18.76 ± 1.21**	20.65 ± 0.75**	31.81 ± 1.84**
VI (Aβ + 200 mg/kg MOH)	**15.34 ± 0.82****	**19.10 ± 0.73***	**35.66 ± 1.17***
VI I (Aβ + 400	**11.65 ± 0.73*****	**21.81 ± 1.84*****	**26.61±**
VIII (Aβ + 200 mg/kg MOA)	17.81 ± 1.26**	18.55 ± 1.17*	39.03 ± 1.38**
IX (Aβ + 400 mg/kg MOA)	13.64 ± 0.85**	19.81 ± 1.64**	32.72 ± 1.85**
X (Aβ +200 mg/kg MOC)	19.36 ± 1.16**	17.47 ± 0.93	45.37 ± 1.29*
XI (Aβ +400 mg/kg MOC)	14.81 ± 1.05**	18.85 ± 1.35*	36.81 ± 2.01*

*p < 0.05, **p < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test

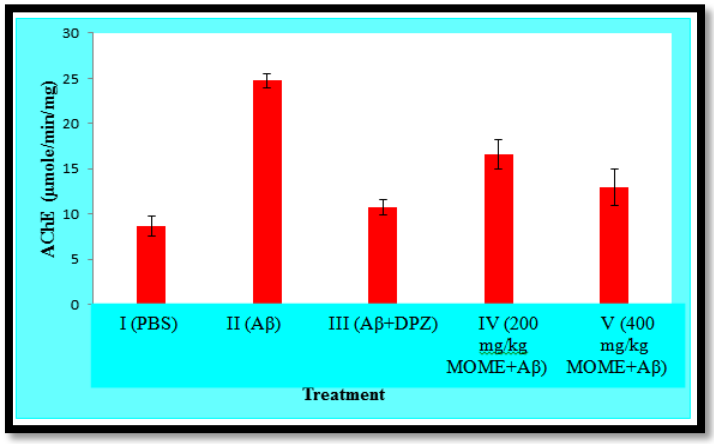

Figure 9: Effect of MOME on AChE levels in amnesia mice (mean±SD, n=6)

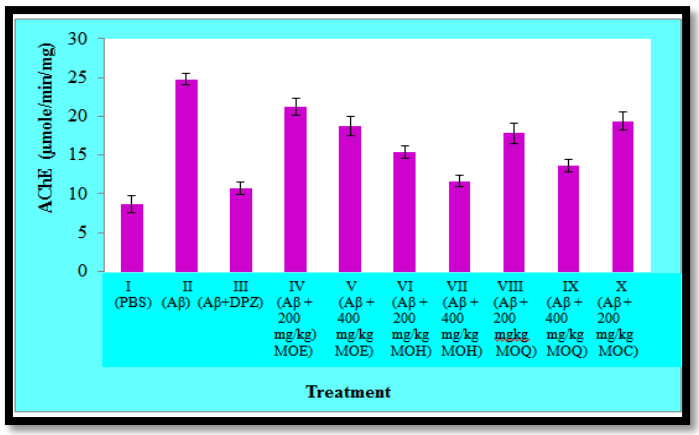

Figure 10: Effect of different fractions of MOME on AChE levels in amnesia mice (mean±SD, n=6)

3.1.5.2. Glutathione peroxidase (GSH)

Glutathione levels are decreased in negative (16.8 ± 0.97) when compared to control (23.69 ± 1.53), increased levels are observed in methanol extract (18.28 ± 1.66 and 20.59± 1.54 for 200 and 400 mg/kg, respectively) (Table 4) The GSH enzyme levels were significantly increased in the treatment groups of MOEA (18.13 ± 0.92 and 20.65 ± 0.75), MOC (17.47 ± 0.93 and 18.85 ± 1.35), MOH (19.10 ± 0.73 and 21.81 ± 1.84) and MOA (18.55 ± 1.17 and 19.81 ± 1.64) fractions. The results are statistically significant compared with negative control ($p<0.05$). Figure 10 and 11 represented the effect of CQME and its fractions on GSH levels.

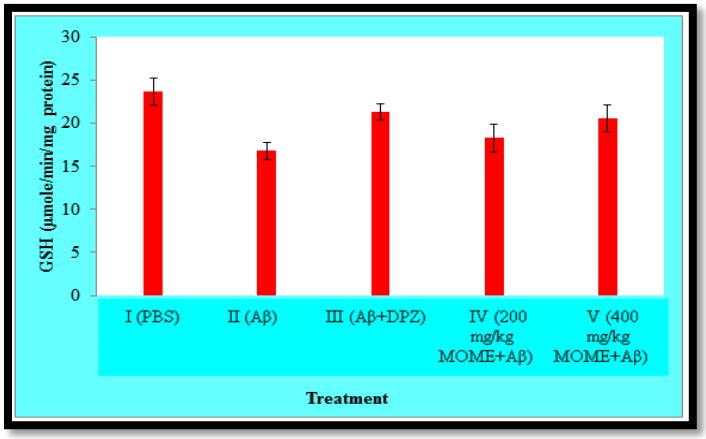

Figure 11: Effect of MOME on GSH levels in amnesia mice (mean±SD, n=6)

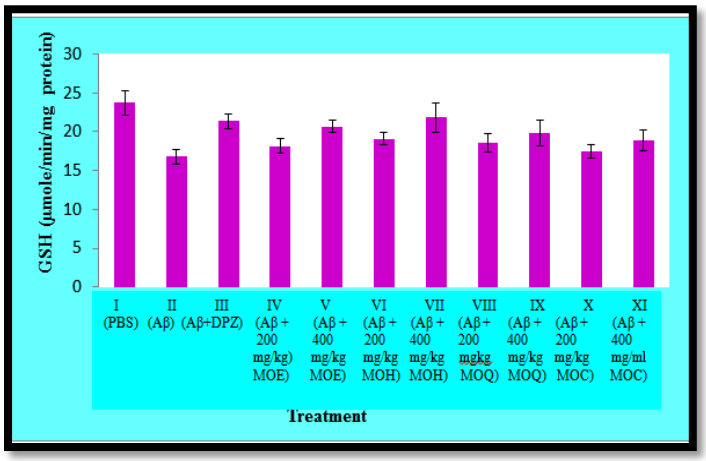

Figure 12: Effect of different fractions of *Moringa oleifera* on GSH levels in amnesia mice (mean±SD, n=6)

3.1.5.3. Estimation of MDA

MDA levels are increased in negative (52.85 ± 2.74) when compared to control (15.64 ± 0.53), and decreased levels are observed in methanol extract was found to be 31.64 ± 0.74 and 29.79 ± 0.93 µg/g/wet tissue for 200 and 400 mg/kg dose respectively. Decreased levels were observed in MOEA (41.60 ± 1.72 and 31.81 ± 1.84 for 200 and 400 mg/kg), MOC (45.37 ± 1.29 and 36.81 ± 2.01 200 and 400 mg/kg), MOH (35.66 ± 1.17 and 26.61± 1.65 200 and 400 mg/kg) and MOA (39.03 ± 1.38 and 32.72 ± 1.85) fractions was observed. The decreased levels of MDA parameter levels were observed in fractions treated group and was the indication of anti-amnesic activity of *Moringa oleifera* fractions. The results are presented in Figure 13 and 14.

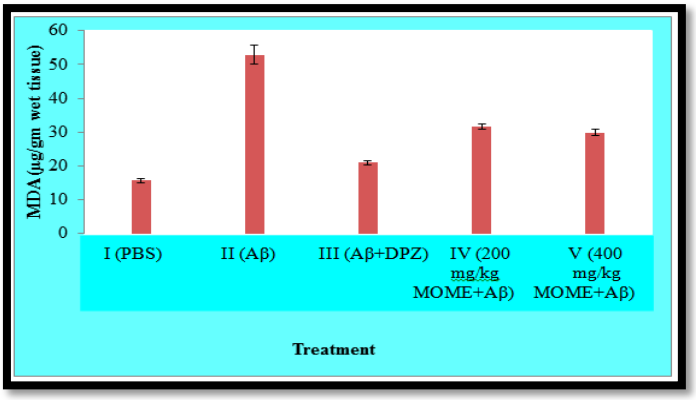

Figure 13: Effect of MOME on MDA levels in amnesia mice (mean±SD, n=6)

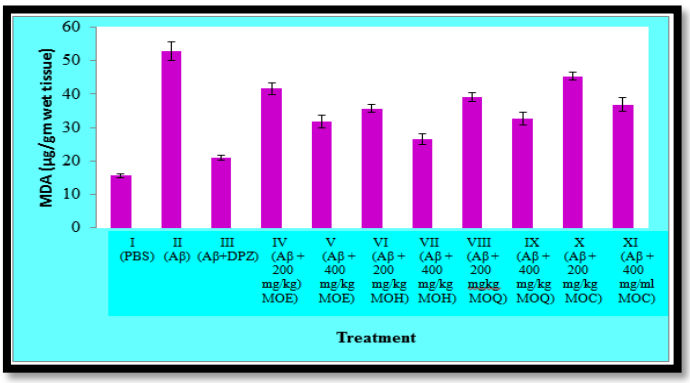

Figure 14: Effect of different fractions of MOME on MDA levels in amnesia mice (mean±SD, n=6)

3.1.5.4. MAO Assay

The neurotransmitter metabolic enzyme responsible for the metabolism of bigenic amines in the brain were significantly (P<0.001) elevated after Aβ injection in negative control animals (31.88 ± 0.53 and 34.60±0.86 for MAO-A and B, respectively) when compared with the control group (20.32 ± 0.82 and 21.33±0.69 for MAO-A and B, respectively). In the treatment groups there was a significant reduction of MAO-A (P<0.001) and B (P<0.05) in low dose (200 mg/kg) treated animals on comparing to the control group. The high dose treated group shown a significant decrease in both MAO-A and B with P<0.001 on comparison with the neurotoxicity induced animals. Among the treated groups, the 400 mg/kg treatment showed a dose dependent significance (P<0.001). These actions of MOME and MOA on MAO-A and B were represented in Table 6 and Figures 15 and 16.

Table 6: Effect of MOME and MOH on MAO-A and B parameters (Mean ± SD, n=6)

Group	MAO (nmol/mg protein.h) Mean ± SD	
	A	B
I (PBS)	20.32 ± 0.82	21.33±0.69
II (Aβ)	31.88 ± 0.53	34.60±0.86
III (Aβ + DPZ)	21.23 ± 0.66***	22.45±0.74***
IV (200mg/kg MOME+	28.41 ± 0.73*	29.34 ± 1.06*
V (400 mg/kg MOME +	23.98 ± 1.70**	25.02±1.31**
VI (Aβ + 200 mg/kg MOA)	27.66 ± 0.69*	26.72±0.88**
VII (Aβ + 400 mg/kg MOA)	22.18 ± 0.53***	23.86±0.76***

*p < 0.05, **p < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test

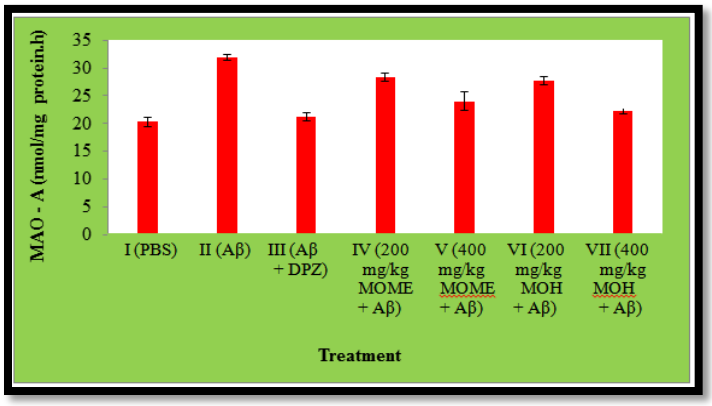

Figure 15: Effect of MOME and MOH on MAO-A levels in amnesia mice (mean±SD, n=6)

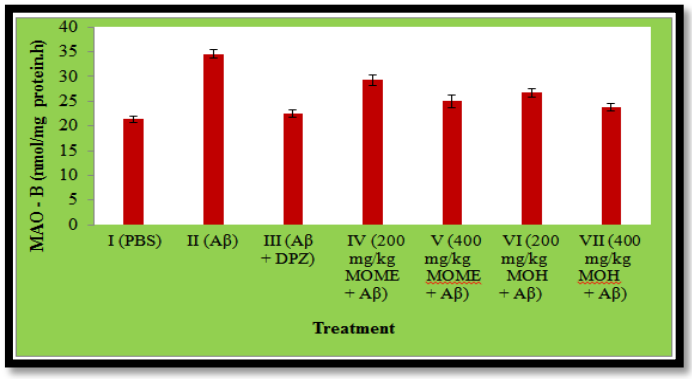

Figure 16: Effect of MOME and MOH on MAO-B levels in amnesia mice (mean±SD, n=6)

3.1.5.5. Estimation of antioxidant enzyme

The levels of SOD in the brain of Aβ induced group was significantly reduced (P<0.001) when compared to the phosphate buffered saline treated group. A significant (P<0.05 and P<0.001) escalation was exhibited in both low (200 mg/kg) and high dose (400 mg/kg) treated animals on comparison with amnesia induced group respectively. Among the treatment groups, there was a significant (P<0.01) dose dependent increase in SOD was shown between 200 mg/kg dose and 400 mg/kg dose. In all the parameters of the antioxidant evaluation such as SOD, Catalase and Vitamin C, there was a significant (P<0.001) reduction in amnesia induced group when compared with that of the control group. The 200 mg/kg and 400 mg/kg treated animals significantly increased the levels of GPx with P<0.001 and P<0.01 respectively when compared with the negative control group. The dose dependent change of the SOD activity between the low dose and high dose indicated a significant (P<0.05) improvement.

In case of catalase enzyme the treatment of MOME reversed the reduction with significance of P<0.05 in low dose and showed extremely significant (P<0.001) increase in high dose. When compared to the low dose treated group of animals, the high dose treated animals shown a significant (P<0.05) changes indicate the dose dependent activity of MOME. The non-enzymic antioxidant, Vitamin C exhibited a significant increase of activity with P<0.01 and P<0.001 in low dose and high dose treated animals respectively on comparing with the neurotoxicity induced group. There is also a significant dose dependent activity in vitamin C was noted in 400 mg/kg treated group when compared to the 200 mg/kg treated group. All the antioxidant properties with various parameters were shown in Table 7 and Figures 17 to 19.

Table 7: Effect of MOME and MOA on free radical scavenging property

Group	SOD (U/min/mg protein)	Catalase (U/mg protein)	VIT.C µg/mg protein
I (PBS)	6.83 ± 0.57	2.17 ± 0.06	0.89 ± 0.07
II (Aβ)	1.73 ± 0.39	0.85 ± 0.09	0.51 ± 0.06
III (200 mg/kg MOME + Aβ)	3.98 ± 0.38*	1.63 ± 0.26**	0.63 ± 0.04*
IV (400 mg/kg MOME + Aβ)	4.96 ± 0.46**	1.98 ± 0.35**	0.75 ± 0.02**
V (Aβ+ 200 mg/kg MOA)	4.23 ± 0.43**	1.86 ± 0.47**	0.74 ± 0.04**
VI (Aβ+ 400 mg/kg MOA)	5.89 ± 0.81***	2.09 ± 0.76***	0.79 ± 0.06***

*p < 0.05, **p < 0.01, ***p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test

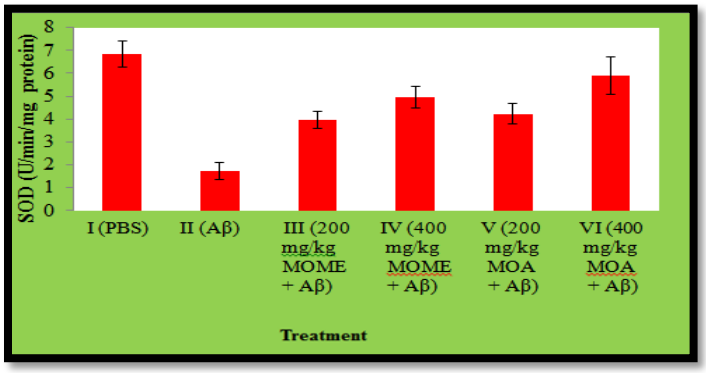

Figure 17: Effect of MOME and MOA on SOD levels in amnesia mice (mean±SD, n=6)

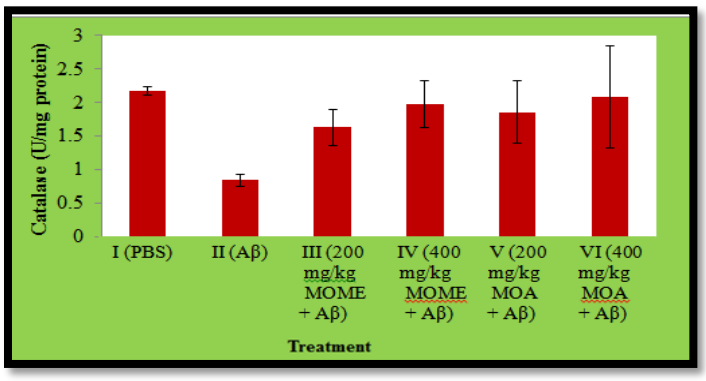

Figure 18: Effect of MOME and MOA on Catalase levels in amnesia mice (mean±SD, n=6)

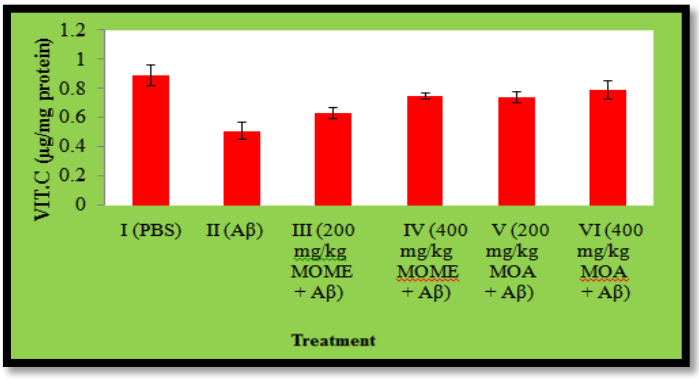

Figure 19: Effect of MOME and MOA on VIT.C levels in amnesia mice (mean±SD, n=6)

The antioxidant property of different MOME and fractions were evaluated by using DPPH free radical scavenging assay and these fractions exhibited dose dependent free radical scavenging activity. The calibration curve of ascorbic acid was plotted in the linearity range of 1-10 nM/mL. The correlation coefficient was 0.999 (Table 8 and Figure 20). The IC_{50} value of MOME, MOEA, MOC, MOH and MOA fractions were found to be 31.37, 29.90, 32.36, 25.48 and 39.38 µg/mL, with respective to IC_{50} value of ascorbic acid and was found to be 12.92 µg/mL.

Table 8: Standard graph of ascorbic acid

Concentration (nM/mL)	Absorbance
1	0.124
2	0.233
4	0.442
6	0.626
8	0.82
10	0.998

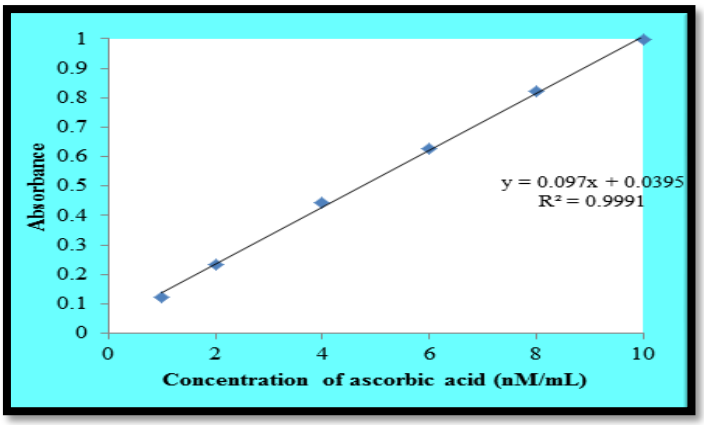

Figure 20: Calibration curve graph of ascorbic acid

3.3. Discussion

Dried leaves of *Moringa oleifera* tested for anti-Alzheimer model in novel object recognition experiment which is employed in animal models of neurological disorders to assess memory, particularly memory recognition. The decision to explore the unfamiliar object represents the utilization of memory for learning and identification. *Moringa oleifera* tested for antialzhiemer model in novel object recognition test showed dose-dependent increase in recognition index. The leaves of *Moringa oleifera* were collected and authenticated. The collected leaves were extracted with methanolic solvent by using Soxhlet apparatus and fractionated with ethyl acetate, n-hexane, chloroform and water. The yield of MEMO, MOE, MOH, MOC and MOQ was found to be 16.8, 7.3, 9.2, 6.6 and 2.6 % w/w, respectively. Neurotoxicity was induced by intra cerebroventricular (i.c.v.) injection of Aβ peptide by identifying bregma point in the skull using stereotaxic apparatus. From the behavioral test results, the latency period of extract and fractions were decreased and % alterations was increased compared to negative control group in jumping box, rectangular maze and Y-maze experiments. In case of biochemical parameters, the elevated levels of AChE was significantly decreased in the treatment groups of MEMO (16.58 ± 1.63and 12.91 ± 2.01for 200 and 400 mg/kg, respectively), MOE (21.20 ± 1.10 and 18.76 ± 1.21), MOC (19.36 ± 1.16 and 14.81 ± 1.05), MOH (15.34 ± 0.82 and 11.65 ± 0.73) and MOQ (17.81 ± 1.26and 13.64 ± 0.85) compared to negative control group (24.77 ± 0.76). In case of MDA levels, the levels are increased in negative (52.85 ± 2.74) when compared to vehicle control (15.64 ± 0.53), and decreased levels were observed in methanol extract (31.64 ± 0.74 and 29.79 ± 0.93 µg/g/wet tissue for 200 and 400 mg/kg dose), MOE (41.60 ± 1.72 and 31.81 ± 1.84), MOC (45.37 ± 1.29 and 36.81 ± 2.01), MOH (35.66 ± 1.17 and 26.61± 1.65) and MOQ (39.03 ± 1.38 and 32.72 ± 1.85) fractions. The decreased levels of MDA parameter in fractions treated group was the indication of anti-amnesic activity of MO fractions. Glutathione levels are decreased in negative (16.8 ± 0.97) when compared to control (23.69 ± 1.53). But, in case of methanolic extract (18.28 ± 1.66 and 20.59± 1.54 for 200 mg/kg and 400 mg/kg), MOE (18.13 ± 0.92 and 20.65 ± 0.75), MOC (17.47 ± 0.93 and 18.85 ± 1.35), MOH (19.10 ± 0.73 and 21.81 ± 1.84) and MOQ (18.55 ± 1.17 and 19.81 ± 1.64) fractions the GSH levels were statistically significant increase when compared with negative control ($p<0.05$). From the DPPH antioxidant activity, The IC_{50} values of MEMO, MOE, MOC, MOH and MOQ fractions were found to be 31.37, 29.90, 32.36, 25.48 and 39.38 µg/mL, with respective to IC_{50} value of ascorbic acid and was found to be

12.92 µg/mL. From the behavioral, biochemical and DPPH antioxidant test results, MEMO and MOH showed statistically significant results, Hence, MEMO and MOH was further used for the estimation of free radical scavenging and MAO levels in amnesia mice. In case of MAO-A and B levels, in negative control group (31.88 ± 0.53 and 34.60±0.86 for MAO-A and B, respectively) the levels are increased when compared with the control group (20.32 ± 0.82 and 21.33±0.69 for MAO-A and B, respectively). In the treatment groups there was a significant reduction of MAO-A (P<0.001) and B (P<0.05) in low dose (200 mg/kg) treated animals on comparing to the control group. The high dose treated group shown a significant decrease in both MAO-A and B with P<0.001 on comparison with the neurotoxicity induced animals. In case of free radical scavenging activity, the levels of SOD, catalase and Vitamin C were significantly ($p<0.05$) increased and also showed dose dependent effect when compared to negative control group.

IV. CONCLUSION

Preliminary phytochemical analysis of an Methanolic extract of *Moringa oleifera* showed the presence of carbohydrates, flavonoids, carotenoids, glycosides, tannins, alkaloids, phenolic compounds. It can be concluded from the study that the Methanolic extract of *Moringa oleifera* leaves possesses significant anti-Alzheimer activity, which is probably due to carotene, mainly β-carotene, which is responsible for showing the antialzhiemer activity, which plays an active role in providing free radical scavenging effect. Thus *Moringa oleifera plant* can be used for the treatment of neurological disorders and may be recommended as a supplement for the anti-Alzheimer activity.

REFERENCES

1. Sanka, N, Santhipriya N and Nadendla RR: 2018. An updated review on Anti-Alzheimer's herbal drugs. Journal of Drug Delivery and Therapeutics., 8: 360-72.
2. Weller, J and Budson A: 2015.Current understanding of Alzheimer's disease diagnosis and treatment. F1000 Research, 7: 2018.
3. Kumar, A, Singh A and Ekavali: 2015 A review on Alzheimer's disease pathophysiology and its management: An update Pharmacological Reports, 67: 195-203.
4. Caroline dos Santos Picanço L: 2018.Current medicinal chemistry the international journal for timely in-depth reviews in medicinal chemistry bentham science send orders for reprints to reprints@benthamscience.ae alzheimer's disease: A Review From The Pathophysiology to Diagnosi New Pers Curr Med Chem., 25: 3141 -59.
5. Howes, M.J.R. Houghton, P.J. 2012. Ethnobotanical treatment strategies against Alzheimer's disease, Current Alzheimer Research, 9, 67-85..
6. Dr. Khursheed Ahmad Ansari. 2017. Sahjana (*Moringa oleifera*), pharmacognosy and pharmacology: A review. Journal of Pharmacognosy and Phytochemistry, 6(1): 442-445.
7. Bhupendra Koul and Neikuozo Chase. 2015*Moringa oleifera* Lam.: Panacea to several maladies. Journal of Chemical and Pharmaceutical Research, 7(6):687-707.
8. Chidambara Murthy KN, Vanitha A, Mahadeva Swamy, M and Ravi Shankar GA. 2003. Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. Journal of Medicine and Food, 6: 99-105.
9. Shirwaikar A, Khan S and Malini S: 2003. Antiosteoporotic effects of ethanol extract of *Cissus quadrangularis* Linn. On ovariectomized rat. Journal Ethnopharmacology, 89: 245-50.
10. Agrawal PK: 2013.Natural product communications editorial. Nat Prod Commun 8.
11. Bum EN:2008. The anticonvulsant and sedative properties of stems of *Cissus quadrangularis* in mice. African Journal Pharmacy and Pharmacology , 2: 042-047.
12. Matsyagiri. L, Prapulla. P, Dr Hemamalini. K.2018. Investigation of *in vitro* anthelminthic activity of bark of *Emblica officinalis*, Indo American Journal of Pharmaceutical Research, 8(08): 390-394.
13. Moto FCO: 2018. Anxiolytic and antiepileptic properties of the aqueous extract of *Cissus quadrangularis* (Vitaceae) in mice pilocarpine model of epilepsy. Front Pharmacol., 9: 1 -10.
14. Toxicity A, Of S, Quadrangularis C and Mice SA: 2018. Acute Toxicity Study of *Cissus Quadrangularis* in , 7.
15. Awodele, O., Oreagba, I. A., Odoma, S., Teixeira Da Silva, J. A., and Osunkalu, V. O. 2012. Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. (Moringaceae). Journal Ethno pharmacology. 139 (2), 330–336.
16. Ravichandran, V. Suresh, B. Sathishkumar, M.N. Elango, K. and Srinivasan, R. 2007."Antifertility activity of hydroalcoholic Extract of *Ailanthus excelsa* (Roxb): an ethnomedicines used by tribals of Nilgiris region in Tamilnadu," Journal of Ethnopharmacology, 112(1):189–191.
17. Upadhyay P: 2018. Revalidation of the neuroprotective effects of a United States patented poly herbal formulation on

scopolamine induced learning and memory impairment in rats. Biomedical Pharmacotherapy, 97: 1046-52.
18. Antunes M and Biala G: 2012. The novel objects recognition memory: Neurobiology, test procedure and its modifications. Cogn

Process, 13: 93-110.
19. Hughes RN: 2004. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations

of memory. Neuroscience Biobehaviour Review., 28: 497-05.
20. Apetz, N. Munch, G. Govindaraghavan, S. Gyengesi, E. 2014. Natural compounds and plant extracts as therapeutics against

chronic inflammation in Alzheimer's disease–a translational perspective, CNS & Neurological Disorders-Drug Targets

(Formerly Current Drug Targets-CNS & Neurological Disorders), 13, 1175-1191.
21. Xia L, Zheng L and Zhou JL: 2017. Effects of ibuprofen, diclofenac and paracetamol on hatch and motor behavior in developing

zebrafish (Danio rerio). Chemosphere, 182: 416-25.
22. Wasik, A., and Antkiewicz-Michaluk, L. (2017). The mechanism of neuroprotective action of natural compounds. Pharmacol.

Rep. 69 (5), 851–860.
23. Khare P, Chaudhary S, Singh L, Yadav G and Verma S: 2014. Evaluation of nootropic activity of *cressa cretica* in scopolamine

induced memory impairment in mice. International Journal Pharmacology and Toxicology, 2: 24-29.
24. Barage SH and Sonawane KD: 2015. Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's

disease. Neuropeptides, 52: 1 -18.
25. Bromley-Brits K, Deng Y and Song W: 2011.Morris water maze test for learning and memory deficits in alzheimer's disease

model mice. Journal Vis Exp, 2-6.
26. Webster SJ, Bachstetter AD, Nelson PT, Schmitt FA and Van Eldik LJ: 2014. Using mice to model Alzheimer's dementia: An

Overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Front Genet, 5: 1 -23.
27. Giacoppo S., Galuppo M., Montaut S., Iori R., Rollin P., Bramanti P., Mazzon E. 2015. An overview on neuroprotective

effects of isothiocyanates for the treatment of neurodegenerative diseases. *Fitoterapia*, 106: 12–21.