

# ANTI-ALZHEIMER'S ACTIVITY OF CISSUS QUADRANGULARIS: Extraction, in vivo Pharmacological, Behavioral, Biochemical and Antioxidant studies

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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 *Cissus quadrangularis* 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. *Cissus quadrangularis* tested for antialzheimer model in novel object recognition test showed dose-dependent increase in recognition index. From the behavioral, biochemical and DPPH antioxidant test results, MECQ and CQE showed statistically significant results, Hence, MECQ and CQE 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 ( $31.88 \pm 0.53$  and  $34.60 \pm 0.86$  for MAO-A and B, respectively) the levels are increased when compared with the control group ( $20.32 \pm 0.82$  and  $21.33 \pm 0.69$  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, *Cissus quadrangularis*, Methanolic extract, 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 (Nieoullon, 2011) [3]. The Alzheimer's disease is the most common and crushing issue of the NDs. It is a serious infection of comprehension and behavioral debilitation that influences social and word related exercises and is additionally a main source of organization in the elderly [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 *Cissus quadrangularis*

*Cissus quadrangularis* (Hadjod) is a perennial plant belonging to the Vitaceae family. It is native to India, Bangladesh and Sri Lanka. are importing it. *Cissus quadrangularis* grows to a height of 1.5 meters, with quadrangular-sectioned branches that are 8 to 10 cm long and 1.2 to 1.5 cm wide. A leathery edge runs along each angle. At the nodes, the toothed tri-lobe leaves, which are 2 to 5 cm broad, appear. Each node has a tendril that emerges from the opposite side [6-7].



Figure 12: Leaves of *Cissus quadrangularis*

### II. MATERIALS AND METHODS

#### 2.1. Plant Material

The leaves of *Cissus quadrangularis* was collected from the Kakatiya University, Warangal, Telangana, India, during month of May and authenticated by botanist Prof. Mustafa, Department of Botany, Kakatiya

University, Warangal, 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

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The Methanolic extract of *Cissus quadrangularis* 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. *Cissus quadrangularis* leaves extract (MECQ) 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 *Cissus quadrangularis* leaves extract (MECQ) 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<sub>2</sub>O<sub>2</sub>), 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 (CPCSEA), New Delhi and The Institutional Animal Ethical Committee (IAEC/05/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 *Cissus quadrangularis* 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 fraction of *Cissus quadrangularis*

#### 3.1.1. Physical nature and percentage yield of extracts

The percentage yield and physical status of the methanolic leaf extract (CQME) and its ethyl acetate (CQEA), n-Hexane (CQH), chloroform (CQC) and aqueous (CQA) fractions *C. quadrangularis* 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

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corresponding methanolic extract.

**Table 1: Percentage yield and physical status of the methanolic extract and fractions of *C. quadrangularis***

Extra ct/Fra ction	C o d e	Y i e l d ( % )
Metha nolic	C Q M E	1 2 .1 4
Ethyl acetate	C Q E A	4 .4
Chloro form	C Q C	3 .1 4
n- hexan e	C Q H	1 .2 2
Aqueo us	C Q A	1 3 .4

### 3.1.2. Acute toxicity studies

The CQME 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<sub>50</sub> of 2000 mg/kg and above is mentioned as unclassified. So further pharmacological screening is carried out. Hence, two doses (1/10th and 1/5th 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 A $\beta$  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 1: Identification of bregma point by Steriotaxic apparatus**

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### 3.2. Behavioral studies

In behavioral studies, the methanolic extract of *Cissus quadrangularis* 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 2.

#### 3.2.1. 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 increase in latency period in negative control group (25.67±2.32) when compared to vehicle control (12.76±1.22) (Table 2, Figure 2, 3). The CQME treated group showed decrease in latency period in groups treated (20.12±1.21 and 14.12±1.09 for 200 and 400 mg/kg).

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

<i>Cissus quadrangularis</i> extract			
Group	J u m p i n g b o x t e s t ( S e c )	R e c t a n g u l a r m a z e s t ( S e c )	Y - m a z e t e s t ( % a l t e r a t i o n s )
<b>I (PBS)</b>	1	4	4
	2	7	7
	.	.	.
	7	3	4
	6	4	5
	±	±	±
	1	3	3
<b>II (Aβ)</b>	.	.	.
	2	1	2
	2	2	4
	2	1	2
	5	5	5
	.	5	.
	6	.	2
7	4	3	
±	5	±	
2	±	2	
.	3	.	
3	.	5	

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	2	2 3	6
<b>III (A<math>\beta</math> + DPZ)</b>	1 3 . 2 3 ± 1 . 5 6	7 0 . 1 2 ± 2 . 7 6	4 3 . 3 4 ± 2 . 3 4
<b>IV (200mg/kg CQME+ A<math>\beta</math>)</b>	2 0 . 1 2 ± 1 . 2 1	9 6 . 2 3 ± 2 . 1 2	3 6 . 1 2 ± 2 . 3 2
<b>V (400 mg/kg CQME + A<math>\beta</math>)</b>	1 4 . 1 2 ± 1 . 0 9	8 0 . 1 2 ± 2 . 2 3	4 0 . 2 3 ± 1 . 8 9
<b>IV (A<math>\beta</math> + 200mg/kg CQEA)</b>	1 9 . 4 5 ± 2 . 2 1	9 4 . 7 8 ± 2 . 1 2	3 8 . 3 2 ± 1 . 7 8
<b>V (A<math>\beta</math> + 400mg/kg CQEA)</b>	1 3 . 9 8 ± 1 . 2 3	7 5 . 1 3 ± 2 . 8 9	4 2 . 9 2 ± 2 . 2 3
<b>VI (A<math>\beta</math> + 200 mg/kg CQH)</b>	2 2 . 1	1 0 9 .	3 0 . 2

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	2 ± 1 . 5 4	3 2 ± 2 2 . 9 8	3 ± 1 . 9 8
<b>VII (Aβ + 400 mg/kg CQH)</b>	2 0 . 4 5 ± 2 . 2 5	9 8 . 2 3 ± 3 . 1 2	3 7 . 1 4 ± 2 . 1 2
<b>VI III (Aβ + 200mg/kg CQA)</b>	2 1 . 1 2 ± 1 . 8 9	9 8 . 1 3 ± 2 . 1 2	3 3 . 4 5 ± 2 . 1 4
<b>IX(Aβ + 400mg/kg CQA)</b>	1 7 . 8 9 ± 2 . 1 3	9 1 . 1 3 ± 2 . 1 2	3 9 . 8 7 ± 2 . 1 2
<b>X (Aβ + 200mg/kg CQC)</b>	2 1 . 8 9 ± 1 . 9 8	9 7 . 1 4 ± 3 . 8 2	3 4 . 7 8 ± 1 . 6 7
<b>XI (Aβ + 400mg/kg CQC)</b>	1 7 . 3 2 ± 2 . 2	8 7 . 1 2 ± 2 . 2	3 7 . 4 5 ± 3 . 2

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	4	1	3
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\*p < 0.05, \*\*P < 0.01, \*\*\*p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test.

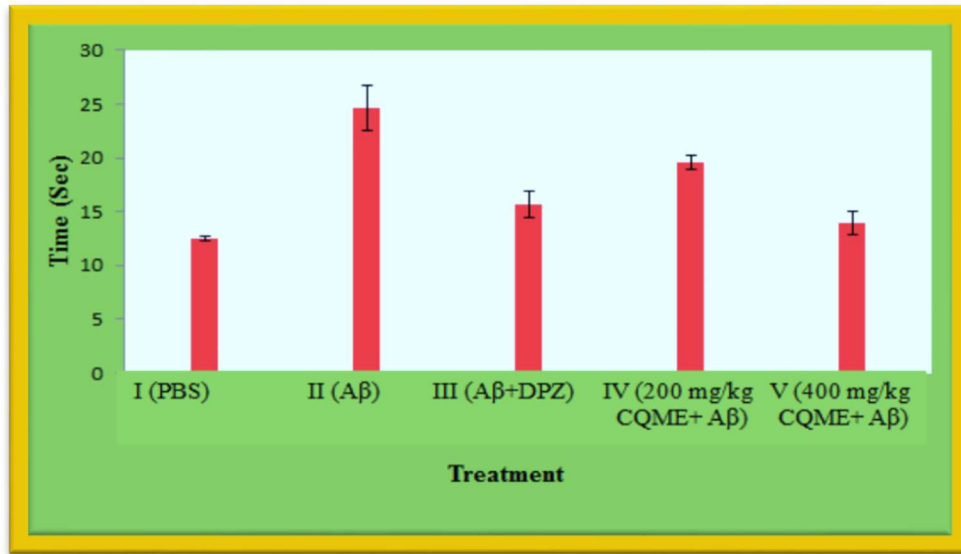


Figure 2: Effect of CQME on jumping box test in amnesia mice (mean±SD, n=6)

In case of fractions treated groups, there is a decrease in latency period in groups treated with CQEA (19.45±2.21 and 13.98±1.23 for 200 and 400 mg/kg), CQC (21.89±1.98 17.32±2.24 and for 200 and 400 mg/kg), CQH (22.12±1.54 and 20.45±2.25 for 200 and 400

mg/kg) and CQA (21.12±1.89 and 17.89±2.13 for 200 and 400 mg/kg). From the results, the CQEA fraction shows statistically significant more activity compared to CQC, CQH and CQA fractions and also all the fractions exhibited dose dependent effect (Table 4.3 and Figure 4.3). \*p < 0.05, \*\*P < 0.01, \*\*\*p < 0.001, when compared to negative control. ANOVA (one-way) followed by Bonferroni's test.

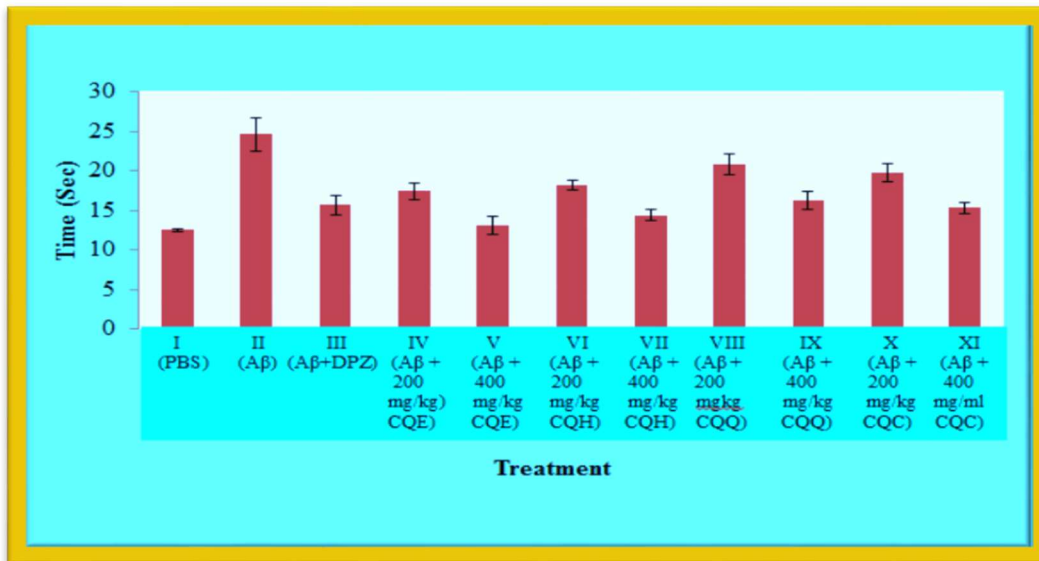


Figure 3: Effect of different fractions on jumping box test in amnesia mice (mean±SD, n=6)

3.2.2. 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 2 and Table 4. From the results, in rectangular maze test there was an increase in maze traverse period in negative control group (155.45±3.23) when compared to control (47.34±3.12), and there is a decrease in traverse period in groups treated with methanol extract (96.23±2.12 and

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80.12±2.23). The fractions treated groups also showed a dose dependent decrease in traverse period in CQEA (94.78±2.12 and 75.13±2.89), CQC (97.14±3.82 and 87.12±2.21), CQH (109.32±2.98 and 98.23±3.12) and CQA (98.13±2.12 and 91.13±2.12) fractions. The results of test were represented in Figures 4, 5 for CQME and fractions of CQME.

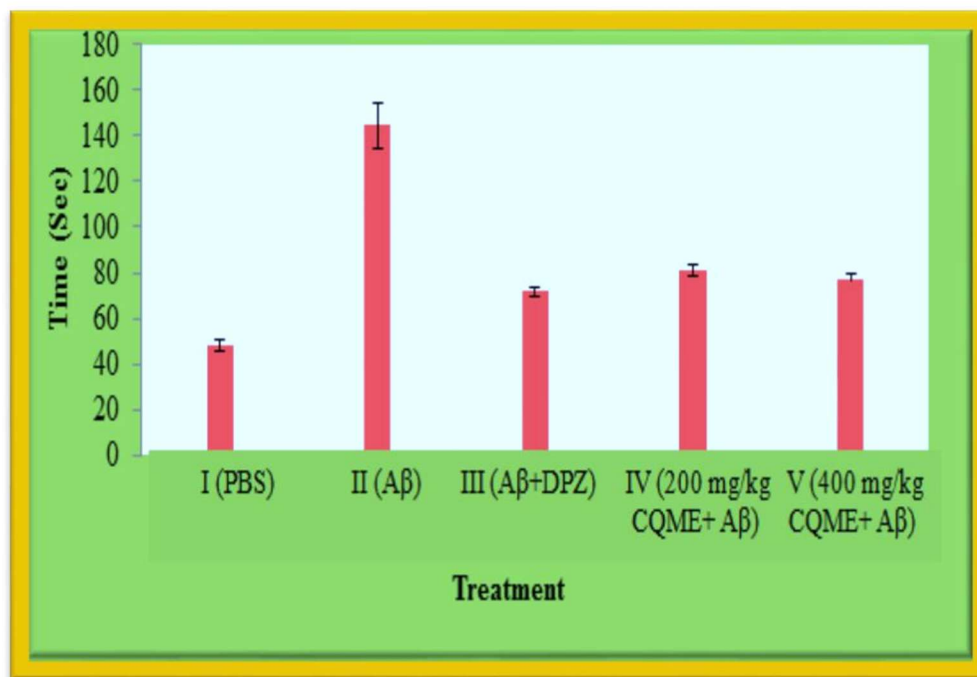


Figure 4: Effect of CQME on rectangular maze test in amnesia mice (mean±SD, n=6)

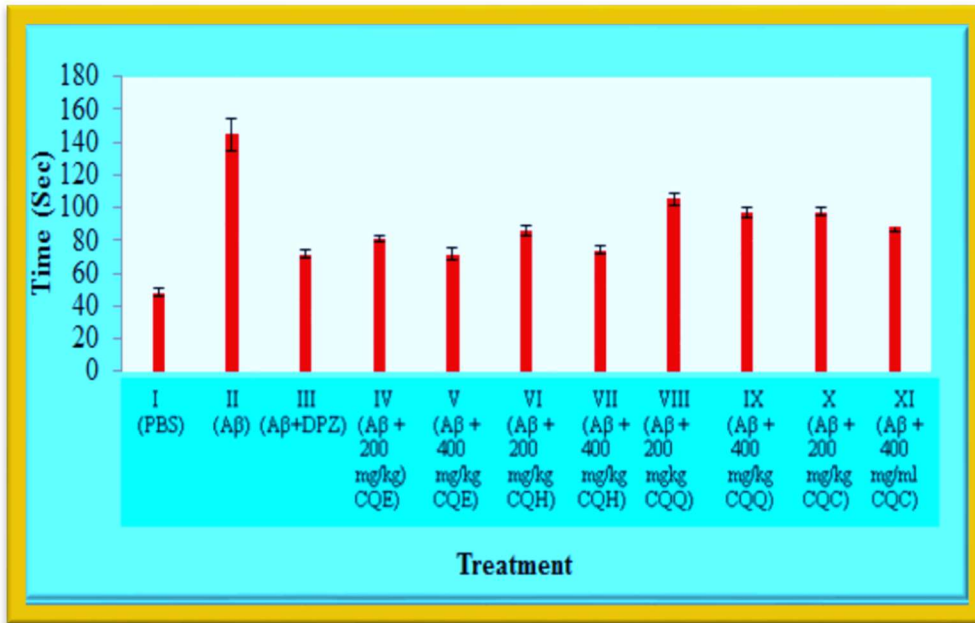


Figure 5: Effect of different fractions on rectangular maze test in amnesia mice (mean±SD, n=6)

3.2.3. Y-maze test

The Y-maze test was performed in amnesia mice and reported the outcomes in % alterations. The results showed in Table 2 and Figures 6 and 7. 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 25.23±2.56 and 47.45±3.24, respectively. CQME showed 36.12± 2.32 and 40.23±1.89 % alterations for 200 and 400 mg/kg respectively, which was statistically significant (p<0001) compared to negative control group. In case of CQEA, CQH, CQC and CQA the % alteration were found to be 38.32±1.78 and 42.92±2.23, 30.23±1.98 and 37.14±2.12, 34.78±1.67 and 37.45±3.23; 33.45±2.41 and 39.87±2.12 for 200 and 400 mg/kg, respectively and was significantly (p<0.001).

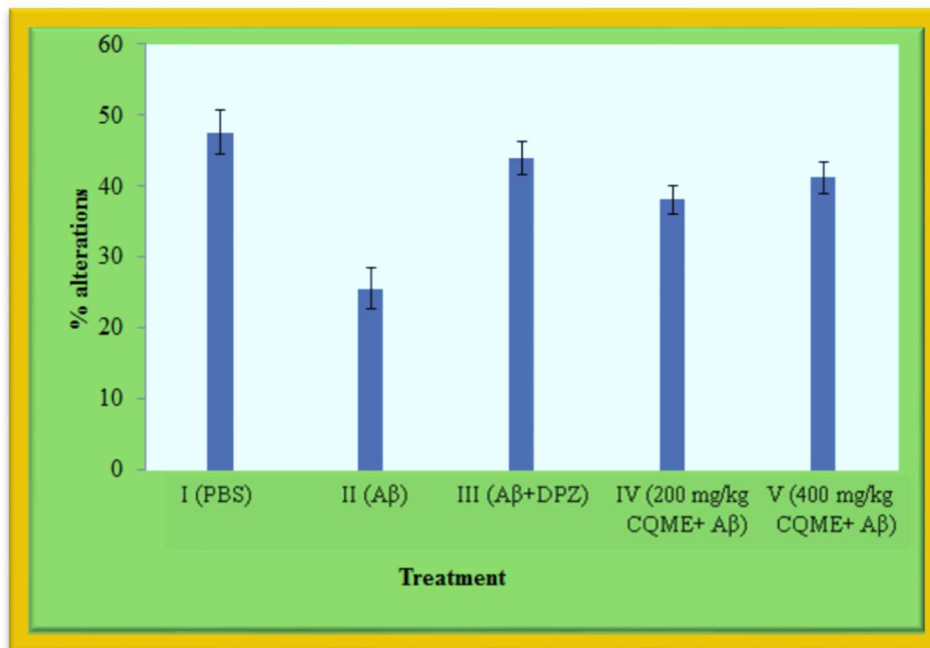


Figure 6: Effect of CQME on Y-maze test in amnesia mice (mean±SD, n=6)

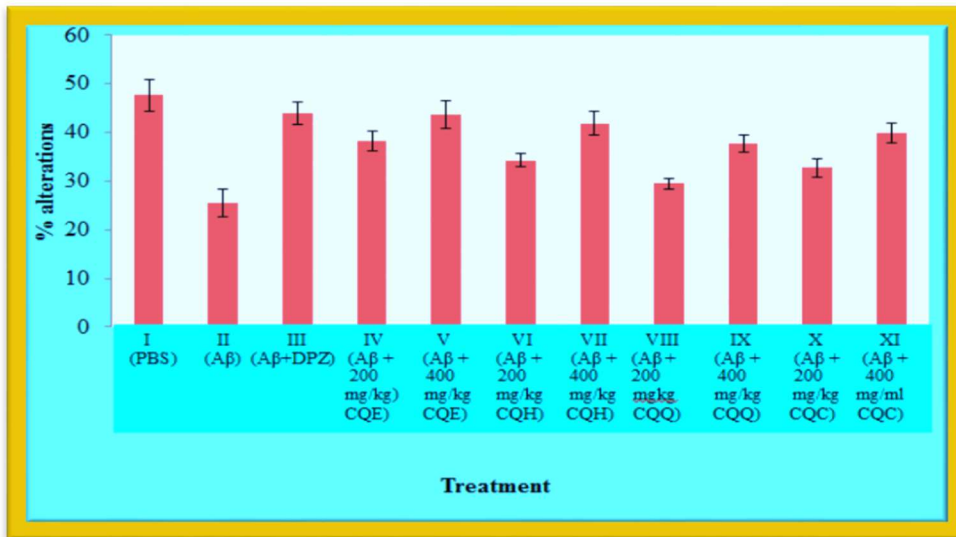


Figure7: Effect of different fractions on Y-maze test in amnesia mice (mean±SD, n=6)

3.2.4. Biochemical estimations

The CQME and fractions (CQEA, CQH, CQC and CQA) were subjected to various biochemical parameters i.e., AChE, GSH and MDA levels. The differences in biochemical parameters in treatment groups were summarized in the Table 3.

3.2.4.1. 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 Ach. E levels. The Ach. E 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  $\mu\text{mole}/\text{min}/\text{g}$ . From the results, it was found that acetyl cholinesterase levels are increased in negative ( $23.43 \pm 2.15$ ) when compared to control ( $10.32 \pm 1.22$ ), and decreased levels are observed in methanol extract ( $16.58 \pm 1.83^{**}$  and  $13.91 \pm 2.21^{***}$  for 200 and 400 mg/kg, respectively) (Figure 8). The AChE enzyme levels were significantly decreased in the treatment groups of CQEA ( $15.15 \pm 3.26$  and  $12.12 \pm 2.23$ ), CQC ( $16.19 \pm 2.25$  and  $13.16 \pm 3.17$ ), CQH ( $19.17 \pm 3.21$  and  $17.13 \pm 3.18$ ) and CQA ( $16.21 \pm 2.46$  and  $14.18 \pm 3.51$ ) 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 9). The decreased levels of AChE were increased along with dose of the extract as well as fractions.

Table 3: Effect of different fractions of CQME on different biochemical parameters (mean±SD, n=6)

Group	A C h E ( $\mu$ m o l e / m i n / g )	G S H ( $\mu$ m o l e / m i n / m g p r o t e i n	M D A ( $\mu$ g / g m w e i t h i s s u e )

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		)	
<b>I (PBS)</b>	1 0 . 3 2 ± 1 . 2 2	2 9 . 1 8 ± 3 . 1 4	2 3 . 4 6 ± 0 . 3 0
<b>II (Aβ)</b>	2 3 . 4 3 ± 2 . 1 5	1 5 . 2 9 ± 2 . 2 3	5 1 . 6 3 ± 0 . 5 4
<b>III (Aβ + Donepezil)</b>	1 1 . 4 5 ± 2 . 1 1 * * *	2 6 . 2 1 ± 3 . 1 2 * * *	2 6 . 6 6 ± 0 . 3 5 * * *
<b>IV (200mg/kg CQME + Aβ)</b>	1 6 . 5 8 ± 1 . 8 3 * *	1 8 . 2 8 ± 1 . 6 6 * *	3 1 . 6 4 ± 0 . 7 4 * *
<b>V (400 mg/kg CQME + Aβ)</b>	1 3 . 9 1 ± 2 . 2	2 4 . 5 9 ± 1 . 5	2 9 . 7 9 ± 0 . 8

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	1 * * *	4 * * *	3 * *
<b>V1 (Aβ + 200mg/kg CQEA)</b>	1 5 · 1 5 ± 3 · 2 6 * *	* 1 9 · 3 6 ± 3 · 1 8	* * 3 0 · 9 8 ± 1 · 0 7
<b>V11 (Aβ + 400 mg/kg CQEA)</b>	1 2 · 1 2 ± 2 · 2 3 * * *	* * 2 5 · 3 9 ± 3 · 2 5	* * * 2 7 · 2 6 ± 1 · 8 8
<b>V11(Aβ + 200mg/kg CQH)</b>	1 9 · 1 7 ± 3 · 2 1 * *	* 1 8 · 2 7 ± 3 · 0 8	* 4 7 · 3 6 ± 1 · 1 6
<b>IX (Aβ + 400mg/kg CQH)</b>	1 7 · 1 3 ± 3 · 1 8 * * *	* * 2 0 · 6 8 ± 2 · 9 4	* * 3 4 · 7 8 ± 1 · 4 3
<b>X (β + 200 mg/kg CQA)</b>	1 6 ·	* 1 7 ·	* 4 3 ·

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	2 1 ± 2 · 4 6	· 1 7 ± 3 · 1 1	7 2 ± 1 · 8 6
<b>XI (Aβ + 400 mg/kg CQA)</b>	1 4 · 1 8 ± 3 · 5 1 *	* * 2 2 · 3 4 ± 2 · 2 4	* * 3 9 · 9 2 ± 2 · 5 4
<b>XII (Aβ + 200mg/kg CQC)</b>	1 6 · 1 9 ± 2 · 2 5 *	* 1 8 · 1 9 ± 2 · 1 3	* 4 3 · 3 1 ± 1 · 6 2
<b>XIII (Aβ + 400mg/kg CQC)</b>	1 3 · 1 6 ± 3 · 1 7 * *	* 2 2 · 5 3 ± 3 · 2 7	* * 3 3 · 6 7 ± 2 · 6 4

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

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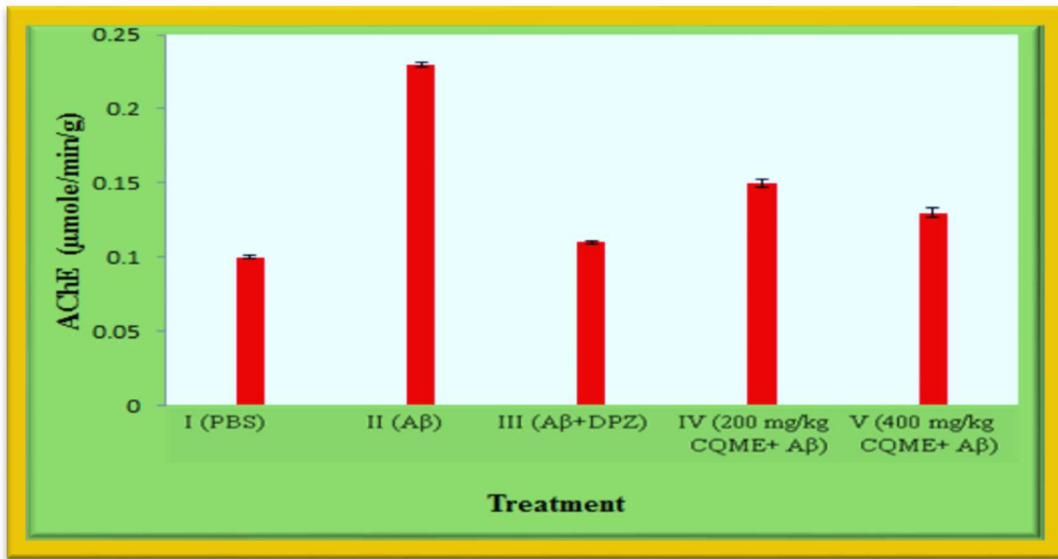


Figure 8: Effect of CQME on AChE levels in amnesia mice (mean±SD, n=6)

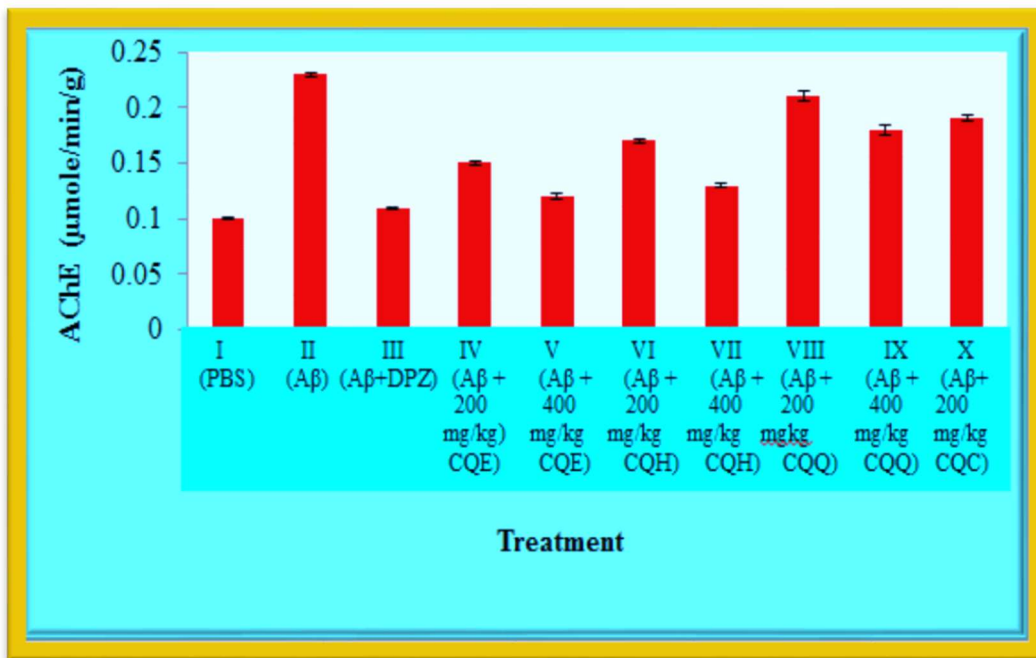


Figure 9: Effect of different fractions of CQME on AChE levels in amnesia mice (mean±SD, n=6)

3.2.4.2. Glutathione peroxidase (GPx)

Glutathione levels are decreased in negative (0.0159±0.00023) when compared to control (0.0298±0.00014), and levels are increased in methanolic extract of 200 mg/kg and 400 mg/kg respectively as follows 0.0266±0.00186 and 0.0275±0.0014. Glutathione levels are decreased in negative (0.0159±0.00023) when compared to control (0.0298±0.00014), and levels are increased in CQEA (0.0256±0.0018 and 0.0279 ± 0.0025), CQC (0.0239±0.0013 and 0.0253±0.0027), CQH (0.0247 ± 0.0008 and 0.0268 ± 0.0004) and CQA (0.0227±0.0011 and 0.0242±0.0024) 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.

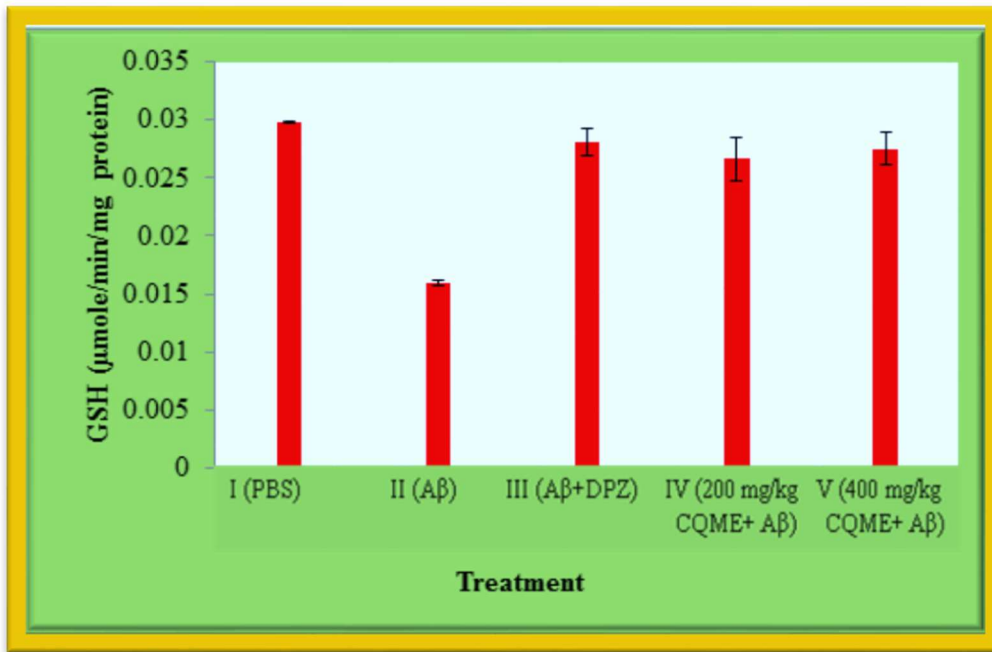


Figure 10: Effect of CQME on GSH levels in amnesia mice (mean±SD, n=6)

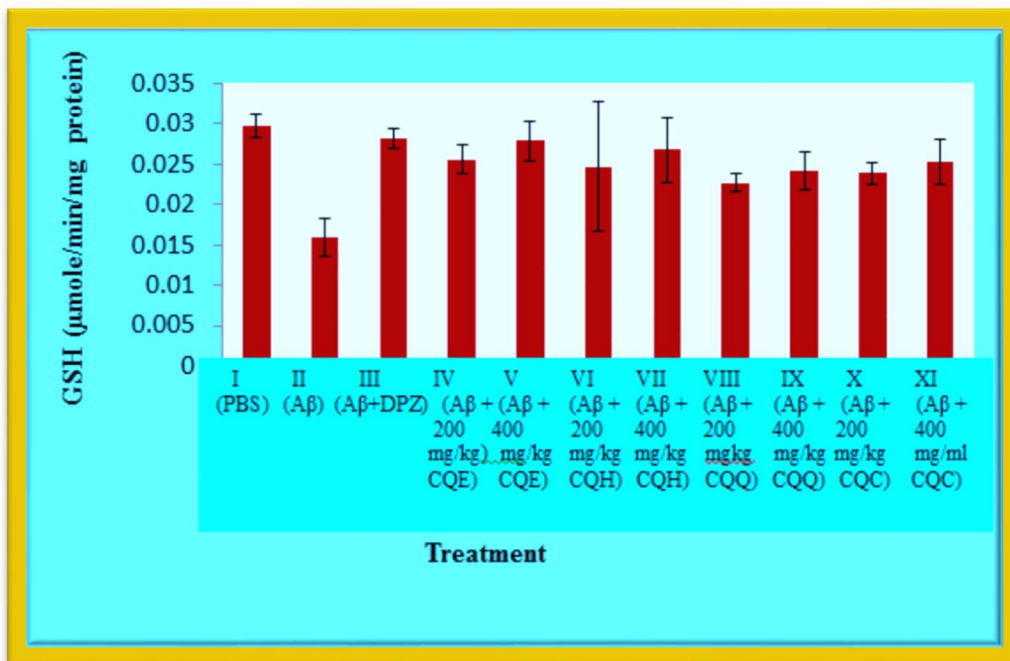


Figure 11: Effect of different fractions of *Cissus quadrangularis* on GSH levels in amnesia mice (mean±SD, n=6)

3.2.4.3. Estimation of MDA

MDA levels are increased in negative (51.63±0.5464) when compared to control (23.46±0.305), and decreased levels are observed in methanol extract was found to be 39.98±0.3012 and 30.14±1.42 μg/g/wet tissue for 200 and 400 mg/kg dose respectively. Decreased levels were observed in CQEA (39.98 ± 1.07 and 31.26 ± 1.88 for 200 and 400 mg/kg), CQC (43.31 ± 1.62 and 33.67 ± 2.64 200 and 400 mg/kg), CQH (41.36 ± 1.16 and 34.78 ± 1.43 200 and 400 mg/kg) and CQA (47.72±1.86 and 39.92±2.54) 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 *Cissus quadrangularis* fractions. The results are presented in Figure 12 and 13.

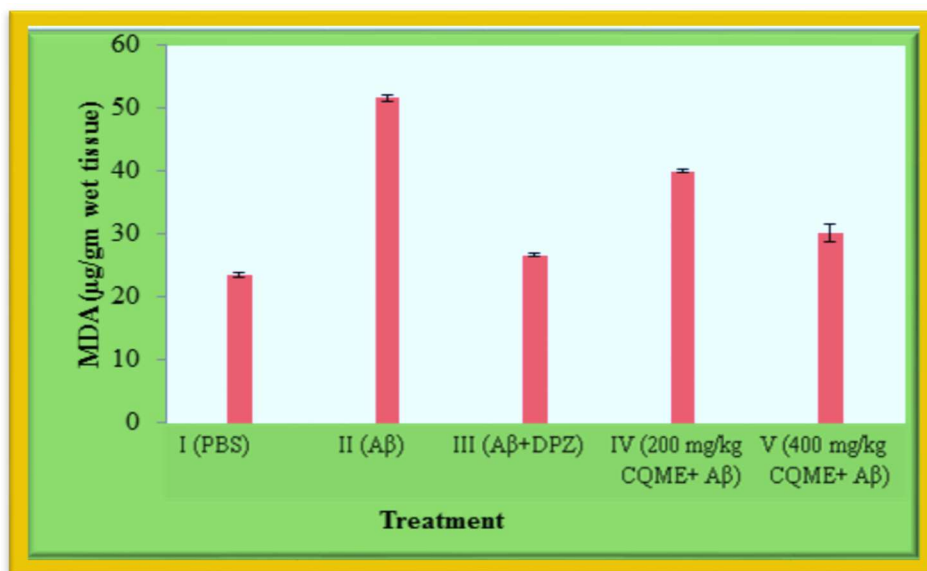


Figure 12: Effect of CQME on MDA levels in amnesia mice (mean±SD, n=6)

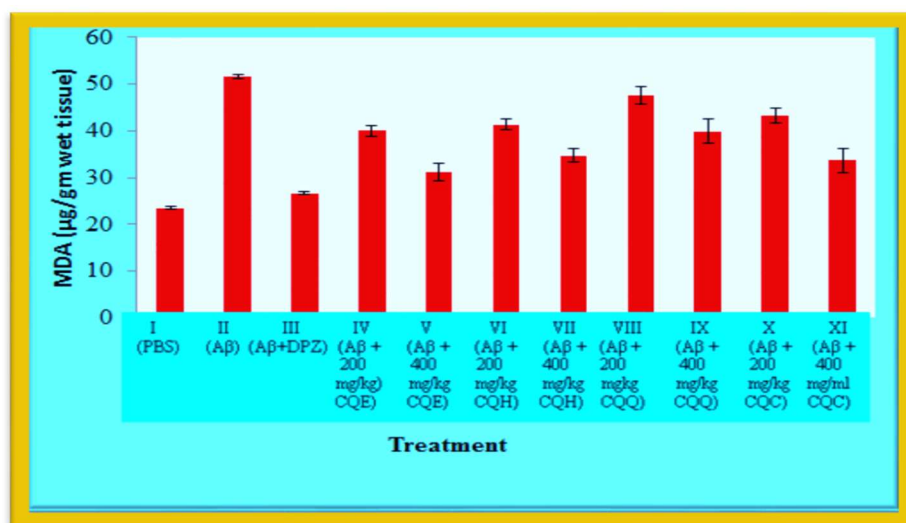


Figure 13: Effect of different fractions of CQME on MDA levels in amnesia mice (mean±SD, n=6)

#### 3.2.4.4. MAO Assay

The neurotransmitter metabolic enzyme responsible for the metabolism of biogenic amines in the brain were significantly ( $P<0.001$ ) elevated after Aβ injection in negative control animals ( $31.88 \pm 0.53$  and  $34.60 \pm 0.86$  for MAO-A and B, respectively) when compared with the control group ( $20.32 \pm 0.82$  and  $21.33 \pm 0.69$  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 CQME and CQEA on MAO-A and B were represented in Table 4 and Figures 14 and 15.

**Table 4: Effect of CQME & CQEA on MAO-A & B parameters (Mean ± SD, n=6)**

Group	MAO (nmol/mg protein.h)	
	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 (200 mg/kg CQME + Aβ)	26.56 ± 0.85 *	31.37 ± 0.59 *
V (400 mg/kg CQ)	23.87 ± 0.70	28.46 ± 0.78 **

ME + Aβ)	66 *	
VI (Aβ + 200 mg/kg CQEA)	25.45 ± 0.77 *	29.05 ± 0.94 *
VII (Aβ + 400 mg/kg CQEA)	22.37 ± 0.89 *	24.18 ± 1.26 **

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

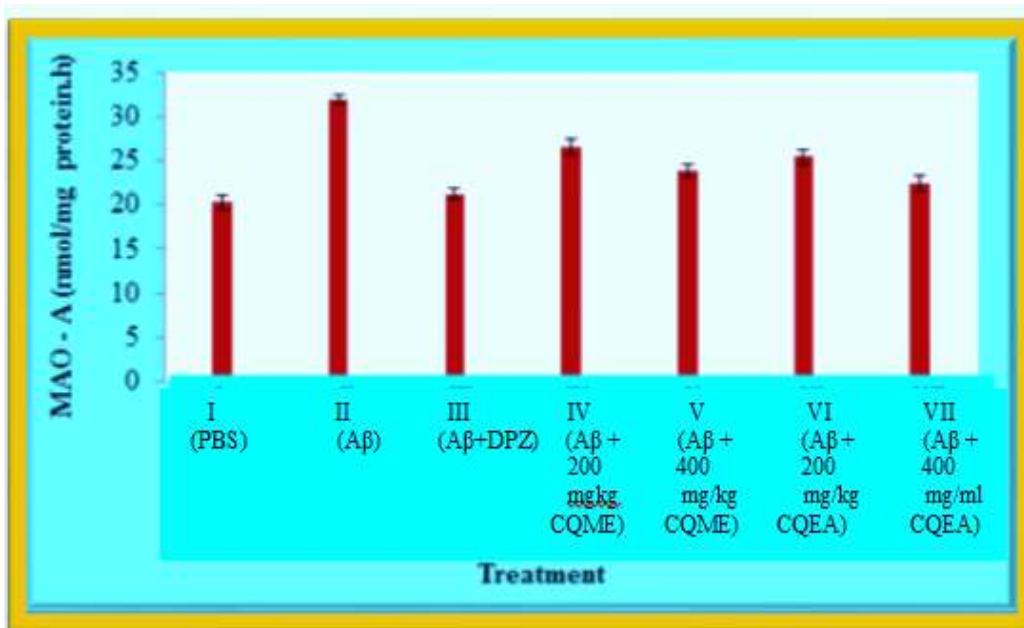


Figure 14: Effect of CQME and CQEA on MAO-A levels in amnesia mice (mean±SD, n=6)

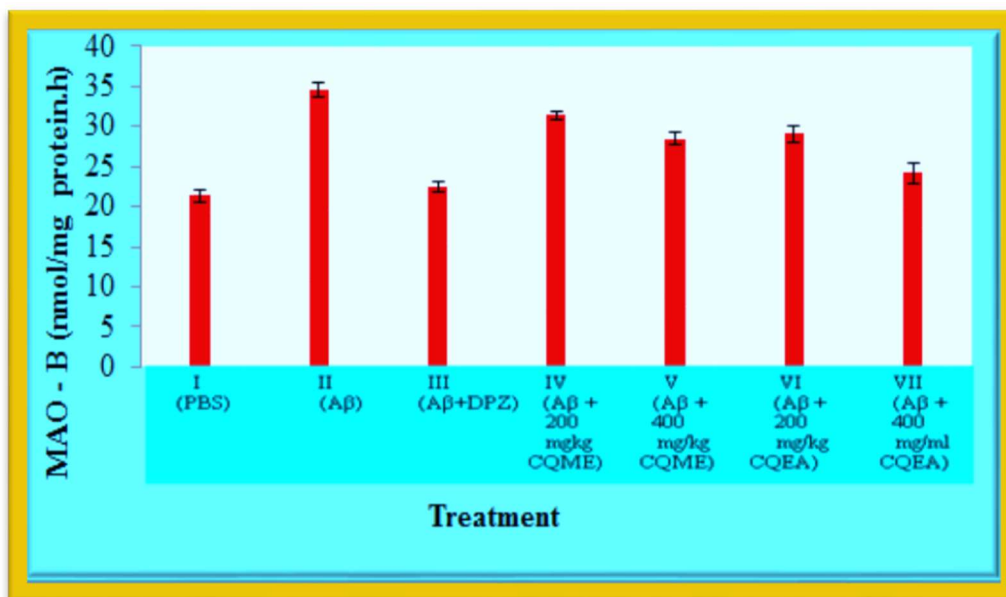


Figure 15: Effect of CQME and CQEA on MAO-B levels in amnesia mice (mean±SD, n=6)

### 3.2.4.5. 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 GSH activity between the low dose and high dose indicated a significant ( $P<0.05$ ) improvement. In case of catalase enzyme the treatment of CQME 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 CQME. 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 5 and Figures 16, 17.

**Table 5: Effect of CQME and CQEA**

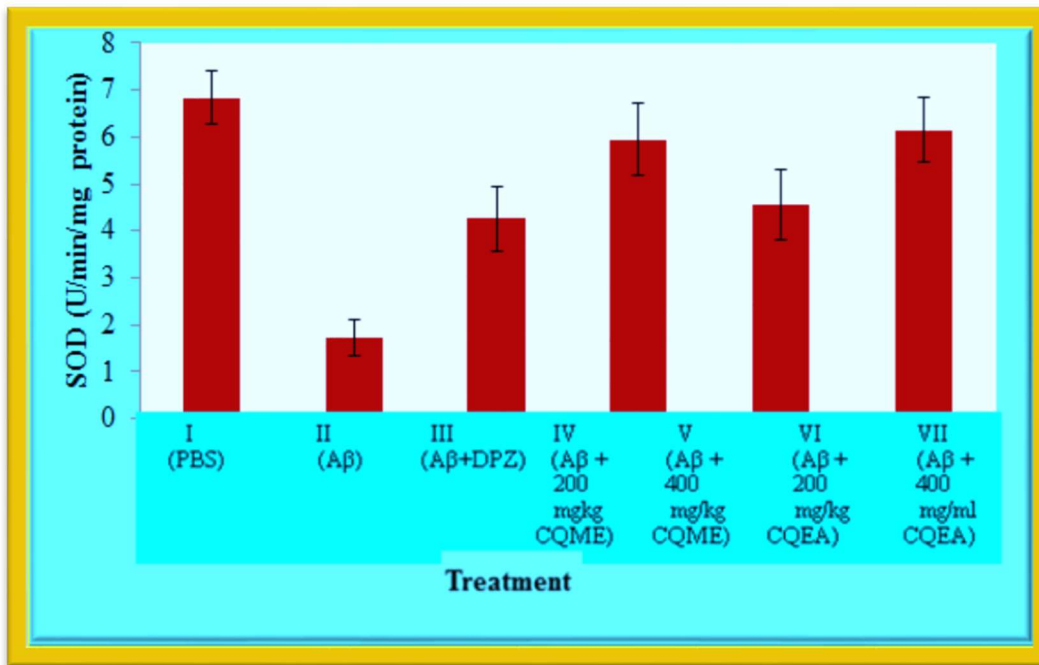
**on free radical scavenging property**

<b>G r o u p</b>	<b>S  O D ( U / m i n / m g p r o t e i n )</b>	<b>C  a t a l a s e ( U / m g p r o t e i n )</b>	<b>V I T . C μ g / m g p r o t e i n .</b>
<b>I ( P B S )</b>	6 .8 3 ± 0 . 5 7	2 .1 7 ± 0 . 0 6	0 .8 9 ± 0 . 0 7
<b>I I ( A β )</b>	1 .7 3 ± 0 . 3 9	0 .8 5 ± 0 . 0 9	0 .5 1 ± 0 . 0 6
<b>I I I ( 2 0 0 m g / k g C Q M E + A β )</b>	4 .2 6 ± 0 . 6 9 *	1 .5 7 ± 0 . 2 7 *	0 .6 7 ± 0 . 0 3 *
<b>I V</b>	5 .	1 .	0 .

<b>( 4 0 0 m g / k g C Q M E + A β )</b>	9 4 ± 0 . 7 7 * * *	8 3 ± 0 . 2 4 * *	7 5 ± 0 . 0 3 * *
<b>V ( A β + 2 0 0 m g / k g C Q E A )</b>	4 .5 4 ± 0 . 7 5 * *	1 .8 1 ± 0 . 2 6 * *	0 .7 1 ± 0 . 0 6 * *
<b>V I I ( A β + 4 0 0 m g / k g C Q E A )</b>	6 .1 5 ± 0 . 6 8 * * *	2 .0 4 ± 0 . 3 8 * * *	0 .8 2 ± 0 . 0 5 * * *

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

Figure 16: Effect of CQME and CQEA on



SOD levels in amnesia mice (mean±SD, n=6)

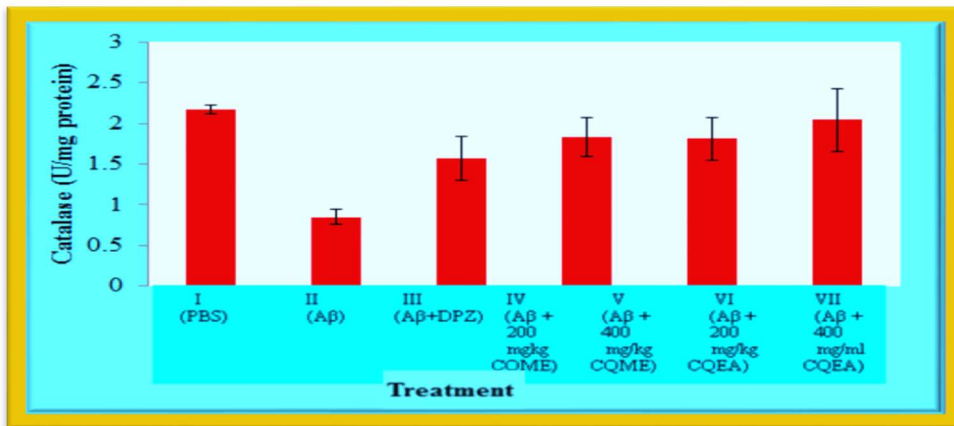


Figure 17: Effect of CQME and CQEA on Catalase levels in amnesia mice (mean±SD, n=6)

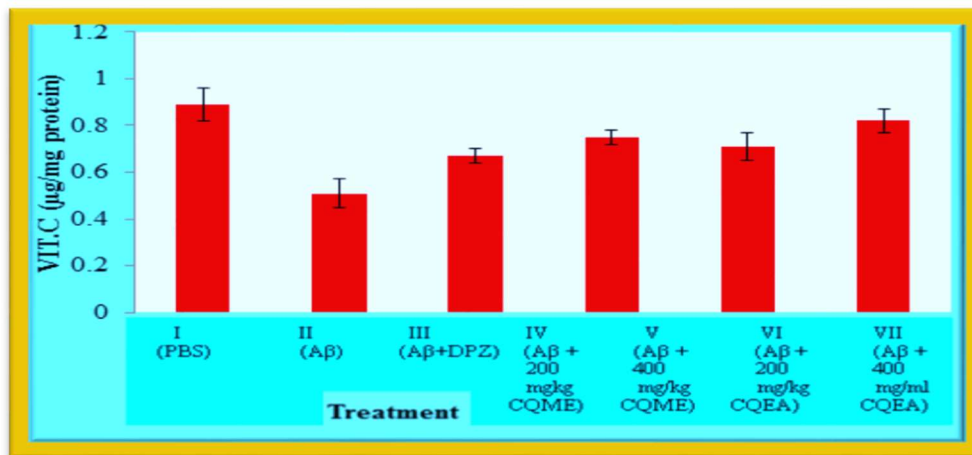


Figure 18: Effect of CQME and CQEA on VIT.C levels in amnesia mice (mean±SD, n=6)

The antioxidant property of different CQME 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 5 and Figure 19). The IC<sub>50</sub> value of

CQME, CQEA, CQC, CQH and CQA fractions were found to be 29.76, 26.42, 33.77, 30.15 and 41.04 µg/mL, with respective to IC<sub>50</sub> value of ascorbic acid and was found to be 12.92 µg/mL.

### 3.3. Discussion

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 24. Dried stems of *Cissus quadrangularis* 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. *Cissus quadrangularis* tested for antialzheimer model in novel object recognition test showed dose-dependent increase in recognition index. From the behavioral, biochemical and DPPH antioxidant test results, MECQ and CQE showed statistically significant results, Hence, MECQ and CQE 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 *Cissus quadrangularis*

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

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