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Nueroprotective and Antioxidant Potential of Methanolic and Aqueous Peel Extract of Citrus Sinesis on B-Amyloid Induced Alzheimers in Mice

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Abstract: ***This study delves into the intricate pathophysiology of alzheimers disease AD, a leading neurodegenerative disorder characterized by significant neuronal loss and cognitive decline, primarily attributed to oxidative stress. Tracing its roots back to Alois alzheimers discovery in 1906, the research the β-amyloid hypothesis, positing that the accumulation of amyloid plaques plays a pivotal role in AD pathogenesis. These plaques, resulting from the abnormal cleavage of amyloid precursor protein APP, alongside hyperphosphorylated tau proteins, disrupt neuronal function and communication, leading to neurodegeneration and dementia. The paper further investigates the potential therapeutic effects of citrus sinensis peel extract, employing an experimental model to amyloid -induced neurotoxicity. Through a comprehensive analysis involving behavioral and biochemical assessments, including acetylcholinesterase and glutathione estimation, the study aims to elucidate the neuroprotective properties of the extract against AD pathology. This research contributes to the ongoing exploration of natural compounds in migrating alzheimers diseases devastating impact, offering insights into novel treatment avenues grounded in the intricate mechanisms underlying neurodegeneration.***

Keywords: citrus sinesis, extraction. DTNB, Anova, GSH

1. Introduction

Alzheimers disease is the one of the neurodegenerative diseases characterized by the loss of neurons and amnesia, intellectual ability. Oxidative stress is one of the reason for Alzheimer's disease. Alzheimer's disease was discovered in 1906 by Alois Alzheimers, a German neurologist and psychiatrist. The disease was initially observed in a 50- year-old women named Auguste D. her family brought her to Dr. Alzheimer in 1901 after noticing changes in her personality and behaviour. The family reported problems with memory, difficulty speaking, and impaired comprehension. Dr. Alzheimer later described Auguste as having an aggressive form of dementia, manifesting in memory, language and behavioural deficits.

Neuropathology of Alzheimer's Disease

Amyloid Hypothesis:
Pathophysiology of AD, debate goes back to the Alzheimer's time 1907 when he observed the neuropathological features of the disease i.e. amyloid plaques and hyperphosphorylated NFTs. β-amyloid plaques are thought to play the central role in AD pathogenesis.

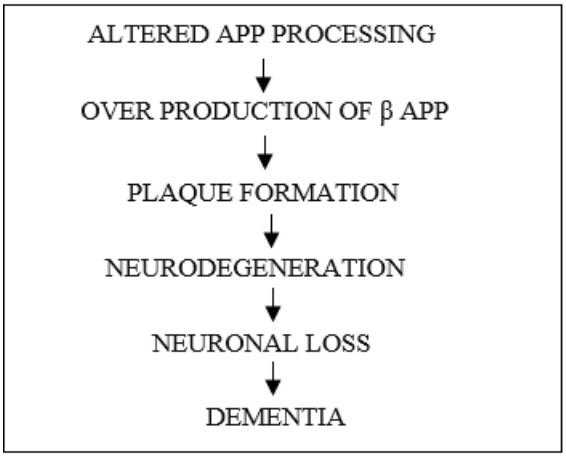
β-amyloid plaques are clumps of insoluble peptides that result from the abnormal cleavage amyloid precursor protein (APP), the exact function of which is unknown. APP is normally cleaved by 3 enzymes; β-secretase, γ-secretase and α-secretase. Cleavage by β-secretase, followed by γ-secretase, yield a soluble 40 amino acid peptide. In AD, a variant form of the γ-secretase cleaves APP at an incorrect place, creating a 42 amino acid peptide called Aβ42 or Aβ, which not soluble and aggregate into identifiable clumps termed as β-amyloid plaques, α- secretase actually serves a protective function as it cleaves APP at a site that prevents Aβ formation.

Genetic studies of AD support role of γ-amyloid plaques and β-secretase in AD pathogenesis. 3 genes have been identified in familial AD (APP, PS1 [presenilin 1], and PS2) and all are known to be involved with formation of Aβ. APP is the gene that codes for APP and is located on chromosome 21. Interestingly, people with Down's syndrome ultimately develop AD at younger age than the general population (Selkoe. 2005). During the AD pathogenesis, tau proteins become hyperphosphorylated, disturbing their bonds to microtubules, thus collapsing microtubule structure and destroying the neurons transport and communication system. Neuronal cell death ensues. Although the causal relationship unclear, hyperphosphorylation of tau is thought to occur after plaques formation

- β-amyloid is a fragment from a larger protein called amyloid precursor protein (APP), a transmembrane protein that penetrates through the neuron's membrane.

- APP is critical to neuron growth, survival and post-injury repair. In Alzheimer's disease, an unknown process causes APP to be divided into smaller fragments by enzymes through proteolysis.
- One of these fragments gives rise to fibrils of β-amyloid, which form clumps that deposit outside neurons in dense formations known as senile plaques.
- Alzheimer's disease has been identified as a protein misfolding disease (proteopathy), caused by accumulation of abnormally folded Aβ and tau proteins in the brain. Plaques are made up of small peptides, 39–43 amino acids in length, called beta-amyloid (Huang *et al.*, 2005).
- These change in calcium channels causes excess influx and cascades the neurotoxicity (Antanitus, 1998). The magnitude of AD has three organized aspects of life cycle with the role as Aβ initiation, construction, its degradation and aggregation. During the process of Aβ in normal cells, in the step of degradation, some molecules of peptides escape from intracellular or extracellular degradation and accumulate to form aggregates of Aβ peptides (oligomers or polymers). These oligomers or polymers are highly cytotoxicity.

AMYLOID CASCADE HYPOTHESIS (John hardy *et al.*, 1991)



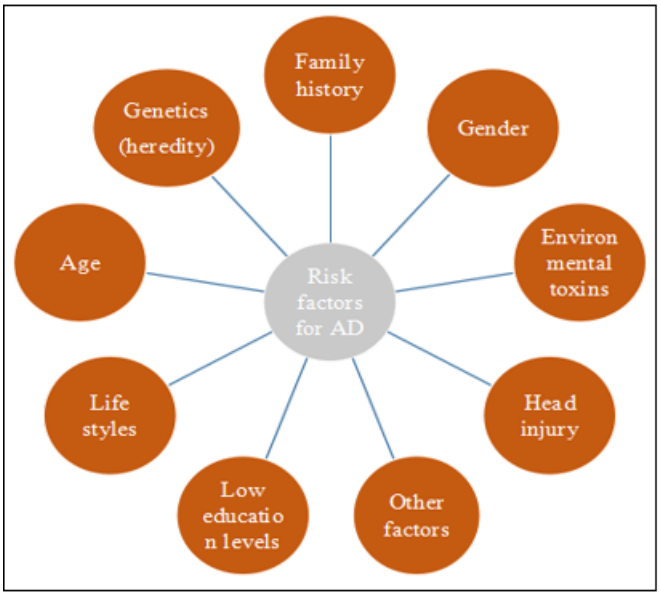


Figure 1: Risk factors for AD

2. Materials and Methods

Peel Material and Extraction:
The peel of *C.sinensis* were collected from kakatiya university, warangal, telangana, india, during month of november and authenticated by botanist Prof. Musthafa, department of botany, kakatiya university, warangal, telangana, india. The collected peels were shade dried, powdered and extracted with methanol by soxhalet apparatus.

Procedure for Extraction of C. Sinensis:
Orange peel (1kg) was extracted exhaustively with 95% methanol in a soxhalet apparatus by continuous heat extraction. The extract was concentrated to a small volume and then evaporated to dryness. The potent yield of *citrus sinensis* extract is then dry extract were subjected to various chemical tests. The potent yield of *citrus sinensis* extract is 28.32%.

Fractionation of Extract:
Dried extract of peel of *citrus sinensis* was taken and treated with methanol and H_2O and separated using a separating funnel. Both the methanol and aqueous fractions are collected.

Animals:
Swiss albino mice (male) are procured from jeva agencies, Ghatkesar, Hyderabad, Animals weighing 18-22 g at the age of 5-6 weeks and the animals were kept under standard conditions maintained at 25±3°C; 35-60% humidity, 12 hr dark light/dark cycle & standard pellet diet and drinking water *ad libitum*. The animals were acclimatized to the laboratory conditions for experiment. The experiment protocol was ethically approved by Institutional Animal Ethical Committee (IAEC/10/Cups/KU/2019) Kakatiya university, Warangal.

Grouping and Induction of Neurotoxicity:
Animals were divided in to five groups, each containing six mice.

Animal group	Receiving materials
Group-1	Phosphate buffer saline
Group-2	Amyloid beta i.e. Injection (3mg/kg)
Group-3	Aβ + Donapezil (5 mg/kg)
Group-4	Aβ + 200 mg/kg M. peel extract of *C.sinensis*
Group-5	Aβ + 200 mg/kg Aq. peel extract *C.sinensis*

Figure 6: Groping of Animals

Neurotoxicity was induced by i.c.v injection of β-amyloid peptide (25-35) by identifying bregma point in the skull (Laursen and Belknap 1986).

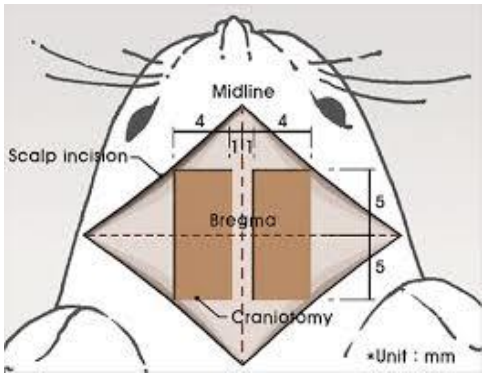


Figure 3: Identification of bregma point and i.c.v injection of β-amyloid.

Drug treatment started on the 14th day of the β-amyloid treatment and continued for six days. On the 7th day of the drug treatment or 21st day of β-amyloid treatment behavioural studies and biochemical parameters were estimated.

Methods determining behavioural parameters:

- Jumping box test
- Rectangular maze test
- Y-maze test

Methods determining biochemical parameters:

- Acetyl cholinesterase estimation
- Glutathione estimation
- DPPH radical scavenging Assay
- Measurement of lipid peroxidation

Behavioural Parameters Estimation

1) Jumping Avoidance Box (Conditioned Avoidance Test):

Box divided into 2 equal chambers by Plexiglas partition, with a gate providing access to adjacent compartment through 14*17 cm space. In each trial animal is subjected to light for 30 seconds followed by a sound stimulus for 10 seconds. Immediately after sound stimulus, mice receive a single low intensity foot shock (0.5mA, 3 sec). Each animal received a daily session of 15 trials with an inter trial duration of 15 seconds for 5 days.

Figure 4: Jumping box

2) Rectangular Maze Test:

Assessment of memory was done using medicraft rectangular maze. The apparatus consisted of three interconnected chambers A, B & C. Chamber B constituted the maze. Food deprived mice were placed in chamber A & challenged to learn & to remember the location of C, after travelling through chamber B. Their presence in chamber C was indicated by a pilot light. Chamber C contained the reward which was food for the hungry animal. The animals were trained which for consecutive daily sessions, & the time required to transverse the maze was noted. They were considered trained when the maze completion time for three consecutive days were more (or) less constant. Maze traversing time was than recorded for each animal before & after drug treatment.

Figure 5: Rectangular maze

3) Y- MAZE Test:

Y. T-maze served as the enteroceptive model to evaluate acquisition of spatial memory in experimental animal models. The apparatus was constructed of plain wood & consist of identical three arms he arms were randomly designated.

a) Start arm in which the rat started to explore (always open)
b) Novel arm: which was blocked during the first trial, but open during the second trial & the other arm was always open.

Each arm was 35cm×6cm×15cm (width×height×length). The maze has an equilateral centre, each arm of the Y beginning from each side of the triangle & extending radially away from the centre at an angle of 120°, forming the letter Y shape of the maze. It was important that the three arms be made similar to prevent preference on the part of the animal when introduced in to the maze. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli. Each animal was placed at the end of the start arm and allowed to move freely through the maze for 8 min. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already visited. The series of arm entries, including possible returns to the same arm, is recorded by a video-tracking system (VJ instruments, Washim, Maharastra, India). Alteration is defined as the successive entries into the three arms, on overlapping triplet sets. The percentage of alteration is calculated as the ratio of actual alterations to possible alterations, defined as the total number of arm entries minus two, and multiplied by hundred. Typically, mice exhibit an alteration percentage of 60-70%, and perform 25-35 arm entries within the 8 min session.

% alteration = {(No. of alterations) / (Total arm entries-2)}*100

Figure 6: Y-maze

Biochemical Estimations

1) Acetylcholinesterase (ACHE) Enzyme Determination
Estimation of Brain Cholinesterase:
Acetyl cholinesterase enzyme activity was estimated by Elman method.

Reagents
0.1M phosphate buffer
Solution A: 5.22gm of potassium hydrogen phosphate and 4.68gm of sodium hydrogen phosphate are dissolved in 150ml of distilled water.

Solution B: 6.2gm of sodium hydroxide dissolved in 150ml of distilled water. Solution B is added to solution A to get the deserved pH (8.0 or 7.0) and then finally the volume is made upto 300ml with distilled water.

DTNB Reagent
39.6mg of DTNB with 15mg of sodium bicarbonate is dissolved in 10ml of 0.1M phosphate buffer (pH 7.0).

Acetylthiocholine (ATC)
21.67mg of acetylthiocholine was dissolved in 1ml of distilled water.

Preparation of Brain Homogenate
The animals were scarified by anaesthetized using thiopentone and brains of the animals were removed quickly and placed in ice cold saline. The tissues were weighed and homogenized in 0.1M phosphate buffer (pH-8) and the brain homogenate was used for the estimation of Brain AchE level.

Estimation of Brain Acetyl cholinesterase level
The esterase activity was measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AchE was allowed to react with the –SH reagent 5, 5'-dithiobis nitro benzoic acid, which is reduced to thio nitro benzoic acid, a yellow coloured anion with an absorption maxima at 412nm.

Assay Procedure
1) 0.4ml of aliquot of brain homogenate was added to a cuvette containing 2.6ml of phosphate buffer (0.1M) and to this 100µl of DTNB was added.
2) The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412nm in spectrophotometer, when absorbance reaches a stable value, it was recorded as the basal reading.
3) 20µl of substrate (ATC) was added and change in absorbance was recorded for a period of 10 minutes at intervals of 2 minutes. Change in the absorbance per minute was determined.

Reagent Sample Blank
Phosphate buffer solution 2.7ml, Supernatant 0.4ml and DTNB 0.1ml. The enzyme activity was calculated using the following formula :
$R = 5.74\ (10^{-4})\ \Delta A/Co$
R = Rate in moles substrate hydrolyzed per min per gm of tissue,
ΔA= Change in absorbance per min,
Co = Original concentration of tissue (mg/ml)

2) Assay of Reduced Glutathione (GSH) (Lawrence and Burk, 1976)
Reagents:
- 0.8 mM EDTA
- 0.32 M Phosphate buffer, (pH 7.0)
- 3 mM Reduced glutathione
- 0.3 M Disodium hydrogen phosphate
- DTNB solution (40 mg of DTNB in 100 ml of 1% sodium citrate)

Preparation of Brain Homogenate:
Mice were decapitated under anesthesia and brains were quickly removed out and homogenized in 100mM Tris HCl buffer of pH 7.4 containing 0.1mM EDTA to yield 5% (w/v) homogenate. The homogenate was then cold centrifuged at 10,000 rpm for 10mins and the resultant supernatant was used to estimate the following biochemical parameters.

Procedure for Standard Graph:
- Reduced Glutathione (Reduced GSH) was used as a standard according to the method described by Ellman et al., (1959).
- A stock solution of 1mM GSH (15.4 mg of GSH dissolved in 50ml of 0.1N HCl) was prepared.
- Serial dilutions were made from the above stock solution of concentrations, 0.2, 0.4, 0.6 and 0.8mM GSH.
- From each concentration, 0.2ml was taken and 2.3 ml of 0.1M Phosphate buffer of H 7.6 and 0.5ml of 1mM DTNB (19.8mg of DTNB was dissolved in 50ml of 0.1M Phosphate buffer of pH 7.6) were added.
- Then this mixture was shaken thoroughly and was incubated for 5mins at room temperature.
- Absorbance of produced yellow colour after the incubation was measured at 412 nm using Systronics Visible Spectrophotometer.

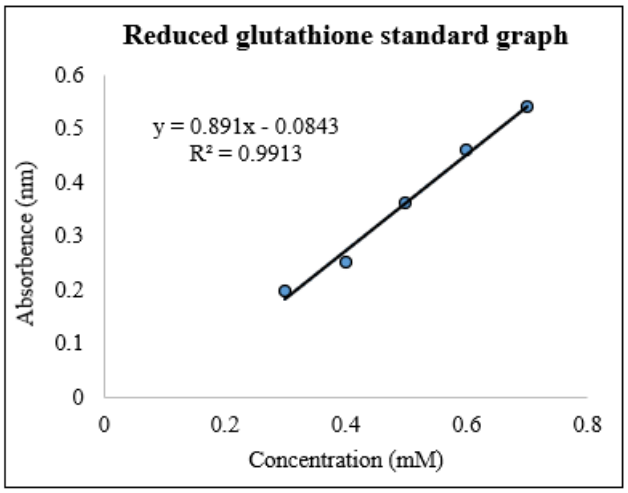


Figure 7: Reduced Glutathione standard graph

Tissue homogenate/aliquot extraction procedure:

- 1ml of supernatant was taken from the tissue homogenate which was prepared by the procedure explained previously.
- This 1ml supernatant was precipitated with 1ml of Sulfosalicylic acid and cold digested for 1hr at 4℃ and then, cold centrifuged at 1200×g at 4℃ for 15mins.
- The supernatant was collected from the above centrifuged mixture and used for assay.

Procedure for estimation of reduced GSH in tissue homogenate extract:

- The estimation procedure described by Ellman et al., (1959) was modified and was used for the reduced GSH estimation.
- Tissue extract was prepared by the above mentioned procedure.
- 0.2ml of tissue extract was added with 2.3ml of 0.1M Phosphate buffer (pH 7.6) and 0.5 of 1mM DTNB. Then, the mixture was incubated at room temperature for 5mins.
- The absorbance of produced yellow colour was observed at 412nm using Systronics Visible Spectrophotometer.

3) DPPH Radical Scavenging Assay:

Procedure for standard graph:

The capacity to scavenge the 'stable' free radical DPPH by ethanol and aqueous fractions were measured according to (Gyamifi *et al.,* 1999) which is based on the reduction of methanolic solution of the colored free radical of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). A methanol DPPH solution (0.1mm, 1ml) was mixed with serial dilutions (10, 20, 40, 60, 80µg/ml) of the methanolic and aqueous fractions incubated for 30min at room temperature. For each concentration the assay was run in triplicate & the absorbance was read at 517nm. Ascorbic acid was used as standard to compare with extracts. IC50 (the antiradical dose required to cause a 50% inhibition) for ascorbic acid, methanol and aqueous fractions were determined. The ability to scavenge the DPPH radical was calculated using the following equation.

% INHIBITION = (A0-A1/A0) × 100

Where; A0 is the absorbance of control & A1 is the absorbance of test.

4) Measurement of Lipid Peroxidation (MDA)

Reagents: Acetic acid (1.5ml, 20%)
Thiobarbituric acid (1.5ml, 0.9%)
Sodium dodecyl sulphate (0.2ml, 9.1%)

Procedure for Standard Graph:

- Standard graph for the lipid peroxidation assay was prepared by the method described by Ohkwa et al., (1979).
- A stock solution of 1,1,3,3-TetraEthoxyPropane (TEP) was prepared by taking 22mg of TEP and dissolved using distilled water and the volume was adjusted to 10ml by distilled water in a 10ml volumetric flask.
- From this prepared stock solution of TEP, 0.2ml, 0.4, 0.6ml, 0.9ml, 1.0ml (2nM, 4nM, 6nM, 9nM and 10nM respectively) were taken in centrifuge tubes.
- 0.2ml of 9.1% SDS, 1.5ml of 20% acetic acid and 1.5ml of 0.9% aqueous solution of TBA were added to above concentrations in centrifuge tubes and were mixed thoroughly.
- The final volume was adjusted to 5ml using distilled water in all tubes and heated in oil bath at 95℃ for 1hr.
- The tubes were cooled then to room temperature and the chromogen resulted was extracted with 5ml of 15:1 v/v n-Butanol and Pyridine mixture by shaking vigorously.
- These tubes were then centrifuged at 4000 rpm for 10mins to separate organic phase and its absorbance was measured at 532nm using Systronics Visible Spectrophotometer.
- The standard graph was prepared by taking MDA concentration on X-axis and corresponding absorbance on Y-axis.

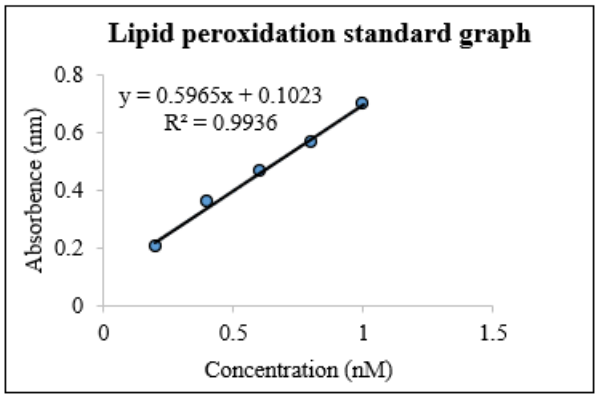


Figure 8: Lipid peroxidation standard graph

Procedure for estimation of MDA levels in Tissue homogenate:

- MDA levels in tissue homogenate was measured by the method developed by Ohkwa et al., (1979).
- 0.2ml of tissue homogenate was taken and was added with 0.2ml of 9.1% SDS, 1.5ml of 20% acetic acid and 1.5ml of 0.9% Aqueous solution of TBA.
- The volume of this mixture was made up to 5ml with distilled water and then heated in an oil bath at 95℃ for 1hr.
- It was cooled and then 5ml of mixture of n-Butanol and Pyridine (15:1 v/v) was added and vigorously shaken.
- This mixture was then centrifuged at 4000rpm for 10 mins. The organic layer was separated and the absorbance was measured at 532nm using Systronics Visible Spectrophotometer.

- The tissue MDA levels were measured from the standard curve and expressed as nmol/g tissue.

3. Results

Behavioural Parameters: Jumping Box Test

The below table consists latency period of Control, Standard (DPZ), Negative (Aβ), M. extract, Aq. extract groups in jumping box test.

Table 7: latency period in jumping box

Group	Mean ± (S.E.M)
Control	7.62 ±0.4250
Negative (Aβ)	27.41 ±1.19541
Standard (DPZ)	9.366 ±0.7670***
M. extract	16 ±0.5832***
Aq. extract	19.32 ±0.9544***

*p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. In jumping box test there was an increased in latency period in negative control group (27.41±1.19541) when compared to control (7.62±0.4250), thers is decrease in latency period in groups treated with aqueous extract (19.32±0.9544), methanolic extract (16±5832).

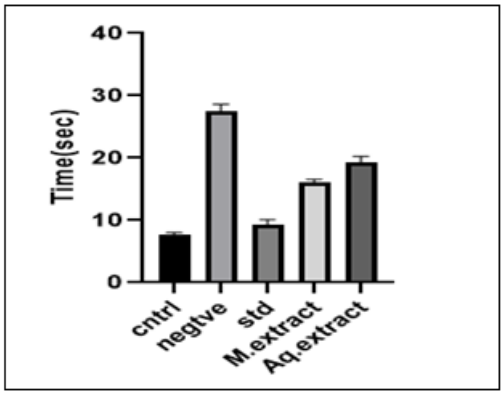


Figure 9: Latency period in jumping box

The graph indicates the latency period of Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract groups.

Rectangular Maze Test:

The table Indicates traverse time of Control, Standard (DPZ), Negative (Aβ), M. extract, Aq. extract groups in Rectangular maze test.

Table 8: Traverse time in rectangular maze

GROUP	Mean ±S.E.M
Control	31.69 ±0.6592
Negative	137.66 ±0.9368
Standard	68.4150 ±0.8631***
M. extract	74.67 ±0.2891***
Aq. extract	82.576 ±0.7421**

*p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. In rectangular maze test there was an increased in maze transverse period in negative control group (137.66±0.9368) when compared to control group (31.69±0.6592) and there is decrease in transverse period in groups treated with aqueous extract (82.57±0.7421), methanolic extract (74.67±0.2891).

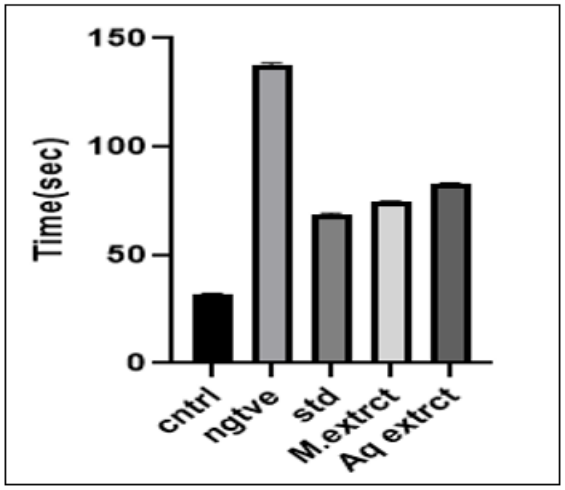


Figure 10: Traversing time in rectangular maze

The graph indicates the maze traversing period of Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract groups.

Y-MAZE TEST:

The table indicates % alteration of Control, Negative (Aβ), standard (DPZ), M. extract, Aq. extract groups in Y-maze test.

Table 9: % alteration in Y-maze

Groups	Mean ±(S.E.M)
Control	74.541 ±1.989
Negative	34.195 ±2.347
Standard	59.529 ±1.619***
M. extract	38.721 ±0.817***
Aq. Extract	45.130 ±1.357**

*p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. In negative control group and control groups the % alteration was found to be (34.195±2.347) and (74.541±1.989) respectively and aqueous extract (45.130±1.357) and methanolic extract (38.721±0.817).

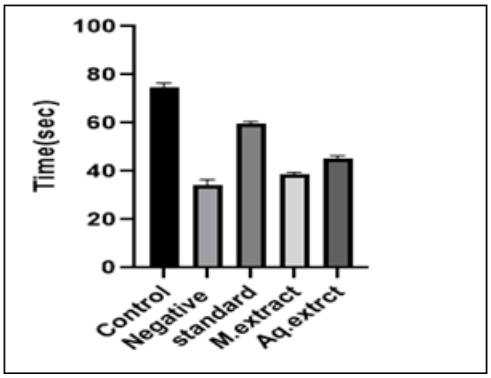


Figure 11: % alteration in Y-maze

Figure 11: Graph indicates the % alteration of Control, Negative (Aβ), Standard (DPZ), M. Extract, Aq. Extract groups.

Biochemical Parameters
Acetyl Choline Esterase Activity

Table 10: Indicates Acetylcholinesterase activity of Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract.

Table 10: Acetylcholinesterase activity

Groups	Mean ±S.E.M
Control	0.3617 ±0.0013
Negative	0.4023 ±0.0017
Standard	0.2351 ±0.007***
M. extract	0.2876 ±0.0031**
Aq. extract	0.2749 ±0.0028**

p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. In biochemical parameters the AChE levels are increased in negative group (0.4023±0.0017), when compared to control group (0.3617±0.0013) and increased levels are observed in aqueous extract (0.2749±0.0028) and methanolic extract (0.2876±0.0031).

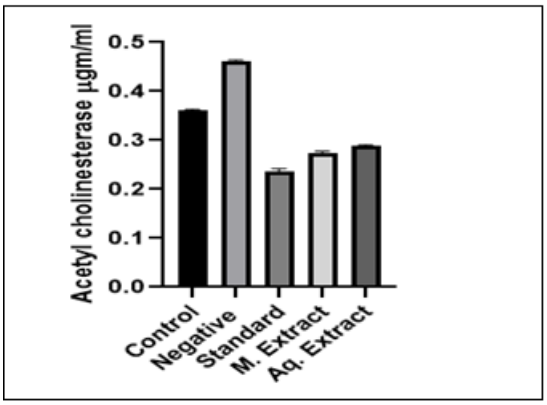


Figure 12: Acetylcholinesterase levels

Figure 12: Graph depicting levels of AChE in Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract groups.

Reduced Glutathione Activity:

Table 11: Indicate glutathione levels of Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract.

Table 11: Reduced Glutathione levels

Groups	Mean ±S.E.M
Control	0.42 ±0.00025
Negative	0.1425 ±0.00270
Standard	0.2587 ±0.005***
M. extract	0.289 ±0.005***
Aq. extract	0.135 ±0.00296***

*p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. Glutathione levels are decreased in negative control group (0.1425±0.00270) when compared to control group (0.42±0.00025) and levels are increased in aqoueus extract (0.135±0.00296) and methanolic extract (0.289±0.005).

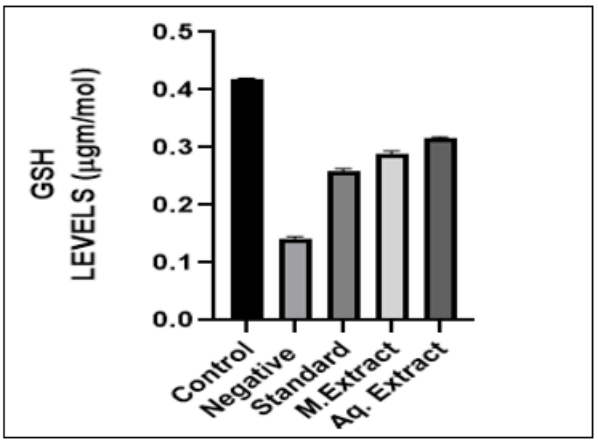


Figure 13: Graph depicting levels of GSH in Control, Negative (Aβ), Standard (DPZ). M. extract, Aq. extract groups.

MDA Estimation:

Table 12: Indicates the levels of malondialdehyde levels of Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract groups.

Table 12: MDA levels

Groups	Mean ±S.E.M
Control	21.4 ±0.4365
Negative	49.54 ±0.5752
Standard	25.67 ±0.3627***
M. extract	27.55 ±0.2375***
Aq. extract	28.98 ±0.3285***

*p<0.05, **p<0.01, ***p<0.001 when negative control compared to control group and treated groups compared to negative control. ANOVA (one-way) followed by Bonferroni's test. MDA levels are increased in negative group (49.54±0.5752) when compared to control group (21.4±0.4365), decreased levels are observed in aqueous extract (28.98±0.3285) and methanolic extract (27.55±0.2375).

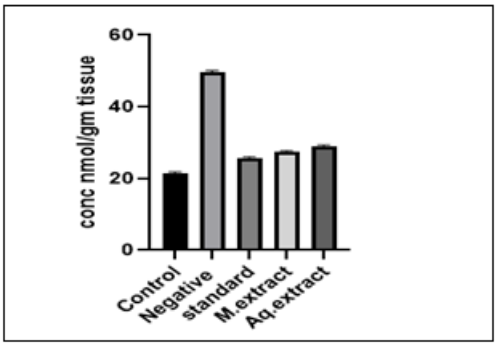


Figure 14: MDA levels

Figure 14: Graph depicting levels of MDA in Control, Negative (Aβ), Standard (DPZ), M. extract, Aq. extract groups.

DPPH Radical Scavenging Activity:
% inhibition of Ascorbic Acid

Table 13: Shows the % inhibition of ascorbic acid at concentration. IC_{50} of ascorbic acid was found to be: 41.19µg/ml

Concentration(µg/ml)	% inhibition
5	17.16
10	19.2
20	26.19
40	49.25
60	72.12
80	85.12

Table 13: % inhibition of ascorbic acid
y = mx+c
y = 0.9654x+10.247 y=50
50-10.247 =39.753
39.753/0.965 =41.19µg/ml

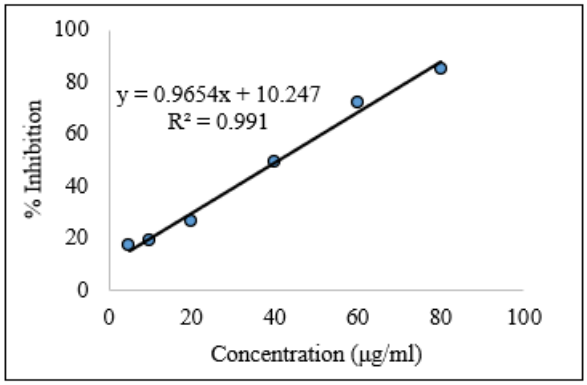


Figure 15: DPPH radical scavenging activity of Ascorbic acid.

Figure 15: The above graph shows DPPH radical scavenging activity of ascorbic acid. The concentration increases % of inhibition increases.

% inhibition of Methanolic Extract:

Table 14: Shows the inhibition of methanolic extract at various concentrations. IC_{50} of methanolic extract was found to be: 27.69µg/ml.

Concentration(µg/ml)	% inhibition
5	29.47
10	39.12
20	44.74
40	61.22
60	74.12
80	87.74

Table 14: % inhibition of M. extra
y = mx+c
y = 0.7451x+29.37 y=50
50-29.37 =20.63
20.63/0.745 =27.69µg/ml
IC_{50} = 27.69µg/ml

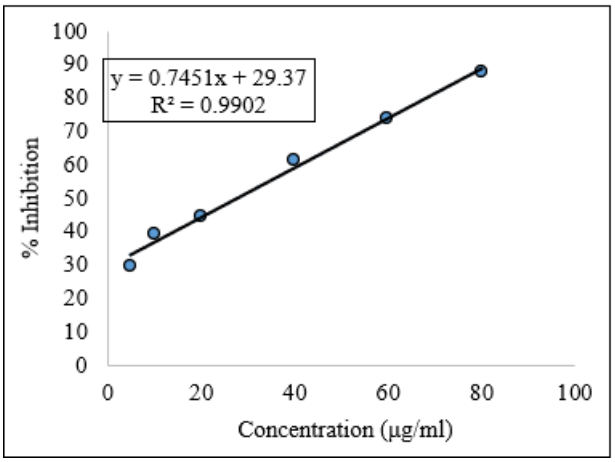


Figure 16: DPPH radical scavenging activity of methanolic extract *C. sinensis*

Figure 16: The above graph shows DPPH radical scavenging activity of methanolic extract of *C. Sinensis.* The Concentration increases % of inhibition increases.

% inhibition of aqueous extract:

Table 15: Shows the % inhibition of Aqueous extract at various concentrations. IC_{50} of Aqueous extract was found to be: **32.731µg/ml.**

Concentration(µg/ml)	% inhibition
5	28.36
10	33.12
20	40.14
40	57.37
60	72.58
80	82.12

Table 15: % inhibition of Aq. Extract
y =mx+c
y=0.7362x+25.903 y=50
50-25.903 =24.097
24.097/0.7362 =32.731µg/ml
IC_{50}=32.731µg/ml

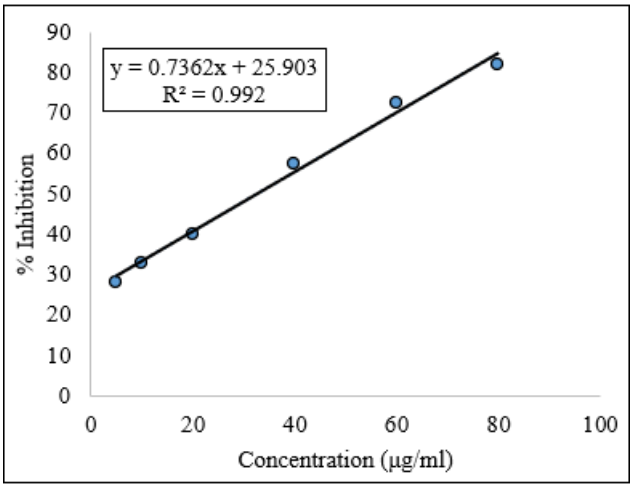


Figure 17: The DPPH radical scavenging activity of Aq. extracts of *C. sinensis*

Figure 17: The graph shows DPPH radical scavenging activity of Aq.extract of *C. sinensis.* The concentration increases % of inhibition increases.

4. Conclusion

From the above results it is found that both methanol and aqueous extracts of *Citrus sinensis* are having significant neuroprotective and anti-oxidant activity. Aqueous extract is having more significant activity when compared to methanol extract. Morevover, greatly reduced in amyloid content was found in brain following aqueous treatment. By fractionating the methanolic extract we can optimize the Alzheimer's disease.

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