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Nueroprotective and Antioxidant Potential of Ethanolic Bulb Extract of Allium Sativum on $Alcl_3$ induced Alzheimers in Rat

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Abstract: ***This study investigates the neuroprotective and antioxidant potential of the ethanolic extract of allium sativum in a rat model of Alzheimers disease induced by $AlCl_3$.The research highlights the significant impact of the allium extract in migrating the effects of Alzheimers, providing a promising avenue for further exploration in the treatment of neurodegenerative diseases.***

Keywords: Allium sativum, extraction. DTNB, Anova, GSH

1. Introduction

Alzheimers disease is the one of the neurodegenerative diseases characterized by theloss of neurons and amnesia, intellectual ability. Oxidative stress is one of the reasons for Alzheimer's disease. In the present study Neuroprotective Potential of *A. sativum* on $AlCl_3$ induced neurotoxicity in rat. Al is reported as a potent neurotoxin and has been associated with AD since exacerbates brain oxidative injury, causes neuronalinflammation, and induces Aβ deposition, which leads to impairment in working memory (Hanna R *et al.*,2020). So Aβ deposition it shown selective potent neurotoxic property, it promotes the inflammatory process and fibrillary aggregation (Pike *et al.*, 1995). In this investigation, we induced the Alzheimer's in rats by injection of $AlCl_3$, which is suitable model for AD (Atsumi et al., 1994).

Survey of literature reveled that *Allium sativum* traditionally used in treatment of constipation, cramps, colic, cough, bronchitis, cold. It has been screened for pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anxiolytic further it has not screened for Alzheimer's activity. So the aim of presentwork was to investigate the potential pharmacological & Neuroprotective effects of *Alliumsativum* on $AlCl_3$ induced Alzheimer's disease in rat.

In biochemical estimations, we found the decreased levels of MDA and AChE and increase in glutathione levels in both extracts. In DPPH radical scavenging activity of ethanolic extracts showed significant % inhibition of free radicals. It shows that ethanolic extract were having significant anti-oxidant activity. So finally ethanolic extract of *Allium sativum* on AD in rats it shows neuro- protective effect by increasing the hippocampal memory in anti-oxidant activity in biochemical parameters.

2. Materials and Methods

Bulb Material and Extraction:
The peel of *Allium sativum* were collected from super market, Hyderabad, Telangana, India, during month of march. The collected bulb were shade dried, powdered and extracted with Ethanolbymaceration.

Procedure for Extraction of *Allium Sativum*
Preparation of Ethanol extract of *A.sativum* bulb The raw *Allium sativum* was slice, dried in air and then pulverized to powder. The extraction was performed by soaking 100g of the pulverized garlic in 600ml of distilled water for 24 hours, The residue and filtrate were obtained by filtering the soaked garlic (*Allium sativum*) using Whatman No.1 filter paper the residue was dried on a cardboard paper and the filtrate was obtained as extract.

Fraction Ation of Extract:
Dried extract of bulb of *Allium sativum* was taken and treated with ethanol and separated using a separating funnel. The ethanol fractions are collected.

Animals:
Wister rats (male) are procured from Mahaveer agencies, Ghatkesar, Hyderabad, Animals weighing 150-200 gm at the age of 12-21 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.

Grouping and Induction of Neurotoxicity:

Animals were divided into four groups, each containing six rats.

Table 1: Grouping of Animals

Animal group	Receiving materials
Group-1	Phosphate buffer saline
Group-2	Inducer ($AlCl_3$) i.c.v injection (100mg/kg)
Group-3	Inducer ($AlCl_3$) + Donapezil (5mg/kg)
Group-4	$AlCl_3$ + 250mg/kg Ehanol bulb extract of *A.sativum*

Neurotoxicity was induced by i.p injection of $AlCl_3$ for 21days. At the end of the study, all the rats were anesthetized; brain samples were quickly harvested for biochemical analysis.

Methods determining biochemical parameters:

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

Biochemical Estimations

1) Acetylc Holinest Erase (ACHE) Enzyme Determination Estimation of Brain Cholinesterase:

Acetylc holinest erase enzyme activity was estimated by Elman method.

Reagents

0.1Mphosphatebuffer

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 up to 300ml with distilled water.

DTNB Reagent

39.6mg of DTNB with 15mg of sodium bicarbonate is dissolved in 10ml of 0.1M phosphate buffer (pH7.0).

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 in0.1M phosphate buffer (pH-8) and the brain homogenate was used for the estimation of BrainAchE level

Estimation of Brain Acetylcholinest erase level

The esterase activity was measured by providing an artificial substrate, acetyl thiocholine (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 benzoicacid, 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 mMEDTA
- 0.32 M Phosphate buffer, (pH7.0)
- 3mM Reduced glutathione
- 0.3 MDi sodium hydrogen phosphate
- DTNB solution (40mg of DTNB in 100ml of1% 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 1mMGSH (15.4mg of GSH dissolved in 50ml of 0.1NHCl) was prepared.
- Serial dilutions were made from the above stock solution of concentrations, 0.2, 0.4, 0.6 and 0.8mMGSH.
- From each concentration, 0.2ml was taken and 2.3 ml of 0.1M Phosphate buffer of H 7.6and 0.5ml of 1mM DTNB (19.8mg of DTNB was dissolved in 50ml of 0.1M Phosphate buffer of pH7.6) were added.
- Then this mixture was shaken thoroughly and was incubated for 5 mins at room temperature.
- Absorbance of produced yellow colour after the incubation was measured 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 ethanolic solution of the colored free radical of 1, 1-diphenyl-2-picryl hydrazyl (DPPH).Amethanol 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-Tetra Ethoxy Propane (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.

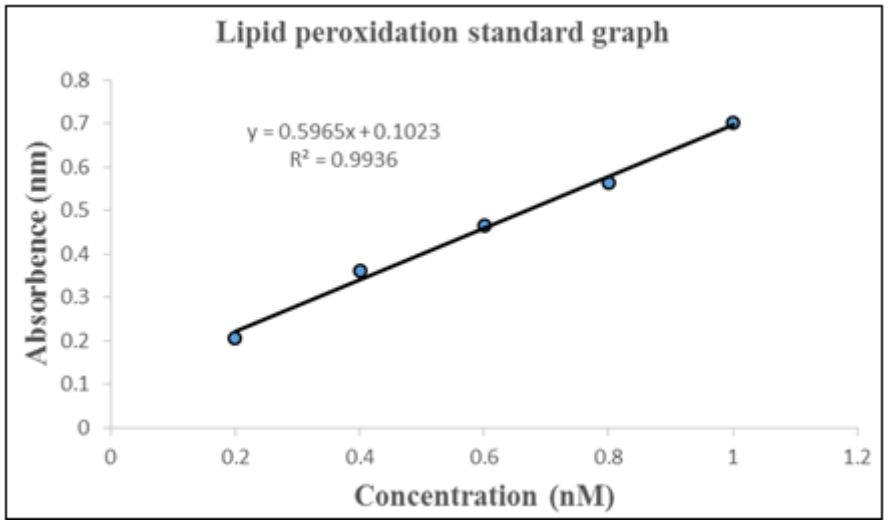

Figure 1: Lipid per oxidation standard graph.

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 upto 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 asnmol/g tissue.

3. Results

Biochemical Parameters Acetylcholineesterase Activity

Table 2: Indicates Acetylcholinesterase activity of Control, Negative (AlCl3), Standard (DPZ), E. extract.

Table 2: Acetylcholinesterase activity

Group	Mean±(SEM)
Control	0.075±0.005
Negative (Alcl3) (100mg/kg)	0.275±0.015
Standard (DPZ,5mg/kg)	0.13±0.010***
Ethanolic fraction(250mg/kg)	0.15±0.01**

*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 (0.275±0.015), when compared to control group (0.075±0.005) and increased levels are observed in ethanolic extract (0.15±0.01**).

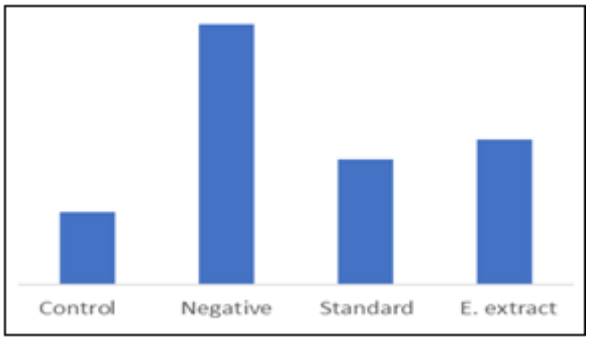

Figure 2: Acetylcholinesterase levels

Figure 2: Graph depicting levels of AChE in Control, Negative ($AlCl_3$), Standard (DPZ), E. extract.

Reduced Glutathione Activity

Table 3: Indicate glutathione levels of Control, Negative (AlCl3), Standard (DPZ), E.extract.

Group	Mean±(SEM)
Control	0.207±0.013
Negative (Alcl3) (100mg/kg)	0.1335±0.0025
Standard (DPZ,5mg/kg)	0.1745±0.003**
Ethanolic fraction (250mg/kg)	0.159±0.003*

Table 3: Reduced Glutathione levels

*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 (0.1335±0.0025) when compared to control group (0.207±0.013) and levels are increased in ehanolic group (0.159±0.003*).

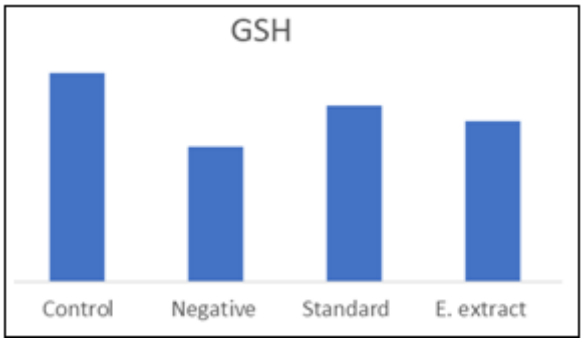

Figure 3: Glutathione levels

Figure 3: Graph depicting levels of GSH in Control, Negative ($AlCl_3$), Standard (DPZ). E. extract groups.

Group	Mean ± (SEM)
Control	17.785 ± 1.565
Negative (Alcl3)(100mg/kg)	57.735 ± 0.0150
Standard (DPZ,5mg/kg)	24.56 ± 0.5600**
Ethanolic fraction (250mg/kg)	27.535 ± 1.565***

Table 4: MDA levels

*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 (57.735±0.0150) when compared to control group (17.785±1.565), decreased levels are ethanolic extract (27.535±1.565).

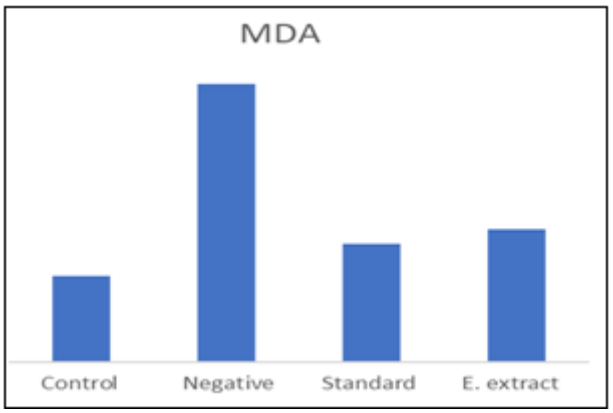

Figure 4: MDA levels

Figure 4: Graph depicting levels of MDA in Control, Negative ($AlCl_3$), Standard (DPZ), E. extract.

DPP Hradical Scavenging Activity:

% Inhibition of Ascorbic Acid

Table 5: Shows the % inhibition of ascorbic acid at concentration. IC50 of ascorbic acid was found to be: 41.19µg/ml.

Table 5: % inhibition of ascorbic acid

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

y=mx+c
y = 0.9654x+10.247y=5050-10.247=39.753
39.753/0.965 =41.19µg/ml
IC_{50}=41.19µg/ml

Figure 5: DPPH radical scavenging activity of Ascorbic acid.

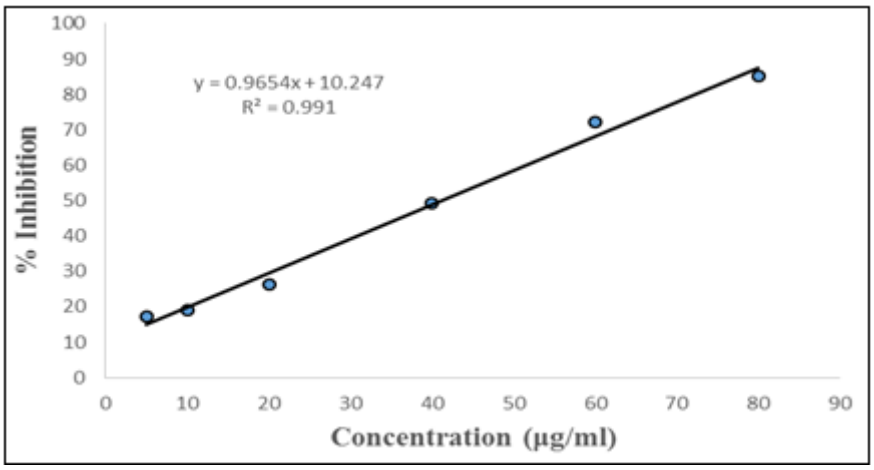

Figure 5: The above graph shows DPPH radical scavenging activity of ascorbic acid. The concentration increases % of inhibition increases.

% Inhibition of Ethanolic Extract:

Table 6: Shows the inhibition of ethanolic extract at various concentrations. IC_{50} of ethanolic extract was found to be: 27.69µg/ml.

Table 6: % inhibition of E. extract

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

y =mx+c
y=0.7451x+29.37y=50
50-29.37=20.63
20.63/0.745=27.69µg/ml

IC_{50}=27.69µg/ml

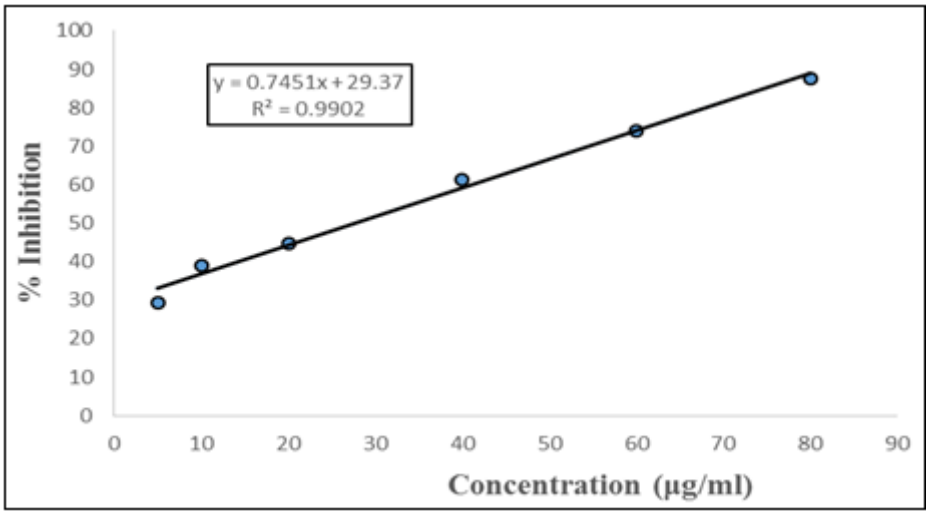

Figure 6: DPPH radical scavenging activity of ethanolic extract *A. sativum*

Figure 6: The above graph shows DPPH radical scavenging activity of ethanolic extract of *A, sativum.*

4. Conclusion

The findings of this study demonstrate the significant neuroprotective and antioxidant properties of the ethanolic bulb extract of allium sativum in a rat model of Alzheimer's disease induced by $AlCl_3$. These results suggest that garlic extract could potentially be utilized as a therapeutic agent in the treatment of Alzheimers disease, warranting further research in this area.a

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