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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

EVALUATION OF NEUROPROTECTIVE EFFECT OF *AMARANTHUS HYBRIDUS* IN BRAIN OF MEMORY IMPAIRED RAT

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KEYWORDS:

Alzheimer's disease, oxidative stress, *Amaranthus hybridus*, Streptozotocin.

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ABSTRACT

Objective: The present study is to investigate the Neuroprotective effect of *Amaranthus hybridus* in brain of memory impaired rat. This study was aimed to evaluate the memory improving effect of *Amaranthus hybridus*. **Methods:** In this present study ICV-STZ at a dose of (1 and 3 mg/ kg) two days produced impaired memory in rats. The impaired rats were treated with *Amaranthus hybridus* for a period of 21 days. Vitamin E was used as a standard drug. At the end of treatment period various behaviour models (Morris water maze and Elevated plus maze) were used for evaluation of memory enhancing effect of the treatment. The brain level of lipid peroxidation, reduced glutathione and superoxide dismutase were estimated to evaluate the role of oxidative stress in AD. **Results:** Administration of *Amaranthus hybridus* with ICV-STZ caused decrease in escape latency time and transfer latency showing improvement of memory in impaired rats. Treatment with Amaranthus hybridus decreased in the level of brain lipid peroxidation and increased in level of brain reduced glutathione and superoxide dismutase indicated for improvement of memory in impaired rats. **Conclusion:** Results conclude that antioxidants like *Amaranthus hybridus* may be used for the treatment on impaired rat as well as for Alzheimer's disease.

INTRODUCTION:

The brain is the most complicated part of the human body that cannot be easily understood. This organ which is three-pound is the centres of skill, translator of the senses, promote of body movement, and regulate of behaviour. This single organ is responsible for every aspect of our body, ranging from heart rate, thinking, reasoning, sexual activity to emotion, language, learning, and memory.

Learning is due to the experience that alters in the behaviour. Which is act of developing recently or modifying and enforcing existing knowledge, behaviours, skill, values or importance and that involve synthesizing distinct types of information. It divided into the long term, mental association and due to experience. The change in the organism by learning and the changes produced are relatively permanent. Human learning may occur as part of education, schooling, training and personal development. It is to be goal-oriented and to be support by motivation. Learning may occur consciously or without conscious awareness [1] [2]. Memory is associated with learning. Memory is a progression that includes different individually separate stages i.e. encoding, consolidation, storage, retrieval and forgetting. Encoding is allows the knowledge from the outer world that is to reach the five senses in the configuration of chemical and physical stimuli [1].

Alzheimer's Disease (AD) - Alzheimer's disease is a neurodegenerative disorder that produces an impairment of cognitive abilities. The common cause of AD is dementia. It impairs the memory and ability to learn, reasoning, judgment, communication and daily routine activities. The pathological attributes in AD are amyloid plaques, neurofibrillary tangles, inflammatory processes and disturbance of neurotransmitters. Basically brain cells wither away and die, causing disorientation, dementia and severe changes in personality and social interactions. Alzheimer's disease (AD) is the most common neurodegenerative disease featuring progressive impairments in memory, cognition, and behaviour and ultimately leads to death. The histopathological changes of Alzheimer's disease include neuronal and synaptic loss, formation of extracellular senile plaques and intracellular neurofibrillary tangles in brain. Multiple lines of evidence indicate that oxidative stress not only strongly participates in an early stage of Alzheimer's disease prior to cytopathology, but plays an important role in inducing and activating multiple cell signaling pathways that contribute to the lesion formations of toxic substances and then promotes the development of Alzheimer's disease. There is currently no cure for most forms of dementia including AD [3]. Brain areas associated with cognitive functions, particularly the neocortex and hippocampus, are the regions that mostly affected by the pathology which is characteristic of AD. Pharmacological treatment strategies in AD include three categories of drug:

1) Their mechanism is based on disease-modifying therapies such as vitamin E;

2) Their mechanism is based on compensation of neurotransmitter such as a cholinesterase inhibitor;

3) Psychotherapy factors that are prescribed for symptoms of conduct disorder impaired neurogenesis indicate poor cognitive function.

Important neuropathological features of AD include deposition of amyloid plaques in brain tissue and meningeal blood vessels as well as presence of neurofibrillary tangles in the hippocampus and the cerebral cortex of the brain. AD is associated with inflammatory processes. Reactive oxidative species can damage cellular components and function as a second messenger in the inflammation. Utilization of antioxidants may be useful in prevention and treatment of AD. One factor that plays an important role in the pathogenesis of AD is oxidative stress that is an imbalance between free radicals and antioxidant systems. Oxygen free radicals can attack proteins, nucleic acids and lipid membranes, therefore disrupt cellular function and integrity. Brain tissue contains large amounts of polyunsaturated fatty acids which are particularly vulnerable to free radical attack. Lipid peroxidation is thought to be destructive form of oxidative degradation that damage cell membrane and produces a number of secondary products, both of the loop and splitting forms of oxygenated fatty acids have neurotoxic effects. Increase in the levels of malondialdehyde (MDA), one of the reactive oxidative species, has recognized as an important lipid peroxidation indicator [4].

Antioxidants - Antioxidants are the compounds that prevent oxidation. They are varying in sizes, compositions and molecular weight. Some are small in size and have low molecular weights; others are enormous in size and can even be macromolecules such as proteins. Antioxidants have several uses, both in physiological systems and in human-made applications. It was found that while natural antioxidants can indeed have many positive physiological effects such as the prevention of DNA oxidation, the sources from which they are consumed must also be carefully considered to maximize absorption. While synthetic antioxidants are harmless in small concentrations, studies in animal models have shown evidence of their toxicity in higher concentrations [5]. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which commence chain reactions that damage cells like proteins, lipids, carbohydrates and DNA. Antioxidants stop these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves[5] [6].

Vitamin E (α-tocopherol) is a lipid-soluble vitamin with high antioxidant properties which decreases free radical- mediated damage in neuronal cells. Many, but not all, monitoring studies have suggested a protective effect of vitamin E with or without other antioxidant vitamins for the prevention of cognitive decline and Alzheimer's disease (AD). Due to of laboratory and population-based data, vitamin E has

been proposed as a treatment to delay neurodegeneration in AD patients [7]. Vitamin E is the lipid soluble, chain-breaking antioxidant that plays a protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals in biological membranes. It also useful in control the toxic effects of insecticides and chemicals [8]. Vitamin E is a potent peroxyl radical scavenger; it is a chain- breaking antioxidant that prevents the propagation of free radical damage in biological membranes. Vitamin E is the collective name for eight naturally occurring molecules, Tocotrienols differ from tocopherols in that they have an unsaturated phytyl side chain; the four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol nucleus [9].

Amaranthus Hybridus - *Amaranthus hybridus* is popularly called "Amaranth or pigweed, slim amaranth", is an annual herbaceous plant of 1- 6 feet high.The plant belongs to the Amaranthaceae family. It is found that the parts of the *Amaranthus hybridus* are used as diuretic antiscorbutic, appetizer, astringent, carminative, laxative, stomachic and tonic, and for jaundice. Some workers have reported *Amaranthus hybridus* pharmacological activities like anti-bacterial and anti-oxidant activities. However, there are no reports on the diuretic and anti-inflammatory activities of the plant. Hence, the present study was designed to evaluate the anti-inflammatory and diuretic potential of ethanol extract of *Amaranthus hybridus* and diuretic properties of ethanol extra leaves using experimental animal models [10]. Leaves of *A. hybridus* contain appreciable amount of proteins, fat, fibre, carbohydrate and calorific value, mineral elements, vitamins, amino acids and generally low level of toxicants [11]. Leaves are simple, broadly tapering at the end to ovate in shape with the lower surface hairless or sparsely covered with hair along the margins and veins [12]. The vitamin compositions of the leaves are β-carotene, thiamine, riboflavin, niacin, pyridoxine, ascorbic acids and α-tocopherol. Seventeen amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalmine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine) are detected. The chemical compositions are alkaloid; flavonoid, saponin, tannins, phenols, hydrocyanic acid and phytic acid [13].

MATERIAL AND METHODS

Experimental Animals: Albino wistar rats either sex weighing about 120- 200 g was procured from Shri Guru Ram Rai Institute of Technology and Sciences, Patel Nagar, Dehradun, Uttarakhand (India). They were acclimatized in animal house with air condition facility room temperature at 23 ± 2 °C with 12/12 h light/dark photo period, with free access drinking water and food. The experiment was approved by Institutional Animal Ethics Committee (IAEC) and carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.264/CPCSEA), New Delhi, India.

Preparation of Methanolic extraction of *Amaranthus hybridus*: The fresh whole leafy vegetables were chopped and dried in shade. The dried masses were blended into fine powder by frequent sieving and 25g powders were extracted by soxhlet process with methanol for 24 h. After extraction the contents were concentrated at maintained proper conditions and dried in desiccators to get corresponding extracts. All the extracts were stored at 4°C in airtight containers until need for further studies [14].

Induction of Alzheimer Disease: Alzheimer's disease (AD) was produced by the Intracerebroventricular (ICV) Streptozotocin (STZ). The Streptozotocin was injected bilaterally with ICV-STZ (3mg/kg) in two divided doses, on days 1 and day 3. Impairment in rat was induced by ICV-STZ. He were anesthetised with anaesthetic chloramphenicol and i.c.v. injections were made with a hypodermic needle of 0.4 mm external diameter attached to a 10 µl Hamilton microliter syringe (Top Syringe, Mumbai, India). The needle was covered with a polypropylene tube except for 3 mm of the tip region so as to insert this portion of the needle perpendicularly through the skull into the brain of the rat. STZ was dissolved in freshly made ACSF (25 mg/ml) solution. The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into the right or left ventricle randomly. Two doses of STZ (1 and 3 mg/ kg) were administered by I.C.V. injection bilaterally. The second dose was administered 48 h after the first dose. The concentration was adjusted so as to deliver a maximum of 5 µl in a single injection. ACSF (147 mM NaCl; 2.9 mM KCl; 1.6 mM MgCl2, 1.7 mM dextrose) [15].

Experimental Design:

The protocol was approved by the Institutional Animal Ethics Committee (Registration No.M.Ph/IAEC/01/2014/ECC-6) and will be carried out in accordance with the CPCSEA guidelines.11 groups, each comprising of 6 animals

Group 1: Control (saline) group (10ml/kg, i.p) 30 min before conducting acquisition trial from day 1 to day 4 & 30 min in before the retrieval trial conducted on day 5.

Group 2: Streptozotocin injected (3mg/kg) in two dosages schedule, i.e. on the 1st & 3rd days and followed by exposure to the Morris water maze test.

Group 3: Streptozotocin injected (3mg/kg) in two dosages schedule, i.e. on the 1st and 3rd days and followed by exposure to the elevated plus maze test.

Group 4: Streptozotocin injected (3mg/kg) in two dosages and vitamin-E (50 mg/kg/day, p.o) will be administered to the rat for 21 days and followed by exposure to the Morris water maze test.

Group 5: Streptozotocin injected (3mg/kg) in the two dosages and vitamin-E (50mg/kg/day, p.o) will be administered to the rat for 21 days & followed by exposure to the elevated plus maze.

Group 6: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (125mg/kg) will be administered to the rat for 21days & followed by exposure to the Morris water maze.

Group 7: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (250mg/kg) will be administered to the rat for 21 days & followed by exposure to the Morris water maze.

Group 8: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (500mg/kg) will be administered to the rat for 21 days & followed by exposure to the Morris water maze.

Group 9: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (125mg/kg) will be administered to the rat for 21 days & followed by exposure to the elevated plus maze.

Group 10: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (250mg/kg) will be administered to the rat for 21 days & followed by exposure to the elevated plus maze.

Group 11: Streptozotocin injected (3mg/kg) in two dosages and *Amaranthus hybridus* (500mg/kg) will be administered to the rat for 21 days & followed by exposure to the elevated plus maze.

Following parameters were estimated: After the evaluation of learning and memory animal was sacrificed by cervical dislocation (under light anaesthesia) then brain was removed after 25 days and various parameters are estimated.

Morris Water Maze:

Morris water maze test was employed to assess learning and memory of the animal. Morris water maze is a swimming based model where the animal learns to escape on to a hidden platform. It consisted of large circular pool (150 cm in diameter, 5 cm in height, filled to a depth of 30 cm with water maintained at 28±1°C). The water was made opaque with white colour non-toxic dye or milk. The tank was divided into four equal quadrants with the help of threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10×10 cm), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. the rat was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 90 sec to locate submerged platform. Then, it was allowed to stay on the platform for 20 sec. If it failed to find the platform within 90 sec, it was guided gently onto platform and allowed to remain there for 20 sec. Day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or learning. Animal was subjected to training trials for four consecutive days, the starting position was

change with each exposure as mentioned below and target quadrant (Q4 in the present study) remain constant throughout the training period.

Day 1 Q1 Q2 Q3 Q4

Day 2 Q2 Q3 Q4 Q1

Day 3 Q3 Q4 Q1 Q2

Day 4 Q4 Q1 Q2 Q3

On fifth day, platform was removed and each rat was allowed to explore the pool for 90 sec. Mean time spent by the animal in target quadrant searching for the hidden platform was noted as index of retrieval or memory. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other subject in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study [16].

Elevated Plus Maze:

Elevated plus maze was used to evaluate the memory. The plus maze consisted of two open (16×5 cm2) and closed (16×5×12 cm) arms, connected by a central platform of 5×5 cm2. The maze was elevated to height of 25 cm. above the floor. A fine line was drawn in the middle of the floor of each closed arm. On the first day (21st day of the treatment) the animal were placed individually 30 min. after oral administration of either vehicle or the test drug at the end of open arms. The time taken by the animal to move from open to closed arm (transfer latency) was noted on the first day. Transfer latency (TL) is elapse time (in sec). Between the time of placement of the animal on the open arm and the time at which all four legs were inside the closed arms. The rat was allowed to explore the maze for 2 min. and return to home case. Retention of this learning task (retention memory) was examined 24 h after the first day trial (i.e. 24 after last dose). Transfer latency measured in plus maze on first day served as an index of learning and acquisition, whereas transfer latency on second day served as an index of retrieval and memory [17].

TBARS Analysis:

This assay is used to determine the lipid peroxidation. Aliquots of 0.5mL distilled water were added with1 mL of 10% trichloroacetic acid and were added with 0.5mL of brain tissue homogenate. This is centrifuged at 3000 rpm for 10 min. To the 0.2mL supernatant, 0.1mL thiobarbituric acid (0.375%) was added. Total solution is placed in water bath at 80◦c for 40 min and cooled at room temperature. Absorbance was read at 532nm [18].

SOD Determination:

Cytosolic superoxide dismutase activity was assayed as per kono. The assay system consisted of 0.1 mM EDTA, 50Mm sodium carbonate and 96 Mm of nitro blue tetrazolium (NBT). In the cuvette, 2 mL of above mixture was taken and to it 0.05mL of post mitochondrial supernatant and 0.05 mL of hydroxylamine hydrochloride were added. The auto-oxidation of hydroxylamine was observed by measuring the change in optical density at 480 nm for two min at 30/60 sec interval [19].

Estimation of Reduced Glutathione:

Glutathione was measured according to the method of Ellman10. An equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2 ml phosphate buffer (pH 7.4), 0.5 ml 5û5-dithiobis (2-nitrobenzoic acid) and 0.4 ml distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 5 min [20].

STATISTICAL ANALYSIS

The statistical analysis would be carried out using Graph Pad Prism 6.0 software. All values were presented as Mean ± SEM. Multiple comparisons between different groups will be performed using Analysis of Variance (ANOVA) followed by Tukey's test for multiple comparisons tests. Difference level at $P<0.001$, $P<0.01$ and $P<0.05$ was considered statistically significant condition.

RESULTS

The present study on "Evaluation of neuroprotective effect of *Amaranthus hybridus* in brain of memory impaired rat" was carried out on albino wistar rats of either sex.

Effect of *Amaranthus hybridus* on escape latency (EL) of albino wistar rats by using Morris Water Maze

Results of present study are summarized in the table 1 and fig 1. During escape latency when we compare the four days trials then there no such significance difference in day first between the control, STZ and *Amaranthus hybridus* groups with various doses. On day 2-4 when the control group is compared with STZ group so that there is a highly significance difference in them ($P< 0.001$), when Stz group is compared with the Stz along with Vit-E (STZ+ Vit- E) group there is a highly significance difference ($P< 0.001$), and when there is a comparison of *Amaranth hybridus* with different doses with the STZ then there is a slight significance in *Amaranthus hybridus* low dose (125 mg/ kg) ($p< 0.05$) on day 3 & ($P< 0.001$) on day 4 and highly significance in medium dose (250 mg/ kg) and (500 mg/ kg) ie; ($P< 0.001$).

So results of this study indicate that the STZ produced impairment of memory in rats as shown in escape latency test. Vitamin E and *Amaranthus hybridus* along with STZ produced significant improvement in learning & memory as shown by decrease in escape latency.

Effect of *Amaranthus hybridus* on time spent in target quadrant (TSTQ) of albino wistar rats by using Morris Water Maze

Results of present study are summarized in the table 2 and fig 2. During time spent in target quadrant (TSTQ) when we compare the trials then there is a significance differences in between the control, STZ and *Amaranthus hybridus* groups with various doses. When the control group is compared with Stz group so that there is a highly significance difference in them (P< 0.001), when STZ group is compared with the STZ along with Vit-E (STZ+ Vit- E) group there is a highly significance difference (P< 0.001), and when there is a comparison of *Amaranth hybridus* with different doses with the STZ then there is a slight significance in *Amaranthus hybridus* low dose (125 mg/ kg) (p< 0.05) and highly significance in medium dose (250 mg/ kg) and (500 mg/ kg) ie; (P< 0.001).

So results of this study indicate that the STZ produced impairment of memory in rats as shown In TSTQ. Vitamin E and *Amaranthus hybridus* along with STZ produced significant improvement in learning & memory as shown by increases in TSTQ.

Effect of *Amaranthus hybridus* on transfer latency (TL) of Albino wistar rat by using Elevated Plus Maze

Results of present study are summarized in the table 3 and fig. 3. STZcaused significance increased (P<0.001) in transfer latency when it compared with the control group which indicates impairment in learning & memory. Animals were deals with the Vitamin E and *Amaranthus hybridus* with medium and higher doses (250 and 500 mg/kg) produced significant decrease in transfer latency as compared with the STZ group (P< 0.001). But when the *Amaranthus hybridus* is compared with the STZ there is no such significance in low dose of *Amaranthus hybridus* (125 mg/ kg).

Effect of *Amaranthus hybridus* on reduced glutathione (nM/ mg of protein) of albino wistar rat

As shown in table 4 and fig.4. STZ induced memory impaired in rats in which produced a significance difference (P< 0.001) in brain glutathione level when compared with the control group. During the comparison of STZ along with Vitamin E with the Stz group there is a significance difference ie; (P< 0.001) and when STZ along with *Amaranthus hybridus* with the STZ group then it is clear that with low dose of *Amaranthus hybridus* (125 mg/ kg) produced low significance value (P< 0.01) and highly significance with medium and high doses of *Amaranthus hybridus* (250 and 500 mg/ kg) (P< 0.001).

Effect of *Amaranthus hybridus* on Thiobarbituric acid reactive substances (TABARS) (nM/ mg of protein) of albino wistar rat

As shown in table 5 and fig. 5. Level of lipid peroxidation (LPO) in brain produced a significance difference ($P< 0.001$) when STZ group is compared with the control group. Administration of Vitamin E along with the STZ group produced significance value ($P> 0.01$) in level of brain LPO as compared with Stz group and when dealing of *Amaranthus hybridus* along with the STZ with all doses (125, 250 and 500 mg/ kg) is compared with STZ group there is also found a significance difference between them ($P<0.001$).

Effect of *Amaranthus hybridus* on Superoxide dismutase (SOD) (nM/ mg of protein) of albino wistar rat

As shown in table 6 and fig. 6 shows that STZ group produced a significant decrease ($P< 0.001$) in brain superoxide dismutase (SOD) level as compared to the control group of animals. Vitamin E along with STZ group produced significance value ($P< 0.001$) in brain of SOD level as compared with the Stz group of animals. Further *Amaranthus hybridus* along with the STZ with all doses (125, 250 and 500 mg/ kg) produced significance value ($P< 0.001$) as compared with the Stz group of animals. Therefore the results were suggested that the effect of *Amaranthus hybridus* showed improvement in learning and memory.

Table 1: Effect of *Amaranthus hybridus* on escape latency (EL) of Albino wistar rat by using Morris water maze.

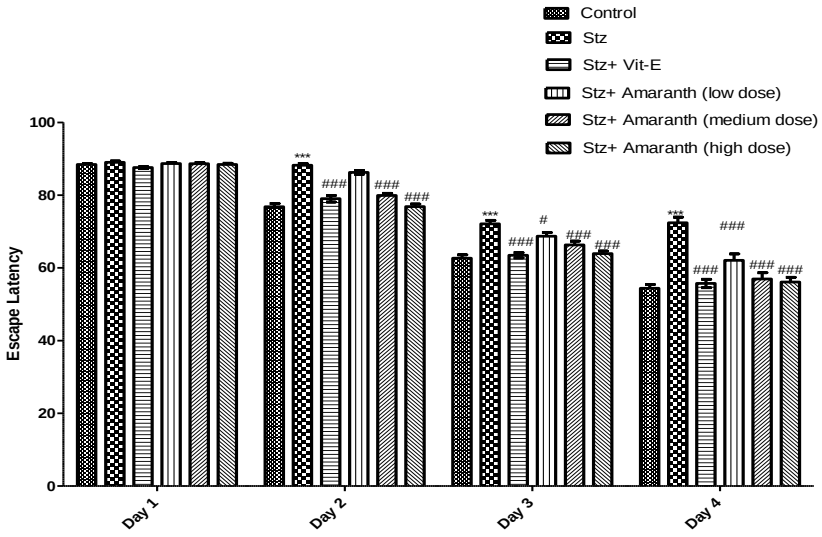
Treatment	EL (sec) Day-1	EL(sec) Day-2	EL (sec) Day-3	EL (sec) Day-4
Control	88.43 ± 0.30	76.80 ± 0.88	62.66 ± 0.89	54.40 ± 1.04
Streptozotocin (STZ)	89.03 ± 0.34	88.23 ± 0.46***	72.11 ± 0.95***	72.400 ± 1.53***
STZ+ Vit-E	87.58 ± 0.28	79.01 ± 0.89$^{###}$	63.46 ± 0.72$^{###}$	55.71 ± 1.14$^{###}$
STZ+ Amaranth (low dose) (125 mg/ kg)	88.66± 0.32	86.26 ± 0.524	68.68 ± 1.03$^{#}$	62.08 ±1.76$^{###}$
STZ+ Amaranth (medium dose) (250 mg/ kg)	88.65 ± 0.28	79.90 ± 0.60$^{###}$	66.30 ± 1.06$^{###}$	56.93 ± 1.75$^{###}$
STZ+ Amaranth (high dose) 500 mg/ kg)	88.41 ± 0.33	76.83 ± 0.71$^{###}$	63.93 ± 0.67$^{###}$	56.10 ± 1.31$^{###}$

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.

$^{***}P < 0.001$ as compared to control;

$^{#}P < 0.05$ as compared to STZ; $^{###}P < 0.001$ as compared to STZ;

Fig. 1: Effect of *Amaranthus hybridus* on escape latency (EL) of Albino wistar rat by using Morris water maze.



STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.

***P < 0.001 as compared to control;

#P < 0.05 as compared to STZ;

###P < 0.001 as compared to STZ;

Table 2: Effect of *Amaranthus hybridus* on time spent in target quadrant (TSTQ) of Albino wistar rat by using Morris water maze.

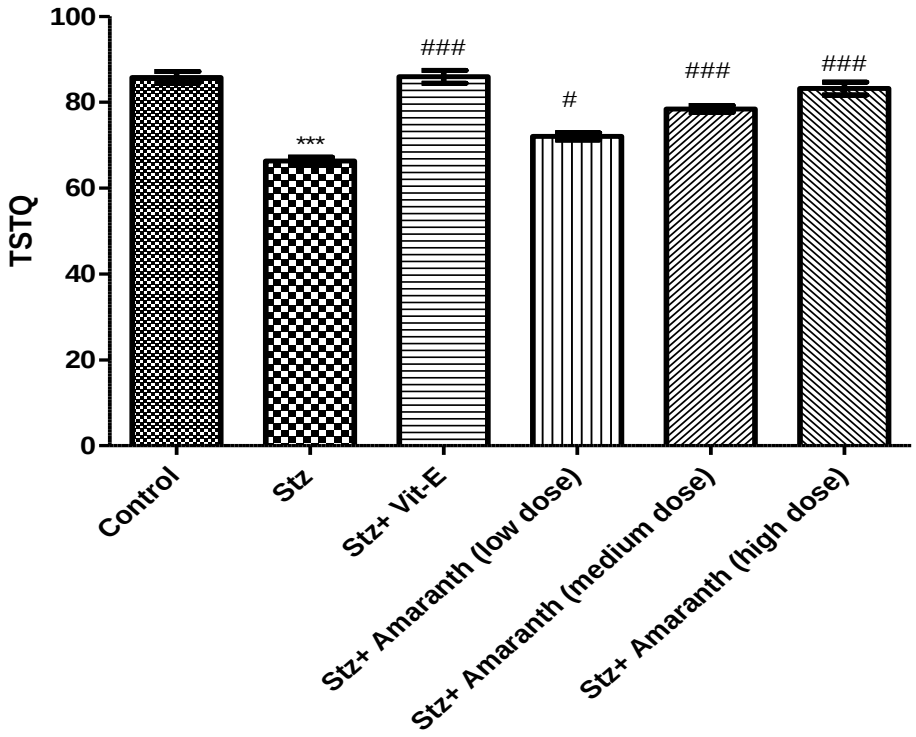
Treatment	**TSTQ (sec) Day 5**
Control	85.76 ± 1.39
Streptozotocin (STZ)	66.300±0.91***
STZ+ Vit- E	85.91 ± 1.47###
STZ+ Amaranth (low dose)	72.03 ± 0.83#
STZ+ Amaranth (medium dose)	78.43 ± 0.79###
STZ+ Amaranth (high dose)	83.20 ± 1.49###

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.

***P < 0.001 as compared to control;

#P < 0.05 as compared to STZ; ###P < 0.001 as compared to STZ;

Fig.2: Effect of *Amaranthus hybridus* on time spent in target quadrant (TSTQ) of Albino wstar rat by using Morris water maze.



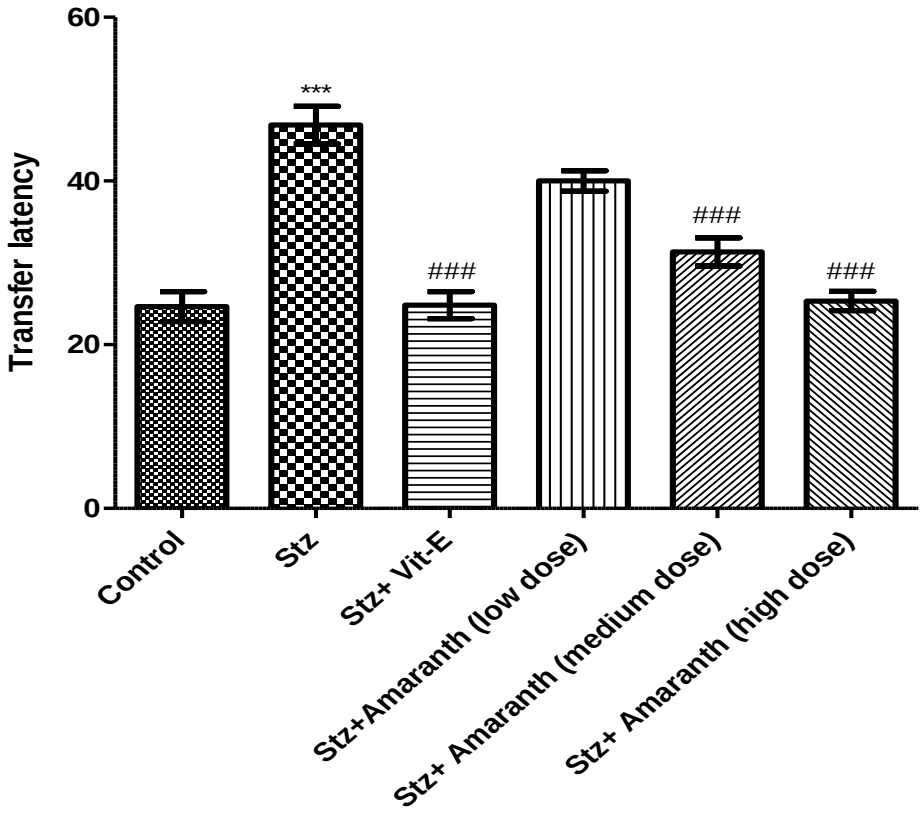
STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*
n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
#P < 0.05 as compared to STZ;
###P < 0.001 as compared to STZ;

Table 3: Effect of *Amaranthus hybridus* on transfer latency (TL) of Albino wistar rat by using elevated plus maze.

Treatment	Transfer latency (sec)
Control	24.66 ± 1.81
STZ	46.83 ± 2.30***
STZ+ Vit-E	25.00 ± 1.77###
STZ+ Amaranth (low dose)	40.00 ± 1.26
STZ+ Amaranth (medium dose)	31.33 ± 1.72###
STZ+ Amaranth (high dose)	24.66 ± 1.17###

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control; ###P < 0.001 as compared to Stz;

Fig. 3: Effect of *Amaranthus hybridus* on transfer latency (TL) of rat by using elevated plus maze.



STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analyzed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.

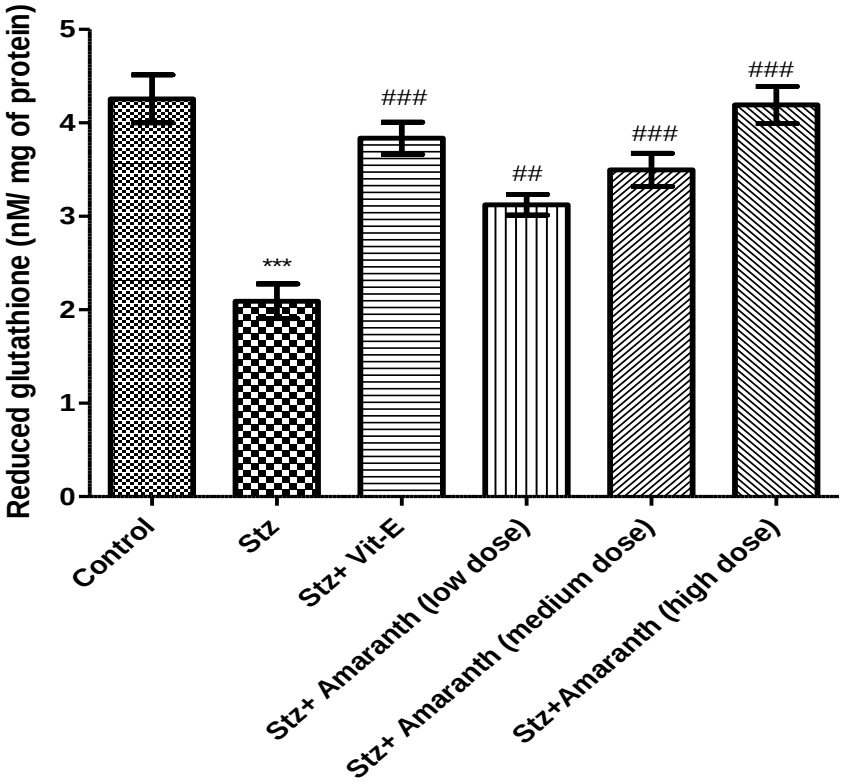
***P < 0.001 as compared to control; ###P < 0.001 as compared to STZ;

Table 4: Effect of *Amaranthus hybridus* on reduced glutathione (nM/ mg of protein) of Albino wistar rat

Treatment	**Reduced glutathione (nM/ mg of protein)**
Control	4.25 ± 0.25
STZ	$2.09 \pm 0.18^{***}$
STZ+ Vit-E	$3.835 \pm 0.17^{###}$
STZ+ Amaranth (low dose)	$3.12 \pm 0.10^{##}$
STZ+ Amaranth (medium dose)	$3.49 \pm 0.17^{###}$
STZ+ Amaranth (high dose)	$4.19 \pm 0.19^{###}$

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
$^{###}$P < 0.001 as compared to STZ; $^{##}$P < 0.01 as compared to STZ;

Fig. 4: Effect of *Amaranthus hybridus* on reduced glutathione (nM/ mg of protein) of Albino wistar rat



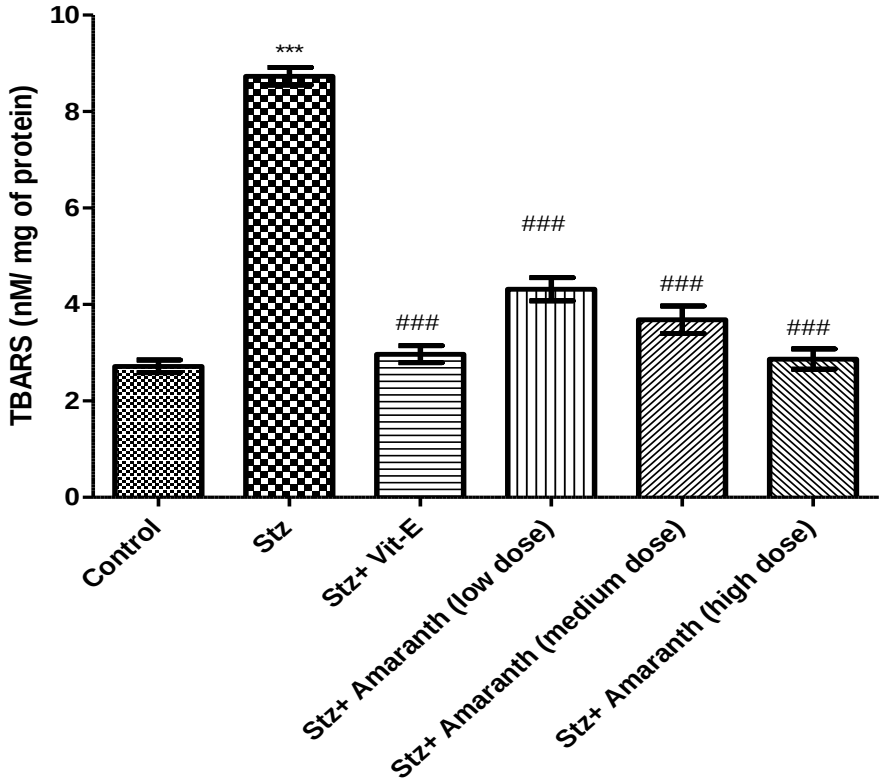
STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*
n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
###P < 0.001 as compared to STZ;
##P < 0.01 as compared to STZ;

Table 5: Effect of *Amaranthus hybridus* on Thiobarbituric acid reactive substances (TBARS) (nM/ mg of protein) of Albino wistar rat

Treatment	TBAR (nM/ mg of protein)
Control	2.71 ± 0.13
STZ	8.73 ± 0.18***
STZ+ vit-E	2.96 ±0.17$^{###}$
STZ+ Amaranth (low dose)	4.31 ± 0.24$^{###}$
STZ+ Amaranth (medium dose)	3.68 ± 0.28$^{###}$
STZ+ Amaranth (high dose)	2.86 ± 0.21$^{###}$

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
$^{###}$P < 0.001 as compared to STZ;

Fig. 5: Effect of *Amaranthus hybridus* on Thiobarbituric acid reactive substances (TBAR) (nM/ mg of protein) of Albino wistar rat



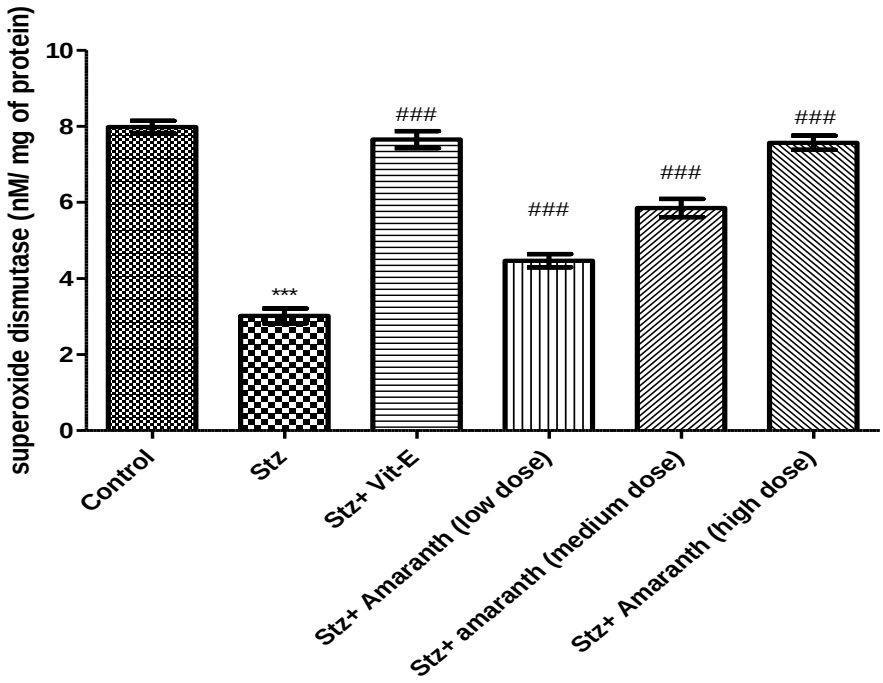
STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*
n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
###P < 0.001 as compared to STZ;

Table 6: Effect of *Amaranthus hybridus* on Superoxide dismutase (SOD) (nM/ mg of protein) of Albino wistar rat

Treatment	SOD (nM/ mg of protein)
Control	7.98 ± 0.15
STZ	3.01 ± 0.19***
STZ+ Vit-E	7.650 ± 0.22###
STZ+ Amaranth (low dose)	4.46 ± 0.17###
STZ+ Amaranth (medium dose)	5.85 ± 0.23###
STZ+ Amaranth (high dose)	7.56 ± 0.18###

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.
***P < 0.001 as compared to control;
###P < 0.001 as compared to STZ;

Fig. 6: Effect of *Amaranthus hybridus* on Superoxide dismutase (SOD) (nM/ mg of protein) of Albino wistar rat



STZ- Streptozotocin**, Vit- E-**Vitamin-E**, Amaranth-** *Amaranthus hybridus*

n = 6 in each group. Values are expressed as Mean ±SEM. Data was analysed by ANOVA followed by Tukey's post-hoc test. Here low, medium and high dose are 125, 250 and 500 mg/ kg.

***P < 0.001 as compared to control;

###P < 0.001 as compared to STZ;

DISCUSSION

Alzheimer's disease is a progressive neurodegenerative disease, characterized by deficits in memory and cognitive function. The incidence of brain disorders is theatrical on the rise as life expectancy increases. Alzheimer's disease (AD) is a complex, multifactorial, progressive, neurodegenerative disease which mostly affecting the elderly population which is estimated for 50–60% of dementia cases in persons over 65 years of age.

According to the World Health Organisation (WHO, 2006), around 35 million people in industrialized countries are suffered from AD by 2010. The disease is characterized by defect of memory and impairment of multiple cognitive and emotional functions. The pathological inherent in AD are amyloid plaques, neurofibrillary tangles, inflammatory processes and disturbance of neurotransmitters. Basically brain cells die, causing disorientation, dementia and severe changes in personality and social

interactions. There is currently no cure for most forms of dementia including AD [21].The present study evaluates the effect of *Amaranthus hybridus* in brain of memory impaired rat.

Presently, it is accepted that free radical mediated lipid peroxidation has a crucial role in the pathogenesis of many disease processes such as atherosclerosis, diabetes mellitus, carcinogenesis, inflammation and many other conditions. Hence, the use of various antioxidant supplements for the prevention or reduction in damage to biological tissues is currently extensively investigated in various disease conditions. The amount of exogenous antioxidants needs by individuals will be influenced by the oxidative stress status of the individual as this will affect the endogenous cellular antioxidant defence system [22].

Vitamin E is essential antioxidant for neurological functions and it has shown in the treatment of neurodegenerative disorders that involve free radical processes and oxidative damage, like Alzheimer's disease. This fact, growing body of evidence indicating that neurodegenerative processes are associated with oxidative stress, lead to the convincing idea that various neurological disorders were prevented and/or cured by the antioxidant properties of vitamin E. also, Vitamin E protected the brain against the seizures and neuronal damage and also reduced percentage of neuronal cell death [23].Result of present study found that Icv-Stz at a dose of 1 and 3 mg/ kg resulted in increase in the escape latency time in acquisition process as evaluated in morris water maze and it also increase transfer latency in elevated plus maze. On the other hand, control rats were well formed memory. It has been reported that vitamin E improved the amnesic and dementia deficits in memory and it also seems that *Amaranthus hybridus* is more effective in improving impaired spatial memory induced by Icv-Stz.

Lipid peroxidation plays a major role in oxidative damage. It has been reported that the level of MDA are generally higher in AD. Administration of Icv-Stz at a dose of 1 and 3 mg/ kg in rats produced the increased MDA level which is more responsible for the oxidative damage in rats when compared to normal group. *Amaranthus hybridus* with high dose (500 mg/kg) on amnesic group produced significance decrease level of brain LPO which indicates that improvement in memory and learning ability rather than the low and medium dose (125 and 250 mg/ kg). This indicating reduces in oxidative stress [24]. Glutathione is an endogenous antioxidant presenting in the reduced form within the cell. It has been shown to react with free with free radicals and prevent generation of hydroxyl free radicals. The decreased level of GSH in Icv-Stz treated animals indicates that there is an increased generation of free radicals and reduced activity of glutathione system in combating oxidative stress. *Amaranthus hybridus* with high dose (500 mg/kg) treatment was able to restore the GSH levels and also cause a significant increase in glutathione than the low and medium dose (125 and 250 mg/ kg) [25]. Further, it

has been also reported in the present study that the level of brain SOD decrease in Icv-Stz treated group of animals which indicated that impairment of memory and learning ability. However, the *Amaranthus hybridus* with high dose i.e. 500 mg/ kg improve the level of SOD and this indicated to improvement in memory than the low and medium dose i.e. 125 and 250 mg/ kg [26].

The results of present study suggest that *amaranthus hybridus* could provide an opportunity to reduce other harmful drugs which having there harmful effects as well as it increase therapeutic effect. Due to the plant antioxidants property it may be useful in the Alzheimer disease or in neurodegenerative disorders thus at the time proper precaution and care could be taken.

CONCLUSION

The purpose of the present study was to evaluate the Neuroprotective effect of *Amaranthus hybridus* in brain of memory impaired rat. In this study *Amaranthus hybridus* showed significant improvement in the learning and memory.

In the present study Streptozotocin at a dose of 3 mg/kg caused induction of Alzheimer disease in rats. Treatment with *Amaranthus hybridus* caused improvement in learning & memories of impaired rat as indicated by the decreased in transfer latency and escape latency in Elevated Plus Maze and Morris Water Maze test respectively. *Amaranthus hybridus* also decreased neurodegeneration and decreased in the level of oxidative stress in the brain. It increases the reduced glutathione and superoxide dismutase level in brain and decreased the TBARS level in brain. It also decreased in the oxidative damage in the brain by increasing the level of antioxidants.

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