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EVALUATION OF ANTIOXIDANT AND NEUROPROTECTIVE EFFECT OF POLY HERBAL COMPOUND AGAINST SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN ANIMAL MODEL

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Abstract:

The present study was designed to evaluate the anti oxidant and neuroprotective effect of ethanolic extract of eleusine corocana, cyperus rotandus and moringa oleifera against scopolamine induced memory impairment in animal model. The study was carried for 21 days.The Alzheimer's disease was induced using scopolamine 2 mg/kg b.w The phytochemical screening showed the presence of flavonoids, steroids, phenols, terpenoids, glycosides and alkaloid compound. Alzheimers disease was screened by observing behavioural parameters using actophotometer, elevated plus maze and biochemical estimations like acetyl cholinesterase, catalase, glutathione, lipid peroxidation and super oxide dismutase The treatment with ethanolic extract of eleusine corocana,cyperus rotandus and moringa oleifera (EEECM) 200 mg/kg and 400 mg/kg effectively decreased the AChE and malondialdehyde levels when compared with the disease control group and increased the anti oxidant enzyme . Histopathological studies were performed where disease control group showed the presence of neurofibrillary tangles and tau proteins accumulations in the hippocampus and apoptic neurons and inflammation in the cerebral cortex.and the treated groups of EEECM 200mg/kg and 400 mg/kg showed similar results to standard group where the cerebral cortex and hippocampus appeared normal and also showed mild proliferations in the region of hippocampus . Thus, the results suggest that EEECM possess anti oxidant and neuro protective properties.

Key words: *Alzheimers disease, Scopolamine, Donepezil, Ethanolic extract, Eleusine corocana, Cyperus rotandus, Moringa oleifera, Acetyl cholinesterase.*

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INTRODUCTION:

Neurodegeneration disease is a range of conditions which primarily affect the neurons in the human brain. This diseases are incurable and debilitating conditions that result in progressive degeneration or death of nerve cells, which causes problems with movement or mental functioning.[1] Alzheimer's disease is one such progressive neurodegenerative disease in which it causes brain cells to waste away degenerate and die. Alzhiemer's disease is the most common cause of dementia. A continuous decrease in thinking, behavioural and social skills disrupts a persons ability to function independently. Alzheimers disease is expected to be caused by the normal build up of proteins in and around brain cells, one of the protein involved is called amyloid which deposits plaques around the brain cells and the other protein is called tau, deposits of which form tangles within brain cells. levels of one neurotransmitter, acetylcholine are particularly low in brains of people affected with alzheimers disease.[2] The other factors that may contribute to alzheimers disease are cardiovascular disease, high blood pressure , obesity, high cholesterol and diabetes.[3] The signs and symptoms include depression, apathy, social withdrawal, mood swings, loss of inhibitions, delusions, irritability and aggressiveness.[4]

The plants extracts have always been good source of treatment in many diseases, like wise the present therapy choses to poly herbal formulation was used for the treatment of alzheimers disease. The poly herbal formulation comprised of three plants namely *eleusine corocana* (dried seeds) belonging to family poaceae , mainly cultivated in india, commonly known as ragi. it is mostly used for the treatment of cardiovascular diseases, diabetes, as nephro protective and in lowering cholesterol levels[5], the other plant is *cyperus rotandus*(dried root tubers) of family cyperaceae , commonly known as nagarmotha, native to asia and Africa , it is mostly used for its anti bacterialproperties, neuro protective and in treating other systemic disorders[6]. The last plant is *moringa oleifera* (leaves) commonly known as sahajna , native to india,belongs to family moringaceae, it is widely used for hypertension, in cardiovascular diseases, as neuroprotective agent and in cancer also .[7]

Alzheimers disease is not a preventable condition,but maintaining healthy diet and exercise regularly can reduce the progression of the disease.The treatment include drugs of cholinesterase inhibitors like donepezil, rivastigmine and galantamine and the NMDA receptor antagonist drugs include memantine and other new drugs include aducanumab , solanezumab, intepirdine, verubecestat, AADvac1 and CSO-1103. The goal of these new drugs is to reduce AD symptoms.[8]

MATERIALS AND METHODS:

Animals:

Swiss albino mice strain (25-30g) were used for the study.The experimental mice were procured from Sainath Animal Agency, Musheerabad, Hyderabad, India. The mice were kept under standard well controlled conditions before and throughout the experimental duration. The temperature was maintained at 22°C (±3°C) and relative humidity was between 50-60%. The animals were given pellet diet and drinking water ad libitum, kept in 12h/h light/dark cycle and maintained for atleast 5 days prior to dosing to allow for acclimatization to laboratory conditions. The experimental protocol was given approval by Institutional Animal Ethical Committe and was carried under the compliance of IAEC guidelines. (IAEC/SUCP/2019/08).

Plant material collection and extraction:

The Plants parts of *eleusine corocana- dried seed grains,cyperus rotandus-dried root tubers and moringa oleifera-fresh leaves* were collected from, identified and authenfied by Dr. Shaik Mohammed Aliuddin Secretary: Hyderabad Unani Research Foundation. Hyderabad, Telangana State. The plants were cleaned , shade dried, coarsely powdered and sieved through sieve no.100. The ethanolic extract of *eleusine corocana,cyperus rotandus, moringa oleifera* This powder was used for solvent extraction . The EEECM was formulated in 2:1:2 ratio(*eleusine corocana:cyperus rotandus:moringa oleifera*)About 250gms of powder poly herbal powder extract was subjected to soxhlet extraction using 500ml solvent ethanol. This cycle was repeated many times until the colour of the solvent in the siphon of the soxhlet faded away. The extract was concentrated on water bath.The EEECM was suspended in CMC and administered orally.

Phytochemical screening:

The EEECM was subjected to various phytochemical tests to identify chemical constituents such as flavanoids, alkaloids, glycosides, polyphenols, essential oils, proteins, amino acids, carbohydrates, steroid etc.[9]

Scopolamine induced memory impairment:

Scopolamine is an anticholinergic agent that acts as a competitive antagonist to muscarinic M1

acetylcholine receptors. It is used as a short-term amnesia model in both animals and human studies. It is shown to impair memory and spatial learning thus producing similar dementia like effects as seen in patients with Alzheimer's disease. Amnesia was induced in the given test animals by administration of scopolamine intraperitoneally at a dose of 2mg/ kg body weight of the animals prior to standard and test drug.

Experimental design:

30 experimental mice were divided into 5 groups of six animals in each and treated as follows:

30 minutes before the administration of the standard and the test drugs, Scopolamine (2mg/kg b.w.) i.p, suspended in 5% w/v CMC was administered to the standard and test groups.

Group 1 (Normal control group) – This group was given 0.5% w/v CMC suspension at the dose of 1ml/kg body wt. p.o. once a day for a duration of 21.

Group 2 (Disease control group**)** – This group was treated with Scopolamine 2mg/kg body wt. i.p. for a duration of 21 days.

Group 3 (Standard) – This group was treated with Scopolamine 2mg/kg body wt. i..p. and Standard Donepezil Hydrochloride 5mg/kg body wt. po for a period of 21 days.

Group 4 (EEECM 200 mg/kg) – This group was treated with Scopolamine 2mg/kg body wt. i.p + EEECM (200mg/kg b.w.p.o.) for a period of 21 days.

Group 5 (EEECM 400 mg/kg) – This group was treated with Scopolamine 2mg/kg body wt. i.p.+ EEECM (400mg/kg b.w., p.o.) for a period of 21 days.

Behavioural screening studies:

Locomotor activity (actophotometer):

Actophotometer is used to assess the locomotor activity. Make sure that all photo cells are working for accurate recording, turn on the actophotometer and place individually each animal in activity cage for 10 min and note there basal activity score. Again re-test each animal after administering the drug for 30 min. Note the difference before and after the administration of drug. Calculate the activity score(no. of counts).[10]

Elevated plus maze:

Elevated plus maze is used as an exteroceptive conduct model to assess memory in mice. Transfer latency (TL) is defined as the time taken by the animal to move from open arm into one of the closed arms. The mice were placed on the end of open arm facing away from central platform, TL was recorded for each animal, they were then allowed for 2 more minutes to explore the maze and then returned to there respective cages. Retention of learned task was examined 24 hrs after trial, drop in there transfer latency on there subsequent maze exposure were taken as index of successful retention.[11]

Dissection and Homogenisation:

On 21st day, the experimental mice were sacrificed by giving overdose of anaesthetic ether. The brain was excised carefully and transferred to ice cold phosphate buffer. Brain tissues were subjected to homogenisation ice bath with 10% NaCl in distilled water at not more than 4^0 followed by centrifuge at 3000rpm. The resultant supernatant was then used for estimating the following parameters.

Estimation of Acetyl cholinesterase:

Also known as Ellman's method. The brain homogenized tissue 0.4ml was added to a cuvette containing 2.6ml phosphate buffer of ph8. Then 100ul DTNB was added to cuvette, and absorbance was measured at 412nm.Then 20ul of acetylthiocholine iodide was added and change in absorbance was recorded with opposite to blank containing 3ml buffer, DTNB and acetylthiocholine iodide. Then the changes in absorption were calculated per min.[12]

Determination of anti oxidant parameters:

Estimation of Catalase activity:

To 2.25 ml of potassium phosphate buffer, 100µl of tissue homogenate was added and incubated at 25^0 C for 30 minutes. Then 0.65ml H_2O_2 was added to initiate the reaction. The blank was prepared using 2.5ml potassium phosphate buffer and 0.65ml H_2O_2. and the change in absorbance at 240 nm for 2-3 min was measured.[13,14]

Lipid peroxidation (LPO)

5ml solution of 1ml of the tissue homogenate, 0.2 ml of solution of SLS, 1.5ml each of TBA and acetic acid (20%) was prepared. This was incubated for couple of minutes and then heated for thirty minutes in a water bath. n butanol- pyridine mixture was utilised to extract the chromagen and centrifuged for ten minutes at 4000 rpm. At 532 nm, the absorance of the organic layer was assessed. Conc. of Malondialdehyde is expressed as Nano moles/mg of protein.[14]

Reduced glutathione (GSH)

The 10% homogenate tissue was deproteinized using equal volume of TCA. Then Allowed to stand at 40C for 1 hr. The contents were then centrifuged at 3000 rpm for 15 min. The supernant (0.5 mL) was added to 2 mL of Tris HCl buffer (0.4 M, pH 8.9) containing

EDTA (0.02 M, pH 8.9) followed by DTNB (0.01 M)the Volume was made to 3 mL by adding distilled water (0.5 mL) the Absorbance was measured at 412 nm.[15]

Superoxide dismutase (SOD)

The supernatant (0.1ml) was added to carbonate bicarbonate buffer (pH 9.7). To this, epinephrine (1ml) was added and absorbance measured at 480nm for 2 min. [16]

Histopathological Analysis:

The animals were sacrificed and there brains were excised. The intact whole brain was transferred to 10% formalin solution and subjected for histopathological studies.

Statistical Significance:

The statistical analysis was carried out using Graph pad prism 8.2.0. All results were expressed as Mean ± SEM. Groups of data were compared with analysis of variance (ANOVA) followed by Dunnett's multiple comparision test to identify significance ($p<0.001$, $p<0.01$, $p<0.05$) among groups.

RESULTS:

Phytochemical screening

The preliminary phytochemical analysis of EEECM confirmed the presence of flavonoids, phenols, amino acids, steroids, essential oils, saponins, terpenoids and alkaloids suggesting its potential antioxidant activity.

Locomotor activity:

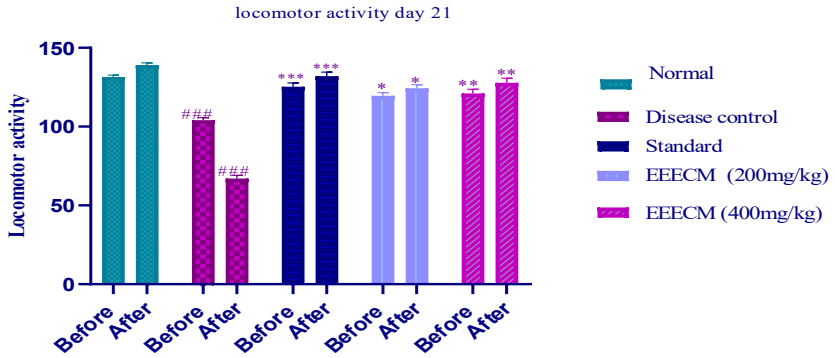

Figure 1. Effect of EEECM on locomotor activity of experimental rats. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: Locomotory activity is evaluated by using Actophotometer. There was decrease in locomotor activity of Scopolamine treated group (77.2 ± 1.72) was increased in EEECM treated group (137.8 ± 2.35) on day 21.

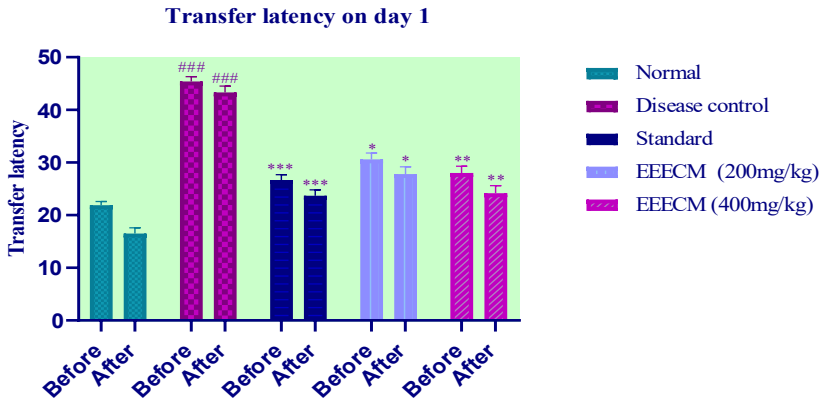

Figure 2: Effect of EEECM on transfer latency of experimental rats. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01

*P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Transfer latency is evaluated by using elevated plus maze. There was decrease in % Alteration in EEECM treated group (27.8 ±1.3) when compared to Scopolamine treated group (43 ± 1.7) on day 1, indicating the decrease transfer latency behaviour in EEECM treated groups.

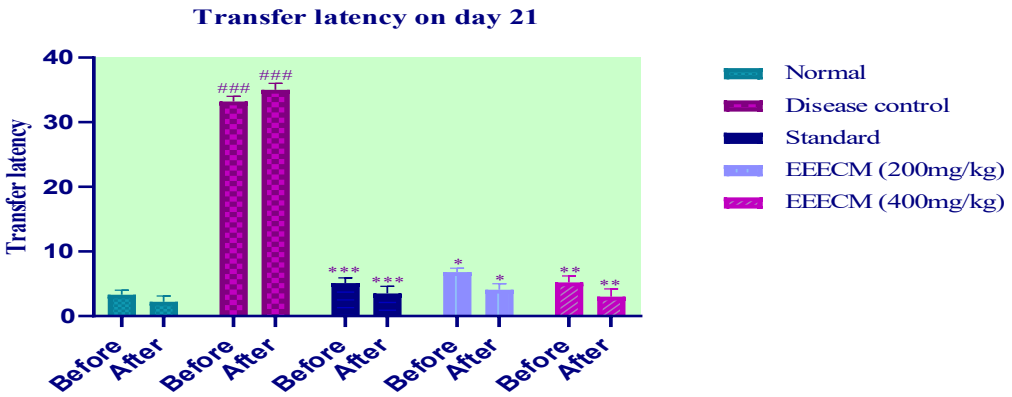

Figure 3: Effect of EEECM on transfer latency of experimental rats . The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Transfer latency is evaluated by using elevated plus maze. There was decrease in % Alteration in EEECM treated group (3.1 ±0.3) when compared to Scopolamine treated group (35± 0.5) on day 21 , indicating the decrease transfer latency behaviour in EEECM treated groups.

Estimation of Acetyl cholinesterase:

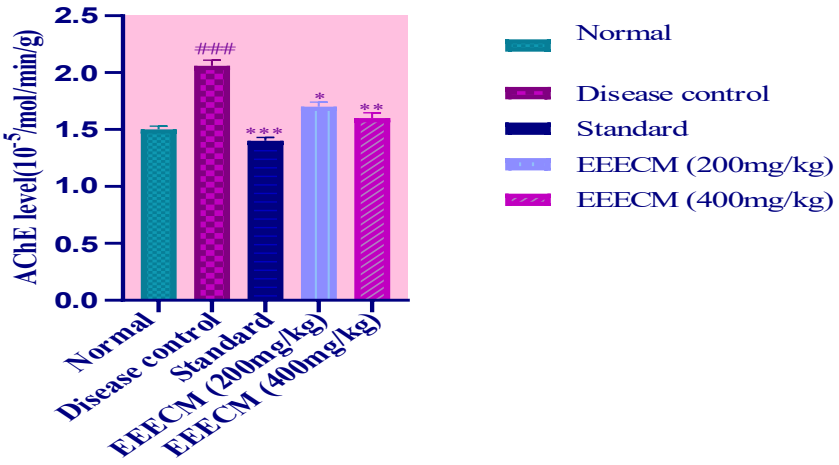

Figure 4.Effect of EEECM on Acetylcholinesterase levels of experimental rats . The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001

**P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Acetylcholinesterase enzyme levels were increased in Scopolamine treated group (2.06 ± 0.05) was significantly decreased in EEECM treated group (1.6 ± 0.04) similar to Donepezil treated group 1.4 ± 0.03) reveals that EEECM may possess anti Acetylcholinesterase activity.

Estimation of Catalase activity:

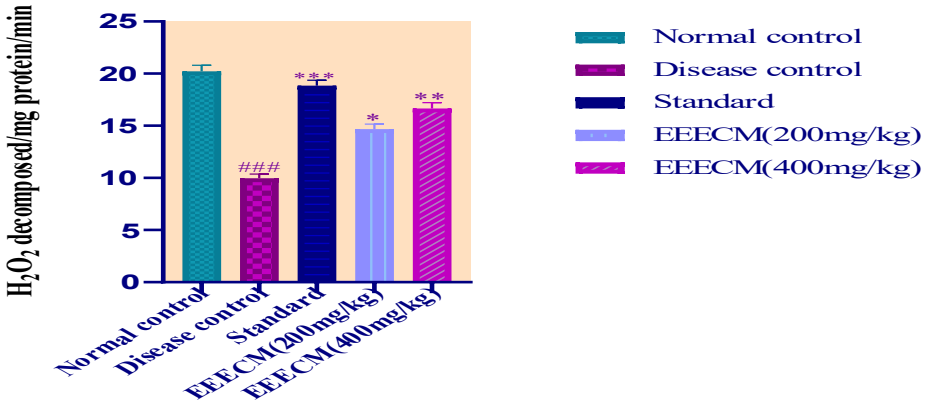

Figure 5.Effect of EEECM on Catalase levels of experimental rats . The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Catalase levels were increased in EEECM treated group (16.6 ± 1.0) similar to Donepezil treated group (18.8 ± 0.6) when compared to Scopolamine treated group (9.9± 0.5) reveals that EEECM possess antioxidant properties.

Estimation of Super oxide dismutase:

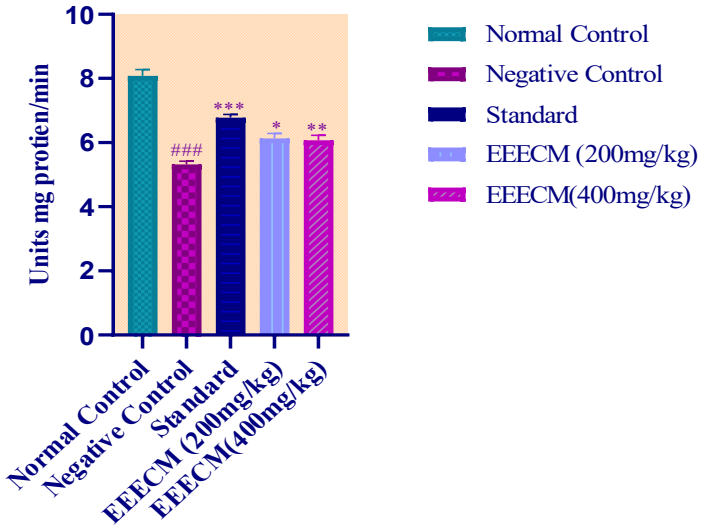

Figure6.Effect of EEECM on SOD levels of experimental rats. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The SOD levels were increased in EEECM treated group (6.3 ± 0.4) similar to Donepezil treated group (6.7 ± 0.2) when compared to Scopolamine treated group (5.3 ± 0.2) reveals that EEECM possess antioxidant properties.

Estimation of Glutathione:

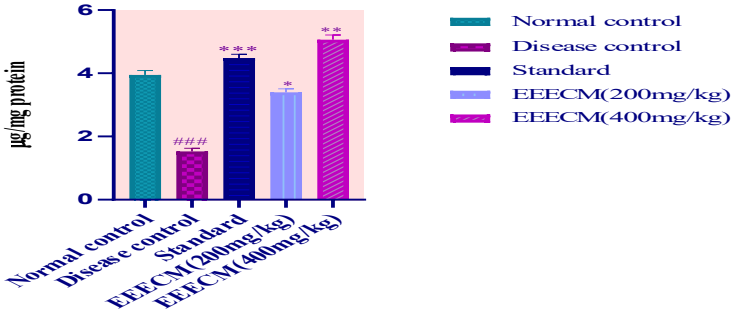

Figure7.Effect of EEECM on Glutathione levels of experimental rats. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Reduced Glutathione levels were increased in EEECM treated group (5.06 ± 0.2) similar to Donepezil treated group (4.4 ± 0.09) when compared to Scopolamine treated group (1.5 ± 0.47) reveals that EEECM possess antioxidant properties.

Estimation of Lipid Peroxidation:

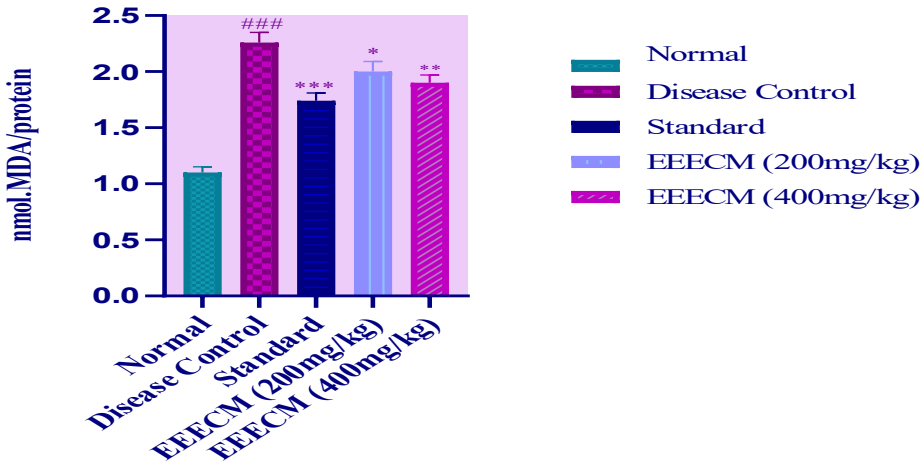

Figure 8.Effect of EEECM on Malondialdehyde levels of experimental rats. The values are expressed as Mean ± SEM (n = 6). One way ANOVA was carried out followed by dunnetts multiple comparison test. ***P < 0.001 **P<0.01 *P<0.05 when compared with disease control group. ###P < 0.001 when compared with vehicle control group. ns – not significant.

Inference: The Malondialdehyde levels (end product of Lipid peroxidation) were increased in Scopolamine treated group (2.26 ± 0.09) was significantly decreased in EEECM treated group (1.9 ± 0.09) similar to Donepezil treated group (1.74± 0.07) reveals that EEECM decreases Lipid peroxidation.

Histopathological results:

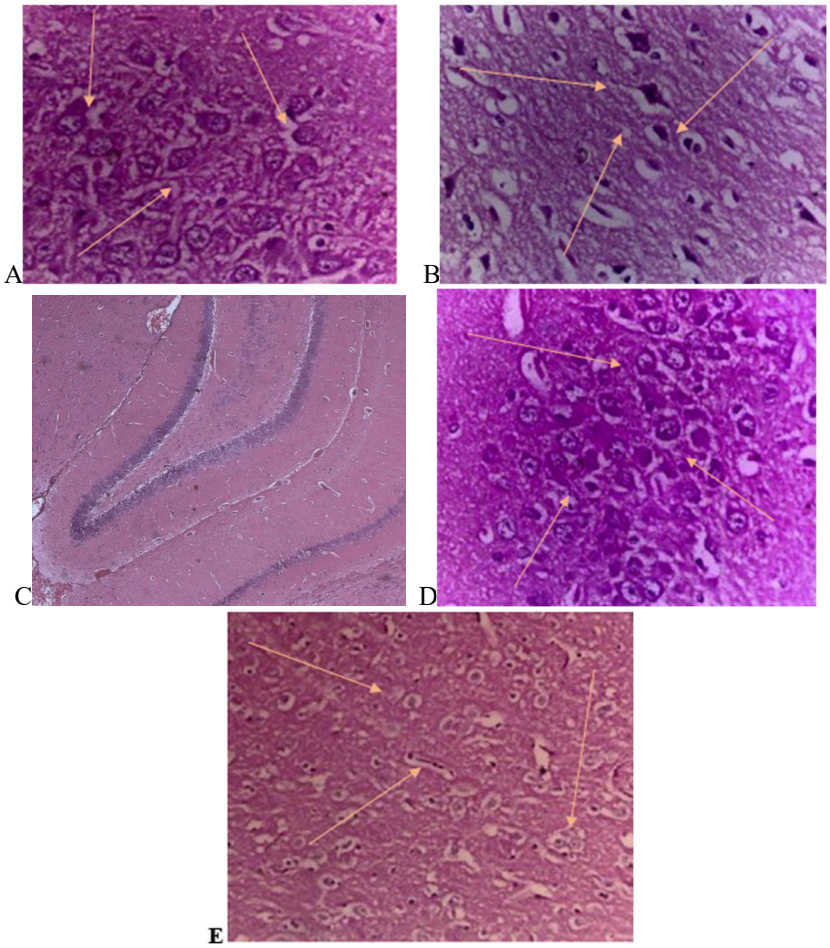

Figure 9: Histopathology of brain A: Normal control B: Disease control C: Standard group D: EEECM 200 mg/kg E: EEECM 400 mg/kg

Inference: A) Control mice brain showing normal histological structure, cortex region appeared normal, normal hippocampus B) Mice exposed to 2mg/kg scopolamine showed the presence of neurofibrillary tangles, amyloid plagues and edematous vacuoles, severe neutrophilic infiltration, congestion in blood vessels and pericellular edema. (C) Hippocampus of brain appeared normal and found that proliferation of neurons was observed entire region. (D)Mice administered with 200mg/kg of EEECM showing mild degenerative changes in hippocampus region. (E) Mice treated with 400mg/kg of EEECM showing the normal histological structure.

DISCUSSION:

Alzheimers disease is the progressive neurodegenerative disorder. The etiology behind its continuous and irreversible vegetative cell degeneration is not fully understood. The current therapy options in AD are limited to two main approaches. One being the use of anticholinesterases to maintain levels of ACh in brain. Another approach has been the use of NMDA receptor antagonists to prevent excito toxicities that are responsible for damage of neurons.

In the current study ethanolic extract of *eleusine corocana, cyperus rotandus and moringa oleifera* (EEECM)were used. The phytochemical test of EEECM confirmed presence of flavonoids, alkaloids,

polyphenols, triterpenes etc showing its potential antioxidant scavenging activity. Swiss albino mice were used . Mice treated with EEECM 200 mg/kg and 400 mg/kg were subjected to actophotometer, increase the locomotor activity to (134.3±1.9) & (137.8±1.9) similar to Donepezil treated group (142±2.7) when compared with scopolamine treated group.The *EEECM* 200 mg/kg & 400 mg/kg decreased the transfer latency to (4.1±1.8) and (3.1±1.8) as similar to donepezil treated group (3.5±1.2) on 21stday.

Acetylcholinesterase, catalyses choline and other choline esters that function as neurotransmitters. AChE levels were decreased in treated groups with *EEECM* 200 mg/kg & 400 mg/kg (1.7±0.04) to (134.3±1.9) when compared with scopolamine treated group (2.06±0.05).These results were in accordance with the study done by Kondumahanti V N Lakshmi. et al.[17]

The EEECM show favourable antioxidant activity. Levels of Catalase, SOD and GSH were increased and decreased level of Malondialdehyde in mice treated with EEECM.

Catalase is an enzyme that catalyses decomposition of hydrogen peroxide to water and oxygen. Levels of catalase were increased in mice treated with *EEECM* 200 mg/kg and 400 mg/kg (14.6±1.4) and (16.6±1.0) respectively when compared to disease control group (9.9±0.5). These results were in accordance with the study done by Kondumahanti V N Lakshmi.et al.[17]

Superoxide dismutase (SOD) catalyses the dismutase of superoxide (O2-) to hydrogen peroxide(H_2O_2) and molecular oxygen(O_2). Level of SOD increased in mice treated with *EEECM* 200 mg/kg & 400 mg/kg (6.1±0.15) and (6.3±0.4) respectively when compared with disease control group (5.3±0.2). These results were in similar accordance with the study done by Nagarjuna S.et al.[18]

Reduced GSH levels impairs the clearance of H_2O_2 and formation of hydroxyl radicals which result in the formation of a oxidative environment Levels of GSH were increased in mice treated with *EEECM 200mg/kg& 400 mg/kg* (3.3±0.1) and (5.06±0.2) respectively when compared to disease control group (1.5±0.47).These result were in accordance with the study done by Ittiyavirah SP ,et al.[19]

The Lipid peroxide radicals are produced due to assault of the free radicals on double bond of unsaturated fatty acid and arachidonic acid. The Levels of Malondialdehyde, showed a decrease in mice *EEECM 200 mg/kg & 400 mg/kg* (2.04±0.09) and (1.9±0.07) respectively when compared to disease control group (2.26±0.09). These results were in accordance with the study done by Kondumahanti V N Lakshmi.et al.[17]

The histopathological reports showed that scopolamine effectively damaged the mice brain which was evident from the presence of neurofibrillary tangles and tau proteins accumulations in the hippocampus and apoptic neurons and inflammation in the cerebral cortex. The donepezil group showed the protective effect when compared to disease group not including any accumulation of tau protein and neurofibrillary tangles. Treatment with EEECM 200 mg/kg & 400 mg/kg showed similar results to standard group where the cerebral cortex appeared normal with mild proliferations in the region of hippocampus.

CONCLUSION:

The treatment with EEECM decreased the Acetylcholinesterase and Malondiadehyde levels and increased the activity of antioxidant enzymes namely-Catalase, Super oxide dismutase and Glutathione. The histopathological studies on mice brain showed significant improvement in the treatment group (EEECM 200mg/kg and 400mg/kg) as that similar to standard, while the brain of negative mice shown accumulations, apoptic neurons and inflammation. The preliminary phytochemical analysis of EEECM shows the presence of flavonoids, alkaloids, phenol and terpenes contributed to its neuroprotective and anti oxidant activity.

The behavioural study of mice treated with EEECM showed increase in locomotor activity when compared to scopolamine treated groups. Eleveted plus maze study demonstrated improvement in retention in mice with EEECM against scopolamine.

Thus it is concluded that *eleusine corocana , cyperus rotandus and moringa oleifera(* EEECM) have antioxidant and neuroprotective activity and is potential potent low cost herbal medicine for neurodegenerative disease like in alzheimers, which is relatively safer than conventional therapy. However the depth research on the neuroprotective potential of poly herbal compound is required using more experimental paradigms and detailed methods to identify mechanism that could be invested for the prevention and treatment of Alzheimer's disease.

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CONFLICT OF INTEREST:
There is no conflict of interest among the authors.

REFERENCE:
1. What is neurodegenerative disease. Available at: https://www.neurodegenerationresearch.eu/authors-guidelines.php [Accessed 09 September 2019].
2. Alzheimer's disease. Available at: https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-2035044/authors-guidelines.php [Accessed 09 September 2019].
3. Causes of alzheimer disease. Available at: https://www.nhs.uk/conditions/alzheimers-disease/causes/authors-guidelines.php [Accessed 09 September 2019].
4. Symptoms of alzheimer's disease. Available at: https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447/authors-guidelines.php [Accessed 09 September 2019].
5. Eleusine corocana. Available at: https://pfaf.org/user/Plant.aspx?LatinName=*Eleusine+coracana*/authors-guidelines.php [Accessed 09 September 2019].
6. Cyperus rotandus. Available at: https://www.cabi.org/isc/datasheet/17506/authors-guidelines.php [Accessed 09 September 2019].
7. Gopalakrishnan L, Kruthidoriya, kumar DS. *Moringa oleifera*: A review on nutritive importance and its medicinal applications. J sci dir, 2016; 5 : 49-56.
8. Medications for memory. Available at: https://www.alz.org/alzheimers-dementia/treatments/medications-for-memory)/authors-guidelines.php [Accessed 09 September 2019].
9. Khandelwal KR. 2005. Preliminary phytochemical screening. In: Khandelwal KR,
10. ed. Practical Pharmacognosy Techniques and Experiments. Pune: Nirali Prakashan 149-56.
11. SK Kulkarni 1999. Handbook of experimental pharmacology. Mumbai: Vallabh Prakashan 131.
12. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc, 2007; 2: 322-328.
13. Ellman GL et al, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol,1961; 7: 88-95.
14. Claiborne A et al, A handbook of method of oxygen free radicle research 1985: 283-285
15. Sajjadian M et al, Protective effects of cannabidol on cuprizone induced demylienation in C57BL/6 mice, J contem Med Sci, 2017; 3: 278-283.
16. Dorval J, Hontela A. Role of glutathione redox cycle and catalase against defense oxidative stress induced by endosulfan in adrenocortical cells in rainbow trout (Oncorhychus mykiss). Toxicol Appl Pharmacol, 2003 ;192: 191-200.
17. Mishra HP, Fridovich I. The Role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The J Biol chem, 1972; 247: 3170-3175.
18. Lakshmi KVN, M R Kumar, ChandraSekhar K, S. Manish, K. T. Sunil kumar, K. Prasad, Kumar V S Nemmani. Pharmacological evaluation of methanolic extract of *desmostachya bipinnata* against scopolamine induced dementia of alzheimers type in albino wistar rats. Indo Am J Pharm Sci, 2019; 6: 14047-14055.
19. Nagarjuna S, Arifullah M, Kumar AS, Srinath B, Reddy KS, Reddy YP. Evaluation of antioxidant and antiparkinsonian activities of *Brassica oleracea* in haloperidol-induced tardive dyskinesia. Int J Green Pharm, 2015; 9: 143-149.
20. Ittiyavirah SP, Ruby R. Effect of hydro-alcoholic root extract of *Plumbago zeylanic*al alone and its combination with aqueous leaf extract of *Camellia sinensis* on haloperidol induced parkinsonism in wistar rats. Annals of Neurosciences,2014; 21:47-50.