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Anti- Alzheimer's and Anti- Oxidant Activity of *Cyamopsis tetragonoloba* Fruit Extract in Mice

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Abstract **Original Research Article**

The present study was designed to investigate the anti-alzheimer's activity of *Cyamopsis tetragonoloba* fruit in mice. The two exteroceptive models, scopolamine (1mg/kg) and diazepam (1mg/kg) induced amnesia were used to study the anti-alzheimer's effect of ethanolic fruit extract of *Cyamopsis tetragoloba* (CTFE) at the oral dose of 200mg/kg and 400mg/kg in Wistar albino mice. Piracetam (400mg/kg) was used as a standard drug. After completion of the 10 days respective treatments, scopolamine induced amnesia model showed the better activity in all the three; Elevated plus maze, Radial arm maze and Y maze parameters. The CFE at a dose of 200mg/kg and 400mg/kg, improved memory in a dose dependent manner. In scopolamine and diazepam induced amnesia model, biochemical parameters like lipid hydroperoxide ($P<0.05$) and malondialdehyde ($P<0.01$) levels were significantly decreased with administration of CFE (400mg/kg) when compared to scopolamine control. Whereas, acetylcholinesterase ($P<0.01$) level was significantly increased when compared to scopolamine control on administration of CFE (400mg/kg). In-vivo antioxidants like superoxide dismutase, reduced glutathione and catalase were decreased on inducing amnesia by scopolamine and diazepam when compared to normal group. Treatment with 400mg/kg of CFE significantly increased the levels of superoxide dismutase ($P<0.05$), reduced glutathione ($P<0.05$) and catalase ($P<0.05$) when compared to negative controls. From the results it was concluded that the ethanolic fruit extract of *Cyamopsis tetragonoloba* elicits anti-alzheimer's activity in experimental animal models.

Keywords: *Cyamopsis tetragonoloba* fruit, anti- alzheimer's, and antioxidants.

INTRODUCTION

Alzheimer's disease is an irreversible, progressive, neurodegenerative disorder. The credit for first time describing a dementing condition, which later became known as Alzheimer's disease, goes to German physiatrist and neuropathologist Dr. Alois Alzheimer in 1906. It is a disorder that causes the gradual loss of brain cells, leading to dementia and is characterised by loss of intellectual ability, memory loss, mental and physical behavioural changes, and the areas of the brain that control memory and thinking skills are affected first. Then, cells die in other regions of the brain. Reduced quality of life for patients, with an important impact on human health. In developed countries, AD is one of the most financially costly diseases. And it generally affects the elderly. It is characterised by the development of acetylcholine depletion, amyloid beta protein aggregation, and neurofibrillary tangles, which are associated with neuronal loss of affecting to greater extent cholinergic neurons.

Herbal medicines sometimes referred as herbalism (or) botanical medicine is the use of herbs for their therapeutic or medicinal value. An herb is a whole plant or plant part valued for its medicinal aromatic or acceptable qualities. Herbal plants contain variety of chemical substances that act upon the body. The origin of *Cyamopsis tetragonoloba* is unknown, since it has never been found in the wild. It is assumed to have developed from the African species *Cyamopsis tetragonoloba*. It was further domesticated in India and Pakistan, where it has been cultivated for centuries. Since these beans are seen in cluster, called cluster beans. Constituents present; Flavonoids- diadzein, genistein, quercetin, kaempherol. Polyphenols - gallotannins, gallic acid, myricetin-7-glucoside-3glycoside, chlorogenic acid, ellegic acid, 2, 4,3-trihydroxy benzoic acid,texasin-7-o-glucoside. Triterpenoidal saponin - 3-epikatonic acid. Sterols - campesterol, avenasterol, stigmasterol, sitosterol. iron,carbohydrates, proteins, fibre, calcium, vitamins.

Pharmacological activities of *Cyamopsis tetragonoloba* fruit are Anti-diabetic activity, Hypocholestremic activity, Anti-ulcer activity, Haemolytic activity, Cytoprotective activity, Anticholinergic activity, Anticoagulant activity, Antimicrobial activity, Anti-asthmatic activity, Anthelmintic activity, Anti-inflammatory activity, Reversible antifertility activity, Wound healing activity, Antioxidant activity, Anti-cataract activity.

MATERIALS AND METHODS

Plant material

The plant material consists of dried powdered fruit of *Cyamopsis tetragonoloba* belonging to the family *Fabaceae*.

Plant collection and authentication

The fruit of *Cyamopsis tetragonoloba* was collected on July 2019 from Nemmara, Palakkad district in Kerala, India. The plant was identified by Dr. C. Murugan, Joint Director, Scientist, C-I/C, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore bearing the reference number BSI/SRC/1/23/2019/Tech.145.

Preparation of plant extract

Fresh fruit of *Cyamopsis tetragonoloba* was collected and dried for one month and later powdered. This powder was then extracted with ethanol for 24 h using soxhlet apparatus.

Drugs and chemicals

DPPH, ascorbic acid, nitro blue tetrazolium, butylated hydroxyl toluene oxidized glutathione; epinephrine and 5 5'Dithiobis-2 nitro benzoic acid were purchased from Himedia Labs., Pvt. Ltd., Mumbai. 2-deoxy-2-ribose, Quercetin, *O*-dianisidine were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai. Trichloroacetic acid and Folin Ciocalteau reagent were purchased from SD Fine Chemicals Ltd., Mumbai. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

ETHICAL CONSIDERATION

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and was approved by the Institutional Animal Ethical Committee (Proposal Number: NCP/IAEC/2019-20/07).

Animals

Mice of both sexes weighing between 25 and 30gm were obtained from the animal house of the Nandha College of Pharmacy and Research Institute, Erode, Tamilnadu, India. For each study, the animals were grouped into five groups each containing six mice and the animals are marked for their identification by using Indian ink. The animals were maintained under standard environmental conditions of 50± 10% relative humidity and 12h light and 12h dark cycle throughout the experiment. The animals were used after an acclimatization period of five days in propylene gages on the laboratory environment. During acclimatization period, mice were provided with standard pellets diet (Hindustan Lever Pvt Ltd., Bangalore) and clean drinking water. All procedure was conducted according to guidelines of the CPCSEA.

EXPERIMENTAL DESIGN

SCOPOLAMINE INDUCED AMNESIA IN MICE

The animals were divided into 5 groups of 6 mice each as follows:

Group I: Animals received normal saline for 10 days.

Group II: Animals received normal saline for 10 days. On the 10th day, scopolamine (1mg/kg, i.p) is injected 30min after normal saline administration

Group III: Animals received piracetam (400mg/kg, i.p) for 10 days. On the 10th day, scopolamine (1mg/kg, i.p) is injected 30min after piracetam administration

Group lV: Animals received CTFE (200mg/kg; p.o) suspended on 1% CMC for 10days. On the 10th day, scopolamine (1mg/kg, i.p) is injected 30min after extract administration.

Group V: Animals received CTFE (400mg/kg; p.o) suspended on 1% CMC for 10days. On the 10th day, scopolamine (1mg/kg, i.p) is injected 30min after extract administration

DIAZEPAM INDUCED AMNESIA IN MICE

The animals were divided into 5 groups of 6 mice each as follows:

Group I: Animals received Normal saline for 10days.

Group II: Animals received normal saline for 10 days. On the 10th day, diazepam (1mg/kg, i.p) is injected 30min after normal saline administration

Group III: Animals received piracetam (400mg/kg, i.p) for 10 days. On the 10th day, diazepam (1mg/kg; i.p) is injected 30min after piracetam administration

Group IV: Animals are received CTFE (200mg/kg; p.o) suspended on 1% CMC for 10days. On the 10th day, diazepam (1mg/kg; i.p) is injected 30min after extract administration.

Group V: Animals are received CTFE (400mg/kg; p.o) suspended on 1% CMC for 10days.On the 10th day, diazepam (1mg/kg; i.p) is injected 30min after extract administration.

PHARMACOLOGICAL EVALUATION

Elevated plus maze

The elevated plus maze for mice consisted of two open arms (16 cm × 5cm) and two covered arms (16cm × 5 cm ×15cm) extended from a central platform (5cm× 5 cm) and the maze was elevated to a height of 25 cm from the floor. The mouse was trained for maze task performance by conducting once a daily training trial along with dosing with the respective drug for ten days. On the first day, each mouse was placed at

the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. Respective Inducing agent [(Diazepam 1mg/kg, i.p), (Scopolamine 1mg/kg, i.p)] was administered before retrieval for induction of retrograde amnesia. Mice were exposed to maze after 45min of inducing agent administration. The TL was recorded on the first day (i.e., 10th day of drug administration) for each animal. If the animal did not enter into one of the covered arm within 90 sec, it was gently pushed into one of the two covered arms and TL was assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h (11th day) after the first day trial.

Radial arm maze

The radial arm maze method serves as exteroceptive behavioural model to evaluate learning and memory of mouse. Each arm 50×12cm in dimension of the eight arm. Radial maze extends from the octagonal shaped central hub of 30cm in diameter. The platform is elevated 40 cm above the floor, small backup metal cups (3cm in diameter & 1cm deep) arm mounted at end of the each arm that serve as receptacles for reinforces food. Each mouse maintained at 85% of its total diet was exposed to the maze daily for training trial with the food pellet in a fixed arm along with respective drug treatment for a period of 10 days. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli.

Respective Inducing agent Diazepam 1mg/kg, i.p, Scopolamine 1mg/kg, i.p was administered before retrieval for induction of retrograde amnesia after 30min of respective drug or vehicle administration. Mice were exposed to maze after 45min of inducing agent administration. The latency to find food was recorded on the first day (i.e., 10th day of drug administration) for each mice. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage.

The latency was examined 24 hours after the first day trial (i.e 11th day) where in food pellet was placed in variable arm for evaluation of working memory. Each mouse placed on the central hub was allowed to choose any of the arms freely to get the food. Latency to find food was recorded as measure of working memory evaluation.

Y maze Test

Short-term memory was assessed by spontaneous alternation behaviour in the Y-maze task. The Y-maze used in the present study consist of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. 30 min after the respective drug administration, mice were placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behaviour was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviours was then the total number of arms entered minus two and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100. The maze was cleaned with a 10% ethanol solution and dried with a cloth before the next animal was tested. Spontaneous alternation behaviour is considered to reflect spatial working memory, which is a form of short-term memory.

BIOCHEMICAL ESTIMATION

Collection of Brain Sample

Immediately after behavioural testing (retrieval) on elevated plus maze, radial arm maze and Y maze and Hebb-williams maze, animals were sacrificed by cervical dislocation under light anaesthesia with diethylether. The whole brain was carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of phosphate buffer (pH 8, 0.1 M). The homogenate was centrifuged using refrigerated centrifuge at 3000 rpm for 10 min at 4◦C and the resultant cloudy supernatant liquid was used for the following tests.

Brain Acetylcholinesterase Activity

Brain acetylcholinesterase was estimated using the method of Ellman *et al*., Briefly, 0.4mL of brain homogenate was added to a test tube containing 2.6mL of phosphate buffer. 0.1mL DTNB reagent was added to the above mixture and absorbance was noted at 12 nm. 0.02mL of acetylthiocholine iodide solution was added and again absorbance was noted 15 min thereafter. Change in absorbance per min was calculated. The rate of hydrolysis of substrate was calculated using following formula:

R = change in absorbance/min×5.74 × 10−4/*C*0,

R = rate of hydrolysis of acetylcholine iodide/min/mgtissue,

C0 =weight of tissue homogenate in mg/mL.

Estimation of Malondialdehyde (MDA)

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of homogenate (Tris HCl buffer, PH 7.4) was treated with 2 ml (1:1:1 ratio) of TBA –TCA-HCL reagent (Thiobarburic acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 rpm at room temperature for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The

values are expressed as nmoles of MDA/min/mg protein.

Estimation of lipid hydroperoxides

About 0.1 ml of homogenate was treated with 0.9 ml of Fox reagent (188 mg Butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250mM sulphuric acid) and incubated for 30 min. the colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/mg protein (Nieshus and Samuelsson, 1986).

DETERMINATION OF *IN-VIVO* ANTIOXIDANTS

Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of tissue homogenate at 480 nm. The reaction mixture contained 150 µl of homogenate, 1.8 ml of 30 mM carbonate buffer (pH 10.2) and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. The activity was expressed as units/mg tissue protein (Misra and Fridovich, 1972).

Estimation of catalase (CAT)

The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1 ml of tissue homogenate and was incubated at 37ºC for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzymatic activity. One unit of catalase activity was defined as the amount of enzymes causing the decomposition of µmol H_2O_2/mg protein/min at pH, 7.0 at 25^{o}C (Aebi, 1984).

Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. Briefly after centrifugation, 0.5 ml of supernatant was taken and mixed with 2.0 ml of 0.3 mol/L disodium hydrogen phosphate (Na_2HPO_4) solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml, 1% sodium citrate) was added and the absorbance was measured immediately after mixing. Results were expressed in µmol GSH/min/mg protein (Sener *et al.*, 2007).

Statistical analysis

Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. P values <0.05 were considered significant.

RESULTS

EFFECT OF FRUIT EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* ON ELEVATED PLUS MAZE

Table 1: Transfer latency of fruit extract of *Cyamopsis tetragonoloba* on Elevated plus maze by scopolamine induced Alzheimer's in mice

Drug Treatment	Transfer Latency (Sec)	
	Acquisition day (10th day)	Retention day (11thday)
Group I- Normal control	29.72±1.2	27.28±1.4
Group II- Negative control (Scopolamine 1mg/kg)	29.49±0.9	39.86±0.8^a
Group III- Positive control (Piracetam 400mg/kg)	27.16±0.8	22.02±1.2^b
Group IV- CTFE (200mg/kg)	28.38±1.4	25.45±1.3^b
Group V- CTFE (400mg/kg)	27.24±0.9	24.76±1.0^b

Values are mean± SEM; n=6.^{a}P<0.01 Vs control. ^{b}P<0.01 Vs Scopolamine control

Table 2: Transfer latency of fruit extract of *Cyamopsis tetragonoloba* on Elevated plus maze by diazepam induced Alzheimer's in mice

Drug Treatment	Transfer Latency (Sec)	
	Acquisition day (10th day)	Retention day (11th day)
Group I- Normal control	29.72±1.2	27.28±1.4
Group II- Negative control (Diazepam 1mg/kg)	29.16±1.1	35.09±2.2^a
Group III- Positive control (Piracetam 400mg/kg)	26.13±0.9	21.24±0.8^b
Group IV- CTFE (200mg/kg)	28.16±1.2	24.16±0.9^b
Group V- CTFE (400mg/kg)	27.39±1.1	23.04±1.2^b

Values are mean± SEM; n=6.^{a}P<0.01 Vs control. ^{b}P<0.01 Vs Diazepam control

EFFECT OF FRUIT EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* ON RADIAL ARM MAZE

Table 3: Working memory evaluation of fruit extract of *Cyamopsis tetragonoloba* on Radial arm maze by scopolamine induced Alzheimer's in mice

Drug Treatment	Latency to find food (Sec)	
	Acquisition day (10th day)	Retention day (11th day)
Group I- Normal control	15.96±3.6	15.82±2.8
Group II- Negative control (Scopolamine 1mg/kg)	15.84±1.2	35.27±2.5[a]
Group III- Positive control (Piracetam 400mg/kg)	14.87±2.1	27.14±1.8 [b]
Group IV- CTFE (200mg/kg)	15.68±1.5	32.34±3.1
Group V- CTFE (400mg/kg)	15.29±1.3	30.09±2.6 [b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05 Vs Scopolamine control

Table: 4: Working memory evaluation of fruit extract of *Cyamopsis tetragonoloba* on Radial arm maze by diazepam induced Alzheimer's in mice

Drug Treatment	Latency to find food (Sec)	
	Acquisition day (10th day)	Retention day (11thday)
Group I- Normal control	15.96±3.6	15.82±2.8
Group II- Negative control (Diazepam 1mg/kg)	16.25±2.8	29.17±3.5[a]
Group III- Positive control (Piracetam 400mg/kg)	14.01±2.4	19.94±2.6 [c]
Group IV- CTFE (200mg/kg)	15.71±3.7	23.52±2.2 [b]
Group V- CTFE (400mg/kg)	15.09±3.5	21.67±1.6 [c]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Diazepam control

EFFECT OF FRUIT EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* ON Y MAZE

Table 5: Percentage spontaneous alteration of fruit extract of *Cyamopsis tetragonoloba* on Y maze by Scopolamine induced Alzheimer's in mice

Drug Treatment	Spontaneous Alteration (%)
Group I- Normal control	66.42±4.7
Group II- Negative control (Scopolamine 1mg/kg)	48.54±3.8[a]
Group III- Positive control (Piracetam 400mg/kg)	58.29±4.1[c]
Group IV- CTFE (200mg/kg)	50.63±3.7
Group V- CTFE (400mg/kg)	53.02±2.5[b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Scopolamine control

Table: 6: Percentage spontaneous alteration of fruit extract of *Cyamopsis tetragonoloba* on Y maze by diazepam induced Alzheimer's in mice

Drug Treatment	Spontaneous Alteration (%)
Group I- Normal control	66.42±4.7
Group II- Negative control (Diazepam 1mg/kg)	36.81±2.6[a]
Group III- Positive control (Piracetam 400mg/kg)	46.96±3.4[c]
Group IV- CTFE (200mg/kg)	41.27±2.9[b]
Group V- CTFE (400mg/kg)	43.18±3.8[b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Diazepam control

EFFECT OF FRUIT EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* ON BIOCHEMICAL PARAMETERS

Table 7: AChE, MDA and LH levels of fruit extract of Cyamopsis tetragonoloba in brain by scopolamine induced Alzheimer's in mice

Drug Treatment	Acetylcholine esterase (µmol/g of tissue)	MDA (nmoles/mg protein)	LH (nmoles/mg protein)
Group I- Normal control	2.18±0.09	1.18±0.04	0.52±0.02
Group II- Negative control (Scopolamine 1mg/kg)	1.27±0.06[a]	2.16±0.07[a]	0.98±0.01[a]
Group III- Positive control (Piracetam 400mg/kg)	1.74±0.05[c]	1.53±0.04[c]	0.69±0.05[c]
Group IV- CTFE (200mg/kg)	1.36±0.07	1.84±0.06[b]	0.82±0.04[b]
Group V- CTFE (400mg/kg)	1.59±0.06[c]	1.61±0.04[c]	0.78±0.03[b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Scopolamine control

Table 8: AChE, MDA and LH levels of fruit extract of *Cyamopsis tetragonoloba* in brain by diazepam induced Alzheimer's in mice

Drug Treatment	Acetylcholine esterase (µmol/g of tissue)	MDA (nmoles/mg protein)	LH (nmoles/mg protein)
Group I- Normal control	2.18±0.09	1.18±0.04	0.52±0.02
Group II- Negative control (Diazepam 1mg/kg)	1.64±0.08[a]	1.92±0.02[a]	0.86±0.03[a]
Group III- Positive control (Piracteam 400mg/kg)	1.92±0.06[c]	1.38±0.04[c]	0.61±0.02[c]
Group IV- CTFE (200mg/kg)	1.71±0.08	1.69±0.03[b]	0.79±0.02
Group V- CTFE (400mg/kg)	1.83±0.07[b]	1.54±0.02[c]	0.68±0.01[b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Diazepam control

EFFECT OF FRUIT EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* ON *IN-VIVO* ANTIOXIDANTS

Table 9: SOD, CAT and GSH levels of fruit extract of *Cyamopsis tetragonoloba* in brain by scopolamine induced Alzheimer's in mice

Drug Treatment	SOD (nmoles/ mg protein)	CAT (µmoles/ mg protein)	GSH (nmoles/min/mg protein)
Group I- Normal control	3.18±0.01	28.4±1.4	10.6±0.6
Group II- Negative control (Scopolamine 1mg/kg)	2.04±0.02[a]	17.1±1.5[a]	7.2±0.4[a]
Group III- Positive control (Piracetam 400mg/kg)	2.81±0.02[c]	25.8±1.2[c]	9.8±0.9[b]
Group IV- CTFE (200mg/kg)	2.28±0.01[b]	19.7±1.6	7.9±0.6
Group V- CTFE (400mg/kg)	2.57±0.04[b]	22.7±1.3[b]	8.7±0.8[b]

Values are mean± SEM; n=6.[a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Scopolamine control

Table-10: SOD, CAT and GSH levels of fruit extract of *Cyamopsis tetragonoloba* in brain by diazepam induced Alzheimer's mice

Drug Treatment	SOD (nmoles/ mg protein)	CAT (µmoles/ mg protein)	GSH (nmoles/min/mg protein)
Group I- Normal control	3.18±0.01	28.4±1.4	10.6±0.6
Group II- Negative control (Diazepam 1mg/kg)	2.72±0.03[a]	21.6±1.2[a]	8.5±0.6[a]
Group III- Positive control (Piracetam 400 mg/kg)	3.07±0.01[c]	27.1±1.8[c]	10.2±0.8[c]
Group IV- CFE (200mg/kg)	2.78±0.03	22.5±1.5	9.2±0.7[b]
Group V- CFE (400mg/kg)	2.96±0.02[b]	25.9±1.7[b]	9.8.7±0.9[b]

Values are mean± SEM; n=6. [a]P<0.01 Vs control. [b]P<0.05, [c]P<0.01 Vs Diazepam control

DISCUSSION

Despite the severity and high prevalence of this disease, allopathic system of medicines is yet to provide a satisfactory antidote. Therefore I was motivated to explore the potential of medicinal plants to manage the AD. From the research and also from the phytochemical analysis and finding of *Cyamopsis tetragonoloba* may be stated that the plant might

possess anti-alzheimer's activity due to the presence of flavones. *Cyamopsis tetragonoloba* is an annual herb, seen as cluster, self- pollinated crop belonging to the family Fabaceae. The *Cyamopsis tetragonoloba* fruit was extracted with ethanol using soxhlet apparatus for 24 hours.

Anti-alzheimer's activity of the ethanolic fruit extract was assessed in mice by using two exteroceptive models such as scopolamine induced amnesia and diazepam induced amnesia. In these two models, behavioural and biochemical parameters were evaluated. Invivo antioxidants of brain homogenate also accessed in both models.

In scopolamine induced amnesia model, behavioural parameters evaluated were elevated plus maze, radial arm maze, Y maze. Scopolamine increased the transfer latency in elevated plus maze as compared to the respective normal control group. Treatment of the CFE significantly reduced the scopolamine induced Alzheimer's in contrast to the normal control. The decrease in the transfer latency indicating the memory improvement. Effect of the doses of 200mg/kg and 400mg/kg CFE were comparable to the standard 400mg/kg piracetam. Therefore this indicates that CFE significantly increases the learning performance.

While in radial arm maze, scopolamine increased the latency to find food as compared to the respective normal control group. Treatment of the CFE significantly reduced the scopolamine induced Alzheimer's in contrast to the normal control. The CFE at a dose of 200mg/kg and 400mg/kg respectively, 400mg/kg showed the values near to the standard drug piracetam 400mg/kg. Therefore this indicates that CFE significantly increases the learning performance.

Finally in Y maze the mice administered with the CFE 400mg/kg and piracetam 400mg/kg showed significant increase in the percentage spontaneous alteration as compared to the control group. But while comparing, piracetam is much more significant than CFE. Thus the extract could be agreed to improve memory performance of the mice.

Similarly in Diazepam induced amnesia model, the above mentioned behavioural parameters were evaluated. Diazepam increased the transfer latency in elevated plus maze as compared to the respective normal control group. Treatment of the CFE significantly reduced the diazepam induced Alzheimer's in a dose dependent manner. The decrease in the transfer latency indicating the memory improvement. The CFE at a dose of 200mg/kg, 400mg/kg showed the values near to the standard drug piracetam 400mg/kg. Therefore this indicates that CFE significantly increases the learning performance.

While in radial arm maze, Diazepam increased the latency to find food as compared to the respective normal control group. The CFE at a dose of 200mg/kg and 400mg/kg respectively, 400mg/kg showed the values near to the standard drug piracetam400mg/kg. Treatment with CFE at a dose of 200mg/kg also reduce latency to find food significantly when compared with scopolamine control. Therefore this indicates that CFE significantly increases the learning performance.

Finally in Y maze the mice administered with the CFE 200mg/kg and 400mg/kg showed significant increase in the percentage spontaneous alteration as compared to scopolamine control group in a dose dependent manner. Piracetam 400mg/kg showed significant increase in the percentage spontaneous alteration as compared to the scopolamine control group. Thus the extract could be agreed to improve memory performance of the mice.

While comparing the inducing agent scopolamine and diazepam, scopolamine induced amnesia in mice better than diazepam in both elevated plus maze and radial arm maze tasks. But in case of Y maze, the exploration period is 8 min. As diazepam induced sedation, the spontaneous alteration gets decreased. The CFE at a dose of 200mg/kg and 400mg/kg, improved memory in a dose dependent manner. But CFE at a dose of 400mg/kg is more significant than 200mg/kg in all tasks.

Biochemical parameters like brain malondialdehyde and lipid hydroperoxide & brain acetylcholine esterase level were evaluated for scopolamine and diazepam induced amnesia models.

The scopolamine treatment significantly decreased the brain acetylcholine esterase levels in the scopolamine-treated group as compared to the normal control group. The CFE at a dose of 200mg/kg and 400mg/kg respectively, 400mg/kg showed the values near to the standard drug piracetam 400mg/kg. Similarly the scopolamine treatment significantly increased the brain MDA levels in scopolamine-treated group as compared to the normal control group. Treatment of the CFE significantly decreased MDA level in a dose dependent manner. The CFE at a dose of 200mg/kg and 400mg/kg respectively, 400mg/kg showed the values near to the standard drug piracetam 400mg/kg. In case of brain LH level, the scopolamine treatment significantly increased the brain LH level in scopolamine-treated group as compared to the normal control group. Treatment of the CFE significantly decreased LH level in a dose dependent manner. Piracetam control showed a significant decrease in brain LH level as compared to scopolamine control.

In diazepam induced amnesia model, the diazepam treatment significantly decreased the brain acetylcholine esterase levels in the diazepam-treated

group as compared to the normal control group. Treatment with CFE at a dose of 400mg/kg showed a significant increase in AChE level when compared to scopolamine control. Piracetam control significantly increased the AChE level as compared to scopolamine control. Similarly, the diazepam treatment significantly increased the brain MDA levels in the diazepam-treated group as compared to the normal control group. Treatment of the CFE significantly decreased MDA level in a dose dependent manner. The CFE at a dose of 200mg/kg and 400mg/kg respectively, 400mg/kg showed the values near to the standard drug piracetam 400mg/kg. In case of brain LH level, the diazepam treatment significantly increased the brain LH level in diazepam-treated group as compared to the normal control group.

Treatment with CFE at a dose of 400mg/kg showed a significant decrease in LH level when compared to diazepam control. Piracetam control showed a significant decrease in brain LH level as compared to diazepam control.

While comparing the effect of inducing agents on biochemical parameters in scopolamine and diazepam induced amnesia models, scopolamine is more effective than diazepam on all three parameters. The CFE at a dose of 200mg/kg and 400mg/kg, improved the alterations in a dose dependent manner. But CFE at a dose of 400mg/kg is more significant than 200mg/kg in all tasks.

After biochemical testing, antioxidants like superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were estimated *in-vivo* using brain homogenate for both scopolamine and diazepam induced amnesia models.

The scopolamine treatment significantly decreased the brain SOD levels in the scopolamine-treated group as compared to the normal control group. Treatment of the CFE significantly increased SOD in a dose dependent manner. Piracetam significantly increased the SOD level compared to scopolamine control. Similarly the scopolamine treatment significantly decreased the brain CAT levels in the scopolamine-control group as compared to the normal control group. Treatment with CFE at 400mg/kg significantly increased the CAT level when compared to scopolamine control. Piracetam significantly increased the CAT level compared to scopolamine control. Finally, in case of reduced glutathione, the scopolamine treatment significantly decreased the brain GSH levels in the scopolamine-treated group as compared to the normal control group. The CFE at a dose of 400mg/kg showed the values near to the standard drug piracetam 400mg/kg.

In diazepam induced amnesia model, the diazepam treatment significantly decreased the brain SOD levels in the diazepam-control group as compared to the normal control group. Treatment with CFE at 400mg/kg significantly increased the SOD level as compared to diazepam control.

Piracetam significantly increased the SOD level compared to diazepam control. Similarly, the diazepam treatment significantly decreased the brain CAT level in the diazepam-control group as compared to the normal control group. Treatment with CFE at 400mg/kg significantly increased the CAT level as compared to diazepam control. Piracetam significantly increased the CAT level compared to diazepam control. Finally, in case of reduced glutathione, the diazepam treatment significantly decreased the brain GSH levels in the diazepam-control group as compared to the normal control group. Treatment with CFE significantly increases the GSH level in a dose dependent manner. Piracetam significantly increased the GSH level compared to diazepam control.

While comparing the effect of inducing agents on brain homogenate antioxidants in scopolamine and diazepam induced amnesia models, scopolamine is more effective than diazepam on all three parameters. The CFE at a dose of 200mg/kg and 400mg/kg, improved the alterations in a dose dependent manner.

The study revealed that induction of amnesia in mice by scopolamine is better than diazepam in behavioural, biochemical and in-vivo brain homogenate antioxidant parameters. The CFE at a dose of 200mg/kg and 400mg/kg improved memory and biochemical and in-vivo antioxidants alterations in a dose dependent manner. Except the case of in-vivo antioxidants estimation, the CFE at the dose of 400mg/kg is more significant than 200mg/kg.

CONCLUSION

The anti-Alzheimer's activity of ethanolic fruit extract of *Cyamopsis tetragonoloba* was evaluated by two exterioceptive models such as scopolamine induced amnesia and diazepam induced amnesia in mice. The fruit extract showed memory enhancement in behavioural parameters such as elevated plus maze, radial arm maze and Y maze. It increased brain acetylcholinesterase and decreased malondialdehyde and lipid hydroperoxide levels. Moreover, it showed increase in superoxide dismutase, catalase and reduced glutathione levels in brain. The study revealed that the *Cyamopsis tetragonoloba* have anti-Alzheimer's activity due to the presence of flavonoids, tannins, saponins, triterpenoids. Further, isolation and characterization of bioactive compounds from the fruit extract, followed by clinical studies are needed to prove *Cyamopsis tetragonoloba* fruit is a potential anti-Alzheimer's agent.

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