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Research Article

Amelioration of Diazepam Induced Memory Impairment by Fruit of *Cucumis sativus* L. in Aged Mice by Using Animal Models of Alzheimer's Disease

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ABSTRACT

Traditionally fruit of *Cucumis sativus* var *sativus* L. (Cucurbitaceae) has been used for its high fiber and vitamin content in gastrointestinal, skin problems and as cooling agent for body as well as brain. The present study assessed the pharmacological effects of cucumber on learning and memory capabilities of mice. Fresh cucumber paste in doses 3, 6, 9 g/kg were fed orally to mice for 15 successive days. Elevated plus maze, sodium nitrite induced metabolic hypoxia and object recognition task models were employed to test learning and memory. Biochemical tests measured anti-cholinesterase activity in brain, serum glucose levels, cholesterol levels, malonadialdehyde levels and reduced glutathione levels in the brain of mice. 9 and 6 g/kg doses reduced TL of mice significantly on 14th ($P < .05$) as well as 15th day ($P < .001$ and $P < .01$ respectively) in EPM whereas in diazepam induced amnesia 6 mg/kg dose reduced transfer latency significantly (14th day $P < .01$ and 15th day $P < .001$). 6 mg/kg dose increased FESC, NESC and DESC in small compartment scores profoundly ($P < .05$) in sodium nitrite induced hypoxia model and depicted significantly ($P < .001$) enhanced d2 index in object recognition task. Brain AChE and serum cholesterol levels were reduced significantly ($P < .01$) by 9 g/kg dose whereas the lower dose (6 g/kg) dose depicted significant ($P < .01$) results on blood glucose, brain GSH and lipid peroxide levels. This is the first report describing that cucumber increased learning and memory in rodents. Various chemical constituents such as agmatine, cucurbitacins, vitamins etc make the cucumber a deserving candidate for studying its effects on brain. Further research is needed for elucidation of its mechanism in brain and specific chemical constituent responsible for its memory modulating activity in aged mice.

Key Words: Hypoxia, acetylcholinestrase, vitamins, learning and memory

INTRODUCTION

The fruit of *Cucumis sativus* var *sativus* L. (Cucurbitaceae) is commonly known as cucumber. It is widely cultivated plant in India, China, Europe, England, Turkey, Russia, Iran and the United States. It is a creeping vine that bears cylindrical fruit that is edible when ripe. It is a great folk medicine used to reduce heat and inflammation in South Africa. [1] Traditionally cucumber has been used as laxative, astringent, anthelmintic, antipyretic, hair tonic; in hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhea, cough, hoarseness of voice and eye diseases. [2] In addition it has been used in gastrointestinal disorders like indigestion, stomach pain, vomiting, constipation, piles, dysentery, cholera, anorexia, liver ailments, intestinal worms, ulcerative colitis. [3,4,5,6] Furthermore, the fruit of *C. sativus* also reported to comprise of pharmacological properties such as hypolipidemic [7], hypoglycemic [8], anti-cancer [9], hepatoprotective [10], anti-inflammatory [11], antioxidant [12], antifungal [13], wound healing [14], antihypertensive, diuretic, protein kinase C inhibitory [15], antacid and carminative. [16] Different chemical compounds such as glycosides, sitosterol, bitter principle cucurbitacins, cucumegastigmanes, lactic acid, agmatine [17], polyamines [18], triterpenoids, polyphenols, flavonoids, many amino acids etc have been identified in cucumber. [19] Besides this cucumber is an excellent source of different vitamins. [19]

Aging leads to functional and anatomical changes in the hippocampus, a brain structure that is important for learning. [20] The ability to learn new tasks decreases with age. [21] On the cellular level, synaptic contacts, synaptic strength, and plasticity are reduced. [22] Poor Memory, lower retention and slow recall are common problems in modern life. Age, stress, emotions are conditions that may lead to dementia, anxiety, high blood pressure to more ominous threat like schizophrenia and Alzheimer's diseases. Alzheimer's disease is a leading cause of dementia in developed countries and primarily affects the elderly persons. [23] The total number of people with dementia worldwide in 2010 is estimated at 35.6 million and is projected to nearly double every 20 years. The

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Table 1: Parameters analyzed in object recognition task

Parameters analyzed	*Index*	*Calculation*
Exploration time for both objects during T1	e1	e1 = a1 + a2
Measure of global habituation from T1 to T2	h1	h1 = e1 – e2
Exploration time for both objects during T2	e2	e2 = a + b
Discrimination between familiar and novel object during T2	d1	d1 = b – a
Discrimination between familiar and novel object during T2, a relative *measure corrected for explorative activity (e2)*	d2	d2 = e2 / d1

Table 2: Effect of cucumber on $NaNO_2$ induced metabolic hypoxia in mice

Treatment	*Dose (kg^{-1})*	*FESC (min^{-1})*	*NESC*	*DESC (sec)*
Control	Normal Diet	2.11 ± 0.25	6.33 ± 0.76	87.66 ± 1.45
$NaNO_2$	75 mg	0.78 ± 0.07*	2.33 ± 0.21*	71.00 ± 1.37#
$NaNO_2$+Piracetam	75 mg + 400 mg	3.67 ± 0.17*	11.00 ± 0.52*	111.50 ± 1.96#
Piracetam	400 mg	4.39 ± 0.50#	13.16 ± 1.49#	121.17 ± 1.66#
$NaNO_2$+C3	75 mg + 3 g	1.67 ± 0.17	5.00 ± 0.52	91.17 ± 1.19
$NaNO_2$+C6	75 mg + 6 g	2.72 ± 0.26	8.30 ± 0.79	94.33 ± 1.54*
$NaNO_2$+C9	75 mg + 9 g	3.50 ± 0.39*	10.50 ± 1.18*	96.83 ± 1.25**

*Values are in mean ± SEM. (n=6). # P < 0.001, ** P < 0.01, * P < 0.05 as compared to control group of mice. DESC, F = 109.52; FESC, F = 18.531; NESC, F = 18.825; P value is <0.0001. C3=Cucumber paste of dose 3g, C6=Cucumber paste of dose 6g, C9=Cucumber paste of dose 9g.*

fastest growth in the elderly population is taking place in China, India, and their south Asian and western pacific neighbors. Most of the dementia's are a progressive, neurodegenerative, debilitating disorder associated with a decline in cognitive abilities, manifested by loss of memory, impaired judgment, aphasia, apraxia, agnosia, disorientation, confusion, impaired judgment, disturbed sleep, loss of interest in life. Hypercholesterolemia, decreased cholinergic inputs to brain, oxidative stress, hyperglycemia, inflammation and aging are the primary risk factors for Alzheimer's disease. The diagnosis of AD can only be confirmed on autopsy - by the presence of amyloid plaque, neurofibrillary tangles, neuronal & synaptic loss and brain atrophy in specific brain areas. [24] Probable diagnosis is made in a living patient (with at least 85% accuracy) on the basis of cognitive tests (especially delayed recall) and exclusion of other conditions such as stroke, hypothyroidism or nutritional deficiency. Besides memory loss, Alzheimer's patients show dramatic personality changes, disorientation, declining physical coordination, and an inability to care for themselves. In the final stages, victims are bedridden, lose urinary and bowel control, and suffer epileptic attacks. [23]

The treatment of cognitive dysfunction is still a hard nut to crack in the field of medicine. Therefore, neuroscientists all over the world are busy exploring the usefulness of alternative systems of medicine (e.g. nature cure, ayurveda, homeopathy etc.). In the light of above introduction, the present study investigates the role of cucumber on learning and memory of mice. Chemical constituents such as agmatine, polyamines, polyphenols, triterpenoids, flavonoids and many vitamins may be responsible for its effect on learning and memory. Cucumber is a dietary substance and consumed by human beings as such. Therefore, cucumber was administered in the diet.

MATERIALS AND METHODS

Animals: Swiss aged male mice (12 - 15 months old) weighing around 35 g were employed, as consequent variation in estrogen levels in female mice may influence the cognitive behavior of animals. [25] All the animals were procured from the Disease Free Small Animal House, CCS Haryana Agricultural University, Hisar (Haryana), India. The animals had free access to food and water, and were housed in an animal room with alternating light-dark cycle of 12 h each at temperature 22 ± 1°C. The animals were acclimatized for at least 7 days to the laboratory conditions before behavioral experiments. The Institutional Animal Ethics Committee approved the experimental protocol and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 0436).

Experimental Design: Fresh cucumbers were collected from local cultivated source and variety was authenticated at Department of Botany, CCS Haryana Agriculture University, Hisar, Haryana (India). The fresh unpeeled cucumber fruit was ground to a paste using an electric grinder. Different concentrations [30%, 60% and 90% (w/w)] of cucumber paste in doses 3, 6 and 9 g/kg were fed *ad libitum* to separate groups of rodents through a specially prepared diet for 15 days successively. The doses were determined on the basis of literature reports. [7, 15] This special diet comprised of a mixture of cucumber paste, standard pellet diet (Ashirwad, Chandigarh, India) and a pinch of salt (sodium chloride); to impart taste. Each animal consumed around 5 g/day of this specially prepared diet. The diet intake was measured daily by weighing the remaining diet (uneaten) in the cages and subtracting this amount from the total feed amount given on the previous day. Control animals received the normal standard pellet diet without cucumber paste. On 14th day, 90 min after the administration of diet, the animals were exposed to object recognition task and sodium nitrite induced hypoxia. The retention of the learning was measured on 15th day after 24 h. After behavioral estimations; the mice were sacrificed

Table 3: Pearson's correlation coefficients (r_p) between different measures of object recognition performance (e1, e2, d1, d2) in mice

	e1	*e2*	*d1*
e2	-0.054	-	-
d1	0.147	0.706	-
d2	0.280	0.430	0.932

Table 4: Effect of cucumber on habituation (f) index e1, e2, d1, d2 values of mice. Effect of cucumber on exploration of individual objects in trial 1 and trial 2 by mice. Paired '*t*' test employed for comparison of a1 and a2 of trial 1

Treatment	*Dose (kg^{-1})*	*f (sec)*	*e1 (sec)*	*e2 (sec)*	*d1 (sec)*	*d2 (sec)*	*a1 (sec)*	*a2 (sec)*	*a1 (sec)*	*b (sec)*
Control	ND	-5.17 ±1.22	10.50±0.76	15.67±1.31	3.00 ± 0.45	0.20 ± 0.04	7.00 ± 0.58**	3.50 ± 0.43	6.33 ± 0.76	9.33 ± 0.61
PCT	400 mg	-7.33 ±1.61	13.67±0.76	21.0 ±0.97*	11.67 ±0.42#	0.55 ±0.01#	9.0 ± 0.73**	4.67 ± 0.33	4.67 ± 0.33	16.33 ± 0.67
C3	3 g	-5.33 ±1.48	14.00 ±1.65	16.33 ±1.12	6.67 ± 0.71*	0.41 ±0.03**	8.00 ± 0.73	6.0 ± 0.93	4.83 ± 0.40	11.55 ± 0.85
C6	6 g	-5.34 ±2.22	12.67±1.54	18.00 ±1.39	7.00 ± 1.15*	0.38±0.06*	8.17 ± 0.90#	4.50 ± 0.62	5.50 ± 0.62	12.50 ± 1.12
C9	9 g	-8.83 ±1.19	11.67±1.02	20.50±0.76*	9.83 ± 0.95#	0.47 ±0.03#	7.50 ± 0.76#	4.17 ± 0.31	5.33 ± 0.33	15.17 ± 0.79

*e2, F = 4.493, P value is 0.0071 (NS); d1, F = 17.611; d2, F = 11.400. P value is <0.0001. # P < 0.001, ** P < 0.01, * denotes P < 0.05 as compared to control group of mice. ND=Normal diet, PCT=Piracetam.*

to measure brain acetylcholinesterase, malonadialdehyde, blood glucose, serum cholesterol and brain reduced GSH levels.

Chemicals and Vehicles Used: 5,5'-dithiobis-2-nitrobenzoic acid and reduced glutathione (Sisco Research Lab. Pvt. Ltd., Mumbai, India). Acetylcholine iodide, eserine salicylate (Hi-Media, India), piracetam (Nootropil; UCB India Limited, India), simvastatin (Krabs Biochemicals and Industries Limited, India), donepezil HCl (Donep; Wockhardt Ltd., Baddi, India), cholesterol diagnostics kit (Erba Diagnostics, Germany), cholinesterase diagnostics kit (Bayer Diagnostics, India). Donepezil injection and piracetam injection were dissolved separately in normal saline and injected intra peritoneal. Simvastatin was suspended with 0.5% w/v carboxymethylcellulose sodium and given orally.

Elevated Plus-Maze: Elevated plus-maze (EPM) served as the exteroceptive behavioral model to evaluate learning and memory in mice. The procedure, technique and endpoint for testing learning and memory were followed as per the parameters described by the investigators working in the area of neuropsychopharmacology. [26] Briefly, the EPM for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extended from a central platform (5 cm × 5 cm), and the maze was elevated to a height of 25 cm from the floor. 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 (in sec) by the animal to move from the open arm into one of the covered arms with all its four legs. If the animal did not enter into the enclosed arms within 90 sec, it was gently pushed into one of the two enclosed arms, and the TL was assigned as 90 sec. TL was recorded on the first day (training) for each animal. The mouse was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h after the first day trial.

Sodium Nitrite Induced Metabolic Hypoxia: The observation area consists of a rectangular box of dimension 35 cm x 20 cm x 25 cm divided into a small chamber ($1/4^{th}$ area) and a large one. Using lower doses of sodium nitrite ($NaNO_2$) i.e. 75 mg/kg, sub cutaneous (s.c.), mice were submitted to a positive reinforcement paradigm. Groups of 6 mice were water deprived for 24 h. The mice were treated with the test compound 45 min before they are placed individually in a large chamber. On one wall of the chamber, there is a small compartment that contains a water bottle. The mouse easily finds the bottle and is allowed to drink for 30 s. Each mouse then received a subcutaneous injection of 75 mg/kg $NaNO_2$ before being returned to the home cage. 24 h later, retention testing was performed by placing the mouse in the large chamber, but at this time, the small compartment was kept empty. The frequency of the mouse exploration of the small compartment (FESC), number of entries of mouse in the small compartment (NESC), duration of exploration of the small compartment (DESC) while searching for water is

Table 5: Effect of cucumber on brain acetylcholinestrase levels, serum cholesterol, lipid peroxide levels in brain and brain reduced glutathione levels

Treatment	*Dose (kg^{-1})*	*AChE activity (µmol/l/min/g tissue)*	*Serum Cholesterol (mg/dl)*	*LPO level (nmolMDA/g wet wt.)*	*GSH levels (µmol/g wet wt.)*	*Blood Glucose (mg/dl)*
Control	Normal Diet	18.16 ± 0.39	136.08 ±4.84	120.07 ± 4.86	240.66±11.40	275.33 ± 9.01
Donepezil	0.1 mg	14.02 ± 0.37#	----------	----------	---------	---------
Simvastatin	5 mg	----------	98.15 ±2.30#	----------	---------	--------
C3	3 g	17.75 ± 0.35	118.19±3.39*	103.65±2.93*	278.99±4.82*	236.17±6.90*
C6	6 g	16.30 ±0.42*	117.35±4.03*	101.87±2.07**	291.17±7.89**	230.72±11.8**
C9	9 g	15.84 ±0.52**	113.73±3.84**	95.48 ± 2.79#	311.39±10.09#	204.78±5.64#

For brain AChE levels F = 15.923; for serum cholesterol levels F = 12.831, P value is <0.0001; for LPO levels F = 11.929, P value is 0.0002; for GSH levels F = 11.174; for blood glucose, F = 11.325 P value is <0.0001

evaluated over a period of 3 min. An increase in duration and frequency correlates with improved memory. [27, 28]

Object Recognition Task (ORT): The observation area consisted of a circular open field, 480 mm in diameter and the wall height 400 mm. Four different sets of objects, made of aluminum were used i) a cone ii) a ball iii) a plate iv) a glass bottle filled with sand. All objects were available in triplicate. They could not be displaced by the mouse nor could the mouse climb onto or hide under the objects. The objects had no natural significance and they were never associated with any kind of rein forcer. Two objects were presented in the first trial and a third one in the recognition trial to prevent odor cues. Objects were cleaned with tap water and detergents after each trial. During two consecutive days, the mice were habituated to the apparatus and the testing procedure. They were allowed to explore the empty apparatus twice for 3 min each day (one morning and one afternoon session). Animal was placed into the apparatus, equidistant from the two objects, facing the wall in front of the experimenter. Duration of exploration was 3 min.

Animals were trained in pairs of two trials that were separated by a retention interval of one hour. During the first trial (T1) the apparatus contained two identical objects, "A1" and "A2". These objects were placed in a symmetrical position about 120 mm (with reference to the centre of the object) away from the wall. During T2 the apparatus contained two different objects, a copy of the familiar one "A" from T1 and a novel object "B". Mean time (in sec) exploring the familiar object A(a) and B(b) during T2; and A(a1) and A(a2) during T1 were measured. Following parameters were measured (Table 1).

Exploration is defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with nose. Sitting on the object is not considered as exploratory behavior.

Location preference was tested by comparing a1 and a2 (paired '*t*' test). A virtual group was constructed with a mean of zero and standard error of mean (SEM) that corresponded with the average SEM of the discrimination parameter. This group can be considered as a group that did not discriminate between the objects with an associated expected sample variation. Although this can be considered as an arbitrary choice, it provides a most optimal manner to evaluate whether the discrimination performance of a group in a specific delay condition differed from zero. For this virtual group, the mean (SEM) calculated for d1 and d2 were 0 (1.37) and 0 (0.06) respectively. Values of d2 below 0.15 can be considered as failure to discriminate. This value will refer to as the 'discrimination level' in the present study. In addition, the interdependency of different measures of object recognition performance (e1, e2, d1 and d2) was assessed using Pearson's correlation coefficient (r_p). [29]

Measurement of Locomotor Activity: The effect of cucumber paste on ambulation (spontaneous locomotor acivity) was recorded using Medicraft photoactometer (INCO, Ambala, India) in different groups of mice.

Estimation of Brain Acetylcholinesterase (AChE): Brain AChE activity was measured by the method of Ellman *et al*. [30] The change in absorbance per minute of the sample was measured on double beam UV-Visible spectrophotometer (Systronics 2203, Bangalore, India) at 420 nm.

Estimation of Serum Total Cholesterol Level: CHOD-PAP method by Allain *et al.* was used for the estimation of serum total cholesterol. [31] The absorbance was read at 510 nm and 630 nm (Filter 1 and Filter 2) against the blank sample by using double beam UV-Visible spectrophotometer.

Estimation of Lipid Peroxide Level: Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Ohkawa *et al.* [32] The MDA content was expressed as nmol/mg protein. The protein concentration was estimated by Lowry method using bovine serum albumin as the standard. [33] Absorbance was measured at 532 nm using double beam UV-Visible spectrophotometer.

Estimation of Reduced Glutathione: Absorbance was measured at 412 nm on double beam UV-Visible spectrophotometer. Data are expressed as µg per g wet weight of brain tissue. [34]

Estimation of Blood Glucose Level: GOD-POD method was used for the estimation of blood glucose using Auto-analyzer. [35] The absorbance was read at 510 nm and 630 nm (Filter 1 and Filter 2) against the blank sample by using Autoanalyzer (Erba Mannheim Chem-5 Plus V2).

Statistical Analysis: Values are in mean ± SEM. (n = 6). For statistical analysis one-way ANOVA was used followed by Dunnett's *t*-test. Pearson's correlation

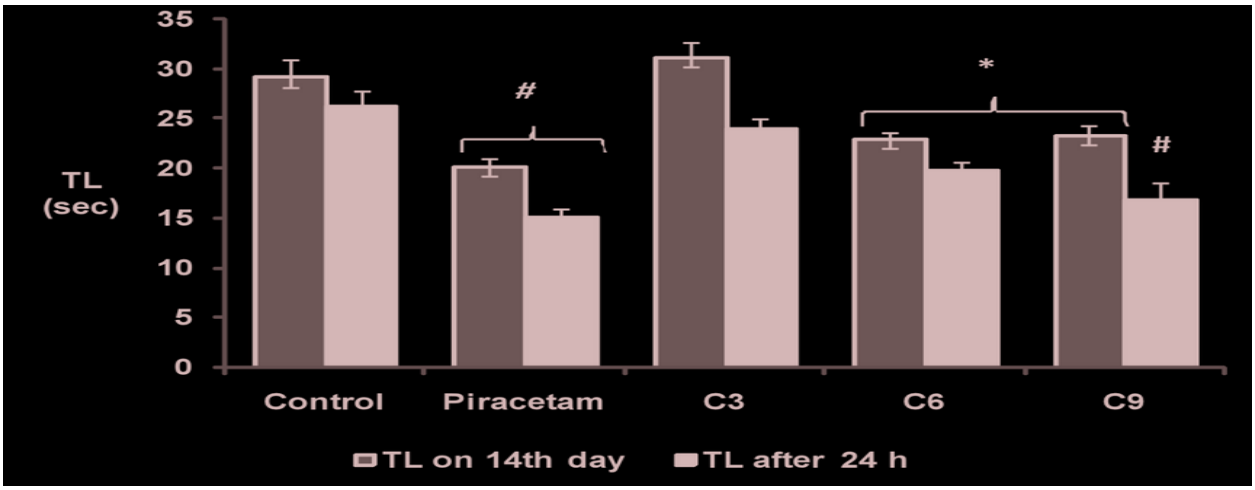

*Fig 1: Effect of cucumber on learning and memory of mice using EPM. # p < .001, ** p < .01, * p < .05 as compared to control group of mice. f = 14.842. C3=cucumber paste of dose 3g, C6=cucumber paste of dose 6g, C9=cucumber paste of dose 9g.*

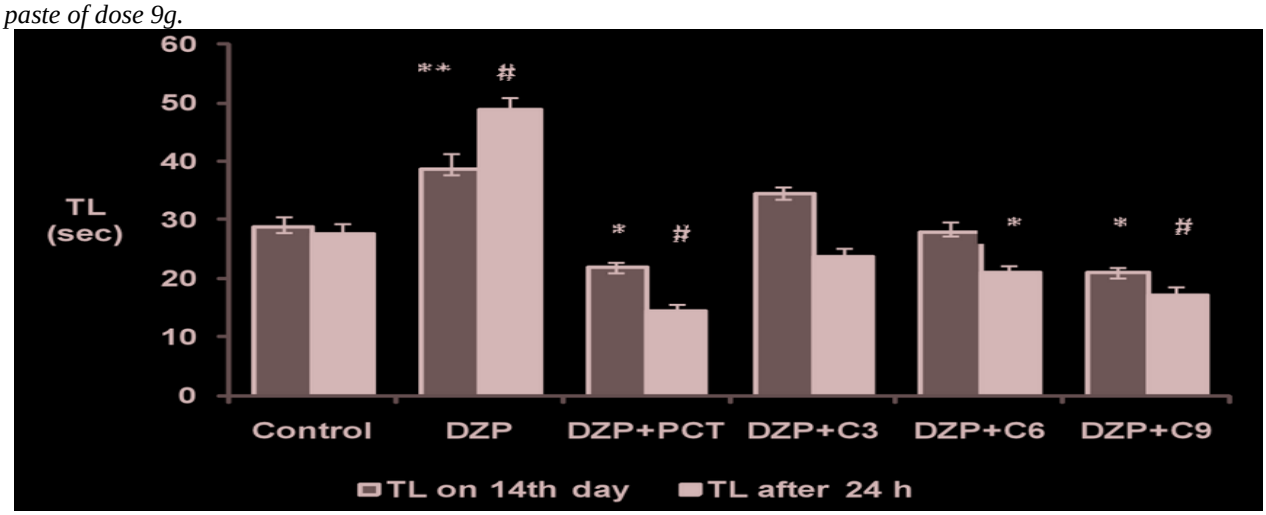

*Fig 2: Effect of cucumber on diazepam induced amnesia in mice using EPM. # denotes p < .001, ** p < .01, * p < .05 as compared to control group of mice. f = 41.356. C3=cucumber paste of dose 3g, C6=cucumber paste of dose 6g, C9=cucumber paste of dose 9g. DZP=diazepam, PCT=piracetam.*

coefficients (r_p) were measured between object recognition performance parameters (e1, e2, d1 and d2). Paired '*t*' test for location preference was employed. $P < 0.05$ - 0.0001 was considered as statistically significant.

RESULTS

Cucumber paste given to mice *ad libitum* has not affected the diet intake and weight of animals in comparison of control group animals.

Effect of cucumber on learning and memory of mice by using EPM: 9 g/kg dose of cucumber diminished the TL (23.33 ± 0.88) recorded on 14th day significantly ($P < .05$) as compared to control (29.17 ± 1.78). Furthermore, this dose of cucumber decreased the TL (16.83 ± 1.74) recorded next day i.e. 15th day, profoundly ($P < .001$), indicating a remarkable improvement in learning as well as memory of mice. Moreover, cucumber at a dose of 6 g/kg decreased the TL (23.00 ± 0.58) significantly ($P < .05$) on day 14th while reducing the TL (19.83 ± 0.87) next day markedly ($P < .01$), indicating significant improvement in learning and memory of mice. The 3 g/kg dose of piracetam improved learning remarkably in mice, by decreasing TL (20.17 ± 0.87) markedly ($P < .01$) on 14th day and enhanced the memory of mice by reducing TL (15.17 ± 0.70) profoundly ($P < .001$) on 15th day (Figure 1).

Effect of cucumber on diazepam induced amnesia in mice using EPM: The TL (38.83 ± 2.65) was elevated markedly ($P < .01$) on day 14th by diazepam (1 mg/kg, i.p.) injected before training while profoundly ($P < .001$) increased TL (48.83 ± 2.14) of day 15th which reflected remarkable impairment in learning and memory in mice. However, the TL (21.18 ± 0.80) recorded on 14th day was reduced significantly ($P < .05$) by 9 g/kg dose of cucumber as compared to control (28.83 ± 1.85), whereas TL (17.17 ± 1.30) of day 15th was reduced profoundly ($P < .001$) as compared to control (27.67 ± 1.74) by cucumber at same dose, which reflected remarkable reversal in diazepam induced impairment of learning and memory. The 6 g/kg dose also reduced TL (21.17 ± 1.01) recorded on day 15th significantly ($P < .05$), indicating reversal of diazepam induced memory deficit. Piracetam caused marked ($P <$

.01) increase in learning (TL 22.0 ± 0.73) and profound (P < .001) enhancement of memory (TL 14.67 ± 0.88) in diazepam treated mice (Figure 2).

Effect of cucumber on $NaNO_2$ induced metabolic/brain hypoxia in mice: $NaNO_2$ administered after training to thirsty mice, caused significant impairment in memory of mice as reflected by significantly (P < .05) decreased score of FESC (0.78 ± 0.07) as compared to control group. 9 g/kg (p.o.) dose significantly (P < .05) enhanced FESC (3.5 ± 0.39) recorded on 14th day in comparison to FESC (2.11 ± 0.25) of control group, which reflected a good reversal of $NaNO_2$ induced memory impairment. Moreover, piracetam (400 mg/kg, i.p.) a nootropic agent, reversed the $NaNO_2$ induced memory deficit and thus improved the memory in mice significantly (P < .05) as reflected by increased score of FESC (3.67 ± 0.17) (Table 2).

We observed a significant (P < .05) impairment in memory of mice, when $NaNO_2$ was administered after training to thirsty mice as indicated by decreased score of NESC (2.33 ± 0.21) in comparison to control group. At 9 g/kg dose the NESC (10.5 ± 1.18) score of mice recorded on 14th day was significantly (P < .05) increased in comparison to NESC (6.33 ± 0.76) of control group, which indicated a good reversal of retrograde memory impairment induced by $NaNO_2$. Moreover, piracetam reversed the $NaNO_2$ induced memory deficit as reflected by significantly (P < .05) increased score of NESC (11.0 ± 0.52) as compared to NESC of control.

A profound (P < .001) decrease in the score of DESC (71.0 ± 1.37) was noted as compared to control group when $NaNO_2$ was administered after training to thirsty mice. The 9 g/kg dose markedly (P < .01) increased score of DESC (96.83 ± 1.25) in comparison to control group (87.66 ± 1.45) thereby reversed the retrograde amnesia. Moreover, DESC (94.33 ± 1.54) score was significantly (P < .05) enhanced as compared to control by 6 g/kg dose of cucumber, which indicated increased memory of mice. Piracetam remarkably increased the memory in mice as reflected by a profoundly (P < .001) increased score of DESC (111.5 ± 1.96) in comparison to control. However, lower dose 3 g/kg produced no significant effect on memory of mice in all three scores (Table 2).

Effect of cucumber on memory of mice using ORT: It can be seen that the habituation / familiarity (f) indices are negative. Negative value reflected an increase in the total exploratory activity from T1 to T2. The exploration times in T1 were found to be not significant as compared to control for any of three doses of cucumber. 9 g/kg dose is the highest effective dose as compared to control group. Results showed a significantly increased time taken by the aged mice for exploration of object a1 in trial 1 as compared to object a2 which depicts the location preference of mice for object exploration. However, in trial 2 the mice spends more time in exploring object b as compared to object a1. The object a1 was placed according to location preference of mice in trial 2 (Table 4).

The Pearson's coefficient depicted the sympathy among all the variables in this procedure. A positive value indicates a positive relationship between variables and a negative value indicates a negative relationship. It depicts whether the variables are in proportional to each other or not (Table 3).

Exploration time (20.5 ± 0.76) in T2 was significantly (P < .05) increased in comparison to exploration time (15.67 ± 1.31) of control group, by 9 g/kg dose (Table 4). Piracetam (400 mg/kg, i.p.) significantly (P < .05) enhanced e2 (21.0 ± 0.97) as compared to e2 of control group. However, 6 g/kg and 3 g/kg dose did not have significant effect on e2 (18.0 ± 1.39, 16.33 ± 1.12) of mice. The observations made by us reflected perspicuously that cucumber dose dependently increased the d1 index and hence increased discrimination ability of mice between two objects. The 9 g/kg dose of cucumber profoundly (P < .001) increased the absolute discrimination index d1 (9.83 ± 0.95) as compared to that of control group (3.0 ± 0.45) thereby indicated that the mice spent more time investigating the new object (Table 4). Furthermore, the 6 g/kg and 3 g/kg doses significantly (P < .05) enhanced the d1 index (7.0 ± 1.15 and 6.67 ± 0.71 respectively) which indicated enhanced discrimination ability of mice. Piracetam increased the d1 index (11.67 ± 0.42) profoundly (P < .001) as compared to d1 index of control group (3.0 ± 0.45) which indicated the improved recognition potential of aged mice. The relative discrimination index d2 was increased profoundly as compared to control group by 9 g/kg dose of cucumber.

Similar to d1 index the cucumber dose dependently enhanced the d2 scores of mice. In comparison to d2 index (0.2 ± 0.04) of control group, the 9 g/kg dose of cucumber resulted in profound (P < .001) increase in d2 index (0.47 ± 0.03), which reflected remarkable increase in recognition ability of mice. The d2 index (0.38 ± 0.06) was enhanced significantly (P < .05) by 6 g/kg dose in addition to increase in d2 index (0.41 ± 0.03) markedly (P < .01) by 3 g/kg dose of cucumber, which indicated enhanced ability of mice to differentiate between two objects. Furthermore, piracetam (400 mg/kg, i.p.) administered to mice for 7 days successively also enhanced the d2 value (0.55 ± 0.01) profoundly (P < .001).

Biochemical tests to identify metabolic effects of cucumber in mice: Results depicted significant decrease in AChE activity in brain of mice by 6 g/kg and 9 g/kg dose. All the three chosen doses reduced serum cholesterol level, lipid peroxide (LPO) level in brain of mice and blood glucose level significantly, while these doses increased the brain reduced glutathione (GSH) levels in mice (Table 5).

Cucumber paste (3, 6, 9 g/kg) when administered for 15 successive days did not show any significant change in locomotor activity of mice (scores: 194 ± 13, 196 ± 13, 203 ± 14) as compared to control animals (scores: 188 ± 11, 182 ± 12, 180 ± 11).

DISCUSSION

Molecular mechanism of AD involves the formation of a peptide known as amyloid beta ($A\beta_{40}$ and $A\beta_{42}$) which clusters into amyloid plaques on the blood vessels and on the outside surface of neurons of the brain which ultimately leads to the killing of neurons. [36] Following amyloid plaque formation two processes play an important role in causing the death of neurons: (1) inflammation and

oxidative damage, and (2) neurofibrillary tangles (NFTs) which contain hyperphosphorylated tau protein.

Elevated plus maze is a neutral model, in which mice show preference towards covered arms and it is particularly useful for assessment of spatial long term memory. In the present study, we administered cucumber for 15 days consecutively to mice. The mice depicted good learning index and prodigious memory score as indicated by abridged TL, when compared to control animals that received only banal diet. Furthermore, pretreatment with cucumber for 15 days protected the mice from learning and memory impairment produced by interoceptive stimuli diazepam given before training. Piracetam, the first representative of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. [37] Piracetam improved learning and memory significantly and also vividly reversed the memory-impairing effect of diazepam in the present study.

$NaNO_2$ induced brain hypoxia or metabolic hypoxia is an interoceptive behavioral model which can be utilized for screening of nootropics and free radical scavengers having memory improving effects. Reduction in oxygen supply to brain as in case of hypoxia, hypercapnia or ischemia is reported to produce retrograde amnesia in rodents. Here are some vital roles of nitric oxide (NO) in brain. Physiological role: Neurotransmission and memory. Pathophysiological role: Degeneration of neurons due to excitotoxicity, generation of free radicals by reaction of NO with oxygen, NO binds to cytochrome c oxidase in mitochondria and fetter cell respiration and release superoxide anions from mitochondrial respiratory chain etc. Reduced glutathione deficiency facilitates NO and peroxynitrite-dependent neurotoxicity by, possibly, increasing the rate of protein nitration and mitochondrial damage at complex I. [38] $NaNO_2$ releases NO *in vivo*. Biosynthesis of NO occurs throughout the CNS particularly in the cerebellum in neurons as well as in glia from L-arginine by NO synthetase (NOS). [39] Memantine protects against excitotoxicity without interfering with glutamate signaling, while also increasing BDNF and exerting anti-oxidant effects. In present study cucumber reversed the NO induced amnesia in mice. Hence, it has same property as that of memantine and may be same mechanism of action also.

ORT is a one-trial learning task having several advantages for assessing the effects of drug treatment on memory processes independently i.e. acquisition, consolidation and retrieval. In the present study, the cucumber significantly enhanced the absolute discrimination index d2 and relative discrimination index d1 as compared to control groups which reflected a remarkable increase in the recognition of new object by mice in trial T2. Cucumber also protracted the exploratory activity of mice significantly in trial T2 as compared to control group, which manifested perspicuously increased ability of mice for object differentiation. In general it is assumed that d2 is more reliable measure of discrimination performance than the d1 because it corrects for the total exploratory activity. But it has to be noted that also d2 should be treated with caution, as can be inferred from our data. A small change in exploration time in T2 will have a large effect on d2 value. But if total exploration is high enough then it is less likely to affect the d2 value i.e. d2 will then be independent of e2.

The neurons being killed in the greatest numbers by NFTs are (1) the large cholinergic neurons in the basal nucleus of Meynart (2) the large pyramidal neurons in the entorhinal cortex (3) output neurons in the CA1 region of the hippocampus. AD is associated with decreased density of muscarinic as well as nicotinic acetylcholine receptors in the cerebral cortex which play an important role in learning & memory. There is increasing evidence, however, that the primary action of AChE inhibitors may not be primarily due to prolongation of action of acetylcholine (Ach). AChE promotes aggregation of amyloid-beta. [40] A mixture of amyloid-beta & AChE shows 3-times more aggregation of amyloid-beta than amyloid-beta alone. Glycation of AChE is increased by amyloid-beta. [41] AChE inhibitors increase the amount of APP that is cleaved by alpha-secretase rather than beta-secretase. In the present study cucumber decreased the levels of AChE in brain of aged mice and thus, may retarded the deposition of Aß by elevating APP. Choline present in cucumber in good amount can also help to improve brain Ach levels.

Both types 1 and 2 diabetes are also important risk factors for decreased performance in several neuropsychological functions. Chronic hyperglycemia and hyperinsulinemia primarily stimulates the formation of Advanced Glucose End products (AGEs), which leads to an overproduction of Reactive Oxygen Species (ROS). Protein glycation and increased oxidative stress are the two main mechanisms involved in biological aging, both being also probably related to the etiopathogeny of AD. AD patients were found to have lower than normal cerebrospinal fluid levels of insulin. Besides its traditional glucoregulatory importance, insulin has significant neurothrophic properties in the brain. Greater insulin resistance with aging leads to higher plasma insulin which stimulates nitric oxide synthetase. Nitric oxide in mitochondria combines with superoxide to produce peroxynitrite. Peroxynitrite inhibits mitochondrial enzymes, antioxidant enzymes, increases free-radical damage and activates a protein kinase that can phosphorylate tau protein leading to NFTs. [42, 43] In the present study cucumber reduced the glucose levels in blood of mice. The hypoglycemic action of cucumber may be due to its extrapancreatic site of action, that is, by direct metabolic effect on tissues, particularly liver. [44]

The direct evidence supporting elevated oxidative stress in AD is: (1) increased brain Fe, Al, and Hg in AD, capable of stimulating free radical generation; (2) enhanced lipid peroxidation and decreased polyunsaturated fatty acids in the AD brain, and increased 4-hydroxynonenal; (3) increased protein and DNA oxidation in the AD brain; (4) reduced energy metabolism and decreased cytochrome c oxidase in the brain in AD; (5) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1,

SOD-1 in senile plaques. Detoxifying enzymes, e.g. glutathione peroxidase, gluthathione reductase, superoxide dismutase and catalase together with antioxidant mechanisms such as the glutathione system and vitamin E, C and A are involved in the defense system against radical injury. [45] Glutathione can protect synapses from damage by peroxynitrite. [46] An imbalance between the formation of oxygen free radicals and the protective mechanisms has been proposed as a major factor for aging and Alzheimer's disease. The present research depicts that cucumber reduced oxidative stress by replenishing the levels of GSH in mice. Moreover, many vitamins present in cucumber in high amount may also serve the purpose of antioxidant.

High level of dietary Vitamin E and Vitamin C is associated with a lowered risk of AD. [47] Vitamin E has been shown to reduce F_2-isoprostane levels in the plasma of rats [48] and to reduce Aß deposition in mice subjected to repetitive concussive brain injury. Cucumber is a good source of these vitamins may protect brain from AD.

The toxicity of $Aß_{42}$ (amyloid-beta) is often attributed to the aggregation of this peptide into a ß-sheet structure of ordered fibrils. [49] Acidic conditions (such as exist in lysosomes and inflammation) enhance amyloid-beta aggregation. The overall alkalizing property of cucumber can eliminate the acidic conditions in brain.

CONCLUSION

The primary risk factors for AD such as hypercholesterolemia, decreased cholinergic inputs to brain, oxidative stress and hyperglycemia are rectified by cucumber paste in aged mice thereby improved the learning and memory of mice. Its potential to vitalize the neurons in ischemic conditions in perspicuous manner may propel its beneficial role in brain injury, environmental toxicity and other hypoxic conditions of brain.

AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no conflicts interest.

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