ISSN:0975-5772

George Lekha *et al* / Journal of Pharmaceutical Science and Technology Vol. 2 (2), 2010, 130-138

Cognitive enhancement and Neuroprotective effect of *Celastrus paniculatus* Willd. seed oil (Jyothismati oil) on male Wistar rats.

George Lekha[1*], Bhagya P Kumar[2], Shankar Narayana Rao[3], Irudaya Arockiasamy[4] and Karthik Mohan[5]

[1]Dept. of Botany, Mount Carmel College, Bangalore, Karnataka, India

[2,3]Dept. of Neurophysiology, NIMHANS, Bangalore, Karnataka,India

[4]Dept. of Plant Biology and Biotechnology, St.Joseph's College (Autonomous), Trichy, Tamil Nadu, India

[5]Dept. of Biochemistry, St.Joseph's College (Autonomous), Trichy, Tamil Nadu, India.

*E-mail:*lekha_tharian@yahoo.co.in

Abstract:

Amongst the various functions of the brain, one of the most interesting is the ability to acquire new information and store them for further retrieval. Learning is a process of acquiring new information, while memory refers to the persistence of a change in behaviour overtime, in a state that can be retrieved later. Several Central Nervous System disorders (CNS) are often associated with impairment in cognitive functions. Alzheimer's disease has a primary impact on learning and memory and other disorders like schizophrenia, bipolar depression are associated with secondary deficits in learning and memory functions. The mechanisms underlying learning and memory include an interaction between the various neurotransmitter systems, amongst which the central cholinergic function is known to play a prominent role. Estimation of Acetyl Choline Esterase (AChE) activity provides a relatively easy and valuable assessment of cholinergic function. In this context the Jyothismati oil from seeds of *Celastrus paniculatus*, widely used in the indigenous medicinal systems to treat brain related disorders was used to determine its effect on the learning process in the adult male Wistar rats. To study effect on learning and memory radial arm maze paradigm was used. The data indicated enhancement in radial arm maze acquisition with chronic administration of CP oil (400 mg/kg body weight). A decrease in AChE activity was noted in the treated animals leading to increased cholinergic activity in the brain. There was significant decrease in the AChE activity assayed from hypothalamus, frontal cortex and hippocampus of the rat brain treated with 400 mg/kg body weight. No side effects were observed with administration of the seed oil.

Key words - *Acetyl choline esterase, Alzheimer's disease, Celastrus paniculatus, Radial arm maze (RAM)*

Introduction:

Loss of memory and of cognitive function affects people worldwide (Artur et al, 2008). Such loss may be the result of different progressive neurological disorders of the brain. It affects both men and women and is common in the elderly (Meyers, 1998). However cognitive decline is not necessarily a function of aging. Several causes drug abuse, allergies, metabolic and neurological disorders are equally responsible for loss of cognitive function of brain. Memory storage and cognitive function in the human brain includes mainly the right and left cerebral hemispheres (Neuwelt, 1991). Memory loss and problem in learning may range from normal to a mild cognitive impairment, or MCI, or to a more severe disturbance, such as dementia. People age 55 to 90 years may have forgetfulness characterized by MCI, but may not be clinically diagnosed as having Parkinson's or Alzheimer's disease. Cognitive function embraces the quality of knowing, which includes all aspects of perception; recognition, conception, sensing, thinking, reasoning, remembering and imagining. Cognitive impairment is the difficulty in dealing with or reacting to new or novel information or situations. The past two decades have seen tremendous advances in the area of brain physiology, learning, memory, and various brain disorders, and a host of mechanisms at molecular level have been delineated. Synapses--the junctions of nerve cells representing the basic interactive unit of neuronal circuits--constitute the fundamental systemic relationship within the brain. Understanding how this interactive multitude of neuronal circuitries established initially, and refined continuously throughout life, is fundamental to understanding the molecular basis of learning and memory. At present, an impressive array of chemical entities affecting synapse formation, neuronal differentiation, neurotransmission, nerve growth and repair, and several other functions are recognized. Approximately 50

neurotransmitters belonging to diverse chemical groups have been identified in the brain. Acetylcholine (Ach), the first neurotransmitter to be characterized, has a very significant presence in the brain; recently it was determined that acetylcholine is essential for learning and memory (Winkler et al, 1998). Acetylcholine has been a special target for investigations for almost two decades because its deficit, among other factors, has been held responsible for senile dementia and other degenerative cognitive disorders, including Alzheimer's disease. Major emphasis has been on acetylcholine, because the number of acetylcholine receptors declines with advancing age. Inhibitors of acetylcholine esterase (AChE), which terminates the action of acetylcholine, have been special targets for development. *Celastrus paniculatus*, a plant belonging to *Celastraceae* was in use from time immemorial to treat brain related disorders and to enhance learning and memory (Gaitonde et al, 1957). The present study envisages the following objectives which were derived from the paradigm explained above with a thrust on the understanding of the effect of *Celastrus paniculatus* seed oil (Jyothismati oil), a potential nervine, on the central nervous system based on behavioural and biochemical study.

Materials and Methods:

Experimental animals

Adult male Wistar rats weighing around 150 – 160 grams were used for the study. The animals were housed in polypropylene cages (22.5 x 35.5 x l5cm) and controlled temperature (25 ± 2°C), humidity (50-55%) and light (12h-light-dark cycle) environment, with food and water ad libitum. The experiments were carried out in accordance to the guidelines of the institutional animal ethical committee.

Drug

Commercially available *Celastrus paniculatus* seed oil. The oil was emulsified with 1% Tween-20 (solubilising agent) and Dimethyl sulphoxide (solvent). Three different doses (100 mg, 200 mg and 400 mg / kg of body weight) of the seed oil was injected intraperitoneally to the grouped animals for 14 days.

Experimental Design

The animals were divided into four groups containing six animals each. The first group was Normal control group, second, third and fourth were pre-treated with 100 mg, 200 mg and 400 mg / kg of body weight the Jyothismathi oil for 14 days respectively. The animals were trained for two consecutive days and their behavioural changes were studied for 16 days.

Assessment of behaviour in the radial arm maze (RAM)

Learning and memory in the 8-arm radial arm maze was assessed earlier (Srikumar et al, 2004; Srikumar et al, 2007; Titus et al, 2007)). The eight arm radial maze consisted of a computer monitored maze (Columbus Instruments, Ohio, USA), with equally spaced arms (42 x 1.4 x 1.4cm) radiating from an octagonal central platform and the maze was kept 80cm elevated from the ground**.** Prior to the training, the animals were kept on a restricted diet and body weight was maintained at 85% of their free feeding weight, with water available *ad libitum*. Before the commencement of the behavioural assessment, all groups of rats were semi-starved for 48 hrs in order to motivate them towards food reward. On subsequent days, 5-8 gm of food was provided to maintain 85% of its initial body weight, through out the experimental period.

Training

To acclimatize the rats to the RAM prior to acquisition all the arms were baited and rats were allowed to explore the maze for 10 minutes and were given two such sessions on two consecutive days.

Partially baited RAM task

In this, four of the eight arms are baited and the rats are trained to choose only the baited arms. This task permits discerning of reference memory and working memory

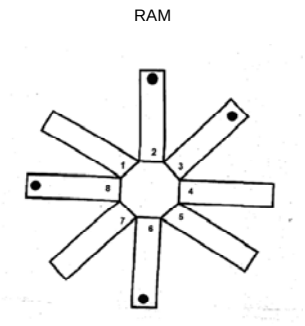
components of spatial memory. An entry into an unbaited arm is regarded as a reference memory error and any re-entry either to a baited or unbaited arm is considered as a working memory error.

Acquisition

Rats were given two trials per day. At the beginning of each trial, the maze was thoroughly cleaned with 70% ethanol and four of the arms (2, 3, 6 and 8) were baited with food reinforcement (Kellogg's Planets and Stars TM, Kellogg India Ltd., Mumbai). The rat was placed in the centre of the octagon and was allowed a free choice. An arm choice was recorded when a rat ate a bait or reached the end of an arm. The maze arms were not rebaited, so only the first entry into the baited arm was recorded as a correct choice. The trial continued until the rat entered all the four baited arms or 5 minutes had elapsed. At the end of the trial, the rats were returned to the home cages and were subjected to second trial after an inter-trial interval of l hour. Training was continued till the rats attained the criteria of 80% correct choice.

Evaluation criteria

Data from 4 trials were averaged and represented as blocks. The data was analyzed for percent correct choice, reference and working memory errors. Percent correct choice was calculated by dividing the number of correct entries by the total number of entries and multiplying by 100. An entry into an unbaited arm was considered a reference memory error (RME) and any re-entry was considered as a working memory error (WME).



A schematic representation of Radial Arm Maze is shown in the Figure. The numbers inside the arms represent the arm number and the brown spheres inside the arms represent the food pellets.

Assay of acetylcholinesterase (AChE) activity in the brain:

After behavioural assessment on these tasks, the alteration in the AChE activity was evaluated in the frontal cortex, hippocampus, septum, hypothalamus and brain stem. Rats were decapitated and the brain regions were quickly dissected out on a chilled petridish. AChE activity was determined using Ellman's method (Ellman et al, 1961) with minor modifications as described elsewhere (Shankaranarayanan et al, 1998; Srikumar et al, 2006; Sunanda et al, 2000). The esterase activity was measured by providing the substrate, acetylthiocholine (ATC). Thiocholine released by the cleavage of ATC by AChE is allowed to react with the -SH reagent 5, 5'.dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm.The extinction co-efficient of the thionitro benzoic acid is 1.36 x 104/mole/centimetre The concentration of thionitro benzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

Results and discussion:

Behavioural Study: *Radial arm maze experiment*

The radial arm maze was introduced in 1976 to study hippocampal dependent learning and memory (Olten and Samuelson, 1976) .The radial arm maze has been widely used in behavioural neuroscience and behavioural pharmacology. Thus RAM tests are useful in evaluating the effect of drugs, stress and various other environmental factors on learning and memory (Bhagya et al. 2008; Srikumar et al, 2006& 2007; Titus et al, 2007). Reference memory and working memory are the two variables that report the physiological status of the brain. Amongst the various functions of the

brain, one of the most interesting one is the ability to acquire new information and store them for further retrieval. Learning is defined as 'an enduring change in mechanisms of behaviour that results from experience with environmental events'. Learning is a process of acquiring new information, while Memory refers to the persistence of a change in behaviour overtime, in a state that can be retrieved later. The effect of *Celastrus paniculatus* on performance of a partially baited RAM task was studied. Results on the correct choice of a partially baited RAM task are given in the table. The data in the table -1 clearly shows that the % of correct choice is 47.45 ± 1.91 in the first block of 4 trials and as the trials increased the % correct choice goes up to 88.33 ± 1.78, i.e., almost 2 fold (1.86) and the increase of % correct choice is in arithmetic progression as the number of trial increases. Another equally important phenomenon revealed in this experiment is that active principle in the *Celastrus paniculatus* has not much role to play in healthy animals which could be observed in CP 100, CP 200 and CP 400. Results of this experiment reveal that as the trials are increased the % correct choice also increased (almost parallel) and hence the RAM experiments could be reliably used for our actual study that is alleviating role of *Celastrus paniculatus* in stress animals.

Observations of the effect of *Celastrus paniculatus* to normal rats on reference memory errors and working memory errors (correct) in partially baited RAM task are expressed in tables – 2 and 3 respectively. The data in table -2 shows a significant decrease in reference memory errors as trials progressed. In normal control animals the reference memory error (RME) is 3.58+ 0.21 in the first block of 4 trials and as trials increased the RME is brought down to 0.50+ 0.08 in the eighth block of four trials. Animals administered with CP seed oil (100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight) also showed a similar trend. But the treated animals did not show a greater decrease than control animals. So this decrease in RME cannot be attributed to CP oil. The table -3 which give the data on working memory error (WME) also showed a decrease in WME as trials progressed. This trend was also observed in animals given the different doses of the drug treatment (100 mg/kg body weight, 200 mg/kg body weight and 400 mg/kg body weight). But this decrease in treated animals was not appreciable compared to normal control. In the present study the higher dose 400mg/kg /day for 14 days showed slightly better performance than 100 and 200 mg/kg doses. Incidentally chronic CP administration was associated with no observable side effects in animals even with the 400 mg/kg dose regimen. Chronic treatment with 400 mg/kg CP alone resulted in a small degree of enhancement. A marked degree of task enhancement was not expected since the rats were young and presumably cognitively unimpaired.

Learning and memory is a complex phenomenon that is affected by various factors. These factors can either enhance the performance by facilitating the learning process or impair by inhibiting the process of learning. Stress is one such factor, which has been found to have a prolonged effect on cognitive functioning and is associated with hippocampus damage. 21 days of restraint stress in rats is known to impair performance on different types of spatial memory tasks like T-maze, Y-maze, radial arm maze and Morris water- maze (McEwen, 2000; Ramkumar, 2001; Ramkumar 2005; Srikumar et al, 2004; Srikumar , 2006; Sunanda, 2000).

In a similar study (Karanth et al, 1980), rats were treated with 400 mg/kg of CP (by oral gavage) once daily for 3 days. The animals were then given 10 trials in a raised platform shock-avoidance task. Each trial was spaced 5 min apart. The CP treated rats exhibited a significantly increased learning curve compared with

vehicle treated animals in the avoidance paradigm. In another study, rats treated daily with 850 mg/kg of CP oil for 15 days exhibited a significant improvement in their retention times in a two-way passive avoidance task. CP also produced a significant decrease in the content of norepinephrine, dopamine and serotonin and certain of their respective metabolites in both brain and urine (Nalini et al, 1995).

Biochemical study

The mechanisms underlying learning and memory include an interaction between the various neurotransmitter systems, amongst which the central cholinergic system is known to play a prominent role. Estimation of acetylcholinesterase (AChE) activity provides a relatively easy and valuable assessment of cholinergic function. In the recent past, the role of ACh in learning and memory has been demonstrated indubitably. Further, pharmacological manipulation of cholinergic function has been found useful in the treatment of CNS disorders like Alzheimer's and Parkinson's disease. Thus assessing cholinergic function is considered as an important tool in neuroscience research. Acetylcholine per se has a very short half-life and direct estimation of ACh is a little difficult in brain homogenates. There are several approaches to evaluate cholinergic function indirectly. Estimating the expression of choline acetyltransferase (ChAT) and acetylcholine esterase (AChE) by immunochemical and histochemical techniques provide information on the cholinergic function, but are tedious and time consuming. Estimation of AChE activity provides a relatively easy and valuable assessment of cholinergic function.

The table - 4 shows the AChE activity in the five brain regions namely frontal cortex, hippocampus, septum, hypothalamus and brain stem.The results clearly show that AChE activity showed a gradual decrease in frontal cortex when treated with an increasing dose of CP oil. There is a marginal increase in activity in the hippocampus when the animal is treated with 200 mg/kg body weight of *Celastrus* seed oil. There is enhanced AChE activity in the septum when treated with 100 and 400 mg/kg body weight of the rats while a decline in activity at higher dose in the hypothalamus is seen. An increase in AChE activity in the brain stem in CP 200 and CP 400 was observed.

At higher dose, CP did appear to inhibit brain cholinesterase activity in the hippocampus and hypothalamus without affecting in frontal cortex. The results of this study are consistent with the possibility that there is a basis for the conflict derived mainly from anecdotal reports that CP may enhance learning and memory in humans.

Furthermore, this plant seed oil may be more effective in individuals who are cognitively impaired as a result of chemical or organic brain damage as compared with normal subjects. In the least, these data may provide the impetus for further study of the material, and isolation of its active components.

The mechanism of action by which CP enhances learning and memory performance in behavioural tasks is as yet unknown. At higher dose, CP did appear to inhibit brain cholinesterase activity in the hippocampus and hypothalamus without affecting in frontal cortex. The results of this study are consistent with the possibility that there is a basis for the conflict derived mainly from anecdotal reports that CP may enhance learning and memory in humans. Furthermore, this plant seed oil may be more effective in individuals who are cognitively impaired as a result of chemical or organic brain damage as compared with normal subjects. In the least, these data may provide the impetus for further study of the material, and isolation of its active components. Studies on brains from patients suffering from Alzheimer's disease have shown reduced AChE activity in the hippocampus and cortex (Nakano et al,1986).

Table 1: Effect of *Celastrus paniculatus* seed oil in normal rats and performance in a partially baited RAM task

BLOCKS	NC	CP100	CP200	CP400
1	47.45 ± 1.91	51.89 ± 4.04	47.96 ± 1.83	50.25 ± 1.00
2	53.28 ± 2.64	55.17 ± 3.34	58.39 ± 2.58	61.62 ± 1.86
3	57.32 ± 2.4	53.81 ± 2.16	62.70 ± 3.91	65.09 ± 2.66
4	64.77 ± 2.25	58.11 ± 2.54	59.69 ± 3.73	73.84 ± 2.45
5	75.38 ± 2.89	64.95 ± 2.43	65.78 ± 5.33	85.37 ± 3.66
6	82.42 ± 1.77	70.00 ± 3.44	70.36 ± 4.13	89.44 ± 3.06
7	85.23 ± 1.93	69.43 ± 4.64	74.18 ± 1.67	87.70 ± 3.02
8	88.33 ± 1.78	80.00 ± 1.58	84.12 ± 2.65	89.44 ± 3.37

Data is represented as Mean ± SEM. NC: normal control (n = 12). CP100, CP200 and CP400 (12): normal rats subjected to 14 days of treatment with *Celastrus paniculatus* 100,200 and 400 mg/kg, i.p., respectively

Table 2: Effect of *Celastrus paniculatus* seed oil in normal rats on reference memory errors in partially baited RAM task

BLOCKS	NC	CP100	CP200	CP400
1	3.58 ± 0.21	3.21 ± 0.45	3.83 ± 0.29	3.16 ± 0.26
2	3.26 ± 0.25	2.78 ± 0.38	2.88 ± 0.15	2.00 ± 0.16
3	2.78 ± 0.22	3.32 ± 0.26	2.55 ± 0.34	1.66 ± 0.18
4	2.08 ± 0.21	2.75 ± 0.18	2.72 ± 0.28	1.27 ± 0.13
5	1.43 ± 0.17	2.28 ± 0.22	2.19 ± 0.45	0.66 ± 0.19
6	0.95 ± 0.12	1.78 ± 0.31	1.86 ± 0.36	0.44 ± 0.15
7	0.72 ± 0.12	1.75 ± 0.30	1.39 ± 0.18	0.40 ± 0.15
8	0.50 ± 0.08	1.28 ± 0.20	0.96 ± 0.16	0.61 ± 0.17

Data is represented as Mean ± SEM. NC: normal control (n = 12). CP100, CP200 and CP400 (12): normal rats subjected to 14 days of treatment with *Celastrus paniculatus* 100,200 and 400 mg/kg, i.p., respectively.

Table 3: Effect of *Celastrus paniculatus* in normal rats on working memory errors in partially baited RAM task

BLOCKS	NC	CP100	CP200	CP400
1	1.03 ± .18	0.97 ± 0.38	0.69 ± 0.2	1.08 ±0.13
2	0.77 ± .14	0.55 ± 0.21	0.62 ± 0.09	0.56 ± 0.14
3	0.77 ± .14	0.55 ± 0.19	0.22 ± 0.09	0.58 ± 0.10
4	0.38 ± .11	0.52 ± 0.12	0.37± 0.18	0.28 ± 0.1
5	0.28 ± 0.09	0.27 ± 0.13	0.22 ± 0.15	0.14 ± 0.07
6	0.29 ± 0.07	0.13 ± 0.06	0.16 ± 0.07	0.11 ± 0.08
7	0.24 ± 0.07	0.22 ± 0.06	0.19 ± 0.06	0.17 ± 0.08
8	0.16 ± 0.06	0.05 ± 0.05	0.16 ± 0.07	0.61 ± 0.17

Data is represented as Mean ± SEM. NC: normal control (n = 12). CP100, CP200 and CP400 (12): normal rats subjected to 14 days of treatment with *Celastrus paniculatus* 100,200 and 400 mg/kg, i.p., respectively.

Table 4: AChE data table

REGIONS	NC CP100		CP200	CP400
Frontal cortex	4.75 ± 0.07	4.48 ± 0.24	4.30 ± 0.16	3.78 ± 0.15
Hippocampus	4.84 ± 0.06	4.53 ± 0.44	4.88 ± 0.27	4.30 ± 0.20
Septum	8.89 ± 0.31	9.71 ± 0.82	8.61 ± 0.55	9.41 ± 0.71
Hypothalamus	5.68 ± 0.17	5.91± 0.35	5.08 ± 0.29	3.21 ± 0.29
Brainstem	6.47 ± 0.27	5.89 ± 0.28	6.66 ± 0.26	6.66 ± 0.26

Data is represented as Mean ± SEM. NC: normal control (n = 12). CP100, CP200 and CP400 (12): normal rats subjected to 14 days of treatment with *Celastrus paniculatus* seed oil 100,200 and 400 mg/kg, i.p., respectively.

Intracranial induced behavioural self-stimulation which is shown to enhance operant performance and reverse the stress deficits is associated with an increase in AChE activity (Ramkumar et al, 2008b; Shankaranarayana et al, 2008b; Yoganarasimha et al, 1998). Thus there is a tight correlation between cholinergic function, AChE activity and cognition. Extensive evidence supports the view that cholinergic mechanisms modulate learning and memory formation. Learning requires combinational participation of multiple neural systems. ACh might be a neuromodulator important for regulating the relative balance in neural systems. Today, the evidence supporting the important role for ACh in modulating cognitive functions includes findings from a host of pharmacological studies showing that interfering with cholinergic function generally impairs and augmenting cholinergic increase or decrease ACh functions in different memory systems.

The experimental plant of our interest *Celastrus paniculatus* (CP) is widely used in the indigenous medicinal systems of India from ancient times for neuropsychological disorders. Hence the plant's seed oil (Jyothismati oil) was selected for scientifically validating its effect on learning and memory and its curative effect in stress by virtue of its antioxidant effect.

In the present study seed extract of *Celastrus paniculatus* was investigated for it's effect on cognitive functions in rats. To study effect on learning and memory radial arm maze paradigm was used. This is a special task where the animals learn to alternate between the arms of the maze that radiate from the central platform. A rat or a mouse is released on the central platform and it has to retrieve maximum food pellets located at the end of each arm - only one pellet per arm and the animals have to remember the arm already visited The data indicated slight enhancement in radial arm maze acquisition with chronic administration of CP oil (400 mg/kg body weight). This marginal improvement could be attributed to the fact that animals were young and cognitively unimpaired. The biochemical aspect was also studied in terms of acetylcholine esterase activity. A decrease in AChE activity leads to an increase in cholinergic activity in the brain. Five brain regions namely Frontal cortex, Hippocampus, Hypothalamus, Septum and Brain stem were chosen for this study.100 mg/kg body weight did not show much significance in all the five regions. Frontal cortex, brain stem and hypothalamus showed slight decrease in AChE activity with 200 mg/kg body weight of *Celastrus* oil. There was significant decrease in the enzyme activity with 400 mg/kg body weight of the oil in the regions of the hypothalamus and frontal cortex followed by the hippocampus. No side effects were observed with administration of the seed oil.

Anxiety and nervousness seems to be growing in prevalence among many individuals in our modern, fast moving society. This tension wears on our mental capacity. Stress, anxiety, tension and

depression are intimately connected with most illness. With the high level of stress now being experienced in our society, consumers are realizing that "adaptogenic" herbs, formulated properly, assist in the prevention of many of the stress related ailments that are engulfing our civilization.

References:

[1] Artur S. d'Avila Garcez, Luis C. Lamb and Dov M. Gabbay. Neural-Symbolic Cognitive Reasoning. Cognitive Technologies. *Springer*, 2008, ISBN 978-3-540-73245-7, 2008.

[2] Meyers C. "Methylphenidate Therapy Improves Cognition, Mood, and Function of Brain Tumor Patients." *J Clin Oncology*. 1998; 16:2522-2527.

[3] Read more at Suite101: Chemotherapy and Cognitive Function: The Mental Impairment Caused by Chemotherapy is Well Understood http://cancer.suite101.com/article.cfm/chemotherapy_and_cognitive_function#ixzz0fFC02jBe

[4] Neuwelt E. "Primary CNS Lymphoma Treated With Osmotic Blood-brain Barrier Disruption: Prolonged Survival and Preservation of Cognitive Function." *J Clin Oncology*. 1991; 9:1580-1590.

[5] Winkler.J., Suhr .S.T., Gage. F.H., Thal.L.J and Fischer .L.J. 1995. Essential role of neocorticat acetylcholine in spatial memory. *Nature*.375: 484-487.

[6] Gaitonde,B.B., Raiker,K.P., Shroff, F.N. Patel, J.R. 1957. Pharmacological studies with malakanguni, an indigenous tranquillizing drug (preliminary report), *Current Medical Practice* 1, 619-621.[*on Celastrus paniculatus* Willd.].

[7] Bhagya,V., Srikumar B.N., Raju, T.R. and Shankaranarayana Rao, B.S. 2008. Neonatal clomipramine induced endogenous depression in rats is associated with learning impairment in adulthood. *Behavioural Brain Research*; 187:190–194.

[8] Srikumar, B.N., Bindu, B., Priya, V., Ramkumar, K., Shankaranarayana Rao, B.S., Raju, T.R. and Kutty, B.M. 2004a. Methods of assessment of learning and memory in rodents. In: *Brain and Behavior* (Raju TR, Kutty BM, Sathyaprabha TN, Shankaranarayana Rao BS, eds), pp 145-151 Bangalore: NIMHANS.

[9] Srikumar, B.N., Raju, T.R. and Shankaranarayana Rao, B.S. 2007. Contrasting effects of bromocriptine on learning of a partially baited radial arm maze task in the presence and absence of restraint stress. *Psychopharmacology* (Berl) 193:363-374.

[10] Titus, A. D. J., Shankaranarayana rao, B. S., Harsha, H. N., Ramkumar, K., Srikumar, B. N., Singh, S. B., Chattarji,S. and Raju, T. R. 2007.Hypobaric hypoxia-induced dendritic atrophy of hippocampal neurons is associated with cognitive impairment in adult rats. *Neuroscience* 145 :265–278

[11] Ellman, G.L., Courtney, K.D., Andres,V, Jr. and Feather Stone, R.M. 1961.A new rapid colorimetric determination of acetylcholine esterase activity. *Biochem Pharmacol* 7: 88-95.

[12] Shankaranarayana Rao, B. S., Raju, T. R. and Meti B. L. 1998. Self-Stimulation of Lateral Hypothalamus and Ventral Tegmentum Increases the Levels of Noradrenaline,Dopamine, Glutamate, and AChE Activity, But Not 5-Hydroxytryptamine and GABA Levels in Hippocampus and Motor Cortex. *Neurochemical Research*, Vol. 23, No. 8, pp. 1053–1059.

[13] Srikumar,B.N., Raju,T.R. and Shankaranarayana Rao, B.S. 2006. The involvement of cholinergic and noradrenergic systems in behavioural recovery following oxotremorine treatment to chronically stressed rats. *Neuroscience* 143:679-688.

[14] Sunanda, Shankaranarayana Rao, B.S. and Raju, T.R. 2000b. Chronic restraint stress impairs acquisition and retention of spatial memory task in rats. *Curr Sci* 79: 1581-1584

[15] McEwen B.S. 2000.The neurobiology of stress: from serendipity to clinical relevance. *Brain Res* 886:172-189.

[16] Ramkumar, K.2001. Self-stimulation rewarding experience reverses stress-induced behavioural deficits and cholinergic dysfunction. M.Phil Dissertation, NIMHANS (Deemed University), Bangalore.

[17] Ramkumar. K.2005. Reversal of stress induced deficits: morphological biochemical and behavioural evaluation. Ph.D. in Neurophysiology Thesis. NIMHANS (Deemed University), Bangalore.

[18] Sunanda, Shankaranarayana Rao, B.S. and Raju ,T.R. 2000a. Restraint stress-induced alterations in the levels of biogenic amines, amino acids, and AChE activity in the hippocampus. *Neurochem Res* 25:1547-1552.

[19] Karanth,K.S., Haridas,K.K., Gunasundari,S. and Guruswami,M.N.1980. Effect of Celastrus paniculatus on learning process. *Aroyga- J. Health. Sci*., Vol.6 (2), pp 137-139.

[20] Nalini, K., Karanth, K. S., Rao, A. and Aroor, A. R. 1995. Effects of *Celastrus paniculatus* on passive avoidance performance and biogenic amine turnover in albino rats. *J. Ethnopharmacol*. 47: 101–108.

[21] Nakano,S., Kato,T., Nakamura,S. and Kameyama,M. 1986. Acetylcholinesterase activity in cerebrospinal fluid of patients with Alzheimer's disease and senile dementia. *J Neurol Sci*. 75(2):213-23.

[22] Nakano,S., Kato,T., Nakamura,S. and Kameyama,M.1986. Acetylcholinesterase activity in cerebrospinal fluid of patients with Alzheimer's disease and senile dementia. *J Neurol Sci*. 75(2):213-23.

[23] Ramkumar, K., Srikumar, B.N., Shankaranarayana Rao, B.S. and Raju, T.R.2008. Self-Stimulation Rewarding Experience Restores Stress-Induced CA3 Dendritic Atrophy, Spatial Memory Deficits and Alterations in the Levels of Neurotransmitters in the Hippocampus. *Neurochem Res*. 33:1651–1662.

[24] Shankaranarayana Rao, B.S., Titus, A.D.J., Bhagya, V., Veena, J., Srikumar, B.N., Deepti, N.V., Ramkumar, K. and Raju,T.R . 2008b. Assay of acetylcholinesterase activity in the discrete regions of the rat brain. In: Modern Neurochemical Techniques to Understand Brain Functions. Shankaranarayana Rao BS, Subhash MN, Panicker MM and Srinivas Bharath MM (eds.), National Institute of Mental Health and Neuro Sciences, Bangalore, India. pp. 32-35.

[25] Yoganarasimha,D., Shankaranarayana Rao,B.S., Raju, T.R. and Meti, B.L.1998. Facilitation of acquisition and performance of operant and spatial learning tasks in self-stimulation experienced rats. *Behav Neurosci*. 112(3):725-9.