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Research Article

EVALUATION OF GREEN TEA FOR ITS NOOTROPIC ACTIVITY

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ABSTRACT

*Green tea (*Camellia sinesis*) has a lot of claims as well as documented evidence of it being used traditionally for a number of health benefits. Free radicals contribute to the aging process as well as to the development of a number of health problems, including cancer, heart disease and cognitive decline as in neurological diseases, (Alzheimer's, Dementia) and in learning disorders such as dyslexia.Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called polyphenols.Polyphenols in green tea can neutralize free radicals and may prevent some of the damage they cause and may help maintain the parts of the brain that regulate learning and memory. The present study was undertaken to establish the memory boosting potential of green tea by preclinical testing in mice. The aqueous extract (AQGT) 5 mg/ kg body wt and 10 mg / kg body wt of green tea was compared with the marketed formulation (Medharasayana) 10 ml/ kg body wt for its nootropic activity. The exteroceptive models like Elevated plus Maze, Morris Water Maze, Passive Avoidance Step down models and interoceptive model of scopolamine induced memory deficit were used to evaluate nootropic activity; green tea decreased the transfer latencies significantly ($p< 0.001$) when compared with the marketed formulation. It also significantly increased brain acetyl cholinesterase enzyme inhibitiory activity when compared with the marketed formulation. Green tea's anti-oxidant content as well as its ability to increase the brain Ach content may be the neurochemical basis for its improved learning and memory.*

Key words: *Green tea, Nootropic, Anticholinesterase, Antioxidants*

INTRODUCTION:

Nootropics are agents that improve mental functions such as memory, intelligence, motivation, attention, concentration, cognition and increase blood circulation to brain. Although there is no proper cure for cognitive impairment, alternative pharmacology treatment modulates can reduce the symptoms of memory loss and slow disease progression[1]. Nootropic agents like piracetam and cholinesterase inhibitors like donepezil are commonly used for improving memory, mood and behavior. However the resulting adverse effects of these drugs such as diarrhoea, nausea, insomnia, muscular cramps and other known side effects have made their use limited. Hence it is worthwhile to explore the utility of traditional medicines in treatment and management of various cognitive disorders [2, 15].The present

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study is aimed to investigate nootropic potential of green tea (*Camellia sinensis*). There is lack of scientific data regarding effect of green tea on learning and memory in preclinical animal studies. This prompted us to investigate the nootropic potential of green tea in in-vivo and in-vitro standard animal models.

MATERIALS AND METHODS:

Drugs and Chemicals: All the drugs and chemicals used in the study were obtained from authorized dealers. Nootropil (Piracetam 150 mg/Kg) and Hyoscine (Scopolamine 0.4 mg/Kg) was purchased from YashodaHospital, Secunderabad. Dithiobisnitrobenzoic acid (DTNB), Acetyl thiocholine iodide (ATCI), Eserineand thiobarbituric acid were purchased from Chem n Chem Stores, Shahpur, Hyderabad.

Plant materials:

Dried authenticated leaves of green tea (Cameliasinensis) belonging to family Theaceae was obtained from Kishanlaldawasaaz near Gulzar house Charminar, Hyderabad. The samples have been deposited in the form of a herbarium at Sri Venkateshwara University, Botanical department, for future reference.

Extract preparation:

Dried green tea leaves were powdered and then extracted in a Soxhlet extractor using distilled water and methanol for a period of 48 hours [5]. The aqueous and methanolic extracts were concentrated using a Rotavap at temp 80^0 C and 60^0 C respectively. An yield of 5.6 g and 0.35 g of aqueous and methonolic extracts were obtained. The extracts so obtained were then used for experimental work and also stored in a well closed container for further use [6].

Acute toxicity studies:

The acute toxicity studies were performed in mice by giving the aqueous and methanol extract of green tea at doses 10, 15, 20, 25 and 30 mg /Kg body weight. The animals did not exhibit any toxic symptoms even at 30 mg/Kg body weight and the dose was fixed at 1mg and 5mg/Kg body weight based on the OECD guidelines 425.

Chemicals:

All the chemicals used were of analytical grade. Nootropil ® (piracetam)-150mg/kg, Hyosine (scopolamine) 0.4mg/kg, were purchased from Apollo healthcare medical centers Hyderabad.

Animals:

Male swiss albino mice (25-30gms) were used throughout experiment. Animals had free access to feed and water ad libitium during quarantine period. Experimentation was carried out according to CPCSEA guidelines and experimental work also approved by Institutional animal ethics committee Exteroceptive and Interoceptive models used for evaluating memory and learning [4].

Elevated plus maze

Elevated plus maze(EPM) is used toevaluate the spatial long-term memory in mice. The procedure for testing learning and memory was followed as per the neuropsychopharmacological principle of retention of learned tasks.The apparatus consisted of two open arms(16cm X 5cm) and two enclosed arms(16cm X 5cm) which extended from a central platform (5cm X 5cm). The maze was elevated to a height of 25cm from the floor. Transfer latency (TL) is the time taken by the mouse to enter with all its legs into one of the enclosed arms.The mouse was placed at the end of the open arm facing away from the central platform and the TL was recorded when the mouse entered one of the enclosed arm. It was then allowed to explore the apparatus for 10 secs.The cut off time for TL was recorded as 90 secs if the animal did not enter the enclosed arm within 90 secs. TL was recorded on the 1^{st}day which was 24 hrs after the 1^{st} exposure to the maze. It was then recorded again on the 8^{th} day. The drugs were administered to various groups for 7 days. The TL was recorded on the days of testing i.e. 1^{st} day and 8^{th} day after 30 mins of administration of the drugs [7].

Morris water maze

Morris water maze consists of a large circular tank with a depth of 30cm, diameter 50cm. In the center a platform of 15cm having dimensions 5cm X 5cm is mounted. The pool is filled with water added with milk in order to make it opaque. Later animals were allowed for training before the experimental day. On the 1st day animals were treated with different doses of standard and test samples. The animal was placed at the corner of the tank and allowed to swim until it identifies the hidden platform. The cut-off time is 90seconds. The transfer latency is the time taken by the mouse to identify the platform. TL was recorded on 1st day and 8th day [8].

Step down

Step down type of passive avoidance test is used to examine long term memory. The apparatus consists of transparent acrylic cage (30X30X40 cm in height) with a grid floor; a platform (4X4X4 cm) is fixed in the centre of the grid floor. Electric shocks of 1Hz, 500msec, 40V DC are delivered to the grid floor. The training was carried out before the experimental day. On the experimental day the mouse was placed on the platform in the center of the cage and when the mouse steps down and places all its paws on the grid floor shock was delivered. Later the animal was placed again on the platform after 60-90 minutes and Step down latency (SDL) was recorded with an upper cut of time of 300 seconds [9].

ESTIMATION OF BRAIN ACETYL CHOLINESTERASE:

Brain acetyl cholinesterase activity (AChE) was measured by the method of colorimetric measurement. 0.5 ml of the cloudy supernatant liquid of the brain homogenate was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB (5,5-dithiobis-2-nitrobenzoic acid) solution (10 mg DTNB in 100 ml of sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4ml portions were pipetted out into two test tubes. Into one of the test tubes, 2 drops of eserine solution was added, 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipette out into both the tubes and incubated for 10 minutes at 30° C. The solution in the tube containing eserine was used for zeroing the colorimeter. The resulting yellow color was due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After calibrating the instrument, change in absorbance per minute of the sample was read at 420 nm [10-12].

Statistical Analysis:

The step-down latency and transfer latency were analyzed using the Student's Paired 't' test.A probability level of $P<0.01$ was considered as significant. The AChE activity and open field behavior of different groups were analyzed using One Way Analysis of Variance(ANOVA),followed by Dunnett's test for individual comparison of groups, viz.; AprobabilitylevelofP<0.0001forOnewayANOVAwasconsideredassignificant, and for posttest (Dunnett's test), aprobability level of $P<0.01$ was considered as significant[7].

RESULTS AND DISCUSSION:

Table1: EPM results of Green tea

S.NO	*Treatment*	*Transfer Latency On 1 St Day Mean± Sem*	*Transfer Lantency On 8 Th Day Mean± Sem*
1	CONTROL (C)	17.1±1.13	16.3±1.2
2	NEG.CONTROL(N.C)	23.3±1.33	23.3±0.91
3	STANDARD (STD)	29.8±2.02	14.6±1.17**
4	MARKETED PRODUCT(M.P)	30.0±1.39	16.0±1.09**
5	GREEN TEA AQUA(GTA)	35.0±2.30	17.1±0.65**
6	GREEN TEA METHONAL(GTM)	29.8±1.51	17.0±0.81**

** indicates the difference with the control group at $p<0.01$. n=6 in each group.

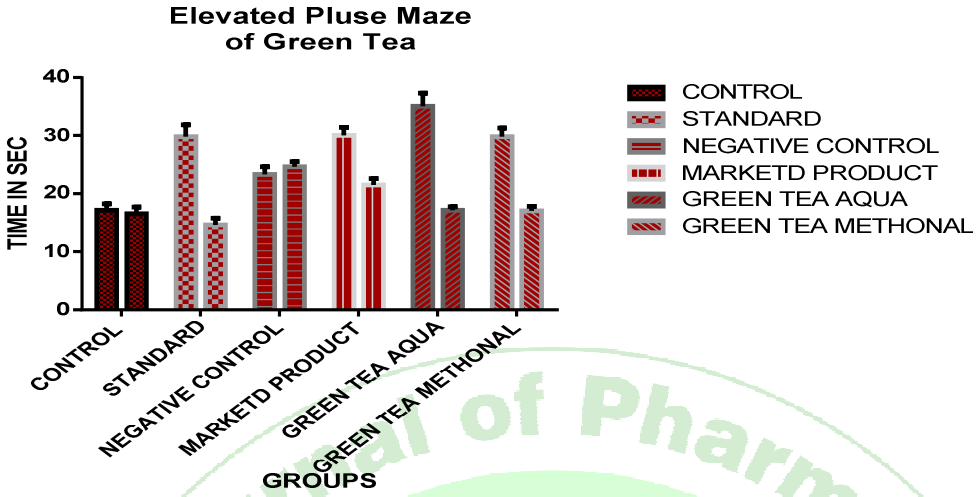

Fig1: Bar charts showing Green tea EPM results

Table 2: WM green tea results

S.NO	*Treatment*	*Transfer Latency On 1 St Day Mean± Sem*	*Transfer Lantency On 8 Th Day Mean± Sem*
1	CONTROL (C)	17.5±1.11	10.50±0.50^{ns}
2	NEG.CONTROL(N.C)	11.8±0.60	9.16±0.47
3	STANDARD (STD)	17.0±1.39	5.3±0.55**
4	MARKETED PRODUCT(M.P)	17.16±1.57	7.5±0.56^{NS}
5	GREEN TEA AQUA(GTA)	17.33±1.33	5.8±0.945**
6	GREEN TEA METHONAL(GTM)	17.66±1.22	6.5±1.057**

** And ^{ns} indicates the difference with control group at p< 0.01 and p>0.05. n=6 in each group.

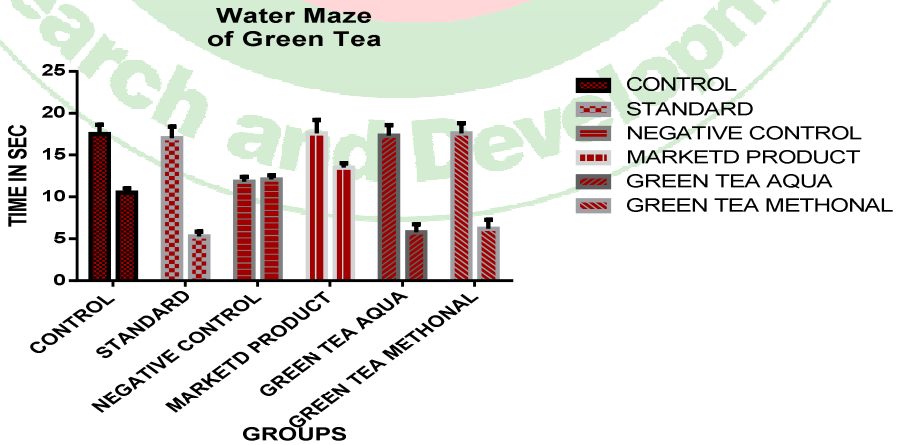

Fig 2: Bar charts showing WM results

Table 3: SD results for green tea:

S.NO	*Treatment*	*Step Down Latency On 1 St Day Mean± Sem*	*Step Down Lantency On 8 Th Day Mean± Sem*
1	CONTROL (C)	10.33±0.421	10.83±0.70^{NS}
2	NEG.CONTROL(N.C)	11.83±0.542	10.16±1.40
3	STANDARD (STD)	9.167±0.792	24.50±0.76**
4	MARKETED PRODUCT(M.P)	10.83±0.83	19.83±0.60**
5	GREEN TEA AQUA(GTA)	8.66±0.843	19.16±1.078**
6	GREEN TEA METHONAL(GTM)	9.667±0.33	14.0±0.44*

*, ** and ^{ns} indicates the difference with control group at p<0.05, 0.01 and p>0.05. n=6 in each group.

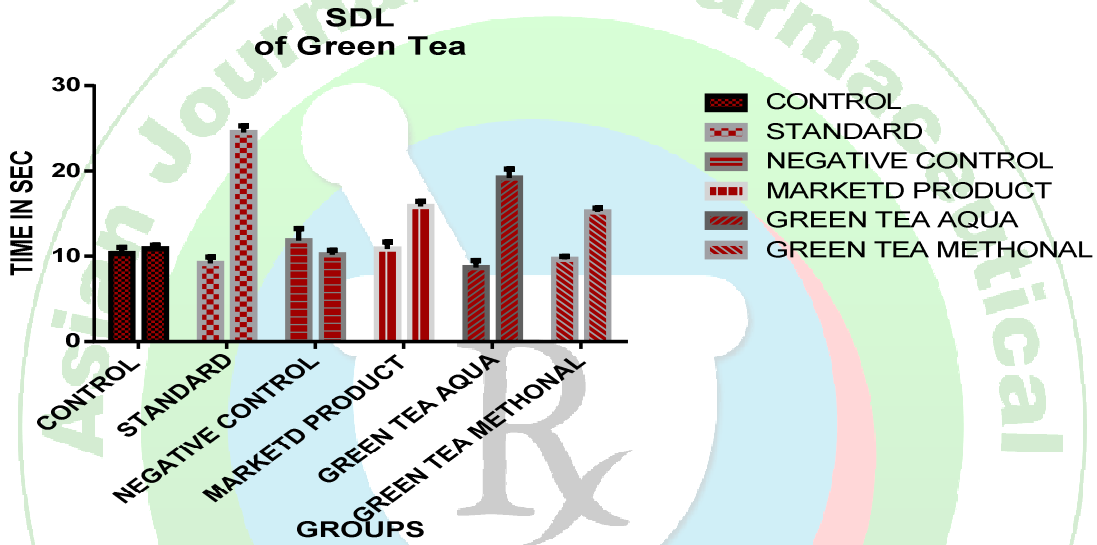

Fig3: Bar charts showing Green tea SDL results

ACHE ESTIMATION RESULTS:

Table 4: Results showing AchE of Green tea

S.NO	*Treatment*	*Dose*	*Acetyl choline esterase enzyme activity. (Mean ± SEM)*
1	CONTROL (C)	10ml/kg	29.5±0.691^{ns}
2	NEG.CONTROL(N.C)	0.4mg/kg	31.19±0.710
3	STANDARD (STD)	150mg/kg	11.01±0.551**
4	MARKETED PRODUCT(M.P)	10ml/kg	30.11±0.712^{ns}
5	GREEN TEA AQUA(GTA)	10ml/kg	14.2±0.610**
6	GREEN TEA METHONAL(GTM)	1ml/kg	16.3±0.630**

** indicates the difference with negative control group at p<0.01. n=6 in each group.

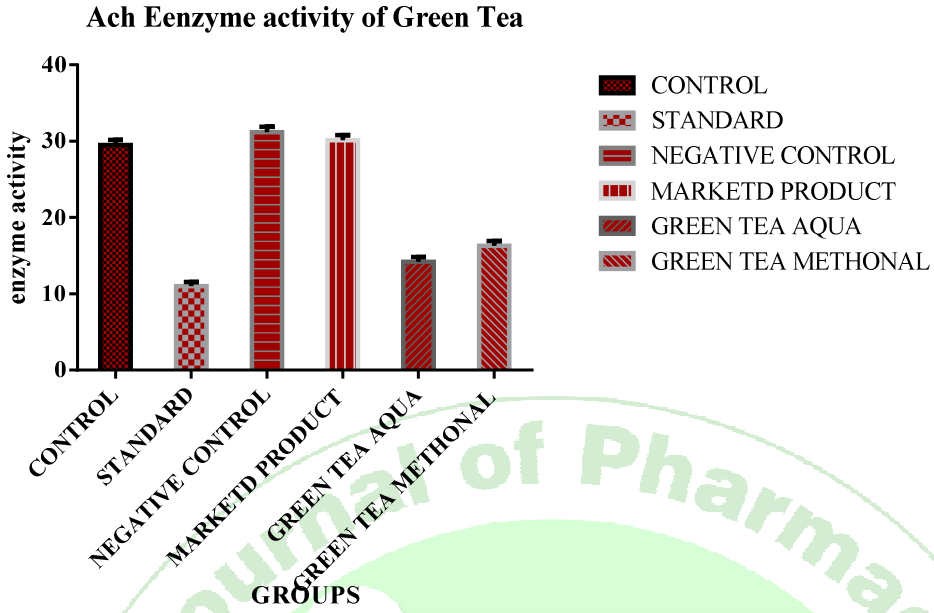

Fig 4: Bar charts showing results Ach E activity

DISCUSSION:

The study demonstrates the effectiveness of green tea extract in improving passive avoidance acquisition and memory retention in mice [14]. Passive avoidance behavior is based on negative reinforcement and is used to examine long term memory. Anti-amnesic effect of extract of plant is manifested as increase in latency to experience shock (acquisition) and decrease in transfer latency in elevated plus maze and Morris water maze, as the animal made to achieve cut off time 60/90 secs as the criterion (memory retention)[16]. Central cholinergic system plays an important role in learning and memory. Piracetam (150mg/kg, p.o.) and green tea extract (10ml/kg, p.o.) significantly lowered AChE activity ($p<0.01$), on the other hand the marketed product did not lower the AChE activity significantly (ns), Nootropic agents have selective facilitatory effect on integrative functions of the central nervous system particularly on intellectual performance, learning capacity and memory. Polyphenols in green tea can neutralize free radicals and may prevent some of the damage they cause and may help maintain the parts of the brain that regulate learning and memory. The beneficial effect of green tea may be the result of antioxidant polyphenols, improvement in cerebral circulation and brain metabolism [17-18].The present study gives the scientific evidence of green tea inhibiting the AChE activity in the mice whole brain homogenate, indicating its potential in the attenuation of symptoms of cognitive deficits.

CONCLUSION:

Green tea extract was found to improve learning abilities and memory capacities in mice. Data obtained from the study shows significant memory enhancement by extract of green teaatadoseof10ml/kg body weight *p.o*. The study proves the modern claims of green tea being a very good remedy for preventing cognitive decline.

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