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Evaluation of Nootropic and Anti-Nociceptive Activity of Green Tea in Comparison with Medhya Rasayana

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Abstract

Green tea (Camellia sinesis) is a known traditional medicinal plant that has been consumed for its putative nutritional and health benefits for centuries. The polyphenols found in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG), comprise 30-40 percent of the extractable solids of dried green tea leaves. Green tea polyphenols have demonstrated significant antioxidant activity. In the present study aqueous extract of green tea (10 ml/kgp.o), methanolic extract of green tea (5 ml/kg p.o) and standard Piracetam (150 mg/kg body weight p.o) were used to evaluate the Nootropic activity. Its behavioral studies on mazes like Elevated plus Maze, Morris Water Maze and avoidance behavior on step down type passive avoidance models were performed, whole brain acetyl cholinesterase enzyme activity was also estimated. For evaluating the analgesic activity, analgesic models like hot plate and acetic acid induced writhing were performed. Methanolic extract of green tea showed significant ($p<0.01$) change in evaluation parameters like transfer latency and step down latency when compared with the aqueous extract of green tea. Methanolic extract of green tea showed better anti-nociceptive activity in acetic acid induced writhing model (41.6%) when compared to hot plate method (6.64%). Whole Brain acetyl cholinesterase enzyme activity was performed and the results indicated that the methanolic extract of green tea ($p<0.01$) has better nootropic activity than aqueous extract. The results proved that the methanolic extract of green tea has better Nootropic and anti-nociceptive activity when compared with the aqueous extract of green tea. Methanolic extract of green tea also proved to be superior to the marketed product, Medha rasayana as a memory enhancer.

Keywords: Nootropic; Anti-nociceptive; Acetyl cholinesterase; Green tea; Cognition

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. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Hence it is worthwhile to explore the utility of traditional medicines in treatment and management of various cognitive disorders [2]. Medhya rasayana is an Ayurvedic memory enhancer consisting of medhya (memory boosting) plants. This Ayurvedic formulation, Medha rasayana was used to compare with the extracts of green tea for its Nootropic and Anti-nociceptive activity.Recent studies have implicated relationships between learning, memory and chronic pain. Learning and memory process has been postulated to be involved in mechanisms of chronic pain [3]. Thus, recent studies support the view that chronic pain is a maladaptive learned phenomenon [4,5].

The purpose of our work was to investigate to see if green tea extract supported the above statements.The present study is aimed to investigate nootropic and Anti nociceptive potential of green tea (Camellia sinensis). There is lack of scientific data regarding effect of green tea on learning and memory and analgesic activity in preclinical animal studies. This prompted us to investigate the Nootropic and analgesic potential of green tea in 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) Tramodal, Aspirin, was purchased from Yashoda Hospital, Secunderabad. Acetic acid (10%), Dithiobisnitrobenzoic acid (DTNB), Acetyl thiocholine iodide (ATCI), Eserine and thiobarbituric acid were purchased from Chem n Chem Stores Shahpur, Hyderabad.

Plant materials

Dried authenticated leaves of green tea (*Camelia sinensis*) belonging to family The aceae 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 [6,7]. The aqueous and methanolic extracts were concentrated using a Rotavap at temp 80°C and 60°C, respectively. A 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 [8].

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.

Animals

Male swiss albino mice (25-30 gms) 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 [9].

Elevated plus maze

Elevated plus maze (EPM) is used to evaluate 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 (16 cm × 5 cm) and two enclosed arms (16 cm × 5 cm) which extended from a central platform (5 cm × 5 cm). 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 90secs if the animal did not enter the enclosed arm within 90 secs. TL was recorded on the 1st day which was 24 hrs after the 1st exposure to the maze. It was then recorded again on the 8th day. The drugs were administered to various groups for 7 days. The TL was recorded on the days of testing i.e. 1st day and 8th day after 30 mins of administration of the drugs [10].

Morris water maze

Morris water maze consists of a large circular tank with a depth of 30 cm, diameter 50 cm. In the center a platform of 15 cm having dimensions 5 cm×5 cm 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 90 seconds. The transfer latency is the time taken by the mouse to identify the platform. TL was recorded on 1st day and 8th day [11].

Step down

Step down type of passive avoidance test is used to examine long term memory. The apparatus consists of transparent acrylic cage (30×30×40 cm in height) with a grid floor; a platform (4×4×4 cm) is fixed in the centre of the grid floor. Electric shocks of 1 Hz, 500 msec, 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 [12].

Estimation of Acetyl Cholinesterase enzyme activity of whole brain

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 4 ml 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 [13-17].

The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation:

$$R=\delta \text{ O.D} \times \text{Volume of assay}$$

$$E \times \text{mg of protein}$$

Where R=Rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/min/mg protein. Δ O.D=change in absorbance/ min and E=Extinction coefficient=13600/M/cm.

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.; A probability level of $P<0.001$ for One way ANOVA was considered as significant, and for post test (Dunnett's test), a probability level of $P<0.01$ was considered as significant.

Analgesic Activity

Hot plate test

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws.

The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is controlled for 55°C to 56°C.

This can be a copperplate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. As per for the experimental design all the groups given the drugs according to their route of administration and dose. Later after 60 minutes, the animals are placed on the hot plate and the observations were recorded and at the time interval of 90, 120 and 180 minutes [18].

Acetic acid-induced writhing test

30min after receiving dose as per the experimental design for every group, each mouse was given intra peritoneally 0.7% aqueous solution of acetic acid (10 ml/kg body weight) [19]. Each animal was placed in a transparent observation cage and the number of writhes per mouse was counted for 30 minutes. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs [20,21].

The percentage of inhibition was calculated using the following ratio:

(Control mean – treated mean) × 100/control mean.

Results and Conclusion

The study demonstrates the effectiveness of green tea extract in improving passive avoidance acquisition and memory retention in mice. 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) [22]. Central cholinergic system plays an important role in learning and memory. Piracetam (150 mg/kg, p.o.) and both extracts of green tea significantly lowered AChE activity ($p<0.01$). On the other hand the marketed product did not lower the AChE activity significantly. 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 extracts may be the result of antioxidant polyphenols, improvement in cerebral circulation and brain metabolism [23] (Figures 1-6).

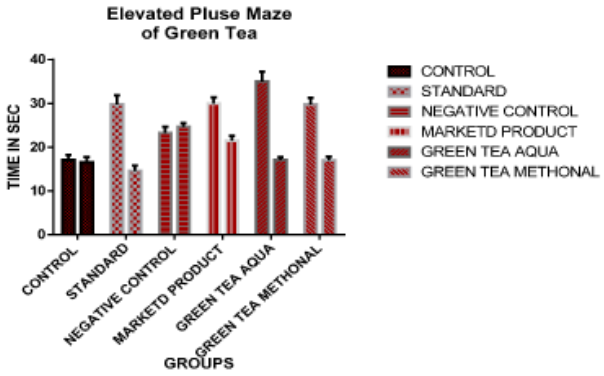

Figure1: Bar charts showing Green tea EPM results.

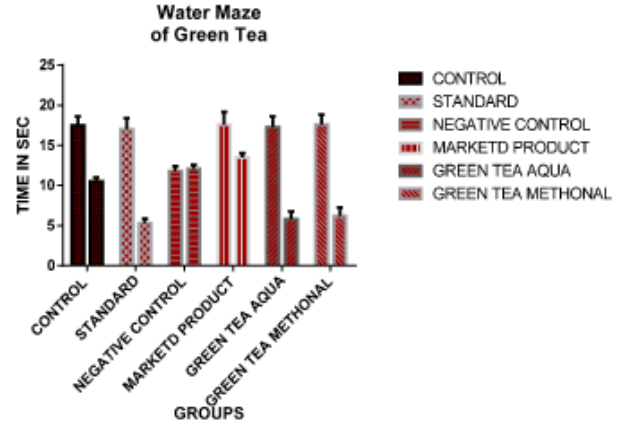

Figure 2: Bar charts showing WM results.

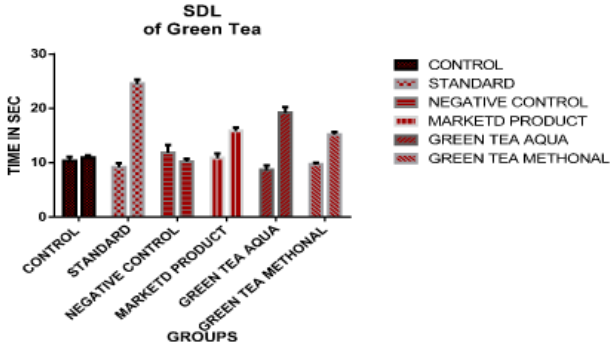

Figure 3: Bar charts showing Green tea SDL results.

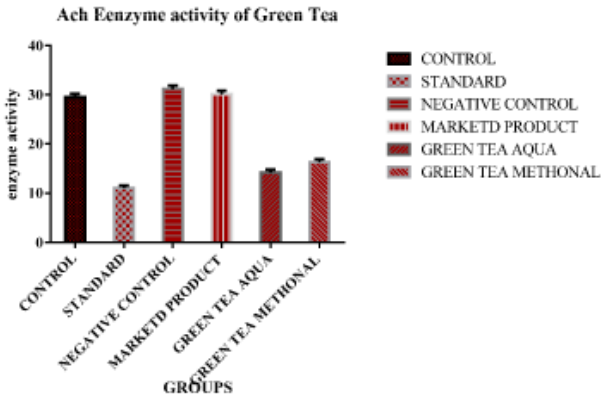

Figure 4: Bar charts showing results Ach E activity.

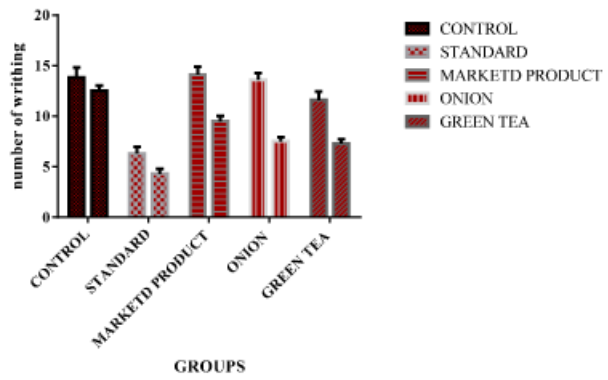

Figure 5: Bar chart showing the writhing results.

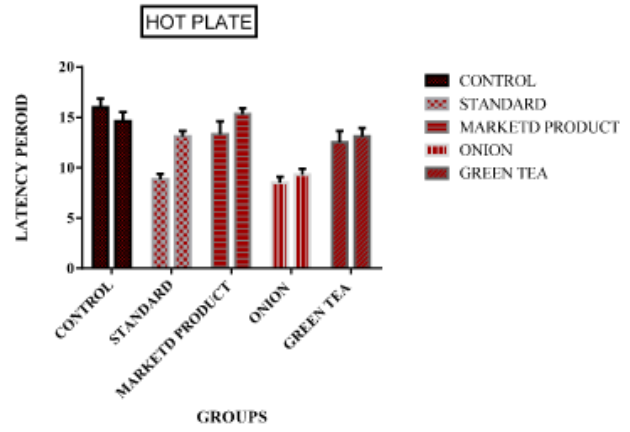

Figure 6: Bar Chart showing Hot plate results.

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. Green tea (Camellia sinesis) elevated Acetylcholine (ACh) level in the brain and ultimately, improved the memory of mice respectively. After seven days of chronic treatment, the animals were dissected to estimate AChE level in whole brain, and that is considered as an additional parameter as a cholinergic marker of learning and memory.Data obtained from the study showed significant memory enhancement at a dose of aqueous extract of green tea (10ml/kg body weight) and methonolic extract of green tea (5 ml/kg body weight).

Medhya rasayana is an Ayurvedic memory enhancer consisting of medhya (memory boosting) plants [24]. Our studies showed that the marketed product was less superior compared to our plant extracts. The conclusion that can be drawn from these studies is that all polyherbal formulations do not necessarily show synergistic effect and have to be evaluated (Tables 1-6).

S. NO	Treatment	Transfer Latency on 1st Day MEAN ± SEM	Transfer Lantency on 8th 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**

Table 1: EPM results of Green tea, ** indicates the difference with the control group at $p<0.01$. n=6 in each group

S.NO	Treatment	Transfer Latency on 1st Day MEAN ± SEM	Transfer Lantency on 8th Day MEAN ± SEM
1	Control (C)	17.5 ± 1.11	10.50 ± 0.50ns

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 ± 0.56NS
5	Green Tea Aqu a(Gta)	17.33 ± 1.33	5.8 ± 0.945**
6	Green Tea Methonal (Gtm)	17.66 ± 1.22	6.5 ± 1.057**

Table 2: WM green tea results, ** And ns indicates the difference with control group at p<0.01 and p>0.05. n=6 in each group.

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 ± 0.70NS
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 Produc t(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*

Table 3: SD results for green tea*, ** and ns indicates the difference with control group at p<0.05, 0.01 and p>0.05. n=6 in each group.

Ache Estimation Results

S.NO	Treatment	Dose	Acetylcholine esterase enzyme activity. (Mean ± SEM)
1	Control (C)	10 ml/kg	29.5 ± 0.691ns
2	Neg. Control (N.C)	0.4 mg/kg	31.19 ± 0.710
3	Standard (Std)	150 mg/kg	11.01 ± 0.551**
4	Marketed Product (M.P)	10 ml/kg	30.11±0.712ns
5	Green Tea Aqua (Gta)	10 ml/kg	14.2±0.610**
6	Green Tea Methonal (Gtm)	1 ml/kg	16.3±0.630**

Table 4: Results showing AchE of Green tea** indicates the difference with negative control group at p<0.01. n=6 in each group.

Writhing Data

S. No.	Treatment	0th day MEAN ± SEM of writhing for 30 min	9 th day MEAN ± SEM of writhing for 30 min	% inhibition on 9th day
1	Control	13.83 ± 0.98	12.5 ± 0.56	-
2	STD	6.3 ± 0.66	4.3 ± 0.49 **	57.6
3	GT	11.6 ± 0.84	7.3 ± 0.42**	41.6
4	MP	14.1 ± 0.79	9.5 ± 0.50**	24

Table 5: Shows the summary results of writhing.

The acetic acid induced abdominal constrictions or writhing was significantly (P<0.01) reduced by onion and green tea extract when compared with the control group. Significance value details are in Table 5 and the graph represented fig 19 shows the difference of analgesic within the groups.

Values are Mean ± SEM** indicates difference with the control group at p<0.01.n=6 in each group. The percentage inhibition on 9th day shows better analgesic results when compared with the control group.

Hot Plate Data

S.NO	Treatment	MEAN ± SEM of lateny period 0th day	MEAN ± SEM of lateny period 9th day	% inhibition on 9th day
1	Control	16 ± 0.85	11.16 ± 0.94	-
2	STD	8.83 ± 0.54	13.16 ± 0.54**	49.04
3	MP	13.33 ± 1.3	15.83 ± 0.60ns	18.75
4	GT	12.5 ± 1.14	13.33 ± 0.84*	6.64

Table 6: Shows the summary results of hot plate model.

The result of the effect of onion and green tea on hot plate induced pain in mice is presented

The result showed that there was significant difference in onion and green tea groups when compared with the control group at p<0.01 and marketed product was non-significant to the control group (Table 6).

Values are Mean ± SEM*, **and ns indicates the difference with control group at p<0.05, 0.01 and p>0.05. n=6 in each group. When 0th day compared with 9th day the results, they had shown the good analgesic activity when compared with the standard and control groups.

The Anti-nociceptive effect of extract of green tea was evaluated in different models of pain viz narcotic model of hot plate and non-narcotic model of acetic acid induced writhing syndrome test. Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve endings. The mechanism of analgesic effect of green tea extracts could probably be due to blockade of the effect of endogenous substances that excite pain nerve endings. The hot plate test is considered selective for opioid like compounds, which are centrally acting analgesics in several animal species. Green tea extracts showed anti-nociceptive activity in hot plate test that may be in part be mediated by opioid receptors. The statement that "Chronic pain is a maladaptive learning phenomenon [3] and that learning and "Memory process has been postulated to be involved in mechanisms of chronic pain" [4,5] has not been supported by our findings with standard animal analgesic models. The mechanism of action of analgesic activity is also mediated through the cholinergic pathway as evidenced by our results which shows increased levels of Ach. We therefore conclude that probably apart from the cholinergic axis playing an important role in anti-nociceptive activity, the phytochemicals of green tea extract help one to withstand the sensation of pain along with enhancement of memory without enhancing the sensitivity to pain which otherwise would take place as a maladaptive process of learning and memory. The results proved that the methanolic extract of green tea has better Nootropic and anti-nociceptive activity when compared with the aqueous extract of green tea. Hence we want to reinforce the statement that nature is a good storehouse of natural remedies for all ailments and promote the use of green tea as a regular beverage of condensed phytochemicals which enhance cognitive processes without enhancing one's sensitivity to pain.

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