

Neurochemical and behavioral effects of green tea (*Camellia sinensis*): A model study

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Abstract: Being rich in polyphenolic compounds such as flavonoids, green tea is suggested to be a potential candidate for the treatment of obesity, stress, depression, Parkinson's and other disorders. Since serotonin has an important role in the pathophysiology of these disorders, present study was designed to monitor the effects of green tea in rats. Green tea extract was provided to the male Albino Wistar rats for 5 weeks, and effects on behaviors were monitored. Results show a decrease in food intake after 5th week but not before. An increase in locomotive activities of the animals was observed, as monitored in novel as well as in familiar environment. Anxiolytic effects were observed in elevated plus maze but not in light dark activity box. An increase in dopamine and serotonin turnover was observed. Our results suggest that beneficial effects of green tea drinking might be due to alteration of serotonin and/or dopamine metabolism. We thereby propose that in further experiments, green tea should be administered in animal model of learned helplessness and effects on the development of adaptation to stress should be monitored. Neurochemical estimations of catecholamine and indoleamine in these animal models of stress exposed to green tea would help in understanding the anxiolytic effects of green tea.

Keywords: Green tea, anxiety, locomotion, elevated plus maze, Skinner's box, open field, Serotonin, dopamine, HPLC-EC.

INTRODUCTION

Green tea is becoming more popular throughout the world for its beneficial effects such as vasodilatation, improved plasma lipid profile and increased insulin sensitivity (Erba *et al.*, 2005; Kim *et al.*, 2007; Venables *et al.*, 2008). Being rich in flavanoids, it is potential candidate for the treatment of cancer, obesity, parkinson's, stress, depression and related disorders (Chantre & Lairon, 2002; Carvalho *et al.*, 2010; Levites *et al.*, 2002; Coimbra *et al.*, 2006; Zhu *et al.*, 2012). Around 50% of the total free amino acid content in tea is L-Theanine, which is a derivative of L-glutamic acid (Yokogoshi *et al.*, 1998) and could exhibit neuroprotective effects (Egashira *et al.*, 2004; Cho *et al.*, 2008; Yamada *et al.*, 2007). It has been reported that theanine increases the levels of neurotrophin mRNA by activating inhibitory neurotransmitters (Yamada *et al.*, 2007). Since 5-HT (5-Hydroxytryptamine; serotonin) plays an important role in the pathophysiology of Parkinsonism, stress and depression, effects of theanine on 5-HT should also be evaluated. To monitor the neurochemical and behavioral effects of repeated administration of green tea, was the aim of present study. Findings would be beneficial in understanding the mechanism of action of green tea as well as its implication in

the treatment of conditions involving altered 5-HT metabolism.

MAETIALS AND METHODS

Animals

Study was carried out on locally breed Albino Wistar rats (150-250g) purchased from HEJ Research Institute of Chemistry. Rats were caged individually in specially designed Perspex cages in a quite room with free access to water and cubes of standard rat food, 1 week before starting the experiment so that they could adopt the laboratory environment.

Experimental protocol

Twenty four rats were randomly assigned to control and test groups (each containing 12 animals). Rats of control and test groups had free access to cubes of standard rodent diet as well as tap water and green tea extract (1.0 g/L), respectively. Food intake and growth rates were monitored on weekly basis for 5 weeks. Activities in Skinner's box, open field, light dark activity box and elevated plus maze were monitored after 5 weeks. After 5 weeks, animals were decapitated in a balanced design and brain samples were collected and stored at -70°C until neurochemical analysis by HPLC-EC.

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Light dark box activity

Light dark box activity was performed as described before (Ikram *et al.*, 2007). Specifically designed two Perspex boxes of equal dimensions (26x26x26 cm) were used to monitor the activity. One box is transparent and other is black walled. There is also an entry between them. To determine light and dark box activity, animal was taken out from home cage and was placed for the first time in the light box. Entries in light compartment as well as time spent in the very same, were monitored for 5 min.

Elevated plus maze activity

The elevated plus maze is also widely used as animal model of anxiety. The apparatus was specially designed in our laboratory and consisted of two closed and two open arms. Width (10 cm) and length (50 cm) of arms was same. A central area 5cm² was joining the arms. At a height of 60 cm, the maze was elevated from floor. Rat was placed in the center of the plus maze, to monitor its activity. Entries and time spent in the open arms were determined for 5 min.

Skinner's box activity

Transparent Perspex cages (26x26x26 cm) with sawdust covered floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. Fifteen minutes later, the animals were injected with drug or vehicle. Numbers of cage crossings were counted 5 min post-injection for 10 min (Ikram & Haleem, 2011).

Open field activity

A square area (76x76 cm) with walls 42 cm high was used to monitor activity in a novel environment. The floor of apparatus was divided by lines into 25 squares of equal size. Animals were injected with drug or vehicle and placed in the central square of the open field immediately after the injection. Numbers of squares crossed with all four paws were counted for 5 min (Ikram *et al.*, 2007).

Brain dissection

After decapitation, skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for neurochemical estimations using High performance liquid chromatography with electrochemical detection (HPLC-EC) (Ikram *et al.*, 2011).

Neurochemical estimations by hplc-ec

HPLC-EC determination was carried out as described earlier (Ikram *et al.*, 2011). A 5µ Shim-pack ODS separation column of 4.0 mm internal diameter and 150mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer of pH 2.9 at an operating potential of

2000-3000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu LEC 6A detector at an operating potential of +0.8V.

STATISTICAL ANALYSIS

Results are represented as means±S.D. Statistical analysis was performed by two-way ANOVA or Student's t-test (whatever applicable). Post hoc comparison of groups was performed by Newman-Keuls test following ANOVA. Values of $p < 0.05$ were considered significant.

RESULTS

Fig. 1 shows effects of repeated green tea administration on weekly food intake. Analysis of the data by two-way ANOVA (repeated measure design) revealed significant effects of green tea ($F=631.92$; $df=4,120$; $p < 0.01$) on food intake. However, effects of repeated monitoring ($F=9.94$; $df=1,120$) as well as interaction between the two ($F=4.09$; $df=1,120$) were non-significant. Post hoc analysis by Newman-Keuls test showed significantly decreased ($p < 0.01$) food intake in green tea treated rats after 5th week but not before.

Fig. 2 shows effects of repeated green tea administration on light dark box activity as monitored after week 5. Analysis of the data by Student's t-test showed no significant effect of green tea on entries as well as time spent in the light compartment.

Fig. 3 shows effects of repeated green tea administration on elevated plus maze activity as monitored after week 5. Analysis of the data by Student's t-test showed significant increase in entries ($p < 0.01$) as well as time spent ($p < 0.01$) in the open arm of the apparatus.

Fig. 4 shows effects of repeated green tea administration on locomotive activities as monitored after week 5. Analysis of the data by Student's t-test showed significantly increased locomotive behavior of green tea treated rats in novel ($p < 0.01$) as well as in familiar ($p < 0.01$) environment.

Fig. 5 shows effects of repeated green tea administration on brain serotonin and dopamine metabolism. Analysis of the data by Student's t-test showed significantly increased metabolism of serotonin as well as dopamine.

DISCUSSION

In the present study, green tea decreased food intake after 5th week but not earlier (fig. 1). Others have reported that green tea and other bioactive ingredients may reduce energy intake by relatively sustaining satiety and suppressing hunger (Reinbach *et al.*, 2009). Decreased food intake (hypophagia) in green tea treated animals

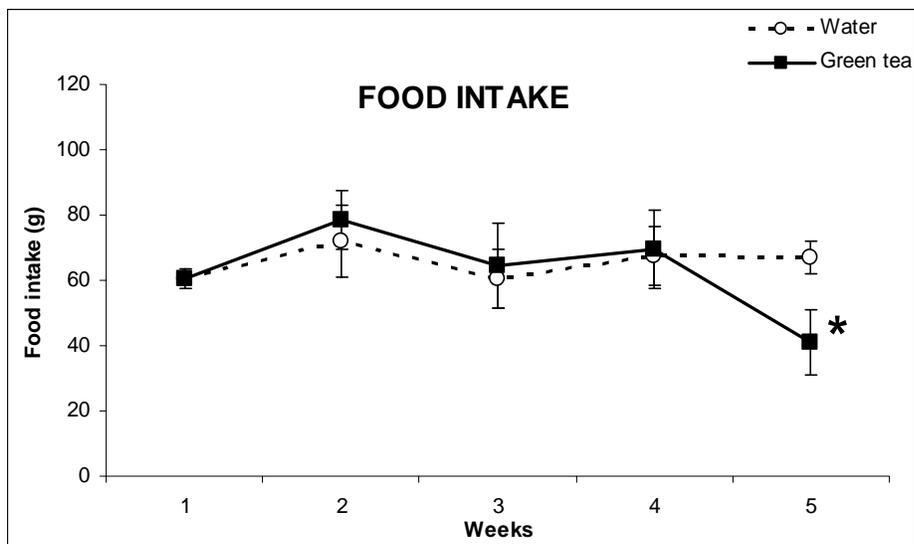


Fig. 1: Effects of repeated green tea administration on weekly food intakes. Values are means \pm SD (n= 12). *p<0.01 from water treated controls following two-way ANOVA (repeated measure design).

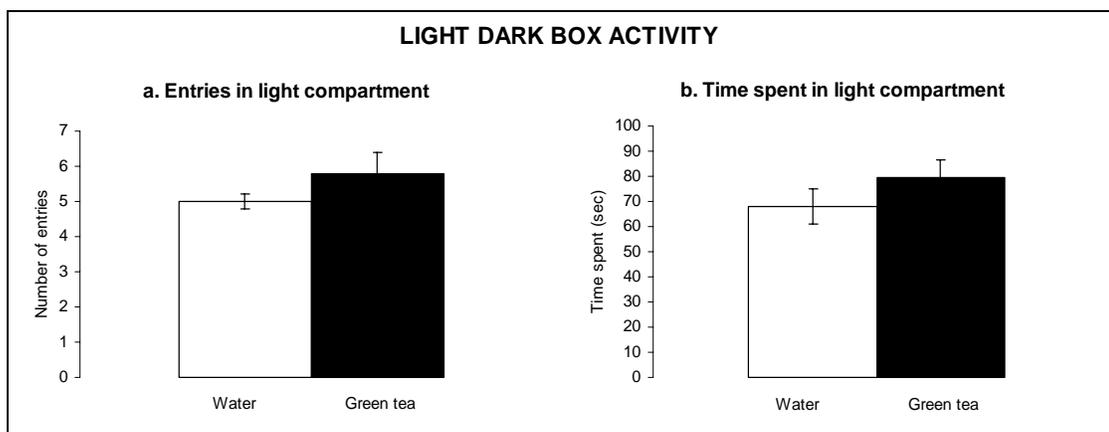


Fig. 2: Effects of repeated green tea administration (for 5 weeks) on light dark box activity. Values are means \pm SD (n=12) as monitored after week 5. Individual differences among the groups were found to be non-significant following Student's t-test.

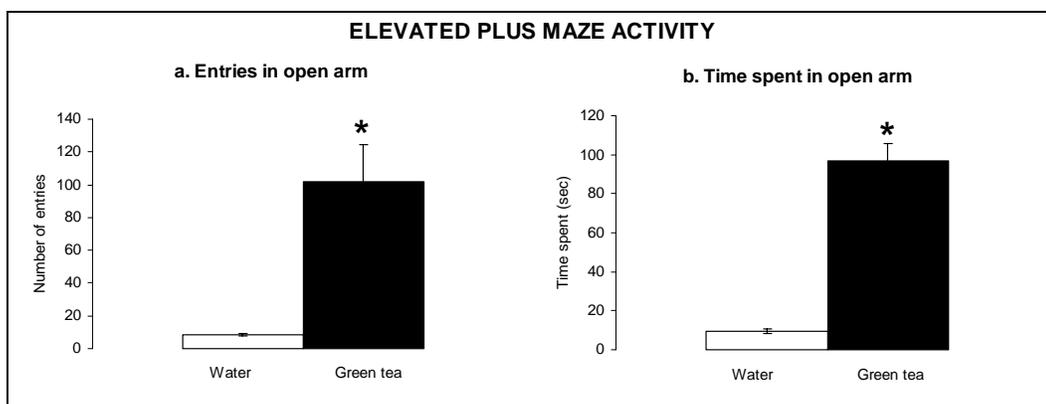


Fig. 3: Effects of repeated green tea administration (for 5 weeks) on elevated plus maze activity. Values are means \pm SD (n= 12) as monitored after week 5. *p<0.01 from water treated controls following Student's t-test.

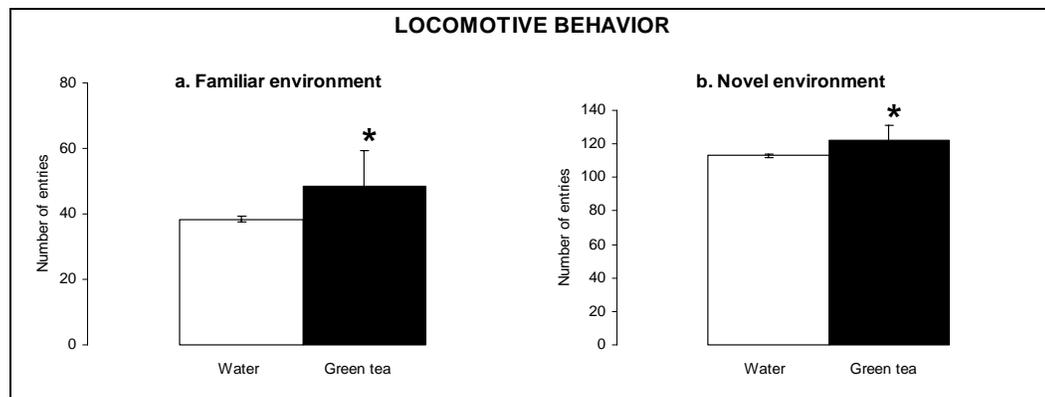


Fig. 4: Effects of repeated green tea administration (for 5 weeks) on locomotive activities as monitored in familiar environment of Skinner's box (a) and novel area of open field (b). Values are means \pm SD (n= 12) as monitored after week 5. *p<0.01 from water treated controls following Student's t-test.

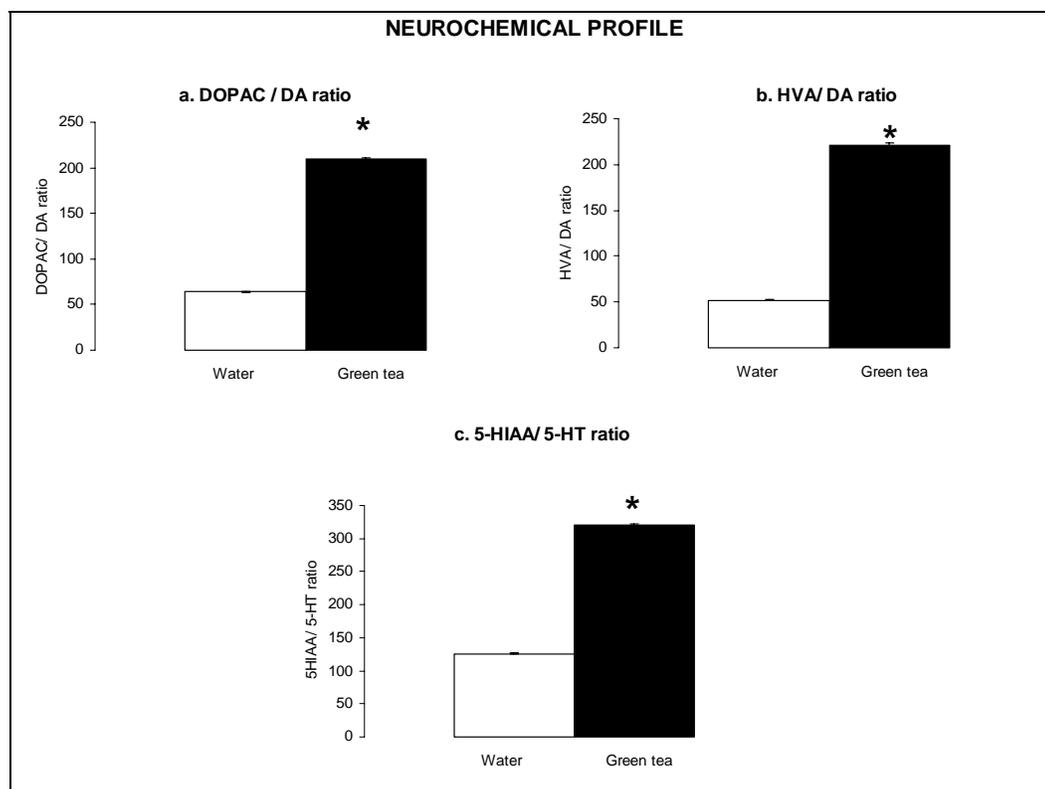


Fig. 5: Effects of repeated green tea administration (for 5 weeks) on brain catecholamine and indolamine metabolism. Values are means \pm SD (n=12) as monitored after week 5. *p<0.01 from water treated controls following Student's t-test

might be due to increased availability of 5-HT at 5-HT_{2C} receptors, as suggested by neurochemical profile (fig 5) in present study. Others also have reported that 5-HT_{2C} agonists produce hypophagia in animals (Simansky *et al.*, 2004; Vickers *et al.*, 2001).

Green tea treated animals did not show significant increase in the exploration of light compartment of light dark activity box (fig. 2). However, exploration of the

open arm of elevated plus maze was increased significantly, in green tea treated animals (fig. 3), suggesting that green tea could produce anxiolytic effects. Vignes *et al.* (2006), have reported that green tea polyphenol (-)-epigallocatechin gallate (EGCG), increased time spent in open arms of the elevated plus maze, in a dose-dependant manner (0.6, 1.25, 2.5, 5.0, 7.5, 15, 30 & 60 mg/kg i.p.). These findings are in accordance with the results obtained in present study. This

anxiolytic effect of green tea could be mediated by flavonoids in it. The ability of flavonoids, to modulate GABA_A receptor activity could be the underlying cause of anxiolytic effects of green tea. Low concentrations of EGCG could also potentiate the effects of benzodiazepine on GABA_A-receptor-mediated currents (Campbell *et al.*, 2004). It seems interesting that increased levels of 5-HT produced hypophagia but not the anxiety, as anxiogenic behavior is produced upon activation of 5-HT_{2C} receptors. 5-HT_{2C} receptor agonists produce anxiolytic effects (Martin *et al.*, 2002). Some other underlying mechanism might be involved in the mediation of anxiolytic effects of green tea.

Green tea increased locomotive behavior in the animals, as elicited in familiar as well as novel environment (fig. 4). This increased activity might be due to alertness and improvement of cognitive functions. Green tea could therefore be used for the treatment of disorders involving impaired locomotive function.

Neurochemical profile showed increased metabolism of dopamine as well as serotonin (fig. 5) upon repeated administration of green tea extract. This shows that green tea could be used for the treatment of disorders involving altered/abnormal metabolism of these neurotransmitters, such as Parkinson's, appetite, cognitive disorders, dementia, stress, depression and others. Further research in this area is needed to further elucidate the mechanism of action of green tea and involvement of different factors.

CONCLUSION

In conclusion, present study shows that green tea could be effective for the treatment of anxiety and hyperphagia. Result could be implicated for the treatment of disorders involving decreased serotonin and or dopamine metabolism.

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