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Injected Haloperidol-Induced Motor Deficits Are Potentiated in Rats Drinking Green Tea as a Sole Source of Water: Relationship with Dopamine Metabolism in the Caudate

TAFHEEM MALIK[1,2]* AND DARAKHSHAN JABEEN HALEEM[1,3]

[1.] *Neurochemistry & Biochemical Neuropharmacology Research Laboratory Department of Biochemistry, University of Karachi, Karachi, Pakistan*

[2.] *Department of Biochemistry, Jinnah Medical & Dental College 22-23, Shaheed-e- Millat Road, Karachi, Pakistan*

[3.] *Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*

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ABSTRACT

The antipsychotic drug "haloperidol" (HAL) has been widely used for the treatment of a range of neuropsychiatric disorders. However treatment also induces extrapyramidal symptoms (EPS) including short term parkinsonism and late complication tardive dyskinesia (TD). These idiopathic symptoms are associated with serious limitations in this therapy. Some studies have suggested that oxidative stress induced during the metabolism of HAL is involved in the elicitation of EPS. We speculated if green tea may prevent HAL-induced EPS because of its antioxidant properties. In the present study, the efficacy of green tea extract (GTE) given as a sole source of water on HAL-induced EPS male albino wistar rats was examined. We found that HAL-induced motor deficits and elicitation of TD were more severe in GTE than water dinking animals. HAL-induced dopamine level was increased and its metabolites concentrations were higher in the nucleus accumbens and lower ($p < 0.01$) in the caudate of GTE-drinking than water-drinking animals. Increased ratios of homovanillic acid (HVA) and 3, 4-dihydroxyphenylacetic acid (DOPAC)/dopamine in the caudate may be involved in the precipitation of HAL-elicited EPS while drinking GTE. Conversely GTE increased the level of dopamine moreover raised its metabolites in the nucleus accumbens may relapsed the schizophrenic symptoms while on the treatment HAL. We thus suggest that patients on HAL therapy should avoid green tea.

Key words: Green Tea, *Camellia sinensis*, Haloperidol, Nucleus Accumbens, Caudate Putamen, Extrapyramidal Symptoms, Parkinsonism, Tardive Dyskinesia, Tardive Myoclonus

INTRODUCTION

Haloperidol treatment has been shown to produce oxidative stress in patients with acute neuropsychiatric symptoms. Oxidative stress has also been implicated with the HAL-induced extrapyramidal symptoms (EPS)[(1-3)].

EPS is characterized with initial parkinsonian-like symptoms, and late complications are dyskinesia syndromes. Studies on animal models showed that acute administration of HAL elicits motor deficits homologous to parkinsonian-like symptoms of humans. Impairment in motor coordination can be quantified in rats using rota rod[(5-10)]. On the other hand rats treated with repeated chronic doses of HAL develop late complication i.e. orofacial movements described as tardive syndromes, such as tardive vacuous chewing movement (tVCMs)[(9)] and tardive myoclonus[(11)]. tVCMs are used for tardive dyskinesia (TD) quantification in rat model[(9)].

HAL is known to have high affinity for dopamine D_2 type receptors. By blocking D_2 type receptors in the corpus striatum it treated psychiatric symptoms besides elicited syndromes[(9,10,12)]. Hence HAL with its metabolites provides both therapeutic action and adverse events through striatal neurons[(3)] which are often suggested to eradicate symptoms of idiopathic EPS with the placement of antioxidative effects on neurons. It could reduce the symptoms that appear in the course and development of EPS[(11,13-14)].

The neuropathology associated with EPS within and around the region is thought to involve excessive production of free radicals and dopamine autoxidation. The detail mechanistic studies have shown that dopamine and serotonin interact antagonistically in the caudate to control motor activity[(6)]. Nucleus accumbens is a region of brain known to be involved in emotional control. Both striatal regions are affected by dopamine autoxidation during HAL therapy[(15)]. The ratios of dopamine metabolites to dopamine are known indicators that depict the clear picture of the HAL-induced

* Author for correspondence. Tel: +92-334-204-3442; Fax: +92-213-5377846; Email: Tafheemm85@gmail.com

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adverse events. Report from our laboratory provided evidence that the increase in dopamine metabolism is directly proportional to the increase in EPS events[6].

Some reports have suggested that the constituents of green tea have antioxidative properties that may have protective effects against chemotoxicants to reduce distressing effects[16-18]. Conversely our recent report[19] and other studies together have found its properties controversial.

Therefore the current study concerns with the effects of green tea extract (GTE) on HAL-induced EPS in rats. In order to understand the role of dopamine and its metabolites in the modulation of HAL-induced EPS, we determined homovanillic acid (HVA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) concentrations and ratios to dopamine concentration in the caudate and nucleus accumbens of the rat brain.

MATERIAL AND METHODS

Male albino Wistar rats (150g-200g) were housed individually under standard laboratory conditions maintained on a 12-hour light/dark cycle with free access to food, rodent diet pellets and water was allowed libidum for the first week. Animals were acclimatized to laboratory conditions before the experiment.

I. *Treatment*

Twenty four animals were randomly divided into four groups (six animals in each group): (I) water plus saline (II) water plus HAL (III) GTE plus saline (IV) GTE plus HAL. The green tea 1 g was extracted with hot (boiling) water (1 L) for 5 minutes. It was freshly made in 24 h and provided as a sole source of water. HAL (*Serenace; Searl*) was injected 1 mg/Kg daily and equal volume of saline 1 mL/Kg daily was injected to the controls intraperitonealy (IP). The doses were administered according to a balanced design between 10:00 and 12:00 h.

II. *Behavioral Experiments*

All behavioral experiments were conducted at room temperature in noise-protected environment. Behavioral assessments were repeated every 6th day of the week.

III. *Motor Function Tests*

(I) *Monitoring Motor Coordination*

Motor coordination was monitored on a Rota-Rod® System (UGO BASILE, Biological research apparatus, COMERIO, Varese, Italy). The Rota-Rod with a drum of 7 cm diameter was adjusted on 2-20 revolution per l min (rpm) speed. Rats were trained to maintain balance on a rotating bar, until they attained 150 s walking on rotating bar. The latency to fall in test session of 150 s was taken as a measure of motor coordination monitored after 1 hour of HAL administration.

(II) *Quantification of Tardive Syndromes, Orofacial Dyskinesia and Myoclonus*

Two movement disorders units were used to establish drugs responsible for tardive syndromes or 'TS' (tardive orofacial dyskinesia, and myoclonus). tVCMs is an analogous model of orofacial dyskinesia, characterized by purposeless spontaneous opening of mouth with or without tongue protrusion[20]. According to the revised model for orofacial dyskinesia in rats, tVCM were described[22]. We reduced methodological discrepancies and used young rats in order to avoid age related movement deficits in this experiment. We also increased cage size that helps to maintain stressless condition as tVCMs are known to be sensitive to stress. The lowered incidence of dyskinesia during stress is well documented. The incidence of tVCMs was monitored both live and with video recording after the 3rd week of i.p. administration of HAL in both GTE and water-drinking animal groups.

Animals were placed individually in a rectangular prespex activity cage (26/26/26 cm) with a sawdust-covered floor and observed for a period of 15-min. Orofacial dyskinesia was quantified during 10-min observation period. Each chewing episode was scored as a "1". A chewing period consists of distinct bursts of three to five masticatory movements and lasts for 2-5 s. Masticatory movements were referred as single mouth openings in the vertical plane not directed toward physical material. Recently Lerner & colleague described "tardive myoclonus", movement disorder as brief jerks of muscles in legs, neck, trunk, and extremities[11]. A rat model of tardive myoclonus was defined as jerky movements in legs and extremities. We observed and counted appearance of tardive myoclonus in separate mode of motor deficits. The individual rat was constantly observed by an observer blind to the treatment. The tardive myoclonus and tVCMs were monitored at the cut-off time of 10 min that began 4 h after HAL administration.

IV. *Dissection of Striatum*

The animals were killed 20 h after the last injection of HAL on the 43rd day to collect brain samples for the neurochemical analysis.

The dissection procedure of the brain was essentially the same as described before[6,19]. A fresh brain was dipped in ice-cold saline and placed with its ventral site up in the molded cavity of a brain slicer (Alto matrices). Fine fishing line wire was inserted into the slots of the slicer to give slices of 2 mm thickness. The slice containing striatum was transferred to a slide kept on iced normal saline. Punches of 2.5 mm diameter were made bilaterally in the striatum to collect the brain regions in both dorsal and ventral regions of the striatum. The regions were isolated and identified according to the rat brain atlas[23].

Samples were obtained and stored at -70°C for neurochemical analysis of dopamine and its metabolites on the 57th day of treatment.

I. *Brain Regional Analysis by HPLC- EC Technique*

A shim-pack XR-ODS Shimadzu separation column of (5 μ pore size; 4.5 mm I. D. × 15 cm) was used. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 14% methanol (HPLC grade), 0.023% OSS, and 0.005% EDTA. Electrochemical detection was done at an operating potential of 0.8 V (glassy carbon electrode vs. Ag/AgCl reference electrode).

II. *Statistical Analysis*

Data are presented as means ± SD. With SPSS 11.5 software, data on rota rod and treatment induced weekly changes were recorded in the motor coordination tVCMs were analyzed by two-way ANOVA (repeated measure design). By using two-way ANOVA for behavior tardive myoclonus, and neurochemical parameters analysis individual comparison was made by Bonferroni test. Probability and ratios were considered significant at values less than 0.05.

RESULTS

The control rats consumed an average of 31 ± 1 mL water/day, while rats on GTE groups consumed 35 ± 6 mL on day 1, then reached maximum intake of 41 ± 3 mL on day 42. The HAL-treated group consumed 19 ± 2 mL water per day and HAL treated GTE drinking group of animals consumed 21 ± 4 mL/day.

The three-way ANOVA of rota rod performance showed significant effects of HAL (F = 42.41 df = 2, 23, $p < 0.01$) and GTE (F = 18.85 df = 2, 23, $p < 0.01$) while weekly effects (F = 56.14 df = 2, 23, $p < 0.01$) were also significant (Figure 1). Rota rod performance was decreased in both HAL-treated groups of animals. HAL-induced decreased performance on rota rod was smaller in water drinking than in GTE drinking animals. Rota rod performance was steady after 3rd weeks of water-drinking HAL-treated animals. However, water intake decreased greatly in GTE plus HAL treated group of animals in this duration.

HAL injection started to elicit tVCMs (df 1,20) after 3-weeks of administration in both GTE and water drinking animals (F = 54, df 4, 40, $p < 0.01$) while tVCMs did not appear in animals drinking GTE or water in the absence of HAL administration. HAL-induced tVCMs was progressively greater ($p < 0.01$) in the GTE drinking animals in comparison with water drinking group (F = 46.7 df = 4, 40, $p < 0.01$) (Figure 2). Weekly effect (F = 34.4 df = 4, 40, $p < 0.01$) was also significant between groups of animals.

HAL injection also elicited tardive myoclonus (Figure 3) parallel to tVCMs (df 1, 20) after 3-week of administration in both GTE (F = 18.7, $p < 0.01$) and water (F = 11.5, $p < 0.01$) drinking animals while tardive myoclonus did not appear in GTE and water drinking animals in the absence of HAL. Interaction of GTE on HAL treatment was also significant (F = 15.3, $p < 0.01$). HAL-induced tardive myoclonus complication was significantly enhanced ($p < 0.01$) in GTE-drinking animals in comparison with water drinking group (Figure 3).

The alteration in dopamine and its metabolites concentrations in caudate region (Figure 4) became (df 1,20) significant after 6-weeks of HAL administration (F = 5.3, $p < 0.01$) and GTE drinking (F = 8.0, $p < 0.01$), while interaction of GTE was also significant (F = 6.3, $p < 0.01$) on dopamine concentration in the caudate region of animals. Post hoc analysis showed that dopamine concentration decreased significantly ($p < 0.01$) in HAL plus GTE group of animals. Dopamine concentration was lower in GTE-drinking than water-drinking control animals. HAL-induced dopamine concentration was more decreased in GTE than in water drinking animals.

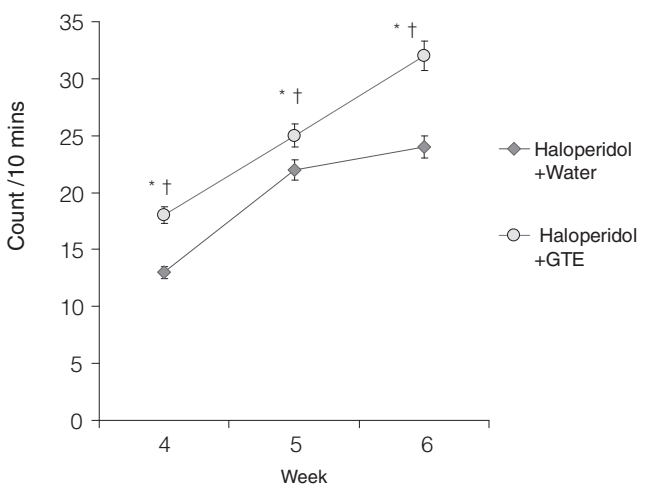

Figure 1. HAL effects on oral administration of water / GTE fluid taking group of animals monitored on rota rod performance. Values are means ± SD (n = 6). *$p < 0.01$ from respective saline treated group, + $p < 0.01$ from respective water treated group following two-way ANOVA repeated measure design.

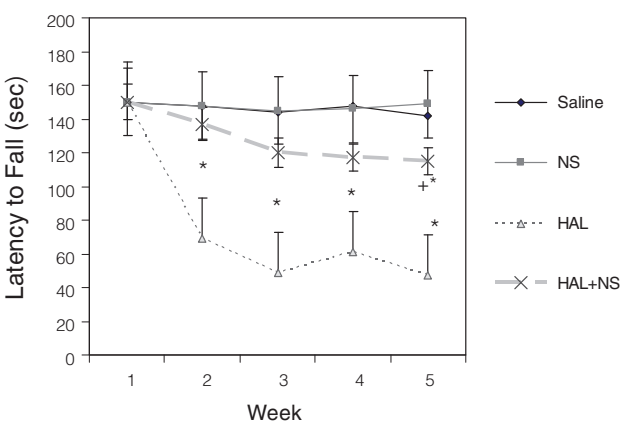

Figure 2. HAL effects on water / GTE fluid drinking group of animals monitored (4th-6th weeks) for HAL-induced tVCMs count. tVCMs appeared after 3rd week of repeated HAL administration. Values are means ± S.D. (n = 6). *$p < 0.01$ from respective weekly differences among groups + $p < 0.01$ from respective water treated group following two-way ANOVA repeated measure design.

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HAL injection elicited the alteration of DOPAC concentration in caudate region of both water and GTE drinking animals (Figure 4), which showed (df 1,20) significant effects after 6 weeks of HAL administration ($F = 14.6$, $p < 0.05$) and GTE drinking ($F = 18.3$, $p < 0.01$). The interaction between GTE and HAL was also significant ($F = 6.8$, $p < 0.05$) on DOPAC concentration in caudate region of animals. Post hoc analysis showed that DOPAC concentration was significantly decreased ($p < 0.01$) in caudate region of HAL plus GTE treated animals. DOPAC concentration was greater in GTE drinking than water drinking controls animals. Either GTE drinking or HAL treated plus water drinking animals showed increased DOPAC concentration in the caudate region. However the increased DOPAC concentration elicited by either HAL or GTE alone was decreased or not altered in GTE plus HAL treated animals.

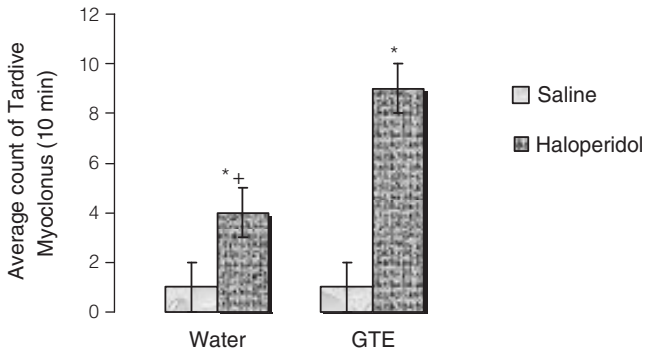

Figure 3. Effects of repeated injection of HAL on tardive myoclonus counts in the group of animals drinking GTE/water fluids. Tardive myoclonus appeared after 3rd week of repeated HAL administration. Values are means ± SD (n = 6). significant difference * $p < 0.01$ from respective controls + $p < 0.01$ from respective water treated group following two-way ANOVA repeated measure design.

HAL injection elicited alteration of HVA concentration in caudate region after 6 weeks of administration in both water and GTE drinking animals (Figure 4) where it showed (df 1,20) significant effects of GTE ($F = 6.9$, $p < 0.01$) HAL ($F = 5.3$, $p < 0.01$). Interaction between GTE and HAL was also significant ($F = 1.9$, $p < 0.05$) on the HVA concentration in caudate region. Post hoc analysis showed that the HVA concentration was significantly ($p < 0.01$) decreased in the caudate region of HAL injected GTE drinking group of animals. HVA concentration was greater in GTE drinking than in water drinking control animals. The increase in HVA concentration by GTE alone was less than that in HAL treated water drinking animals. The increased HVA concentration induced by HAL was significantly diminished in GTE drinking HAL administered group of animals.

HAL injection elicited the alteration in dopamine and its metabolites after 6 weeks in the nucleus accumbens in water and GTE drinking animals (df 1, 20) which showed (Figure 5) significant effects of GTE ($F = 5.7$, $p < 0.01$) HAL ($F = 7.3$, $p < 0.01$). Interaction of GTE and HAL treatment was also significant ($F = 6.63$, $p < 0.01$) on dopamine concentration in the nucleus accumbens. Post hoc analysis showed that the concentration of dopamine was significantly increased

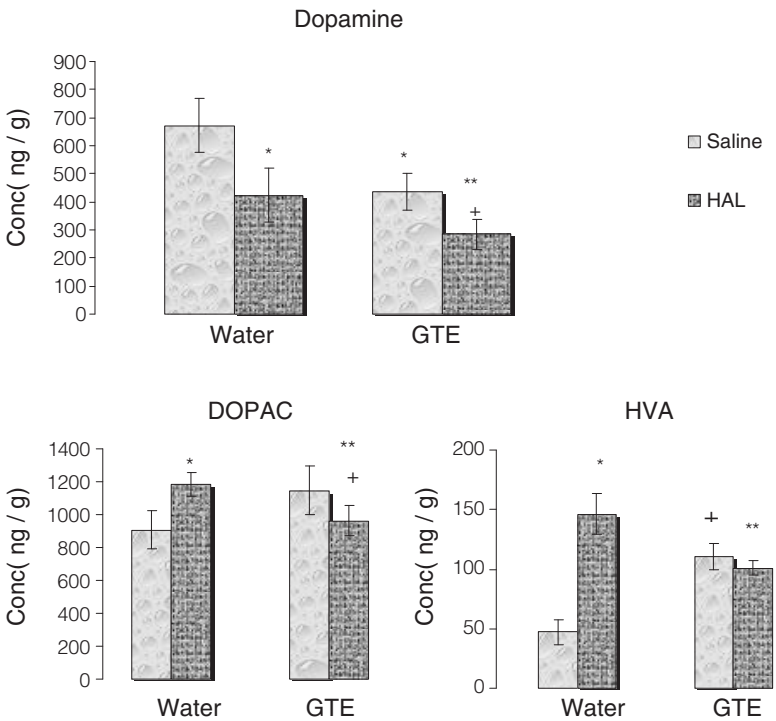

Figure 4. Effects of oral administration of GTE/water intake on dopamine and its metabolites (DOPAC and HVA) concentration in the caudate of HAL-treated group of animals. Values are means ± SD. After 20 h of the last administration of the HAL, + $p < 0.01$ from respective saline injected rats from ** $p < 0.05$ * $p < 0.01$ the respective water treated rats following two-way ANOVA.

($p < 0.01$) in the nucleus accumbens of HAL injected GTE drinking group of animals while dopamine concentration was greater in GTE drinking than in water drinking control groups. Both groups of animals drinking GTE showed increase in dopamine concentration in the nucleus accumbens. Dopamine concentration was decreased in HAL treated water drinking animals. HAL-induced decreased dopamine concentration was reversed in GTE drinking HAL treated group of animals.

HAL injection elicited the alteration in DOPAC concentration which was observed after 6 weeks of treatment in the nucleus accumbens of both water and GTE drinking animals (df 1,20) as shown in Figure 5 with the effects of GTE ($F = 3.2$, $p > 0.05$) and HAL ($F = 9.4$, $p > 0.05$). Interaction between GTE and HAL was significant ($F = 6.8$, $p < 0.05$) on DOPAC concentration in the nucleus accumbens. Post hoc analysis showed that DOPAC concentration was significantly ($p < 0.01$) increased in the HAL-injected GTE drinking animals where DOPAC concentration not altered by HAL in water drinking animals. DOPAC concentration was not altered in GTE drinking control animals. Combination of HAL with GTE elicited much increase of DOPAC concentration in the nucleus accumbens of treated animals.

HAL injection also elicited the alteration of HVA concentration, which was observed after 6-weeks in the nucleus accumbens of both water and GTE-drinking animals (df 1,20), as shown in Figure 5 with significant effects of GTE ($F = 6.9$, $p < 0.01$) and HAL ($F = 5.3$, $p < 0.01$). Interaction of GTE with HAL injection was also significant ($F = 4.9$, $p < 0.05$) on the change of HVA concentration in nucleus accumbens. Post hoc analysis showed HVA concentration in the nucleus accumbens was remarkably increased ($p < 0.01$) in HAL injected GTE drinking animals. HVA concentration was increased by HAL treatment in water drinking groups while HVA did not change in the absence of HAL either in GTE or water-drinking group of animals. HAL-induced elevation of HVA concentration was even greater in GTE drinking group.

HAL-induced DOPAC/dopamine turnover ratio was 2-3 folds greater ($p < 0.01$) in GTE drinking (-82%) than water drinking animals in the caudate region (Table 1). In the absence of HAL, DOPAC/dopamine turnover ratios of GTE drinking group were not significantly ($p > 0.5$) altered to the control group animals, whereas in the HAL injected groups this ratio was significantly increased. HAL-induced HVA/dopamine turnover ratio was 3 folds greater ($p < 0.01$) in the GTE drinking (-62%) than water drinking animals. In the absence of HAL, HVA/dopamine turnover ratios of GTE drinking group were not significantly ($p > 0.5$) altered to the control group of saline treated animals, whereas in HAL injected group this ratio was significantly ($p < 0.01$) increased (-102%) compared to the control group.

In nucleus accumbens, the DOPAC/dopamine turnover ratio was significantly ($p < 0.01$) increased (-88%) in HAL-injected GTE drinking than HAL treated water drinking animal group (Table 1). DOPAC/dopamine turnover ratios were greater (-53%) in GTE drinking as compared to HAL injected GTE drinking group, where these ratios were 8% greater ($p > 0.5$) in controls. HVA/dopamine turnover was ($p < 0.01$) significantly (4 folds) greater in HAL-injected GTE drinking group than water drinking HAL injected animals. An increase of HAL-induced HVA/dopamine

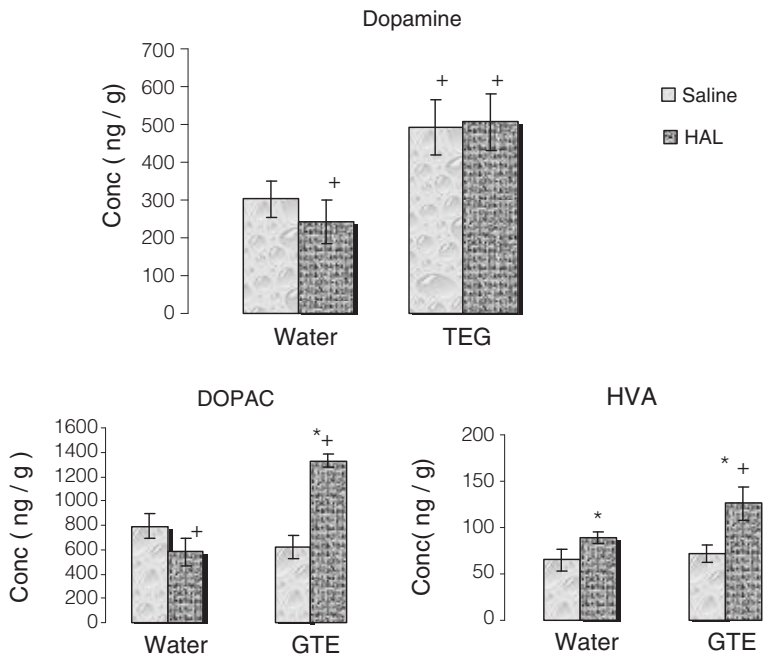

Figure 5. Effects of oral administration of GT intake on dopamine and its metabolites (DOPAC and HVA) concentration in the nucleus accumbens of HAL-treated group of animals. Values are means ± SD. After the 20 h of last administration of the HAL significant differences from + $p < 0.01$ respective saline injected rats from *$p < 0.01$ respective water treated rats following two-way ANOVA.

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Table 1. Dopamine turnover ratios in caudate and nucleus accumbens regions of rat brain

	Caudate (n = 6)				Nucleus Accumbens (n = 6)			
DOPAC / Dopamine	Control		HAL		Control		HAL	
	Water	GTE	Water	GTE	Water	GTE	Water	GTE
	1.8 ± 0.3	2.1 ± 1.1	2.8* ± 0.5	3.4*$^{+}$ ± 0.0	1.5 ± 0.7	1.3 ± 0.04	2.4* ± 0.0	2.8*$^{+}$ ± 0.0
HVA / Dopamine	Control		HAL		Control		HAL	
	Water	GTE	Water	GTE	Water	GTE	Water	GTE
	0.07 ± 0.0	0.16 ± 0.0	0.34* ± 0.0	0.37*$^{+}$± 0.0	1.2 ± 0.7	1.4 ± 0.0	1.8* ± 0.01	2.7*$^{+}$ ± 0.0

Shown means ± SD in ng/mg net weight and means of ratios.
*$p < 0.01$ from respective saline injected controls.
+$p < 0.01$ from respective water drinking.

turnover in the nucleus accumbens region became even more increased in GTE drinking HAL injected animals, which was distinctly different from water drinking controls. In GTE drinking group, the HVA/dopamine turnover ratio was ($p < 0.05$) significantly smaller than the other GTE group of HAL injected animals. However the HVA/dopamine turnover ratios of GTE drinking group were not significantly different to controls.

DISCUSSION

Fluid intake was reduced in both HAL-treated GTE/water drinking groups of animals; however, it was interesting to observe that GTE intake was greater than water intake in HAL treated animals. It has been reported that the low doses of HAL lead to lower water consumption[24].

Data in this study showed that HAL-injected animals drinking either GTE or water for six weeks exhibited significant EPS. As it has been demonstrated in our pilot study that oral administration of HAL also impaired motor coordination significantly, whose extent was more in rats drinking GTE as compared to water-drinking ones[19]. Our present data suggested for the first time the effects on metabolism in the animals drinking GTE and treated with HAL at the same time. Increased HAL-induced parkinsonian-like locomotor deficit was observed in parallel in both HAL treated groups. However, marked difference in both HAL treated groups was also observed on the 5th to 6th weeks. A report has suggested that the HAL treatment produces tolerance in motor deficits on the 3rd week following HAL treatment[9]; conversely there was no tolerance observed in HAL treated GTE drinking animals in this study. HAL treated water drinking group showed tolerance at the 3rd week in comparison and some levels of drug tolerance also appeared in this group of animals. Hence it was contradictory to our hypothesis that GTE may reduce the HAL-elicited movement deficits.

Results in Figure 2 demonstrated significant difference in motor coordination during 4th to 6th weeks of HAL injection in animal group drinking water and GTE. A report where the mice model of Parkinson's diseases (PD) was employed showed that the GTE alone has no significant effect on PD. However it was interesting to note that 5 mg/Kg GTE caused an elevation in dopamine metabolism that was significantly amplified at MPTP (N-ethyl-4 Phenyl-1, 2, 3, 6-tetrahydropyradine) effects of PD elicitation[25].

In current study design, GTE potentiated the HAL-induced parkinsonian like symptoms that was monitored on rota rod. Martins *et al*,[26] have reported neuroleptic elicited high altitude of dopaminergic metabolism produce reactive oxygen species (ROS) and subsequent neuroleptic-induced neurodegeneration. Here it may be possible that HAL bound the free radical of scavenging GTE properties and had potential to make GTE a pro-oxidative agent. A report has demonstrated that the green tea acts as a pro-oxidative agent itself and significantly increases hydrogen peroxide (H_2O_2) levels in a dose dependent manner[27]. Linking from that study, we can postulate that HAL might have potential to alter the anti-oxidative properties of GTE and exert its effects in a manner of converting into potential pro-oxidant-like effects that could result in the increase of metabolism in animals showing severe EPS.

This study also validated Haleem's report that tVCMs are a late-appearing complication of HAL which precipitating in doses-dependent manners overtime[9]; these effects were definite in both HAL treated groups shown in the current study. Data demonstrated that high rate of early and late complications in the movement syndromes appeared at both acute and chronic treatment of HAL. HAL-induced precipitation in tVCMs appeared in timely manners in GTE drinking HAL treated animals. tVCMs symptoms were greater in GTE drinking than in water drinking HAL treated group of animals. It is well documented[9] that the increased precipitation of late-appearing tVCMs complication simultaneously appears with myoclonus symptoms on the 3rd week of HAL treatment, which is also evidently shown in this study.

High differential rates of deficits were recorded in later weeks of HAL treatment in GTE and than in water drinking animals. It has been documented HAL-induced apoptosis upon oxidative stress produced by antagonizing dopaminergic receptors in the striatum would elicit tVCMs symptoms,[28] and we here postulated that green tea may enhance HAL-induced mechanism that depicted higher movement symptoms. To our knowledge this is first time the database

of myoclonus movement disorder unit was reported drug responsible extrapyramidal symptoms. Both tardive dyskinesia and myoclonus are clinically presented differently in patients over the HAL therapy. Suenaga *et al.*[28] have described HAL responsible tardive types that were clinically presented in patients with persistent EPS such as dyskinesia, choreatic movement and *myoclonus.* We observed and counted the appearance of tardive myoclonus as a separate mode of movement deficits in rats (presented in Figure 4). Reporting myoclonus type-symptoms in rat model of EPS, HAL-induced jerky movements of extremities was monitored mainly in legs. HAL-elicited EPS were more found sever in GTE-drinking animals as compared with water drinking ones.

Our study is the first presenting data on the striatal behavior as well as dopamine turnover rates with a pronounced alteration via GTE drinking in the rodent's brain regions. Studies have clearly shown a direct relationship between dopaminergic metabolism and elicitation of EPS in rodents[6,19]. The ratio of striatal HVA/DA, as a measure of the synaptic dopamine turnover was already observed increased in patients with mild symptoms of movement deficits[29]. HAL-treated animals with increased motor deficits and have also shown motor-related events in the striatal region. Anatomical site of HAL action in the striatum is well documented, that has demonstrated regional involvements in uncontrolled motor-nerves. Clinically effective doses of HAL occupy 60-80% of brain dopamine D_2 type receptors, as measured by Positron Emission Tomography (PET) in the human striatum[30,31]. Occupancy at striatal dopaminergic receptors caused motor deficits at HAL-elicited threshold acute side effects parkinsonism and chronic threshold-induced tardive symptoms. Striatum receives input from dopaminergic neurons[32]. Supersensitive responsiveness of the post synaptic D_2 type receptors contributed to the emergence of these syndromes[7].

Interestingly, an increased of dopamine level and its metabolites causes Schizophrenia[33-35]. Results from GTE + HAL group have shown that increased levels of dopamine and its metabolites in the nuclease accumbens may cause relapse of Schizophrenia.

Evidence has supported that the status of this regional dopaminergic metabolism may possibly antagonize HAL effects and also may lead to the relapse of the schizophrenic symptoms[11,33-35]. HAL is not effective in negative symptoms of schizophrenia[33]. The negative symptom complex of schizophrenia has been associated with low dopamine and high metabolites levels. Also it has been reported that increase in dopamine metabolism was found in post-mortem patient of schizophrenia. Low level of dopamine and high level of metabolites in schizophrenic patients were found in caudate[34] which are more likely to lead to a relapse of hallucination due to drinking GTE with the HAL treatment. Therefore, it enhances the possibility that GTE may exaggerate negative deficits complex plus hallucination relapse in schizophrenic patients. Motor impairment can be correlated with dopamine, DOPAC and HVA concentrations in caudate. DOPAC is often considered as an index of intraneuronal dopaminergic catabolism[35]. Studies have recommended that acute administration of HAL increases DOPAC[36] and HVA levels[37]. It can be suggested that dopamine availability to dopamine metabolism ratio augmented motor impairment. In dose-dependent manners these symptoms last at greater magnitude and longer duration in the brain striatum[38]. This study consolidated previous results to understand GTE and HAL effects on dopamine metabolism. Here it was very interesting to note that solely GTE effects on dopamine and its metabolism on rodent brains that mimicked to the human brain[19].

The long term administration of HAL is involved in an increase of sensitivity of the D_2 receptors. Dopamine D_2 receptors are intensely positioned both presynaptically and postsynaptically in striatal dopaminergic neurons[7,37]. It is likely that GTE has presynaptical and postsynaptical roles in processing and incorporating incoming inputs in nucleus accumbens but blocks the networking in caudate of the basal ganglion. We speculate that the delay of incoming inputs of D_2 receptors from the nucleus accumbens toward the caudate could be the cause of increased intensity of EPS. It will be interesting to investigate the role of GTE in pre-and post-synaptical signaling events during integrating and processing networking between regions.

The present study showed that both HAL and GTE decreased dopamine level in the caudate region of striatum. An increase in metabolism of dopamine was in the caudate region of the striatum may be involved in the potentiations of EPS. HAL increases and HAL + GTE inversely decreases dopamine levels in caudate region. It was interesting to note that the GTE itself increases the dopamine levels in the nucleus accumbens but did not produce any motor deficits probably due to normal metabolism of the dopamine in the region. The different effects of HAL and GTE on motor functions and dopamine are largely explainable in terms of their activities at dopamine receptors. Our results suggested that an increase in the dopamine levels to their metabolite ratio, particularly in the striatum, may be taken as an increased neurochemical indexed activity. The ratios of dopamine turnover to the HVA are known indicators that depict the clear picture of the HAL-induced adverse events. As found previously[19] dopamine metabolism in the dorsal striatum was greatly decreased by GTE, supporting that decreases in dopamine metabolism is directly proportional to the precipitation of EPS events which was also conformed in this study.

Our results (Figure 4 and 5) showed the effect of HAL on DOAPC levels, which tended to induce large fraction of dopamine catabolism intraneuronally in the nucleus accumbens. On the other hand an increase level of HVA was also observed in nucleus accumbens but not in the caudate at the repeated dose HAL in GTE-drinking animals. It is well documented that small dose of HAL increases the dopamine release in striatal regions[39]. That is explicable in terms of increased dopamine release and obstructs respectively in these brain regions.

The effects on nucleus accumbens are also known to be involved in the emotional control besides movement control.

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Our unpublished data showed HAL produce effects on the nucleus accumbens connected to the mood related influence and GTE had influence on mood events[40] indicating that GTE along HAL enhances the dopamine and its metabolites levels in the nucleus accumbens that may have some impact on mood responsiveness essentially. It could be hypothesized that the GTE alone and along with HAL may produce some effects on mood receptivity to some extent; however, the behavioral assessment is necessary in order to see the effect on mood impression was not performed in this study.

Authors generally agreed that the clinical response of neuroleptic is associated with an increase in HVA concentration with adverse events of the HAL therapy[41-43]. The proposed mechanism of HVA changes involves a strong blockade of pre- and post-synaptic D_2 receptors. This blockade increases the amount of dopamine release on subsequent action potentials and leads to attenuated dopamine transmission at the post-synaptic D_2 receptors. As biochemical investigations showed that tolerance developed to the increase of striatal HVA after chronic treatment of HAL[41] GTE failed to affect the HVA (Figure 5) content effects. No tolerance developed to the increase to these events while persistence in latency to fall from rota rod increased (Figure 1), count of tVCMs increased (Figure 2) and myoclonus event increased (Figure 3). From these results, it is confirmed that the elevated HVA concentrations was caused by both HAL and GTE treated nucleus accumbens (Figure 5) whereas HVA/dopamine turnover ratio was found significantly (3-4 folds) greater in GTE drinking HAL treated compared to water drinking HAL treated animals. As shown from previous studies of HAL, schizophrenic symptoms appeared at increased dopamine concentration that potentially precipitated as dopamine turnover occurs over HAL administration[44] with the concomitant increases of dopamine release and later turnover in the caudate[44,45]. However, GTE along HAL administration significantly decreased the HAL-induced deceases of dopamine concentration and concomitant increases of dopamine metabolites by means of turnover in the caudate region. HAL-induced increase dopamine was reversed along with its metabolites HVA and DOPAC[46], indicating the antagonism of HAL effects. Here we predict that the antischizophrenic effects of HAL were antagonized by the GTE administration. In the present study, results suggested that the GTE along with the HAL administration produces even worse effect on movements and the possibility of the relapse of the schizophrenic symptoms while antagonizing the HAL effects; however this study lacked the behavioral monitoring of schizophrenic relapse.

A rodent study showed that GTE exerts its paralytic effects on skeletomotor function that suggested denervated diaphragm in rodents in a concentration dependent manner[47]. It is evident from our study that GTE interaction with less concentration of HAL produced greater HAL-induced motor deficits in animals resulting none of facilitatory motor function in rats.

Despite phytotherapeutic reputation of green tea, some studies have shown the controversial results[47-52]. Major components of GTE potentiate cytotoxicity-induced by benzyl-isothiocyanate and H_2O_2 in human Jurkat T lymphocytes[48]. The hydroalcoholic extract of green tea used as a weight reducing agent, it produces significant hepatotoxicity causing fulminant hepatitis in human who may need liver transplantation[49,50]. It has been reported that GTE increased the onset and duration of convulsion thus causing mortality in rodent[51]. These studies focused on the adverse effects and characterized green tea as a health hazardous beverage. GTE was also associated with neurological events like seizures[51] confusion and insomnia[52] proven by clinical trials. Effects on neuromuscular junction-induced fatigue[51] as well as paralytic effects observed in modifying skeletomotor junction affected motor nerve terminals and inhibited acetylcholine release which were observed at frequent intake of green tea[47]. Nevertheless it is quite often used as social drink to enhance performance and mood states[51]. This study does not specify any green tea component but the whole intake of GTE may interact with HAL and trigger neurochemical events on central dopaminergic system. Conversely, it will be interesting to define the component interaction with HAL. Aforementioned report[19] and current study measured the adverse events of GTE that are certainly shown a level of importance for precautionary setup during HAL therapy.

CONCLUSIONS

This study showed that intra-peritoneal injection of HAL and oral consumption of GTE potentiated acute parkinsonian-like effects that increased over time. The evidence of central nervous system nucleus accumbens and caudate may be important to the unique therapeutic effect of this typical antipsychotic drug in the treatment of symptoms, especially the deficit symptoms of schizophrenia. GTE has positive impact at the alteration of dopamine metabolites and its turnover ratios by antagonizing HAL action in the nucleus accumbens that potentiate HAL-induce tVCMs and motor deficits in animals. It is suggested that GTE may not exert its antioxidative effects in support of EPS inhibition, and remarkably potentiate HAL-induced EPS instead. Conversely HAL-induced an increase in HVA/dopamine turnover ratios was persistent in the nucleus accumbens at GTE combination. That may preserve schizophrenic symptoms or sustain recurrence of schizophrenic symptoms. We suggest that patients on either HAL therapy, or suffering from schizophrenia should avoid green tea.

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