Green Tea *(Camellia sinensis)* Potentiates Haloperidol – Induced Extrapyramidal Symptoms and Decreases Dopamine Metabolism in the Dorsal Striatum of Rats

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Abstract: Schizophrenia, a psychiatric illness, has been treated with typical antipsychotic drug haloperidol (HAL). Although the treatment is associated with a high rate success but Extrapyramidal Symptoms (EPS) associated with the treatment is a serious limitation of therapy. Some studies have suggested that oxidative stress induced during the metabolism of HAL is involved in the elicitation of EPS. The components of green tea have been demonstrated to have therapeutic efficacy in reducing oxidative stress. It has been reported that flavoniods of green tea have free radical scavenging properties thus antioxidant in nature. Therefore, it can be speculated that green tea may prevent the EPS induced by haloperidol. In the present study we examine the prophylactic efficacy of Green Tea Extract (GTE) pretreatment on HAL induced EPS. Results revealed that haloperidol–induced impairment of motor coordination were greater (p<0.01) in GTE than water treated animals. The elicitation of Vacuous Chewing Movements (VCMs) was also greater and the levels of dopamine (DA) and its metabolites were greater (p<0.01) in the ventral striatum and smaller in the dorsal striatum of green tea plus haloperidol than water plus haloperidol treated animals. The results suggest that significantly decreases of DA metabolism in the dorsal striatum may be involved in the elicitation of greater EPS induced by HAL in GTE treated group. Conversely an increase in DA metabolism observed in the ventral striatum may potentiate schizophrenic symptoms. We suggest that patients on haloperidol therapy should avoid green tea. The mechanism by which green tea may potentiate haloperidol - induced EPS is discussed.

Key Words: *Camellia sinensis*, haloperidol, ventral striatum, dorsal striatum, extrapyramidal symptoms, Parkinsonism, Tardive Dyskinesia.

INTRODUCTION

Schizophrenia is one of the most sever chronic psychiatric problem with 1% prevalence in total population [1-2]. The symptoms of Schizophrenia are clinically cured with haloperidol by mostly oral treatment. The treatment is associated with Extrapyramidal Symptoms (EPS), initial Parkinsonian like symptoms, and delayed adverse events are Tardive Dyskinesia, akathisia. These distressing adverse events were observed in 20 - 75% patients treated with neuroleptic. Studies on animal models showed that acute administration of haloperidol elicits Parkinsonian like symptoms which were quantified in rats by impaired motor coordination [3-7]. On the other hands rats treated chronically with high dose of haloperidol develop orofacial movements described as vacuous chewing movement (VCMs). These parameters are widely used for EPS quantification in animal models [8].

Haloperidol, the drug is known to have high affinity for dopamine D_2 receptor. It blocks D_2 receptors in the rat corpus striatum [3, 8-9] which produces oxidative stress in striatal neurons [10]. It is often suggested that oxidative stress is involved in the course and development of idiopathic EPS induced by typical neuroleptic [11-14]. It has been reported that the constituents of green tea have free radical scavenging properties that determine a neuroprotective effect against neurotoxicants and reduce distressing effects [11].

Dopamine and Serotonin interact antagonistically in the dorsal striatum (caudate) to control motor activity [4]. Ventral striatum (nucleus accumbens) is a region of brain known to be involved in emotional control. Both striatal regions are affected during haloperidol therapy [15]. The ratios of dopamine metabolites to dopamine are known indicators that depict the clear picture of the haloperidol induced adverse events. Report from our laboratory provided evidence that increase in DA metabolism is directly proportional to increase in EPS events [8].

Therefore present pilot study concerns with the effects of GTE on haloperidol induced EPS in rats. In order to understand the role of dopamine in the modulation of haloperidol -induced EPS the metabolism of dopamine in the dorsal and ventral striatum was also determined.

MATERIALS AND METHODS

Male albino Wistar young rats (150g - 200g) were housed individually. The animals were housed under standard laboratory conditions maintained on a 12-hour

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light/dark cycle with free access to food rodent diet pellets, water was allowed libidum for the first week. Animals were acclimatized to laboratory conditions before the test.

I. Treatment Schedule

Treatment period was divided into two different durations; in first phase three weeks were considered as a control treatment or (pretreatment) period and in second phase 5 weeks were considered as experimental part of study.

Commercially available, Green tea (Brand *Tapal Jasmeen*) was used to prepare in boiling water extract. Green tea leaves (1 gm) soaked and boiled in 1 liter water at the boiling temperature. Freshly prepared green tea extract was provided as a sole source of water to the treated groups. After 3 weeks of pretreatment with GTE, the twelve animals were treated with Haloperidol (*Serenace; Searl*) 1mg/Kg/ day. Using feeding tube both groups of animals was provided haloperidol orally besides continuous oral fluids (GTE /water) intake for 5 weeks in this study cohort.

II. Behavioral Experiments

All behavioral experiment was conducted at room temperature, in noise protected environment. Behavioral assessments were repeated every 6th day of a week in a balanced design.

III. Motor Function Tests

1. (A) Monitoring Motor Coordination

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

2. (B) Quantification of Orofacial Dyskinesia

According to revised model of Vacuous Chewing Movements (VCMs) described [16,17] earlier, we reduced methodological discrepancies to utilized old rats. We also fixed cage sizes that help to maintain stress less condition as it is well known that VCMs are sensitive to stressful handling situations and more likely diminished in count during stress. VCM is an analogous model of orofacial dyskinesia, characterized by purposeless spontaneous opening of mouth with or without tongue protrusion [18]. The incidence of VCMs was monitored both live and with video records after 3rd week of oral haloperidol administration in both GTE and water treated groups of rats.

Animals were placed individually in a rectangular Prespex activity cage (26/26/26 cm) with a sawdust-covered floor and allowed to equate within observation cage for a period of 15 min. Orofacial dyskinesias were quantified during 10-min observation period. Each chewing episode was scored as "one". A chewing period consists of distinct bursts of three of about five masticatory movements and lasts for 2-5 seconds. Masticatory movements were referred to as single mouth openings in the vertical plane not directed toward physical material. Each rat was constantly observed by an observer blind to the treatment. Orofacial dyskinesia and motor coordination of both groups were monitored weekly at 9:00-11:00 am in a balanced design.

IV. Dissection of Striatum

At the end of the experiment animals were sacrificed and the brains were removed immediately. The decapitation was done after 8 weeks from 8 am – 10 am. The dissection procedure of the brain was essentially same as described before [4]. 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 both dorsal and ventral regions of the striatum. The regions were isolated and identified according to the rat brain atlas [19]. Samples were obtained stored at -70°C for neurochemical analysis of dopamine and its metabolites by High Performance Liquid Chromatography with Electrochemical (HPLC- EC) detection system [4].

V. Brain Regional Analysis by HPLC- EC Technique

A 5 µ shim-pack XR- ODS Shimadzu separation column of 4.5 mm internal diameter & 15 cm length was used. The mobile phase was 0.1 M sodium phosphate buffer (pH 2.9) containing 14% HPLC grade methanol, 0.023% OSS, 0.005% EDTA. Electrochemical detection was done at an operating potential of 0.8 V (glassy carbon electrode Vs Ag/AgCl reference electrode) [4].

VI. Statistical Analysis

Data presented as means ± SD. Statistical analysis were performed using two way- Analysis of Variance (ANOVA) (repeated measures design) for each behavioral assessment; with SPSS 11.5 software. A value of alpha was chosen less than 0.05 (Figs. **1-3**). Neurochemical data were analyzed by Student's t- test (Figs. **4-5**).

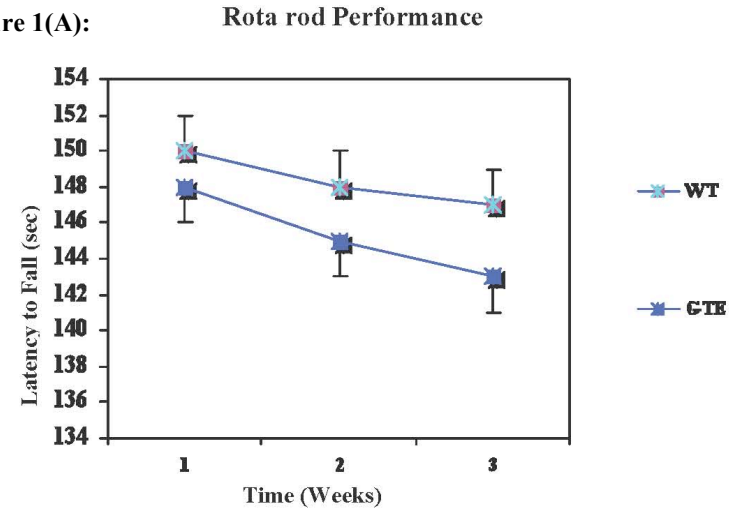
RESULTS

Fig. (**1A**) shows the effects of WT and GTE intake on motor coordination. Data analyzed by two-way ANOVA at repeated measure design. Motor coordination during first three weeks showed df =1, 10, week (F=1.9 $p > 0.05$) and GTE (F=5.9, $p > 0.05$).

Fig. (**1B**) shows haloperidol induced motor impairment in WT and GTE drinking groups during six weeks of treatment. Data analysis by two-way ANOVA repeated measure design showed significant effects of (df =7, 40,) haloperidol group (F=50.9, p<0.01) GTE + HAL (F = 5.1, p < 0.01). Data performed on *post- hoc* analysis showed haloperidol induced- activity scores were significantly smaller (p <0.01) in GTE than WT drinking group of animals. Green tea drinking Haloperidol treated animals performed 39% worse performance in repeated session [Weekly effect] (p<0.01). Haloperidol induced- motor impairment was observed in water drinking animals showed tolerance at 4th -6th week, that marked increases in last three weeks, however green tea drinking group presented persistent in effects of haloperidol induced impairment showed markedly increased in last three weeks. Green tea drinking animals showed no tolerance to haloperidol induced effects during 4th (p<0.01) to 6th week (p<0.01) of haloperidol treatment. *Post hoc* analysis showed that the treatment with green tea potentiates haloperidol induced motor deficits (19% - 23% per week) observed on rota-rod performance, where GTE interaction with haloperidol was observed significant (p<0.01).

Post hoc analysis showed haloperidol induced motor impairment was greater in green tea drinking rats as compared to water drinking rats. Tolerance in motor deficits produced during 7th (p < 0.01) and 8th week (p< 0.01) of Haloperidol treatment. Pretreated with green tea and later

Figure 1(A):



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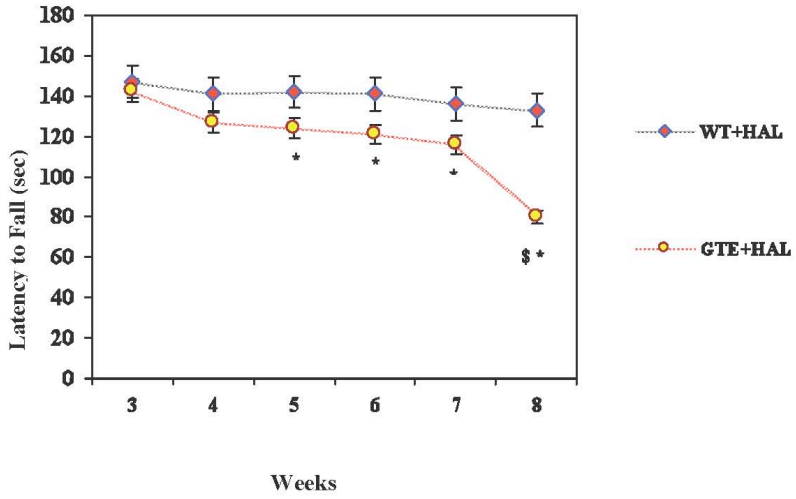

Fig. (1), Time course (1st – 3rd weeks) of water (WT) and green tea (GTE) effects of on rota rod performance.

Differences were observed by two-way ANOVA (repeated measure design). Motor coordination observed was not significant (A).

(B). Haloperidol induced impairment of motor coordination in GTE and WT treated rats for fourth- eight weeks. Values are means ± SD (n = 6). Significant differences by Bonferoni- test *P < 0.01 following two-way ANOVA (repeated measure design). Potent impairment in GTE plus HAL group was observed. HAL+ WT shown some tolerance developed on 7and 8 weeks, but in GTE group showed more sever effects and no tolerance "$" was observed to the HAL effect.

green tea plus haloperidol potentiates motor deficits observed on rota-rod performance.

Fig. (**2**) shows the effects of GTE on haloperidol induced VCMs in rats. Two-way ANOVA (repeated measure design) showed the significant effects (df 1, 15) haloperidol (F = 53.0, $p<0.01$) and GTE (F=33.9, $p<0.01$) observed during 4-6 weeks. *Post hoc* analysis reveals significant ($p<0.01$) increase of VCMs in GTE drinking animals.

Fig. (**3**) shows the effects of haloperidol on DA metabolism in green tea and water drinking rats. The DA levels in dorsal striatum and its metabolites DOPAC, HVA in the WT+ HAL and GTE + HAL treated groups showed a significant decrease of dopamine ($p<0.01$) in the GTE+ HAL treated rats compared to WT+ HAL group.

Dopamine metabolism was recorded significantly decreased and found in haloperidol treated GTE drinking group of animals. Concentration of DOPAC and HVA in the dorsal striatum was found less in GTE+ HAL treated group.

Fig. (**4**) shows the effects of GTE and haloperidol on DA and its metabolites DOPAC and HVA levels the ventral striatum.

Analysis revealed significantly ($p<0.01$) higher levels of DA metabolites, HAV and DOPAC levels were observed greater in haloperidol treated green tea drinking group, compare to water drinking group of animals.

DISCUSSION

In order to establish possible prophylactic effects of GTE *in vivo,* the EPS model was employed. Rats were pretreated with GTE before haloperidol treatment. During three weeks of GTE intake, GTE was not found a sole factor that may significantly alter the motor coordination on rota-rod. Control study duration was taken three weeks (Fig. **1A**), however it will be interesting to monitor long term green tea effects in future studies.

Present findings showed increase in haloperidol induced Parkinsonian like locomotor deficit in both groups monitoring on rota rod. Major difference in both groups was observed on fifth to eight weeks of treatment. However it has reported that haloperidol treatment produce tolerance in motor deficits on third week [8]. HAL+ WT group showed tolerance on third week and no tolerance to treatment was observed in GTE+ HAL treated group of animals. Hence it was contradictory to this hypothesis that GTE may reduce the haloperidol side effects. On the other hand halo-peridolalter the antioxidative properties of GTE and exert its effects a in manner of converting into oxidative stress that leads to sever EPS. Significantly seventh and eighth weeks showed larger differences (Fig. **1B**).

It is well documented that when the model of Parkinson diseases (PD) was employed, GTE alone has no significant effect recorded in mice [21]. However it was interesting to note that 5 mg/kg GTE caused an elevation in DA metabolism that significantly amplify MPTP (*N-methyl - 4Phenyl-1, 2, 3, 6-tetrahydropyradine*) effects in PD model [20]. In the present study design 1mg/liter extract of GTE potentiates the haloperidol induced Parkinsonian like symptoms that were monitor on rota rod. It has been shown that neuroleptic elicited high altitude of dopaminergic metabolism ultimately produced reactive oxygen species (ROS) and subsequent neurodegeneration [21] hence it may be possible that haloperidol bound the GTE free radical scavenging properties and had potential to make green tea a pro-oxidative agent. Studies showed a direct relationship that document in other studies that haloperidol administration enhances DA metabolism and elicits EPS [4-6].

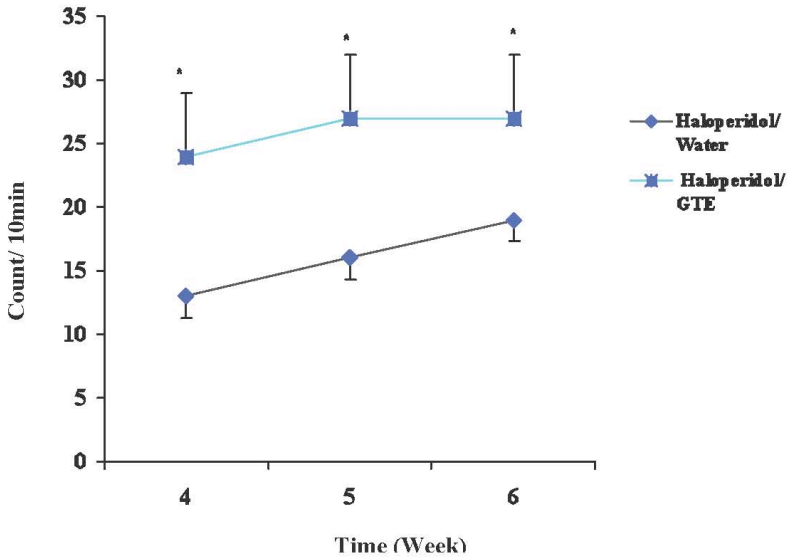

Fig. (2). Time course (6^{th} - 8^{th} week) of the Haloperidol induced Vacuous Chewing Movements (VCMs) observe after 3^{rd} week of HAL administration significantly *P < 0.01 potent in GTE treated group. Significance observed following repeated measures ANOVA. Values are means ± SD.

Our data shows that haloperidol - induced increase of DA metabolism that was greater in GTE treated animals. Combination of GTE with haloperidol potentiates DA metabolism that might lead on peak after 3rd week of the treatment, it can be postulated that DA synthesis to DA metabolism ratio augmented motor impairment.

Haleem *et al.* reported that the VCMs increase in a dependent manners [8]. This study validate the report that haloperidol treated both groups showed high rate of VCMs that increased over time. Parkinsonian like symptoms were greater that may correlate this motor deficit VCMs appeared simultaneously at third week of haloperidol treatment.

Higher differential rates in deficits were recorded in seventh to eighth weeks of GTE + haloperidol treatment that elicited greater VCMs that greater than WT + HAL treated group. It has been reported that apoptosis in the striatum elicit VCMs and it can be postulated that green tea enhance the neuronal death and it depict higher VCMs in the GTE+ HAL treated groups [22].

Anatomical site of haloperidol action, striatum has a known involvement of motor nerves. Clinically effective doses of haloperidol occupy 60% - 80% of brain dopamine D_2 type receptors, as measured by Positron Emission Tomography (PET) in the human striatum [23-24]. Occupancy of striatal DA receptors cause haloperidol elicited threshold in motor deficits; acute Parkinsonism and chronic treatment induced Tardive dyskinesia. Striatum receives input from dopaminergic neurons [25]. Supersensitivity of the post synaptic D_2 type receptors contribute in expression of the syndrome [5].

Fig. (**4**) show that GTE group has low level of dopamine with high metabolites in ventral striatum (nucleus accumbens) and less DA in dorsal striatum (CPu region). The negative symptom complex of schizophrenia can be associated with low dopamine and high metabolites. It has been reported that increased DA metabolism found in post-mortem schizophrenic patient. Low level of DA in schizophrenic patients and higher its metabolites were found in CPu that may induce hallucination [26, 27] could be

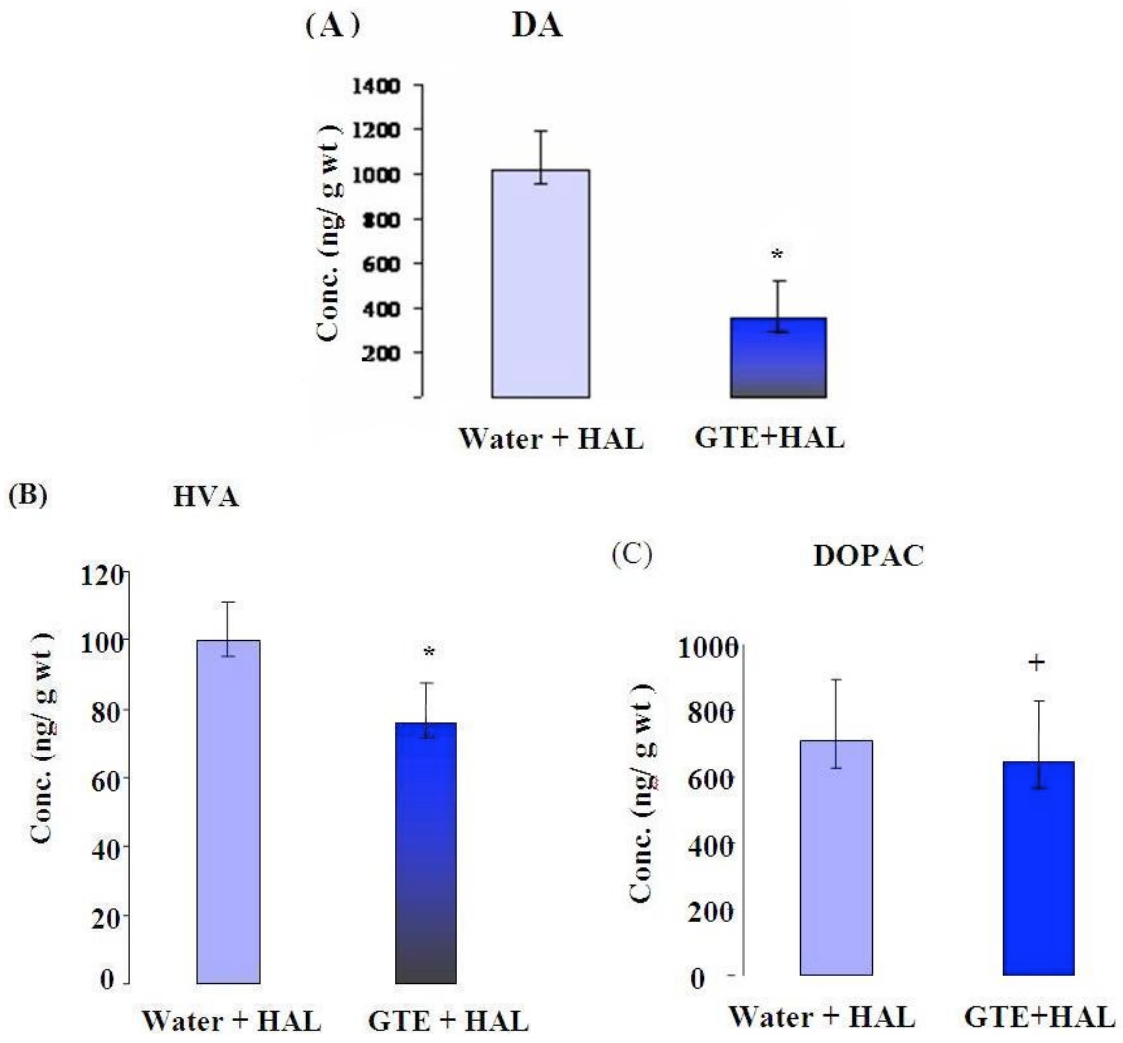

Fig. (3). (ABC) Dopamine metabolism in the dorsal striatum of WT+ HAL and GTE + HAL treated animals. Values are means ± SD. After the last administration of HAL significant differences was observed by student t- test *P< 0.01 $^{+}$ P = 0. 5.

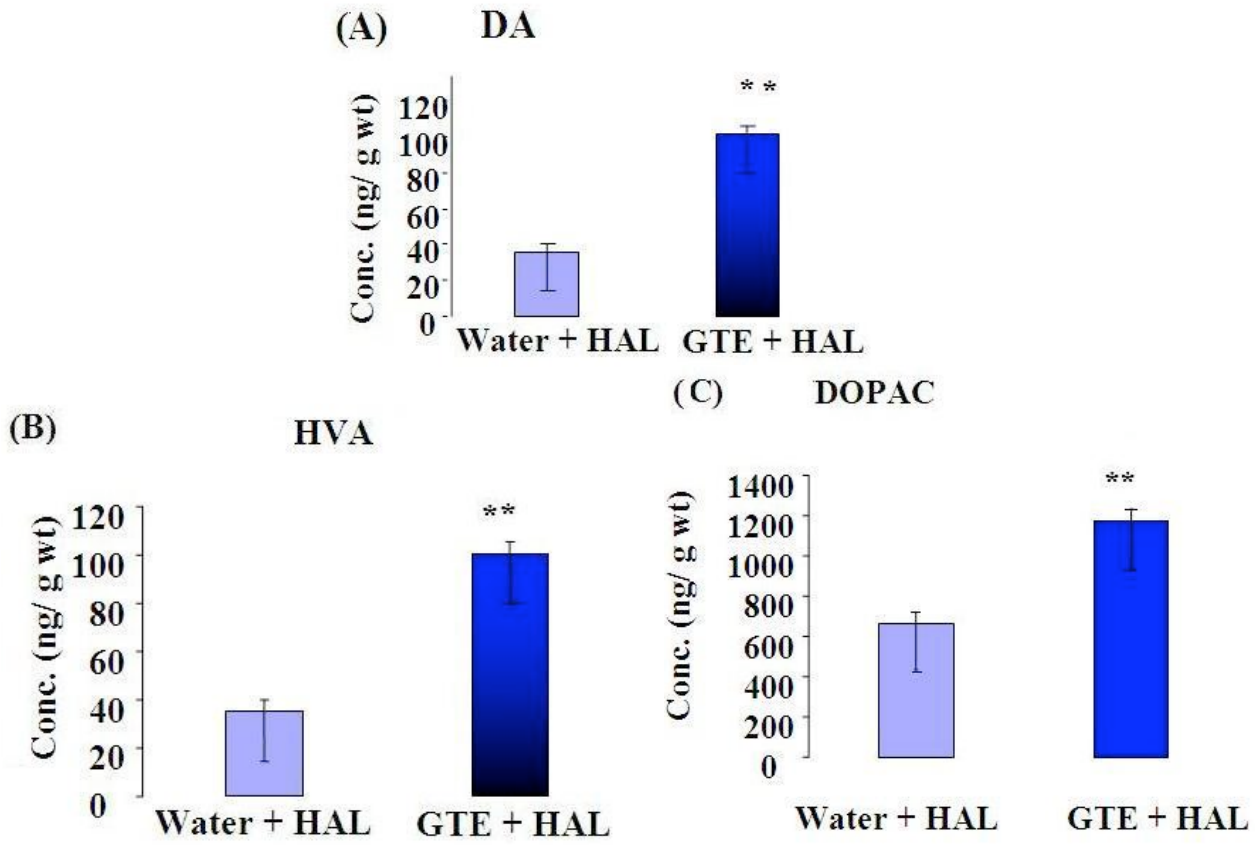

Fig. (4). (ABC) Dopamine metabolism in the ventral striatum of WT+ HAL and GTE + HAL treated animals. Values are means ± SD. After the last administration of HAL significant differences was observed by student t- test * $P < 0.05$, **$P < 0.01$.

predicted in GTE treatment. Therefore it is more likely that GTE may exaggerate negative deficits complex and relapse hallucination in schizophrenic patient. Motor impairment can be correlated with DA, DOPAC and HVA concentrations in CPu. DOPAC is often considered an index of intraneuronal DA catabolism [28] . Studies suggested that acute administration of haloperidol increased DOPAC [29] and HVA [30] levels, in a dose dependent manners. These symptoms last at greater magnitude and longer duration in the brain striatum [31].

An increase in sensitivity of DA D_2 receptors has been reported fowling long term administration of Haloperidol. D_2 receptors are intensely distributed both presynaptically and postsynaptically in striatal dopaminergic neurons [29, 30] and it is more likely that GTE has presynaptic and postsynaptic role in processing and integrating incoming input in nucleus accumbens (AcN) but not towards caudate putamen (CPu) of the basal ganglion network. It will be interesting to find out GTE role in pre and post synaptic events.

Our results (Figs. **3** and **4**) showed the effect of haloperidol on DOAPC levels tend to induce large fraction of DA catabolism occur intraneuronally in the ventral striatum. On the other hand an increase level of HVA was also observed in AcN but not in the CPu at repeated dose haloperidol and GTE intake in rats. That is explicable in terms of increase DA release and obstructs respectively in these brain regions [32]. However the effects on ventral striatum are known to be involved in the emotional control. Haloperidol produce effects on the ventral striatum and substantia Nigra [34], impression that GTE+ HAL enhances the DA and its metabolites levels in ventral striatum may have some impact on mood modification.

Recent findings have proven that motor deficits can be reduced with L- arginine (a NO donor) in dose dependent manners and demonstrated as a possible therapeutic option to reverse VCMs and increased dopamine levels [10]. It is also well documented that GTE polyphenols are a good scavenger of NO ions [32], Epigalocatechin-3-gallate (EGCG) has proven selective inhibitor of inducible nitric oxide synthase (iNOS) in human chondrocytes [33]. Polyphenols of GTE have been shown to decrease plasma nitrite levels significantly via reversed L-arginine effects, inhibited NO production favoring antinociceptive effect in rodent [34 -35] and NO may enhance oxidative stress in entire dorsal striatum of the brain intensify motor impairment .

Green Tea have also associated with neurological events like seizures [36], confusion and insomnia [36] proven by clinical trials. Effects on neuromuscular junctions induce fatigue [37] also reported severe post game fatigue in players and sportsmen [38], convulsion [39] as well as paralytic

effects observed in modifying skeletomotor function, affecting motor nerve terminals and inhibited acetylcholine release these effects observed at frequent intake of green tea [39] and components are proven cytotoxic [40-41] component at lack of antioxidative properties [41] nevertheless it is quite often use as social drink to enhance performance and mood [44]. Aside from water the green tea has per capita world wide consumption approximately 0.12 L per year [38]. Green tea as the important drink is generally used because of its good commercial reputation moreover there is a need to monitor its reported side effects. This study does not specify green tea component that may interact with haloperidol and triggers DA metabolism. Conversely it will be interesting to define component interaction with haloperidol. There is further need to monitor a long term GTE effects in future study design. Though more comprehensive designed study is currently in the pipeline however aforementioned studies and our current measure of adverse events are certainly showed a level of importance. Although there is room to bring this study at clinical relevance however at this stage, green tea interaction with haloperidol is setting up a precautionary measure.

CONCLUSIONS

This study shows that pre and co- treatment of GTE may potentiat acute parkinsonian like effects of haloperidol that increase over time. GTE has positive impact on neurodegenerative mechanism, VCMs induction and motor coordination during haloperidol treatment. It is suggested that GTE has no prophylactic effects to inhibit EPS, but remarkably potentiat EPS by haloperidol and more likely with other antipsychotic drugs. Conversely an increase in DA metabolism observed in ventral striatum may potentiate schizophrenic symptoms. It is suggested that patients on either haloperidol therapy, or suffering schizophrenia should avoid green tea.

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