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Original Article

Chlorogenic acid, a polyphenolic compound, ameliorated haloperidol-induced orofacial dyskinesia and catalepsy in rats

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ABSTRACT

Chlorogenic acid, a polyphenolic bioactive compound, has been reported to possess protective effect against reserpine-induced orofacial dyskinesia in rats, but the effect of chlorogenic acid on haloperidol-induced orofacial dyskinesia has not been reported in the literature. Therefore, the aim of the current study was to investigate the effect of chlorogenic acid on haloperidol-induced orofacial dyskinesia and catalepsy in Wistar male albino rats. Haloperidol (1 mg/kg) was injected by intraperitoneal route to rats for 21 consecutive days to induce orofacial dyskinesia and catalepsy. Chlorogenic acid (10, 20 and 40 mg/kg) was administered orally 45 min prior to haloperidol administration to separate groups of rats for 21 consecutive days. Haloperidol significantly increased vacuous chewing movements (VCMs) and tongue protrusions in rats, indicating induction of orofacial dyskinesia. It also increased catalepsy time and decreased locomotor activity of rats. Haloperidol-induced VCMs, tongue protrusions, catalepsy and hypolocomotion were significantly ameliorated by chlorogenic acid. Haloperidol significantly decreased brain dopamine and serotonin levels which were significantly restored by chlorogenic acid administration. The results of present study indicated significant protective effect of chlorogenic acid against haloperidol-induced orofacial dyskinesia and catalepsy, probably through increase in brain dopamine and serotonin levels.

Key Words: Chlorogenic acid; Catalepsy; Tardive dyskinesia; Vacuous chewing movements
Abbreviations: VCMs – Vacuous chewing movements; *po* – per oral; *ip* – intraperitoneal

INTRODUCTION

Tardive dyskinesia is a late onset adverse effect caused after prolonged treatment with classical or typical antipsychotic drugs, such as haloperidol [1]. It is characterized by increased vacuous chewing movements (VCMs), tongue protrusions and cataleptic behavior [2]. Tardive dyskinesia may be caused due to dopamine super sensitivity and oxidative stress in basal ganglia and striatum of the brain [3, 4]. Classical neuroleptics remain bound to dopamine D_2 receptors which cause hypersensitization of dopamine D_2 receptors. This results in increased density of dopamine D_2 receptors and increased uptake of dopamine, which in turn, disturbs the dopamine levels in brain regions responsible for motor disturbances [5]. Haloperidol is metabolized by an oxidase, which generates large quantities of oxyradicals and a toxic pyridinium-like metabolite [6] and induces oxidative stress [7]. Chronic blockade of dopamine D_2 receptors by neuroleptics in nigrostriatal neurones of the brain, leads to increase in dopamine turnover in basal ganglia, and this may result in overproduction of free radicals such as dopamine quinone and hydrogen peroxide. Tardive dyskinesia is caused due to a neurotoxic effect of these free radicals [2]. Vitamin E and melatonin have antioxidant properties and have been reported to reverse the symptoms of tardive dyskinesia, in clinical studies [8, 9]. Thus, substances possessing antioxidant activities can be explored for prevention and treatment of tardive dyskinesia.

Chlorogenic acid, a polyphenolic compound, is present in many fruits and vegetables [10]. Fruit sources include cherries [11], plums [12], berries [13], and tomatoes [14]. The common vegetable source includes potatoes [15]. Chlorogenic acid is also found in beverages like tea [16] and coffee [17]. This bioactive compound has been reported to possess neuroregenerative [18], nootropic [19], anxiolytic and antioxidant [20], anti-inflammatory and analgesic [21], anti-diabetic and antilipidemic

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[22], antihypertensive [23] and antitumor [24] activities. Recently, we have reported protective effect of chlorogenic acid against reserpine-induced orofacial dyskinesia in rats [25]. However, the effect of chlorogenic acid on haloperidol-induced orofacial dyskinesia has not been reported in the literature. So, the aim of the current study was to investigate the effect of chlorogenic acid on haloperidol-induced orofacial dyskinesia and catalepsy in rats by employing behavioral models and carrying out biochemical estimations in brain.

MATERIALS AND METHODS

Experimental animals: Animals used for the present study were Wistar male albino rats, weighing 100-150 g and 2-3 months age. The animals were purchased from Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Since estrogens have been reported to possess neuroprotective activity [26], so only male rats were used in the study. The animals were housed under standard laboratory conditions with 12 h light-dark cycle. They had free access to food and water, but were deprived of food 1 h before and 1 h after drug administration, so as to maximize absorption of the drugs. The animals were acclimatized to laboratory conditions before start of the experiments, which were carried out between 9:00 and 16:00 h. The study was approved by Institutional Animal Ethics Committee in its 26th meeting held on 9th May, 2014. The animals were properly handled and used for the experiments as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate change (Animal welfare division), Government of India, New Delhi.

Drugs and chemicals: Haloperidol (Serenace®, RPG Life Sciences Ltd, Mumbai, India), chlorogenic acid and dopamine hydrochloride (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), serotonin creatinine sulfate monohydrate (Sigma-Aldrich, USA) were employed in this study. The remaining chemicals used were of analytical grade. Haloperidol injection was diluted with water for injection. Chlorogenic acid was dissolved in normal saline (0.9% w/v sodium chloride). The volume of drug administration was 0.5 ml per 100 g of body weight of rats.

Selection of doses: Haloperidol was injected at a dose of 1 mg/kg, *ip* [27] and chlorogenic acid was administered orally at doses 10, 20 and 40 mg/kg [20].

Induction of orofacial dyskinesia and catalepsy: Haloperidol was administered for 21 consecutive days to induce orofacial dyskinesia and catalepsy. VCMs, tongue protrusions, catalepsy and locomotor activity were measured weekly, but last behavioral assessments were done 24 h after the last dose of haloperidol, that is on 22nd day [27].

Measurement of VCMs and tongue protrusions: Rats were placed individually in a small cage and acclimatized to it for 10 min before behavioral assessments. VCMs and tongue protrusions were measured as reported earlier [28] and followed in our laboratory [29]. The observer was blind to the drug treatments.

Measurement of catalepsy: The catalepsy was recorded using 3 and 9 cm wooden blocks as per the procedure mentioned [30] and followed in our laboratory [29].

Measurement of locomotor activity: The horizontal locomotor activity of each animal was recorded for a period of 10 min [30] using photoactometer (INCO, Ambala, India).

Experimental protocol

The animals were grouped (n=6 each) as follows:

Group I: Vehicle (0.9% w/v sodium chloride) was administered for 21 successive days.

Group II: Haloperidol (1 mg/kg, *ip*) was administered for 21 successive days.

Groups III, IV and V: Chlorogenic acid (10, 20, 40 mg/kg respectively, *po*) was administered 45 min prior to haloperidol administration for 21 successive days.

VCMs, tongue protrusions, catalepsy and locomotor activity were recorded weekly i.e. on 7th and 14th day before administration of chlorogenic acid and on 22nd day (24 h after the last dose of haloperidol).

Biochemical estimations

After behavioural testing on 22nd day, rats were sacrificed by cervical dislocation and forebrain [28] was dissected out. Dopamine and serotonin levels were estimated by the method of Schlumpf et al. [31] and as followed in our laboratory [29].

Statistical analysis

The results were mentioned as mean ± SEM. The data were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison test using Graph Pad Instat. $p < 0.05$ was considered as statistically significant.

RESULTS

Effect on VCMs and tongue protrusions: Haloperidol (1 mg/kg, *ip*) significantly increased VCMs and tongue protrusions on 7th, 14th and 22nd days as compared to respective vehicle treated control. Chlorogenic acid (10, 20 and 40 mg/kg, *po*) significantly reversed haloperidol-induced VCMs and tongue protrusions on 7th, 14th and 22nd days as compared to haloperidol treated group. The effect of chlorogenic acid on reduction of VCMs was same ($p<0.01$) at all the 3 doses on 7th day; dose-dependent ($p<0.05$, $p<0.01$ and $p<0.001$ respectively) on 14th day and same ($p<0.001$) at 20 and 40 mg/kg doses on 22nd day. But on the other hand, effect of chlorogenic acid on reduction of tongue protrusions was same ($p<0.05$) at all the 3 doses on 7th, 14th and 22nd days (Fig. 1 and 2).

Effect on catalepsy: Haloperidol (1 mg/kg, *ip*) significantly increased cataleptic scores of rats on day 7, 14 and 22 as compared to respective vehicle treated control. Chlorogenic acid (20 and 40 mg/kg, *po*) administered for 21 successive days significantly reversed haloperidol-induced catalepsy on 7th, 14th and 22nd days. But the lowest dose (10 mg/kg) of chlorogenic acid significantly reversed haloperidol-induced catalepsy on 14th and 22nd days. The effect of chlorogenic acid on reduction of catalepsy was same ($p<0.001$) at 20 and 40 mg/kg doses on 7th and 14th days; but the middle dose (20 mg/kg) of chlorogenic acid was more effective ($p<0.01$) than lowest and highest doses on 22nd day (Fig. 3).

Effect on locomotor activity: Haloperidol (1 mg/kg, *ip*) significantly decreased locomotor activity on 7th, 14th and 22nd days as compared to vehicle treated control. Chlorogenic acid (10, 20 and 40 mg/kg, *po*) administered for 21 successive days significantly reversed haloperidol-induced hypolocomotion as compared to haloperidol treated group. The effect of chlorogenic acid on locomotor activity was same ($p<0.001$) at all the 3 doses on 7th day; best ($p<0.001$) at the highest dose (40 mg/kg) on 14th and 22nd days (Fig. 4).

Effect on brain dopamine and serotonin levels: Haloperidol (1 mg/kg, *ip*) significantly decreased brain dopamine and serotonin levels as compared to vehicle treated control. Chlorogenic acid significantly reversed haloperidol-induced decrease in brain dopamine and serotonin levels. The effect of chlorogenic acid on reduction of brain dopamine and serotonin levels was best ($p<0.001$) at 20 and 40 mg/kg doses (Table 1).

DISCUSSION

The results of the present study indicated significant amelioration of haloperidol-induced orofacial dyskinesia and catalepsy in rats by chlorogenic acid administered for 21 successive days. This is the first study showing protective effect of chlorogenic against haloperidol-induced orofacial dyskinesia and catalepsy. Haloperidol-induced orofacial dyskinesia is widely accepted model to evaluate effect of drugs on tardive dyskinesia [27, 28]. In the current study, haloperidol significantly increased VCMs and tongue protrusions in rats, indicating induction of orofacial dyskinesia. Chlorogenic acid significantly reversed haloperidol-induced orofacial dyskinesia in rats as indicated by decrease in VCMs and tongue protrusions in rats as compared to haloperidol treated animals. Haloperidol blocks dopamine D_2 receptors and leads to increase in dopamine turnover. This may result in increased hydrogen peroxide production and other toxic metabolites of dopamine, which results in increased oxidative stress [2, 4]. Reported antioxidant activity [20, 32] of chlorogenic acid might also be responsible for its protective effect against haloperidol-induced orofacial dyskinesia. Haloperidol treatment significantly induced catalepsy in rats, which is also supported by the literature [33]. Chlorogenic acid significantly decreased haloperidol-induced catalepsy in rats. There was significant decrease in locomotor activity of rats by haloperidol, which might be due to dopamine supersensitivity [27]. Chlorogenic acid significantly reversed haloperidol-induced hypolocomotion.

In the current study, haloperidol administered for 21 consecutive days significantly decreased brain dopamine and serotonin levels, which is also supported by the literature [27, 34]. Prolonged haloperidol treatment may result in dopamine supersensitivity which may increase the number of dormant receptors. This leads to decrease in dopamine levels in extracellular spaces. Metabolites of haloperidol get accumulated in brain after its chronic administration and these may lead to the death of dopaminergic neurons. Formation of quinone species is also responsible for decrease in dopamine levels [34]. The serotonergic system might be involved in inhibitory modulation of dopaminergic neurons activity [35]. Haloperidol also decreased brain serotonin levels, which is also supported by the literature [27]. Chlorogenic acid significantly restored haloperidol-induced decrease in brain dopamine and serotonin levels.

In conclusion, chlorogenic acid administered for 21 successive days significantly ameliorated haloperidol-induced orofacial dyskinesia and catalepsy in rats possibly though increase in brain dopamine and serotonin levels; and also through alleviation of oxidative stress. Therefore, chlorogenic acid may be explored further for its potential in the management of neuroleptic-induced tardive dyskinesia and Parkinsonism.

Table 1: Effect of chlorogenic acid on haloperidol-induced changes in brain dopamine and serotonin levels

Drug Treatment (mg/kg)	Dopamine levels (pg/mg) (mean ± SEM)	Serotonin levels (pg/mg) (mean ± SEM)
Vehicle (0.9% w/v saline)	957.98 ± 78.63	1501.64 ± 125.69
Haloperidol (1)	558.87 ± 40.12 [a]	851.48 ± 52.39 [a]
Chlorogenic acid (10) + Haloperidol (1)	939.82 ± 68.15 [b]	1298.75 ± 143.81 [b]
Chlorogenic acid (20) + Haloperidol (1)	1210.43 ± 117.78 [c]	1912.30 ± 78.45 [c]
Chlorogenic acid (40) + Haloperidol (1)	1247.58 ± 78.16 [c]	1573.2 ± 111.34 [c]
F (4, 25)	11.726	13.201
p value	<0.05	<0.05

n= 6 each group. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. [a] $p<0.05$ as compared to vehicle treated control, [b] $p<0.05$ and [c] $p<0.001$ as compared to haloperidol treated group.

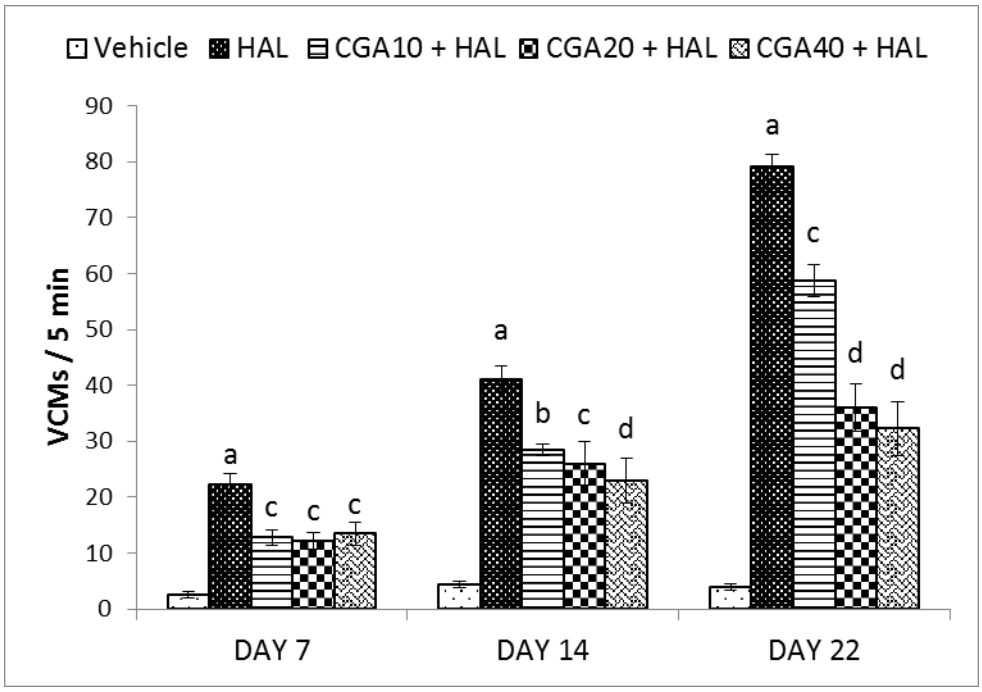

Figure 1: Effect of chlorogenic acid on haloperidol-induced vacuous chewing movements in rats

n= 6 each group. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. *F* (4, 25), Day 7= 19.79; Day 14= 23.61; Day 22= 75.54. $p< 0.05$. [a] $p<0.001$, as compared to vehicle treated control, [b] $p<0.05$, [c] $p<0.01$ and [d] $p<0.001$ respectively as compared to haloperidol treated group. HAL stands for haloperidol; CGA stands for chlorogenic acid.

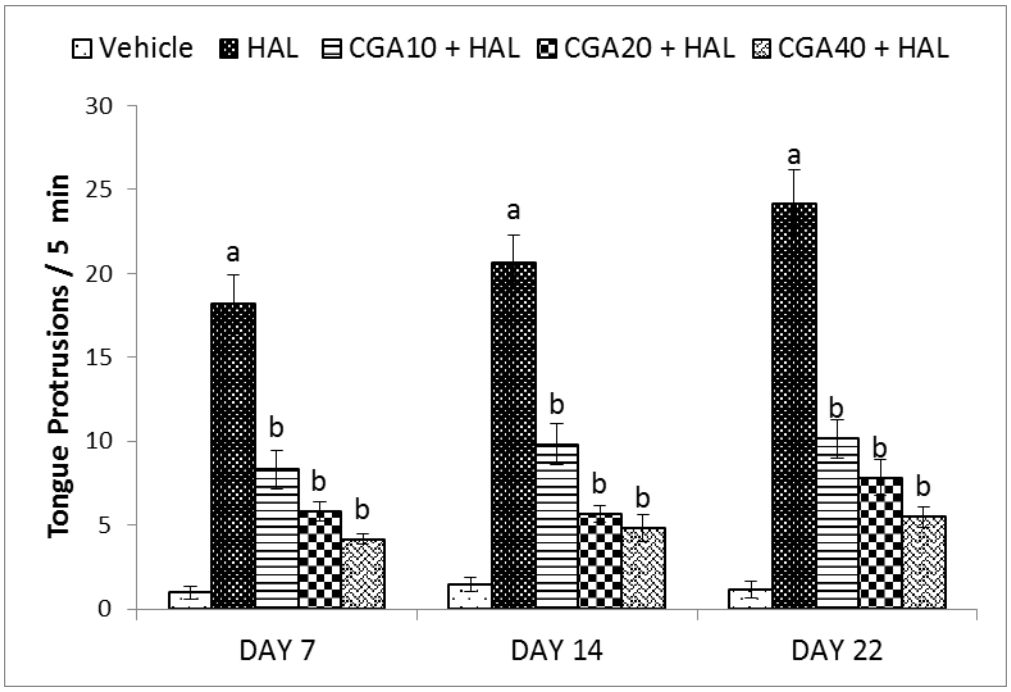

Figure 2: Effect of chlorogenic acid on haloperidol-induced tongue protrusions in rats

n= 6 each group. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. *F* (4, 25), Day 7 = 43.28; Day 14 = 53.01; Day 22 = 54.49. $p< 0.05$. [a] $p<0.001$ as compared to vehicle treated control, [b] $p<0.001$ as compared to haloperidol treated group. HAL stands for haloperidol; CGA stands for chlorogenic acid.

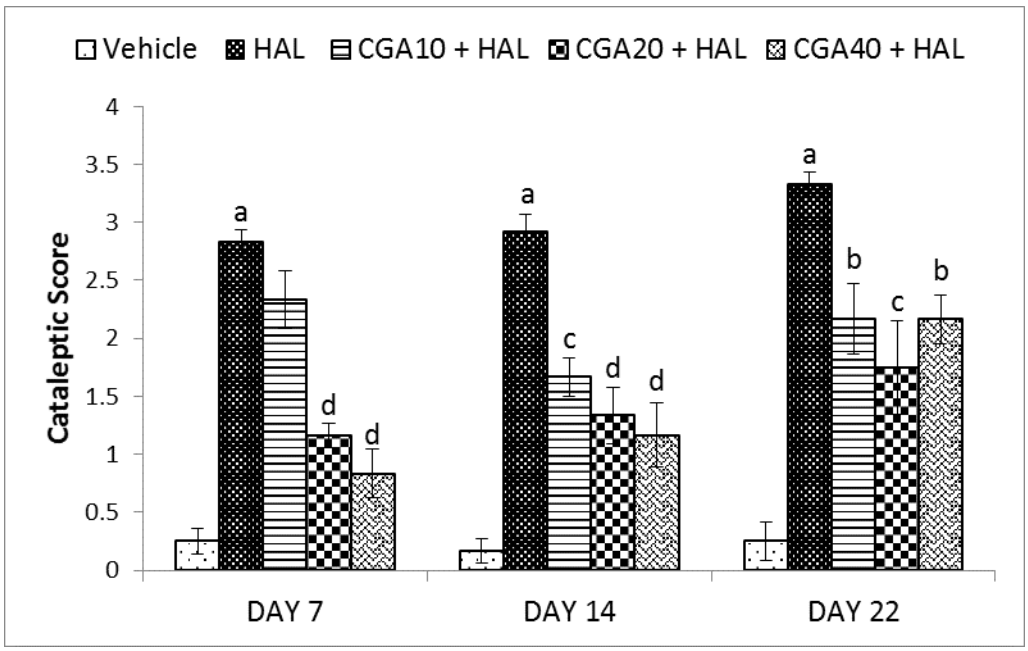

Figure 3: Effect of chlorogenic acid on haloperidol-induced catalepsy in rats

n= 6 each group. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. *F* (4, 25), Day 7= 40.89; Day 14= 24.45; Day 22= 18.06. $p< 0.05$. [a] $p<0.001$ as compared to vehicle treated control, [b] $p<0.05$, [c] $p<0.01$and [d] $p<0.001$ as compared to haloperidol treated group. HAL stands for haloperidol; CGA stands for chlorogenic acid.

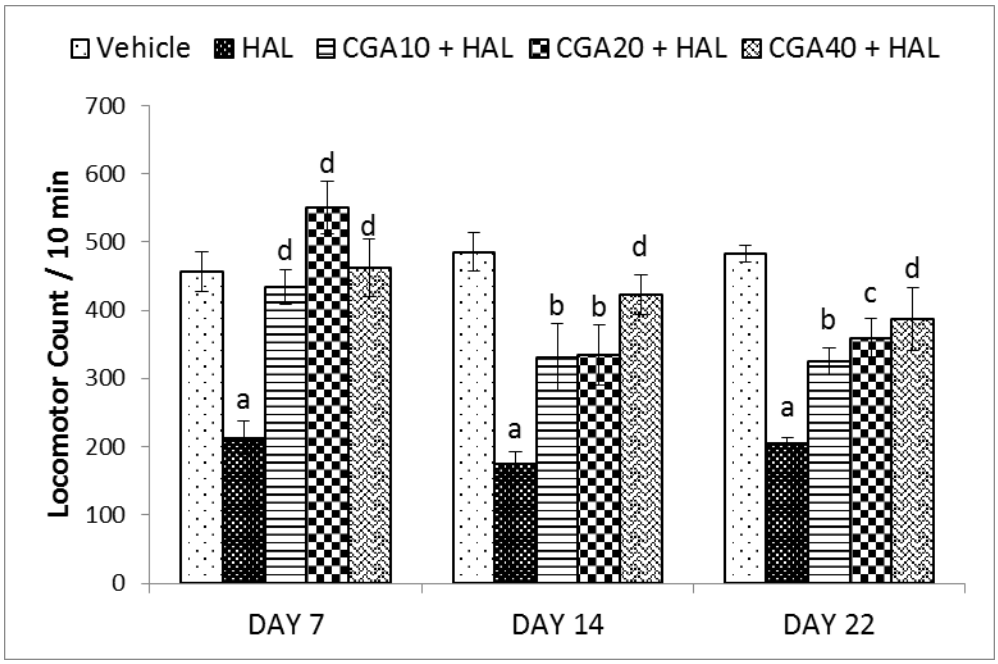

Figure 4: Effect of chlorogenic acid on locomotor activity of rats

n= 6 each group. Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test. *F* (4, 25), Day 7= 14.83; Day 14= 10.77; Day 22= 14.335. $p< 0.05$. [a] $p<0.001$ as compared to vehicle treated control, [b] $p<0.05$, [c] $p<0.01$ and [d] $p<0.001$ as compared to haloperidol treated group. HAL stands for haloperidol; CGA stands for chlorogenic acid.

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