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Research Article
Reversal of Reserpine-induced Orofacial Dyskinesia by Chlorogenic Acid in Rats

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Abstract

Background and Objectives: Chlorogenic acid, a natural polyphenolic compound has been reported to possess neuroprotective and antioxidant activities, but the effect of chlorogenic acid on orofacial dyskinesia has not been explored till date, so the aim of the present study was to investigate the effect of chlorogenic acid on reserpine-induced orofacial dyskinesia in Wistar male albino rats. **Methodology:** Reserpine (1 mg kg^{-1}) was injected by intraperitoneal route for 3 alternate days (day 1, 3 and 5) to induce orofacial dyskinesia in rats. Chlorogenic acid (10, 20 and 40 mg kg^{-1}, p.o.) was administered from 6-20th day (for 15 successive days) to separate groups of rats pretreated with reserpine. **Results:** Administration of reserpine for 3 alternate days significantly increased vacuous chewing movements and also caused hypolocomotion in rats. Treatment with chlorogenic acid (10, 20 and 40 mg kg^{-1}) significantly ameliorated reserpine-induced vacuous chewing movements and hypolocomotion. Reserpine significantly increased lipid peroxidation and decreased the levels of reduced glutathione and activities of catalase and superoxide dismutase in rat brain. Chlorogenic acid significantly reversed reserpine-induced oxidative stress, as indicated by decrease in lipid peroxidation and increase in reduced glutathione levels, catalase and superoxide dismutase activities. **Conclusion:** Chlorogenic acid showed significant protective effect against reserpine-induced orofacial dyskinesia, probably through amelioration of oxidative stress in rat brain.

Key words: Chlorogenic acid, orofacial dyskinesia, reserpine, tardive dyskinesia, vacuous chewing movements

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oral dyskinesia is one of the most serious iatrogenic movement disorders. It is generally characterized by increased vacuous chewing movements, tongue protrusions and cataleptic behavior[1]. Tardive dyskinesia is a late onset adverse effect caused after prolonged treatment with classical antipsychotic drugs[2]. It may be due to abnormalities in basal ganglia and striatum of the brain[3]. In addition, oxidative stress and free radicals may lead to neurodegeneration associated with tardive dyskinesia[4]. Reserpine-induced orofacial dyskinesia is used as an animal model for tardive dyskinesia[5,6]. Reserpine may lead to tardive dyskinesia by depleting catecholamines[7], such as dopamine which leads to an increase in dopamine levels. The metabolism of dopamine by monoamine oxidase in basal ganglia can lead to overproduction of free radicals such as highly reactive hydroxyl radicals and auto-oxidation of dopamine into dopamine quinones (free radicals themselves) and superoxide anions which cause neurotoxicity[5,8,9]. Increased production of lipid peroxidation byproducts and decreased antioxidant enzyme activities in striatum had been reported in reserpine treated animal model and it has been suggested that antioxidants could be promising candidate in treating tardive dyskinesia[10]. Peripheral administration of reserpine (1-10 mg kg^{-1}) produced robust depletion of monoamines (75-90%) in different brain regions[1,11,12] which starts 30 min after injection of reserpine and may last up to 14 days and after 21 days of retrieval finally returns to normal levels[12]. Reserpine (1-10 mg kg^{-1}) also increases lipid peroxidation and deceases reduced glutathione content and activities of catalase and SOD[5,6,13].

Chlorogenic acid, a polyphenolic compound, is found in a wide variety o f fruits and vegetables[14.] The fruit sources include apples[14,15], cherries[16], plums[17], berries[18], apricots[19] and tomatoes[20], while a common vegetable source is potatoes[15,21]. Chlorogenic acid is also found in beverages including tea[15,22], coffee[23] and wine[15]. Chlorogenic acid has been reported to possess various biological activities such as neuroregenerative[24], cytoprotective[25], nootropic[26], anxiolytic and antioxidant[27], anti-inflammatory and analgesic[28], antidiabetic and antilipidemic[29], etc.

As mentioned above, chlorogenic acid has been reported to possess neuroprotective and antioxidant activities, but the effect of chlorogenic acid on orofacial dyskinesia has not been explored till date, so the aim of the present study was to investigate the effect of chlorogenic acid on reserpine-induced orofacial dyskinesia in rats.

MATERIALS AND METHODS

Animals: Wistar male albino rats, weighing 100-200 g and 2-3 months age were purchased from Disease-Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana, India). Female rats were not employed in the present study since estrogens in females possess neuroprotective action, which may mask development of tardive dyskinesia[30]. The animals were housed under standard laboratory conditions with 12 h light-dark cycle. They had free access to food and water *ad libitum*. The animals were acclimatized to laboratory conditions prior to experimentation. The experiments were carried out between 9:00 and 16:00 h. The animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. The experimental protocol was approved by Institutional Animal Ethics Committee.

Experimental protocol: Animals were randomly distributed in five groups (n = 6 for each group). Group I: Vehicle (0.9% saline p.o.)+0.1% v/v acetic acid solution i.p. (vehicle for reserpine), group II: Reserpine (1 mg kg^{-1}, i.p.), group III: Reserpine (1 mg kg^{-1}, i.p.)+chlorogenic acid (10 mg kg^{-1}, p.o.), group IV: Reserpine (1 mg kg^{-1}, i.p.)+ chlorogenic acid (20 mg kg^{-1}, p.o.) and group V: Reserpine (1 mg kg^{-1}, i.p.)+chlorogenic acid (40 mg kg^{-1}, p.o.). Reserpine was administered on alternate days (day 1, 3 and 5) for total three days. Chlorogenic acid was administered for 15 successive days (from 6-20th day). The VCMs were recorded 1 h after administration of the drugs on 20th day.

Drugs and chemicals: Reserpine, chlorogenic acid and glacial acetic acid (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) were used in the present study. Reserpine was dissolved in 0.1% acetic acid[9] and then diluted in distilled water. Chlorogenic acid was suspended in 0.9% saline and administered orally. All the drugs were administered in a volume of 0.5 mL per 100 g of body weight of rats.

Selection of doses: Different doses of various drugs were selected on basis of literature i.e., 1 mg kg^{-1} reserpine[9], 10, 20 and 40 mg kg^{-1} chlorogenic acid[27].

Behavioral models

Reserpine-induced orofacial dyskinesia: To measure orofacial dyskinesia, rats were placed individually in

observation cage (20×20×19 cm^3). The observation cage was fixed with mirrors under the floor and behind the back wall of the cage. The animals were acclimatized for 10 min to the observation chamber before behavioral assessment. The VCMs are operationally defined as single mouth openings in the vertical plane not directed towards physical material. If VCMs occurred during a period of grooming, they were not taken into account. The VCMs were measured continuously for a period of 5 min, using hand operated counters[9].

Measurement of locomotor activity: Horizontal locomotor activities of control and test animals were recorded for a period of 10 min[31] using Medicraft Photoactometer, Model No. 600-6D (INCO, Ambala, India) which operates on photocell that are connected with circuit with counter. When a beam of light falling on photocell is cut-off by the animal, a count is recorded.

Biochemical estimations: Animals were sacrificed by decapitation immediately after behavioral testing. Their forebrains were removed, rinsed with isotonic saline and weighed. The brain homogenate (10% w/v) was prepared in 0.1 M phosphate buffer (pH 7.4), which was divided into three fractions. First fraction was used for estimation of malondialdehyde, reduced glutathione and total protein. The second fraction was centrifuged (Remi instruments, Mumbai, India) at 1000 g for 20 min at 4°C and the supernatant (post-mitochondrial supernatant) was used for estimation of catalase. The third fraction was centrifuged at 12000 g for 60 min at 4°C and this supernatant (post-mitochondrial supernatant) was used for estimation of superoxide dismutase[9,13].

Estimation of lipid peroxidation: Malondialdehyde levels, an index of lipid peroxidation were estimated according to the method of Ohkawa *et al.*[32] and as followed earlier in laboratory[13].

Estimation of reduced glutathione: Reduced GSH in the brain tissue was estimated by the method of Ellman[33] and as followed earlier in laboratory[13].

Estimation of total protein: The total protein concentration was estimated in brain homogenate by biuret method[34], using total protein kit (Crest Biosystems, Coral Clinical Systems, Goa, India).

Estimation of catalase activity: The catalase activity was estimated using method of Aebi[35] and as followed earlier in laboratory[13].

Measurement of superoxide dismutase activity: The SOD was estimated according to the methods of Kono[36] and as followed earlier in laboratory[13].

Statistical analysis: All data were presented as Mean±SEM. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test using Graph Pad Instat. The $p<0.05$ was considered as statistically significant.

RESULTS

Effect of chlorogenic acid on reserpine-induced vacuous chewing movements: The effect of reserpine administration on VCMs in rats is depicted in Fig. 1. Reserpine (1 mg kg^{-1}, i.p.) administered for a period of 3 alternate days (day 1, 3 and 5) significantly ($p<0.001$) increased VCMs in rats compared to vehicle treated group. Chlorogenic acid (10, 20 and 40 mg kg^{-1}, p.o.) administered for 15 successive days significantly reversed reserpine-induced VCMs (Fig. 1).

Effect of chlorogenic acid on reserpine-induced hypolocomotion: The locomotor activity in reserpine treated group was significantly ($p<0.01$) decreased as compared to vehicle treated control. Treatment with chlorogenic acid (10, 20 and 40 mg kg^{-1}, p.o.) significantly reversed reserpine-induced hypolocomotion in rats (Fig. 2).

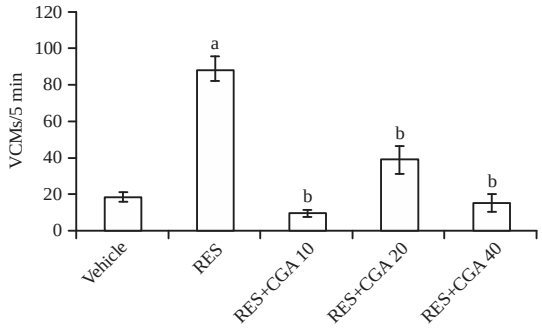

Fig. 1: Effect of chlorogenic acid on reserpine-induced vacuous chewing movements in rats n = 6 each group. Data were analysed by using one-way ANOVA followed by Tukey's multiple comparison test, F (4, 25) = 34.015, p-value<0.05, [a]p<0.001, as compared to vehicle treated control, [b]p<0.001 as compared to reserpine treated group. RES: Reserpine and CGA: Chlorogenic acid

Table 1: Effect of chlorogenic acid on reserpine-induced changes in brain malondialdehyde levels, reduced glutathione (GSH), catalase and superoxide dismutase activities of rats

Treatments (mg kg^{-1})	Malondialdehyde (nmoles /min/mg protein)	GSH (nmoles of GSH/mg protein)	Catalase (μmoles/ min/mg protein)	SOD (% inhibition) (Units/min/g FW)
Vehicle (5 mL kg^{-1})	0.33±0.03	2.02±0.17	9.35±0.91	77.78±3.51
Reserpine (1)	1.05±0.07[b]	1.07±0.08[b]	5.36±0.77[a]	30.56±5.12[b]
Reserpine (1)+chlorogenic acid (10)	0.33±0.06[d]	1.09±0.03	6.73±0.61	63.89±5.56[d]
Reserpine (1)+chlorogenic acid (20)	0.29±0.03[d]	1.72±0.04[d]	8.81±1.01[c]	75±3.73[d]
eserpine (1)+chlorogenic acid (40)	0.12±0.02[d]	1.86±0.06[d]	7.32±0.67	58.33±9.38[c]
F (4, 25)	56.338	24.341	3.97	11.104
p-value	<0.05	<0.05	<0.05	<0.05

n = 6 each group, data were analysed by using one-way ANOVA followed by Tukey's multiple comparison test. [a]p<0.05 and [b]p<0.001 as compared to vehicle treated control, [c]p<0.05 and [d]p<0.001 as compared to reserpine treated group, FW: Fresh Weight

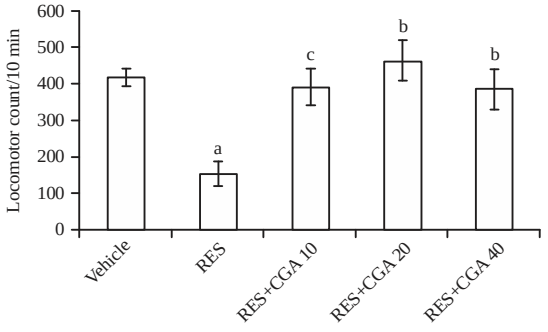

Fig. 2: Effect of chlorogenic acid on locomotor activity of rats using actophotometer n = 6 each group. Data were analysed by using one-way ANOVA followed by Tukey's multiple comparison test, F (4, 25) = 7.062, p-value<0.05, [a]p<0.01, as compared to vehicle treated control, [b]p<0.05, [c]p<0.01 and [d]p<0.001, respectively, as compared to reserpine treated group. RES: Reserpine and CGA: Chlorogenic acid

Effect of chlorogenic acid on reserpine-induced changes in brain lipid peroxidation, reduced glutathione, catalase and superoxide dismutase activities: Levels of malondialdehyde, a measure of lipid peroxidation, were significantly (p<0.001) increased in reserpine treated group. All the three doses (10, 20 and 40 mg kg^{-1}, p.o.) of chlorogenic acid significantly reversed reserpine-induced increase in lipid peroxidation. The content of reduced glutathione was depleted significantly (p<0.001) in reserpine administered group as compared to vehicle treated control. The middle (20 mg kg^{-1}) and highest dose (40 mg kg^{-1}) of chlorogenic acid significantly (p<0.001) restored reserpine-induced decrease in reduced glutathione levels. Reserpine showed significant decrease in activity of catalase (p<0.05) and SOD (p<0.001) as compared to vehicle treated control. Only middle dose (20 mg kg^{-1}, p.o.) of chlorogenic acid significantly reversed reserpine-induced decrease in catalase activity. On the other hand, all the three doses (10, 20 and 40 mg kg^{-1}, p.o.) of chlorogenic acid significantly reversed reserpine-induced decrease in SOD activity (Table 1).

DISCUSSION

The results of present study indicated significant reversal of reserpine-induced orofacial dyskinesia and hypolocomotion by chlorogenic acid in rats. This is the first study showing protective effect of chlorogenic acid against reserpine-induced orofacial dyskinesia. Reserpine-induced orofacial dyskinesia has been suggested as a putative animal model of tardive dyskinesia[1,13]. Reserpine administration significantly increased VCMs and produced hypolocomotion in rats, which is also supported by the literature[10]. Striatal oxidative stress has been strongly implicated in the development of reserpine-induced orofacial dyskinesia[6]. In the current study, reserpine significantly increased lipid peroxidation in rat brain as indicated by increased levels of malondialdehyde levels. Furthermore, it also decreased levels of reduced glutathione and antioxidant enzymes (catalase and SOD) activities in rat forebrain, indicating involvement of free radicals in development of tardive dyskinesia. Reserpine, a monoamine depletor, interferes with vesicular monoamine transporter and inhibits dopamine storage in synaptic vesicles. As a consequence, monoamine oxidase activity is enhanced and cytosolic dopamine oxidative metabolism is also accelerated[37]. This metabolism further leads to production of reactive metabolites and hydrogen peroxide which increase oxidative stress in dopaminergic neurons[10]. Basal ganglia, including striatum, are more susceptible to free radical damage as monoamines are abundant in these regions[8]. Therefore, the agents with antioxidant properties could be effective candidate in suppressing the development of orofacial dyskinesia. Chlorogenic acid has greater ability to pass through blood brain barrier either in its intact form or in its metabolite forms[24], which may inhibit deleterious effects of free radicals in cellular and vascular compartment of brains. In the present study, chlorogenic acid showed significant

reversal of lipid peroxidation by decreasing levels of malondialdehyde levels and restored the GSH levels and antioxidant enzymes (catalase and SOD) activities, which is also supported by the literature[27]. Furthermore, chlorogenic acid has been reported to be cytoprotective against dopamine-related toxicity[25], which also indirectly supports the protective effect of chlorogenic acid against reserpine-induced orofacial dyskinesia in rats.

CONCLUSION

Chlorogenic acid administration significantly ameliorated reserpine-induced orofacial dyskinesia and hypolocomotion in rats, possibly through alleviation of oxidative stress in rat brain. Thus, chlorogenic acid may be explored further for its potential in management of tardive dyskinesia.

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