Original Article

Attenuation of haloperidol induced orofacial dyskinesia by ginkgo biloba extract

Jamal Shams[*1], Ali Shabahang Saber Tehrani[2], Alireza Nourozi[3], Behrouz Rahmani[4], Alireza Zahiroddin[5], Saeed Sadr[6], Mahdi Hedayati[7]

1 . Neuroscience Research Center, Shahid Beheshti University (M.C.)
(Corresponding Author: Dr Jamal Shams. Email: j_shams@yahoo.com)
2 . Neuroscience Research Center, Shahid Beheshti University (M.C.)
3 . Neuroscience Research Center, Shahid Beheshti University (M.C.)
4 . Neuroscience Research Center, Shahid Beheshti University (M.C.)
5 . Dept. of Psychiatry, Shahid Beheshti University (M.C.)
6 . Behavioral Sciences Research Center, Shahid Beheshti University (M.C.), Tehran, Iran
7 . Research Institute for Endocrine Science (RIES)

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Abstract

Introduction: Tardive dyskinesia is one of the major side effects of long-term neuroleptic treatment. Increased reactive oxygen species and oxidative stress has been proposed as possible etiopathologic mechanisms. Ginkgo biloba extract (EGB) is a natural antioxidant. We investigated the effects of ginkgo biloba extract on neuroleptic-induced orofacial dyskinesia in rats, a potential animal model for tardive dyskinesia.

Methods: Orofacial dyskinesia was induced by chronic administration of haloperidol (1 mg/kg i.p) for a period of 21 days. On 22nd day, animals were assessed for development of oral dyskinesia. Malon di aldehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) and catalase levels were evaluated in animal forebrain homogenate.

Results: Chronic haloperidol (1 mg/kg) treatment significantly increased the vacuous chewing movement's frequency, increased MDA and decreased SOD and catalase levels. Co-administration of EGB 25 mg/kg along with haloperidol suppressed the haloperidol induced vacuous chewing movements (Pvalue < 0.05). EGB 100 mg/kg reversed haloperidol induced reduction in SOD level. It also reversed the increment in MDA level observed in haloperidol treated rats.

Conclusion: The present study suggested that oxidative stress plays a significant role in neuroleptic-induced orofacial dyskinesia and EGB co-administration reverses these behavioral and biochemical changes.

Declaration Interest: None.

Keywords: Movement Disorders, Neuroleptics, Gingo Biloba, Dyskinesia, Psychomotor Disorders.

Introduction

Tardive dyskinesia (TD) is one of the major side effects of long-term neuroleptic treatment. Neuroleptics are extensively used in the treatment of schizophrenia and other affective disorders. TD, which occurs in 20–40% of patients taking chronic neuroleptic medication, is a major limitation of neuroleptic therapy (1-4). Oxidative stress and products of lipid peroxidation are implicated in the pathophysiology of various neurological disorders (5). A role for increased reactive oxygen species and oxidative stress in the etiopathology of neuroleptic-induced TD has been proposed (6-8). Chronic treatment with neuroleptics increases free radical production and oxidative stress (9) and also forebrain malondialdehyde (MDA) level, as a marker of lipid peroxidation (10). Additionally, Elkashef *et al.* (11) have reported that rats with vacuous chewing movement had significantly higher thiobarbituric acid reactive substances (TBARS) in the striatum, suggesting increased lipid peroxidation and free radical production in these animals.

Chronic use of neuroleptics is also reported to cause decrease in the activity of antioxidant defense enzymes, superoxide dismutase (SOD) and catalase (6, 12). Free radicals are thought to play a role in the aging process, and age is one of the risk factors for the development of TD. Richardson *et al.* (13) had reported a positive correlation between increasing age and the development of TD, further supporting the role of free radicals in the pathogenesis of TD. Vitamin E, an antioxidant and free radical scavenger has been reported to be effective in the treatment of TD (5, 11, 14-16). All these observations strongly support the role of free radicals in TD.

Ginkgo biloba extract (EGB), a natural antioxidant, is an extract from green leaves of the Ginkgo biloba tree. The main ingredients of EGB are ginkgo-flavone glycosides (24%) and terpenoids (6%). It is well known for its cheap prices and negligible side effects. EGB has various biological activities and different pharmacologic effects, including antioxidation, anti-inflammatory and modulation of immune response. For its few side effects, EGB is extensively used in the therapy of central neural system disorders, acute pancreatitis, myocardial, and intestinal ischemia/reperfusion injury which are associated with inflammatory mediators(17-20). It has also been reported that EGB could scavenge oxidative-free radicals and down-regulate some of the inflammatory mediators involved in inflammatory responses, including TNF-α and IL-6(21).

In the present study we investigated the effects of ginkgo biloba extract on neuroleptic-induced orofacial dyskinesia in rats, a potential animal model for tardive dyskinesia.

Methods

Male Sprague-Dawley rats (250-300 g) were used in this study. The animals were housed under standard laboratory conditions, maintained on a 12-h light and dark cycle and had access to food and water *ad libitum*. Animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were carried out between 09:00 a.m. and 15:00 p.m. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the International Guidelines for the use and care of experimental animals.

Haloperidol (1 mg/kg i.p) was administered chronically to rats for a period of 21 days to induce oral dyskinesia (22, 23). All the behavioral assessments were carried out after 24 h of the last dose of haloperidol (22^{nd} day).

On the test day (22^{nd}) rats were placed individually in a small (30 × 20 × 30 cm) Plexiglas cage for the assessment of oral dyskinesia. Animals were allowed 10 minutes to get used to the observation cage before behavioral assessments. Tongue protrusion and vacuous chewing movements (frequencies) were counted with the help of a hand operated counter by trained observers. Vacuous chewing movements (VCM) are referred to as single mouth openings in the vertical plane not directed toward a physical object. If tongue protrusion and vacuous chewing movements occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming was over. Orofacial dyskinesia (tongue protrusions and VCMs) was measured continuously for a period of 5 min. In all the experiments the scorer was unaware of the treatment given to the animals.

On the 22^{nd} day of haloperidol treatment, the animals were sacrificed by decapitation immediately after behavioral assessment. The brains were removed, forebrain was dissected out and rinsed with isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction for catalase assay was obtained by centrifugation of the homogenate at 1000 × g for 20 min, at 4 °C and for other enzyme assays centrifuged at 12,000 × g for 60 min. at 4 °C.

The quantitative measurement of lipid peroxidation in forebrain was performed using Colorimetric kit (TBARS Assay Kit- Cayman Chemical, Michigan, USA). The dynamic range of the kit was 0-50µM MDA. Intra-assay coefficient of variation was 5.5%.

Reduced glutathione in the forebrain tissue was estimated using Colorimetric kit (Glutathione Assay Kit- Cayman Chemical, Michigan, USA). The dynamic range of the kit was 0-16µM GSH (or 0-8 µM GSSG). Intra-assay coefficient of variation was 1.6%.

Superoxide dismutase activity was assayed using Colorimetric kit (Superoxide Dismutase Assay

Kit- Cayman Chemical, Michigan, USA). The dynamic range of the kit was 0.025-0.25 units/ml SOD. Intra-assay coefficient of variation was 3.2%.

Catalase activity was assayed using Colorimetric kit Catalase Assay Kit- Cayman Chemical, Michigan, USA). The dynamic range of the kit is limited only by the accuracy of the absorbance measurement. Intra-assay coefficient of variation was 3.8%.

The following drugs were used in the present study. Haloperidol (IPDIC co., Iran) was diluted with distilled water. Ginkgo biloba extract (Goldaroo co., Iran) was suspended in vehicle (2% tween solution 80°). All drugs were administered intraperitoneally in a constant volume of 0.5 ml per 100 g of body weight of rat. Animals were divided into the following groups. Vehicle: saline; vehicle: haloperidol; haloperidol: EGB (25 mg/kg); haloperidol: EGB (50 mg/kg); haloperidol: EGB (100 mg/kg) groups respectively. Haloperidol was administered once daily (1.5 mg/Kg) in the morning (9 am) and this group also received vehicle for EGB twice daily. EGB was given once daily (9 am) for a period of 21 days and behavioral assessments were measured 24 h after the last dose. Drug doses were selected on the basis of previous studies reported in the literature (3, 23- 24).

Statistical data analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats (n=6). All the values are expressed as means ± S.E.M. The data were analyzed by using analysis of variance (ANOVA) followed by Tukey's test. In all tests, the criterion for statistical significance was $P<0.05$.

Results

Effect of Ginkgo biloba extract on haloperidol-induced vacuous chewing movements and tongue protrusions

Chronic haloperidol (1mg/kg) treatment significantly increased the vacuous chewing movements (VCMs) frequency but did not have a statistically significant effect on tongue protrusion frequency when compared with vehicle treated controls. Chronic co-administration of EGB 25 mg/kg along with haloperidol suppressed the haloperidol induced vacuous chewing movements (Pvalue<0.05). Other dosages of EGB (50 and 100 mg/Kg) did not change VCM or tongue protrusion frequencies significantly in comparison with the control group (Fig. 1, 2).

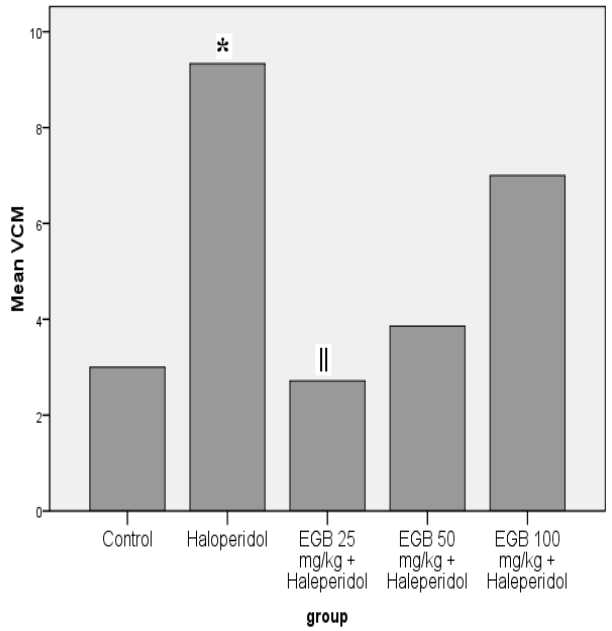
Effect of Ginkgo biloba extract on the forebrain MDA level in chronic haloperidol treated rats

Chronic administration of haloperidol for 21 days induced lipid peroxidation as indicated by a rise in forebrain MDA level compared with vehicle treated rats(P value<0.05). Coad ministration of EGB (100 mg/ Kg) along with haloperidol significantly reversed the extent of lipid peroxidation as compared to haloperidol only treated rats(P value<0.01). Other doses of EGB (25 and 50 mg/Kg) did not have a significant effect on forebrain MDA levels compared with the control group (fig. 3).

Effect of Ginkgo biloba extract on the forebrain glutathione (GSH) levels in chronic haloperidol treated rats Statistical analysis of forebrain GSH levels did not show a significant difference between vehicle-treated and haloperidol treated rats. Co-administration of EGB (25– 100 mg/kg) for 21 days did not change GSH levels compared to haloperidol only treated animals (fig. 4).

Effect of Ginkgo biloba extract on the forebrain antioxidant enzyme levels in chronic haloperidol treated rats. Chronic haloperidol treatment reduced levels of forebrain antioxidant enzyme SOD significantly compared with control group (P value<0.01). Co-administration of EGB 100 mg/kg along with haloperidol significantly increased SOD level compared with haloperidol only treated group(P value<0.05). Other doses of EGB (25 & 50 mg/kg) failed to reverse haloperidol induced reduction of forebrain SOD (Fig 5).

Chronic haloperidol treated rats showed decreased levels of catalase in their forebrain homogenates (P value<0.001). Various doses of EGB (25-50 mg/kg) co administered with haloperidol did not affect forebrain catalase levels significantly (Fig 6).



*: P value<0.05 compared with control group

‖ :P value<0.05 compared with haloperidol only treated group

Figure 1. Effect of Ginkgo biloba extract on haloperidol-induced vacuous chewing movements (VCM)

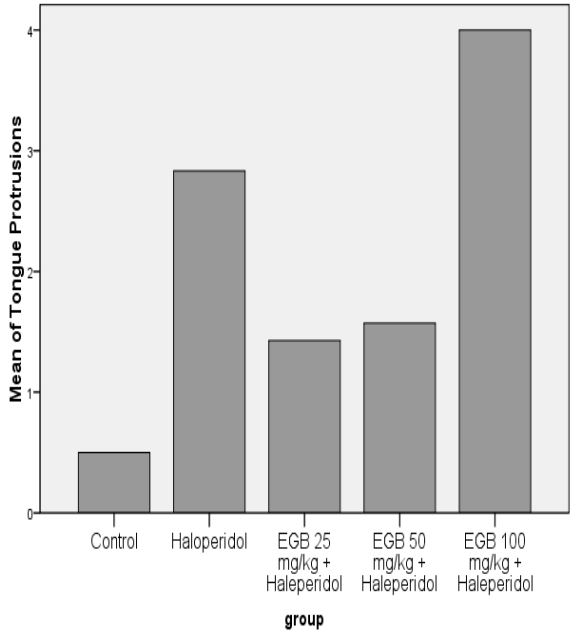
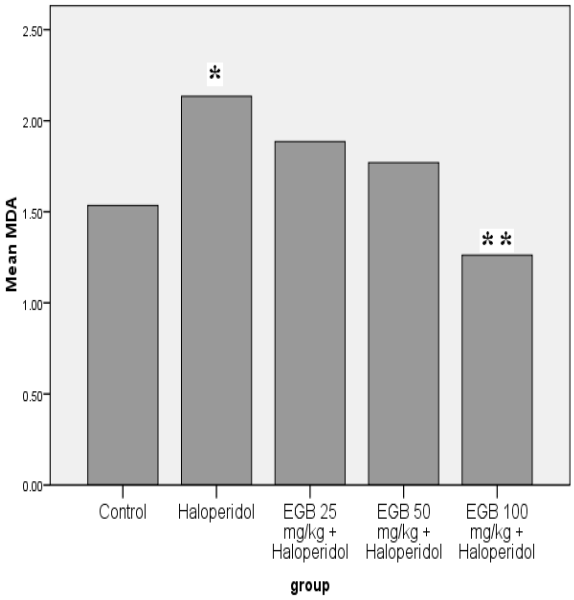


Figure 2. Effect of Ginkgo biloba extract on haloperidol-induced tongue protrusions



*: P value <0.05 compared with control group.

**: P value <0.01 compared with haloperidol only treated group.

Figure 3. Effect of Ginkgo biloba extract on the forebrain MDA level in chronic haloperidol treated rats

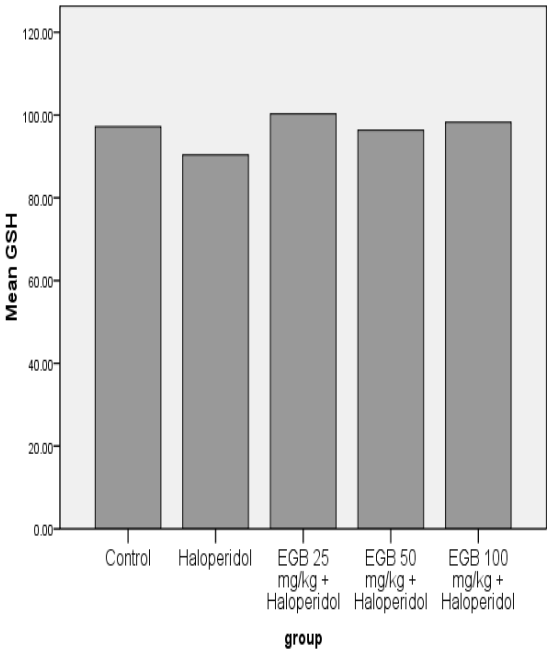
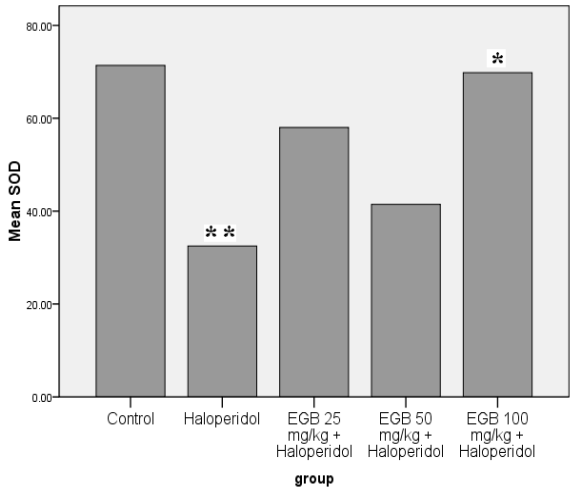
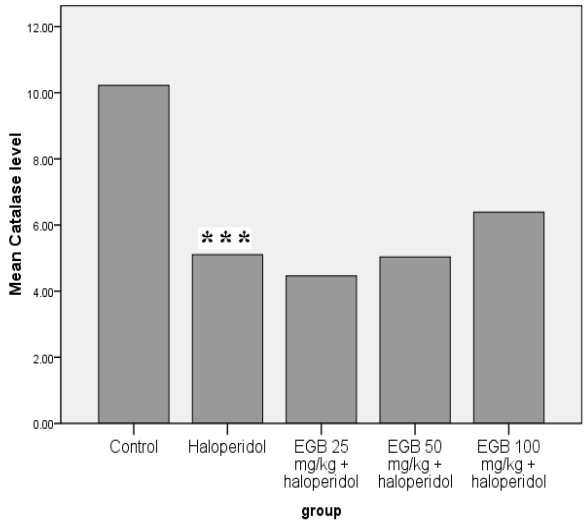


Figure 4. Effect of Ginkgo biloba extract on the forebrain glutathione (GSH) levels in chronic haloperidol treated rats



*: P value <0.05 compared with haloperidol only treated group.
**: P value <0.01 compared with control group.

Figure 5. Effect of Ginkgo biloba extract on the forebrain SOD level in chronic haloperidol treated rats.



***: P value < 0.001 compared with the control group.

Figure 6. Effect of Ginkgo biloba extract on the forebrain Catalase level in chronic haloperidol treated rats.

Conclusion

Existing evidences indicate that production of free radicals is associated with chronic neuroleptic use and this might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonias and Parkinsonism (6, 16). This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH levels (25) and low levels of antioxidant defense enzymes such as SOD and catalase respectively (11). Neuroleptics act by blocking dopamine receptors (26). Such blockade results in increased dopamine turnover (27), which in turn could conceivably lead to an increased production of hydrogen peroxide, resulting in oxidative stress (11, 28). However, this does not seem to be the only mechanism responsible for the GSH depletion observed during haloperidol treatment (29). Another possibility is that neuroleptics suppress the activity of certain detoxifying enzymes, leaving cells unprotected especially if basal enzyme activity is low or the free radical-scavenging mechanisms are less effective. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (30), and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptics (31).

In this study, chronic haloperidol treated animals showed increased forebrain MDA level and vacuous chewing movements and decreased SOD and catalase activity. These results correspond with earlier studies further supporting the role of lipid peroxidation and production of free radicals in neuroleptic induced TD (6, 11).

EGB was shown to have a dose dependent beneficial effect in the TNBS (trinitrobenzene sulfonic acid) induced colitis in rats (21). As a possible mechanism, Yan-Hong Zhou *et al.* (21) suggested that EGB can scavenge oxidative-free radicals and down-regulate some of the inflammatory mediators involved in the intestinal immune and inflammatory responses, including TNF-α, NF κBp65 and IL-6 resulting in the improvement of ulcerative colitis (21).

Additionally, Bridi *et al.* demonstrated that EGB could increase the antioxidant enzyme catalase and SOD activities in the hippocampus, striatum and substantia nigra in rats and decrease lipid peroxidation in the hippocampus (32). Furthermore, Chao *et al.* suggested that Ginkgo biloba extract can increase PGE2 level, SOD activity, and reduce oxidative damage by the actions of cytoprotection and antioxidation to improve the repair of the duodenal mucosa in duodenal ulcer rats (33).

EGB pretreatment was shown to ameliorate ethanol-induced damage in rat live. It was suggested that heme oxygenase-1 up-regulation by EGB was involved in the prophylactic effect (34).

Pattipati *et al.* (35) demonstrated that administration of quercetin (an active ingredient of EGB) dose dependently reduced lipid peroxidation and restored the decreased glutathione levels in haloperidol-treated animals. Quercetin (50–100 mg/ kg) was also shown to reverse the haloperidol - induced decrease in forebrain SOD and catalase levels in rats.

In this study, EGB (100mg/kg) co-administration along with haloperidol reversed the haloperidol induced increase in forebrain MDA level, and restored SOD depletion. Furthermore, EGB 25 mg/kg significantly reduced VCM in haloperidol-treated animals. These results, though not dose dependent, further support the positive anti-oxidant properties of EGB. Accordingly, one or several of these actions of EGB may contribute to the reversal of haloperidol-induced oxidative injury and orofacial dyskinesia.

In conclusion, the present study confirms that oxidative stress plays a significant role in neuroleptic - induced orofacial dyskinesia. Moreover, it demonstrates that chronic EGB treatment reverses the haloperidol induced increase in the frequency of orofacial dyskinesia, as well as haloperidol-induced increase in brain lipid peroxidation. EGB also reverses the effect of haloperidol in reduction of forebrain SOD level. These results further strengthen the oxidative stress hypothesis of tardive dyskinesia (36-38) and suggest a beneficial clinical use of EGB in preventing this motor disorder.

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