Brazilian Journal of Medical and Biological Research (2005) 38: 1649-1654
ISSN 0100-879X Short Communication

Ginkgo biloba leaf extract (EGb 761) enhances catalepsy induced by haloperidol and L-nitroarginine in mice

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Abstract

Ginkgo biloba extract EGb 761 has been reported to have therapeutic effects which have been attributed to anti-oxidant and free radical-scavenging activities, including a direct action on nitric oxide production. L^G-nitro-arginine (L-NOARG), a nitric oxide synthase inhibitor, and haloperidol, a drug that blocks dopamine receptors, are both known to induce catalepsy in rodents. Nitric oxide has been shown to influence dopaminergic transmission in the striatum. The purpose of the present study was to evaluate the effect of the extract obtained from leaves of *Ginkgo biloba* tree EGb 761 on catalepsy induced by haloperidol or by L-NOARG. Albino Swiss mice (35-45 g, N = 8-12) received by gavage a single or repeated oral dose (twice a day for 4 days) of EGb 761 followed by *ip* injection of haloperidol or L-NOARG. After the treatments, the animals were submitted to behavioral evaluation using the catalepsy test. Acute treatment with 80 mg/kg EGb did not modify the catalepsy induced by L-NOARG but, the dose of 40 mg/kg significantly enhanced haloperidol-induced catalepsy measured at the 10th min of the test. After repeated treatment with 80 mg/kg EGb 761, a significant increase in the cataleptic effect produced by both haloperidol and L-NOARG was observed. These data show that repeated EGb 761 administration increases the effects of drugs that modify motor behavior in mice. Since the catalepsy test has predictive value regarding extrapyramidal effects, the possibility of pharmacological interactions between haloperidol and *Ginkgo biloba* extracts should be further investigated in clinical studies.

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Publication supported by FAPESP.

Received September 29, 2004
Accepted April 26, 2005

Key words
- Nitric oxide
- *Ginkgo biloba*
- Catalepsy
- Herbal medicine
- Neuroleptic interaction
- Extrapyramidal system

The *Ginkgo biloba* extract EGb 761 (Tebonin®, Byk Química, São Paulo, SP, Brazil) is a standardized mixture of active compounds, including flavonoid and terpenoid substances, obtained from green leaves of the *Ginkgo biloba* tree. Chronic administration of *Ginkgo biloba* extracts has been proposed to improve some aspects of cognitive performance. Clinical trials support the potential therapeutic usefulness of EGb 761 in the treatment of cerebral insufficiency, cognitive impairment, peripheral and central cir-

culatory disease, and an apparent neuroprotective role after various neuronal insults (for a review, see Ref. 1).

Although the mechanisms underlying the effects of EGb 761 have not been fully established, experimental evidence has indicated that its beneficial therapeutic effects probably occur via antioxidant and free radical-scavenging activities. These activities have been primarily attributed to the flavonoid fraction of the *Ginkgo biloba* extracts (1). The terpenoid fraction, comprising two classes of compounds, ginkgolides and bilobalides, contains antagonists of platelet-activating factor. Several studies have shown that the terpenoid components of the *Ginkgo* extract can reduce ischemia-induced neurotoxicity and inhibit glutamate-induced excitotoxicity (2). Other mechanisms have also been proposed for the effects of *Ginkgo biloba* extracts. For example, the extract may act directly as a nitric oxide (NO) scavenger as well as inhibit NO production in activated macrophages, or may act via its antioxidant effect (1).

EGb 761 has been shown to scavenge various reactive oxygen species including superoxide, hydroxyl, peroxyl radicals, and NO (3). Furthermore, EGb 761 may directly interfere with NO production. For instance, Sharma et al. (4) have shown that pretreatment with EGb 761 orally administered significantly attenuates up-regulation of nitric oxide synthase (NOS) in the brain of rats exposed to heat stress. NOS-positive neurons are located throughout the basal ganglia and NO has been proposed to regulate dopaminergic neurotransmission in the striatum (5). This raises the possibility that NO may play a role in the control of motor behavior. Knockout mice for the neuronal NOS isoform show altered locomotor abilities (6). Mice treated with various NOS inhibitors show impairment in fine motor control. NOS inhibitors reduce spontaneous locomotor activity (7). Acute *ip* administration of 5-80 mg/kg L^G-nitro-arginine (L-NOARG) induces catalepsy in a dose-dependent manner in mice (8). Furthermore, the acute effect of L-NOARG is additive with catalepsy induced by haloperidol (9).

Catalepsy is defined as failure to correct an externally imposed posture. This test is widely used to evaluate motor effects of drugs that act on the extrapyramidal system (10). For example, haloperidol is known to induce catalepsy through the blockade of dopamine receptors in the striatum and nucleus accumbens. In the same way, catalepsy induced by NO inhibition has been proposed to be partially due to interference with striatal dopaminergic neurotransmission (8). Considering the interaction between NO- and dopamine-mediated neurotransmission in the striatum, and the possible effects of EGb 761 on the former, we hypothesized that EGb 761 would interfere with the motor behavior changes caused by L-NOARG, a NOS inhibitor, or by haloperidol, a D2 antagonist.

Male albino-Swiss mice (35-45 g) were housed in groups of 5 per cage with free access to food and water in a temperature-controlled room (23 ± 1ºC) with a 12-h light-dark cycle (lights on at 6:00 am). The behavioral experiments took place in a sound-attenuated, temperature-controlled (24 ± 1ºC) room between 7:00 and 12:00 am. The experiments were carried out according to the National Institute of Health Guide for Care and Use of Laboratory Animals (Institutional Ethics Committee Protocol No. 028/2002), and all efforts were made to minimize animal suffering. In experiment I, mice (8-12/group) received acutely water or 40, 80, or 160 mg/kg EGb 761, *po* (Tebonin®, Byk Química), followed, 30 min later, by an *ip* injection of saline (controls), 2 mg/kg haloperidol (Haldol®, Janssen-Cilag, São Paulo, SP, Brazil), or 40 mg/kg L-NOARG (Sigma, St. Louis, MO, USA). In experiment II, repeated treatment with EGb 761 was performed for 5 days. On day 1, animals (N = 8-12) received water or 80 mg/kg EGb 761,

po, followed 30 min later by an *ip* injection of saline (controls), 2 mg/kg haloperidol or 40 mg/kg L-NOARG. The animals were then treated twice a day with either water or 80 mg/kg EGb 761, for 4 days. On the 5th day, they received again water or 80 mg/kg EGb 761, followed 30 min later by the same *ip* injection as that administered on day 1. Catalepsy was evaluated on day 1 and day 5 after drug injection according to the standard bar-hanging procedure by placing the animals with both forelegs over a horizontal glass bar (0.5 cm OD), elevated 4.5 cm from the floor (10). The time during which the mouse maintained this position was recorded for up 300 s, with three attempts allowed to replace the animal in the cataleptic position. Catalepsy was considered to have ended when the forepaw touched the floor or when the mouse climbed the bar. Measurements were made 10, 60, and 120 min after vehicle, haloperidol or L-NOARG administration.

EGb 761 was dissolved in distilled water and orally administered by gavage (10 ml/kg) with a guide cannula (4 cm x 1 mm OD). The doses used (40-160 mg/kg) were chosen based on literature reports showing effects on the central nervous system and on a pilot study.

The data obtained, reported as means ± SEM, were analyzed by multivariate analysis of variance (MANOVA), followed, when significant interactions were found, by one-way analysis of variance (ANOVA) at each assessment point. The repeated measure factors were time (10, 60, and 120 min) and day of treatment (day 1 or day 5). Multiple comparisons were performed by the Duncan test.

As shown in Figure 1, acute administration of 40, 80, and 160 mg/kg EGb 761 did not induce catalepsy. Catalepsy time was changed by treatment ($F_{3,37} = 5.13$, $P = 0.005$), time ($F_{2,74} = 46.79$, $P < 0.001$) and treatment by time interaction ($F_{6,74} = 46.79$, $P < 0.001$) in the haloperidol-treated groups. Regardless of the EGb dosage, 2 mg/kg haloperidol induced catalepsy throughout the experiment ($F_{4,45\text{-}46}$ ranging from 11.35 to 188.78, $P < 0.001$) except for the EGb dose of 80 mg/kg at 10 min. The cataleptic effect of haloperidol was enhanced by previous administration of 40 mg/kg EGb 761 at 10 min ($P < 0.05$) when compared with the water/haloperidol group.

L-NOARG induced a significant increase in catalepsy time after 60 and 120 min ($F_{4,44}$ ranging from 10.10 to 21.40, $P < 0.001$). However, acute treatment with EGb 761

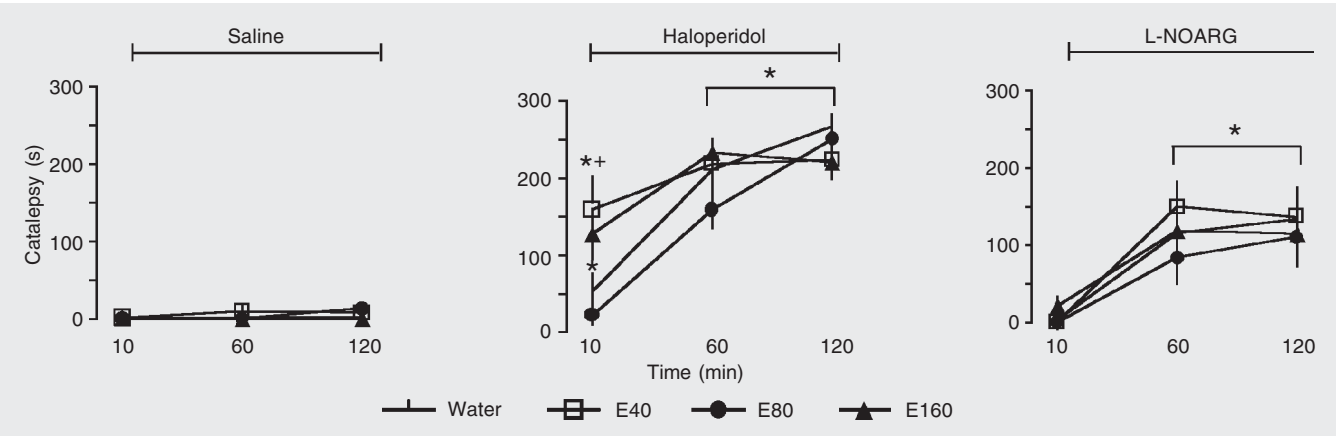

Figure 1. Effects of acute *Ginkgo biloba* (EGb 761) administration on catalepsy induced by haloperidol or L^{G}-nitro-arginine (L-NOARG). Animals (N = 8-12) received a single dose of EGb 761 by gavage, 40 (E40), 80 (E80) or 160 (E160) mg/kg, followed 30 min later by an *ip* injection of saline, 2 mg/kg haloperidol or 40 mg/kg L-NOARG. The animals were submitted to the catalepsy test 10, 60 and 120 min after the treatments. *$P < 0.05$ compared to water/saline and $^{+}P < 0.05$ compared to water/haloperidol (ANOVA followed by the Duncan test).

(40-160 mg/kg) did not modify the effect of L-NOARG (treatment effect, $F_{3,32} = 1.11$, $P > 0.05$; Figure 1).

In experiment II, haloperidol induced a significant increase in catalepsy time (treatment effect: $F_{3,39} = 111.49$, $P < 0.0001$; treatment by day interaction: $F_{3,39} = 7.79$, $P < 0.0001$, and treatment by day by time interaction: $F_{6.78} = 5.48$, $P < 0.001$). This effect was potentiated by repeated administration of 80 mg/kg EGb, at 10 min (treatment by day interaction: $F_{3,39} = 13.45$, $P < 0.0001$; Figure 2, panel A).

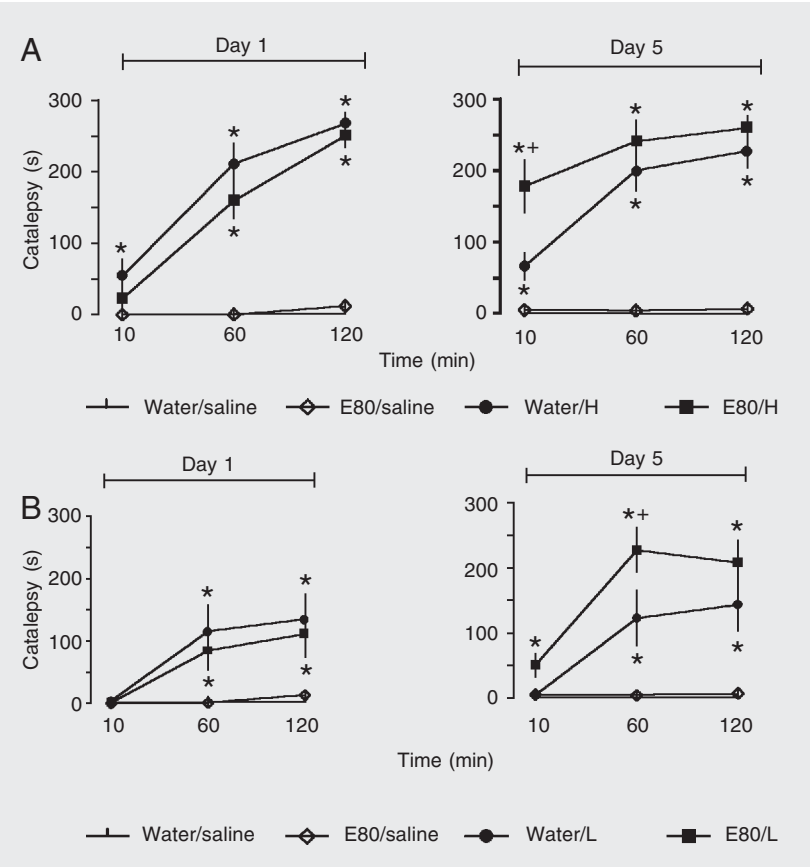

Figure 2. Effects of acute (day 1) or repeated (day 1 and day 5) *Ginkgo biloba* (EGb 761) administration on catalepsy induced by haloperidol (panel A) or L-NOARG (panel B). Animals (N = 8-12) received a single dose of EGb 761, 80 mg/kg (E80) by gavage, followed 30 min later by an *ip* injection of saline, 2 mg/kg haloperidol (H) or 40 mg/kg L-NOARG (L). Ten minutes later they were submitted to the catalepsy test (day 1). The animals were then treated twice a day for 4 days with either E80 or water. On the fifth day (day 5), they again received a single dose of saline. H or L *ip* injection and catalepsy were evaluated 10, 60, and 120 min. *$P < 0.05$ compared to water/saline and E80/saline groups, +$P < 0.05$ compared to water/haloperidol group (ANOVA followed by the Duncan test).

As seen in Figure 2, panel B, 40 mg/kg L-NOARG induced catalepsy in mice (treatment effect: $F_{3,34} = 27.86$, $P < 0.0001$, and treatment by day interaction: $F_{3,34} = 11.00$, $P < 0.0001$). Repeated oral administration of 80 mg/kg EGb 761 significantly increased L-NOARG-induced catalepsy at 10 ($F_{3,37} = 6.9$, $P < 0.001$) and 60 min ($F_{3,37} = 20.63$, $P < 0.0001$). There was a significant treatment by day interaction at 10 min ($F_{3,34} = 7.22$, $P < 0.001$).

The present study confirmed previous findings, showing that haloperidol and NOS inhibitors produce catalepsy in mice. However, acute or repeated treatment with EGb 761, even at the high doses, failed to show any behavioral effect. This suggests that, under these conditions, the extract does not interfere with NO- or dopamine-mediated neurotransmission to the same extent as haloperidol or L-NOARG. These results agree with the lack of locomotor changes in rats acutely or chronically treated with a wide dose range (8-96 mg/kg) of EGb 761 observed by Chermat et al. (11).

A single administration of EGb 761 enhanced the cataleptic effect of haloperidol, an effect that was even more evident after repeated treatment. In the case of L-NOARG, the potentiation of the cataleptic effect appeared only after repeated administration of the extract.

The reason for the high effectiveness of EGb 761 after repeated administration is not clear. Similar findings, however, have been reported in several studies. Brailowsky and Montiel (12) reported that the beneficial effects of EGb treatment on motor function in hemiplegic rats were apparent only after 7-21 days of treatment. Repeated administration of 50 and 100 mg/kg EGb 761 has been also shown to reduce avoidance deficits induced by unavoidable shock and to increase food consumption in mice in a novel situation (13), as well as to exert an anti-stress effect in a discrimination-learning task con-

ducted under stressful conditions (14). Pharmacokinetic mechanisms such as residual effects due to storage of EGb 761 in a slowly releasing body compartment (e.g., fat tissue) or the presence of an active metabolite with a longer half-life could be responsible for these delayed effects (12). Another possibility is related to a proposed gene-regulatory effect requiring repeated administration (15).

The effect of acutely administered EGb 761 on haloperidol-induced catalepsy was not dose-dependent. The reason for the lack of dose-dependent responses is not clear. To date, a systematic pharmacokinetic evaluation of all of the EGb 761 constituents and also of its brain concentration following various routes of administration remains to be conducted.

The mechanisms involved in the potentiating effect of EGb 761 on catalepsy induced by haloperidol or L-NOARG are not known. Catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors (10). This test is a useful method to evaluate the propensity of antipsychotic agents to cause extrapyramidal effects. For neuroleptics such as haloperidol these effects are related to their clinical efficacy. It is therefore interesting that, similar to the facilitation of the cataleptic effect of haloperidol detected in this study, Zhang et al. (16) showed an improvement of positive symptoms after the administration of EGb 761 plus haloperidol to patients with chronic refractory schizophrenia. The authors attributed this therapeutic effect to a plausible antioxidant action of EGb 761, since it was correlated with a decrease in blood levels of superoxide dismutase, one of the scavenging enzymes that detoxifies free radicals.

NO has been proposed to regulate dopamine neurotransmission in the striatum. Although there are contradictory results, most studies suggest that, under physiological conditions, NO increases striatal dopamine by facilitating its release and/or by decreasing its reuptake (5). Centrally acting NO donors increase dopamine efflux via a mechanism dependent on extracellular calcium, and possibly for this reason they are able to antagonize catalepsy induced by haloperidol or L-NOARG. Furthermore, NOS inhibitors have been proposed to decrease locomotor activity by interfering with dopamine neurotransmission (8). A possible action of EGb 761 as an NO scavenger, therefore, could be related to the motor effects found in the present study.

Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine, or opioids have been implicated in catalepsy behavior along with dopamine (10,17-19), and the interference of EGb 761 with these systems cannot be ruled out.

The present results showed that repeated treatment with EGb 761 enhanced catalepsy caused by L-NOARG or haloperidol. It remains to be determined which components or metabolites of EGb are responsible for this effect. Considering that this test has predictive value regarding extrapyramidal effects caused by typical neuroleptics, the possibility of pharmacological interactions between haloperidol and *Ginkgo biloba* extracts should be further investigated in clinical studies.

Acknowledgments

We thank Dr. Francisco S. Guimarães, Faculty of Medicine of Ribeirão Preto, USP, Brazil, for revising the manuscript.

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