Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 76 (2003) 487–492
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of *Ginkgo biloba* on corticosterone stress responses after inescapable shock exposure in the rat

C. Rob Markus[a,b,*], Jan H.C.M. Lammers[b]

[a]*Department of Experimental Psychology, University of Maastricht, Maastricht, The Netherlands*
[b]*Organization for Applied Scientific Research (TNO), Department of Target Organ Toxicology, Zeist, The Netherlands*

Received 19 June 2003; received in revised form 12 August 2003; accepted 3 September 2003

Abstract

Extracts from the leaves of the *Ginkgo biloba* tree (GBE) are found to be clinically effective in neuroprotection, cerebral and cardiovascular function and cognitive processing. Recent animal findings suggest that GBE also may improve stress adaptation and prevent learned helplessness, as evidenced by its reduction of behavioral acquisition deficits of active avoidance after inescapable shock exposure. In the present report, the effects of two doses of GBE were studied on corticosterone stress responses and acquisition of active avoidance after inescapable shock exposure. Forty-eight rats were divided into three groups: either receiving a daily dose of 50 mg/kg or 150 mg/kg of GBE (containing 24% flavonoid and 6% terpenoid) or vehicle for 2 weeks. After 2 weeks of administration, animals were trained for active-avoidance acquisition following inescapable shock exposure (stress induction) or nonshock exposure (nonstress). Administration of 150 mg/kg but not of 50 mg/kg of GBE significantly prevented a corticosterone stress response after inescapable shock exposure ($P < .0001$) without any beneficial behavioral effect on active avoidance. Repeated administration of GBE particularly improves biological adaptation to noxious stimuli without beneficial behavioral consequences. Present findings do not support previous claims about the benefits of *G. biloba* on improving behavioral stress adaptation and acquisition of active avoidance and on reducing behavioral deficits indicative of "learned helplessness."

Keywords: *Ginkgo biloba*; Learned helplessness; Active avoidance; Corticosterone; Stress adaptation

1. Introduction

It has been generally accepted that stress can have a detrimental effect on motivational and cognitive behavior and often precedes the development of learned helplessness or depression (e.g., Mandler, 1984; Brown et al., 1987). During the last decade, there has been increased interest in the beneficial effects of dietary nutrients on cognitive information processing, stress and performance. In particular, the effects of natural products like botanicals and vitamins have received a lot of attention. One interesting product in this respect is the botanical *Ginkgo biloba*. Extracts from the green leaves of the *G. biloba* tree appear to be clinically effective with beneficial effects on neuroprotection, cardiovascular function and cerebral information processing. In accordance, a variety of studies have been published showing the learning- and memory-enhancing effects of standardized *G. biloba* extracts (GBEs) (containing 24% flavonoid and 6% terpenoid) in animal research (e.g., Continella and Drago, 1985; Porsolt et al., 1990; Winter, 1991; Petkov et al., 1993; Rodriguez de Turco et al., 1993; Rapin et al., 1994; Stoll et al., 1996), as well as in clinical and healthy subjects (Kleijnen and Knipschild, 1992; Field and Vadnar, 1998; Kidd, 1999).

In a recent animal study, it has been demonstrated that administration of the GBE also exerts beneficial effects on behavioral stress adaptation during an experimental procedure typically used to study learned helplessness (Porsolt et al., 1990). These authors studied the preventive effect of a GBE (50 or 100 mg/kg/day) on behavioral deficits during acquisition of active avoidance in rats after exposure to inescapable electric shocks. Half of the animals were first placed in Plexiglas boxes in which they received 60 inescapable electric shocks (stress induction condition), whereas the other half of the animals were

* Corresponding author. Department of Experimental Psychology, University of Maastricht, P.O. Box 6200 MD Maastricht, The Netherlands. Tel.: +31-43-3882474; fax: +31-43-3884199.
E-mail address: R.Markus@psychology.unimaas.nl (C.R. Markus).

0091-3057/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2003.09.002

placed in identical boxes without receiving electrical shocks (control condition). Two days later, animals had to perform an active-avoidance acquisition task for three consecutive days. Results revealed that administration of *G. biloba* at both doses prevented the occurrence of stress-induced escape deficits when given repeatedly before the animals were exposed to a series of inescapable shocks.

Although findings from this study suggest that administration of GBE may reduce behavioral deficits indicative of learned helplessness by improving stress adaptation, further evidence is needed to strengthen this assumption. In addition, it is necessary to investigate the effects of GBE on hormonal alterations as a biological indication of stress adaptation.

The main purpose of the present study was to investigate whether the administration of GBE may reduce a stress-induced corticosterone response and behavioral acquisition deficits of active avoidance after inescapable shock exposure in rats. Forty-eight rats were trained for avoidance acquisition following a stressful (receiving inescapable shocks) or a nonstressful (not receiving shocks) condition. Before active-avoidance training, animals received a daily dose (50 or 150 g/kg) of GBE or vehicle for a total period of 2 weeks. Alterations in plasma corticosterone concentrations were measured as a biological measure of stress adaptation.

2. Materials and methods

2.1. Animals

Forty-eight young (10 weeks old) adult male Wistar outbred rats (mean body weight: 200 ± 11.5 g) were obtained from a colony maintained under SPF conditions at Charles River Deutschland (Sulzfeld, Germany). Animals were group housed (four rats per cage) in transparent macrolon cages with wood shavings on the floor under conventional conditions in a temperature (23 °C) and humidity (58%) controlled room. All animals were acclimatized to their laboratory conditions for 5 days before randomization and commencing experimentation. Lighting was artificial by fluorescent tubes, which were time switch controlled at a sequence of 12:12-h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). Animals were kept on a standard diet (Rat and Mouse Breeding Diet, SDS Special Diets Services, Witham, England), and drinking water was provided ad lib from the arrival of the rats until 1 week before the start of the experiment.

The study was conducted at the TNO institute (TNO-Voeding Zeist, The Netherlands), approved by the institutional Review Committee for the use of animal subjects and is carried out in accordance with the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [as revised in 1997, Paris, ENV/MC/CHEM(98)17] and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Test substances

A standardized GBE was used containing 24% flavonoids and 6% terpenoids (Xuzhou Lubao Biochemical Products, Xuzhou, China). The experiment was performed in blind conditions using coded solutions of the test or vehicle substances.

2.3. Apparatus

The experiment was carried out in soundproof automated two-way shuttle boxes ($32 \times 30 \times 28$ cm) with Plexiglas walls containing a stainless steel grid floor (rods are spaced 1.0 cm apart). Each shuttle box was divided into two equal-size compartments containing a stainless steel partition with a gate that provides access to the adjacent room through a 7×7-cm space. The grid floor was connected with an electrical constant-current shock generator able to deliver electrical shocks. Each day before the start of an experimental session, a test program was run to insure proper operation of the equipment.

2.4. Procedure

Animals were randomly assigned ($n = 16$ animals per group) to three groups. The two treatment groups received a daily oral injected (po) of either 50 mg/kg or 150 mg/kg GBE (volume 5 ml/kg, dissolved in distilled water as a vehicle) starting 2 weeks before the onset of active-avoidance testing and continued throughout the experiment. The control group received an equal volume of the vehicle. Following 2 weeks of acclimatization and drug administration, an inescapable stress condition was introduced to half of the animals of each group. One half of the animals of each group was placed in a shuttle box and received a series of 60 scrambled randomized inescapable shocks (15 s in duration, 0.8 mA intensity, every minute $\pm$ 15 s). The other half of the animals of each group was placed under identical conditions without receiving electrical shocks. In order to measure stress-induced changes of corticosterone in plasma, blood samples (0.1 ml/sample) were taken from the tail vein (by tail nick) after exposing the animals to the stress (inescapable shock) or nonstress (nonshock) condition.

Forty-eight hours (Day 3) after the stress or nonstress pretreatment condition, the animals were trained for acquisition of active avoidance for three consecutive days. Animals were placed singly for 5 min into the shuttle box with the gate at the center opened in order to habituate to the test environment. At the start of each test session, the animal was placed in the left compartment of the shuttle box with the gate at the center closed. The first

trial started by the opening of the gate and a light signal (the conditioned stimulus or CS) was presented each consecutive trial for 4 s. If an avoidance response did not occur within this time, a 0.8-mA shock was supplied to the animal for 4 s. If the animal did not react by escaping, the shock and CS was terminated and an escape failure was recorded. On each active-avoidance testing day, the animals were exposed to a daily session consisting of 40 avoidance trials (with intertrial intervals of 30 ± 10 s). Only 4 s were permitted for the animal to escape since only the first few seconds following shock onset seem to be critical to trace interference effects of preexposure to inescapable shocks (Porsolt et al., 1990).

2.5. Assay of plasma corticosterone

Plasma corticosterone concentrations were determined by the coat-a-count procedure using an in-house competitive radioimmunoassay (RIA) kit. In short, 125I-labeled rat corticosterone competed for a fixed time with sample corticosterone for the antibody sites. These antibodies were immobilized to the wall of a polypropylene tube. Counting the tube in a gamma counter yielded a number, which was converted by calibration standards in plasma concentrations.

2.6. Statistical analyses

The main research questions were analyzed by means of repeated measures univariate analyses of variance procedures using the general linear model (GLM; SPSS 7.5 for Windows, SPSS, Chicago). Analyses were performed, with group (vehicle, 50 mg GBE, 150 mg GBE) and stress (inescapable shocks vs. no shocks) as between-subjects factors and time (test days 1–3) as within-subjects factor. Significant results revealed by these procedures were further examined by individual post hoc (Student's *t* tests) analyses. All statistics were evaluated at a significance level of 5% (two tailed). Data are reported as means ± SD.

3. Results

3.1. Effect of GBE on stress-induced plasma corticosterone concentrations

To examine whether the introduction of an inescapable shock condition increases plasma corticosterone in animals receiving different doses of the test substances, an analysis of variance was performed, with group (vehicle, 50 mg/kg GBE, 150 mg/kg GBE) and stress (inescapable shocks vs. no shocks) as between-subjects factor on plasma corticosterone concentrations. Analysis revealed a significant interaction effect of Group × Stress [$F(2,42) = 15.72$; $P < .0001$], indicating a change in plasma corticosterone concentrations after inescapable shock exposure that depended on test substance. As shown in Fig. 1, corticosterone concentrations increased after inescapable shock exposure as compared with the no-shock condition after administration of the vehicle (from 78 ± 31 to 160 ± 72 ng/ml), as well as after 50 mg/kg GBE (from 93 ± 75 to 198 ± 60 ng/ml), whereas this corticosterone response was prevented after administration of 150 mg/kg GBE (81 ± 41 ng/ml). Note that after administration of 150 mg/kg in the absence of shock exposure, there was a significant increase in corticosterone (199 ± 78 ng/ml) as compared with the vehicle or 50/mg/kg.

3.2. Learned helplessness: effect of GBE on escape responses

To examine whether the introduction of inescapable shocks may decrease escape responses depending on the test substances, we first conducted a repeated measures analysis of variance, with group (vehicle, 50 mg/kg GBE, 150 mg/kg GBE) and stress (inescapable shocks vs. no shocks) as between-subjects factors and time (test days 1–3) as within-subjects factor on the number of escape responses. Analysis only revealed a significant main effect of stress [$F(1,42) = 4.0$; $P = .05$] and time [$F(2,41) = 4.64$; $P = .015$] and a significant interaction effect of Time × Stress [$F(2,41) = 11.0$; $P < .0001$], indicating a significant effect of stress on the amount of escape responses regardless of test substances. Further analysis on the data of the different group conditions pooled revealed that the interaction effect originated from the first polynomial contrast [$F(1,42) = 19.87$; $P < .0001$], indicating a linear change in escape responses across test days that depended on shock exposure. As shown in Fig. 2, after no-shock exposure, the number of escapes where highest during the first test day (30 ± 3) and decreased across the remaining second test day (19 ± 4) and third test day (18 ± 4). After inescapable shock exposure, escape responses remained approximately

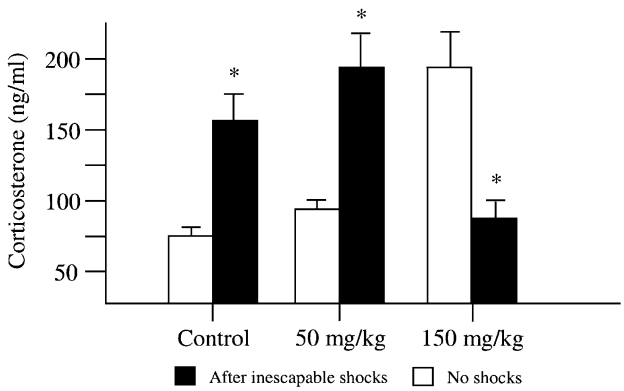

Fig. 1. Significant increase in plasma corticosterone concentrations after inescapable shock exposure in rats was prevented for by administration of 150 mg/kg GBE (* $P < .01$).

stable across test days (from 16 ± 10 to 19 ± 11 to 19 ± 10). No GBE or group effects were found on the amount of escape responses.

Note that a reducing effect of inescapable shock exposure, as compared with no shocks, on the amount of escape responses is particular found on the first test day. In addition, further post hoc testing with group (vehicle, 50 mg/kg GBE, 150 mg/kg GBE) and stress (inescapable shocks vs. no shocks) as between-subjects factors on the separated test days revealed a significant effect of stress [$F(1,42) = 29.37$; $P < .0001$] and group [$F(2,46) = 4.63$; $P = .015$] on the first test day, whereas there were no significant effects, nor any interaction effect between group and stress, on the remainder test days ($P > .5$). As shown in Fig. 3, the amount of escape responses during the first test day was significantly lower after inescapable shock exposure, as compared to the no-shock condition, in the vehicle (17 ± 11 vs. 32 ± 7; $P < .01$), 50 mg/kg (21 ± 10 vs. 32 ± 6; $P < .01$) and after the 150 g/kg (16 ± 10 vs. 30 ± 8; $P < .01$) group condition. In addition, the amount of escape responses was most profoundly reduced after 150 mg/kg in both the inescapable-shock and no-shock condition.

3.3. Effect of GBE on acquisition of active avoidances

We also examined whether the introduction of inescapable shocks may deteriorate acquisition of active avoidance depending on the test substances. However, repeated measures analysis of variance with group (vehicle, 50 mg/kg GBE, 150 mg/kg GBE) and stress (inescapable shocks vs. no shocks) as between-subjects factors and time (test days 1–3) as within-subjects factor did not reveal a significant effect of group ($P = .44$) or stress ($P = .41$), nor was there an interaction effect of Group × Stress (0.88). Analysis only revealed a significant effect of time [$F(2,41) = 47.3$; $P < .0001$] that originated from the first polynomial contrast [$F(1,42) = 94.48$; $P < .0001$], suggesting a linear increase in the number of avoidance responses across test days. Hence, the number of successful avoidances significantly increases from 8 ± 2 (Day 1) to 18 ± 4 (Day 2) and 21 ± 4 (Day 3). No interaction effects where found of Time × Stress ($P > .9$), Time × Group ($P > .3$) or Time × Stress × Group ($P > .8$).

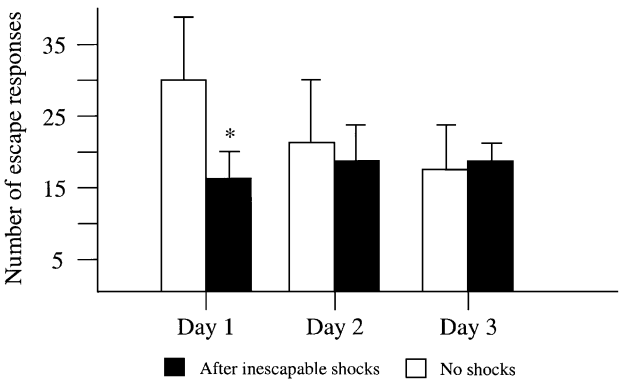

Fig. 2. A significant difference in the number of escape responses (with the data of treatment pooled) after exposure to inescapable shocks, as compared with the no-shock condition, was particularly found during the first test day (* $P < .01$).

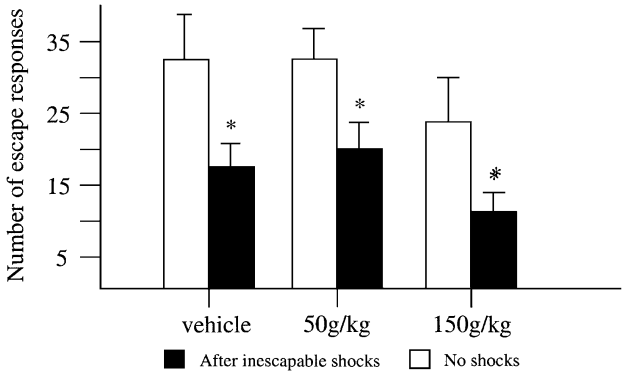

Fig. 3. The amount of escape responses was mostly reduced after 150 mg/kg GBE regardless of the inescapable-shock or no-shock condition (* $P < .01$).

4. Discussion

The aim of the present study was to investigate the positive effects of two doses of *G. biloba* on reducing acquisition deficits of active avoidance in rats after exposure to inescapable electrical shocks. Acquisition deficits following exposure to noxious stressors like inescapable shocks are generally interpreted as an indication of learned helplessness. A significant stress-induced increase in plasma corticosterone concentrations, found after inescapable shock exposures, was prevented by administration of 150 mg/kg GBE. However, no beneficial preventive effects were found of GBE on stress-induced acquisition deficits or learned helplessness.

4.1. Effect of GBE on a corticosterone stress response

Increases in the activity of the hypothalamic–pituitary–adrenocortical axis (HPA), indicated by a rise in plasma corticosterone concentration, during exposure to noxious or threatening stimuli are well-established biological index of distress (e.g., Henry and Meehan, 1981; Frankenhaeuser, 1986; Ursin and Olff, 1993). In addition, increased HPAC activity provides extra glucose for sympathetic action and, on the other hand, suppresses the stress response in order to reestablish physiological balance (Levine and Ursin, 1991; Ursin and Olff, 1993; Maes and Meltzer, 1995). As expected, exposing rats to inescapable shocks in the present study resulted in a significant increase in plasma corticosterone concentrations as compared with the nonshock

condition. These results indicate that the introduction of inescapable shocks was successful in causing high stress and a subsequent increase in sympathetic activation necessary for stress adaptation.

Based on previous findings that *G. biloba* produced beneficial effects on behavioral stress adaptation in rats as evidenced by reduced learned helplessness behavior after stressful inescapable shock exposure (e.g., Porsolt et al., 1990), it was expected that administration of GBE should also improve biological stress adaptation and prevent a corticosterone stress response. Findings of the present study indeed reveal that administration of 150 mg/kg GBE, but not 50 mg/kg GBE, reduces a stress-induced corticosterone response after inescapable electric shock exposure.

Preventive effects of GBE on biological stress responses have also been demonstrated in a study by Rapin et al. (1994). However, in this latter study, 50 mg/kg as well as 100 g/kg GBE (EGb 761) was found to reduce a corticosterone stress response. This inconsistency in dose–response effects may be due to the different research paradigm used. Hence, Rapin et al. (1994) did not use the learned helplessness procedure of introducing a severe inescapable stressor but investigated the effectiveness of GBE in preventing behavioral deficits on a visual discrimination task using auditory perturbation as an escapable stressful distracter. Since the magnitude of a corticosterone stress response seems to be a function of the severity or uncontrollability of the stressor, a difference in dose dependency between the present study and the study of Rapin et al. (1994) might be expected.

Although administration of 150 mg/kg GBE under stress exposure prevented a corticosterone response, corticosterone concentrations markedly increased after administration of 150 mg/kg but not 50 mg/kg GBE in the absence of a stressor. This effect was rather surprising and seems to contradict previous findings that chronic administration of 50–100 mg/kg *G. biloba* reduces basal corticosterone secretion by direct action upon the hypothalamus HPA level (Marcilhac et al., 1998). Although administration of a high dose of GBE seems to be sufficient in the reestablishing stress-induced neuroendocrine activity, it may be suggested that in the absence of a stressor, such an increase in sympathetic activity may act as a stressor as well. However, this hypothesis seems to be rather premature and certainly needs further investigation.

4.2. Effect of GBE on behavioral stress adaptation

A first hypothesis was that exposure of rats to a series of inescapable shocks (seen as an uncontrollable stress condition) should increase the occurrence of acquisition deficits (learned helplessness) during subsequent learning of active avoidance. Results of the present study indeed reveal a successful induction of learned helplessness; evidenced by a reduced number of escape responses after inescapable shock exposure, particularly after the first test day, as compared with the no-shock condition. These results appear to be comparable with the literature indicating behavioral escape deficits (learned helplessness) induced by prior exposure to inescapable electric shocks (e.g., Porsolt et al., 1990; Maier, 1995). However, it remains intriguing why exclusively in no-shock control animals there is a decrease in the amount of escape responses on Days 2 and 3. During the last day, these no-shock controls became as bad at learning the escape responses as the learned helplessness animals. These findings are opposite to what one would expect in a day-to-day learning task. Since we used, approximately, comparable research parameters as those used by Porsolt et al. (1990), we could not explain this deviation in our results.

A second hypothesis was that administration of GBE in rats exposed to inescapable shocks should prevent the occurrence of behavioral deficits indicative of learned helplessness during subsequent active-avoidance learning. This hypothesis was based on prior findings indicating that preventive treatment for 5 days with GBE (EGb 761) could diminish or block the negative impact of inescapable stressful stimulation on subsequent learning capacity (Porsolt et al., 1990). However, results of the present study do not confirm these previous findings. Although it seems rather counterintuitive, it is possible that the use of a longer *G. biloba* treatment period of 2 weeks in the present study, as compared with the shorter treatment period as applied by Porsolt et al. (1990), might have contributed to the current inconsistent findings. Obviously, this needs to be explored in further studies.

The present findings also do not confirm previous findings that *G. biloba* improves acquisition of active avoidances in the absence of a stressor (Petkov et al., 1993). In this latter study, administration of three (10, 30, 90 mg/kg) doses of *G. biloba* (GK 501) for 7 days before training all improved the percentage of avoidances on the second training day, with the most pronouncing effects occurring after the highest doses. Yet, this beneficial effect of *G. biloba* was not confirmed in the present study. Contrary to a positive effect of *G. biloba*, it even appeared that administration of 150 mg/kg GBE most profoundly reduced the amount of escape responses during the first test day in stressed as well as in nonstressed animals. However, it remains to be seen whether a difference between the CS–US interval of 9 s, as was used in the study of Petcov et al. (1993), and an interval of 4 s used in the present study, or a difference in US durance (12 vs. 4 s), might have caused these differences in data.

In addition to some previously mentioned minor differences in research parameters used in the current study, as compared with those of Petcov et al. (1993), Porsolt et al. (1990) and Rapin et al. (1994), different types of GBEs have been used. Although these different extracts all containing 24% flavonoid and 6% terpenoid, which is believed to be the responsible composition for the often reported pharmacological and behavioral effects, it may be

possible that a difference in chemical composition between extracts may at least partly be responsible for these incomparable behavioral findings. However, this requires additional research.

4.3. Stress-reducing effects of GBE

The absence of a beneficial behavioral effect of GBE on stress adaptation in combination with the prevention of a stress-induced corticosterone response seems to be counter-intuitive. Hence, since the reduction of a stress-induced corticosterone is commonly interpreted as a biological measure of improved stress adaptation (e.g., Frankenhaeuser, 1986; Ursin and Olff, 1993), one should also expect to find a subsequent reduction in the negative behavioral consequences of prior stress (inescapable shock) exposure. The absence of a combined beneficial behavioral effect may be interpreted as an indication that administration of GBE exclusively alters the secretion of corticosterone concentrations through a subcortical neuronal route, preventing or overriding the initiation of a conditioned adrenocortical stress response without any interference on higher cortical processes. In support of this notion, a direct inhibitory action of *G. biloba* on corticosterone secretion has previously been demonstrated (Marcilhac et al., 1998). In addition, the beneficial effects of GBE may particularly be restricted to reducing the adverse neurotoxic and immunosuppressive effects of chronic exposure to high concentrations of corticosterone.

5. Conclusion

In summarizing the present findings, this study does not support previous claims about the benefits of *G. biloba* on the prevention of behavioral deficits after inescapable stress exposure in rats. Exposure to inescapable shocks prior to active-avoidance learning induces learned helplessness as evidenced by subsequent increases in acquisition deficits. However, even though administration of 150 mg/kg but not 50 mg/kg of GBE significantly prevented a stress-induced corticosterone response, this was not accompanied by improved acquisition of active avoidance. Results of the present study define a problem with the assumption of the beneficial behavioral effects of *G. biloba* that requires further research. In addition, the biological advantage of these findings on the preventive effects of *G. biloba* on corticosterone hypersecretion needs to be investigated in further research.

References

Brown GW, Bifulco A, Harris TO. Life events, vulnerability and onset of depression: some refinements. Br J Psychiatry 1987;150:30–42.

Continella G, Drago F. Behavioral effects of Ginkgo-biloba extract. In: Agnoli A, Rapin JR, Scapagnini V, Weitbrecht WV, editors. Effects of *Ginkgo biloba* extract on organic cerebral impairment. London: John Libbey; 1985. p. 35–42.

Field BH, Vadnar R. *Ginkgo biloba* and memory: an overview. Nutr Neurosci 1998;1:255–67.

Frankenhaeuser M. A psychobiological framework for research on human stress and coping. In: Appley M, Trumbull R, editors. Dynamics of stress. New York: Plenum; 1986. p. 101–15.

Henry JP, Meehan P. Psychosocial stimuli, physiological specificity, and cardiovascular disease. In: Weiner H, Hofer MA, Stunkard AJ, editors. Brain, behavior, and bodily diseases. New York: Raven Press; 1981. p. 305–33.

Kidd PM. A review of nutrients and botanicals in the integrative management of cognitive dysfunction. Altern Med Rev 1999;4(3):144–61.

Kleijnen J, Knipschild P. *Ginkgo biloba* for cerebral insufficiency. Br J Clin Pharmacol 1992;34:352–8.

Levine S, Ursin H. What is stress? In: Brown MR, Rivier C, Koob G, editors. Neurobiology and neuroendocrinology of stress. New York: Marcel Decker; 1991. p. 3–21.

Maes M, Meltzer H. The serotonin hypothesis of major depression. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press; 1995. p. 933–44.

Maier SF. Learned helplessness: relationship with fear and anxiety. In: Stanford SC, Salmon P, editors. Stress, from synapse to syndrome. London: Academic Press; 1995. p. 207–14.

Mandler G. Mind and body; psychology of emotion and stress. New York: Norton Press; 1984.

Marcilhac A, Dakine N, Bourhim N, Guillaume V, Grino M, Drieu K, et al. Effect of chronic administration of *Ginkgo biloba* extract or Ginkgolide on the hypothalamic–pituitary–adrenal axis in the rat. Life Sci 1998;62(25):2329–40.

Petkov VD, Kehayov R, Belcheva S, Konstantinova E, Petkov VV, Getova D, et al. Memory effects of standardized extracts of Panax ginseng (G115), *Ginkgo biloba* (GK 501) and their combination gincosan (PHL-00701). Planta Med 1993;59:106–14.

Porsolt RD, Martin P, Lenègre A, Fromage S, Drieu K. Effects of an extract of *Ginkgo biloba* (EGb 761) on "learned helplessness" and other models of stress in rodents. Pharmacol Biochem Behav 1990;36:963–71.

Rapin JR, Lamproglou I, Drieu K, Defeudis F. Demonstration of the "anti-stress" activity of an extract of *Ginkgo biloba* (EGb 761) using a discrimination learning task. Gen Pharmacol 1994;25(5):1009–16.

Rodriguez de Turco EB, Droy-Lefaix MT, Bazan NG. EGb 761 inhibits stress-induced polydipsia in rats. Physiol Behav 1993;53:1001–2.

Stoll S, Scheuer K, Pohl O, Muller WE. *Ginkgo biloba* extract (EGb 761) independently improves changes in passive avoidance learning and brain membrane fluidity in the aging mouse. Pharmacopsychiatry 1996;29:144–9.

Ursin H, Olff M. The stress response. In: Stanford SC, Salmon P, editors. Stress, from synapse to syndrome. London: Academic Press; 1993. p. 4–20.

Winter E. Effects of an extract of *Ginkgo biloba* on learning and memory in mice. Pharmacol Biochem Behav 1991;38:109–14.