D. JEZOVA[1,2], R. DUNCKO[1], M. LASSANOVA[2], M. KRISKA[2], F. MONCEK[1].

REDUCTION OF RISE IN BLOOD PRESSURE AND CORTISOL RELEASE DURING STRESS BY GINKGO BILOBA EXTRACT (EGB 761) IN HEALTHY VOLUNTEERS

[1]Laboratory of Pharmacological Neuroendocrinology,
Institute of Experimental Endocrinology, Slovak Academy of Sciences

[2]Department of Pharmacology, Medical Faculty, Comenius University, Bratislava, Slovakia

The standardized extract of Ginkgo biloba (EGb 761) was found not only to improve memory and aging associated cognitive deficits but also to exert beneficial effects on mood. An antistress action of the extract has been suggested but not directly proven. The present study was aimed to evaluate the effects of EGb 761 on salivary cortisol and blood pressure responses during stress in healthy young volunteers (n=70) in a double blind placebo controlled design. A stress model involving a combination of static exercise (handgrip) and mental stimuli was used. Single treatment with EGb 761 (120 mg) reduced stress-induced rise in blood pressure without affecting the heart rate. Salivary cortisol responses showed differences with respect to the gender and the time of day of the stress exposure, with the activation only in male subjects in the afternoon. This activation was absent if they were treated with EGb 761. The performance in a short memory test with higher scores achieved by women remained unaffected by EGb 761 treatment. Thus, this study provides evidence that EGb 761 has an inhibitory action on blood pressure and it may influence cortisol release in response to some stress stimuli.

K e y w o r d s : *EGb 761, stress, memory, hormones, cardiovascular*

INTRODUCTION

Standardized extract of Ginkgo biloba (EGb 761) is supposed to be a neuroprotective drug with significant and therapeutically useful effects on the central nervous system (CNS). It was found not only to improve memory and aging

associated cognitive deficits but also to exert beneficial effects on mood (1-4). Some authors observed antidepressive and anxiolytic effects of EGb 761 (5, 6) and an improvement of the ability of geriatric patients to cope with the demands of daily life (7). Thus, an interaction of EGb 761 with mechanisms involved in the stress response and adaptation to stressful conditions may be suggested (8).

Stress exposure evokes a broad spectrum of neuroendocrine changes, which depend on the nature and intensity of the stress stimulus as well as on the actual state of the subject. Stress stimuli (stressors) may include mental, somatic (hypoglycemia, exercise) or, most frequently, combined physicoemotional components. Important modifying factors are the gender and the time of day of stress exposure (9). The two main characteristics of the stress response are the activation of the sympathoadrenal system leading to a rise in blood pressure and stimulation of the hypothalamic-pituitary-adrenocortical (HPA) axis (9, 10). The individual components of the HPA axis are thought to play a crutial role in stress and its consequences. A significant proportion of patients suffering from depression or dementias exhibit increased cortisol secretion and glucocorticoids were found to induce detrimental effects on the CNS, particularly in the region of the hippocampus (11-13).

Nevertheless, the influence of EGb 761 on stress hormone release is very little understood. Amri et al. (14, 15) have shown an inhibitory effect of EGb 761 treatment on glucocorticoid biosynthesis. Chronic administration of EGb 761 attenuated surgically-induced increase in corticotropin-releasing hormone (CRH) secretion (16). Other preclinical studies in experimental animals, which indicate antistress actions of EGb 761 are based on the measurements of behavioral responses, such as learned helplesness (17), discrimination learning task (18) or stress-induced polydipsia (19). No data are available on possible interference of EGb 761 with cortisol release or its response to stress exposure in humans.

The present study was aimed to evaluate the effects of EGb 761 administration on salivary cortisol and blood pressure responses during stress in healthy young volunteers in a double blind placebo controlled design. Since there are not many mental stress models inducing apparent and reproducible rise in cortisol release (20), an attempt was made in the protocol used to combine mild mental (memory test) and somatic (static exercise) stressors.

SUBJECTS AND METHODS

Subjects

The study group consisted of 70 (33 male and 37 female) healthy volunteers (aged 20-30 years) recruited from students at the Medical Faculty of Comenius University in Bratislava. Subjects were included in the study if they were not currently taking any medication and had no history of using a preparation containing extracts from Ginkgo biloba. Subjects were excluded from the study if they were suffering from any somatic or mental disseases, or had control blood pressure higher than

140/90 mmHg. Women who were in luteal phase of their estrous cycle, pregnant or lactating were also excluded. The subjects gave written informed consent to participate after procedures and possible side effects were explained to them. The study has been carried out in accordance with the Declaration of Helsinki. The protocol was approved by the Ethical Committee of the Medical Faculty of Comenius University in Bratislava, Slovakia.

Drug treatment

The study was designed as a parallel, randomized, double blind, placebo-controlled trial. A single p.o. dose of 120 mg of standardized extract of Ginkgo biloba, EGb 761 (Tanakan sol, 40 mg/ml, Ipsen, France) or placebo were administered. The extract was mixed with a combination of fruit syrups to make the taste and aroma indistinguishable from placebo, prepared in a similar manner. Subjects were allowed to drink another glass of water.

Stress model

As the stressor, a combined stimulus consisting of mental load (memory test) and static exercise (handgrip) was applied. The study was performed during regular courses in pharmacology, with nonparticipating students present, which may have added to the mental component of the stress stimulus.

Handgrip. Each subject performed two bouts of 3 minutes (one bout with each hand) of static handgrip at 30% of maximum voluntary contraction which was previously determined. There were no rest intervals between consecutive bouts of exercise.

Short memory test. Memory performance was measured by the Subtest No. 9 of the Intelligence-Structure-Test (21), modified for local population. Subjects were provided with a table containing 25 words ordered into 5 categories and had to learn the content of the table in 3 minutes. During the evaluation period, which lasted 6 minutes, volunteers received a list of questions examining the number of remembered words. Number of correct answers is presented as raw score. According to Intelligence-Structure-Test Manual this parameter was further transformed into weight score for corresponding population group (university students between 20-35 years of age).

Procedures

Measurements were performed at 0730-1130 h (morning) and 1200-1730 h (afternoon) with the subjects in a sitting position during the whole trial. Control measurements were made just prior to the administration of EGb 761 or placebo. About 1 ml of saliva was collected into plastic tubes and kept frozen until analyzed, blood pressure and heart rate were recorded (Dinamap, Criticon, Stampa, USA), and the maximum voluntary contraction force of each hand was determined (dynamometer). In the 27th minute after the drug administration the subjects were asked to memorize 25 words in 3 minutes. Thirty minutes after the administration of the drug the second blood pressure and heart rate measurement was made and each subject performed isometric handgrip for 6 minutes. Blood pressure and heart rate were measured 3 and 6 min after beginning of handgrip with the cuff being positioned on the hand not doing the work. Immediately after handgrip the subjects were asked to complete the short memory test recalling the memorized set of words. One hour after the drug administration the second sample of saliva was obtained. Salivary cortisol was measured by a slight modification of the method described previously for plasma cortisol (22). Briefly, freshly thawed samples of saliva were centrifuged (2000g, 15 min) and cortisol concentrations were measured by radioimmunoassay in 200 μl of supernatant. ^{3}H-cortisol was used as the radioligand and antiserum prepared to cortisol-21-hemisuccinate-BSA was kindly

provided by C. Oliver, Marseille, France. Dextran coated charcoal was used to separate free and bound fractions.

Statistical analysis

Statistical analysis of blood pressure, heart rate, cortisol and short memory test score was performed using two-way repeated measures analysis of variance. When comparing two values of cortisol concentration in the same subject a paired t-test was used. Calculations were made using Jandel SigmaStat statistical software.

RESULTS

Blood pressure and heart rate

The stress model used resulted in a significant rise in both systolic ($F = 134.1$, $p<0.001$) and diastolic ($F = 155.7$, $p<0.001$) blood pressure. Single administration of EGb 761 had no effect on pre-exercise values, but resulted in a significant inhibition of blood pressure responses to handgrip. Two way Anova revealed a statistically significant effect of EGb 761 treatment on both systolic ($F = 4.1$, $p<0.05$) and diastolic ($F = 5.1$, $p<0.05$) blood pressure (*Fig. 1*). Heart rate responses to static exercise were similar in placebo and EGb 761 treated groups (data not shown).

Salivary cortisol

The pattern of cortisol response was dependent on the time of investigation. A stress-induced increase in salivary cortisol levels was observed in male subjects investigated in the afternoon but not in the morning (*Fig. 2*). This difference was statistically significant only using paired t-test ($p<0.05$). In women, salivary cortisol levels decreased after the stress exposure if the trial was performed in the morning and remained unchanged in the afternoon. Because the second (after stress) sample of saliva was collected 60 min later than the first (before stress) sample, the results are consistent with the usual daily rhythm of cortisol and indicate a lack of adrenocortical activation.

Administration of EGb 761 (*Fig. 3*) did not modify the pre-stress hormone levels, but in contrast to the placebo-treated group, the stress-induced rise in salivary cortisol concentration in male subjects investigated in the afternoon was prevented. In the same time period of investigation, no effect of stress exposure and no effect of EGb 761 treatment was observed in women.

Short memory test

As shown in Table 1, women exhibited a better performance in the Intelligence-Structure-Test compared to men after presentation of the data by both raw ($F = 9.6$, $p<0.01$) and weight ($F = 11.0$, $p<0.001$) scores. Administration of EGb 761 failed to modify the memory performance.
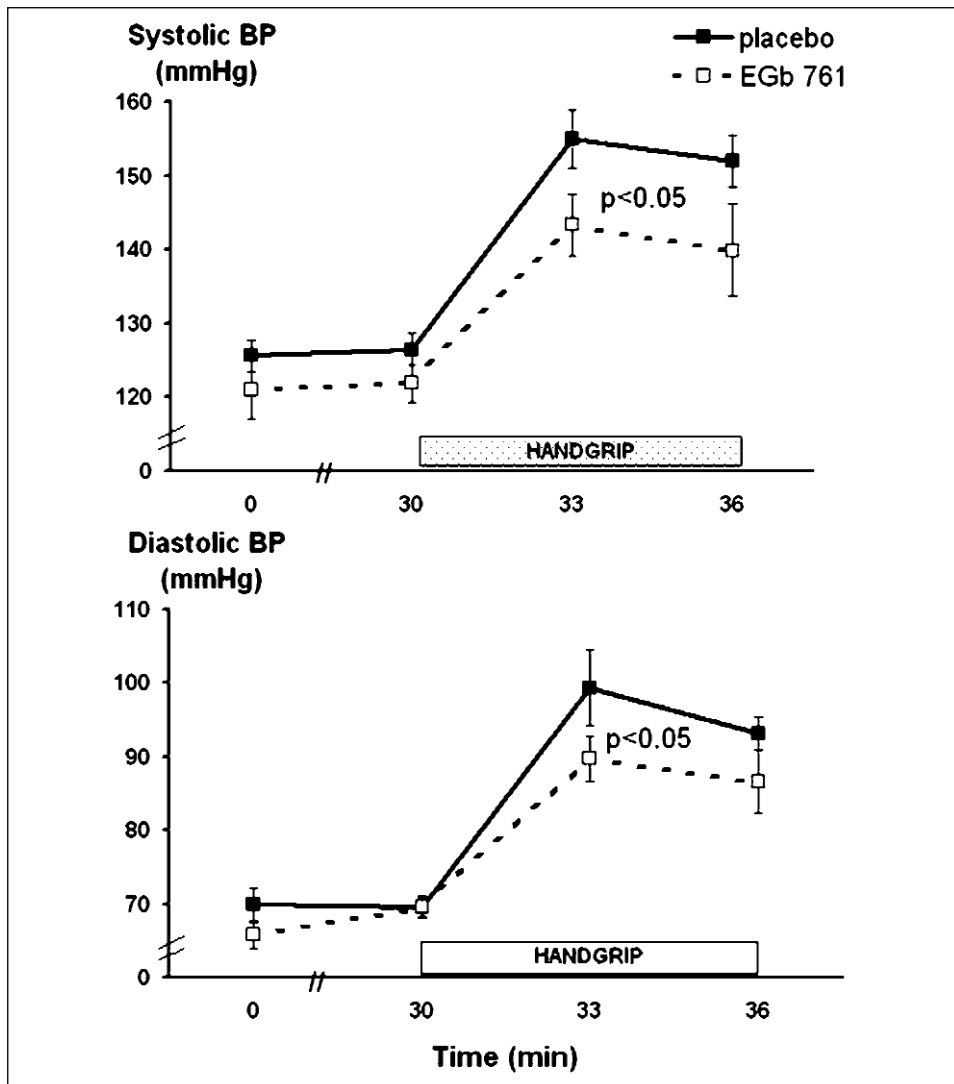
Fig. 1. The inhibitory effect of a single dose of EGb 761 (120 mg, p.o.) on stress induced elevation of systolic and diastolic blood pressure in healthy volunteers. Results represent means ± SEM.

Table 1. Performance in memory test (the Intelligence-Structure-Test) in healthy volunteers treated with a single dose of EGb 761 (120 mg, p.o.) or placebo.

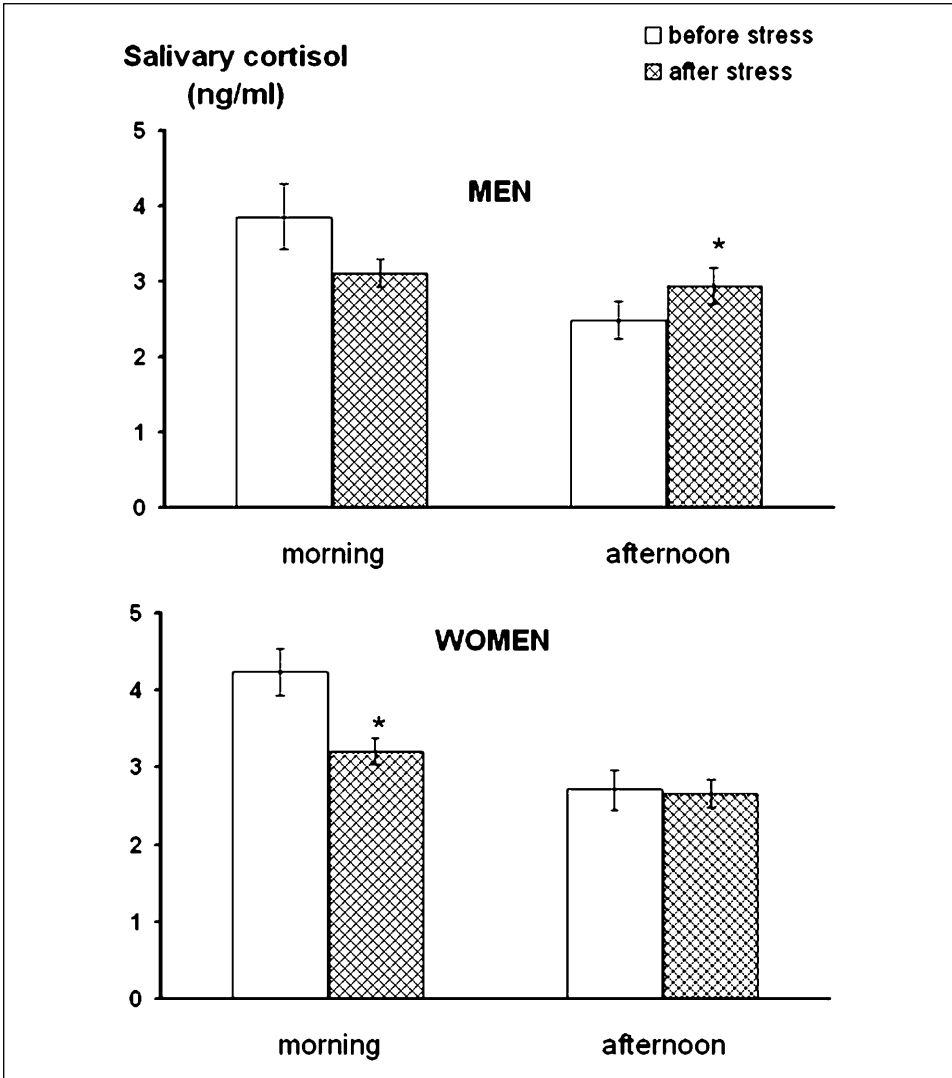|	Raw score		Weight score	
	placebo	EGb 761	placebo	EGb 761
men	12.1 0.7	12.4 0.8	91.2 3.6	97.2 2.8
women	14.9 0.9*	14.5 0.8*	106.6 3.8*	104.8 3.4*

* - men vs. women; p < 0.01

Fig. 2. Gender differences in stress-induced salivary cortisol release at different times of the day in healthy volunteers. Results represent means ± SEM. Statistical significance: * p<0.05 vs. appropriate before stress values.

DISCUSSION

The presented data demonstrate an inhibitory action of EGb 761 treatment on some components of the stress response. Single administration of EGb 761 resulted in a reduction of blood pressure elevation and inhibited the small rise in salivary cortisol levels after exposure of healthy volunteers to a combined stress stimulus consisting of mental load and static exercise.
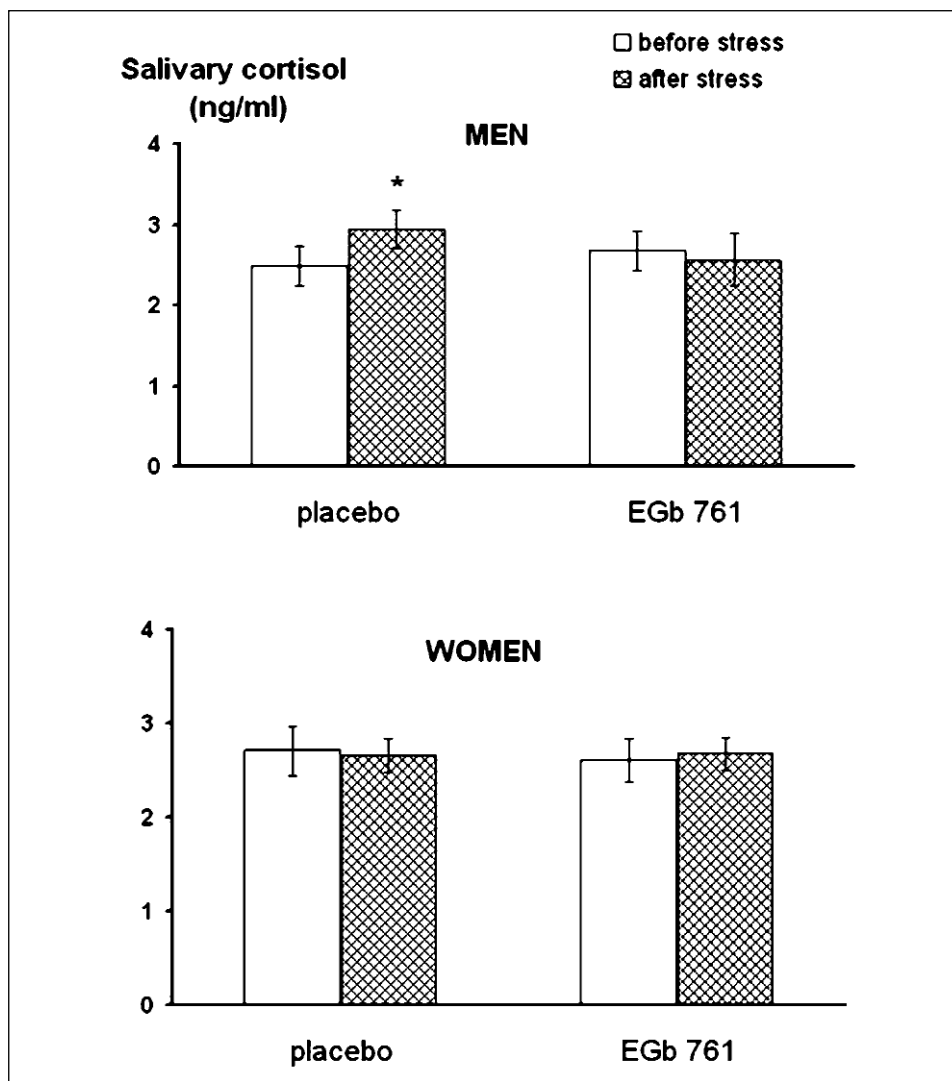
Fig. 3. The inhibitory effect of EGb 761 (single dose of 120 mg, p.o.) on stress-induced release of salivary cortisol in healthy volunteers investigated in the afternoon. Results represent means ± SEM. Statistical significance: * $p < 0.05$ vs. appropriate before stress values.

The effects of extracts of Ginkgo biloba on cardiovascular and hormone responses to stress stimuli in humans have not been studied previously, though an antistress action of EGb 761 has been suggested on the basis of clinical observations and studies in experimental animals (8). A well known component of the stress response is the sympathoadrenal activation with consequent rise in blood pressure and heart rate, which also occured during static exercise used in the present study (23). Single dose of EGb 761 reduced the blood pressure responses

without affecting the rise in heart rate. It should be noted that this action was induced after a single application of EGb 761, while the clinically useful effects of the drug on CNS function are generally expected after repeated treatment (24). However, single administration of EGb 761 was shown to induce other effects, namely to influence the performance in a memory scanning test (25), even though the dose of the drug used was relatively high (600 mg). The dose used in the present study corresponds to the suggested daily dose of the drug (120 mg) used in the treatment of cerebrovascular insuficiency. Accordingly, the performance in the short memory test remained unaffected. Higher scores achieved by women are in agreement with studies documenting sex differences in certain cognitive abilities (26, 27).

Another stress system approached in this investigation was the HPA axis, evaluated by measurement of salivary cortisol concentrations. Levels of cortisol in saliva reflect the free, biologically active fraction of plasma cortisol levels (28) and there is no need of venepuncture or other invasive sampling procedure. Cortisol release may be enhanced by a variety of somatic stressors (28, 30, 31), but does not exhibit profound changes in response to many mental stress model situations (20, 32). In the present study, a novel stress model involving a combination of somatic and mental stimuli was used. Two sub-threshold stimuli were applied, namely completion of a short memory test, which would not be suggested to induce cortisol release, and isometric handgrip shown previously to increase cardiovascular activity but not plasma cortisol levels (23). The mental component of this stress model was strengthened by the motivation to achieve the best performance possible because of the presence and encouragement by nonparticipating students. However, the model was not found to be intensive enough to induce a significant rise in salivary cortisol concentration in all subjects receiving placebo treatment. However, the posibility that there were changes at other time intervals cannot be excluded.

A different picture was obtained if some factors, known to modulate the stress response were considered. Neuroendocrine response during stress is specific, depending not only on the stress model used (33, 34) but also on several modifying factors, such as gender or the time of day of stress exposure (9). Adrenocortical function has an endogenous circadian rhythm with highest cortisol concentrations early in the morning, a decline during the day and a nadir at around midnight hours (35). As to the stress, enhanced hormone responses were observed if the stimulus was applied later in the day (36, 37, 31). As documented by the results of the present investigation, cortisol activation in response to the mild stress model used was enhanced in the male subjects in afternoon hours. It is an interesting finding, as cortisol response to other stress stimuli was previously found to be gender dependent, but higher activation was observed in women (29, 9). It is evident that the impact of gender on cortisol response during stress is specific with respect to the stress model used. The activation of cortisol release in male subjects investigated in the afternoon was

absent if they were treated with EGb 761, demonstrating an inhibitory potential of the drug on stress-induced cortisol release.

The mechanisms involved in the inhibitory action of EGb 761 on the stress response are unclear. As repeated administration of EGb 761 in experimental animals was found to reduce corticosterone biosynthesis via peripheral benzodiazepine receptors (14), it is possible that these receptors are involved also in the inhibition of stress-induced cortisol release in humans. However, in the mentioned study a single administration of EGb 761 failed to modify corticosterone levels. Other possibility is a central modulation of neurotransmitter system involved in the stress response. In vitro studies have shown that similar to antidepressants (38), EGb 761 inhibits the uptake of norepinephrine, dopamine and serotonine in rat brain synaptosomes (39). In addition, the mentioned in vitro study demonstrated that EGb 761 was active as inhibitor of radioligand binding to glutamate receptors of N-methyl-D-aspartic acid (NMDA) subtype and excitatory amino acids were found to participate in stress-induced activation of HPA axis (40). In accordance with the present findings, acute administration of some antidepressant drugs attenuated stress response in experimental animals (41, 42), while repeated antidepressant treatment was associated with an enhancement of stress induced neurochemical and hormonal changes (42, 43).

Though the mechanisms involved and the efficacy under repeated treatment remain to be elucidated, the present study provides evidence that EGb 761 has an inhibitory action on cardiovascular and neuroendocrine responses during stress.

REFERENCES

1. Christen Y, Costentin J, Lacour M. *Effects of Ginkgo biloba Extract (Egb 761) on the Central Nervous System.* Paris, Elsevier, 1992.
2. Hofferberth B. The effect of Ginkgo biloba on neurophysiological and psychometric measurement results in patients with psychotic organic brain syndrome. A double-blind study against placebo. *Arzneimittelforschung* 1989; 39: 918-922.
3. Itil T, Martorano D Natural substances on psychiatry (Ginkgo biloba in dementia). *Psychopharmacol Bull;* 1995 31: 147-158
4. Subhan Z, Hindmarch I. The psychopharmacological effects of Ginkgo biloba extract in normal healthy volunteers. *Int J Clin Pharmacol Res* 1984; 4: 89-93.
5. Halama P, Bartsch G, Meng G. Disorders of brain performance of vascular origin. Randomized double-blind study of the effectiveness of Gingko biloba extract. *Fortschritte Med* 1988; 106: 408-412.

6. Schubert H, Halama P. Depressive episode primarily unresponsive to therapy in elderly patients: efficacy of Ginkgo biloba extract (Egb 761) in combination with antidepressants. *Geriatrische Forschung* 1993; 3: 45-53.

7. Wesnes K, Simmons D, Rook M, Simpson P. A double-blind placebo-controlled trial of Tanakan in the treatment of idiopathic cognitive impairment in the elderly. *Human Psychopharmacology* 1987; 2: 159-169.

8. Clostre F. Ginkgo biloba extract (EGb 761). State of knowledge in the dawn of the year 2000. *Ann pharmaceutiques francaises* 1999; 57: 1S8-88.

9. Jezova D, Jurankova E, Mosnarova A, Kriska M, Skultetyova I. Neuroendocrine response during stress with relation to gender differences. *Acta Neurobiol Exp* 1996; 56: 779-785.

10. Stratakis CA, Chrousos GP. Neuroendocrinology and pathophysiology of the stress system. In *Stress. Basic mechanisms and clinical implications*, GP Chrousos, R McCarty, K Pacak, G Cizza, E Sternberg, PW Gold, R Kvetnansky (eds). New York, New York Academy of Sciences,1995, pp. 1-18.

11. Holsboer F, Barden N, Antidepresants and hypothalamic-pituitary-adrenocortical regulation. *Endocrine Rev* 1996; 17: 187-205.

12. Lupien SJ, Nair NP, Briere S, Maheu F, Tu MT, Lemay M, McEwen BS, Meaney MJ. Increased cortisol levels and impaired cognition in human aging: implication for depression and dementia in later life. *Rev Neurosci* 1999; 10: 117-139.

13. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology* 2000; 22: 108-124.

14. Amri H, Ogwuegbu SO, Boujrad N, Drieu K, Papadopoulos V. In vivo regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by Ginkgo biloba extract EGb 761 and isolated ginkgolides. *Endocrinology* 1996; 137: 5707-5718.

15. Amri H, Drieu K, Papadopoulos V. Ex vivo regulation of adrenal cortical cell steroid and protein synthesis, in response to adrenocorticotropic hormone stimulation, by the Ginkgo biloba extract EGb 761 and isolated ginkgolide B. Endocrinology 1997; 138: 5415-5426.

16. Marcilhac A, Dakine N, Bourhim N, Guillaume V, Grino M, Drieu K, Oliver C. Effect of chronic administration of Ginkgo biloba extract or Ginkgolide on the hypothalamic-pituitary-adrenal axis in the rat. *Life Sci* 1998; 62: 2329-2340.

17. Porsolt RD, Martin P, Lenegere 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: 963-971.

18. Rapin JR, Lamproglou I, Drieu K, DeFeudis FV. Demonstration of the "anti-stress" activity of an extract of Ginkgo biloba (EGb 761) using a discrimination learning task. *Gen Pharmacol* 1994; 25: 1009-1016.

19. Rodriguez de Turco EB, Droi-Lefaix MT, Bazan N. EGb 761 inhibits stress induced polydipsia in rats. *Physiol Behav* 1993; 53: 1001-1002.

20. McCleery JM, Bhagwagar Z, Smith KA, Goodwin GM, Cowen PJ. Modeling a loss event: effect of imagined bereavement on the hypothalamic-pituitary-adrenal axis. *Psychol Med* 2000; 30: 219-223.

21. Amthauer R. *Intelligenz-Struktur-Test, Handanweisung für Durchfürung und Auswertung.* Göttingen, Verlag für Psychologie-Dr.C.J. Hogrefe,1955.

22. Jezova D, Vigas M. Apomorphine injection stimulates beta-endorphin, adrenocorticotropin, and cortisol release in healthy man. *Psychoneuroendocrinology* 1988; 13: 479-485.

23. Nazar K, Jezova D, Kowalik-Borowka E. Plasma vasopressin, growth hormone and ACTH responses to static handgrip in healthy subjects. *Eur J Appl Physiol* 1989; 58: 400-404 .

24. Stough C, Clarke J, Lloyd J, Nathan PJ. Neuropsychological changes after 30-day Ginkgo biloba administration in healthy participants. *Int Neuropsychopharmacol* 2001; 4: 131-134.

25. Allard M. Effects of Ginkgo biloba extract 761 on normal or altered memory function in man. In *Effects of Ginkgo biloba Extract (Egb 761) on the Central Nervous System,* Y Christen, J Costentin, M Lacour (eds). Paris, Elsevier, 1992, pp. 147-157.

26. Anooshian LJ, Seibert PS. Conscious and unconscious retrieval in picture recognition: a framework for exploring gender differences. *J Personality Soc Psychol* 1996; 70: 637-645.

27. Mann VA, Sasanuma S, Sakuma N, Masaki S. Sex differences in cognitive abilities: a cross-cultural perspective. *Neuropsychologia* 1990; 28: 1063-1077.

28. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology* 1989; 22: 150-169.

29. Jezova D, Kvetnansky R, Vigas M. Sex differences in endocrine response to hyperthermia in sauna. *Acta Physiol Scand* 1994; 150: 293-298.

30. Jezova D, Kvetnansky R, Nazar K, Vigas M. Enhanced neuroendocrine response to insulin tolerance test performed under increased ambient temperature. *J Endocrinol Invest* 1998; 21: 412-417.

31. Scheen AJ, Buxton OM, Jison M, Van Reeth O, Leproult R, L'Hermite-Baleriaux M, Van Cauter E. Effects of exercise on neuroendocrine secretions and glucose regulation at different times of day. *Am J Physiol* 1998; 274: E1040-1049.

32. Skosnik PD, Chatterton RT Jr, Swister T, Park S. Modulation of attentional inhibition by norepinephrine and cortisol after psychological stress. *Int Psychophysiol* 2000; 36: 59-68.

33. Jezova D, Skultetyova I. Neuroendocrine response in stress - a specific activation by individual stressors. *Arch Physiol Biochem* 1997; 105: 233-237.

34. Vigas M, Kvetnansky R, Jurcovicova J, Jezova D, Tatar P. Comparison of catecholamine and adenopituitary hormone responses to various stress stimuli in man. In *Stress: The Role of Catecholamines and Other Neurotransmitters,* E Usdin, R Kvetnansky, J Axelrod (eds). New York, Gordon and Breach Science Publishers, 1984, pp.865-882.

35. Krieger DT. Rhythms in CRF, ACTH, and corticosteroids. In *Endocrine Rhythms,* DT Krieger (ed). New York, Raven Press, 1979, pp. 132-141.

36. Nathan RS, Sachar EJ, Langer G, Tabriyi MN, Halpern FS. Diurnal variations in the response of plasma prolactin, cortisol, and growth hormone to insulin induced hypoglycemia in normal men. *J Clin Endocrinol Metab* 1979; 49: 231-235.

37. Sauseng-Fellegger H, Koenig EM, Hinghofer-Szalkay H, Jezova D, Vigas M. Testing of neuroendocrine function in astronauts as related to fluid shifts. *Acta Astronautica* 1992; 27: 55-60.

38. Stahl SM. Basic psychopharmacology of antidepressants, part 1: Antidepressants have seven distinct mechanisms of action. *J Clin Psychiatry* 1998; 59: 5-14.

39. Taylor JE. In vitro interactions of EGb 761 with biogenic amine uptake sites and NMDA receptors. In *Effects of Ginkgo biloba Extract (Egb 761) on the Central Nervous System,* Y Christen, J Costentin, M Lacour (eds). Paris, Elsevier, 1992, pp.1-6.

40. Jezova D, Tokarev D, Rusnak M. Endogenous excitatory amino acids are involved in stress-induced adrenocorticotropin and catecholamine release. *Neuroendocrinology* 1995; 62: 326-32.

41. Connor TJ, Kelliher P, Harkin A, Kelly JP, Leonard BE. Reboxetine attenuates forced swim test-induced behavioural and neurochemical alterations in the rat. *Eur J Pharmacol* 1999; 379:125-133.

42. Lino-de-Oliveira C, Sales AJ, Del Bel EA, Silveira MC, Guimaraes FS. Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression. *Brain Res Bull* 2001; 55: 747-754.

43. Jezova D, Duncko R. Enhancement of stress-induced pituitary hormone release and cardiovascular activation by antidepressant treatment in healthy men. *J Psychopharmacol* 2002; 16: (in press).

Author's address: Daniela Jezova, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, Bratislava 83306, Slovakia. Telephone: 421-2-54773800, fax: 421-2-54774247,
E-mail: ueenjezo@savba.savba.sk