

## Regular Article

# The effect of extract of ginkgo biloba addition to olanzapine on therapeutic effect and antioxidant enzyme levels in patients with schizophrenia

MURAD ATMACA, MD,<sup>1</sup> ERTAN TEZCAN, MD,<sup>1</sup> MURAT KULOGLU, MD,<sup>1</sup>  
BILAL USTUNDAG, MD<sup>2</sup> AND OZLEM KIRTAS, MD<sup>1</sup>

*Departments of <sup>1</sup>Psychiatry and <sup>2</sup>Clinical Biochemistry, Firat University, School of Medicine, Elazig, Turkey*

### Abstract

It has been suggested that the extract of ginkgo biloba (EGb) may enhance the efficiency of the classic antipsychotic haloperidol in patients with chronic schizophrenia, especially on positive symptoms, and reduce serum superoxide dismutase (SOD) levels. Therefore, we decided to evaluate the therapeutic effect of EGb and to examine the effect of it on the levels of antioxidant enzymes in schizophrenic patients on olanzapine treatment. We hypothesized that EGb would have the beneficial effects on schizophrenic symptoms and might cause reductions in antioxidant enzymes. The subjects were randomly assigned to the two groups: olanzapine plus EGb (group I) ( $n = 15$ ) and olanzapine alone (group II) ( $n = 14$ ). The patients were evaluated at baseline and at week 8 with respect to the Positive and Negative Syndrome Scale (PANSS), serum SOD, catalase (CAT), and glutathion peroxidase (GPX) levels. At baseline, no statistically significant difference regarding the mean total PANSS scores between treatment groups was found. At the evaluation of week 8, a significant difference in mean Scale for the Assessment of Positive Symptoms (SAPS) scores but not in Scale for the Assessment of Negative Symptoms scores between groups was found. Total patients had statistically significant higher serum SOD, CAT and GPX levels compared to control groups at baseline. At 8 weeks, there were significant differences in the mean decrease in SOD and CAT levels but not in GPX levels between treatment groups. The changes in SOD and CAT levels were correlated with the change in SAPS in group I, but not in the group II. The present study supported the findings of the previous study demonstrating that EGb might enhance the efficiency of antipsychotic in patients with schizophrenia, particularly on positive symptoms of the disorder.

**Key words** antioxidant enzymes, free radical, ginkgo biloba, olanzapine.

## INTRODUCTION

Free radicals are oxygen-containing chemical substances which are generated under physiological conditions during aerobic metabolism.<sup>1</sup> A major source of radicals in biological systems is dioxygen ( $O_2$ ). The radicals originating from molecular oxygen are generally named as reactive oxygen species (ROS). When free

radicals are generated in excess or the enzymatic and non-enzymatic antioxidant defense systems are inefficient, they can stimulate some chain reactions causing cellular injury or even death of cells.<sup>2</sup> Therefore, a number of investigations have examined free radicals in the pathophysiology of neurodegenerative and psychiatric disorders and suggested that excess free radical formation occurs in schizophrenia and depression,<sup>3,4</sup> although limited studies have been carried out in anxiety disorders (e.g. obsessive-compulsive disorder),<sup>5</sup> and social phobia.<sup>6</sup> Numerous trials have shown that scavenging enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathion peroxidase (GPX) and antioxidant agents may attenuate the damaging effects

Correspondence address: Murad Atmaca, MD, Firat (Euphrates) Universitesi, Firat Tip Merkezi, Psikiyatri Anabilim Dalı, 23119, Elazig, Turkey. Email: matmaca\_p@yahoo.com

Received 8 November 2004; revised 12 May 2005; accepted 26 June 2005.

of free radicals. To date there have been only a few studies carried out on antioxidant treatment of schizophrenia.<sup>7</sup> In contrast, extract of ginkgo biloba (EGb), an important antioxidant agent, has not been examined enough. In only one study, it has been suggested that EGb may enhance the efficiency of the classic antipsychotic haloperidol in patients with chronic schizophrenia, especially on positive symptoms.<sup>8</sup>

Therefore, we decided to evaluate the therapeutic effect of EGb and to examine its effect on the levels of antioxidant enzymes in schizophrenic patients on olanzapine treatment and hypothesized that EGb would have the beneficial effects on schizophrenic symptoms and might cause the reductions in antioxidant enzymes.

## METHODS AND MATERIALS

Of the patients who had applied to Firat University School of Medicine Department of Psychiatry (Elazig, Turkey) and were given olanzapine, with the diagnosis of schizophrenia according to DSM-IV, 37 who gave written informed consent after complete description of the study participated in the study. The study protocol was approved by the Local Ethics Committee of the Firat University School of Medicine. Clinical evaluation was performed by one trained psychiatrist for all patients. A semistructured interview was carried out in order to establish DSM-IV diagnosis. The patients with comorbid axis I disorder were excluded. All patients received monotherapy consisting of olanzapine, and the only concomitant medications permitted were biperiden hydrochloride ( $n=0$ ) and benzodiazepine ( $n=3$ ) derivatives. The daily dose of olanzapine ranged from 5 to 20 mg (the mean dose = 16.8 mg/day). The doses were stabilized 1 month ago before the participation of the study. Before being treated with olanzapine, 19 patients were treated with conventional antipsychotics, six patients were treated with risperidone, and four patients had no pharmacological treatment. In contrast, no patient had taken depot antipsychotics before olanzapine administration.

Twenty-nine available healthy control subjects according to exclusion criteria were chosen from among the hospital staff. None had had any personal and family history of psychiatric disorder.

Exclusion criteria included alcohol and substance abuse or dependence, presence of severe organic condition, users of any antioxidant agent (i.e. E and C vitamins), presence of epilepsy and severe neurologic disorder, presence of infectious disease, and excessive obesity. No difference was noted in the number of cigarettes smoked per day and the number of smokers among the groups. From the 37 patients constituting

the initial sample, eight were excluded from the study because of exclusion criteria.

The subjects were randomly assigned to two groups: olanzapine plus EGb (group I) ( $n=15$ ) and olanzapine alone (group II) ( $n=14$ ). The assessor of Scale for the Assessment of Negative Symptoms (SANS) and Scale for the Assessment of Positive Symptoms (SAPS) was knowing that the subject received EGb or not. The present study was single-blind. EGb (150 mg b.i.d) was added to olanzapine.

All subjects were evaluated using a semistructured questionnaire which was arranged by the authors in accordance with clinical experience and available information sources. The patients were evaluated at the baseline and week 8 with respect to the Positive and Negative Syndrome Scale (PANSS),<sup>9</sup> SOD, CAT and GPX levels.

Venous blood samples from left forearm vein were collected into 5 mL vacutainer tubes containing potassium EDTA between 07.00 and 08.00 h after overnight fasting. Some hematological parameters were measured by using an autoanalyzer (Coulter Max M, Coulter Electronics Ltd, Luton, UK). Data on smoking were obtained from each patient using a questionnaire 1 day before blood drawing. Smoking was not permitted after 23.00 h, 1 day before blood drawing.

The blood samples were centrifuged at 4000 r.p.m. for 10 min at 4°C to remove plasma. The buffy coat on the erythrocytes sediment was separated carefully after plasma was removed and was used in the assay of MDA levels. The erythrocyte sediment was washed three times with 10-fold isotonic NaCl solution to remove plasma remnant.

Hemolysates of erythrocytes were used for measurement of total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity levels according to the method of Sun *et al.*<sup>10</sup> This method is based on reduction of superoxide, which is produced by the xanthine oxidase enzyme system, by nitroblue tetrazolium. GPX (EC 1.6.4.2) activity levels in hemolysates of erythrocytes were measured using the method of Paglia and Valentine<sup>11</sup> in which GPX activity was coupled to the oxidation of NADPH by glutathione reductase. CAT (EC 1.11.1.6) activity was determined by the method Aebi.<sup>12</sup> The principle of the assay is based on the determination of the rate constant  $k$  (dimension:  $s^{-1}$ ) of the hydrogen peroxide decomposition.

Statistical analysis was performed using the statistical package for social sciences (SPSS/PC 9.05 version, 1998; SPSS Inc, Chicago, IL, USA). The  $\chi^2$  test was used to compare categorical variables. Group mean differences were examined by means of the Student's  $t$ -test or analysis of variance (ANOVA). Correlation analysis was performed by Pearson's correlations and

Spearman's rank correlations test, whenever appropriate. Differences were considered significant at  $P < 0.05$  for all these tests.

## RESULTS

All but two patients (one from group I and one from group II) completed the 8-week treatment period. The mean age was  $27.1 \pm 7.3$  years in group I and  $28.7 \pm 8.8$  years in group II ( $P > 0.05$ ). The mean duration of illness was  $5.6 \pm 3.2$  and  $6.1 \pm 4.1$  years in groups I and II, respectively ( $P > 0.05$ ). With respect to the sex distribution, there were seven females and eight males in group I, and eight females and six males in group II ( $P > 0.05$ ). As can be seen, there were no significant differences among groups from the point of general characteristics of the patients. At the week 8 evaluation, the mean doses were  $14.7 \pm 6.8$  mg/day for group I and  $13.8 \pm 5.4$  mg/day for group II ( $P > 0.05$ ).

At the beginning of the study, total patients had statistically significant higher serum SOD ( $P < 0.01$ ), CAT ( $P < 0.05$ ) and GPX levels ( $P < 0.05$ ) compared to control groups. No statistically significant differences were found between group I and II subjects with regard to SOD, CAT and GPX levels ( $P > 0.05$ ). The SOD levels decreased  $224.7 \pm 29.3$  U/g Hb, from a mean of  $1211.1 \pm 234.2$  U/g Hb to  $986.4 \pm 200.1$  U/g Hb in group I ( $P < 0.01$ ), and decreased  $48.6 \pm 18.5$  U/g Hb, from a mean of  $1238.5 \pm 211.9$  U/g Hb to  $1189.9 \pm 284.8$  U/g Hb in group II ( $P > 0.05$ ). The CAT levels decreased  $58.9 \pm 12.1$ /kg Hb, from a mean of  $298.6 \pm 42.3$ /kg Hb to  $239.7 \pm 37.1$ /kg Hb in group I ( $P < 0.01$ ), and decreased  $13.8 \pm 11.6$ /kg Hb, from a mean of  $285.3 \pm 34.9$ /kg Hb to  $271.5 \pm 28.9$ /kg Hb in group II ( $P > 0.05$ ). The GPX levels decreased  $10.1 \pm 2.8$  U/g Hb, from a mean of  $34.4 \pm 4.8$  U/g Hb to  $24.3 \pm 3.7$  ng/mL in group I

( $P < 0.05$ ), and decreased  $7.1 \pm 1.9$  U/g Hb, from a mean of  $33.2 \pm 5.3$  U/g Hb to  $26.1 \pm 5.2$  U/g Hb in group II ( $P < 0.05$ ). At the week 8 evaluation, there were significant differences in the mean decrease in SOD ( $P < 0.01$ ) and CAT ( $P < 0.05$ ) levels but not in GPX ( $P > 0.05$ ) levels between the treatment groups (Table 1).

At baseline, no statistically significant difference regarding the mean total PANSS scores between groups, with also no differences on SANS and SAPS was found ( $P > 0.05$ ). The mean changes in SAPS for groups I and II were  $-9.4$  (SD = 1.6) and  $-3.8$  (SD = 0.8), respectively ( $P < 0.05$ ). At the week 8 evaluation, a significant difference in mean SANS between groups was found ( $P < 0.05$ ). The mean changes in SANS for groups I and II were  $-3.4 \pm 1.1$  and  $-2.7 \pm 0.8$ , respectively ( $P > 0.05$ ). At the week 8 evaluation, no significant difference in the mean SANS scores between groups was found ( $P > 0.05$ ).

The changes in SOD and CAT levels were correlated with the change in SAPS in group I ( $r = 0.56$ ,  $P < 0.05$  for SOD, and  $r = 0.51$ ,  $P < 0.05$  for CAT), but not in group II ( $r = 0.10$ ,  $P > 0.05$  for SOD, and  $r = 0.04$ ,  $P > 0.05$  for CAT). No significant correlation was observed between changes in GPX levels and SANS or SAPS scores in either group ( $r = 0.21$ ,  $P > 0.05$  for group I, and  $r = 0.07$ ,  $P > 0.05$  for group II).

## DISCUSSION

It was found that antioxidant enzyme levels (all of SOD, CAT and GPX) were elevated in patients with schizophrenia compared to healthy controls and that the mean decrease in SAPS scores for the EGb addition group was significantly higher than that in the placebo addition group. In contrast, at the week 8 eval-

**Table 1.** Antioxidant enzyme levels and scale scores in groups

Groups	SOD (U/g Hb)	GPX (U/g Hb)	CAT (/kg Hb)	SANS	SAPS
Group I ( $n = 15$ )					
Baseline	$1211.1 \pm 234.2^*$	$34.4 \pm 4.8^{**}$	$298.6 \pm 42.3^*$	$47.6 \pm 7.1$	$30.3 \pm 7.1^{**}$
Week 8	$986.4 \pm 134.2$	$24.3 \pm 3.7$	$239.7 \pm 37.1$	$44.2 \pm 5.7$	$20.9 \pm 6.0$
Group II ( $n = 14$ )					
Baseline	$1238.5 \pm 211.9$	$33.2 \pm 5.3^{**}$	$285.3 \pm 34.9$	$45.4 \pm 7.2$	$31.2 \pm 6.7$
Week 8	$1189.9 \pm 284.8$	$26.1 \pm 5.2$	$271.5 \pm 28.9$	$42.7 \pm 6.1$	$27.4 \pm 8.5$
Controls ( $n = 15$ )	$961.6 \pm 100.2^{***}$	$24.7 \pm 3.9^{****}$	$260.8 \pm 27.8^{****}$	–	–

Comparison between before and after treatment:  $*P < 0.01$ ,  $**P < 0.05$ . Comparison among treatment groups and controls at baseline:  $***P < 0.01$ ,  $****P < 0.05$ .

SOD, superoxide dismutase; GPX, glutathion peroxidase; CAT, catalase; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Postive Symptoms.

uation, in EGb group there were significant higher decreases in the mean SOD and CAT levels but not in GPX levels compared to placebo addition group.

The finding that antioxidant enzyme levels (all of SOD, CAT and GPX) were elevated in patients with schizophrenia compared to healthy controls in the present study was in accordance with earlier reports. Yao *et al.* showed that SOD activity but not GPX and CAT activities in schizophrenia during the drug-free period was significantly higher compared to normal controls.<sup>13</sup> In another study, it was reported that the levels of plasma uric acid, a potent antioxidant, in patients with schizophrenia were significantly lower than those of healthy controls.<sup>14</sup> It has also been reported that the most important source of free radicals is glial cells and free radicals produced by this cell group are related to neuropsychiatric disorders such as Sydenham's Korea and Parkinson disease, etc.<sup>15</sup> Herken *et al.* who investigated the importance of free radicals in schizophrenia subtypes reported that oxidative stress might have a pathophysiological role in all subtypes of schizophrenia.<sup>16</sup> Another study demonstrated that antioxidative defense mechanisms might be impaired in schizophrenic patients.<sup>17</sup> In our previous study, significant differences between lipid peroxidation product (MDA) and antioxidant enzymes (SOD and GPX) activity levels in schizophrenia and bipolar disorder patients compared to controls led us to believe that these differences might be related with the heterogeneities in etiologies of these disorders.<sup>3</sup> The catecholamines including dopamine and norepinephrine are associated with the production of free radicals, thus conditions causing increased catecholamine metabolism may increase the radical burden, as in hyperdopaminergic hypothesis of schizophrenia.<sup>18</sup> In contrast, it was reported that increased free radical production might cause the destruction of phospholipids and altered viscosity of neuron membranes, change in membrane viscosity may affect serotonergic and catecholaminergic receptor functions.<sup>19</sup>

Another major finding of the present study is that subchronic EGb treatment has caused reductions in the SAPS score and correlated antioxidant enzyme reductions in the EGb addition group compared to the other group. In view of the involvement of free radicals in the etiopathogenesis of schizophrenia, it has been suggested that the antioxidant treatment of schizophrenia can reduce the damaging effect of free radicals. So far, limited studies regarding the efficacy of antioxidant treatment of schizophrenia have been carried out. First, an important reduction in positive symptoms using vitamin E has been obtained by Lohr and Browning.<sup>7</sup> Afterwards it has been reported that EGb has a positive effect in conditions associated with excess free

radical formation including cerebral insufficiency, etc.<sup>20</sup> In contrast, there is only one study evaluating the efficiency of EGb addition in patients with schizophrenia on haloperidol treatment.<sup>8</sup> The present study has confirmed the effectivity of antioxidant agent addition in patients ongoing antipsychotic treatment. The oxidation of catecholamines such as dopamine, and norepinephrine by monoamineoxidase may result in increased radical burden. Increased free radical formation in schizophrenia, therefore, may be explained by means of the classical hyperdopaminergic concept in that disorder. As a result, it may be speculated that the decreases in especially SAPS scores and correlated antioxidant enzyme levels could be part of a cascade of reactions initiated by the EGb because positive symptoms are frequently associated with hyperdopaminergic activity. The finding that the changes in SOD and CAT levels were correlated with the change in SAPS supports opinions aforementioned.

There are some methodological limitations to this study that must be acknowledged. First, the relatively small sample size might not be completely representative of schizophrenic patients on schizophrenia. Second, we could not control some confounding factors related to outpatient habits (i.e. exercise, lifestyle, etc), which might be related to antioxidant enzymes levels. Third, dietary changes may affect the production of free radicals although the patients and controls came from a similar socioeconomic level and had similar characteristics regarding age, female/male ratio, and smoking status. Fourth, we did not use a placebo-control group. In addition, we did not evaluate the effect of EGb on the side-effects of olanzapine (extrapyramidal side-effects and others). Fifth, the condition that we did not measure the products of lipid peroxidation such as malondialdehyde was another limitation of the study. Last, another important limitation is that our study did not have a wash-out period before starting olanzapine or olanzapine plus ginkgo biloba treatment. In conclusion, the present study supported the findings of a previous study<sup>8</sup> which is single trial about this subject and proposed that EGb might enhance the efficiency of antipsychotics in patients with schizophrenia on olanzapine treatment, particularly on positive symptoms of the disorder. The study requires replication using larger number of patients and controls.

## REFERENCES

1. Mahadik SP, Mukherjee S. Free radical pathology and antioxidant defense in schizophrenia: a review. *Schizophr. Res.* 1996; **19**: 1–17.
2. Stadtman ER. Protein oxidation and aging. *Science* 1992; **257**: 1220–1224.

3. Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan E, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels of patients with schizophrenia and bipolar disorder. *Cell Biochem. Funct.* 2002; **20**: 171–175.
4. Bilici M, Efe H, Koroglu MA, Uydu HA, Bekaroglu M, Deger O. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J. Affect. Disord.* 2001; **64**: 43–51.
5. Kuloglu M, Atmaca M, Tezcan E, Gecici O, Tunckol H, Ustundag B. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. *Neuropsychobiology* 2002; **46**: 27–32.
6. Atmaca M, Kuloglu M, Tezcan E, Ustundag B. Antioxidant enzyme and malondialdehyde levels in patients with social phobia. *Psychiatry Res.* (in press).
7. Lohr JB, Browning JA. Free radical involvement in neuropsychiatric illnesses. *Psychopharmacol. Bull.* 1995; **31**: 159–165.
8. Zhang XY, Zhou DF, Su JM, Zhang PY. The effect of extract of ginkgo biloba added to haloperidol on superoxide dismutase in patients with schizophrenia. *J. Clin. Psychopharmacol.* 2001; **21**: 85–88.
9. Andreasen NC. *Scale for the Assessment of Negative Symptoms (SANS)*. Department of Psychiatry College of Medicine, The University of Iowa, Iowa City, 1983.
10. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin. Chem.* 1988; **34**: 497–500.
11. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 1967; **70**: 158–168.
12. Aebi H. Catalase. In: Bergmeyer HU (ed.). *Methods of Enzymatic Analysis*. Academic Press, New York, 1974; 673–677.
13. Yao JK, Reddy R, McElhinny LG, van Kammen DP, 1998. Reduced status of plasma total antioxidant capacity in schizophrenia. *Schizophr. Res.* 1998; **32**: 1–8.
14. Yao JK, Reddy R, van Kammen DP. Reduced level of plasma antioxidant uric acid in schizophrenia. *Psychiatry Res.* 1998; **80**: 29–39.
15. Lohr JB. Oxygen radicals and neuropsychiatric illness: some speculations. *Arch. Gen. Psychiatry* 1991; **48**: 1097–1106.
16. Herken H, Uz E, Özyurt H, Sođüt S, Virit O, Akyol O. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. *Mol. Psychiatry* 2001; **6**: 66–73.
17. Altuntas I, Aksoy H, Coskun I, Caykoylu A, Akcay F. Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. *Clin. Chem. Lab. Med.* 2000; **38**: 1277–1281.
18. Graham DG. On the origin and significance of neuromelanin. *Arch. Pathol. Lab. Med.* 1979; **103**: 359–362.
19. Van der Vliet A, Bast A. Effect of oxidative stress on receptors and signal transmission. *Chem. Biol. Interact* 1992; **85**: 95–116.
20. Kleijnen J, Knipschild P. Ginkgo biloba for cerebral insufficiency. *Br. J. Clin. Pharmacol.* 1992; **34**: 352–358.