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Ginkgo biloba Enhances the Anticonvulsant and Neuroprotective Effects of Sodium Valproate Against Kainic Acid-induced Seizures in Mice

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ABSTRACT

Multiple side effects of commonly used antiepileptic drugs which become obvious with high doses and in combination therapy directed researches toward the possible use of natural products in management strategies of epilepsy. *Ginkgo biloba* extract (GbE-761) is a herbal product that has promising anticonvulsant and antioxidant properties. The aim of this work was to study the effect of GbE-761 on the anticonvulsant and neuroprotective effects of Sodium Valproate (SVA) and studying the possible mechanisms of this effect. The anticonvulsant activity of SVA (200 mg kg^{-1}, i.p.) and its combination with GbE-761 (25 and 50 mg kg^{-1}, orally), was tested against Kainic Acid (KA)-induced seizures in mice. The corresponding changes in brain glutamate, lipid peroxidation, Glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity were investigated. Moreover, levels of serum Neuron-specific Enolase (NSE), serum and brain 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured. Addition of GbE-761 to SVA enhanced the anticonvulsant activity of the latter against KA-induced seizures. This effect was accompanied by a decrease in brain glutamate and lipid peroxidation and increase in brain GSH levels and GSH-Px activity relative to its levels in SVA-treated animals. Moreover, serum NSE and serum and brain 8-OHdG levels significantly decreased by combined SVA and GbE-761 treatment than its levels in animals treated with SVA alone. Results of this study indicate that GbE-761 augments the anticonvulsant and neuroprotective effects of SVA against KA-induced seizures. This effect may be mediated by multiple mechanisms that include modulation of glutamate/GABA-ergic system, inhibition of free radical generation, scavenging of reactive oxygen species and reactivation of antioxidant defenses.

Key words: *Ginkgo biloba*, sodium valproate, anticonvulsant, kainic acid, glutamate, DNA damage

INTRODUCTION

Sodium valproate (SVA) is a broad spectrum antiepileptic drug clinically used several years ago. It is effective in treatment of many types of epilepsy including absence (Potera, 2010), myoclonic (Nejad *et al*., 2009), partial (Rowley and White, 2010) and tonic-clonic (Abend *et al*., 2010) seizures. The use of SVA in relatively high doses is mostly accompanied by side effects like transient gastrointestinal symptoms, including anorexia, nausea and vomiting (Nikalje *et al*., 2011). Also

effects on the CNS are common including sedation, ataxia and tremor in addition to elevation of hepatic transaminases in plasma and others (Chateauvieux *et al*., 2010).

In order to reduce the risk of side effects, overcome resistance and achieve safety and effectiveness, the use of anticonvulsant drugs combination is a fundamental strategy in management of epilepsy (Czuczwar *et al*., 2009). In the last years, a number of herbal products have been demonstrated to have promising anticonvulsant activity (Samuels *et al*., 2008). These herbal products are candidates to be included in combination therapy of epilepsy due to their considerable safety and lower side effects. Among these herbal products is *Ginkgo biloba* L. extract (GbE-761). The two main pharmacologically active groups of compounds present in GbE-761 are the flavonoids and the terpenoids (Smith and Luo, 2004). The flavonoid content in the *Ginkgo* leaf is known to act mainly as antioxidants/free radical scavengers (Moreno *et al*., 2004), enzyme inhibitors and cation chelators (Sarhan *et al*., 2007). Two types of terpenoids are present in GbE-761 as lactones (non-saponifiable lipids present as cyclic esters): Ginkgolides and the bilobalide (Smith and Luo, 2004). GbE-761 has shown beneficial effects in treating neurodegenerative diseases like Alzheimer's, cardiovascular diseases, cancer, stress, memory loss, tinnitus, geriatric complaints like vertigo, age-related macular degeneration and psychiatric disorders like schizophrenia (Mahadevan and Park, 2008). The anticonvulsant effect of GbE-761 was not sufficiently studied and the available data about the effect of GbE-761 on the effect of anticonvulsant drugs are insufficient. Manocha *et al*. (1996) indicated that, GbE-761 decreases the protective effect of both sodium valproate and carbamazepine.

On the other hand, other studies indicated that GbE-761 may have anticonvulsant activity. Ilhan *et al*. (2006) found that GbE-761 protects against development of seizures and increases the anticonvulsant activity of valproic acid against pentylenetetrazole (PTZ)-induced kindling in mice. Sasaki *et al*. (1997) reported that bilobalide a constituent of GbE-76, has an anticonvulsant activity and correlated this effect with bilobalide ability to stimulate drug metabolizing enzymes.

Kainic Acid (KA) is a glutamate analogue used widely to induce seizures that mimic the pathological state of epilepsy and other convulsive disorders (Borlongan *et al*., 1995). It acts as an agonist on the excitatory glutamate non-NMDA (kainite) receptors (Hosseinzadeh *et al*., 2007) causing severe CNS stimulation and induces seizures, neurotoxicity and neuronal damage (Jarvela *et al*., 2011).

Hence, the objective of this study was to investigate the effect of GbE-761 on the anticonvulsant effect of SVA against KA-induced seizures in mice. In addition to investigate the corresponding changes in brain glutamate, lipid peroxidation and antioxidant defenses. Moreover, the combined neuroprotective effect of SVA and GbE-761 against KA-induced neuronal injury and DNA damage was studied.

MATERIALS AND METHODS

The work of this article was carried out in the College of Medicine, Najran University, Najran, Saudi Arabia. The practical part of this study started in 6th June 2011 for two weeks.

Animals: Male adult Swiss-Webster mice weighing 22-30 g from the Animal house of King Saud University were used in all experiments. Mice were housed in plastic cages with stainless steel mesh covers under a 12 h light/dark cycle at 25°C and allowed free access to water and food (laboratory chow) *ad libitum*. All experiments were carried out between 9.00 a.m. and 15.00 p.m. The research was conducted in accordance with the internationally accepted guidelines for the use and care of

experimental animals. The experiments reported in this work were approved by institutional Animal Ethics Committee.

Chemicals: Sodium valproate, kainic acid, thiobarbituric acid, reduced glutathione (GSH), Ellman's reagent [(5,5-dithiobis (2-nitrobenzoic acid), DTNB] and Bovine Serum Albumin (BSA) were purchased from Sigma, (Germany). Standard *Ginkgo biloba* L. extract (GbE-761-761) was purchased from Beaufour Ipsen International, (France). Serum neuron-specific enolase mouse ELISA kit purchased from USCN life science, (Germany). 8-hydroxy-2'-deoxy Guanosine (8-OHdG) assay kit purchased from Cayman's Chemical Co., (USA). All other chemicals were of analytical grade.

Experimental protocol: Animals were divided into seven groups. Each group consisted of 10 mice. SVA in a dose (200 mg kg^{-1}) was injected intraperitoneally (i.p.) either alone or in combination with different doses of GbE-761 (25 and 50 mg kg^{-1}). The dose of ginkgo biliosa standard extract (GbE-761) was calculated for each animal, suspended in normal saline and given orally (p.o.) by stomach tube. Control animals were treated, likewise, with normal saline. Thirty minutes later, animals were subjected for testing of anticonvulsant activity. One hour later, animals in each group were sacrificed by decapitation and blood and brain were obtained from each sacrificed animal for biochemical measurements.

Kainic acid-induced seizure test: The test was performed according to Gupta *et al.* (2002).

Biochemical measurements

Collection of samples: Blood samples were kept at 4°C for 30 min for clotting. Clear serum was obtained by centrifugation of the blood samples after clotting at 3,000 rpm for 15 min and kept frozen until used for 8-OHdG measurement. The brain was rinsed in ice-cold saline; with ice-cold saline, washed with ice-cold saline, blotted carefully, weighed and then homogenized in a phosphate buffer (pH 7.4). The homogenate was divided into two parts. The first part was centrifuged at 3,000 rpm at 4°C for 15 min and the supernatant was collected for determination of lipid peroxidation, Nitric Oxide (NO), reduced Glutathione (GSH) levels and Glutathione Peroxidase (GSH-Px) activity. The second part was mixed with equal volume of perchloric acid (1 mol L^{-1}) and mixed by vortexing. The mixture was allowed to stand for 5 min at 25°C. After centrifugation at 3,000 rpm at 4°C for 5 min the supernatant was collected and used for determination of GSH and 8-OHdG levels.

Determination of lipid peroxidation: The quantitative measurement of lipid peroxidation in hippocampal homogenate was performed according to the method previously described by Ohkawa *et al.* (1979) and Prasanna and Purnima (2011). The amount of Malondialdehyde (MDA), a measure of lipid peroxidation was measured by reaction with thiobarbituric acid at 532 nm using Optima SP 3000 plus spectrophotometer (Indogama, Japan).

Determination of GSH and glutamate levels: Determination of intracellular Glutathione (GSH) was carried out according to the method of Griffith (1980) and Al-Yahya (2006). The glutamate content in the brain homogenate was measured spectrophotometrically according to the method described by Lund (1986).

Determination of GSH-Px activity: Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine (1967) and Chantiratikul *et al.* (2008). The enzymatic reaction which contained β-nicotinamide adenine dinucleotide phosphate (NADPH), GSH, glutathione reductase and a sampler or a standard was initiated by addition of hydrogen peroxide. The change in the absorbance was measured spectrophotometrically. A standard curve was plotted for each assay.

Determination of serum Neuron Specific Enolase (NSE): NSE was assayed according to Sankarab *et al.* (1997) using the mouse serum neuron specific enolase ELISA assay kit (USC life science, Germany). All procedures were carried out according to the provider manual. NSE concentrations were calculated as ng mL^{-1} serum.

Determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG) level: Cayman's 8-hydroxy-2'-deoxyguanosine (8-OHdG) assay kit purchased from Cayman's Chemical Co., (USA) was used. It is a competitive assay that can be used for the quantification of 8-OHdG in serum and tissue homogenate. It recognizes both free 8-OHdG and DNA-incorporated 8-OH-dG. This assay depends on the competition between 8-OHdG and 8-OHdG-acetylcholinesterase (AChE) conjugate (8-OHdGTracer) for a limited amount of 8-OHdG monoclonal antibody. All procedures were carried out in accordance with the provider manual.

Determination of protein content: Total protein in brain homogenate was estimated using method of Lowry *et al.* (1951). The absorption was read spectrophotometrically at 750 nm. The bovine serum albumin was used as standard.

Statistical analysis: The variability of results was expressed as the Mean±SEM. The significance of differences between mean values was determined using one-way analysis of variance (ANOVA) followed by Tukey's post hoc comparison between groups. The $p<0.05$ represents the level of significance.

RESULTS

Effect of SVA, GbE-761 and their combination against KA- induced seizures: Results of anticonvulsant activity of SVA and GbE-761 and their combination against KA-induced convulsions are shown in Table 1. Administration of SVA and GbE-761 or their combination led to significant decrease in seizure activity [$F(6, 63) = 56.22$, $p<0.001$]. SVA in a dose 200 mg kg^{-1}, i.p., significantly reduced the seizure onset, percent of seizures and percent of mortality of tested animals relative to KA control (saline-treated). GbE-761 administered orally 30 min before testing anticonvulsant activity significantly increased the KA seizure onset in doses 25 and 50 mg kg^{-1}. It also reduced the percent of seizures and percent of mortality of animals in the two dose levels relative to KA control (saline-treated). Combined treatment of SVA with GbE-761 led to a significant increase in the seizure onset relative to KA control. This effect was of higher significance level ($p<0.01$) with the dose 50 mg kg^{-1} compared with SVA-treated group. The percent seizures also reduced by the combination of SVA with GbE-761 with complete protection against mortality with the two dose levels of GbE-761.

Effect of SVA, GbE-761 and their combination on brain glutamate: Induction of KA-induced seizures and the effect of tested drugs led to significant changes in the brain glutamate

Table 1: Effect of sodium valproate (200 mg kg^{-1}, i.p.), *Ginkgo biloba* extract (25 and 50 mg kg^{-1}, p.o.) and their combination on seizure onset, percent of seizures and percent of mortality of kainic acid-(10 mg kg^{-1}, i.p.) induced seizures in mice

Treatment (mg kg^{-1})	Onset of seizure (sec)	Seizure (%)	Mortality (%)
KA (Control)	57.5±4.6	100	40
GbE-761 (25)	78.5±3.4^{a}	70	30
GbE-761 (50)	82.6±4.6^{b}	50	20
SVA (200)	79.7±4.7^{a}	50	20
SVA (200)+GbE-761 (25)	100.4±5.8^{c,d}	40	0
SVA (200)+GbE-761 (50)	104.1±6.7^{c,e}	20	0

SVA: Sodium valproate, GbE-761: *Ginkgo biloba* extract, KA: Kainic acid, Results represent Mean±SEM. $^{a}p<0.05$ vs. KA (control), $^{b}p<0.01$ vs. KA (control), $^{c}p<0.001$ vs. KA (control), $^{d}p<0.05$ vs. SVA, $^{e}p<0.01$ vs. SVA

Table 2: Effect of sodium valproate (200 mg kg^{-1}, i.p.), *Ginkgo biloba* extract (25 and 50 mg kg^{-1}, p.o.) and their combination on brain glutamate, malondialdehyde, reduced glutathione levels and glutathione peroxidase activity in kainic acid-induced seizure model in mice

Treatment (mg kg^{-1})	Glutamate (μmol g^{-1} protein)	MDA (μmol g^{-1} protein)	GSH (nmol g^{-1} protein)	GSH-Px (IU g^{-1} protein)
Naïve	2.63±0.28	298.37±14.42	38.52±3.34	30.34±3.45
KA (control)	4.36±0.37^{b}	366.44±12.43^{b}	19.54±3.64^{b}	17.58±2.73^{a}
SVA (200)	2.66±0.34^{d}	294.36±13.45^{d}	24.73±3.86	19.65±2.56
GbE-761 (25)	4.33±0.37	307.26±13.34^{c}	35.34±3.75^{c}	30.45±2.44^{c}
GbE-761 (50)	2.88±0.25^{c}	293.76±12.54^{d}	37.71±3.66^{d}	33.64±2.82^{d}
SVA (200)+GbE-761 (25)	2.84±0.24^{d}	226.57±11.66^{e,f}	41.56±2.57^{e,f}	31.46±2.63^{c,f}
SVA (200)+GbE-761 (50)	1.37±0.12^{d,e}	220.65±13.64^{e,f}	43.94±3.32^{e,g}	33.33±2.22^{d,f}

SVA: Sodium valproate, GbE-761: *Ginkgo biloba* extract, KA: Kainic acid, MDA: Malondialdehyde, GSH: Reduced glutathione, GSH-Px: Glutathione peroxidase, Results represent Mean±SEM. $^{a}p<0.05$ vs. naïve, $^{b}p<0.01$ vs. naïve. $^{c}p<0.05$ vs. KA (control), $^{d}p<0.01$ vs. KA (control). $^{e}p<0.001$ vs. KA (control). $^{f}p<0.05$ vs. SVA, $^{g}p<0.01$ vs. SVA

level [F (6, 63) = 12.72, p<0.001] as shown in Table 2. Corresponding to development of seizures, KA in dose 10 mg kg^{-1}, i.p., increased the brain glutamate level to 4.36 μmol g^{-1} protein relative to 2.63 μmol g^{-1} protein in naïve animals. Pretreatment with SVA significantly reduced brain glutamate elevated by KA to 2.66 μmol g^{-1} protein. In addition, GbE-761 in the dose 50 mg kg^{-1} significantly reduced the brain glutamate level to 2.88 μmol g^{-1} protein. Combined treatment of SVA with GbE-761 significantly decreased brain glutamate to 2.84 μmol g^{-1} protein with dose 25 mg kg^{-1} and 1.37 μmol g^{-1} protein with the dose 50 mg kg^{-1} of GbE-761 relative to 4.36 μmol g^{-1} protein in KA-control and 2.66 in SVA-treated animals.

Effect of SVA, GbE-761 and their combination on brain oxidative stress: Effect of KA-induced seizures and tested drugs on brain lipid peroxidation product, Malondialdehyde (MDA), intracelleular glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity is shown in Table 2.

Results showed significant changes in brain MDA level by KA and tested drugs [F(6, 63) = 14.58, p<0.001]. Injection of KA, in the dose 10 mg kg^{-1}, i.p., significantly increase brain MDA level to 366.44 μmol g^{-1} protein relative to 298.37 μmol g^{-1} protein in naïve animals. SVA in 200 mg kg^{-1} dose significantly reduced the KA-induced increase in brain MDA level to 294.36 μmol g^{-1} protein. In addition, GbE-761 significantly decreased the brain glutamate to 307.26 μmol g^{-1} protein with dose 25 mg kg^{-1} and 293.76 μmol g^{-1} protein with dose 50 mg kg^{-1}. Combined treatment of SVA and GbE-761 significantly reduced brain MDA level to 226.57 and

220.65 μmol g^{-1} protein with doses 25 and 50 mg kg^{-1} of GbE-761, respectively relative to 366.44 μmol g^{-1} protein in KA-control group and 294.36 μmol g^{-1} protein in the SVA-treated group.

In addition, results showed significant changes in brain GSH level by KA-induced seizures and tested drugs [F(6, 63) = 6.710, p<0.001]. Injection of KA, in the dose 10 mg kg^{-1}, i.p., significantly decreased brain GSH level to 19.54 nmol g^{-1} protein relative to 38.52 nmol g^{-1} protein in naïve animals. GbE-761 significantly increased the brain GSH level to 35.34 nmol g^{-1} protein with dose 25 mg kg^{-1} and 37.71 nmol g^{-1} protein with dose 50 mg kg^{-1}. Combined treatment of SVA and GbE-761 significantly increased the brain GSH level to 41.56 nmol g^{-1} protein with the dose 25 mg kg^{-1} and 43.94 nmol g^{-1} protein with the dose 50 mg kg^{-1} of GbE-761 relative to 19.54 nmol g^{-1} protein in KA-control group and 294.36 nmol g^{-1} protein in SVA-treated group.

Moreover, the results showed significant changes in brain GSH-Px activity produced by KA-induced seizures and tested drugs [F(6, 63) = 5.915, p<0.001]. Injection of KA, in the dose 10 mg kg^{-1}, i.p., significantly decreased brain GSH-Px activity to 17.58 IU g^{-1} protein relative to 30.34 IU g^{-1} protein in naïve animals. Single GbE-761 treatment significantly increased the brain GSH-Px activity to 30.45 IU g^{-1} protein with the dose 25 mg kg^{-1} and 33.64 IU g^{-1} protein with the dose 50 mg kg^{-1}. Combined treatment of SVA and GbE-761 significantly increased the brain GSH-Px activity to 31.46 IU g^{-1} protein with the dose 25 mg kg^{-1} and 33.33 IU g^{-1} protein with the dose 50 mg kg^{-1} of GbE-761 relative to 17.58 IU g^{-1} protein in KA-control group and 19.65 IU g^{-1} protein in SVA-treated group.

Effect of SVA, GbE-761 and their combination on serum NSE: The level of serum NSE was significantly changed [F (6, 63) = 13.62, p<0.001] by induction of KA seizures and the effect of the tested drugs as shown in Fig. 1. Injection of KA in dose 10 mg kg^{-1}, i.p., significantly increased serum NSE level (p<0.01) relative to its level in naïve animals. Administration of SVA in the dose 200 mg kg^{-1}, i.p., significantly decreased the serum NSE level (p<0.01) compared with KA-control. Single GbE-761 treatment also decreased the serum NSE level at the significance levels p<0.05

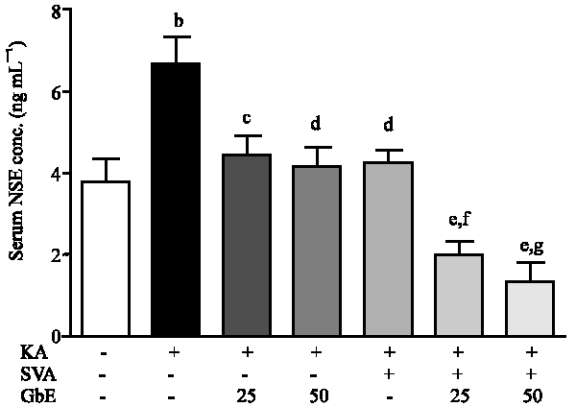

Fig. 1: Effect of sodium valproate (200 mg kg^{-1}, i.p.), *Ginkgo biloba* extract (25 and 50 mg kg^{-1}, p.o.) and their combination on serum neuron-specific enolase level in kainic acid-induced seizure model in mice. SVA: Sodium valproate, GbE-761: *Ginkgo biloba* extract, KA: Kainic acid, NSE: Serum neuron-specific enolase, - : was not given, + : was given. Results represent Mean±SEM. [a]p<0.05 vs. naïve, [b]p<0.01 vs. naïve. [c]p<0.05 vs. KA (control), [d]p<0.01 vs. KA (control). [e]p<0.001 vs. KA (control). [f]p<0.05 vs. SVA, [g]p<0.01 vs. SVA

with the dose 25 mg kg^{-1} and $p<0.01$ with the dose 50 mg kg^{-1}, p.o. relative to KA-control level. Combined treatment of SVA with GbE-761 significantly reduced the serum NSE level ($p<0.001$) in the two dose levels of GbE-761 relative to KA-control level. At the same time, this decrease in serum NSE level was significant compared with its level in SVA-treated group at the significance levels $p<0.05$ with the dose 25 mg kg^{-1}, p.o. and $p<0.01$ with the dose 50 mg kg^{-1}, p.o. of GbE-761 (Fig. 1).

Effect of SVA, GbE-761 and their combination on serum and brain 8-OHdG: The level of serum 8-OHdG was significantly changed [$F(6, 63) = 10.04$, $p<0.001$] by induction of KA seizures and the effect of the tested drugs as shown in Fig. 2. Injection of KA in dose 10 mg kg^{-1}, i.p., significantly increased serum 8-OHdG level relative to its level in naïve animals. Administration of SVA in the dose 200 mg kg^{-1}, i.p., significantly decreased the serum 8-OHdG level compared with KA-control level. In addition, single GbE-761 treatment decreased the serum 8-OHdG with the dose 50 mg kg^{-1}, p.o., relative to KA-control level. Combined treatment of SVA with GbE-761 significantly reduced the serum 8-OHdG level with the two dose levels of GbE-761 at the significance level, $p<0.001$ relative to KA-control level and $p<0.05$ relative to SVA-treated group level (Fig. 2).

Moreover, the level of brain 8-OHdG was significantly changed [$F(6, 63) = 14.67$, $p<0.001$] by induction of KA seizures and the effect of SVA and GbE-761 as shown in Fig 3. Induction of clonic seizures by injection of KA in dose 10 mg kg^{-1}, i.p., significantly increased brain 8-OHdG level relative to its level in naïve animals. Pretreatment with SVA in the dose 200 mg kg^{-1}, i.p., significantly decreased the brain 8-OHdG level ($p<0.01$) compared with KA-control level. In addition, single GbE-761 treatment decreased the brain 8-OHdG significantly at the levels $p<0.05$ with the dose 25 mg kg^{-1} and $p<0.01$ with the dose 50 mg kg^{-1}, p.o. relative to KA-control level.

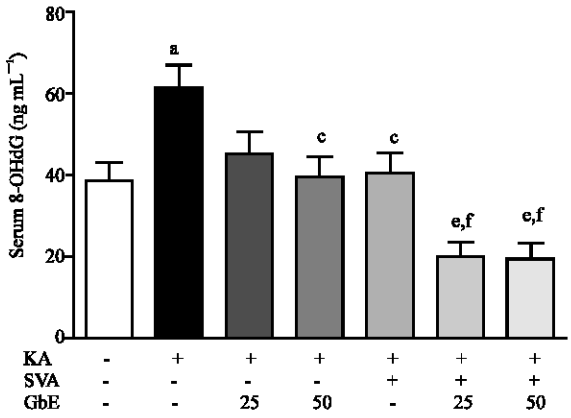

Fig. 2: Effect of sodium valproate (200 mg kg^{-1}, i.p.), *Ginkgo biloba* extract (25 and 50 mg kg^{-1}, p.o.) and their combination on serum 8-hydroxy-2'-deoxyguanosine level in kainic acid-induced seizure model in mice. SVA: Sodium valproate, GbE-761: *Ginkgo biloba* extract, KA: Kainic acid, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, - : was not given, + : was given. Results represent Mean±SEM. [a]$p<0.05$ vs. naïve, [b]$p<0.01$ vs. naïve. [c]$p<0.05$ vs. KA (control), [d]$p<0.01$ vs. KA (control). [e]$p<0.001$ vs. KA (control). [f]$p<0.05$ vs. SVA, [g]$p<0.01$ vs. SVA

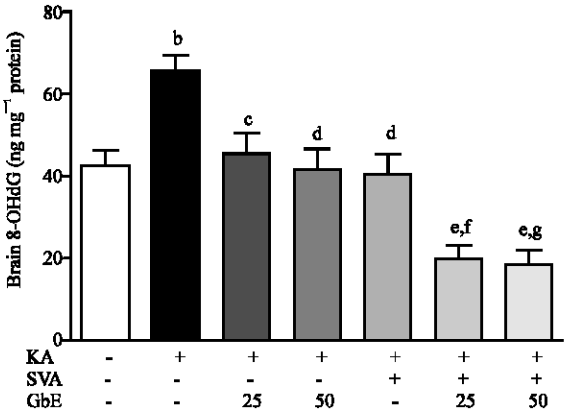

Fig. 3: Effect of sodium valproate (200 mg kg^{-1}, i.p.), *Ginkgo biloba* extract (25 and 50 mg kg^{-1}, p.o.) and their combination on brain 8-hydroxy-2'-deoxyguanosine level in kainic acid-induced seizure model in mice. SVA: Sodium valproate, GbE-761: *Ginkgo biloba* extract, KA: Kainic acid, 8-OHdG: 8-hydroxy-2'-deoxyguanosine, - : was not given, + : was given. Results represent Mean±SEM. [a]$p<0.05$ vs. naïve, [b] $p<0.01$ vs. naïve. [c]$p<0.05$ vs. KA (control), [d]$p<0.01$ vs. KA (control). [e]$p<0.001$ vs. KA (control). [f]$p<0.05$ vs. SVA, [g]$p<0.01$ vs. SVA

Combined treatment of SVA with GbE-761 significantly reduced the brain 8-OHdG level with the two dose levels of GbE-761 at the significance levels, $p<0.001$ relative to KA-control level and $p<0.05$ relative with the dose 25 mg kg^{-1} and $p<0.01$ with the dose 50 mg kg^{-1} relative to SVA-treated group level (Fig. 3).

DISCUSSION

In the present study, the effect of GbE-761 on the anticonvulsant and neuroprotective effects of the widely used broad spectrum anticonvulsant drug SVA was investigated. The anticonvulsant activity of single and combined treatment of SVA in a dose 200 mg kg^{-1}, i.p. and GbE-761 in doses 25 and 50 mg kg^{-1}, p.o., against KA-induced seizure in mice, was studied.

The anticonvulsant effect of GbE-761 was not sufficiently studied and the available data about the effect of GbE-761 on the effect of anticonvulsant drugs are controversial. In contradiction to the results of this study, Ivetic *et al.* (2008) indicated that GbE-761 facilitates the induction of seizures in kindling model of seizures in rabbits induced by hippocampal electrical stimulation. Moreover, Manocha *et al.* (1996) indicated that, GbE-761 decreases the anticonvulsant effect of both SVA and carbamazepine. On the other hand and in agreement with the results of this study, Ilhan *et al.* (2006) found that GbE-761 protects against development of seizures and increases the anticonvulsant activity of SVA against pentylenetetrazole (PTZ)-induced kindling in mice.

In the present study, combined treatment of SVA with GbE-761 in the tested dose levels enhanced the anticonvulsant effect of SVA against KA-induced seizures. As a broad spectrum anticonvulsant drug, SVA is effective against generalized and partial seizures and effectively protects against KA-induced seizures (Velisek *et al.*, 1992). In our results, single GbE-761 treatment in doses 25 and 50 mg kg^{-1}, p.o., showed a protective effect against KA-induced seizures. These findings are in agreement with previous studies that related the anticonvulsant effects of GbE-761 to its main constituent bilobalide (Sasaki *et al.*, 1997).

The accepted mechanism that may contribute to SVA antiseizure actions involves its effect on the glutamate/GABA-ergic transmission in the CNS. Although, valproate has no effect on responses to GABA, it increases the amount of GABA that can be recovered from the brain and decreases the available amount of glutamate. Valproate can stimulate the activity of the GABA synthetic enzyme, Glutamate Decarboxylase (GAD) and inhibit GABA degradative enzymes, GABA-transaminase and succinic semialdehyde dehydrogenase (Atmaca, 2009). Excessive release of glutamate mediates neuronal excitability and cell death. Glutamate via activation of its receptors increases the intracellular calcium concentration ($[Ca^{2+}]i$) and generation of Reactive Oxygen Species (ROS) that is involved in neuronal disorder and degeneration. Both phenomena induced by glutamate are related to cell injury and death (Coyle and Puttfarcken, 1993).

These data are in agreement with our results that showed a significant decrease in glutamate level in brain of animals treated with SVA, the effect that was potentiated by its combination with GbE-761 in KA-induced seizure model. These changes produced by GbE-761 may be attributed to enhancement the ability of SVA to stimulate GAD activity which converted glutamate to GABA. This may lead to the observed reduction in brain glutamate leading to elevation in brain GABA which has anticonvulsant and neuroprotective effects. The neuroprotective effect of GbE-761 is attributed to its major constituent bilobalide which has the ability to decrease brain glutamate and increase brain GABA via stimulation of GAD activity (Arushanian and Beler, 2008). In addition, bilobalide has the ability to stimulate GABA release in cerebral neurons (Sasaki *et al*., 1999) and exerts a direct inhibitory action on kainate receptor (Kanada *et al*., 2005).

In addition, the role of the effect of SVA and GbE-761 combination on the seizure-induced oxidative stress and antioxidant defenses in their anticonvulsant and neuroprotective activity cannot be neglected. Previous studies indicated that KA-induced seizures, which are associated with an increase in extracellular glutamate levels, appear to be associated with generation of ROS and with a decrease in residual antioxidant effects (Ueda *et al*., 2002).

In the present study, the combined treatment with SVA and GbE-761 decreased the MDA and increased the GSH level and GSH-Px activity than their levels in the SVA-treated group. These results indicated that addition of GbE-761 to SVA added the benefits of inhibition of lipid peroxidation and stimulation of GSH and GSH-Px inhibited by KA to the effects of SVA.

GbE-761 is a potent antioxidant. Numerous studies have shown that GbE-761 has an antioxidant, free radical scavenging and neuroprotective effects on neurons suffering from oxidative stress (Arushanian and Beler, 2008). Hence, the observed enhancement in the anticonvulsant effect of SVA mediated by GbE-761 and the corresponding changes in neuronal damage can be, at least partly, related to the antioxidant effect of GbE-761.

Neuron Specific Enolase (NSE), is a marker of acute brain injury and blood–brain barrier dysfunction, elevates in seizure activity. This enzyme is a very sensitive marker for many types of neurological injury. Studies have demonstrated a relationship between the degree of cell damage in the CNS and the serum concentration of NSE (Pandey *et al*., 2011).

Moreover, 8-OHdG is a specific marker for oxidative DNA damage. In normal conditions, ROS attack nuclear and mitochondrial DNA causing oxidized nucleosides and consequently, mutagenic DNA lesions. One of these lesions is 8-OHdG, the end product of the hydroxylation of guanine. Once eliminated, the 8-OHdG lesions may be found in the plasma and are excreted in the urine (Wu *et al*., 2004). Increased oxidative stress-induced by KA-induced seizures may increase the incidence of neuronal and DNA damage. This was clear in our results that showed a marked increase in both serum NSE and 8-OHdG and brain 8-OHdG levels in KA-treated animals.

Both SVA and GbE-761 decreased the serum NSE level and 8-OHdG levels in both serum and brain while, there was a significant reduction of NSE and 8-OHdG levels after their combined treatment compared with that observed after treatment with SVA alone. This indicated that, addition of GbE-761 to SVA not only enhanced its anticonvulsant activity but also enhanced its neuroprotective effect against neuronal damage and oxidative DNA damage induced by ROS produced during KA-induced seizures. The observed enhancement in the neuroprotective effect of SVA by GbE-761 may be attributed to different mechanisms, which can be synergistic, exerted by the active constituents of GbE-761 with SVA. These mechanisms may include the protection against glutamate-induced neuronal injury and death, inhibition of free radical generation, scavenging of reactive oxygen species and regulation of mitochondrial gene expression encoding synthesis of antioxidant defenses.

CONCLUSIONS

GbE-761 in small to moderate doses (25-50 mg kg^{-1}, p.o.) enhances the anticonvulsant and neuroprotective effects of SVA against KA-induced seizures. This effect may be attributed to different mechanisms, including reducing the brain glutamate and increasing brain GABA levels, inhibition of free radical generation, scavenging of reactive oxygen species and reactivation of antioxidant defenses. Results of this study direct the light toward the possible use of SVA and GbE-761 combination in treatment of epilepsy in human.

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