ENHANCEMENT OF THE ANTICONVULSANT AND NEUROPROTECTIVE EFFECTS OF SODIUM VALPROATE BY GINKGO BILOBA AGAINST KAINIC ACID-INDUCED SEIZURES IN MICE

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ABSTRACT

Sodium valproate (SVA) is a widely used broad spectrum antiepileptic drug. Ginkgo biloba extract (GbE-761) is an herbal product that has promising anticonvulsant and antioxidant properties. The aim of this study is to investigate the effect of GbE-761 on the anticonvulsant and neuroprotective activity of SVA. **Methods**: The anticonvulsant activity of SVA (200 mg/kg, i.p.) and its combination with GbE-761, in doses 25 and 50 mg/kg, p.o. 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, serum levels of neuron-specific enolase (NSA) and 8-hydroxy-2-deoxyguanosine (8-OHdG) and brain 8-OHdG level were measured. **Results**: Addition of GbE to SVA enhanced the anticonvulsant activity of SAV against KA-induced seizures, the fact that appeared in the form of an increase in seizure onset and decrease in percent seizures and mortality. This effect was accompanied by a significant decrease in brain glutamate and lipid peroxidation and increase in brain GSH level and GSH-Px activity relative to its levels in KA-treated animals and its levels in SVA-treated animals. In addition, serum NSE and serum and brain 8-OHdG levels significantly decreased by combined SVA and GbE treatment than its levels in animals treated with SVA alone. **Conclusions**: GbE-761 enhances 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.

Keywords: Ginkgo biloba, Sodium valproate, Anticonvulsant, Kainic acid, Glutamate, Antioxidant.

1. INTRODUCTION

Epilepsy is a common and serious neurological disorder. Despite recent advances in drug therapy, treatment of epilepsy is still largely empirical and rational prescribing based on the mechanism of action in an individual patient is generally not possible (Anderson and Moor 2010). Sodium valproate has been used as an antiepileptic drug for several years and there have been many reports of its efficacy in most types of epilepsy including treatment of absence (Potera 2010), myoclonic (Crespel et al., 2009), partial (Rowley et al., 2010), and tonic-clonic (Abend et al., 2010) seizures. Like other antiepileptic drugs, the use of SVA in relatively high doses
is mostly accompanied by side effects like transient gastrointestinal symptoms, including anorexia, nausea, and vomiting in about 16% of patients, and effects on the CNS include sedation, ataxia, tremor 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). A number of herbal products that have been demonstrated to have anticonvulsant activity (Samuels et al., 2008) are candidates to be included in such combination therapy particularly due to their considerable safety and lower side effects. Among these herbal products is *Ginkgo biloba* L. extract (GbE). The 2 main pharmacologically active groups of compounds present in GbE 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, enzyme inhibitors, and cation chelators (DeFeudis and Drieu 2000). Two types of terpenoids are present in GbE as lactones (non-saponifiable lipids present as cyclic esters): ginkgolides and the bilobalide (Smith and Luo 2004). GbE 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 was not sufficiently studied and the available data about the effect of GbE on the effect of anticonvulsant drugs are insufficient. Manocha et al., (1996) indicated that, GbE decreases the protective effect of both sodium valproate and carbamazepine.

On the other hand, other studies indicated that GbE may have anticonvulsant activity. Ilhan et al. (2006) found that GbE 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 bilobalid a constituent of GbE-761, has an anticonvulsant activity and correlated this effect with bilobalid ability to stimulate drug metabolizing enzymes.

Kainic acid (KA, a glutamate analogue) has been used by many researchers to induce seizures that are thought to mimic the pathological state of epilepsy and other convulsive disorders (Armstrong et al. 1986). It acts as an agonist on the excitatory glutamate non-NMDA (kainite) receptors causing severe CNS stimulation and induces seizures, neurotoxicity and neuronal damage (Järvelä et al., 2010).

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

2. MATERIALS AND METHODS

2.1. 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 ^{o} C and allowed free access to water and food (laboratory chow) *ad libitum*. All experiments were carried out between 9.00 AM and 15.00 PM. 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.

2.2. 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 extract (GbE-761) was purchased from Beafour 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.

2.3. Experimental protocol

Animals were divided into seven groups. Each group consisted of 10 mice. SVA in a dose (200 mg/kg) was injected intraperitoneally (i.p.) either alone or in combination with different doses of GbE (25 and 50 mg/kg). The dose of GbE 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. 30 min 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.

2.4. Kainic acid-induced seizure test

The test was performed according to Gupta et al. (2002). 30 min after treatment of animals with SVA, GbE or their combination, mice in both control and tested groups were injected with KA at a dose 10 mg/kg i.p., pH was adjusted to 7.2 ± 0.1. Animals were then observed for behavioral changes (piano-playing forelimb clonic seizures, grooming, rearing, hind limb scratching, wet dog shakes, jaw movements, salivation, and head nodding). Time before the onset of clonic convulsions and the percentage of seizures and mortality over a total period of 1 h was recorded.

2.5. Biochemical measurements

2.5.1. 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× g for 15 min and kept frozen until used for 8-OHdG measurement. The brain was rinsed in ice-cold saline; the whole hippocampus was separated, 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 10,000× g at 4 °C for 15 min and the supernatant was collected for determination of LP, NO, TAC levels and GST activity. The second part was mixed with equal volume of perchloric acid (1 mol/l) and mixed by vortexing. The mixture was allowed to stand for 5 min at 25 °C. After centrifugation at 10,000× g at 4 °C for 5 min the supernatant was collected and used for determination of GSH and 8-OHdG levels.

2.5.2. 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). 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).

2.5.3. Determination of GSH and glutamate levels

For determination of intracellular glutathione (GSH) and glutamate, an equal volume of perchloric acid (1 mol/l) was added to a part of brain homogenate and mixed by vortexing. The mixture was allowed to stand for 5 minutes at room temperature. After centrifugation for 5 minutes, the supernatant was collected. The GSH content of the neutralized supernatant was assayed using Ellman's reagent [5,5'-dithiobis-2-nitrobenzoic acid (DTNB solution)] according to the method of Griffith (1980).

The glutamate content in the supernatant was measured spectrophotometrically according to the method described by Lund (1986). Assay depends on enzymatic dehydrogenation with conversion of NAD^+ to NADH. The concentration of glutamate in the samples is calculated as nmol/mg protein. A standard reference curve was plotted for each assay.

2.5.4. Determination of GSH-Px activity

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine (1967). 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.

2.5.5. 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 serum.

2.5.6. Determination of 8-hydroxy-2'-deoxyguanosine (8-OHdG) level

Cayman's 8-hydroxy-2'-deoxy Guanosine (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.

2.6.6. Determination of protein content

Total protein in brain homogenate was estimated using method of Lowry et al. (1951). Theabsorption was read spectrophoto-metrically at 750 nm. The bovine serum albumin was used as standard.

2.6. 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. $P< 0.05$ represents the level of significance.

3. RESULTS

3.1. Effect of SVA, GbE and their combination against KA- induced seizures.

Results of anticonvulsant activity of SVA and GbE and their combination against KA-induced convulsions are shown in Table 1. Injection of KA in dose 10 mg/kg/ i.p., led to development of clonic seizures in 200% of animals at onset 57.5 ± 4.6 sec. and 40%

mortality. Administration of SVA and GbE or their combination led to significant decrease in seizure activity [F(6, 63) = 56.22, p< 0.001]. Administration of SVA in a dose 200 mg/kg, i.p., significantly reduced the seizure onset (p< 0.05), percent of seizures to 50% and percent of mortality to 20% relative to KA control (saline-treated).GbE administered orally 30 min. before testing anticonvulsant activity significantly reduced the KA seizure onset at significant levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg. It also reduced the percent of seizures to 70 % and percent of mortality to 30% with the dose 25 mg/kg and reduced the percent of seizures to 50 % and percent of mortality to 20% with the dose 50 mg/kg. Combined treatment of SVA in the dose 200 mg/kg with GbE led to a significant reduction in the seizure onset at significant level p< 0.001 with the two dose level relative to KA control. This effect was significant compared with SVA-treated group at significance levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg. The percent seizures also reduced with the combination to 40% with the combination of SVA with GbE dose 25 mg/kg and 20% with GbE dose 50 mg/kg with complete protection against mortality with the two dose levels.

3.2. Effect of SVA, GbE 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 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, i.p., increased the brain glutamate level (p< 0.01) relative to naïve animals. Pretreatment with SVA significantly reduced brain glutamate elevated by KA (p< 0.01). In addition, GbE in the dose 50 mg/kg significantly reduced the brain glutamate level. Combined treatment of SVA with GbE significantly decreased brain glutamate (p< 0.01) relative to KA-control. This decrease was also significant compared with SVA (p< 0.01) level, with GbE dose 50 mg/kg.

3.3. Effect of SVA, GbE 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, i.p., significantly increase brain MDA level (p< 0.01) relative to its level in naïve animals. SVA in 200 mg/kg dose significantly reduced the KA-induced increase in brain MDA level. In addition, GbE significantly decreased the brain glutamate at significance levels p< 0.05 with dose 25 mg/kg and p< 0.01 with dose 50 mg/kg. Combined treatment of SVA and GbE significantly reduced brain MDA level (p< 0.001) relative to KA-control group in the two dose levels of GbE. This decrease was also significant relative to 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, i.p., significantly decreased brain GSH level (p< 0.01) relative to its level in naïve animals. GbE significantly increased the brain GSH level at the significance levels p< 0.05 with dose 25 mg/kg and p< 0.01 with dose 50 mg/kg, while single SVA treatment showed an insignificant effect on brain GSH level. Combined treatment of SVA and GbE significantly increased the brain GSH level (p< 0.001) relative to KA-control group in the two dose levels of GbE. This increase was also significant relative to SVA-treated group at significant levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg.

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, i.p., significantly decreased brain GSH-Px activity relative to its level in naïve animals. Single GbE treatment significantly increased the brain GSH-Px activity at the significance levels p< 0.05 with dose 25 mg/kg and p< 0.01 with dose 50 mg/kg, while results after single SVA treatment showed an insignificant change in brain GSH-Px activity. Combined treatment of SVA and GbE significantly increased the brain GSH-Px activity at the significant levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg relative to KA-control group. This increase was also significant

relative to SVA-treated group with the two dose levels of GbE combined with SVA.

3.4. Effect of SVA, GbE 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, 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, i.p., significantly decreased the serum NSE level (p< 0.01) compared with KA-control. Single GbE treatment also decreased the serum NSE level at the significance levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg, p.o. relative to KA-control level. Combined treatment of SVA with GbE significantly reduced the serum NSE level (p< 0.001) in the two dose levels of GbE 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, p.o., and p< 0.01 with the dose 50 mg/kg, p.o. of GbE (Fig 1).

3.5. Effect of SVA, GbE 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, i.p., significantly increased serum 8-OHdG level relative to its level in naïve animals. Administration of SVA in the dose 200 mg/kg, i.p., significantly decreased the serum 8-OHdG level compared with KA-control level. In addition, single GbE treatment decreased the serum 8-OHdG with the dose 50 mg/kg, p.o., relative to KA-control level. Combined treatment of SVA with GbE significantly reduced the serum 8-OHdG level with the two dose levels of GbE 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 as shown in Fig 3. Induction of clonic seizures by injection of KA in dose 10 mg/kg, i.p., significantly increased brain 8-OHdG level relative to its level in naïve animals. Pretreatment with SVA in the dose 200 mg/kg, i.p., significantly decreased the brain 8-OHdG level (p< 0.01) compared with KA-control level. In addition, single GbE treatment decreased the brain 8-OHdG significantly at the levels p< 0.05 with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg, p.o. relative to KA-control level. Combined treatment of SVA with GbE significantly reduced the brain 8-OHdG level with the two dose levels of GbE at the significance levels, p< 0.001 relative to KA-control level and p< 0.05 relative with the dose 25 mg/kg and p< 0.01 with the dose 50 mg/kg relative to SVA-treated group level (Fig 3).

LIST of Tables

Table 1. Effect of SVA (200 mg/kg, i.p.), GbE (25 and 50 mg/kg, p.o.) and their combination on seizure onset, percent of seizures and percent of mortality of kainic acid- (KA, 10 mg/kg, i.p.) induced seizures in mice.

Treatment (mg/kg)	Onset of seizure (sec.)	Seizure %	Mortality %
KA (Control)	57.5 ± 4.6	100	40
GbE (25)	78.5 ± 3.4[a]	70	30
GbE (50)	82.6 ± 4.6[b]	50	20
SVA (200)	79.7 ± 4.7[a]	50	20
SVA (200) + GbE (25)	100.4 ± 5.8[c,d]	40	00
SVA (200) + GbE (50)	104.1 ± 6.7[c,e]	20	00

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 SVA (200 mg/kg, i.p.), GbE (25 and 50 mg/kg, p.o.) and their combination on brain glutamate, malondialdehyde (MDA), intracellular glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity in in kainic acid (KA, 10 mg/kg, i.p.)-induced seizure model in mice.

Treatment (mg/kg)	Glutamate (µmol/g protein)	MDA (µmol/g protein)	GSH (nmol/g protein)	GSH-Px (IU/g 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 (25)	4.33 ± 0.37	307.26 ± 13.34[c]	35.34 ± 3.75[c]	30.45 ± 2.44[c]
GbE (50)	2.88 ± 0.25[c]	293.76 ± 12.54[d]	37.71 ± 3.66[d]	33.64 ± 2.82[d]
SVA (200) + GbE (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 (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]

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.

Figures

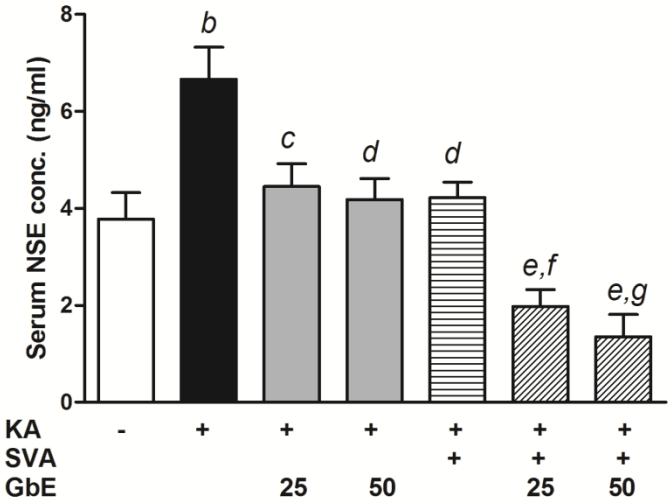

Fig 1. Effect of SVA (200 mg/kg, i.p.), GbE (25 and 50 mg/kg, p.o.) and their combination on serum neuron-specific enolase (NSE) level in kainic acid-induced seizure model in mice. Results represent mean ± SEM. [a] p< 0.05 vs. naïve, [b] p< 0.01 vs. naïve. [c]p< 0.05 vs. KA (control), [d]p< 0.01 vs. KA (control). [e]p< 0.001 vs. KA (control). [f] p< 0.05 vs. SVA, [g] p< 0.01 vs. SVA.

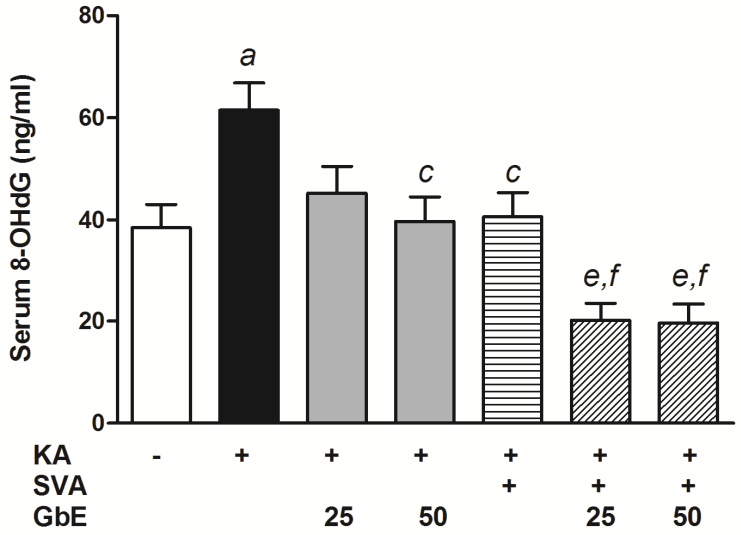

Fig 2. Effect of SVA (200 mg/kg, i.p.), GbE (25 and 50 mg/kg, p.o.) and their combination on serum 8-hydroxy-2-deoxyguanosine (8-OHdG) level in kainic acid-induced seizure model in mice. Results represent mean ± SEM. [a] p< 0.05 vs. naïve, [b] p< 0.01 vs. naïve. [c]p< 0.05 vs. KA (control), [d]p< 0.01 vs. KA (control). [e]p< 0.001 vs. KA (control).
[f] p< 0.05 vs. SVA, [g] p< 0.01 vs. SVA.

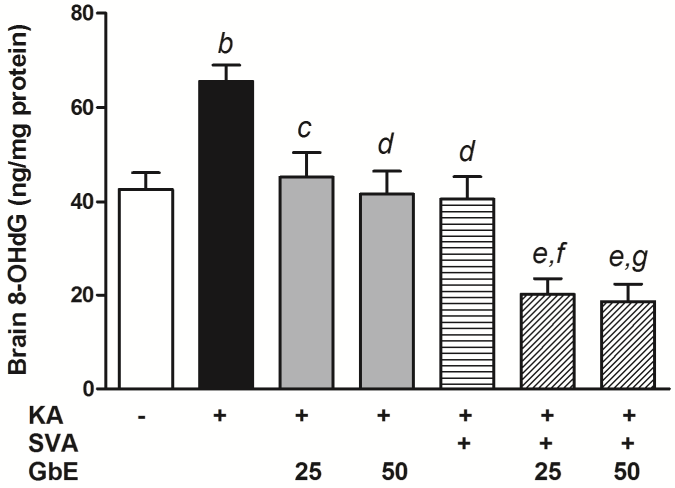

Fig 3. Effect of SVA (200 mg/kg, i.p.), GbE (25 and 50 mg/kg, p.o.) and their combination on brain 8-hydroxy-2-deoxyguanosine (8-OHdG) level in kainic acid-induced seizure model in mice. Results represent mean ± SEM. [a] p< 0.05 vs. naïve, [b] p< 0.01 vs. naïve. [c]p< 0.05 vs. KA (control), [d]p< 0.01 vs. KA (control). [e]p< 0.001 vs. KA (control). [f] p< 0.05 vs. SVA, [g] p< 0.01 vs. SVA.

4. DISCUSSION

In the present study, we investigated the effect of ginkgo biloba extract (GbE) on the anticonvulsant and neuroprotective effects of the widely used broad spectrum anticonvulsant drug SVA. The anticonvulsant activity of single and combined treatment of SVA in a dose 200 mg/kg, i.p., and GbE in doses 25 and 50 mg/kg, p.o., against KA- (10 mg/kg, i.p.,) induced seizure in mice, was studied. In addition, the effects of the tested drugs on brain glutamate, lipid peroxidation and the antioxidant defenses GSH and GSH-Px were tested. Moreover, the corresponding changes in

serum level of neuron specific enolase (NSE), a marker of acute brain injury and blood–brain barrier dysfunction, which is elevated in seizure activity and both serum and brain levels of 8-hydroxy-2-deoxyguanosine a specific marker for oxidative DNA damage were investigated.

The idea that an excessive release of glutamate mediates neuronal cell death was proposed, as the excitotoxicity hypothesis, by Olney and de-Gubareff (1978). Glutamate activates two types of its receptor, *N*-methyl-D-aspartate (NMDA) type and non-NMDA one (Dingledine et al., 1999). Excessive activation of glutamate receptors, resulting in an increase in intracellular calcium concentration ($[Ca^{2+}]i$) and generation of reactive oxygen species (ROS), is involved in neuronal disorder and degeneration.(Choi 1992). Both phenomena induced by glutamate are related to cell injury and death (Coyle and Puttfarcken 1993).

Results of the present study indicated that, SVA in a dose 200 mg/kg, i.p. successfully protected animals against KA-induced seizures. This was reflected in a form of a decrease in the percentage of seizures and morality and significant prolongation of the seizure latency of KA-induced seizures. As a broad spectrum anticonvulsant drug, SVA is effective against generalized and partial seizures (Ma et al., 2010) and effectively protects against KA-induced seizures (Velísek et al., 1992).

In addition, in the present study, GbE in doses 25 and 50 mg/kg, p.o., showed a protective effect against KA-induced seizures. GbE is one of the most extensively researched medicinal plants in the world, used by medical professionals to aid the treatment of many health problems. The most important constituents of GbE are flavone glycosides (quercetin, kaempferol, isorhamnetin) and terpene lactones (ginkgolides and bilobalide) (Mahadevan and Park 2008). Bilobalid is present in GbE in measurable concentrations and has a significant anticonvulsant activity (Sasaki et al., 1997).

Combined treatment of SVA with GbEin doses 25 and 50 mg/kg, p.o enhanced the anticonvulsant effect of SVA against KA-induced seizures. This effect appeared in a form of reduction in percentage of seizures and mortality, and increase in clonic seizures onset in KA seizure model. This effect was with highest significance with the high dose (50 mg/ kg) of GbE-761. In support of our results, Ilhan et al., (2006) found that GbEprotects against development of seizures and increases the anticonvulsant activity of valproic acid against pentylenetetrazole (PTZ)-induced kindling in mice.

Previous studies indicated that a potential mechanism that may contribute to SVA antiseizure actions involves metabolism of GABA. Although valproate has no effect on responses to GABA, it does increase the amount of GABA that can be recovered from the brain and decreases the amount of glutamate after the drug is administered to animals. In vitro, valproate can stimulate the activity of the GABA synthetic enzyme, GAD, and inhibit GABA degradative enzymes, GABA-transaminase and succinic semialdehyde dehydrogenase (Atmaca 2009).

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 in KA-induced seizure model. These changes produced by GbE-761may 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 (Mula 2011).

According to Mahady (2001), the neuroprotective effect of GbE is attributed to its major flavonoid constituent bilobalid. Bilobalid has the ability to decrease brain glutamate and increase brain GABA via stimulation of GAD activity (Sasaki et al., 1999). In addition, Jones et al (2002) showed that, bilobalid has the ability to stimulate GABA release in cerebral neurons. Moreover, GbE and its constituents inhibit the response mediated by activation of NMDA receptor (Li et al., 2011) and exert a direct inhibitory action on kainate receptor (Kanada et al., 2005).

Although the effect of SVA and GbE on the glutamate represent a major part in their anticonvulsant and neuroprotective effects, but their effects on the seizure-inducd oxidative stress and antioxidant defenses are also important. In the present study, KA increased the brain level of MDA, the lipid peroxidation

product and decreased the activity of antioxidant defenses GSH and GSH-Px. 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).

GbE in the tested doses decreased the MDA level and increased the GSH level and GSH-Px activity in brains of animals exposed to KA-induced seizures. In addition, the combined treatment with SVA and GbE decreased the MDA and increased the GSH level and GSH-Px activity than their levels in SVA-treated group. These results indicated that addition of GbE to SVA added the benefits of inhibition of lipid peroxidation and stimulation of GSH and GSH-Px to the effects of SVA.

GbE has a potent antioxidant effect (Rhein et al., 2010). Numerous studies have shown that GbE has an antioxidant (Arushanian and Beĭer, 2008), free radical scavenging (Louajri et al., 2001) and neuroprotective effects on neurons suffering from oxidative stress (Saleem et al., 2008). Hence, the observed enhancement in the anticonvulsant effect of SVA mediated by GbE and the corresponding changes in neuronal damage can be, at least partly, related to the antioxidant effect of GbE-761. On the other hand, some studies indicated that, antioxidant effects do not contribute in the anticonvulsant activity against KA-induced seizures (Akcay et al., 2005)

Neuron specific enolase (NSE), is a marker of acute brain injury and blood–brain barrier dysfunction, elevates in seizure activity. NSE is released into both the cerebrospinal fluid and serum after CNS damage. 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 concentration of NSE. Thus, it appears that NSE may be a marker of CNS damage (Pandey et al., 2011).

In addition, 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. The DNA lesions are consequently, removed by the base excision repair (BER) pathway, which prevents replication of DNA lesions. Moreover, ROS inhibit the BER system through direct interactions with cellular repair proteins (Feng et al., 2006). Since the BER pathway removes the mutagenic 8-OHdG lesions, the inhibitory effects of ROS pathways on BER activity may potentiate mutagenesis and DNA damage. 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 (Valavandis et al 2009). This was clear in our results that showed a marked increase in both serum NSE and 8-OHdG and brain 8-OHdG levels, biomarkers for neuronal damage and oxidative DNA damage in KA-treated animals.

In the present study, both SVA and GbE could decrease 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 combined treatment of SVA with GbEcompared with that observed after treatment with SVA alone. This indicated that, addition of GbEto SVA not only enhanced its anticonvulsant activity but also enhanced its neuroprotective effect against neuronal damage and oxidative DNA damage induced by ROS during KA-induced seizures the benefit that obtained by addition of GbE, which by itself has the ability to protect the neurons against glutamate-induced injury (Xu et al., 2010) to SVA.

Hence, the observed enhancement in the neuroprotective effect of SVA by GbE may be attributed to different mechanisms, which can be synergistic, exerted by the active constituents of GbE. Both EGb 761 and bilobalide are protective against glutamate-induced neuronal death in vitro by synergistic mechanisms involving anti-excitotoxicity, inhibition of free radical generation, scavenging of reactive oxygen species, and regulation of mitochondrial gene expression (Chandrasekaran et al., 2003).

CONCLUSION

GbE in small to moderate doses 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 combination in treatment of epilepsy in human.

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