



Evaluation of the neuroprotective effect of taurine and green tea extract against oxidative stress induced by pilocarpine during status epilepticus

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Abstract Status epilepticus (SE) has functional and structural consequences resulting in brain damage. The present study aims to investigate the role of taurine and green tea extract in the neuroprotection against oxidative stress and changes in acetylcholinesterase (AChE) and Na^+ , K^+ -ATPase activities during SE induced by pilocarpine in the hippocampus of adult male rats. Animals received an oral administration of either taurine (100 mg/kg) or green tea extract containing 100 mg/kg epigallocatechin gallate for 3 days before the induction of SE with pilocarpine (380 mg/kg, i.p.) and were sacrificed 1 h after pilocarpine injection. Data indicated that a state of oxidative stress has evolved during SE as evident from the significant increase in lipid peroxidation level and significant decrease in reduced glutathione (GSH) level. Significant decreases in AChE and Na^+ , K^+ -ATPase activities were also recorded. Pretreatment of rats with taurine exaggerated the increase in lipid peroxidation and failed to prevent the decrease in Na^+ , K^+ -ATPase activity resulting from pilocarpine. However, taurine pretreatment prevented the reduced activity of hippocampal

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; EC, (–)-epicatechin; ECG, (–)-epicatechin gallate; EGC, (–)-epigallocatechin; EGCG, (–)-epigallocatechin gallate; GSH, reduced glutathione; GST, glutathione-S-transferase; LP, lipid peroxidation; MDA, malondialdehyde; NO, nitric oxide; ROS, reactive oxygen species; SE, status epilepticus; SOD, superoxide dismutase; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy

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AChE induced by pilocarpine during SE. Pretreatment of rats with green tea extract prevented the increase in lipid peroxidation occurring during SE. However, it failed to inhibit the decrease in Na^+, K^+ -ATPase activity. In conclusion, taurine pretreatment failed to reduce the oxidative stress induced during SE. In contrast, pretreatment of rats with green tea extract ameliorated the oxidative stress induced by pilocarpine and this may assist in reducing the insults of hyperexcitability and excitotoxicity that occur during SE and thereby reduce neuronal damage.

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Introduction

Status epilepticus (SE) is a severe clinical manifestation of epilepsy and has functional and structural consequences resulting in brain damage (Matzen et al., 2008). In this condition, a single seizure or a series of seizures lasts for 30 min or more without restoration of consciousness, and is associated with a significant morbidity and mortality, including neuronal damage and dysfunction (Fountain, 2000). SE is the first phase that occurs during the evolution of temporal lobe epilepsy (TLE). SE is followed by a silent phase and spontaneous recurrent seizures (SRS) (Cavalheiro et al., 1994).

The hippocampus has been a focus of interest in temporal lobe epilepsy research because it contains several well-described neuronal circuits linked to seizure onset and because it develops, in the time course of the disease, a severe loss of pyramidal cells in CA1, CA3 and the dentate gyrus (Pitkanen and Lukasiuk, 2009).

It is well known that when reactive oxygen species (ROS) production is excessive, the intrinsic antioxidant scavenging capacity is overwhelmed resulting in the development of oxidative stress which can induce tissue injury and may activate apoptosis processes (Todorova et al., 2004). The hippocampus is particularly susceptible to lipid peroxidation due to the simultaneous presence of high levels of polyunsaturated fatty acids and iron (Halliwell and Gutteridge, 1989). In addition, the hippocampus presents a low number of antioxidant systems (de Freitas, 2010).

Oxidative stress can dramatically alter neuronal function and has been associated with neurochemical changes observed during SE and SRS induced by pilocarpine (Barros et al., 2007). Moreover, oxidative stress and mitochondrial dysfunction could be acute consequences of SE that are related to the mechanism of seizure-induced neuronal cell death and subsequent epileptogenesis (Patel, 2004). Therefore, antioxidant compounds have been of great interest as potential therapies for treatment of epilepsy (Golden and Patel, 2009).

Taurine (2-aminoethansulfonic acid) is present at high concentrations in the mammalian brain (Guidotti et al., 1972). Several functions of taurine have been reported, including neuroprotection (Chen et al., 2001), neuromodulation (Kuiyama, 1980) and neurotransmission (Taber et al., 1986).

Animal studies have shown that taurine has anticonvulsant action (Huxtable and Nakagawa, 1985). In some animal studies, a significant reduction in seizure frequency was observed (König et al., 1977; Takahashi and Nakane, 1978).

The intracerebroventricular injection of taurine restored the normal-like electroencephalographic features during the interictal period in pilocarpine-treated rats during spontaneous recurrent seizures (Radwan, 2001) whereas no benefit was seen in other studies (Mantovani and DeVivo, 1979). Moreover, taurine has been shown to hyperpolarize neurons in the hippocampus (del Olmo et al., 2000) by opening chloride channels through its interaction with GABA_A receptors (Bureau and Olsen, 1991), glycine receptors (Hussy et al., 1997), or taurine receptors (Wu et al., 1992). Moreover, taurine has antioxidant properties (Chen et al., 2012), due to its ability to improve mitochondrial function by stabilizing the electron transport chain and inhibiting the generation of reactive oxygen species (Jong et al., 2012).

Green tea is one of the plant products that have significant effects on human health. It contains many polyphenolic compounds, including (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate, and (–)-epicatechin. Of these, EGCG is the most abundant and most effective antioxidant having one diphenolic and two triphenolic groups (Kondo et al., 1999). Green tea polyphenols can penetrate the blood–brain barrier and remain in the brain for more than 24 h (Suganuma et al., 1999). Green tea, its extract and its isolated constituents were found to be effective in preventing oxidative stress (Babu et al., 2006) and brain problems (Unno et al., 2007). Several studies suggested that green tea polyphenols might protect against Parkinson's and Alzheimer's diseases and other neurodegenerative diseases (Pan et al., 2003; Weinreb et al., 2004). Previously, in an electroencephalographic study, Yokoi et al. (1989) examined the effects of EGC or EGCG on the occurrence of epileptic discharges in EEGs induced by the injection of iron ions into the sensorimotor cortex of rats. The authors found that pretreatment with EGC or EGCG prevented the occurrence of epileptic discharges in some rats and slowed them in other rats. They suggested that EGC or EGCG scavenged active oxygen free radicals to prevent the formation of an epileptic focus. A recent study of Kang et al. (2010) showed that EGCG has a strong protective effect against hippocampal neuronal oxidative stress and cell death both *in vitro* and *in vivo*.

The main objective of the present study was to gain further insight into the role of taurine and green tea in the neuroprotection against oxidative stress and changes in acetylcholinesterase (AChE) and Na^+, K^+ -ATPase enzyme activities resulting from pilocarpine-induced SE in adult male rats.

Materials and methods

Experimental animals

The experimental animals used in the present study were adult male Wistar albino rats weighing 200–250 g. The animals were purchased from the animal house of the National Research Center (Giza, Egypt). They were given standard laboratory diet supplied from National Research Center (Giza, Egypt) and water *ad libitum*. The diet consisted of 10% casein, 4% salt mixture, 1% vitamin mixture, 10% corn oil, 5% cellulose and was completed to 100 g with corn starch. The animals were maintained under fixed appropriate conditions of housing and handling. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt, which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Drugs and chemicals

Pilocarpine was obtained from Macfarlan Smith Ltd. (Edinburgh). It was dissolved in saline. Atropine sulfate was obtained from Boehringer Ingelheim (Germany). Green Tea Mega EGCG (Veggie capsules) was purchased from Pro-Health, Inc. (USA). Each capsule contains 725 mg of Opteava® Green Tea 95% Extract (standardized to provide 95% polyphenols). The polyphenol breakdown gave a total polyphenol concentration of 688.8 mg, total catechins 543 mg, EGCG 290 mg and other polyphenols 145 mg. The contents of one capsule were suspended in 5.8 ml distilled water. Taurine supplied by Sigma (USA) was dissolved in distilled water. Trichloroacetic acid, thiobarbituric acid, 1-chloro-2,4-dinitrobenzene, acetylthiocholine iodide and glutathione were also obtained from Sigma (USA).

Design of experiment

Rats were divided into 4 groups. Group 1 consisted of control animals which were administered orally with saline for 3 consecutive days. Group 2 consisted of untreated pilocarpinized animals which were administered orally with saline for 3 consecutive days and then injected intraperitoneally with a single dose of pilocarpine (380 mg/kg) according to [Turski et al. \(1983\)](#) 60 min after the last saline administration. Atropine sulfate was injected subcutaneously at a dose of 5 mg/kg, 30 min before the induction of epilepsy, to prevent peripheral muscarinic stimulation ([Williams and Jope, 1994](#)). Group 3 consisted of treated animals which were administered orally with taurine (100 mg/kg) for 3 consecutive days and then injected intraperitoneally with a single dose of pilocarpine (380 mg/kg) 60 min after the last taurine administration. Group 4 consisted of treated animals which were administered orally with green tea extract (Green Tea Mega EGCG) containing 100 mg/kg of EGCG and then injected intraperitoneally with a single dose of pilocarpine (380 mg/kg) 60 min after the last green tea extract administration. Green tea extract was used as it is more stable than pure EGCG because

of the presence of other antioxidant constituents in the extract ([Osada et al., 2001](#)).

All the animals were sacrificed after 1 h of pilocarpine injection. After decapitation, the brain of each animal was transferred rapidly to an ice-cold Petri dish where it was dissected to remove the hippocampus. The hippocampal samples were weighed (~0.05 g) and kept at -56°C until analyzed. Each brain sample was then homogenized in 4 ml 5%w/v 20 mM phosphate buffer, pH 7.6. The supernatant was used for the assay of the different measured parameters.

Determination of nitric oxide level

The assay of nitric oxide (NO) was carried out using Biodiagnostic Kit No. NO 25 33 (Biodiagnostic Co., Egypt). This method is based on the spectrophotometric method of [Montgomery and Dymock \(1961\)](#) in which endogenous nitrite concentration is measured as an indicator of NO production. It depends on the addition of Griess Reagent which converts nitrite into a deep purple azo compound whose absorbance is read at 540 nm in a Helios Alpha UV-Visible spectrophotometer (Thermo Spectronic, England).

Determination of lipid peroxidation

Lipid peroxidation (LP) was determined by measuring the level of thiobarbituric reactive species according to the method of [Ruiz-Larrea et al. \(1994\)](#). The thiobarbituric acid reactive substances, the most important of which is malondialdehyde, react with thiobarbituric acid to produce a red colored complex with peak absorbance at 532 nm.

Determination of reduced glutathione level

The measurement of reduced glutathione (GSH) levels was performed using Biodiagnostic Kit No. GR 25 11 (Biodiagnostic Co., Egypt) which is based on the spectrophotometric method of [Beutler et al. \(1963\)](#). It depends on the reduction of 5,5'-dithiobis 2-nitrobenzoic acid by GSH to produce a yellow color whose absorbance is measured at 405 nm.

Determination of enzyme activities

Superoxide dismutase (SOD) was measured using Biodiagnostic Kit No. SD 25 21 (Biodiagnostic Co., Egypt) which is based on the method of [Nishikimi et al. \(1972\)](#). This method depends on the ability of the enzyme to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye. The change in absorbance is read at 560 nm over 5 min after addition of phenazine methosulfate.

Glutathione-S-transferase (GST) was determined by the method of [Habig et al. \(1973\)](#) which measures the conjugation of 1-chloro-2,4-dinitrobenzene with GSH. This conjugation is accompanied by an increase in absorbance at 340 nm, the rate of increase being directly proportional to GST activity.

The procedure used for the determination of AChE activity in the hippocampus was a modification of the method of [Ellman et al. \(1961\)](#) as described by [Gorun et al. \(1978\)](#). The method is based on the measurement of the thiocholine

produced as acetylthiocholine is hydrolyzed. Thiocholine is allowed to react with the -SH reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow colored anion whose absorption was read immediately at 412 nm.

Na^+, K^+ -ATPase and Mg^{2+} -ATPase activities were measured spectrophotometrically according to Bowler and Tirri (1974) as described by Tsakiris et al. (2000). Na^+, K^+ -ATPase activity was calculated as the difference between total ATPase activity ($\text{Na}^+, \text{K}^+, \text{Mg}^{2+}$ -dependent) and Mg^{2+} -dependent ATPase activity.

Statistical analysis

The data were expressed as means \pm S.E.M. The n value for each experimental group was 6. Data were analyzed by analysis of variance (ANOVA) followed by the Duncan multiple range test when the F -test was significant ($p < 0.05$). All analyses were performed using the Statistical Package for Social Sciences (SPSS) software in a compatible personal computer.

Results

After 30 min of pilocarpine injection, the animals became hypoactive and then displayed orofacial automatisms, salivation, eye-blinking, twitching of vibrissae and yawning. This was followed by generalized convulsions and limbic SE after about 40 min from pilocarpine injection. Animals pretreated with taurine before SE showed mild to severe tremors with facial automatisms, hair erection, eye-blinking, dyskinesia and difficulty in breathing. However, there were no signs of convulsions. On the other hand, animals pretreated with green tea extract before induction of epilepsy exhibited mild tremors at the beginning then moderate sedation with no clear manifestation of the other symptoms of SE.

The data on the effect of the pretreatment of rats before induction of SE by pilocarpine with green tea extract or taurine for 3 days on some oxidative stress parameters in the hippocampus are presented in Fig. 1.

ANOVA revealed significant differences in hippocampal SOD, MDA (as a measure of lipid peroxidation), GSH, GST and NO between control rats, pilocarpine-treated rats, rats pretreated with taurine (100 mg/kg) for 3 days before pilocarpine injection and rats pretreated with green tea extract for 3 days before pilocarpine. The i.p. injection of pilocarpine resulted in a significant increase in SOD activity and MDA levels in the hippocampus after 1 h, i.e., during SE. In addition, a significant decrease in hippocampal GSH and NO levels and GST activity occurred in pilocarpinized rats during the same period. Pretreatment of rats with taurine (100 mg/kg) before induction of SE exaggerated the increase in lipid peroxidation and failed to ameliorate the changes in SOD and GSH obtained after pilocarpine. However, taurine prevented the decrease of NO levels and GST activity induced by pilocarpine during SE. On the other hand, pretreatment of rats with green tea extract before pilocarpine injection prevented the increase in MDA levels and SOD activity obtained during SE. It also attenuated the decreases in GSH and NO levels and GST activity in SE-induced rats.

As illustrated in Fig. 2 and indicated from ANOVA, there were significant differences in the activities of AChE and

Na^+, K^+ -ATPase in the hippocampus between the different experimental groups. A significant decrease in the activities of both AChE and Na^+, K^+ -ATPase was recorded after 1 h in unprotected pilocarpinized rats. Taurine pretreatment for 3 days before the induction of SE prevented the decrease in AChE activity induced by pilocarpine in the hippocampus but failed to prevent the decrease in Na^+, K^+ -ATPase activity. The decrease in AChE activity was attenuated in rats pretreated with green tea extract before pilocarpine. However, green tea extract pretreatment had no effect on the pilocarpine-induced decrease in Na^+, K^+ -ATPase activity.

Discussion

The present results showed a significant increase in hippocampal lipid peroxidation level and SOD activity in pilocarpine-treated rats during SE. This was accompanied by significant decreases in GSH level, GST activity and NO level. This indicates that a state of oxidative stress has evolved during SE induced by pilocarpine. This oxidative stress could be attributed to the imbalance between the production of ROS and the antioxidants of the hippocampus. These results agree with the findings of other investigators during SE in the pilocarpine model (Tejada et al., 2006; de Freitas, 2010; Khadrawy et al., 2013). This is correlated with a state of hyperexcitability arising from the massive release of glutamate which stimulates NMDA receptors resulting in a massive influx of Ca^{2+} and the formation of ROS and RNS which cause lipid peroxidation, mitochondrial and nuclear DNA damage (Fonnum and Lock, 2004).

GSH is a nonenzymatic cellular antioxidant defense system (Galleano and Puntarulo, 1995). In addition, it may act as a substrate in various enzymatic antioxidant defense mechanisms (Dringen, 2000). The significant decrease in GSH level may be due to its exhaustion to mitigate the state of oxidative stress that evolved during SE. The present decrease in GST activity could be due to the decrease in its substrate GSH as GST catalyzes the addition of the tripeptide GSH to endogenous and xenobiotic substances (Yousef, 2004).

Superoxide dismutase (SOD) converts superoxide anion (O_2^-) to hydrogen peroxide (Akyol et al., 2002). The present increase in SOD may reflect an early attempt of the brain to resist the generation of free radicals produced during SE induced by pilocarpine.

The role of NO during seizures is controversial. While some authors believe that NO may be an endogenous anticonvulsant (Przegalinski et al., 1996), others suggest a proconvulsant role for NO (Rajasekaran, 2005). The anticonvulsant effects of NO are achieved by its modulatory effect on NMDA receptors where NO donors reduce NMDA-evoked ion current (Schuman and Madison, 1994). Thus, the attenuation of the action of NMDA receptors may underlie the neuroprotective role of NO (Manzoni et al., 1992). Accordingly, the significant decrease in hippocampal NO levels in the present study could facilitate the induction of SE seizures induced by pilocarpine.

Acetylcholinesterase (AChE) has a crucial role in terminating cholinergic neurotransmission by hydrolyzing acetylcholine (ACh) (Duysen et al., 2002). Thus the significant decrease in hippocampal AChE activity, in the present study, may underlie the excessive content of acetylcholine during SE (Frantseva et al., 2000) induced by the cholinergic agonist pilocarpine.

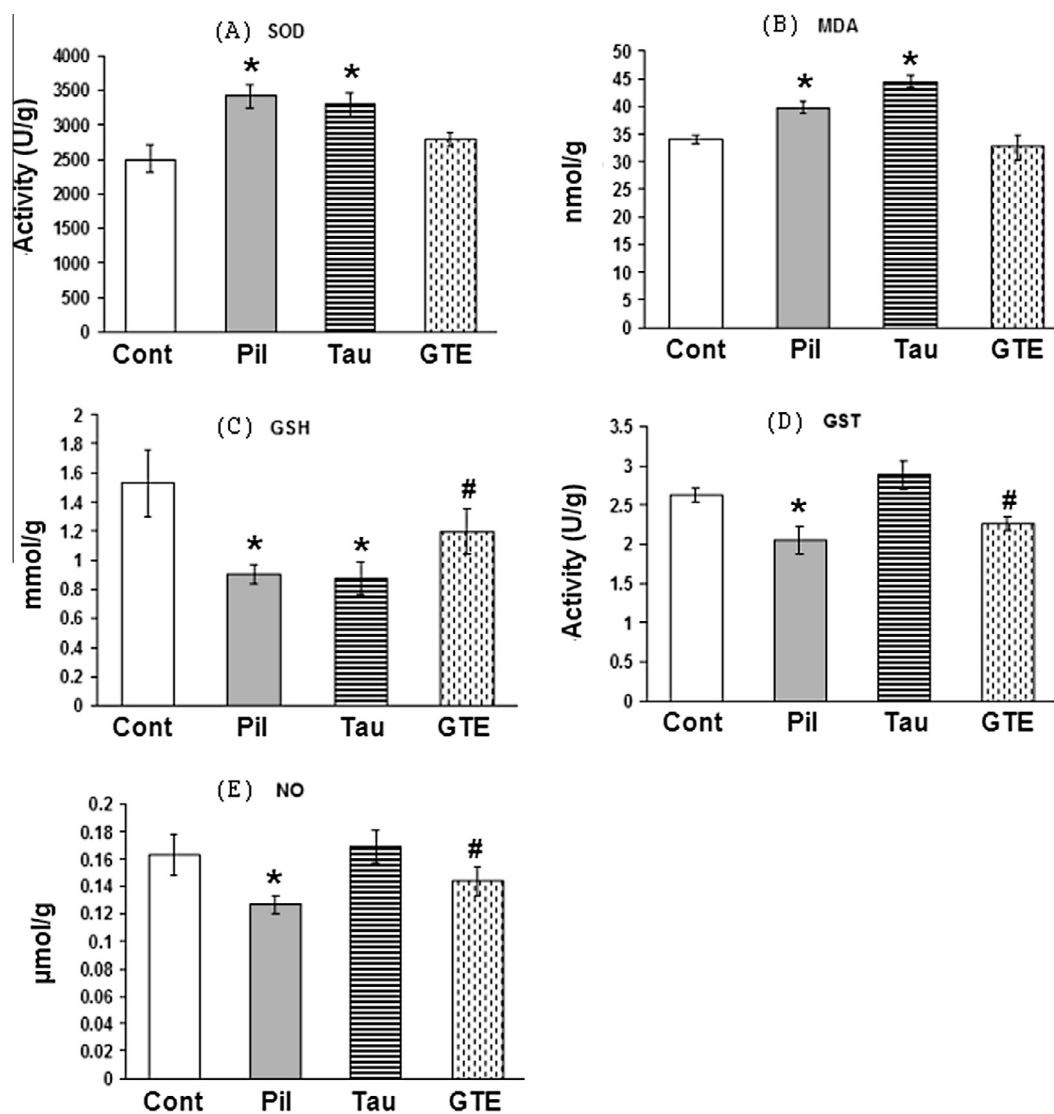


Figure 1 Effect of taurine (Tau) and green tea extract (GTE) on (A) SOD, (B) MDA, (C) GSH, (D) GST and (E) NO in the hippocampus of pilocarpine-treated rats. □ Control, ■ Pilocarpine-treated rats (Pil), ▨ Pilocarpinized rats treated with taurine (Tau), ▩ Pilocarpinized rats treated with green tea extract(GTE), * sign indicates significant change from control, # sign indicates non significant changes between control, GTE and pilocarpine-treated animals.

On the other hand, the significant inhibition in the activity of hippocampal Na^+, K^+ -ATPase, in the present investigation, is an evidence of the state of hyperexcitability or depolarization arising from the influx of Na into the cell as Na^+, K^+ -ATPase acts to maintain the ionic gradient for neuronal excitability (Erecińska and Silver, 1994). Kamboj et al. (2006) reported that seizures alter membrane lipid composition and this can affect membrane-bound enzymes and this in turn may have serious consequences on neuronal functioning. Moreover, Chuang et al. (2009) reported that during a prolonged seizure, neuronal cells may exhibit a temporary drop in ATP production. Thus, the reduced Na^+, K^+ -ATPase activity could be due to the decreased concentration of its substrate (ATP) on the one hand and the increased membrane lipid peroxidation as a result of the attack of the cell membrane by free radicals generated during SE on the other hand.

Although many studies have shown that taurine has antioxidant activity (Chen et al., 2012), the present study

revealed that pretreatment of rats with taurine (100 mg/kg) for 3 days exaggerated the increase in lipid peroxidation induced by pilocarpine during the SE period. In addition, it failed to prevent the increase in SOD activity and the decrease in GSH levels resulting from pilocarpine. This suggests that the daily pretreatment of rats with taurine for 3 days showed no evidence of antioxidant activity in this model during SE. This can be attributed to the severe oxidative stress during SE together with the stimulating effect of taurine on the release of glutamate and other neurotransmitters (Suárez and Solís, 2006). Supporting this explanation is the inability of taurine to prevent the inhibition of Na^+, K^+ -ATPase activity which ensures the presence of a state of hyperexcitability arising from pilocarpine.

However, the present investigation revealed that taurine pretreatment prevented the reduced activity of hippocampal AChE and NO levels induced by pilocarpine during SE. This may explain the reported anticonvulsant effect of taurine.

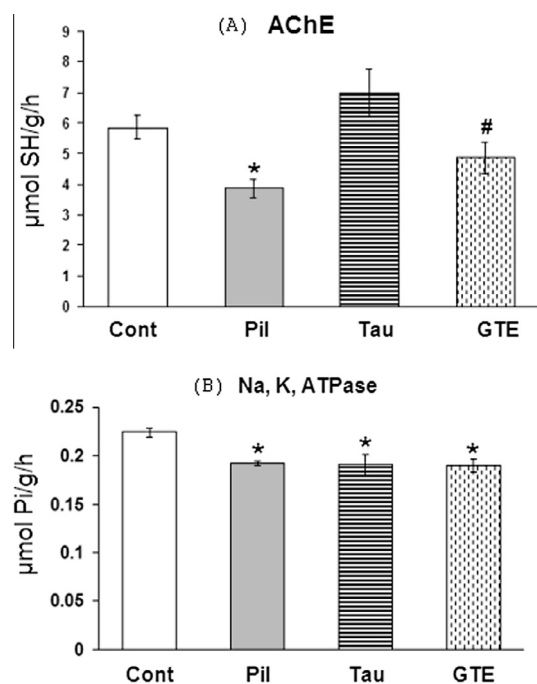


Figure 2 Effect of taurine (Tau) and green tea extract (GTE) on (A) AChE and (B) Na^+ , K^+ -ATPase activity in the hippocampus of pilocarpine-treated rats. □ Control, ■ Pilocarpine-treated rats (Pil), ▨ Pilocarpinized rats treated with taurine (Tau), ▩ Pilocarpinized rats treated with green tea extract (GTE), * sign indicates significant change from control, # sign indicates non significant changes between control, GTE and pilocarpine-treated animals.

The ability of taurine to prevent the decrease in NO obtained during SE may help to reduce NMDA receptor activation and thus reduce the excitotoxic effects of glutamate.

In the present study, taurine administration for 3 days before induction of SE prevented the decrease in AChE activity in the hippocampus of pilocarpine-treated rats. This may help in reducing the cholinergic activity and thus attenuate the drastic symptoms of SE.

Thus, it may be concluded that taurine may have an anticonvulsant effect which is mediated by its ability to prevent the excessive cholinergic and glutamatergic activity by maintaining AChE activity and NO levels. However, this study rules out an antioxidant effect of taurine since taurine was unable to prevent lipid peroxidation or increase antioxidant levels. In line with the present findings, it has been found that taurine was relatively ineffective in scavenging peroxynitrite in cultured neurons treated with NO donors (Mehta and Dawson, 2001). Similarly, in cultured cerebellar neurons, taurine prevented the kainate-induced cell death without reducing the free radical levels (Boldyrev et al., 1999).

In the present study, the daily pretreatment of rats with the green tea extract for 3 days before pilocarpine injection prevented the increase in lipid peroxidation and SOD activity obtained during pilocarpine-induced SE. Green tea extract also attenuated the decreases in NO and GSH levels and GST and AChE activities occurring during SE. However, it failed to inhibit the decrease in Na^+ , K^+ -ATPase activity.

Several compounds of green tea have been reported to suppress lipid peroxidation in model systems and in several

biological systems such as mitochondria and microsomes (Middleton et al., 2000). The structure of polyphenols in green tea suggests that O-dihydroxyphenyl (catechol) or 1,2,3-trihydroxyphenyl (pyrogallol) structure is responsible for the most effective property of green tea which is inhibition of lipid peroxidation (Jovanovic et al., 1996). Catechins react with peroxy radicals in phospholipid bilayers via a single electron transfer followed by deprotonation (Jovanovic et al., 1996). Moreover, green tea polyphenols have been shown to possess relatively potent metal-chelating properties (Grinberg et al., 1997). This is important in view of the presence of highly active catalytic metal ions, i.e., iron and copper that are known to produce oxygen radicals in situ via Fenton reaction in the hippocampus (Zaleska and Floyd, 1985). The presence of hydroxyl groups attached to the ring structure of the polyphenols (or catechins) of green tea facilitates the interaction with metal ions by hydrogen bonding. This in turn may prevent lipid peroxidation by restricting the access of the metal ion toward lipid hydroperoxides in biomembranes (Morel et al., 1993).

Thus, the structure of the catechins present in green tea extract may be responsible for its efficient prevention of lipid peroxidation in rats subjected to SE induced by pilocarpine. The chelation of ions by green tea catechins may also lead to a decrease in Fenton reaction and increase in hydrogen peroxide in the brain (Skrzydewska et al., 2002). This may explain the inability of green tea extract to raise the decreased levels of GSH after SE to control levels since GSH may be consumed in scavenging the excessive hydrogen peroxide generated during SE. It follows that pretreatment with green tea extract failed to maintain GST activity in SE-induced rats due to the reduced concentration of its substrate GSH.

Moreover, the attenuation of the increased SOD activity after pretreatment with green tea extract, in the present study, was also expected in view of the metal-chelating ability of green tea catechins which permit these compounds to protect SOD and glutathione peroxidase from the actions of metal-generated free radicals as suggested by Skrzydewska et al. (2002).

As lipid peroxidation is the main hallmark of brain oxidative stress due to the high content of brain lipids, the absence of lipid peroxidation in SE model pretreated with green tea emphasizes the potent antioxidant activity of green tea extract in preventing the oxidative stress induced by pilocarpine.

However, the state of hyperexcitability induced by pilocarpine persisted in rats pretreated daily with green tea extract for 3 days. The inability of green tea to suppress the state of hyperexcitability was evident from the reduced activity of Na^+ , K^+ -ATPase.

On the other hand, an improvement in the decreased AChE activity in SE-induced rats occurred, in the present investigation, due to the pretreatment of rats with green tea extract for 3 days. Although the enzyme activity was not maintained at control values, this improvement may be useful in hydrolyzing the increased ACh levels to some extent and thus reduce the severity of the symptoms of SE as observed in the present study.

Lin et al. (1998) suggested that changes in the brain might be expected after a long time of oral administration of green tea. This may explain the inability of green tea extract to completely prevent the changes in the antioxidant parameters induced by pilocarpine, in the present study.

In conclusion, it is clear from the present findings that the daily pretreatment of rats with green tea extract for 3 days ameliorated the state of oxidative stress during SE induced by pilocarpine. This may be helpful in reducing the insults of hyperexcitability and excitotoxicity that occur during SE and thereby reduce neuronal damage. Green tea extract also attenuated the decrease in AChE activities occurring during SE but failed to prevent the decrease in Na^+/K^+ -ATPase activity. In contrast, daily taurine pretreatment for 3 days failed to show any significant effect on the oxidative stress induced in the hippocampus by the present pilocarpine model of SE. However, the present investigation revealed that taurine pretreatment prevented the reduced activity of hippocampal AChE and NO levels during SE induced by pilocarpine which may explain the reported anticonvulsant effect of taurine.

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Conflict of interest

No conflict of interest.

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