

Effects of Lycopene and Sodium Valproate on Pentylenetetrazol-Induced Kindling in Mice

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What's Known

- It is well known that the serious adverse effects of conventional antiepileptic drugs render epilepsy treatment difficult and challenging. Antioxidants such as lycopene have a protective role in the treatment of epilepsy by inhibiting the oxidative mechanism.

What's New

- For the first time, we evaluated lycopene in combination with sodium valproate to treat pentylenetetrazol-induced epilepsy and found significant improvement in animals.

Abstract

Sodium valproate and tomato extract have been studied in different experimental models of epilepsy individually. The aim of the present study was to evaluate the effect of lycopene on the antiepileptic effects of sodium valproate against pentylenetetrazol-induced kindling in mice. Swiss albino mice of either sex were randomly divided into 5 groups, with each group containing 8 mice. These groups were treated with pentylenetetrazol (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14 day, i.p.); sodium valproate (200 mg/kg, p.o.) + pentylenetetrazol; lycopene (2 mg/kg, p.o.)+sodium valproate(200 mg/kg,p.o.)+pentylenetetrazol; and lycopene (4 mg/kg, p.o.) + sodium valproate (200 mg/kg, p.o.) + pentylenetetrazol, for 14 days, respectively. After treatment, the animals were observed for 30 minutes for behavioral analysis. Subsequently, the animals were sacrificed, and their brain was removed for the biochemical estimations of thiobarbituric acid reactive substances, catalase, superoxide dismutase activity, reduced glutathione, and gamma-aminobutyric acid. Significant pentylenetetrazol-induced seizure was characterized by alteration in the seizure score and latency as well as a significant increase in the levels of brain thiobarbituric acid reactive substances and a significant decrease in reduced glutathione, catalase, superoxide dismutase, and gamma-aminobutyric acid levels. Treatment with sodium valproate and lycopene significantly restored the seizure score, latency, thiobarbituric acid reactive substance, reduced glutathione, catalase, superoxide dismutase, and gamma-aminobutyric acid levels near to normal compared to pentylenetetrazol. The present study provides experimental evidence that a combination therapy of lycopene along with sodium valproate attenuated seizure and oxidative stress against pentylenetetrazol-induced kindling in mice.

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• Pentylenetetrazol • Epilepsy • Gamma-aminobutyric acid

Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, which are sudden, unprovoked, and transitory, and recurrent episodes of abnormal hypersynchronous neuronal discharge.¹ Epilepsy is the most common primary neurological disorder. It is estimated that over 50 million people worldwide are epileptic (1–2% of the

world's population), out of whom 40 million are living in developing countries.² Epilepsy is the second most common neurological disorder in India.^{3,4} Regardless of advances in epilepsy research, the pharmacotherapy of epilepsy remains largely pragmatic due to a lack of basic pathology. The use of synthetic anticonvulsants such as phenytoin, carbamazepine, and sodium valproate (SVP) is associated with adverse effects such as negative impacts on learning and memory.⁵ Furthermore, about 30% of patients with epilepsy have seizures that do not react adequately to conventional antiepileptic drugs (CAEDs).⁶ These limitations with CAEDs alone highlight the need for exploring drugs that could potentiate the action of CAEDs so as to make the treatment of epilepsy more effective. It might be useful to assess the use of natural remedies possessing antioxidant activity against epileptic seizures. SVP is one of the mostly prescribed antiepileptic drugs in the treatment of many different types of partial and generalized epileptic seizures and also for other neuropsychiatric problems.⁷

SVP increases the turnover of gamma-aminobutyric acid (GABA) and thereby potentiates GABAergic functions in some specific brain regions thought to be involved in the control of seizure generation and propagation. SVP causes neuronal excitation mediated by the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors for its anticonvulsant effect. It also blocks sodium ion channels.⁸

Lycopene (LYC), the red pigment in tomatoes, is an unsaturated carotenoid with well-known health benefits.⁹ Many studies have shown that LYC is involved in the protection of various disorders such as cardiovascular disease, prostate cancer, and respiratory and endothelial cancer.^{10,11} LYC exerts potent anti-inflammatory effects as an antioxidant and free-radical hunter, which may reduce cellular harm.^{12,13} LYC stimulates antioxidant enzymes such as superoxide dismutase, glutathione (GSH) peroxidase, and GSH reductase.¹⁴ It also inhibits H₂O₂-induced lipid peroxidation and lipoprotein modification.¹⁵

Whereas SVP is used in the treatment of epilepsy, LYC is a potent antioxidant and reduces the generation of reactive oxygen species. Hence, the present study was undertaken to determine whether LYC along with SVP could provide superior seizure control in pentylenetetrazol (PTZ)-induced kindling in mice.

Materials and Methods

Drugs and Chemicals

SVP was used as a standard drug in the present study. The marketed preparation of SVP (TORVATE Tablet) was purchased from Torrent Pharmaceuticals Ltd., Ahmedabad, India. LYC was procured from Moraceae Pharmaceuticals, Uttarakhand, India. All the other chemicals and solvents used were of AR grade.

Animals

Swiss albino mice of either sex weighing 25–30 g were procured from the Animal House Facility, KIET School of Pharmacy, Ghaziabad (UP), India. The animals were kept in polypropylene cages under standard laboratory conditions. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KIET School of Pharmacy (Registration #1099/C/07/CPCSEA and approval #IAEC/KSOP/2013-14/03), Ghaziabad.

Experimental Protocol

The mice were randomly divided into 5 groups and treated as follows: normal control group – treated with normal saline (2 mL/kg on days 8, 10, 12, and 14, i.p.), PTZ control group – treated with PTZ (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14, i.p.), SVP200 + PTZ – treated with SVP (200 mg/kg, p.o) daily for 14 days and PTZ (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14, i.p.), LYC2 + SVP200 + PTZ – treated with LYC (2 mg/kg,) and SVP (200 mg/kg) daily for 14 days plus PTZ (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14, i.p.), LYC4+ SVP200 + PTZ – LYC (4 mg/kg) and SVP (200 mg/kg) daily for 14 days plus PTZ (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14).

Behavioral Analysis

The kindling score and the latency of kindling were measured according to the method reported by Agarwal et al.¹⁶ A subconvulsant dose of PTZ (45 mg/kg) was given intraperitoneally on every second day (i.e., day 8, day 10, and day 12), and then 70 mg/kg was administered on day 14. The PTZ injection was stopped when the animal showed adequate kindling. The convulsive behavior was observed for 30 minutes (i.e., a seizure score of 5 on 4 consecutive injections). After each injection, convulsive behavior was observed as follows:

Stage 0 - No response

Stage 1 - Hyperactivity, restlessness, and vibrissae twitching

- Stage 2 - Head nodding, head clonus, and myoclonic jerks
- Stage 3- Unilateral or bilateral clonus
- Stage 4 - Forelimb clonic seizures
- Stage 5 - Generalized clonic seizures with loss of righting reflex.

Biochemical Analysis in the Brain Tissue

The quantitative estimation of lipid peroxidation was done by determining the concentration of thiobarbituric acid reactive substance (TBARS) in the brain using the method of Ohkawa et al.¹⁷ Catalase activity was assayed by the method of Clairborne.¹⁸ Reduced GSH was estimated according to the method of Ellman.¹⁹ Superoxide dismutase activity (SOD) was assayed according to the method of Kono.²⁰

Biochemical Estimation of Gamma-aminobutyric Acid in the Brain Tissue

GABA in the brain tissue was measured according to the method reported by Maynert et al.²¹

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad software; San Diego, CA). The data were expressed as mean±SEM. All the values were analyzed using the one-way analysis of variance, followed by the Dunnett *t*-test. The results were significant if $P < 0.05$.

Results

Assessment of the Seizure Score

All the mice in each group survived without any complications at the end of the kindling period. In the PTZ group, a repeated administration of a dose of PTZ (45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14, i.p.) resulted in increasing convulsive activity leading to generalized clonic-tonic seizure. Figure 1 shows that a pre-administration of SVP (200 mg/kg) along with LYC (2 and 4 mg/kg, respectively) significantly inhibited the seizure scores compared to PTZ. None of the animals could achieve a score of 5 with 4 injections of PTZ.

Assessment of Latency

With the administration of PTZ, there was a significant decrease in the latency of seizure at the dose of 45 mg/kg on days 8, 10, and 12 and 70 mg/kg on day 14. Pre-treatment with SVP (200 mg/kg) + LYC (2 and 4 mg/kg) significantly ($P < 0.01$ and $P < 0.001$) increased seizure latency compared to PTZ. Maximum increase in seizure

latency was observed at 4 mg/kg of LYC along with SVP (figure 2).

Assessment of Brain Tissue Thiobarbituric Acid Reactive Substance

Table 1 illustrates the brain levels of oxidative stress markers in the kindled and non-kindled mice. PTZ-induced kindling produced a significant ($P < 0.01$) increase in the brain tissue TBARS content, an index of lipid peroxidation, as compared with the normal control group. Pre-treatment with SVP (200 mg/kg) + LYC (2 and 4 mg/kg) significantly ($P < 0.01$) decreased TBARS levels compared to PTZ. LYC decreased TBARS levels in a dose-dependent manner.

Assessment of Brain Tissue Reduced Glutathione

Table 1 shows the brain tissue reduced GSH levels. There was a significant ($P < 0.01$) decrease in the GSH level in the PTZ group. Pre-treatment with SVP (200 mg/kg) + LYC (2 and 4 mg/kg, respectively) significantly ($P < 0.01$) increased the GSH levels as compared to PTZ. This effect was more prominent in SVP + LYC (4 mg/kg).

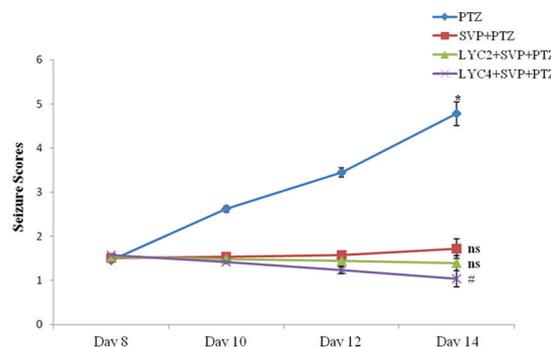


Figure 1: Effects of lycopene and sodium valproate on pentylenetetrazol-induced mean kindling score as assessed on days 8, 10, 12, and 14 of the study are depicted here. PTZ, Pentylenetetrazol; SVP, Sodium valproate (200 mg/kg); LYC, Lycopene (2 and 4 mg/kg). Data are expressed as mean±SEM. * $P < 0.01$ as compared to day 8, # $P < 0.01$ as compared to the PTZ control group. Ns: Non-significant.

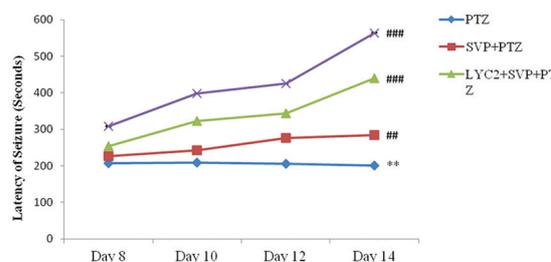


Figure 2: Effects of sodium valproate and lycopene on the latency of seizure are depicted here. Data are expressed as mean ± SEM. ** $P < 0.01$ as compared to day 8. ### $P < 0.01$ and #### $P < 0.001$ versus PTZ.

Assessment of Brain Tissue Catalase

Table 2 shows the brain tissue catalase levels. There was a significant ($P<0.01$) decrease in the catalase level in the PTZ group. Pre-treatment with SVP (200 mg/kg) + LYC (2 and 4 mg/kg) significantly ($P<0.05$ and $P<0.01$) increased the catalase levels as compared to PTZ. This effect was observed more prominently in SVP + LYC (4 mg/kg).

Assessment of Brain Tissue Superoxide Dismutase

Table 2 depicts the brain tissue superoxide dismutase levels. There was a significant ($P<0.01$) decrease in the superoxide dismutase level in the PTZ group. Pre-treatment with SVP (200 mg/kg) + LYC (2 and 4 mg/kg) significantly ($P<0.01$) increased the superoxide dismutase levels as compared to PTZ. This effect was observed more prominently in SVP + LYC (4 mg/kg).

Assessment of Brain Tissue Gamma-Aminobutyric Acid

Table 2 shows the brain tissue GABA levels. There was a significant ($P<0.01$) decrease in the GABA level in the PTZ group. Pre-treatment with SVP (200 mg/kg + LYC [2 and 4 mg/kg]) significantly ($P<0.01$) increased the GABA levels as compared to PTZ. This effect was observed more prominently in SVP + LYC (4 mg/kg).

Discussion

It was observed in the present study that an administration of a subconvulsant dose of PTZ on alternate days resulted in a near-to-stage 5 seizure. PTZ-induced kindling in the mice showed an increase in the level of lipid peroxidation and a decrease in the levels of SOD, catalase, GSH, and GABA. PTZ kindling is a standardized animal model for epilepsy and refers to a phenomenon in which repeated injections of a convulsant causes gradual seizure development culminating in generalized tonic-clonic seizures; it is an appropriate model resembling epilepsy in humans.²² In the present study, the PTZ-treated mice showed a significant increase in the score of seizure and latency, which chimes in with the results of a previous study.¹⁶

SVP was used in the present study as it is one of the mostly prescribed antiepileptic drugs in the treatment of many different types of partial and generalized epileptic seizures and also for other neuropsychiatric problems such as bipolar disorders, schizoaffective disorder, social phobias, and neuropathic pain as well as for the prophylaxis of migraine headache.⁷ LYC is potent antioxidant which stimulates antioxidant enzymes such as superoxide dismutase, GSH peroxidase, and GSH reductase.¹⁴ It also inhibits H_2O_2 -induced lipid peroxidation and lipoprotein modification.¹⁵ In addition, LYC plays an important role in protecting cell membranes from lipid peroxidation, neutralizing hydroxyl radicals,

Table 1: Effects of lycopene and sodium valproate on TBARS and reduced glutathione levels in pentylenetetrazol-induced changes in the brain tissue

S. No.	Groups	TBARS (nmol MDA/mg protein)	Reduced glutathione (nmol/mg of protein)	P value
1	Normal Control	0.52±0.22	1.012±0.06	-
2	PTZ Control	3.49±0.36 ^a	0.344±0.05 ^a	0.009
3	SVP+PTZ	2.28±0.32 ^b	0.899±0.07 ^b	0.049
4	LYC2+SVP+PTZ	1.32±0.14 ^c	0.923±0.07 ^c	0.009
5	LYC4+SVP+PTZ	1.09±0.13 ^c	0.952±0.07 ^c	0.008

TBARS: Thiobarbituric acid reactive substance; PTZ: Pentylenetetrazol; SVP: Sodium valproate (200 mg/kg); LYC: Lycopene (2 and 4 mg/kg). Data are expressed as mean±SEM. ^a $P<0.01$ versus control; ^b $P<0.05$; ^c $P<0.01$ versus the PTZ control

Table 2: Effect of lycopene and sodium valproate on GABA, SOD, and catalase levels in pentylenetetrazol-induced changes in the brain tissue

S. No.	Groups	GABA (ng/g of brain tissue)	SOD (IU/mg protein)	Catalase (nmol H_2O_2 consumed/min/mg protein)	P value
1	Normal Control	32.25±1.23	42.34±1.31	12.15±1.11	-
2	PTZ Control	8.286±1.46 ^a	21.57±1.91 ^a	6.84±0.28 ^a	0.009
3	SVP+PTZ	28.27±1.16 ^b	34.12±1.23 ^b	11.35±0.92 ^b	0.049
4	LYC2+SVP+PTZ	29.32±1.19 ^c	39.42±2.17 ^c	12.13±1.27 ^c	0.009
5	LYC4+SVP+PTZ	31.21±1.20 ^d	45.36±2.21 ^c	14.24±1.63 ^d	0.008

GABA: Gamma-aminobutyric acid; SOD: Superoxide dismutase activity. Data are expressed as mean±SEM. ^a $P<0.01$ versus control; ^b $P<0.05$; ^c $P<0.01$ versus the PTZ control

and promoting further protection beyond antioxidant activity.

Treatment with both SVP and LYC significantly decreased the seizure score and increased the latency period. SVP and LYC at a higher dose conferred better protection against PTZ-induced convulsion than that shown by these drugs at a lower dose.

In the pathogenesis of seizure, free radicals have an important role. Accordingly, the protective effects of antioxidants have been studied against different types of seizures.²³⁻²⁵ Some research findings suggest that free radicals are produced during a PTZ-induced kindling model in rats and may contribute to the biochemical sequelae of events leading to seizure-induced cell death.^{26,27} As a result, LYC was used in the present study as it exerts potent anti-inflammatory effects through its action as an antioxidant and free-radical scavenger by reducing cellular damage.^{12,13}

In the present study, PTZ treatment in the mice significantly increased the levels of TBARS, indicating that oxidative stress occurred as a consequence of seizures and thus contributed to seizure-induced brain damage. The results of the present study are similar to those of a previous study.²⁸ Treatment of the mice with PTZ along with SVP and LYC significantly prevented PTZ-induced elevations in lipid peroxidation. It is also known that convulsions followed by an increase in lipid peroxidation in the brain tissue decrease the levels of antioxidant enzymes.²⁹ Furthermore, free radicals are normal products of cellular aerobic metabolism involved in the development of seizures.³⁰ Free-radical-scavenging enzymes such as GSH, CAT, and SOD are the first-line cellular defense against oxidative stress, eliminating reactive oxidative stress such as GSH, catalase, superoxide, and hydrogen peroxide and preventing the formation of more reactive hydroxyl radicals. In the present study, the GSH, SOD, catalase, and GABA levels were significantly decreased in the PTZ-treated mice, while these levels were significantly increased in the mice treated with SVP and LYC. Treatment with SVP in the PTZ-treated mice elevated the GABA levels. The results of the present study are concordant with those of a previous study by Akbas et al.³¹ SVP has antiepileptic effects and acts as an antioxidant according to Ozerol et al.³² LYC is a potent antioxidant insofar as the administration of petroleum ether and alcoholic tomato extracts significantly increased the tissue GSH and decreased the malondialdehyde and protein level in the brain.³³ The findings of the present study are consistent with those of a previous study where SVP with N-acetyl

cysteine at the fixed dose ratios was found to be synergistic.³⁴ These findings indicate that LYC potentiates the antiepileptic effects of SVP against PTZ-induced kindling in mice and that these effects may be attributed to its cellular antioxidative defense mechanism and the synergistic antioxidant effects of LYC and SVP.

There was no funding source for the present study. Our research facilities were too limited to carry out the research work at molecular level in the present study.

Conclusion

Treatment with SVP and LYC significantly restored behavioral and biochemical alterations. Thus, LYC potentiates the antiepileptic effect of SVP by attenuating the seizure score and oxidative stress in mice.

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Conflict of Interest: None declared.

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