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INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF PHYSIOLOGY

EFFECTS OF RESVERATROL AND NITRIC OXIDE INTERACTION ON PENICILIN INDUCED EPILEPTIFORM ACTIVITY

PhD Thesis

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ÖZET

RESVERATROL-NİTRİK OKSİT ETKİLEŞİMİNİN PENİSİLİNLE OLUŞTURULAN EPİLEPTİFORM AKTİVİTE ÜZERİNE ETKİSİ

MOHAMED HASSAN MOHAMUD

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Doktora, Kasım/2021
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Penisilin kaynaklı epilepsi modeli en yaygın kullanılan fokal epilepsi modellerinden birisidir. Resveratrol, üzüm başta olmak üzere birçok farklı bitkide bulunan doğal bir fitoaleksindir. Nitrik oksit (NO), insan vücudunda doğal olarak üretilen ve ikinci haberci olarak kullanılan çok önemli bir düzenleyici moleküldür. Bu çalışmanın amacı, Resveratrol-nitrik oksit etileşiminin penisilinle oluşturulan epileptiform aktivite üzerine etkisini araştırmaktır.

Çalışmamızda 84 adet erkek Wistar rat kullanılmıştır (n: 6). Epileptiform aktivite, 2,5 mikrolitre hacminde 500 IU Penisilin-G'nin Hamilton mikro enjektör ile kortekse (intrakortikal) uygulanmasıyla oluşturulmuştur. Elektrofizyolojik analiz için, Resveratrol 25, 50 ve 100 mg/kg, L-NAME 60mg/kg, L-Arginin 500mg/kg, Aminoguanidine 100mg/kg ve 7-Nitroindazole (7-NI) 40mg/kg, penisilin enjeksiyonundan 30 dakika sonra intraperitoneal olarak uygulanmıştır. Online olarak alınan elektrokortikografi (ECoG) kayıtlarında, offline olarak frekans ve amplitüd analizi yapılmıştır.

Resveratrolün 50 mg/kg dozu, penisilin ile oluşturulan epileptiform aktivitenin spike frekansını azalttı ($p<0.05$). Bu nedenle etkin doz olarak belirlendi. 7-NI (40 mg), aminoguanidin (100 mg) ve L-Arginin (500 mg) tek başına uygulandığında, penisilin ile oluşturulan epileptiform aktivitenin spike frekansını azaltırken ($p<0.05$), L-NAME (60 mg) spike frekansı üzerinde etkisizdi ($p>0.05$). 7-NI, aminoguanidin ve L-Arginin; Resveratrol ile kombine edildiğinde, tek başlarına uygulandıkları zaman oluşturdukları antikonvulsif etkilerini potansiyalize ederek istatistiksel olarak daha güçlü antikonvulsif etki gösterdiler ($p<0.05$). Resveratrol (50 mg/kg) ve L-NAME (60 mg) birlikte uygulandığında ise, tek başına uygulandığında etkisiz olan L-NAME, Resveratrolün antikonvulsif etkisini büyük ölçüde blokladı. Tüm deney grupları birbiriyle kıyaslandığında spike amplitüdü bakımından istatistiksel olarak anlamlı bir farklılık bulunamadı.

Resveratrol, Penisilin kaynaklı epileptik aktivite üzerine antikonvülsan etki göstermiştir. Bu etki; NO, nNOS ve iNOS yoluyla gerçekleşmiştir. NO ve Resveratrol etkileşimi birbirini potansiyelize etmiş ve daha fazla antikonvülsan etki ortaya çıkmıştır. NO ve Resveratol etkileşiminin moleküler mekanizmasını açıklanabilmesi için daha ileri tetkiklerle yapılacak çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Resveratrol; Epilepsi, Penisillin; Aminoguanidin; 7-NI; L-Arjinine; L- NAME

ABSTRACT

EFFECTS OF RESVERATROL AND NITRIC OXIDE INTERACTION ON PENICILIN INDUCED EPILEPTIFORM ACTIVITY
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The penicillin-induced epilepsy model is one of the most widely used focal epilepsy models. Resveratrol is a natural phytoalexin found in many different plants, especially grapes. Nitric oxide (NO) is a very important regulatory molecule naturally produced in the human body and used as a second messenger. The aim of this study is to investigate the effect of Resveratrol and nitric oxide interaction on Penicillin induced epileptiform activity.

84 male Wistar rats were used in our study (n: 6). Epileptiform activity was created by applying 500 IU Penicillin-G in a volume of 2.5 microliter to the cortex (intracortical) with a Hamilton microinjector. For electrophysiological analysis, Resveratrol 25, 50 and 100 mg / kg, L-NAME 60mg / kg, L-Arginine 500mg / kg, Aminoguanidine 100mg / kg and 7-Nitroindazole (7-NI) 40mg / kg, were administered intraperitoneally 30 minutes after Penicillin injection. The Electrocorticography was taken as Online (ECoG) record, the frequency and amplitude analysis were done offline.

Resveratrol 50 mg / kg dose reduced the spike frequency of epileptiform activity induced by Penicillin ($p < 0.05$). Therefore, it was determined as an effective dose. When 7-NI (40 mg), Aminoguanidine (100 mg) and L-Arginine (500 mg) were administered alone, spike frequency of epileptiform activity induced by Penicillin decreased ($p < 0.05$), while L-NAME (60 mg) was no effect on spike frequency. ($p > 0.05$). When 7-NI, aminoguanidine and L-Arginine combined with resveratrol, they showed a statistically stronger anticonvulsive effect by potentiating their anticonvulsive effects when applied alone ($p < 0.05$). When resveratrol (50 mg / kg) and L-NAME (60 mg) were administered together, L-NAME, which was ineffective when administered alone, largely blocked the anticonvulsive effect of resveratrol. When all experimental groups were compared with each other, no statistically significant difference was found in terms of spike amplitude.

Resveratrol showed anticonvulsant effect on Penicillin-induced epileptic activity. This effect was via NO, nNOS and iNOS. The interaction of NO and resveratrol potentiated each other and more anticonvulsant effects emerged. Further studies are needed to explain the molecular mechanism of NO and resveratrol interaction.

Key words: Resveratrol; Epilepsy; Penicillin; Aminoguanidine; 7-NI; L-Arginine; L-NAME

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LIST OF ABBREVIATIONS/ACRONYMS

7 NI	: 7- Nitroindazole
AED	: Anti-epileptic drug
AEDs	: Anti-epileptic drugs
AG	: Aminoguanidine
Ca + 2	: Calcium ion
cAMP	: Cyclic AMP
DMSO	: Dimethyl Sulfoxide
EcoG	: Electrocorticography
FBM	: Felbamate
GABA	: Gamma amino butyric acid
GABAA	: GABA-A Receptor
GABAB	: GABA-B Receptor
HIC	: High income countries
i.c.v	: Intracerebroventricular
i.p	: Intraperitoneally
ILAE	: International epilepsy-war association
IU	: International unit
L- Arg	: L- Arginine
LMIC	: Low- and middle-income countries
L-NAME	: N(gamma)-nitro-L-arginine Methyl Ester
LTG	: Lamotrigine
LTP	: Long term potentiation
MES	: Maximal electroshock-induced seizure
Mg.	: Milligram
Min	: Minutes
Ml.	: Milliliters
mV.	: Millivolt
Na +	: Sodium ion
NMDA	: N-Methyl-D-aspartate
NMDA.	: N-Methyl D-Aspartate
NO	: Nitric Oxide
NOS	: Nitric Oxide Synthetase
OXC	: Oxcarbazepine

PTZ	: Pentylenetetrazol
RSV	: Resveratrol
SEM	: Mean standard error
SWD	: Spike wave discharge
TBI	: Traumatic brain injury
TPM	: Topiramate
TRP	: Transient receptor potential channels
WAG/Rij	: Wistar albino glaxo from rijswijk
µl	: Microliter
µV	: Microvolt
SE	: Status epilepticus
CBZ	: Carbamazepine

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1. INTRODUCTION

Epilepsy is chronic neurological disorder in which is the third most common following Alzheimer's / dementia and migraine (Fisher Robert S. et al., 2005). Epileptic seizure or convulsion are a clinical manifestation that occurs as a result of excessive and abnormal electrical discharge of a group of neurons in the brain. This clinical picture includes sudden and transient abnormal events involving changes in consciousness, motor, sensory, autonomic or psychic symptoms. Many events such as infection, trauma, tumors intracranial lesions,, etc. cause epilepsy and profoundly affect human life (Bambal et al., 2011). Epilepsy is predicted to have a prevalence of 1 per cent in the world. In 2009 the World Health Organization published data on 50 million patients with epilepsy worldwide. (Williams T. J. et al., 2017) .

Regardless of etiology, the common feature of all epileptic seizures is the increase in neuronal excitation (Engelborghs et al., 2000). Increased excitation of the neurons is due to decreased inhibitory neurotransmitters, mainly gamma-aminobutyric acid (GABA) or increased excitatory neurotransmitters, particularly glutamate, resulting in a shift in the ending balance in the direction of excitation, change in voltage-gated ion channel, intracellular or extracellular distribution of excitatory ion-channels. Even with the best drugs available in about 30 per cent of patients with epilepsy seizure cannot be prevented (Cascino et al., 1994).

Consequently , the quality of life is increasing with the emergence of new treatment modalities (Avanzini et al., 2003). Many drugs used experimentally to treat epilepsy have been found to have beneficial effects. Before these substances are used in humans, the effect of the test animals under laboratory conditions must be understood.

Experimental epilepsy models have been created to have a deeper understanding in pathophysiology of epileptic seizures and to find new anti-epileptic medications. Experimental seizures caused by chemical convulsant or electrical stimulation, reflex epilepsies, and idiopathic epilepsies are the three types of animals epilepsy models (Akdogan et al., 2008). Chemical convulsant are commonly used as a quick and easy way to induce seizures. Penicillin is a commonly used chemical convulsant. Penicillin given topically, intracerebrally, or systemically causes abnormal and paroxysmal activity, which can progress to seizures, which has been used in the study of epilepsy mechanisms (Wanleenuwat et al., 2020).

Resveratrol (3,5,4'-tri-hydroxy stilbene, RSV) is a polyphenolic compound found in black grape skin, raspberry, red wine, plum, mulberry, blueberry peanut and cranberry. Every gram of skin in fresh grape contains 50 to 100 mg of resveratrol and wine concentrations can vary between 0.2 and 7.7 mg per liter. The epidemiological finding of an inverse correlation between the red wine consumption and the occurrence of heart disease resulted in "French paradox," Its proven activity has been associated with it. Studies have found that in a variety of neurological conditions, RSV has neuroprotective effects (Shin et al., 2010).

Ethemoglu et al. (2017) claim that coating RES with liposome improves the antioxidant action, resulting in fewer epileptic seizures. In penicillin-induced epilepsy model, resveratrol incorporated into liposomes exhibited greater anticonvulsant and antioxidant effects than resveratrol alone.

In a cerebral ischemic stroke model, RSV reduces total and cortical infarct volumes (as reduced by formation of reactive oxygen species), depending on time and sex (Shin et al., 2010). In Alzheimer's model, administration of 100 mM RSV for 7 days to rats reduced beta-amyloid-induced neuronal damage, strengthened spatial memory and antioxidant activity (Huang et al., 2011), and in mice, administration of 50 mg / kg RSV for 21 days via gavage resulted in neurodegeneration and inflammatory activity (Lofrumento et al., 2014), and Increases nerve cell survival rate in the midbrain (Wang S.-j. et al., 2013).

The studies on the effect of RSV on epilepsy are limited. In the PTZ-ignition model, RSV pre-administering also reduces seizure and oxidative stress (Saha et al., 2014). Pre-administration of RSV was shown to be protective against seizures caused by pentylenetetrazol (PTZ) (Gupta et al., 2002). The 10 day RSV therapy protects the hippocampal neurons in the CA1 and CA3 regions from death in PTZ ignition model (Meng et al., 2014) and in the temporal lobe epilepsy caused by kainite acid (Wu et al., 2009). RSV pre-treatment performed with pilocarpine-induced status epilepticus also reduces early inflammatory reaction. The underlying mechanism of RSV's molecular neuroprotective effects are still unknown. (Shetty, 2011).

Nitric oxide (NO) is a free radical molecule act as neurotransmitter or neuronal messenger in the central and peripheral nervous system and it is synthesized from L-arginine by nitric oxide synthase (NOS) (Bredt et al., 1990), (Bredt et al., 1994), (Bredt, 1999). NOS is divided into three isoforms.: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) (Yoshida et al., 1995). It has been proposed a contradictory role for NO in seizure development and pathogenesis (Ayyildiz M. et al., 2007; Garthwaite et al., 1995).

With the discovery of NO as a neuronal messenger, its effect on neuronal functions has been the subject of intense research. However, the role of the nitrenergic system in experimental epilepsy has not been fully understood, despite many studies conducted so far.

L-NAME (non-specific NOS inhibitor) enhanced N-methyl-D-aspartate (NMDA)-induced convulsions in mice implying that NO has an anticonvulsant role (Buisson A et al., 1993). PTZ and strychnine-induced seizures in rats were inhibited by L-NAME and NG-nitro-L-arginine (LNNA, non-specific NOS inhibitor), indicating that NO plays a convulsive role. (Kaputlu et al., 1997).

7-nitroindazole (7NI, selective nNOS inhibitor) inhibited convulsions elicited by voice, pilocarpine and kainic acid in animals (Jones et al., 1998), while in various animal models the anti-epileptic drugs and anti-convulsant substances enhanced the anti-seizure effect. In experimental epilepsy, the frequency of epileptiform activity was reduced by 7-NI in Penicillin model (Bosnak et al., 2007).

In contrast to saline-injected isolated conditioned mice, L-Arginine substantially decreased the seizure threshold of isolated conditioned animals ($p<0.01$) (Amiri et al., 2014). Combined of low doses of thalidomide and morphine, the anticonvulsant effects were significantly decreased (P 0.01) after pretreatment with a non-effective dose of the NO precursor L-arginine (Pourshadi N. et al., 2020).

In acute treatment with 100 mg/kg Aminoguanidine, a non-selective inhibitor of iNOS, on the seizure caused by intravenous pentylenetetrazol showed a significant effect on the clonic seizure threshold and also inhibited the onset of the seizure induced by atorvastatin (Moezi et al., 2012).

As all these data suggest, RSV plays in a variety role of neurological diseases, involving in epilepsy. On the other hand, the nitric oxide system appears to play many

roles in neurological diseases, such as epilepsy. Despite studies showing that both RSV and NO are involved in epilepsy in studies that are independent of each other, there is no study in the literature regarding the role of both in playing a role in epilepsy. However, there are a few studies on this interaction other than epilepsy. NO was found to contribute to RSV's protective effects in renal ischemic conditions (Chander et al., 2005). Studies show that RSV mediates the downregulation of various inflammatory biomarkers including cyclooxygenase 2, interleukins, tumor necrosis factor and inducible NOS (Foti Cuzzola et al., 2011).

With this study, it will be possible to determine the effect of RSV and NO on epileptiform activity induced by Penicillin for the first time and the findings of this study make a significant contribution to the literature about the effect of Resveratrol and nitric oxide interaction in Penicillin induced epileptiform activity.

2. BACKGROUND

2.1. Seizure and epilepsy

An epileptic seizure is conceptually described as: "a transient onset of signs and/or symptoms in the brain due to abnormal excessive or synchronous neuronal activity (Falco-Walter et al., 2018). Epileptic seizures generally indicate the existence of an imbalance between inhibitory (GABAergic) and excitatory (glutamatergic) neurotransmission. Furthermore, the epileptogenic mechanism remains unclear (Taskiran et al., 2018). At some point in their lives, up to 3% of the population will suffer from epilepsy (Henshall et al., 2005) and Around 65 million people worldwide are affected (Nieoczym et al., 2019).

When an individual has epilepsy, it is diagnosed:

1. At least two unprovoked or reflex seizures apart from at least 24 hours
2. One unprovoked or reflex seizure and the likelihood of another seizure similar to the general risk of recurrence following two unprovoked seizures (≥60 %) over the next 10 years; or
3. an epilepsy syndrome (Falco-Walter et al., 2018).

Updated classification in 2017, International League Against Epilepsy (ILAE) Classified of the Epilepsies into three groups according to seizure type, epilepsy type and epileptic syndrome (figure 2.1).

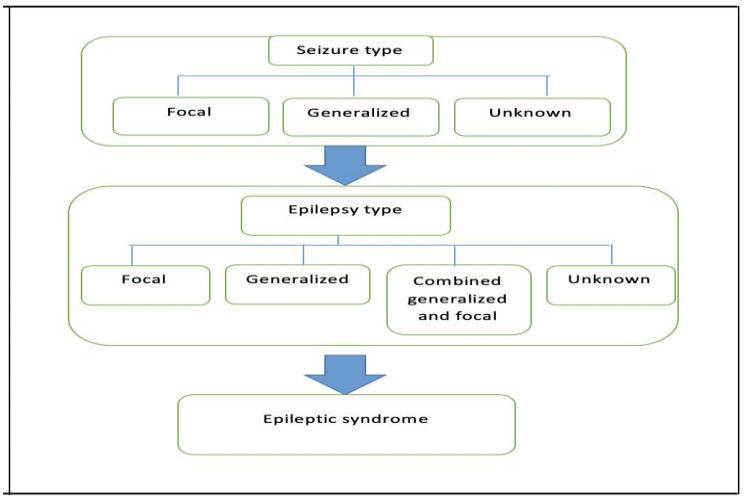


Figure 2.1 ILAE classification of epilepsies (Fisher Robert S. et al., 2017)

2.1.1. Classification of epileptic seizure

In 1981, the classification of seizures was first revised, in response to the increasing use of video electroencephalography (EEG), which had affected clinical practice. The term "generalized," "complex partial," "simple partial," and "unclassifiable" were introduced in 1981 and are still used today. The ILAE updated seizure classification again in 2010 with slight modifications (Berg et al., 2011). Although the need for revised classifications has been obvious for some time, it was challenging to attain consensus. The ILAE decided to set up a new task force in response to this need, which in 2014 established and released a revised concept of epilepsy (Fisher R. S. et al., 2014), the final seizures classification follows (Fisher Robert S. et al., 2017).

A) Focal onset

These previously called partial seizures originating in networks limited to one hemisphere. For each form of seizure ictal onset is consistent from seizure to seizure, with specific patterns of propagation that may include the ipsilateral and/or contralateral hemisphere. The symptoms and signs that develops during a seizure will describe the specific region of the brain, or the lobe or hemisphere involved in the onset and progression of seizures (Kumar A. et al., 2020b).

Focal seizure is classified into:

- **Aware:** Awareness during a seizure is characterized as the patient, even though immobile, is completely aware of himself and the environment throughout the seizure. If consciousness is retained, the seizure is a focal aware seizure. Previously, this form of seizure was referred to as a "simple partial seizure."
- **Impaired awareness:** When awareness is impaired at a certain point during the seizure. The degree of awareness loss can vary. Previously, the words 'complex partial seizure' and 'focal dyscognitive seizure' were used to describe a focal impaired awareness seizure.
- **Focal to bilateral seizure:** A seizure that begins from one side or even a part of the brain and afterward spreads for both sides, previously referred to as secondarily generalized seizures, is now ideally referred to as "focal to bilateral seizure" (Kumar A. et al., 2020a).

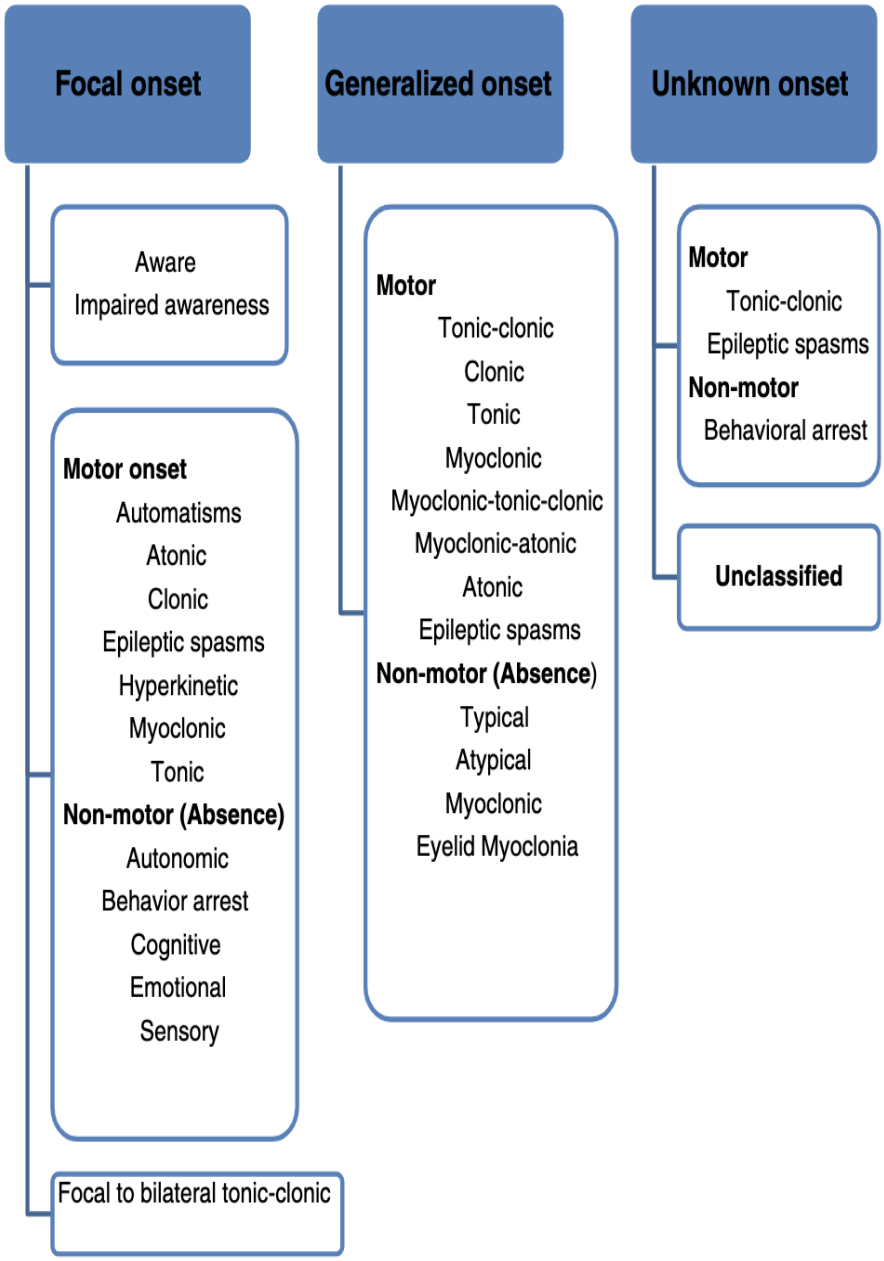


Figure 22.2. Classification of seizure type in ILAE 2017 (Bhasin et al., 2019)

B) Generalized seizure

a. Motor onset: generalized seizure with motor symptoms that effects muscle activity and corresponds to "grand mal." it could be:

1. Tonic
2. Clonic
3. Tonic- clonic
4. Myoclonic

5. Myoclonic-tonic-clonic
6. Atonic
7. Epileptic spasm

b. Non-motor onset: these are primarily absence seizures, and the term refers to the old term "petit mal."

1. Typical
2. Atypical
3. Myoclonic
4. Eyelid myoclonic

2.1.2. Etiology of epilepsies

Structural etiology

An abnormality visible on structural neuroimaging is considered as a structural etiology where the electroclinical evaluation along with the image findings which leads to a reasonable inference that the abnormality of the image is the likely cause of the seizures. Structural etiologies can be acquired such as trauma, stroke, and infection, or genetics such as several cortical developmental malformations (Scheffer et al., 2017).

Genetic etiology

The genetic contribution of causes to the origin of variety of epilepsy was demonstrated in family studies, epidemiological studies, twin studies and studies of Mendelian heritage disorders where epilepsy is a significant symptom. Various mutations have been identified in genes that cause or potentially lead to the development with certain types of epilepsy in recent years. Single-gene epilepsies are caused by the majority of these genes and result in a small number of epileptic syndromes that are autosomal dominant uncommon, familial and. Majority of these single-gene epilepsy are initiated by gene mutations encoding neuronal ion channel subunits and neuronal maturation and migration-related proteins during embryonic development (Sanchez-Carpintero Abad et al., 2007). Genetic disease that causes variants often develops de novo and is not hereditary, so there is often no family history of epilepsy, despite the genetic cause of the patient's epilepsy (Falco-Walter et al., 2018).

Infectious etiology

The world's most common etiology for epilepsy, particularly in developing countries, is infectious. CNS infections can lead in both acute symptomatic seizures (which are closely related to the initial infection's timing) and epilepsy. Neurocysticercosis, subacute sclerosing panencephalitis, cerebral malaria, HIV, cerebral toxoplasmosis, Tuberculosis, are among the infectious etiologies. The infectious process, however, is thought to be the major cause of epilepsy, these infections sometimes have a structural correlation (International League Against Epilepsy, 2020, March 30)

Metabolic etiology

It's the direct consequence of a proven or suspected metabolic disorder in which seizures are a key symptom of the disorder. Those who have a temporary metabolic imbalance that results in acute symptomatic seizures are not considered to have epilepsy because their seizures were not provoked. Metabolic disorders may in certain cases do have such a genetic defect but others can be acquired, like cerebral folate deficiency and pyridoxine-dependent seizure (Parikh et al., 2012).

Immune etiology

The immune etiology may be described as one in which there is proof of inflammation in the CNS through autoimmune mediation. The diagnosis of these autoimmune encephalitis becomes increasing rapidly, especially with increased access to antibody tests. Autoimmune encephalitis and epilepsy were linked to both neuronal cell surface antibodies (NMDAR, VGKC complex, GABA-B,AMPA, and GluR5) and intracellular neuronal antibodies (ANNA-1, GAD65 and Ma) (Correll, 2013).

Unknown etiology

The term "unknown" refers to the cause of epilepsy remains unclear and relevant diagnoses cannot be made apart from the basic electroclinical semiology.

2.1.3. Epidemiology

Incidence of Acute Symptomatic Seizures

The median frequency of acute symptomatic seizures is between 29-39 per 100,000 annually (Hauser W. A. et al., 2008). Among the youngest age class (< 1 year) and in elderly population, acute symptomatic seizures are predominated. The most common precipitating factors are traumatic brain injury (TBI), Fever, cerebrovascular disease, infection, drug withdrawal, and metabolic disorders (Beghi Ettore, 2020)

Incidence and prevalence of Epilepsy

In low and middle-income (LMIC) countries the incidence was higher than in high-income countries (HIC), the incidence rate of epilepsy was 61.4 per 100,000 persons per year (Fiest et al., 2017). The incidence is also higher among the lowest socio-economic groups in HIC and people of different ethnic origin within the same population (Beghi E. et al., 2014).

The prevalence of epilepsy varies significantly between countries based on the local distribution of severity and etiological factors, the number of diagnosed seizures and considering either active epilepsy (active prevalence) or also including cases of remission (life-time prevalence). The average lifetime prevalence of epilepsy was 7.60 per 1,000 population and lower for HIC (5.18 per 1,000) than for LMIC (8.75 per 1,000) and also males are slightly higher than females (Fiest et al., 2017). Prevalence rates often differ in selected populations and appear to be higher for individuals of certain ethnicities, people with poor health and economically disadvantaged subjects (Kaiboriboon et al., 2013; Kelvin et al., 2007) .

Incidence and Prevalence by Seizure Type

Focal seizures are the most common form of seizure in children as well as adults. A most common type of focal seizure is focal impaired awareness seizure, which affects approximately 36% of all people who have seizures. (Hauser W. A. et al., 1993; Wirrell et al., 2011).

2.1.4. Pathophysiology of epilepsy

The human cerebral cortex constitutes 82% of the total brain mass although it contains just 19% of neurons (Jenrow et al., 2019). Epileptogenesis is the mechanism of transforming a non-epileptic brain to one that can generate recurrent, spontaneous

seizures. The mechanism arises from an imbalance between inhibitory and excitatory activity in a neuronal network, which makes it probable to behave in a hypersynchronous, excessive, oscillatory manner that, if continued, disrupts with normal neuronal processing and may disrupt other neuronal networks (Fisher Robert S. et al., 2005; Pitkänen A. et al., 2014). The epileptogenic networks are widely distributed for generalized epilepsy, involving bilateral thalamocortical structures. For focal epilepsy, networks involve neuronal circuits, usually limbic or neocortical, in one hemisphere (Crunelli et al., 2002).

The difference among inhibition and excitation occurring in epileptogenic networks is not necessarily only an increase in excitation or a lack of inhibition; in certain cases, such as absence seizures, an abnormal increase in inhibition can be pro-epileptogenic (Cope et al., 2009; Pinault et al., 2005) or limbic epilepsies in immature brain (Galanopoulou, 2008). Most generalized epilepsy is assumed to have a genetic origin (Helbig et al., 2008), whereas focal epilepsy was thought to be illustrated mainly by structural cerebral anomalies, especially in drug-resistant epilepsy (Berg et al., 2009; Li L. M. et al., 1995). Seizures mainly arise from abnormal behavior in the cortical neurons, while axons and glial cells in the white matter may be secondarily involved (Thijs et al., 2019).

The distinctive characteristic of all epileptic syndromes is a recurrent increase in excitability of the neurons. A number of causative factors such as oxygen deprivation, trauma, infection, tumors and metabolic derangements may be associated with unusual cellular discharges. In around half of patients suffering from epilepsy, however, no specific causative factors are identified. However, insight into the epilepsy pathophysiology and the underlying neurochemical and histological changes has led to appropriate approaches for the development of new anti-epileptic drugs (AEDs) (Engelborghs et al., 2000).

Functional and maybe even structural changes that occur in the post-synaptic membrane, thereby changing the character of a receptor protein –conductance of channels, thus facilitating the paroxysmal depolarizing shift (PDS) development and increased excitability. As extracellular K+ concentrations rise (as during seizure activity), the equilibrium of K+ around the neuronal membrane decreases, leading in decreased currents outward of K+ and the overall current will become inward, depolarizing the neuron to a point that it activates the Ca2 + currents thus resulting in

a PDS and spike burst (Dichter, 1997). Increased extracellular K+ depolarizes the neurons which results in spike discharge. Glia is capable of removing neurotransmitters from the extracellular space and buffering K+ to correct the increased extracellular K+ concentrations during seizures (Bordey et al., 1998).

2.1.5. Electroencephalography (EEG) and Electrocorticography (ECoG)

EEG and ECoG are by far the most common methods used in epilepsy-related research. EEG is the process of recording neuron-generated electrical potentials in the brain with electrodes mounted on the scalp; This is used to distinguish between different phases of sleep, diagnose different organic brain injuries including coma, and neurological disorders such as epilepsy, and assess response rates to treatment. Hans Berger first used electroencephalogram (EEG) to successfully measure human brain waves in 1924 (Haas, 2003). The purpose was to show that the human brain's electromagnetic fields could be used for telepathy. Although the signals he observed for this reason were ineffective, clinicians and scientists have widely adopted the EEG. This is because the measurements are easy to make, and the recorded rhythms are brain state informative (Jia et al., 2011). Usually, the recording electrodes are positioned over the major anatomical structures of the brain such as the frontal, temporal or parietal lobes in standardized positions. Electrocorticography (ECoG) is the method of recording electroencephalographic signals directly from surgically exposed cerebral cortex (Paranjape et al.).

For EEG recording two types of electrodes are used. One of them is the active electrode and the positioned in the area to be recorded. The other electrode is positioned in an area (such as the earlobe) far from the active electrode and considered to be zero. This electrode is referred to reference electrode. A large number of active electrodes are placed in different parts of the brain when recording EEG in the clinic. The potential difference between a reference electrode and an active electrode is measured (monopolar recording), or the potential difference is measured between two active electrodes (bipolar recording). Recording electrodes are generally mounted in the skull according to a certain scheme, in the frontal, parietal, occipital, and temporal regions. These very weak electrical potentials are usually registered by electrodes mounted on the scalp and reinforced with amplifiers (Haas, 2003; Pillai et al., 2006).

Previously, EEG waves were considered to be the sum of neuronal action potentials within the cortex. It was later seen that deep anesthesia and hypoxia lost their potential for action but their slow EEG potential remained. These action potentials contribute to the formation of EEG waves, but it is very small. In a normal individual, the frequency of potentials measured from the scalp is 1 to 30 Hz per minute; its amplitude is 20-100 microvolts. Skull and skin have the effect of reducing EEG wave amplitude. The frequency and amplitude of EEG waves are relatively complex and therefore can change under different conditions. Although, when EEG is being taken, there is no special circumstance, the patient must not be anesthetized and fasted for a long time. Since the normal rhythm of hunger-related hypoglycemia and anesthetic drugs may alter. While testing EEG the most important problem is that it can differentiate artifacts from actual disorders. Artifacts may be the result of different mechanical-electrical potentials in EEG recording, such as eye movements not originating from brain, movement and muscle artefacts, electrode shift, and sweating. EEG waves are divided into five broad groups, by their frequency (Timofeeva et al., 2001).

a. Alpha waves

The human brain's best studied rhythm is the normal alpha-rhythm. The best observed region of the alpha wave is occipital region with an amplitude of around 50 μV and the frequency ranges from 8-13 Hz. They appear rhythmically on both sides of the head, however on the non-dominant side, the amplitude is often slightly higher, especially in right-handed persons and seem to be particularly prominent with eyes closed and relaxation. Attention such as mental arithmetic, stress, eye opening the alpha activity usually disappears.

There are also many variants of the alpha rhythm, including temporal alpha, characterized by independent alpha activity over the temporal regions shown in elderly patients, frontal alpha, comprising of alpha activity over the frontal head regions, which can be correlated with medications, anesthesia or following sleep arousal. alpha coma pattern, this type is pathological and reflects when a comatose patient is invariant and unresponsive to any stimuli, or paradoxical alpha, that is a return of alpha activity with an eye opening or an alerting stimulus (Britton et al., 2016)

b. Beta waves

It is typically shown in symmetrical distribution on both sides and is most apparent in frontal region. Beta waves are the lowest of EEG waves in amplitude (2-20 μV), but the highest in frequency (13-30 Hz). Decreases or entirely vanishes in compromised areas of the brain. In the presence of stimuli, it is more intense and when there is excessive activity in the mind. Sedative-hypnotic medications in particular the benzodiazepines and the barbiturates accentuate this. In areas of cortical damage, it can be missing or decreased. It's usually considered to be a normal rhythm. For patients who are alert or nervous, or have their eyes open, it is the dominant rhythm. Increased beta activity in quantitative analysis may be linked to brain-over-arousal symptoms such as anxiety, obsessiveness, sleep disturbance, hyperactivity. Impaired beta activity in quantitative analysis may be linked to brain-under-arousal symptoms such as difficulty concentrating, problem solving (Roy Sucholeiki, 2019; Steriade et al., 1990).

c. Gamma waves

Waves beyond 30 Hz are commonly called gamma wave. Human experiments in particular have revealed that 40 Hz activity is important for cognitive functions and the integration of sensory information. Animals also experience gamma-oscillations that follow high-level mental activities. A prominent rhythm of gamma provides a hallmark of dedicated networks. Gamma has been found in various species in a variety of cortical regions, as well as subcortical structures. With sensory motivation and a wide variety of cognitive phenomena, including perceptual grouping and attention, gamma power rises in the sensory cortex(Fries et al., 2001; Henrie et al., 2005; Tallon-Baudry et al., 1999). Through higher cortex, gamma intensity is increased during memory (Pesaran et al., 2002) and learning activities (Bauer et al., 2007).

d. Theta waves

They are slow waves with a 4-7 Hz frequency and a 20-100 microvolt amplitude. It is not seen in healthy adults while awake. It's common to see them in children. They arise in the presence of sub-focal lesions of the cortex, in metabolic encephalopathy and also in hydrocephalus. Often, they are found in brain degenerative disorders. Theta waves are usually seen at any age while in sleep and those waves are abnormal in awake adults if they occur in excess. Collectively, theta and delta waves are known as slow waves (Vyazovskiy et al., 2005).

e. Delta waves

It has highest amplitude ranges 20-200 μV and smallest brain wave frequency 0.5-3.5 Hz. This happens during the third and fourth stages of sleep. It suggests brain abnormalities such as 'tumor' when it happens in an awake person. It is the predominant rhythm in babies up to age 1 and representation of subconscious thought (Timofeeva & Gordon, 2001).

Table 2.1. Characteristic of EEG waves

EEG waves	Frequency	Brain state
Alpha	8-12 Hz	Very relaxed, passive attention
Beta	12-35 Hz	Anxiety dominant, active, external attention, relaxed
Gamma	>35 Hz	Concentration
Theta	4-8 Hz	Deeply relaxed, inward focused
Delta	0.5-4 Hz	Sleep

2.1.6. Experimental Models of seizure and epilepsy

Different models of epilepsy were used to describe the entire complex events underlying epilepsy disorder, evaluate new anti-epileptics, improve appropriate diagnostic strategies and treatment modalities, or incorporate new strategies to resolve epilepsy-related issues. Despite the unavoidable structural and functional variations in phylogenetic terms between humans and other mammals, the presence of common basic mechanisms is the principal reason for using these models.

A. Chemical models

1. Pilocarpine model

Pilocarpine model of epilepsy is a widely used model for studying temporal lobe epilepsy (TLE) in rodents. TLE is by far the most common type of epilepsy in humans and has been characterized by an initial injury occurrence (early-life febrile seizures, trauma), a latent period accompanied by the onset of recurrent spontaneous seizures (epilepsy) and mesial temporal lobe sclerosis (Luby et al., 1995). Pilocarpine is an agonist of the acetylcholine receptors which stimulates muscarinic receptors. M1 muscarinic receptors have been proposed to be involved in seizure initiation, while glutamate receptors as N-methyl-d-aspartic acid (NMDA) are involved in seizure maintenance as well (Hamilton et al., 1997).

Classic protocol requires the administration of 1 mg / kg of scopolamine methylnitrate intraperitoneal (i.p.) 30 min before i.p. 350 to 385 mg / kg pilocarpine injection. The methylnitrate of scopolamine is given for reducing its peripheral effects of cholinergic action. The rats experience behavioral and electrographic seizures which can last for many hours approximately 45 min after pilocarpine injection. The length of the status epileptic may be regulated through benzodiazepine or phenobarbital medication. Most adult rats treated with pilocarpine experience spontaneous recurring seizures after 1-2 weeks of latent time (Arida et al., 1999; Goffin et al., 2007; Raol et al., 2006). Other variants have also been used in the treatment protocol of pilocarpine and the pilocarpine administration route. Some labs applied pilocarpine either directly into the cerebral ventricle or hippocampus (Furtado et al., 2011). Lithium treatment (3 mEq / kg, i.p.) 14–24 h before pilocarpine treatment is a widely used status epilepticus induction protocol (Clifford et al., 1987; Kubová et al., 2004).

2. Kainic acid model

Kainic acid is a glutamate analog and an agonist kainate glutamate receptor and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), in which was first isolated from seaweed Digenea Simplex. To induce epileptic status, kainic acid might be given intracerebroventricularly, directly into specific brain region or systemically. A single high-dose kainic acid injection (10–18 mg / kg) causes stereotypical behavioral seizures beginning with gazing periods, accompanied by head shaking and wet-dog shakes. Rats exhibit recurrent limbic motor seizures such as falling, forelimb,

rearing, tremor facial clonus, and mastication, approximately 1 hour after kainic acid injection, before entering epileptic status, which can last for hours (Ben-Ari, 1985).

Many laboratories administer several lower doses of kainic acid (5 mg / kg) instead of a single large dose, till an animal establishes epileptic status. This method decreases animal mortality dramatically during the status period (Hellier et al., 1998). As with the pilocarpine model, the kainic acid model often imitates several essential human TLE characteristics. Rats treated with repeated low dose of kainic acid develop spontaneous recurrent seizures after a latent duration of about 1 week. The spontaneous recurrent seizure phenotype varies from non-convulsive electrographic seizures to a very noticeable seizure marked by rearing, forelimb clonus and lack of reflex correction (Williams P. A. et al., 2009).

3. Flurothyl model

Flurothyl (trifluoroethyl ether) is a volatile liquid and a potent convulsant to its vapour. Flurothyl is a non-competitive antagonist at GABAA receptors (Krasowski, 2000) and is most frequently used as a model for recurrent generalized tonic-clonic neonatal seizures (Hoffmann et al., 2004). Flurothyl may, however, also be used in adult rats as well as mice for seizure induction. An animal is placed inside a fume hood in an airtight plastic cylindrical chamber for the induction of seizures in rat pups. Flurothyl is then injected into the chamber using a syringe pump at a rate of 50ml / min. After an average flurothyl exposure of 2,22 min P10 rat pups develop hindlimb tonic extension and forelimb (Holmes, 1997). When the pup is taken out of the chamber and returned to room air, seizures cease almost instantly. Immature rats with recurrent flurothyl-induced seizures have no cell loss (Riviello et al., 2002) and do not develop later-life spontaneous seizures; nevertheless, they are more susceptible to seizures (Isaeva et al., 2010).

4. Penicillin Model

The Penicillin model has been used to study the neurodevelopmental effects of epilepsy to answer important questions about the nature of epilepsy. It was initially based on recordings of the cat neocortex and was presented as a simple and elegant model that could be used to study the spread of seizure activity using intracellular recordings.

Penicillin’s are among the oldest classes of antibiotics that are still widely used in the world. They are highly effective against bacteria and are associated with psychological and psychiatric side effects. Disorientation, confusion, myoclonus, seizures, non-convulsive status epilepticus (NCSE), and encephalopathy have all been linked to Penicillin G, piperacillin, ticarcillin, ampicillin, amoxicillin, and oxacillin. Penicillin's neurotoxic effects were first recorded after intraventricular administration of Penicillin G, which resulted in myoclonic twitching.

The basic structure of penicillin’s is 6-aminopenicillanic acid, which consists of a thiazolidine ring and a β-lactam ring with an amino group at the C-6 position. After enzymatic cleavage of the β-lactam ring, epileptogenic activity was lost, implying that it plays an important role in convulsion induction. The pathogenesis of β-lactam-induced central nervous system excitation is thought to be caused by interference with inhibitory synaptic transmission caused by inhibition of GABA binding to the GABAAR. The chloride influx is modulated by ligand binding to different binding sites in transmembrane GABAAR complexes, which are ligand-gated chloride channels made up of multiple subunits. Reduced GABA-mediated chloride influx inhibition can occur through a variety of mechanisms, including direct blockage by binding to chloride ionophores, decreased chloride conduction by allosteric inhibition, binding of the binding site to GABA, or benzo-diazepine binding site.

Regardless of its concentration in the cerebrospinal fluid (CSF), Penicillin G has the greatest epileptogenic potential. The proconvulsant property of Penicillin G was significantly reduced when the benzylic hydrogen in the structure was replaced with a sulfonic or amino group. In experimental models, toxic doses of Penicillin given intravenously triggered myoclonic jerks. Penicillinase has been used to suppress the seizures (Wanleenuwat et al., 2020).

B. Electrical Stimulation Models

1. Kindling model

Kindling is a mechanism in which minimal-intensity electrical stimulation or a small-dose chemical convulsant such as pentylenetetrazol is continuously applied to a region of the brain before an epileptic seizure is trigged. First a stimulating electrode is implanted stereotaxically in the region of interest in order to electrically kindle an

animal. Some of the most frequently illuminated regions are the perirhinal cortex, hippocampus, amygdala and piriform cortex. The kindling rate varies between the brain regions and between different strains of rats (Löscher et al., 1998; Racine, 1972).

Kindling-induced seizures that arise from the stimulation site and afterwards spread repeatedly to other areas, represent the clinical profile of partial seizures with secondary generalization. Kindling is one of the widely known models for studying TLE mechanisms. The key benefit of the kindling is that a particular brain region can be stimulated to research its role in seizure generation and epileptogenesis in a regulated manner. The kindling model is often used to evaluate the efficacy of AEDs preclinically. The drug is given to completely kindled rats to test the anticonvulsant effect and its ability to prevent seizures is evaluated. To seek out if the medication will prevent the development process of epilepsy, the medication is given during the ignition process and its efficacy is determined to suppress the kindling (Löscher, 2002).

2. Self-sustaining status epilepticus (SSSE) model

Repeated electrical stimulation of the limbic system or of hippocampal circuits including perforant pathway can cause status epilepticus in rodents which persists even after stimulus is removed, such that, the seizures become self-sustaining (Lothman et al., 1989). SSSE is being used as a model for human mesial temporal lobe epilepsy (MTLE). SSSE produces neuronal loss and hippocampal synaptic rearrangement. The majority of rats develop spontaneous seizures after a latent duration of about 3 weeks. The model's disadvantage relative to chemo convulsant models of status epilepticus is that the implantation of the electrode is labor intensive and cumbersome (Mazarati et al., 2002).

3. Maximal Electroshock Seizures (MES) model

MES are a generalized of tonic – clonic seizures model. MES is caused by electroshock delivery by (most widely used) corneal electrodes, transcranial electrodes, or ear clips. The eyes are anesthetized for corneal stimulation by local application of 0.5 per cent tetracaine just before electrodes are placed into the cornea (Mares et al., 2006). The stimulation causes seizures that comprise of hindlimb and forelimb tonic extensions. Due to its user sincerity, MES testing is regularly used to potential screening of AEDs. The phenytoin anticonvulsant activity was established

using the MES model. The length of the hind leg's maximal tonic extension is usually analyzed to measure the drug 's efficacy (Putnam et al., 1937).

C) Genetic model

Epilepsy can be caused by mutations in many genes. Epilepsy has been reported to cause dysfunction of the ion channels, receptors, enzymes, and transporters caused by mutations in their genes (Reid et al., 2009). In many strains of the mouse, genetic mutations occur spontaneously which lead to epilepsy. These models have made an immense contribution to our fundamental understanding of the epilepsy pathophysiology. Tottering, ducky, lethargic, and stargazer mice, for example, all display an absence-type epilepsy and have calcium ion channel mutations. Mutation in a voltage-gated calcium channel leads to decreased calcium currents within neurons causing neurotransmitter release in tottering mice (Wakamori et al., 1998). In simple terms, a decreased presynaptic glutamate release has been reported in tottering mice. It was stated that decreased glutamate release in thalamus could enhance GABA-mediated inhibition resulting to synchronized thalamocortical circuit firing and thus spike-wave on EEG and absence seizures. In addition, a reduction in calcium currents and hence a reduction in neurotransmitters release, may affect the formation of proper synaptic connectivity and brain development (Caddick et al., 1999). Certain strains of rat also exhibit inherited of the absence type seizures. WAG / Rij (Wistar Albino Glaxo strain inbred in the UK) and the genetic absence epilepsy of rats from Strasbourg (GAERS) strain of rats develop absence epilepsy spontaneously. The EEG reported from these rats' cortex shows spike-and-wave discharges which correlate behaviorally with immobility (Coenen et al., 2003).

D) Developmental model

1. Febrile seizure model

Febrile seizures often happen while fever affect 2-5 per cent of children aged 6 months to 5 years which are the most common type of seizures in infants and children (Hauser W Allen, 1994). Retrospective studies indicate that most TLE patients had also undergone prolonged early-life febrile seizures (Cendes et al., 1993). Animal studies of febrile seizures include a steady increase in mice or rats' core body temperature till convulsions occur (Holtzman et al., 1981). For instance, a P10 rat pup is one of commonly used animal models of febrile seizures and put in a 3-l jar, and a

steady stream of moderately hot air is sprayed over the rat using a commercially available hairdryer till seizure starts. At about 41 C of body temperature recorded using a rectal probe, nearly all rat pups experienced stereotyped behavioral seizures comprising of wet-dog shaking, body tonic and flexion facial automatisms (Baram et al., 1997).

2. Hypoxic ischemic (HI) seizure model

The frequency of seizures during the neonatal period is greatest, and HI is among the most common causes of neonatal seizure. Neonatal HI raises by five times the risk of developing epilepsy in later life and induces serious cognitive and behavioral deficits. Most adult patients who have had mild neonatal HI experience cognitive impairment (Glass et al., 2009). A variety of animal models demonstrate fundamental human hypoxic-ischemic encephalopathy (HIE) features (Epsztein et al., 2008). Throughout hypoxic exposure, the pup becomes hypocapnic due to hyperventilation with no systemic pH change. Cerebral blood flow decreases the ligation during hypoxia by 40–60 percent of control values in the hemisphere ipsilaterally and returns to control values immediately upon return to normoxic scenario (Vannucci S. J. et al., 2004). It involves a combination in ligation of carotid and prolonged hypoxia over 90 min to cause neuropathological damage, infarction, and later-life epilepsy development. This model displays many of the popular characteristics of human HI such as extent brain damage, epilepsy development to certain animals not all and cognitive impairment (Kadam et al., 2010; Vannucci R. C. et al., 1997).

3. West syndrome/ Infantile spasm model

Infantile spasms or West syndrome most often occurs between the ages of 4 and 9 months in infancy. It is a severe disease that affects live births of 1 person per 2000–6000. Epileptic spasms characterize a West syndrome, a peculiar interictal EEG pattern called hypsarrhythmia, and poor developmental outcome. The seizures comprise of a sudden brief flexion or limbs and body extension. Some of the oldest models of infantile spasms required direct injection of corticotropin-releasing hormone (CRH) into rat pups' brains. CRH administration triggered severe seizures. The seizures, however, were not like spasms and mimics limbic seizures (Baram et al., 1991). Some other model of infant spasms includes intraperitoneal administration of

NMDA, a glutamate receptor agonist, into P15 rat pups that had previously been treated to prenatal betamethasone. In this model, the acute seizures were close to human infantile spasms (Velíšek et al., 2007).

E) Trauma model

A common cause of the acquired epilepsy is traumatic brain injury (TBI), which affects around 1.5 million Americans annually. The incidence of TBI-following epilepsy directly correlates with severity of the injury, and most likely the TBI patients with the penetrating head injury will develop epilepsy. Clinical findings show that several regions of the brain, such as thalamus, hippocampus, and basal forebrain, become neurodegenerated after TBI. Many approaches were used to cause TBI in animals, such as weight drop, cortical undercut, fluid percussion and guided cortical impact (CCI).

In the lateral fluid percussion injury (FPI) model, an injury is caused by saline application forcefully over a small exposed cortical surface. The impact force can be modified to achieve the severity of the injury desired (McIntosh et al., 1989). Long-term monitoring of video-EEG in critically injured adult rats showed that 50% of the injured rats acquire epilepsy. Histological study shows both hippocampus and damage cortex (Pitkänen Asla et al., 2006). CCI is a closed head injury model for which has been updated to specifically regulate the severity of injury from the original weight drop model. On anesthetized animals, first craniotomy is performed to expose the cortex. A computer-controlled air-driven piston then compresses the cortex to induce injury (Hunt et al., 2009).

F) Emerging epilepsy models

1. Zebrafish seizure model

Epilepsy researchers around the globe most commonly study epilepsy using rats or mice. Small size, faster growth, high breeding rate and many similarities between rodent and human brain are the obvious reasons for this. Even so, an easier preparation such as cell cultures or organism including flies, worm, fish that is easy to grow, maintain and manipulate is needed for certain studies. Zebrafish use has increased for studying mechanisms of neurological disorders (Bandmann et al., 2010). Zebrafish is a basic vertebrate, small and easy for large populations to maintain. It produces large embryo clutches, which are both easy and transparent to see fluorescent reporters and

undergo rapid growth outside of utero. It takes 3–4 months for Zebrafish to achieve maximum maturity after fertilization (Hortopan et al., 2010b).

In order to establish a model of zebrafish seizure, Baraban and colleagues subjected zebrafish larvae to different concentration of pentylenetetrazol 7 days after fertilization and observed its effects on zebrafish brain electrical activity and swimming behavior. When zebrafish larvae are exposed to pentylenetetrazol resulting in distinct seizure-like behaviors including an increase in swimming activity, accompanied by brief clonus-like activity and fast circular swimming leading to a loss of posture. During exposure to pentylenetetrazol, an epileptiform-like electrographic activity was also observed from the fish's optic tectum (midbrain). (Baraban et al., 2005).

Comparably, intraperitoneal kainic acid injection as well caused behavioral seizures in adult zebrafish (6–9 months-old) (Alfaro et al., 2011). Zebrafish were now been used to determine the important pathways involving epilepsy, to show the effects of AED on seizure-induced learning deficits and to describe the genetic attributes of epilepsy (Hortopan et al., 2010a).

2.2. Resveratrol

Resveratrol 's history, the active ingredient in red grapes, peanuts, berries and many other food plants, dates indirectly back to the Ayurveda, the ancient Indian treatise on longevity science. The first known use of grape extracts may be dated to 2500 BC or earlier for human health. Grape juice (*Vitis vinifera* L.) was the main component of "darakchasava" (fermented red grape juice), a well-known Indian herbal remedy prescribed as cardiotonic and often administered for other disorders. This has now been shown that, the principal components of darakchasava using high-performance liquid chromatography research are the Resveratrol and pterostilbene polyphenols, which account for their various medicinal properties. (Paul B. et al., 1999). Resveratrol had first been identified as a component of the white hellebore roots (Veratrum grandiflorum O. Loes) in 1940, and afterwards in the dried roots of *Polygonum cuspidatum*, known in Japanese as Ko-jo-kon, being used traditional Japanese and Chinese medicine to treat hyperlipidemia, favus, suppurative dermatitis and athlete's foot (tinea pedis) (Lee S. K. et al., 1998; Vastano et al., 2000).

Resveratrol is therefore known as a naturally occurring phytoalexin developed in response to ultraviolet (UV) irradiation, stress, injury and fungal (Botrytis cinerea) infection by a wide variety of plants other than grapes such as mulberries and peanuts as part of their protection mechanism. Resveratrol also was found in the epidermis of the leaf and the skin of grape berries in 1976 although not in the flesh (Langcake PWVM et al., 1979; Langcake P et al., 1976). Fresh grape skins comprise 50 to 100 mg of Resveratrol each gram, and wine concentrations can range between 0.2 to 7.7 mg a liter. An epidemiological discovery of an inverse association between red wine consumption and the incidence of heart disease has led to the "French paradox," which is associated with its established activity (Kopp, 1998; Sun A. Y. et al., 2002).

Among other phytochemicals, many bioactive molecules were reported to contain phytoestrogens, mostly found in vegetables, soy and fruits. These molecules can be divided into four main classes, namely flavonoids, isoflavonoids, lignans and stilbenes. Stilbenes, especially trans-Resveratrol and its glucoside, are reported widely to have beneficial health effects, despite showing anticarcinogenic, antioxidant, antitumor and estrogen / antiestrogenic activity (Kalantari et al., 2010) . Resveratrol is currently marketed as a dietary supplement with such a broad range of pharmacological properties including cell protection against oxidative stress (Aschemann-Witzel et al., 2015).

2.2.1. Resveratrol Chemistry

Resveratrol is a polyphenol of stilbenoid, containing two rings of phenols bound by an ethylene bridge. Resveratrol's chemical structure (trans 3,5,40 - trihydroxystilbene) is recognized in two isomeric forms, cis- and trans Resveratrol (figure 2.3). Transform is significant in terms of its prevalence and is due to various biological properties, namely inducing cellular responses such as differentiation, apoptosis, cell cycle arrest and improving anti-proliferation cancer cells. When exposed to UV irradiation, the transform can undergo isomerization in cis form (Akinwumi et al., 2018; Anisimova et al., 2011).

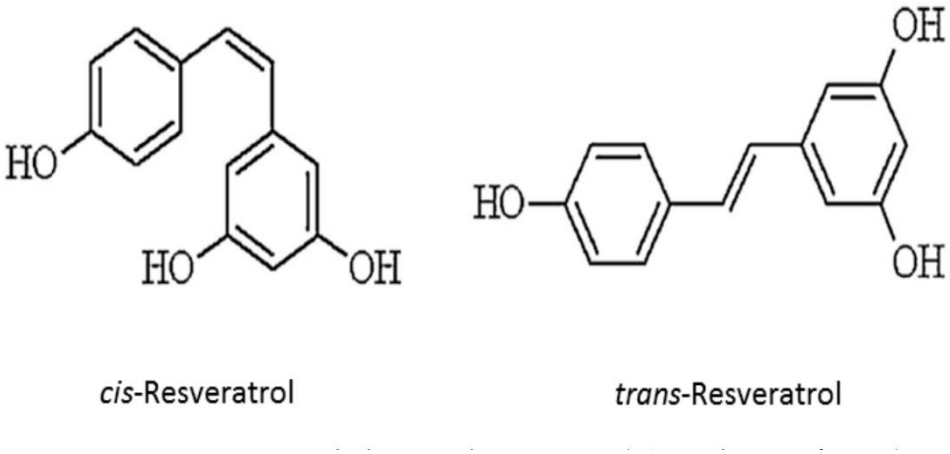


Figure 2.3 Chemical structure of Resveratrol (Salehi et al., 2018)

Resveratrol structure modification has therefore received particular interest from scholars, and several Resveratrol derivatives were synthesized and These derivatives, such as hydroxylated, methoxylated and halogenated, all have beneficial therapeutic benefits (Cichewicz et al., 2002; Park E. J. et al., 2015). Resveratrol is available as glycosylated forms, called piceid, in dietary products. While plants and pathogens, and perhaps even human gastrointestinal tracts, contain enzymes capable of triggering oxidation and eventual inactivation of polyphenols, glycosylation avoids Resveratrol's enzymatic oxidation, thus preserving its biological effects and greatly increasing stability and bioavailability (Walle, 2011). In addition, since the intestinal cells could only absorb Resveratrol aglycone form, the process of absorption needs glycosidases. The relative proportions of aglycone and glycosylated Resveratrol in foods and drinks may therefore modulate its absorption rate (Fan et al., 2009).

The main antibacterial compounds were identified as three glycosylated Resveratrol analogues, piceid, piceatannol glucoside and Resveratroloside isolated from the invasive plant species *Polygonum cuspidatum*. Glycosylated Resveratrol analogs have similar biological effects after transepithelial passage because Resveratrol in the intestine can be hydrolyzed into deglycosylated forms (Jacob et al., 2014; Shan et al., 2008). In vitro studies, however, have shown that the glycosylated analogs show even more potent bioactivities. Resveratrol and piceid, for example, have similar antioxidant ability, but piceid seems more powerful than Resveratrol because of its radical form reaction (Su et al., 2013). Resveratrol glycoside was reportedly more successful against hepatitis B virus than Resveratrol (Park S. et al., 2017). Piceatannol

has already been reported to have stronger immunomodulatory, anti-inflammatory, anti-leukemia anti-proliferative, anti-leishman, and protein-tyrosine kinase inhibitory activity (Fan et al., 2009).

Pterostilbene first was obtained from *Pterocarpus santalinus*, a plant used in the treatment of diabetes in traditional medicine (Lee P. S. et al., 2018). This active constituent of *Pterocarpus marsupium* is found primarily in blueberries, grapes, and other plant woods (Yeo et al., 2013). Pterostilbene has a Resveratrol-like structure except that a methoxyl group was substituted in A ring 3 and 5 position (Lee P. S. et al., 2018). This pro-lipophilicity compound, greater than Resveratrol, improves its bioavailability resulting in stronger bioactivities, including anti-lipidemic, antidiabetic, anticancer, and cardioprotective effects than Resveratrol (Kuršvietienė et al., 2016; Yang et al., 2017). Resveratrol nanoformulation was conceived in the same manner as a promising approach to preserving biological function, where polycaprolactone forms a hydrophobic core, while polyethylene glycol forms a hydrophilic shell of an encapsulated Resveratrol micelles (Moyano-Mendez et al., 2014). Lipid nanoparticles and nanostructured lipid carriers are two special Resveratrol nanodelivery methods that have been established for nutraceutical purposes to enhance oral bioavailability of Resveratrol (Gokce et al., 2012). Resveratrol nanoparticles have actually improved its solubility and enhanced its antioxidative activity unlike free form (Chen J. et al., 2017). For example, Resveratrol nanoformulation displayed an in vivo absorption increases, duration of activity increased and bioavailability is improved by 3.516 times further, while compared to raw form (Shen et al., 2017). Furthermore, Resveratrol's hydrophobic nature contributes considerably to its limited bioavailability, resulting from its low water solubility. Resveratrol encapsulated in methylated-β-cyclodextrins has therefore increased its water solubility and, subsequently, its bioavailability, preserving its antioxidant and antibacterial activity while promoting its further use in the food industry, aimed at controlling foodborne pathogens as well as nutraceuticals (Duarte et al., 2015).

2.2.2. Biological function of Resveratrol

Resveratrol has a broad variety of biological characteristics, including antioxidant, neuroprotective, anti-inflammatory, cardioprotective, and anticancer activities.

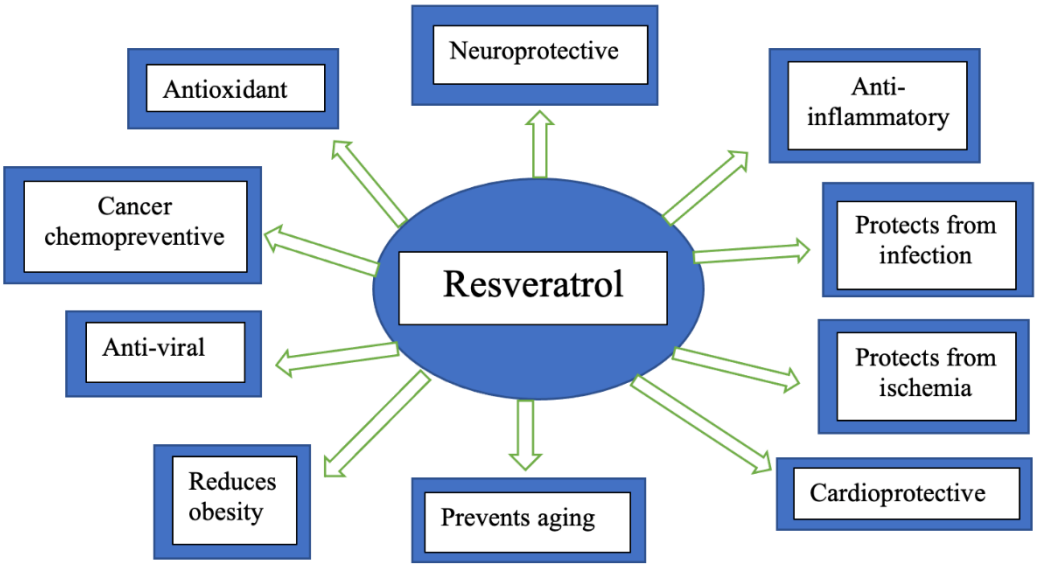


Figure 2.4 Biological function of Resveratrol (Galiniak et al., 2019)

Antioxidant effect of Resveratrol

In the last few years, a numerous study has shown that reactive oxygen species (ROS) or reactive oxygen metabolites (ROM) play a significant role in facilitating the development of oxidative stress. The term ROS belongs to molecules or parts of molecules in valence orbitals which contain unpaired electrons. This state of electron deficiency serves to make these agents highly reactive and then they can damage adjacent molecules by extracting an electron by them or by donating an electron (Bergamini et al., 2004).

They are typically formed in aerobic environments by a variety of mechanisms, including electron 'leakage' while biological oxidation, the activity of flavin dehydrogenases and complex membrane-associated secretion, as well as the direct activation of oxygen by irradiation. Hydroxyl (OH^-), superoxide anion (O_2^-), peroxyl (RO2) and alkoxyl (RO) radicals are the most significant of these (figure 2.5). Also, there are highly harmful nitrogen-based products, such as nitric oxide (NO) and its metabolite peroxynitrite, this term refers both to anion oxoperoxynitrate as well as its conjugate acid hydrogen oxoperoxynitrate, as result of the rapid radical-radical

reaction along With NO and O redicals. Additionally, some non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2), ozone (O_3), or hypochloric acid (HOCl) are oxidizing agents rapidly converted in to radicals (Beckman et al., 1990; Lorenz et al., 2003).

Physiological ROS concentrations are involved in cellular functions as well as transcriptional and posttranscriptional regulation. ROS has indeed been viewed as significant intermediate signal transduction controls for cell differentiation, immune activation, gene expression apoptosis (Nose, 2000).

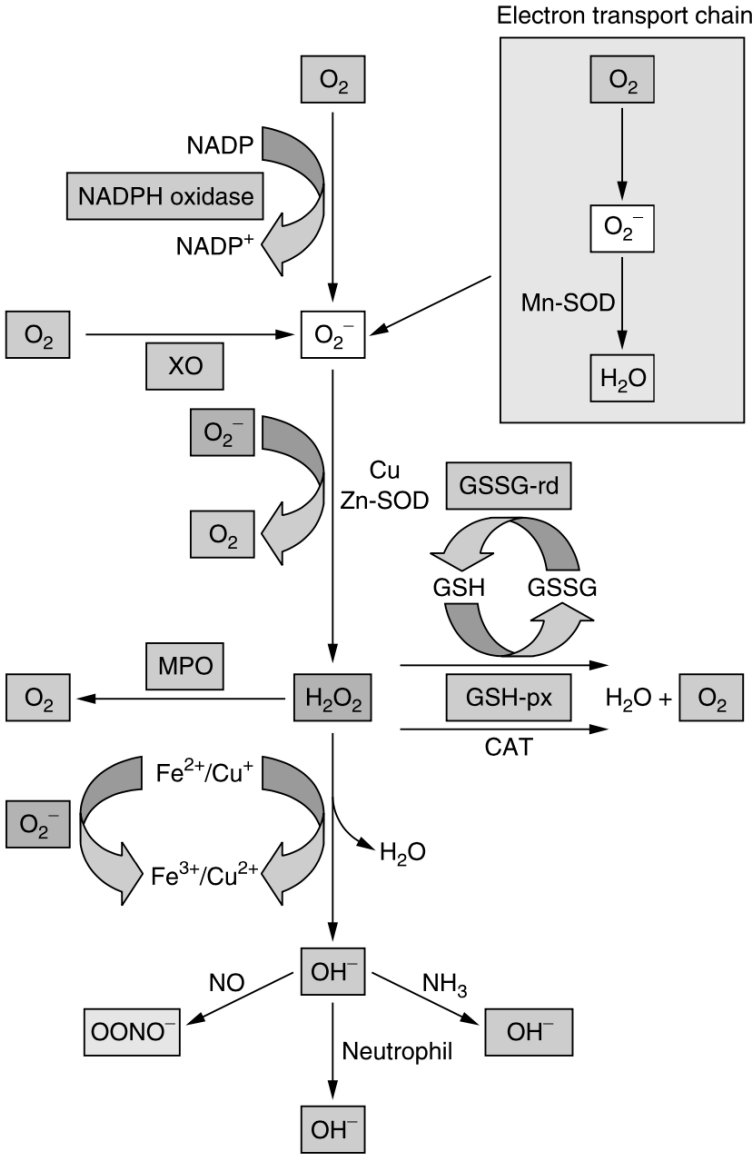


Figure 2.5 Mechanism of generation of free radicals (Lorenz et al., 2003).

Potential sources of ROS inside the cells include mitochondria, myeloperoxidase (MPO), ischemically activated xanthine oxidase (XO), nitric oxide synthase (NOS),

cyclooxygenase (COX), and reduced of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Resveratrol has several bioactivities, but its ability to act as a potent antioxidant is the best defined Resveratrol property (Malhotra et al., 2015). Resveratrol antioxidant properties depends on the nuclear structure being organized by the functional groups. Moreover, numbers of configuration, substitution, and total hydroxyl groups greatly affect many antioxidant activity mechanisms, including radical scavenging and chelation abilities of metal ions (Stivala et al., 2001). Resveratrol could also be used to prevent or reduce lipid oxidation in pharmaceutical products, postpone the formation of toxic oxidation products and maintain both nutritional quality and extend the shelf-life of pharmaceuticals (Papuc et al., 2017). Additionally, the antioxidant activities Resveratrol is being effectively used to prevent cells against oxidative stress caused by hydrogen peroxide, in which pretreatment with Resveratrol encouraged cell survival and defense toward Ultraviolet-irradiation-induced cell death. cellular protection of Resveratrol can be accomplished, at least in part, through its ability to serve as a direct antioxidant and an indirect cellular antioxidant system enhancer by modulating multiple antioxidant cellular pathways, thus maintaining the cellular redox status (Means et al., 2017).

Anticancer effect of Resveratrol

Multiple studies it has been shown that Resveratrol has antitumor action, as well as being a candidate for a variety of cancers for treatment and prevention (Bishayee, 2009). Several in vitro and in vivo studies have confirmed the anticancer properties of Resveratrol, which indicate that Resveratrol is capable of inhibiting all stages of carcinogenesis including promotion, initiation and progression (Zykova et al., 2008). Numerous researches have also shown that Resveratrol not only acts as a chemopreventive agent and also shows chemotherapeutic properties associated with its anti-inflammatory, pro-apoptosis, antioxidant and anti-proliferative action (Van Ginkel et al., 2007). In fact, Resveratrol is thought to affect intracellular signaling pathway components including cell survival and apoptosis regulators, pro-inflammatory mediators, , and metastatic and angiogenic tumor switches through modulating a distinctive collection of upstream kinases, transcription factors and their regulators (Kundu et al., 2008). Resveratrol has shown apoptotic and anti - proliferative activity on human cervical carcinoma by inducing cell shrinkage in HeLa cells and apoptosis via activating caspase-3 and -9, upregulation of the expression of

the pro-apoptotic B-cell lymphoma (Bcl)-2-associated X protein, and downregulation of the expression of the anti-apoptotic Bcl-2 and Bcl-extra-large proteins (Li L. et al., 2018).

Resveratrol is indeed a histone deacetylase inhibitor which exhibits its antiproliferative action by initiating cell cycle arrest, inducing apoptosis, angiogenesis inhibition, increasing the formation of reactive oxygen species that induce oxidative stress, and mitotic cell death in tumor cells (Singh et al., 2018). The existence of 40`-OH in trans-conformation along with the stereoisomer (40-hydroxystyryl moiety) is absolutely necessary for inhibition of cell proliferation. Enzymatic assays showed that suppression of DNA synthesis was caused by a direct interaction between Resveratrol and DNA polymerases (Szekeres et al., 2010). Another in vitro work has also shown that Resveratrol improves the effectiveness of chemotherapy by inactivating NF-γB protein; a transcription factor formed by cancer cells that controls the expression of certain genes. Because this factor appears, cancer cells are immune to chemotherapy which then helps them to reproduce. Resveratrol blocks this transcription factor, allowing chemotherapeutics to act on its target sites (Shukla et al., 2011).

Additionally, as an upcoming preventive and therapeutic agent against breast cancer, the phytoestrogen, Resveratrol has received considerable attention (Sinha et al., 2016). Resveratrol has also demonstrated promise as part of the combination therapy, especially in breast cancer (Alamolhodaei et al., 2017). In combination with other chemotherapeutic agents, this compound has been shown to reverse drug resistance in a wide variety of in vitro cell technologies by sensitizing tumor cells to drug-mediated effects (Varoni et al., 2016). Resveratrol shows the ability of pancreatic cancer cells to become more sensitive to gemcitabine therapy (Cheng et al., 2018). Resveratrol decreases the chance of high nephrotoxicity of cisplatin, a cancer chemotherapy agent against bladder, ovaries, testicular and many other cancers (Valentovic, 2018).

Effect of Resveratrol in cardiovascular system

In developed countries, coronary heart disease (CHD) and stroke are the leading causes of disability and death. Most CHDs are caused by atherosclerosis, an arterial degenerative mechanism that is induced by oxidative stress and chronic inflammatory state. The role of smoking, arterial hypertension, diabetes mellitus,

hypercholesterolemia, lack of physical activity, overweight/ obesity, and genetic factors in assessing cardiovascular risk is well known (Castaldo et al., 2019).

Resveratrol protective role has been shown to enhance cardiovascular function in diabetic rats by maintaining the functional capacity of cardiac stem / progenitor cell compartments and mature cardiac cells, improving cardiac condition by decreasing inflammatory activity, and reducing unfavorable ventricular remodeling of a diabetic heart, leading to a significant recovery of ventricular function (Delucchi et al., 2012). Resveratrol demonstrated beneficial effect in cardiac insufficiency by enhancing left ventricular function, decreased cardiac hypertrophy, contractile dysfunction and remodeling, interstitial fibrosis and plasma BNP concentrations (Riba et al., 2017).

Yan et al. (2018) proposed that, Resveratrol works by suppressing endothelial nitric oxide synthase expression, vascular endothelial growth factor and suppressing p38 phosphorylation in diabetes-related myocardial infarction in rats. Resveratrol also significantly reduced blood glucose, body weight, plasma triglyceride levels, heart rate and alanine transaminase (ALT) /aspartate transaminase (AST) ratio in myocardial infarction-related diabetic rats, while significantly increasing total plasma insulin levels (Öztürk et al., 2017).

Additionally, Resveratrol greatly decreased inflammatory factors and the levels of malondialdehyde, a marker of oxidative stress. This finding found that treatment with Resveratrol would enhance cardiovascular function by reducing myocardial ischemia-reperfusion injury, vasodilatation, and atherosclerosis. Contrary to this, Resveratrol induces vasodilation at physiological concentrations and consequently reduces the risk of hypertension and cardiovascular diseases (Hung et al., 2000).

In other hand, those latter findings also have confirmed the use of *Polygonum cuspidatum* as a source of Resveratrol in traditional chinese medicine to treat and prevent arteriosclerosis and hyperlipidemia (Zhang H. et al., 2013). Conclude, Resveratrol 's cardiovascular protective role has been related to several molecular targets and may be beneficial in creating novel therapies for metabolic syndrome atherosclerosis, ischemia and heart failure (Rauf et al., 2017).

Neuroprotective effect of Resveratrol

Seizures could result in neuronal death, leading to the development of an abnormal neural network which exacerbates seizures and increases the probability of future seizures. Thereby, it is particularly important to protect the neurons by treating epilepsy (Pitkänen Asla et al., 2011). In neurodegenerative disease models, Resveratrol can improve pathological neuronal damage, cell survival by inhibiting apoptosis, inflammation, and oxidative stress, and therefore improve cognitive dysfunction and the decline of motor function associated with these diseases (Kumar A et al., 2007). Resveratrol is thought to produce its neuroprotective effects by regulating the heme oxygenase-1 and peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α), reducing the expression of Nrf-2 and activating the Sirt-1 uncoupling protein 2 pathways (Mudo et al., 2012; Ren et al., 2011).

Friedman et al.,(2013) used Resveratrol as a therapy for a rat model of epilepsy caused by kainic acid. They discovered that Resveratrol weakened kainic acid-induced convulsions and related hippocampal neuronal excitotoxicity in adult mice, increased neuronal survival, and reduced lipid peroxidation, findings suggest that Resveratrol may prevent excitotoxic neuronal injury, possibly through its antioxidant property. Albani et al., (2010) reported a neuroprotective effect of Resveratrol on seizures through the Sirt-1 pathway. Other scientists have discovered that Resveratrol's neuroprotective function in acute seizures may be linked to inhibition of voltage-gated potassium channels, discharge of neurons in the CA1 area of the hippocampus and excitatory synaptic transmission in hippocampal neurons mediated by glutamate postsynaptic receptors. This Resveratrol neuroprotective effect in models of epilepsy could contribute to the development of an epilepsy treatment (Gao et al., 2005; Li M. et al., 2005).

Resveratrol has many neuroprotective effects in different neurodegenerative disorders, such as Huntington's, Alzheimer's and Parkinson 's diseases, neurodegenerative alcohol-induced disorders and lateral amyotrophic sclerosis (Wahab et al., 2017). Resveratrol protective effects have been demonstrated not restricted to antioxidant and anti-inflammatory activity and also enhanced mitochondrial functions and biogenesis via the pathway SIRT1/AMPK / PGC1α and vitagenes that prevent oxidative stress-induced deleterious effects (Bastianetto Stéphane et al., 2015; Sun Albert Y et al., 2010).

Resveratrol reduces cholinergic neurotransmission, expression of the neurotrophic factor derived from the brain, and oxidative stress, facilitates clearance of β-amyloid peptides and anti-amyloid cleavage of APP, and decreases neuronal apoptosis (Rege et al., 2014). A meta-analysis has shown that Resveratrol has reduced significantly the Mood States Profile (POMS) including vigor and fatigue (Farzaei et al., 2018). Resveratrol is also capable of improving rat motor skills and deactivating neuroinflammatory response after intracerebral hemorrhage. It can be used as a therapeutic target agent for the treatment of intracerebral bleeding (Cai et al., 2018).

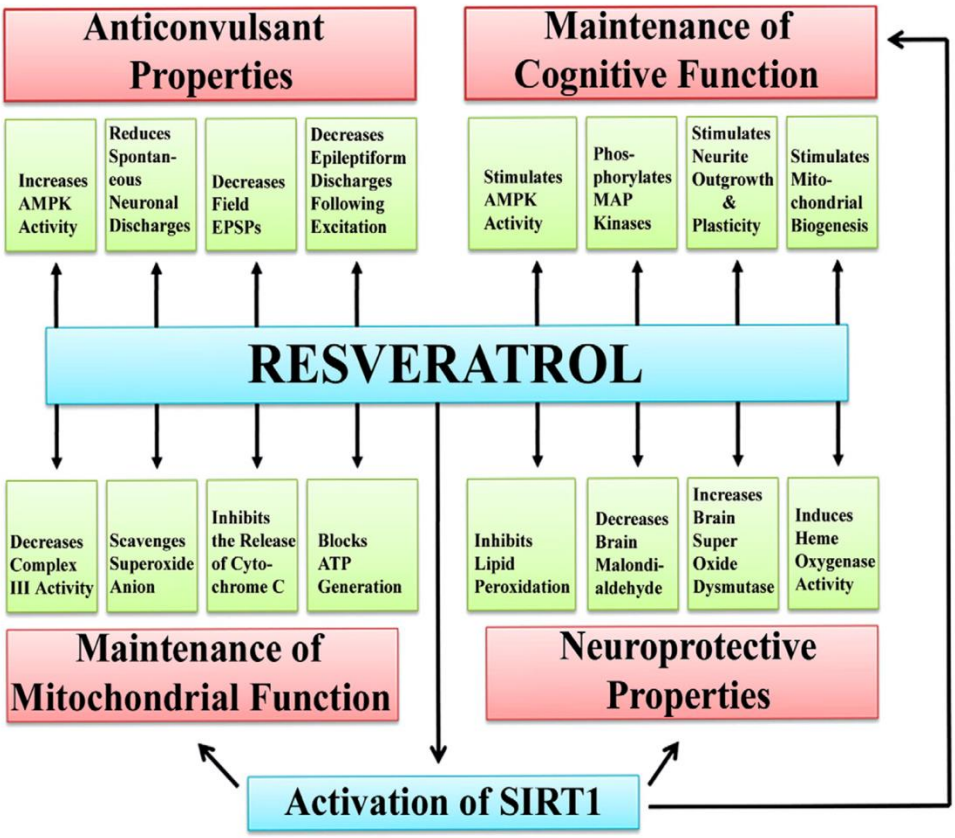


Figure 2.6 Neuroprotective mechanism of Resveratrol (Bastianetto S. et al., 2015)

Anti-inflammatory effect of Resveratrol

Inflammation is considered to be the principal physiological protection mechanism that helps the body to fight against infection, burns, toxic chemicals, allergens as well as other noxious stimuli. Unrestrained and persistent inflammation can, however, have harmful effects. While inflammation is essentially a defense mechanism, multiple chronic diseases are underpinned by the harmful sequelae of inflammation. Inflammation was identified as an etiological factor in such a broad variety of human diseases including neurodegenerative disorders, respiratory, cardiovascular, cancer, rheumatoid arthritis, diabetes, and so on. In both acute and chronic inflammatory responses, flare by vascular and cellular reactions in response to inflammatory stimuli mediated by chemical factors. Inflammation mediators include a wide range of chemical compounds, including vasoactive amines (e.g., serotonin and histamine), plasma proteases (e.g., complements protein and kinins), cytokines (e.g., tumor necrosis factor (TNF) and interleukins (IL)), arachidonic acid metabolites (e.g., prostaglandins (PGs), leukotrienes, thromboxane's), growth factors such as platelet-derived growth factor. Inflammatory vascular and cellular reactions typically include vasodilatation, increased vascular permeability to proteins, increased penetration into the inflamed tissue of inflammatory cells such as granulocytes and lymphocytes, production of chemokines and cytokines, activation of leukocytes and so on (Coleman, 2010).

Resveratrol's immunomodulatory function was suggested 18 years ago, with an investigation showing how it prevents the proliferation of spleen cells triggered by concanavalin A (ConA), interleukin-2 (IL-2), or alloantigenes, and much more effectively inhibits the formation of IL-2 and interferon-gamma (IFNγ) in lymphocyte and the formation of tumor necrosis factor alpha (TNF-α) or IL-12 by macrophages (Malaguarnera, 2019). Resveratrol regulates innate and adaptive immunity by interacting with multiple molecular targets (Švajger et al., 2012). Nonetheless, its properties sometimes seem to contrast. Resveratrol has been reported for the attenuation of immune cell-mediated release of various inflammatory cytokines. Resveratrol has also been documented to modulate immune function in a dose-dependent manner, while Resveratrol activates the immune system at low doses, while at high doses it induces immunosuppression (Sharma et al., 2007). Its role for an immunomodulator was already demonstrated in different animal models and cell lines.

Resveratrol decreases inflammatory responses in peritonitis in mice, reverses immunosenescence in the elderly and increases immunological activity toward cancer cells (Yuan et al., 2012). Resveratrol prevented IL-6 release from cortical mixed glial cells induced by hypoxia / reoxygenation, findings suggest the compound is successful in managing the inflammatory process in the stroke caused by ischemia (Wang M. et al., 2001). Resveratrol inhibits microglia activation which results in the release of different proinflammatory factors, the development of reactive oxygen species, as well as the activation of signal pathways that lead to neuroinflammation (Zhang F. et al., 2010). In vitro, Resveratrol modulates the inflammatory reaction in the intestinal cells at moderate to high concentrations by decreasing NF-κB expression and preventing dysfunctions of mitochondria. This finding has been stated in vivo in which Resveratrol inhibits NF-κB activation and TNF-α production, reduces neutrophil infiltration throughout the intestinal mucosa, and suppresses intestinal tumorigenesis (Nunes et al., 2018).

Effect antimicrobial activity of Resveratrol

Resveratrol has also been researched for its ability to inhibit the growth of certain pathogenic microorganisms, such as Gram-negative and Gram-positive bacteria and fungi, in addition to the biological activities mentioned above (Salehi et al., 2018). Resveratrol demonstrated antibacterial activity against Gram-positive bacteria, and the time-kill assay indicated that its results were due to its bacteriostatic action. The mechanism which underlies antibacterial activity, however, is not clearly understood. Resveratrol also had the potential to influence cells with altered cellular morphology and DNA content (Paulo et al., 2010).

Pseudorabies virus is one of the deadly swine pathogens for which no cure is available and which also contributes to economic losses. Resveratrol demonstrated antiviral activity by inhibiting the replication of the Pseudorabies virus and effectively improving growth efficiency and reducing mortality of piglets infected by Pseudorabies virus (Zhao et al., 2018).

Pterostilbene is a methoxylated Resveratrol derivative that showed superior pterostilbene antibacterial activity against drug-resistant Staphylococcus aureus (MRSA) with minimum inhibitory concentration (MIC) compared with Resveratrol. Pterostilbene anti-MRSA effectiveness has been associated with bacterial membrane

leakage, upregulation of ribosomal protein and downregulation of chaperone protein and can be applied topically to treat skin MRSA infection with less toxicity to eukaryotic cells (Yang et al., 2017). Resveratrol is a particularly important agent on pneumonia caused by Staphylococcus aureus and Treatment of the infectious diseases caused by S. aureus. Resveratrol may also ease diarrhea caused by rotavirus (Abba et al., 2015).

2.3. Nitric Oxide (NO)

NO has been identified as one of the most significant factors responsible for air pollution used in modern communities, in numerous industrial areas and automobiles. In 1980, acetylcholine was found to have caused isolated vessel enlargement, but this effect was not observed when the vascular endothelium was extracted. A compound called endothelium relaxing factor (EDRF) was subsequently produced from the endothelial cells induced by acetylcholine, and it was determined to vasodilate the vessels (Furchgott et al., 1980) and In 1987, nitric oxide was found to be this vasodilating factor (Ignarro et al., 1987; Palmer et al., 1987). He was awarded the Nobel Prize for Medicine in 1988 to Robert E. Furchgott, Louis J. Ignarro and Ferid Murad for their work and important contributions.

NO has become the focus of interest in the disciplines investigating physiological and pathological processes after recognizing that NO can be synthesized in mammalian cells as both a physiological and cytotoxic agent. The discovery of NO considered a trans-synaptic retrograde transmitter involved in a wide range of physiological and physiopathological processes in the body, and particularly in the nervous system, has led to changes in information and views on the communication among nerve cells. Before the discovery of NO, known neurotransmitters are usually amino acid and peptide-structured compounds. Even so, NO is not stored in vesicles, which is a very small molecule, and have no unique protein receptor and secretory mechanism. Once formed, it enters the target cells and rapidly diffuses. (Moncada et al., 1991).

2.3.1. Nitric Oxide biosynthesis

NO is a reactive, free radical gas which has no special mechanism for synaptic secretion and it is not stored in vesicles. NO is a small molecule, with 30 g/mol of molecular weight. It is a lipophilic substance which can easily move through the membranes and does not need a receptor to establish a cellular response (Moncada, 1992). Literature review indicates that NO can serve as a neurotransmitter, neuromodulator and second messenger, having both protective and toxic effects. NO most important roles include immune response, control of blood-vessel tone and neuronal functions (Calabrese et al., 2007).

Though in the presence of O2 it has a half-life of over 4 minutes in the liquid solution, its half-life in biological systems is less than 30 s. It can diffuse into a distance of 0.4-0.5 mm. Nitric oxide in the presence of molecular oxygen is relatively unstable and can auto-oxidize rapidly and spontaneously to create a variety of nitrogen oxides.

1 $2NO^{\cdot} + O_2 \rightarrow 2NO_2^{\cdot},$

2 $NO^{\cdot} + NO^{\cdot} \rightarrow N_2O_3$

3 $NO_2^{\cdot} + NO_2^{\cdot} \rightarrow N_2O_4$

Figure 2.7 Different varieties of nitrogen oxides. 1. Nitrogen dioxide, 2. Dinitrogen trioxide, 3. Dinitrogen tetroxide

NO is formed during the conversion of L-arginine (L-Arg) to citrulline by a group of isozymes, the nitric oxide synthases (NOS) (Snyder, 1992). There are three isoforms in the NOS family: endothelial (eNOS), inducible (iNOS), and neuronal (nNOS). In addition, the mitochondrial NOS, previously thought to be an iNOS isoform, is a subtype of nNOS related to the inner mitochondrial membrane (Eissa et al., 1996; Elfering et al., 2002). The three human NOS genes are very distinct: the iNOS gene is located on chromosome 17 (Geller et al., 1998) , the nNOS gene is localized on chromosome 12 (Kishimoto et al., 1992) and the eNOS gene is mapped to chromosome 7 (Marsden et al., 1993).

Hypoxia, insulin, estradiol, high salt concentration, receptor ligands of the epidermal growth factor, corticosterone, hypertension, amyotrophic lateral sclerosis, angiotensin II or gamma-amino butyric acid (GABA) can stimulate nNOS transcription(Guix et al., 2005). For their activation, both constitutive nNOS and eNOS require the formation of Ca2 + -calmodulin complexes, whereas iNOS is de novo synthesized after inflammatory stimulation (Ebadi et al., 2003; Mungrue et al., 2003). Such agents as heme, tetrahydrobiopterin, flavin mononucleotide, flavin adenine dinucleotide and reduced nicotinamide-adenine dinucleotide phosphate are co-factors necessary for the catalytic activity of all three NOS isoforms (Calabrese et al., 2007). NOS it has two sub-units: the catalytic site capable of oxidizing L-arginine, and the L-arginine binding site; Both subunits contain a reductase domain as well as an oxygenase domain, which can bind calmodulin in a Ca^{2+} -dependent way. Only nNOS and eNOS, are Ca^{2+} -calmoduline complex dependent while the iNOS shows separate independent $Ca2^{+}$ calmoduline (Ferraro et al., 2004).

NO passes into the presynaptic region, there activating the Guanine Cyclase (GC) enzyme and thus increasing cGMP production. There are 2 GC-types. One is found in the cytoplasm in dissolved form and is activated by NO. The other one is bound to the cell membrane, and is activated by peptide hormones (Friebe et al., 2003).

2.3.2. Pathophysiology of nitric dioxide

Central nervous system and Nitric oxide

NO is considered to play a part in many CNS functions. There are 3 types of NOS isoforms in the brain: present in neurons of type I (nNOS), glia cells of type II (astrocyte), and endothelial cells of type III (eNOS). The best known of these is nNOS. ENOS is more related to vascular activity in the endothelial cells of the cerebral blood vessels and somewhat distinct from peripheral endothelial NOS. Although some participate in hippocampal neurons with long term potentiation (LTP). Type II NOS locations are limited to glial cells, but there is not yet adequate research on them. Throughout the central nervous system, nNOS functions include neurotoxicity, neuroprotection, synaptic plasticity including control of sensory behaviors such as learning, and pain expression, long term depression (LTD) and LTP. Differing findings have been stated about the function of NO in both of these categories, and this is likely based on both the mechanism of action of nitric oxide and the experimental models

and conditions (James, 1998). NO is a distinctive regulator for neurogenesis and synaptogenesis that has positive or negative effects on different signal pathways and locations (Hu et al., 2014). Dr. Bredt and collaborators in 1990 identify nNOS localization suggesting a neural role for NO. They showed nNOS in the brain that was primarily associated to distinct neuronal populations including autonomic nerve fibers in the retina, neural innervation of the posterior pituitary, adrenal medulla, cell bodies and nerve fibers in the intestine's myenteric plexus, and endothelial vascular cells. These transcendental findings therefore provide the first conclusive evidence for a strong association between NO and neurons. In particular, several findings indicated that the postsynaptic release of NO dependents on Ca^{2+} (Bredt et al., 1990). NO release is critically related to cerebral blood flow regulation, synaptic plasticity and the establishment and activity-dependent refinement of axonal projections during later developmental stages (Gally et al., 1990). Nowadays it is well recognized that NO is involved in controlling a number of physiological and pathological processes. Low NO concentrations are usually neuroprotective and mediate physiological signaling whereas higher concentrations are neuroinflammatory and neurotoxic (Balez et al., 2016).

Neurotoxicity

NO has neurotoxic effect in the central and peripheral nervous systems, as well as neurotransmitter function. It is thought that glutamate-induced neurotoxicity occurs via nitric oxide in the central nervous system. Under normal physiological conditions, calcium entry into the cell increases when glutamate binds to the NMDA receptor in the post-synaptic region. NNOS enzyme is activated by increased calcium in the neuron. The NO produced after which suppresses the NMDA receptor activity, either via cGMP or S-nitrosylation. As a consequence, more calcium entry into the cell stops and neurotoxicity is prevented. Recent studies have suggested NO as a vital regulator of neuroinflammation, indicating a potential role for major depressive disorder pathophysiology.

NO has also long been used as part of the neurotoxic insult triggered by neuroinflammation in the brain of Alzheimer's. Until cognitive symptoms arise, however, the perception is changing. This has thus highlighted a compensatory, neuroprotective function for NO which protects synapses by increasing the excitability of the neurons. Here an underlying mechanism for increasing excitability by NO

through modulation of voltage gated potassium channel activity was suggested (Balez & Ooi, 2016). Additionally, low NO production is linked to schizophrenia pathogenesis. Neither donor could be a promising class of compounds for schizophrenia treatment. In addition, existing analyzes show that both NO donors and NOS inhibitors are involved in object recognition memory and indicate that NO may be a promising cognitive impairment target (Pitsikas, 2015a, 2015b).

The peroxynitrite (ONOO-) formed by the mutual reaction of these two radicals (hydrogen peroxide and nitrogen dioxide) is as reactive as the radical hydroxyl and causes nitration and oxidation to biomolecules. In the cells where NO synthesis actively continues, arginine amino acid concentration decreases and becomes the momentum limiting stage for NO synthesis. In this case, the enzyme NOS starts to synthesize superoxide rather than NO. Nitric oxide's neurotoxic effects are only observed in cases where its synthesis is well above the concentration required for its work. Nitric oxide neurotransmitter functions are seen in nanomolar concentrations and in such concentrations, do not cause any toxic effects (Dawson, 1994).

Long-term potentiation (LTP):

This can be characterized as a sustained increase of synaptic transmission effectiveness, or at least hours or days of increase in synaptic power due to activity. Various excitatory synapses in CNS are typical of LTP. Bliss and Lomo mentioned it for the very first time and these researchers stated that when they tested granule cells for a few seconds at high frequency and tested with one shock, EPSP increased. This synaptic plasticity is claimed to be a synaptic model of memory and learning. In higher brain centers that play a role in cognitive functions, particularly in the cerebral cortex and hippocampus, this phenomenon is much stronger (Dinerman et al., 1994).

The LTP mechanism is not fully understood but it is acknowledged that glutamate and excitatory amino acids play a significant role. Normal EPSP is regulated by glutamate secretion from the presynaptic nerve. AMPA receptors are activated when glutamate is release, sodium ion channels are opened, and the post-synaptic cell is depolarized and at the same time, during tetanic stimulation, NMDA receptors are activated which causes Ca2 to enter the postsynaptic cell. This calcium inflex in the postsynaptic cell induces changes in the function of protein kinase, phospholipase, protease, and NOS. In particular, protein tyrosine kinase and calcium / Calmodulin-

dependent kinase II (CaMKII) play a role in LTP (Malinow et al., 1989) . When NOS inhibitors are administered LTP formation is prevented (Böhme et al., 1991). The overall effect of NO on synaptic transmission depends on Presynaptic stimulus frequency. NO induces long-term potency (LTP) when the stimulus is given at high frequency and long-term depression (LTD) when the stimulus frequency is low (Zhuo et al., 1999).

Perception of pain

The primary afferent fibers and posterior root ganglia in the periphery contain NOS. Some sensory structures contain NOS in the brainstem and thalamus, as well. For certain studies NO has been reported to play a role in allodynia, hyperalgesia, and hyperesthesia, for glutaminergic pathways regulated by NMDA receptors (Budai, 2000). An intrathecal administration of L-NNA in rats suppressed the behavior of scare and fleeing through formalin injection into the foot sole. L-arginine reverses the L-NNA effect and this finding shows that NO plays a role in the spinal cord nociceptive pathways. The effect of NO on pain pathways has been reported to be bilateral, based on the dosage. Thermal pathways involve activation of the NMDA receptor induced predominantly by NO synthesis, whereas mechanical pathways involve non-NMDA glutamate receptors in which cyclooxygenase activity and subsequent metabolism of arachidonic acid are induced. (Cury et al., 2011; Lincoln et al., 1997).

Nitric oxide and peripheral nervous system

Peripheral nerves provide important connections to muscles, autonomic systems, and sensory organs within the central nervous system. Nitric oxide (NO) is involved in essential actions that include many aspects of peripheral nerve function and disease. It provides significant roles in "natural" afferent pain signaling through the spinal cord's dorsal horn, and in autonomic regulation via nitrergic innervation. During the fundamental processes of Wallerian peripheral nervous degeneration, NO is generated following injury which can bear on subsequent regenerative events. NO takes part in microvascular changes following injury through its actions on vasa nervorum, the blood supply to the nerves, but also has direct roles in axon and myelin breakdown and clearance prior to regeneration. NO contributes to the production of neuropathic pain. Increased local NO levels can damage the axons and growth cones during

inflammation. Small persistent increases in NO may also lead to peripheral nerve damage, or diabetes neuropathy (Zochodne et al., 2005).

NO plays an important role in the gastrointestinal tract as an inhibitory neuromuscular transmitter, which acts on the reflexive relaxation of the stomach and inhibitory pathways that provide tonic inhibition of the small intestines. But not always, it also interacts with nonadrenergic, noncholinergic neurotransmitters such as VIP and A TP. It often functions as a neuromodulator in the gastrointestinal tract by increasing or decreasing the release of acetylcholine and substance P. NO generally plays an inhibitory role in other peripheral nervous systems. For instance, it induces relaxation of the lower urinary tract, the neck of the bladder, the cavernous tissue of the penis and the retractor muscle of the penis. It causes relaxation by neurogenic vasodilatation in endothelial cells and also it may reduce vasoconstrictive effect sympathetic nervous system. Preganglionic neurons in peripheral parasympathetic and sympathetic ganglia have the ability to secrete NO (Lincoln et al., 1997; Lychkova, 2013).

Epilepsy and nitric oxide

The association between NO and epileptic seizures, especially since 1991, has been the subject of growing numbers of studies. There has been several research performed to establish the effect of nitric oxide in experimental epilepsy and new studies are now being applied to these studies. A few of the findings achieved so far show that NO is an endogenous proconvulsant substance, whereas others say that has an anticonvulsant effect (Ferraro & Sardo, 2004).

Anticonvulsant role of NO: In an experimentally induced of generalized epilepsy, caused by NMDA intraventricular injection, the pharmacological blockage of nNOS resulted in an increase in both the frequency and duration of seizures (Buisson A. et al., 1993). Consequently, a direct association was demonstrated between the reduction of cerebral NO levels and a facilitative effect on the development and/or progression of both focal and generalized seizures (Kabuto et al., 1996; Przegaliński et al., 1996). In addition, the use of a precursor such as L-arginine demonstrated an effective action in reducing the susceptibility to seizure, similar to common antiepileptic medications, indicating a possible involvement of NO as an

anticonvulsant Nitric oxide synthesis, epileptic seizures and kindling (Herberg et al., 1995).

Proconvulsant role of NO: It was hypothesized that NO could also act as a pro-convulsive agent, inducing seizures through a complex mechanism involving a functional change in vascular motility control. In addition, the functional role of NO in the neurotoxicity phenomenon caused by NMDA receptor activation during the epileptic disorder linked to cerebral blood flow (CBF) alteration was speculated (Penix et al., 1994; Rigaud-Monnet et al., 1994). In contrast, some disorders associated with the onset of vasodilation-related seizures tend to be avoided or delayed by NOS inhibitors in preliminary treatment (Bitterman et al., 1998). A rise in NOS activity was demonstrated of an experimental epilepsy model, obtained by int rahippocampal injection of kainic acid. This finding showed a significant link between NOS cerebral level and epileptiform intensity (Yasuda et al., 2001). All these research findings illustrate a functional involvement of NO in proconvulsant mechanisms in the CNS which indicate a potential significance in a new therapeutic approach (Lamas et al., 1998).

Even previous research regarding the role played by nitric oxide in epileptic seizures are far from solving the issue. These studies include varying and conflicting findings, depending on the model of seizure used and the concentration of bioactive substances applied.

3. MATERIALS AND METHODS

3.1. Ethical Approval

This study approval was granted by the Ondokuz Mayis University Ethical Committee for Animal Experiments (number: 2017/13, date: 31.03.2017). Experiments in this study were carried out on the basis of the rules and regulations (protocols) of the OMU Animal Research Ethics Committee.

3.2. Test Animals

180-220 gr Male Wistar Albino rats were obtained from the Ondokuz Mayis University Experimental Animal Application and Research Center in these studies and kept under control with the same conditions in the animal laboratory of Physiology Department. 84 Wistar Albino rats, aged 8-12 weeks, were used. At the center, animals were raised without limitation of food and water in the natural light-dark cycle.

3.3. Electrocorticogram (ECoG) recordings

The research was conducted in Ondokuz Mayis University, Faculty of Medicine Physiology Department. The rats, which were fasted one day before the surgery, were anesthetized with 1.25 g / kg urethane (intraperitoneal, i.p) in 25% solution and the scalp was opened with a length of 2-5 cm in the rostro-caudal direction. Bregma was identified after removing soft tissue on the somatomotor cortex.

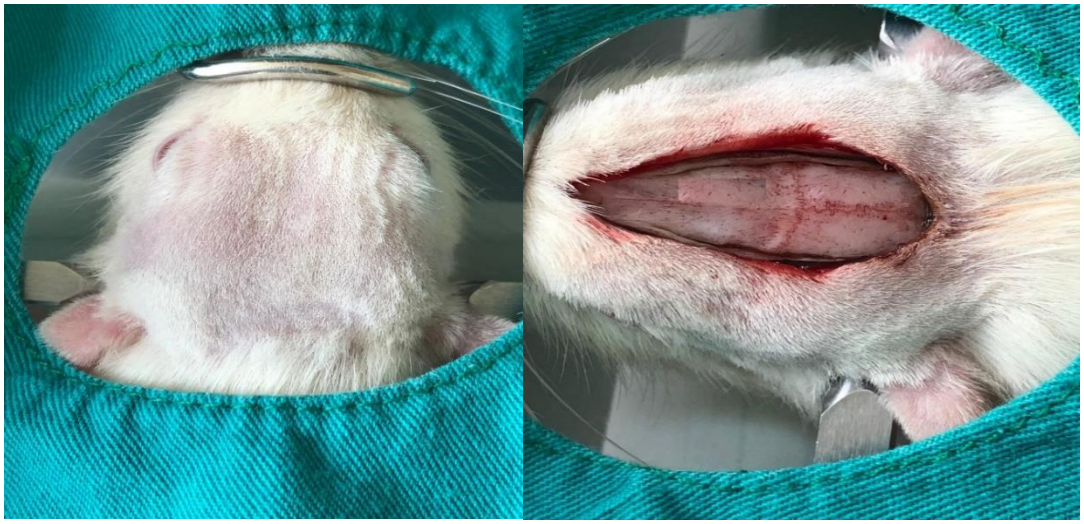


Figure 3.1. Removal of soft tissue from the skull

With the assistance of a hand drill, three holes with a diameter of 0.2 mm were opened for electrodes with a stereotaxic device (positive electrode, negative electrode and i.c. injection). Custom-made stainless-steel screws were installed in the openings. Screws were advanced 1 mm deep in vertical direction.

These screws were then connected to the Power lab data acquisition system by winding copper wires in the following coordinates:

1. Positive electrode of 4 mm anterior from bregma and 3 mm left lateral to the midline
2. Negative electrodes of 4 mm posterior from bregma and 3 mm left lateral to the midline
3. The ground electrode was wrapped around the mosquito clamp and then attached to the ear

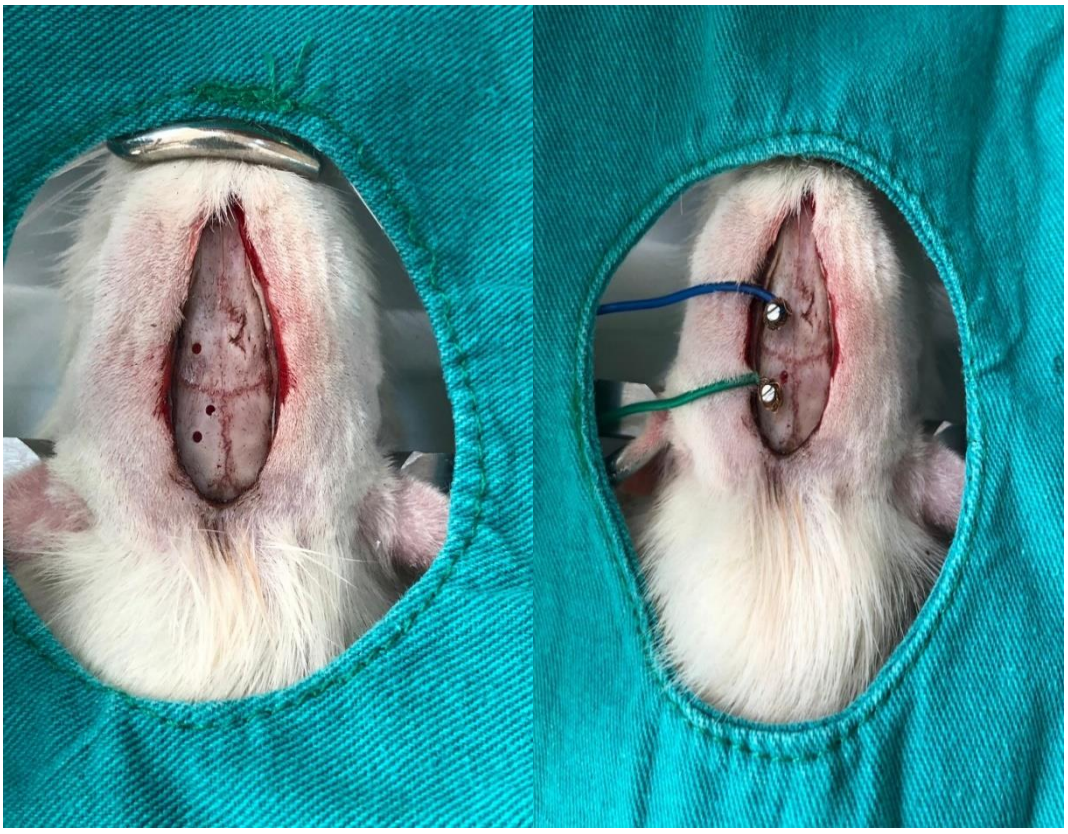


Figure 3.2. Removal of soft tissue from the skull

Figure 3.3. Power lab acquisition System

A third hole was opened for of 500 IU Penicillin-G injection in 1.5 mm caudal and lateral 1.5 mm from the bregma. Hamilton microinjector injection of Penicillin-G 500 IU into the cortex (i.c) in 2.5 microliters in a volume to generate epileptiform activity.

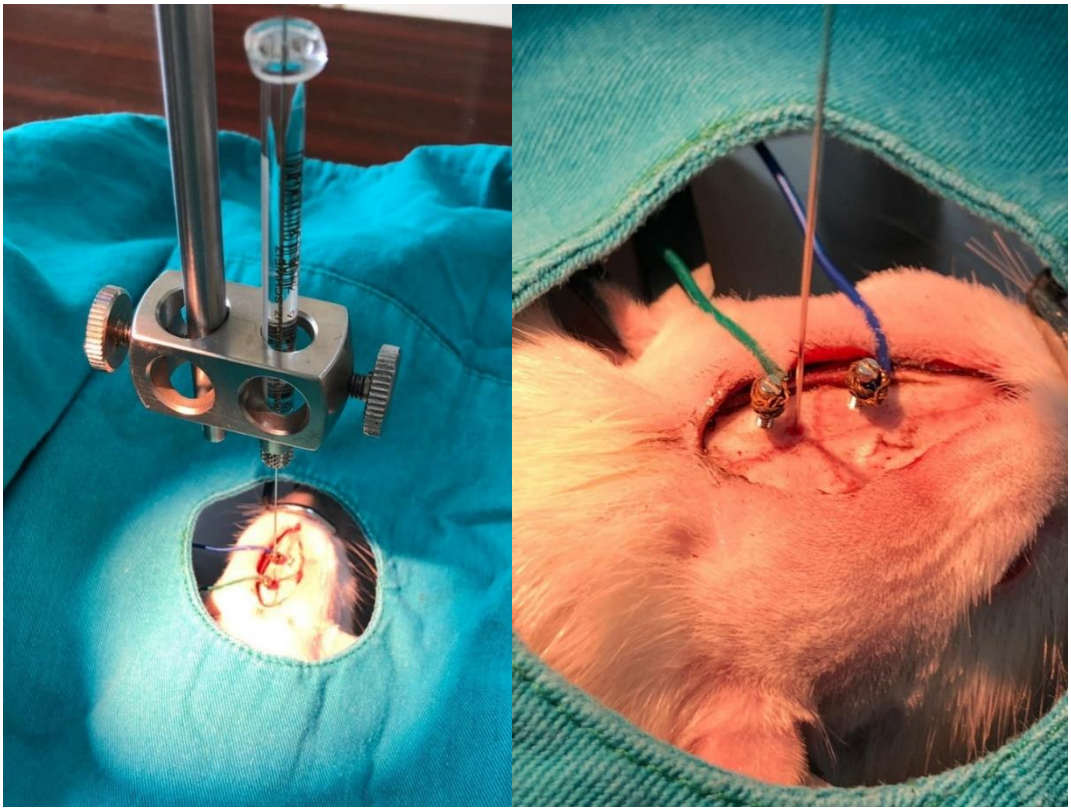


Figure 3.4. Injection of Penicillin G by Hamilton microinjector

With the Power Lab data acquisition system, the ECoG (electrocorticography) record, which was taken for 180 minutes was recorded online and was analyzed offline after the experiment was completed. After 30 min of epileptic activity, rats were given Resveratrol (25mg, 50mg and 100mg/kg), L-NAME (60mg/kg), 7-Nitroindazole (40 mg /kg), L-Arginine (500mg/kg), Aminoguanidine (100 mg / kg) intraperitoneally.

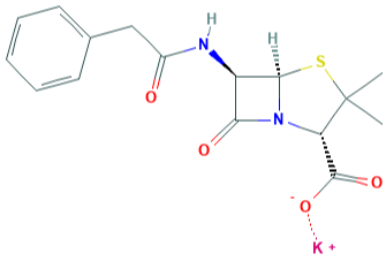
3.4. Applied Chemical Substances

Penicillin G Potassium

Molecular Formula: $C_{16}H_{17}KN_2O_4S$

Molecular Weight: 372.5 g/mol

Molecular Structure:



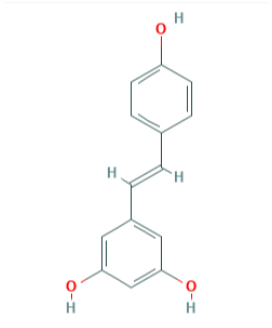
Application methods: 0.0670 g of Penicillin was weighed and dissolved in 0.5 ml of distilled water. A 2.5 µl volume of 500 IU Penicillin G injected in the cortex (i.c) with Hamilton microinjector.

Resveratrol

Molecular Formula: $C_{14}H_{12}O_3$

Molecular Weight: 228.24 g/mol

Molecular Structure:



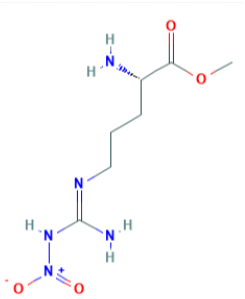
Application methods: after thirty minutes of Penicillin G injection, Resveratrol 25 mg/kg, 50 mg/kg and 100 mg/kg was given intraperitonially and DMSO was used as solvent.

N(gamma)-nitro-L-arginine Methyl Ester (L-NAME)

Molecular Formula: $C_7H_{15}N_5O_4$

Molecular Weight: 233.23 g/mol

Molecular Structure:



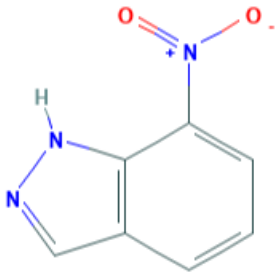
Application methods: DMSO was used to dissolve 60 mg/kg of L-NAME and intraperitonially applied after 30 minutes of Penicillin G in both single and with effective dose of Resveratrol (50mg/kg).

7-Nitroindazole

Molecular Formula: $C_7H_5N_3O_2$

Molecular Weight: 163.13 g/mol

Molecular Structure:



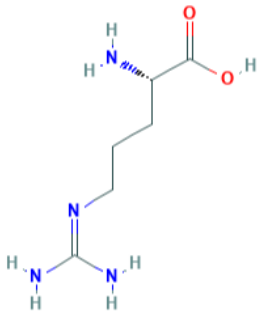
Application methods: 40 mg/kg of 7-Nitroindazole was dissolved in DMSO and applied intraperitonially after 30 minutes Penicillin G and in both single dose and effective dose of Resveratrol (50mg/kg)

L-Arginine

Molecular Formula: $C_6H_{14}N_4O_2$

Molecular Weight: 174.2 g/mol

Molecular Structure:



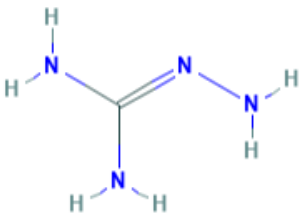
Application methods: L-Arginine 500 mg/kg was applied after 30 minutes of Penicillin G in both single dose and effective dose of Resveratrol (50mg/kg) intraperitonially and dissolved in saline.

Aminoguanidine

Molecular Formula: CH_6N_4

Molecular Weight: 74.09 g/mol

Molecular Structure:



Application methods: Aminoguanidine 100 mg/kg was dissolved in DMSO and applied intraperitoneally after 30 minutes of Penicillin G in a single dose and with Resveratrol (50mg/kg).

3.5. Experimental Animal Groups

1. **Control Group:** Baseline activity was recorded without the administration of any substance for 180 minutes (n= 6)
2. **Penicillin (500 units) group:** Penicillin G was injected intracortically and ECoG activity was recorded for 180 minutes (n= 6).
3. **DMSO (RSV Solvent) group:** 0.25 ml of Dimethyl sulfoxide (DMSO) was administered intraperitoneally and ECoG was recorded for 180 minutes (n= 6).
4. **Penicillin (500 IU i.c) + Resveratrol (25 mg/kg i.p) group:** After 5 minutes of base line recording, Penicillin was administered intracortically and injected after 30 minutes after Penicillin G injection, Resveratrol 25mg/kg was administered intraperitonially (n= 6).
5. **Penicillin (500 IU i.c) + Resveratrol (50 mg / kg i.p) group:** After 5 minutes of base line recording, Penicillin was administered intracortically and injected 30 minutes after Penicillin G injection, Resveratrol 50mg/kg was administered intraperitonially (n= 6).
6. **Penicillin (500 IU i.c) + Resveratrol (100 mg / kg i.p) group:** 5 minutes After base line recording, Penicillin was administered intracortically and 30 minutes after Penicillin G injection, Resveratrol 50mg/kg was administered intraperitonially (n= 6).

   Among the experimental groups above, Resveratrol 25 mg/kg and 100 mg /kg did not effect in both spike frequency and spike amplitude while Resveratrol 50 mg/kg decrease significantly in spike frequency and thus it considered as effective dose.
7. **Penicillin (500 IU i.c) + L-NAME (60 mg /kg, i.p) group:** L-NAME 60 mg/kg was administered intraperitoneally after 30 min of Penicillin injection and ECoG was recorded for 180 minutes (n= 6).
8. **Penicillin (500 IU i.c) + Resveratrol (50 mg/kg, i.p.) + L-NAME (60 mg / kg, i.p) group:** 30 minutes after Penicillin G intracortical injection, Resveratrol 50mg/kg was applied intraperitoneally and 5 minutes after that L-NAME 60mg/kg was administered intraperitonially and ECoG was recorded for 180 minutes (n= 6).

9. **Penicillin (500 IU i.c) + L-Arginine (500 mg / kg, i.p) group:** 30 minutes after Penicillin injection, L- Arginine 500 mg/kg were applied intraperitonially and ECoG was recorded for 180 minutes (n= 6).
10. **Penicillin (500 IU i.c) + Resveratrol (50 mg/kg, i.p.) + L-Arginine (500 mg / kg, i.p) group:** 30 minutes after Penicillin G intracortical injection, Resveratrol 50mg/kg was applied intraperitoneally and 5 minutes after that L-Arginine 500mg/kg was administered intraperitonially and ECoG was recorded for 180 minutes (n= 6).
11. **Penicillin (500 IU i.c) + Aminoguanidine (100 mg / kg, i.p.) group:** Aminoguanidine 100 mg/kg was administered intraperitonially 30 minutes after Penicillin injection, and the data was recorded for 180 minutes (n= 6).
12. **Penicillin (500 IU i.c) + Resveratrol (50mg/kg, i.p.) + Aminoguanidine (100 mg / kg, i.p.) group:** after 30 minutes of Penicillin G is injected intracortically, Resveratrol 50mg/kg was applied intraperitoneally and 5 minutes after that Aminoguanidine 100mg/kg was administered intraperitonially and ECoG was recorded for 180 minutes (n= 6).
13. **Penicillin (500 IU i.c) + 7-Nitroindazole (40 mg / kg, i.p.) group:** Penicillin was administered intracortically after the base line recording, 30 minutes later 7-NI 40 mg/kg was applied intraperitoneally and ECoG was recorded for 180 minutes (n= 6).
14. **Penicillin (500 IU i.c) + Resveratrol (50 mg/kg, i.p.) + 7-Nitroindazole (40 mg / kg, i.p.) group:** after 30 minutes of Penicillin G is injected intracortically, Resveratrol 50mg/kg was applied intraperitoneally and 5 minutes after that 7-Nitroindazole 40mg/kg was administered intraperitonially and ECoG was recorded for 180 minutes (n= 6).

3.1. Evaluation of electrophysiological recordings

Basal activity recordings of all rats were taken for 5 minutes and intracortical Penicillin G injection was performed. Epileptiform activity appeared in the ECoG within 2-4 minutes after injection of Penicillin (500 IU, 2.5 µl, i.c). Within 20-30 minutes, the frequency and amplitude of epileptiform activity was stabilized, which lasted 3-4 hours. After Penicillin injection, the first substance was administered 30 minutes later. After the last substance was injected, the recording was taken for 2.5 hours and then was terminated. ECoG recordings were recorded in Power Lab data acquisition system and stored for offline analysis on the PC.

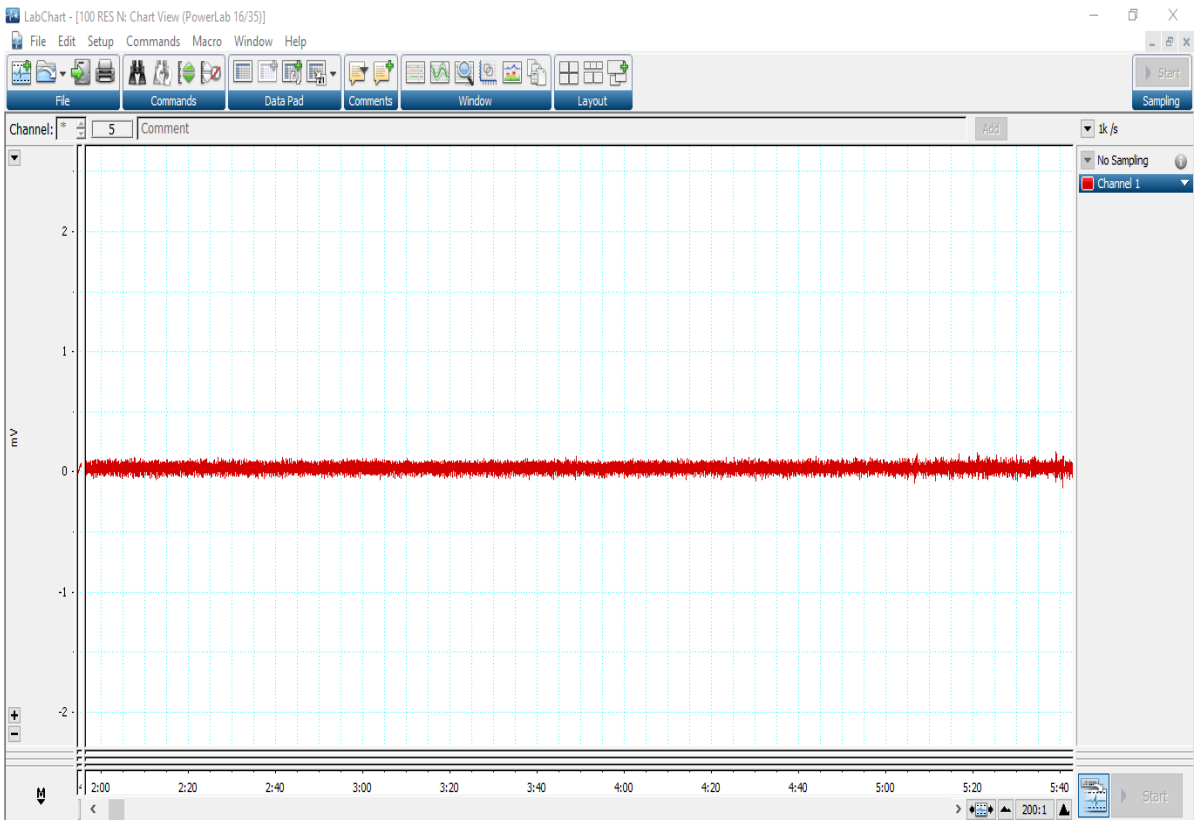


Figure 3.5. Basal activity of ECoG recording before Penicillin G injection

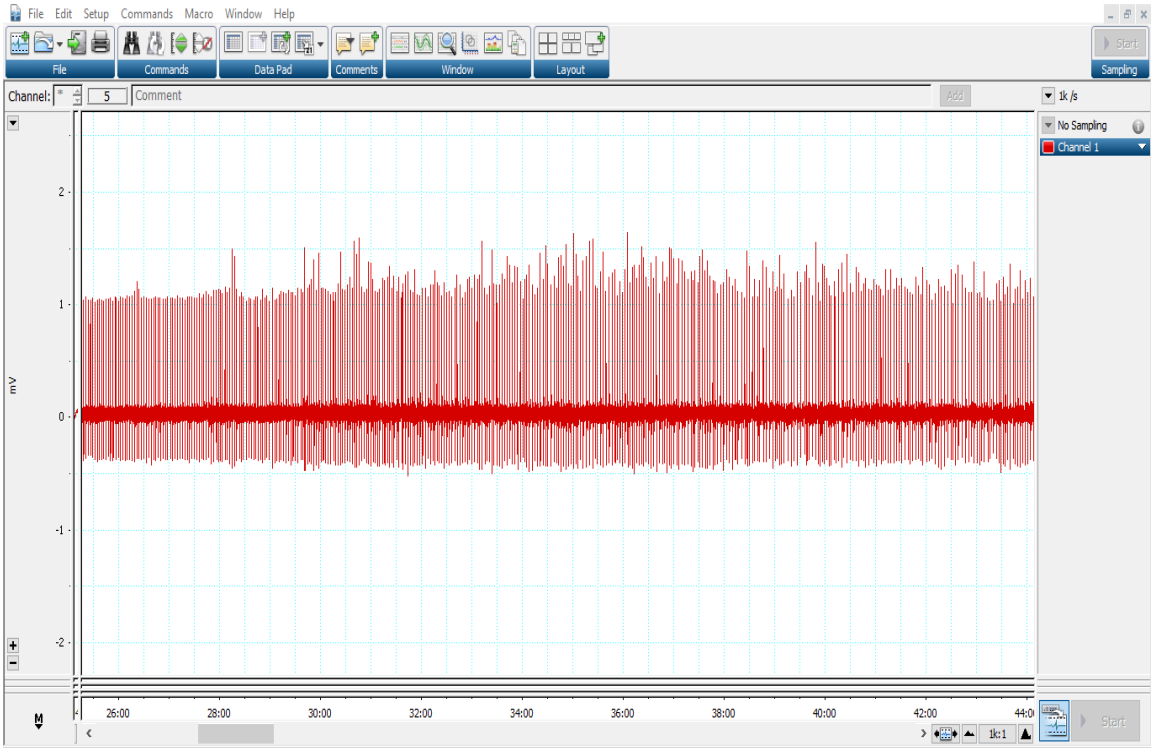


Figure 3.6. Epileptiform activity of ECoG recording after Penicillin G injection

The epileptiform activity obtained from experiments were analyzed offline for spike frequency and amplitude. ECoG recordings are divided into one-minute segments using Chart v7.0.3 (AD Instruments, Australia) software and its features. The average spike amplitudes and number of spikes per minute were automatically calculated with the help of this software. For the recordings acquired from all animals used in the experiment, this calculation was made individually.

3.6. Statistical Analysis

Following all electrophysiological recordings, the data were converted into numerical and then were evaluated statistically using the Statistical Package for Social Sciences (SPSS) 17.0 software. One-way analysis of variance (ANOVA) was used after the data were found to fit normal distribution. Post-Hoc Tukey test was used to determine differences between the groups (One-Way Anova Post-Hoc Tukey Test). The values of the experimental groups used in graphics and text were expressed as mean ± standard error (SEM). According to the results obtained from the tests, significant differences were considered as those with p values less than 0.05.

4. RESULTS AND DISCUSION

4.1.RESULTS

In this research, the effect of different doses of Resveratrol in penicillin-induced experimental epilepsy model were examined and its most the most effective dose was determined. Furthermore, the interaction between Resveratrol and nitric oxide on penicillin-induced epileptiform activity was investigated. The effect of studied substances (Resveratrol, Nitric Oxide agonists and antagonists) was calculated separately for each dose on spike frequency and spike amplitude, and the mean ± standard error (SEM) values were determined and statistically analyzed.

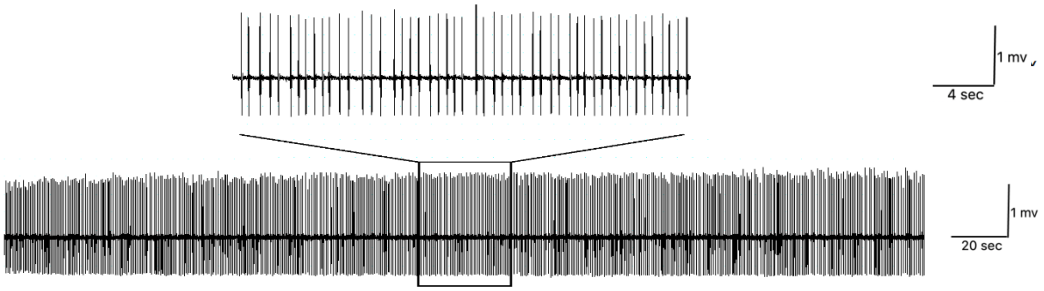
4.1.1. Penicillin epileptiform activity

In present study, epileptiform activity produced by intracortical administration of Penicillin occurred within 2-5 minutes of injection. Spike amplitudes and frequencies of epileptiform activity achieved stable level at 20-30 minutes after injection and proceed 3 hours after injection. The overall period of ECoG recording is 180 minutes from Penicillin injection in the experiments, the first 30 minutes of this period is when the epileptiform activity produced by Penicillin reaches a stable level. The remaining minutes are the period during which the items used in the experiment are evaluated.

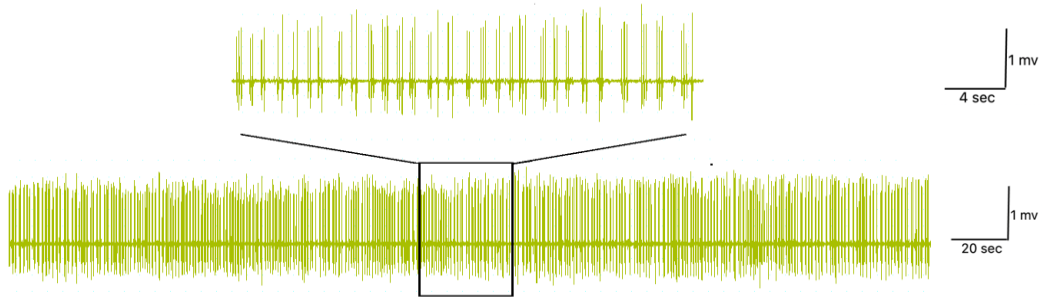
There was no noticeable difference in terms of the spike frequency and amplitude of epileptiform activity recorded between the experimental groups within 30 minutes of Penicillin injection ($p>0.05$)

Only Penicillin G (500 IU, i.c.) was given for the Penicillin group. For the DMSO group, DMSO was given intraperitoneally after 30 minutes of Penicillin G (500 IU, i.c.) administration. When the control groups (Penicillin and DMSO) were compared between each other, there was no statistically significant difference in terms of spike number and amplitude values of epileptiform activity ($p>0.05$). Basal activity is observed in the records obtained from Resveratrol 50 mg/kg (effective dose) group administered alone without Penicillin administration. Since there is no spike activity (spike), amplitude or frequency cannot be mentioned in this group. (Figure 4.1-4.3, Table 4.1, 4.2).

A) Penicillin G 500 IU



B) DMSO



C) 50 mg/kg Resveratrol

Figure 4.1. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU, B) DMSO, C) Resveratrol 50 mg/kg

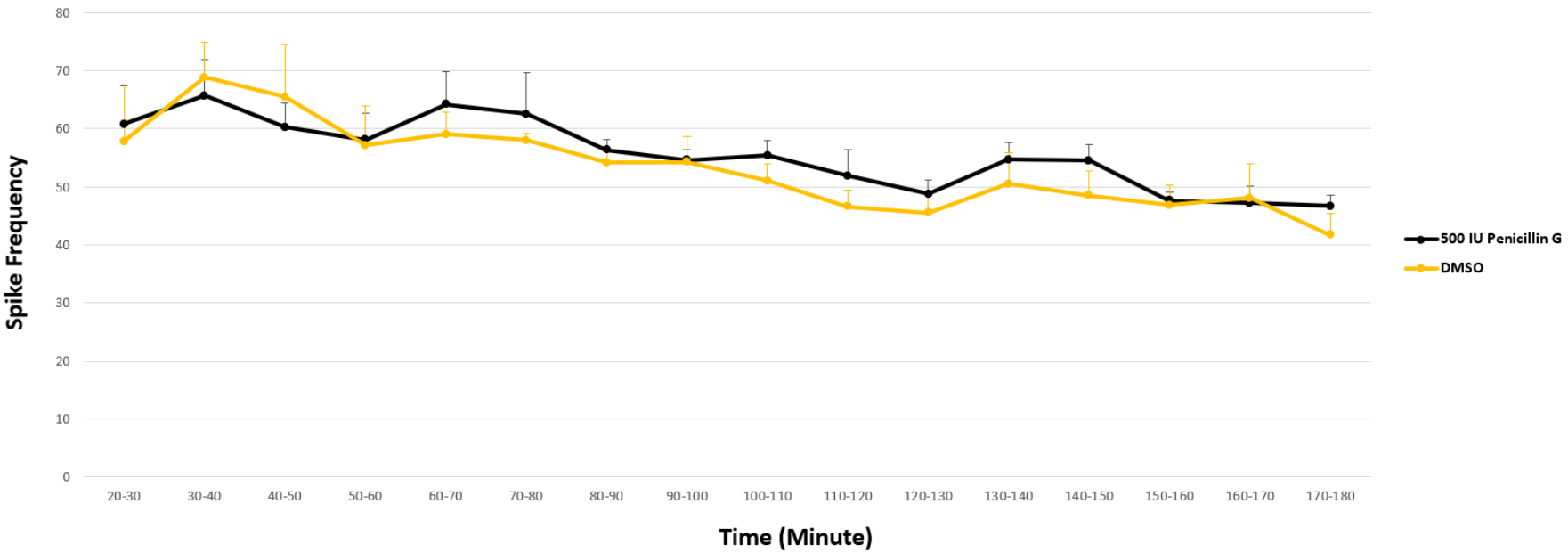


Figure 4.2. Effects of DMSO on spike frequency after 30 min Penicillin injection. Mean spike number in 10 min ± SEM

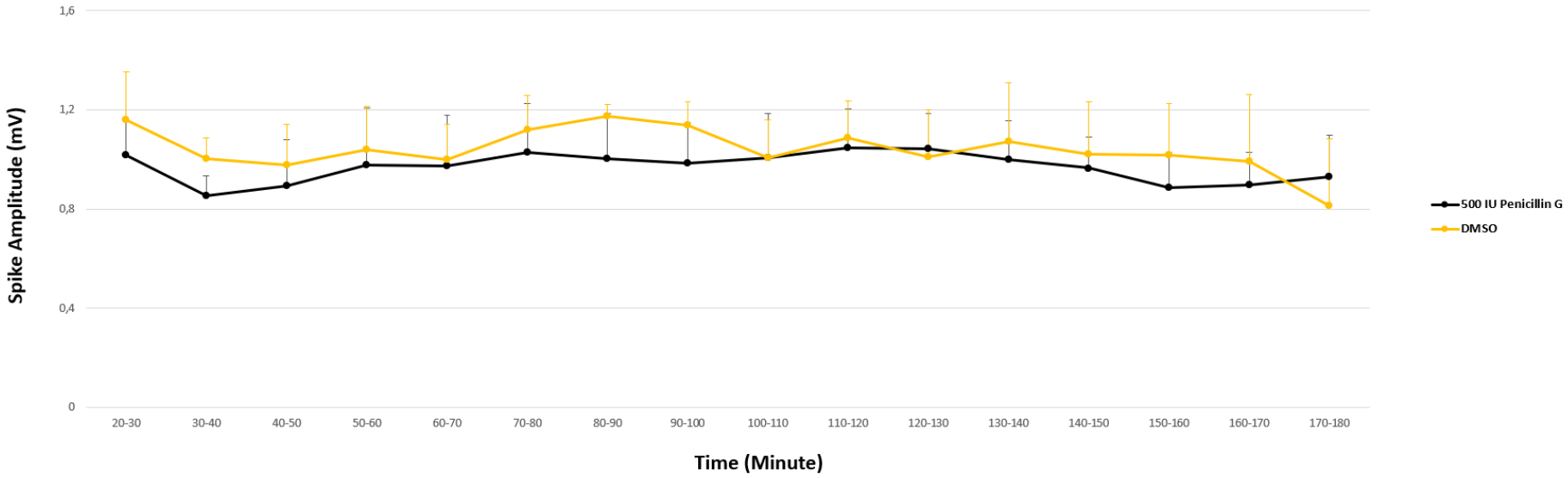


Figure 4.3. Effects of DMSO on spike amplitude after 30 min Penicillin injection. Mean spike amplitude in 10 min ± SEM

Table 4.1. Mean values of spike frequencies obtained from penicillin, 500 IU and DMSO groups in every 10 minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	DMSO (Mean ± SEM)
20-30 min	60,8 ± 6,6	57,9 ± 9,6
30-40 min	65,7 ± 6,3	68,9 ± 6,1
40-50 min	60,3 ± 4,2	65,5 ± 9,1
50-60 min	58,1 ± 4,7	57,1 ± 6,8
60-70 min	64,2 ± 5,6	59 ± 3,8
70-80 min	62,6 ± 7,2	58,1 ± 1,2
80-90 min	56,4 ± 1,7	54,2 ± 2,3
90-100 min	54,6 ± 1,8	54,3 ± 4,4
100-110 min	55,4 ± 2,5	51 ± 3
110-120 min	51,9 ± 4,5	46,6 ± 2,9
120-130 min	48,8 ± 2,4	45,6 ± 2,9
130-140 min	54,7 ± 2,9	50,5 ± 5,4
140-150 min	54,6 ± 2,7	48,5 ± 4,3
150-160 min	47,6 ± 1,4	46,9 ± 3,5
160-170 min	47,2 ± 2,9	48,1 ± 5,9
170-180 min	46,7 ± 1,8	41,7 ± 3,7

.

Table 4.2. Mean values of amplitude obtained from penicillin, 500 IU and DMSO groups in every 10 minutes interval (mean spike number ± SEM)

Time	500 IU Penicillin G (Mean ± SEM)	DMSO (Mean ± SEM)
20-30 min	1,02 ± 0,14	1,16 ± 0,19
30-40 min	0,85 ± 0,08	1 ± 0,08
40-50 min	0,89 ± 0,19	0,98 ± 0,16
50-60 min	0,98 ± 0,23	1,04 ± 0,18
60-70 min	0,98 ± 0,2	1 ± 0,14
70-80 min	1,03 ± 0,2	1,12 ± 0,14
80-90 min	1 ± 0,18	1,17 ± 0,05
90-100 min	0,98 ± 0,17	1,14 ± 0,1
100-110 min	1,01 ± 0,18	1,01 ± 0,15
110-120 min	1,05 ± 0,16	1,09 ± 0,15
120-130 min	1,04 ± 0,14	1,01 ± 0,19
130-140 min	1 ± 0,16	1,07 ± 0,24
140-150 min	0,96 ± 0,13	1,02 ± 0,21
150-160 min	0,89 ± 0,14	1,02 ± 0,21
160-170 min	0,9 ± 0,13	0,99 ± 0,27
170-180 min	0,93 ± 0,17	0,81 ± 0,27

4.1.2. Effect of Resveratrol on epileptiform activity

After 30 minutes of Penicillin injection (500 IU intracortically), three separate experimental group were created and epileptiform activity were examined in each group by applying Resveratrol at dose of 25, 50 and 100 mg/kg intraperitoneally.

4.1.2.1. Effect of Resveratrol at 25 mg/kg dose

After 30 minutes of Penicillin injection, Resveratrol 25 mg/kg was administered intraperitoneally. There was no statistically significant change in both spike frequency and spike amplitude during whole study ($p>0.05$) (Figure 4.4-4.6, Table 4.3, 4.4).

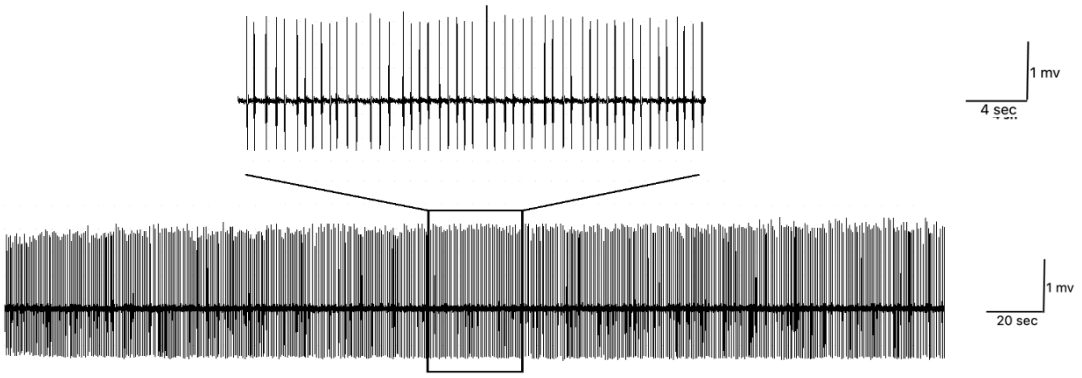
4.1.2.2. Effect of Resveratrol at 50 mg/kg dose

The 50 mg/kg dose of Resveratrol given 30 minutes after Penicillin injection, decreased spike frequency significantly starting at 100 minutes till the end of the recording ($p<0.05$) and we considered 50 mg/kg as effective dose. Most reduction was significantly between 130-150 minutes ($p<0.01$); and 150-170 minutes was considered highly significant ($p<0.001$). During the 180 minutes recording period, there were no statistically significant difference in spike amplitude compared to penicillin group ($p>0.05$) (Figure 4.4-4.6, Table 4.3, 4.4).

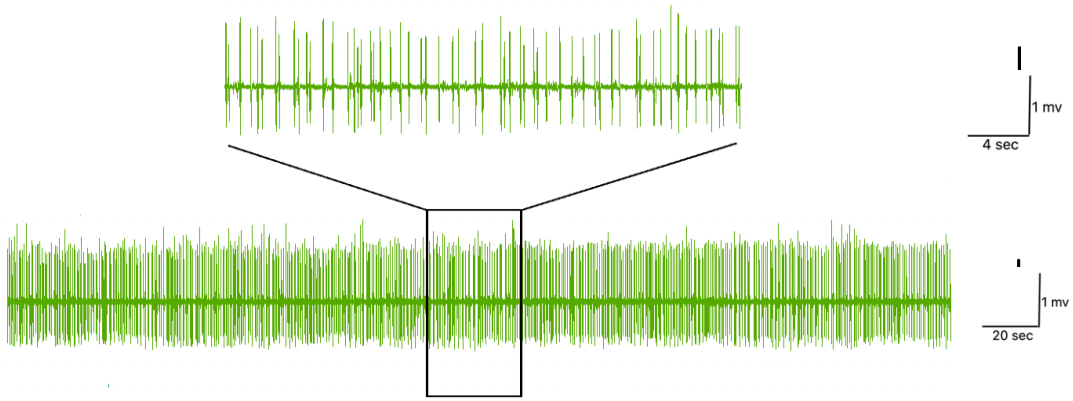
4.1.2.3. Effect of Resveratrol at 100 mg /kg dose

After 30 minute of Penicillin injection intracortically, Resveratrol 100 mg/kg was injected intraperitoneally. There was no significant change in both spike frequency and spike amplitude compared to penicillin group ($p>0.05$) (Figure 4.4-4.6, Table 4.3, 4.4).

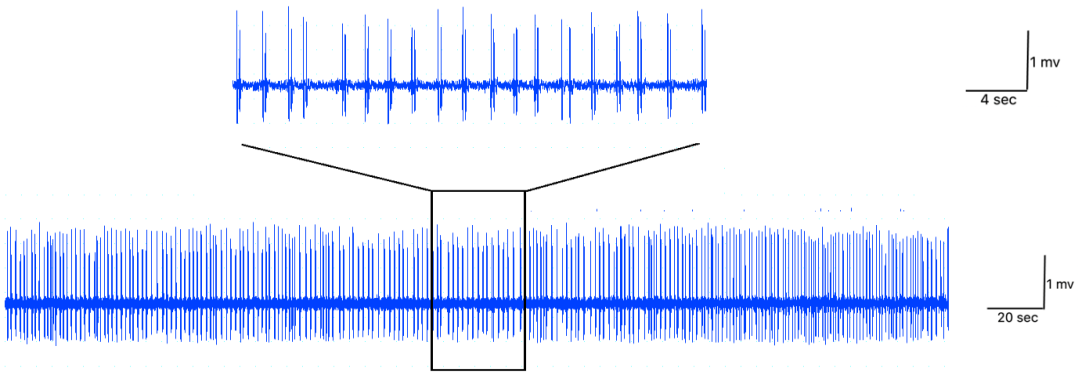
A) Penicillin G 500 IU



B) Resveratrol 25 mg/kg



C) Resveratrol 50mg/kg



D) Resveratrol 100 mg/kg

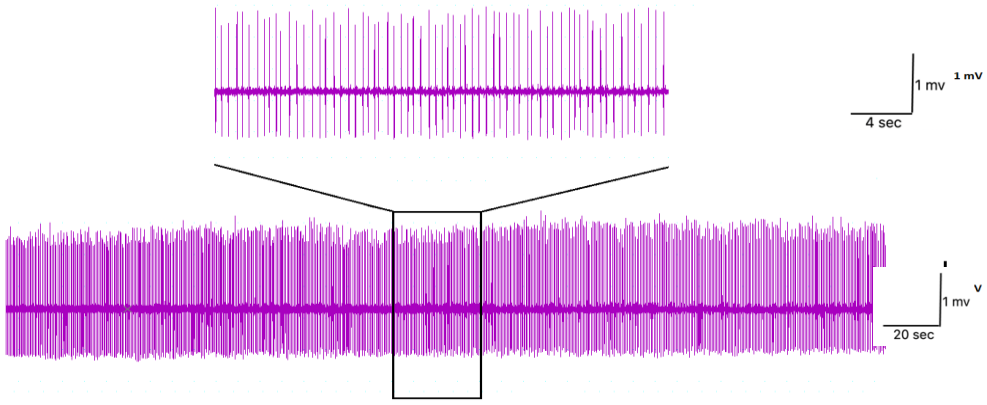


Figure 4.4. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU. B) Resveratrol 25 mg/kg + Penicillin G 500 IU C) Resveratrol 50 mg/kg + Penicillin G 500 IU. D) Resveratrol 100 mg/kg + Penicillin G 500 IU Sample images from ECoG recoding

Table 4.3. Mean values of spike frequencies obtained from penicillin G 500 IU and Resveratrol (25 mg/kg, 50 mg/kg, 100 mg/kg) groups in every 10 minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	25 mg Resveratrol (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	100 mg Resveratrol (Mean ± SEM)
20-30 min	60,8 ± 6,6	68,5 ± 8,1	60,2 ± 5,4	57,3 ± 4,8
30-40 min	65,7 ± 6,3	69,7 ± 5,6	58,9 ± 5,1	49,3 ± 1,5
40-50 min	60,3 ± 4,2	62 ± 8,4	52,9 ± 5,3	55,8 ± 3,9
50-60 min	58,1 ± 4,7	64,1 ± 4,5	54 ± 7,9	57 ± 3,8
60-70 min	64,2 ± 5,6	67,9 ± 4,7	60,8 ± 7,6	54,1 ± 6,9
70-80 min	62,6 ± 7,2	66,4 ± 3,5	57,6 ± 6,3	64,6 ± 3,9
80-90 min	56,4 ± 1,7	59,3 ± 5,7	45,4 ± 4,6	58,3 ± 3,8
90-100 min	54,6 ± 1,8	54,3 ± 5	44,2 ± 3,5	62,8 ± 7,7
100-110 min	55,4 ± 2,5	56,9 ± 4,2	43 ± 2,8	65,2 ± 1,2
110-120 min	51,9 ± 4,5	60,7 ± 2,1	37,6 ± 2,3	61 ± 3
120-130 min	48,8 ± 2,4	50,8 ± 2,4	39,1 ± 1,4	58,6 ± 3
130-140 min	54,7 ± 2,9	53,2 ± 4,4	35,7 ± 2,5	59,7 ± 3,5
140-150 min	54,6 ± 2,7	48,7 ± 2,7	34,4 ± 2,1	51,5 ± 4,5
150-160 min	47,6 ± 1,4	47,1 ± 2,9	30,7 ± 1,5	54,1 ± 3,5
160-170 min	47,2 ± 2,9	45,2 ± 1,9	26,9 ± 2	49,1 ± 3,5
170-180 min	46,7 ± 1,8	42,3 ± 1,8	37,3 ± 1,7	50,2 ± 3,2

Table 4.4. Mean values of amplitude obtained from penicillin G 500 IU and Resveratrol (25 mg/kg, 50 mg/kg, 100 mg/kg) groups in every 10 minutes interval (mean spike amplitude ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	25 mg Resveratrol (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	100 mg Resveratrol (Mean ± SEM)
20-30 dk	1,02 ± 0,14	1,22 ± 0,33	0,99 ± 0,24	1,25 ± 0,47
30-40 dk	0,85 ± 0,08	1,08 ± 0,25	1,02 ± 0,3	1,09 ± 0,3
40-50 dk	0,89 ± 0,19	0,95 ± 0,18	1,05 ± 0,32	1,04 ± 0,39
50-60 dk	0,98 ± 0,23	0,84 ± 0,06	1,05 ± 0,3	0,97 ± 0,36
60-70 dk	0,98 ± 0,2	0,8 ± 0,03	0,98 ± 0,18	0,95 ± 0,37
70-80 dk	1,03 ± 0,2	0,88 ± 0,11	0,88 ± 0,03	0,8 ± 0,29
80-90 dk	1 ± 0,18	0,85 ± 0,08	0,86 ± 0,01	0,83 ± 0,25
90-100 dk	0,98 ± 0,17	0,75 ± 0,03	0,82 ± 0,02	0,78 ± 0,23
100-110 dk	1,01 ± 0,18	0,68 ± 0,04	0,78 ± 0,02	0,72 ± 0,21
110-120 dk	1,05 ± 0,16	0,69 ± 0,08	0,77 ± 0,06	0,77 ± 0,23
120-130 dk	1,04 ± 0,14	0,76 ± 0,04	0,75 ± 0,04	0,74 ± 0,19
130-140 dk	1 ± 0,16	0,75 ± 0,01	0,72 ± 0,02	0,71 ± 0,12
140-150 dk	0,96 ± 0,13	0,82 ± 0,05	0,69 ± 0,01	0,75 ± 0,1
150-160 dk	0,89 ± 0,14	0,79 ± 0,05	0,67 ± 0,05	0,74 ± 0,002
160-170 dk	0,9 ± 0,13	0,83 ± 0,001	0,65 ± 0,08	0,73 ± 0,0002
170-180 dk	0,93 ± 0,17	0,72 ± 0,03	0,65 ± 0,05	0,74 ± 0,01

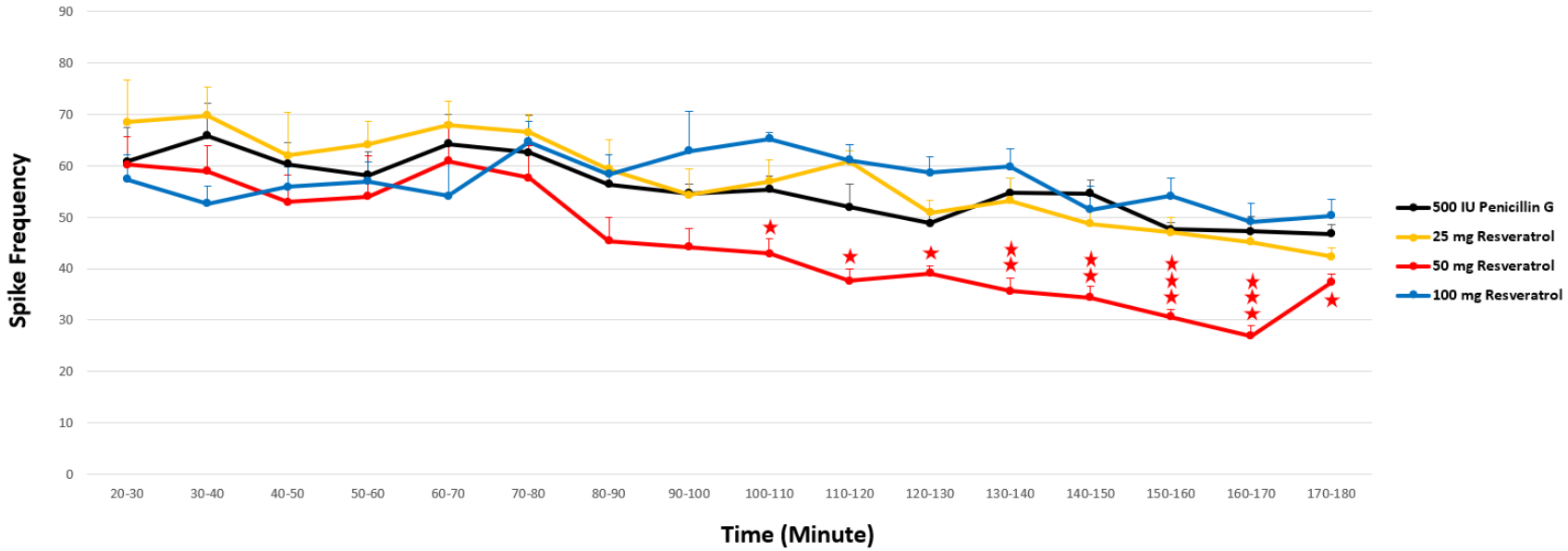


Figure 4.5. Results of various Resveratrol dosages on spike frequency 30 min after injection of Penicillin. Mean spike number in 10 min ± SEM (★=p<0.05, ★★=p<0.01, ★★★=p<0.001).

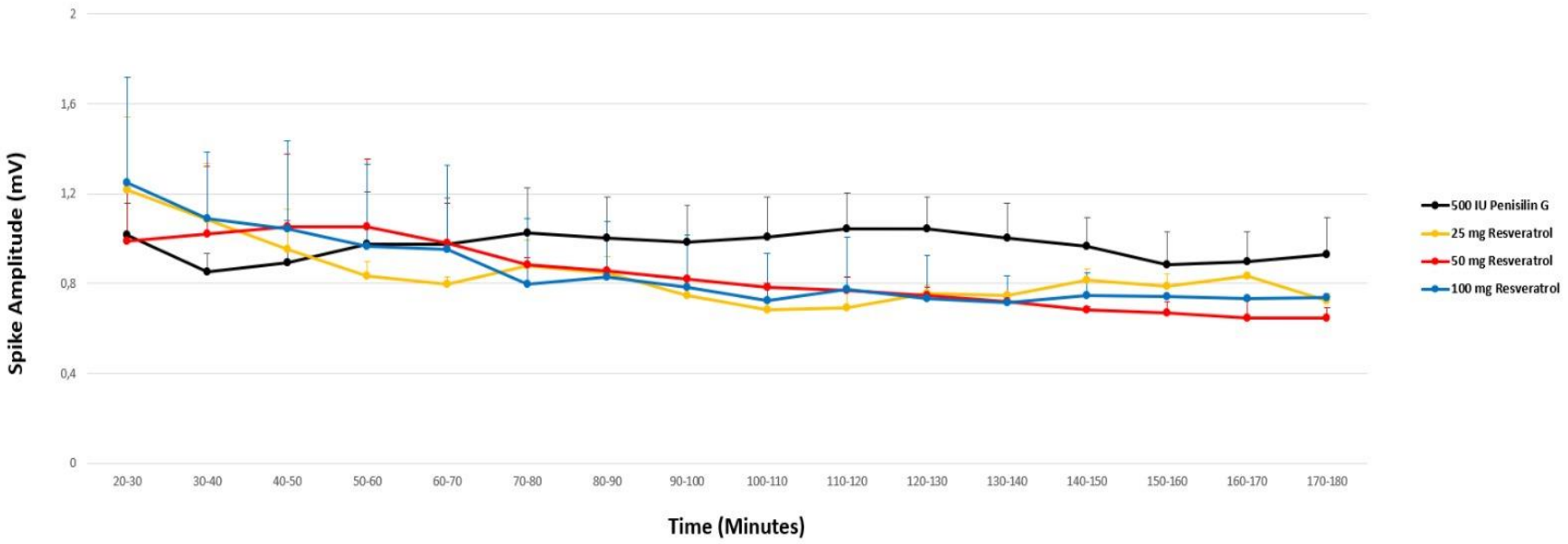


Figure 4.6. Effects of various doses of Resveratrol on spike amplitude 30 min after injection of Penicillin (mean spike amplitude ± SE).

4.1.3. Effect of interaction between Resveratrol and nitric oxide on epileptiform activity

In this research, we examined the relationship between NO and Resveratrol using the experimental epilepsy model produced with penicillin. 30 minutes after administration of penicillin, L-NAME 60 mg/kg, L-Arginine 500 mg/kg, Aminoguanidine 100 mg/kg and 7-Nitroindazole 40 mg/kg was applied intraperitoneally alone and with Resveratrol 50 mg/kg, in each with different experimental groups.

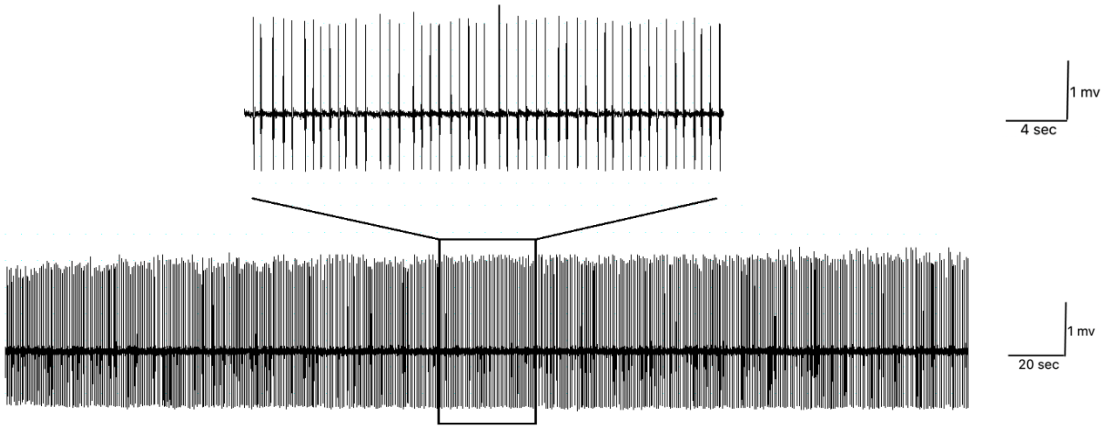
4.1.3.1. Effect of 7 Nitroindazole on epileptiform activity

7 Nitroindazole 40 mg/kg was applied 30 min after Penicillin G 500 IU intracortical injection, the spike frequency was reduced significantly at 60 minutes till the end of the recording period (180 minutes) ($p<0.05$) (Figure 4.7-4.9, Table 4.5, 4.6). This decrease is significant between in 60 -70, 110 -140. and 150-170 minutes ($p<0.01$); and highly significant between 100 -110, 140-150 and 170-180 minutes ($p<0.001$). In comparison to the penicillin group, there is no significant difference in spike amplitude ($p>0.05$).

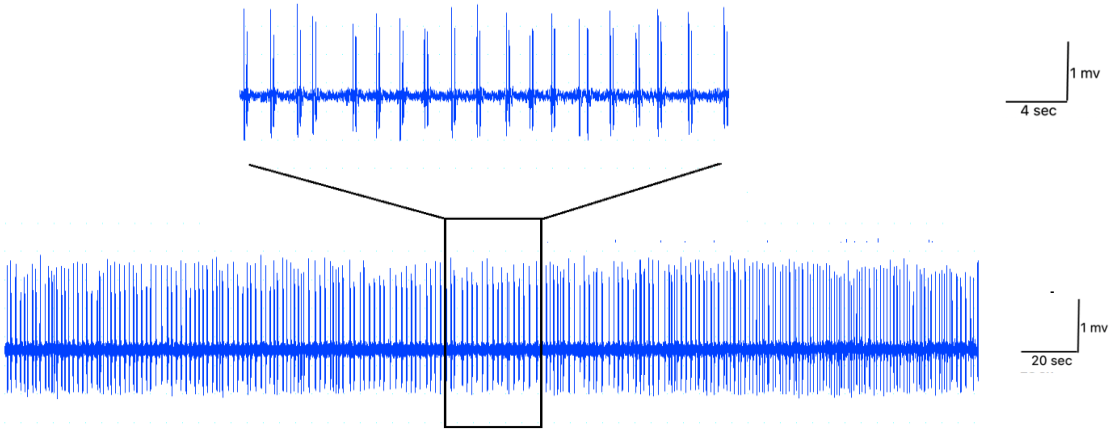
4.1.3.2. Effect of 7 Nitroindazole 40 mg/kg and effective dose of Resveratrol 50 mg/kg on epileptiform activity

Thirty minutes after Penicillin G 500 IU intracortical application, the effective dose of Resveratrol 50 mg/kg and 40 mg/kg 7-NI was administered intraperitoneally. The spike frequency decreased significantly at 60 minutes till 180 minutes ($p<0.5$) (Figure 4.7-4.9, Table 4.5, 4.6). This decrease became highly significant between 60-90 minutes ($p<0.01$); and become highly significantly in between 100-180 minutes ($p<0.001$). In comparison to the penicillin group, there no significant difference in spike amplitude

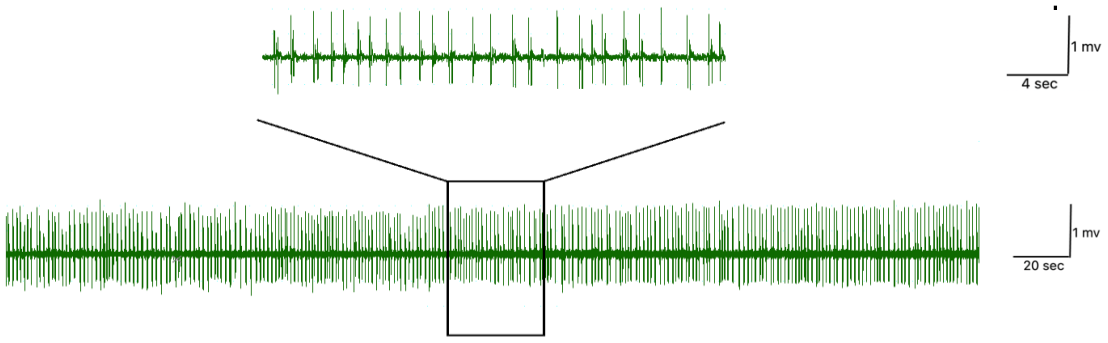
A) Penicillin G 500 IU



B) Resveratrol 50 mg/kg



C) 7 Nitroindazole 40 mg/kg



D) Resveratrol 50mg/kg + 7 NI 40 mg/kg

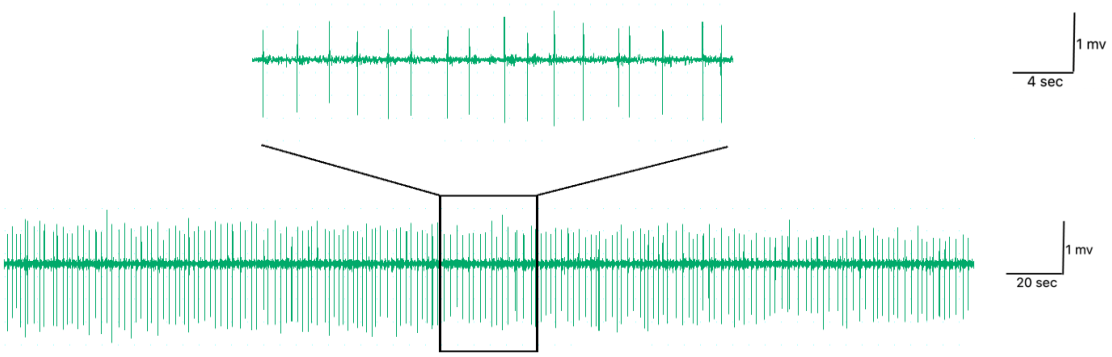


Figure 4.7. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU. B) Penicillin G 500 IU + resveratrol 50 mg/kg C) Penicillin G 500 IU + 7 NI 40 mg/kg. D) Penicillin G 500 IU + Resveratrol 50 mg/kg + 7 NI 40 mg/kg.

Table 4.5. Mean values of spike frequencies obtained from penicillin G 500 IU, Resveratrol 50 mg/kg and 7 Nitroindazole 40 mg/kg in every 10minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	40 mg 7-NI (Mean ± SEM)	Resveratrol + 7-NI (Mean ± SEM)
20-30 min	60,8 ± 6,6	60,2 ± 5,4	58,7 ± 4	51,8 ± 6,3
30-40 min	65,7 ± 6,3	58,9 ± 5,1	48 ± 5,5	50,3 ± 2,2
40-50 min	60,3 ± 4,2	52,9 ± 5,3	46,1 ± 6,3	47,5 ± 5,2
50-60 min	58,1 ± 4,7	54 ± 7,9	40,4 ± 3,7	42,3 ± 6,6
60-70 min	64,2 ± 5,6	60,8 ± 7,6	35,6 ± 2,9	33,4 ± 3,2
70-80 min	62,6 ± 7,2	57,6 ± 6,3	37,9 ± 3,5	33 ± 1,2
80-90 min	56,4 ± 1,7	45,4 ± 4,6	37,7 ± 4,4	33 ± 1,6
90-100 min	54,6 ± 1,8	44,2 ± 3,5	36,4 ± 6,2	34,9 ± 2,1
100-110 min	55,4 ± 2,5	43 ± 2,8	34,9 ± 3,2	31,3 ± 1,4
110-120 min	51,9 ± 4,5	37,6 ± 2,3	31,3 ± 3,1	25,7 ± 2,4
120-130 min	48,8 ± 2,4	39,1 ± 1,4	37,1 ± 2,5	21,5 ± 1,8
130-140 min	54,7 ± 2,9	35,7 ± 2,5	33 ± 2,9	21,3 ± 3,8
140-150 min	54,6 ± 2,7	34,4 ± 2,1	29,4 ± 1,8	23,9 ± 2,7
150-160 min	47,6 ± 1,4	30,7 ± 1,5	26,2 ± 4,7	20,1 ± 3,6
160-170 min	47,2 ± 2,9	26,9 ± 2	30,8 ± 3,5	14,1 ± 2,3
170-180 min	46,7 ± 1,8	37,3 ± 1,7	27,8 ± 1,5	9 ± 2,1

Table 4.6. Mean values of amplitude obtained from penicillin G 500 IU, Resveratrol 50 mg/kg and 7 Nitroindazole 40 mg/kg in every 10minutes interval (mean spike amplitude ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	40 mg 7-NI (Mean ± SEM)	Resveratrol + 7-NI (Mean ± SEM)
20-30 min	1,02 ± 0,14	0,99 ± 0,24	1,37 ± 0,5	0,97 ± 0,3
30-40 min	0,85 ± 0,08	1,02 ± 0,3	1,09 ± 0,47	0,9 ± 0,41
40-50 min	0,89 ± 0,19	1,05 ± 0,32	0,98 ± 0,32	0,97 ± 0,26
50-60 min	0,98 ± 0,23	1,05 ± 0,3	0,87 ± 0,14	0,79 ± 0,26
60-70 min	0,98 ± 0,2	0,98 ± 0,18	0,86 ± 0,14	0,71 ± 0,23
70-80 min	1,03 ± 0,2	0,88 ± 0,03	0,84 ± 0,1	0,75 ± 0,29
80-90 min	1 ± 0,18	0,86 ± 0,01	0,79 ± 0,08	0,82 ± 0,22
90-100 min	0,98 ± 0,17	0,82 ± 0,02	0,79 ± 0,1	0,8 ± 0,16
100-110 min	1,01 ± 0,18	0,78 ± 0,02	0,85 ± 0,16	0,76 ± 0,07
110-120 min	1,05 ± 0,16	0,77 ± 0,06	0,87 ± 0,26	0,75 ± 0,06
120-130 min	1,04 ± 0,14	0,75 ± 0,04	0,85 ± 0,3	0,8 ± 0,03
130-140 min	1 ± 0,16	0,72 ± 0,02	0,78 ± 0,26	0,72 ± 0,01
140-150 min	0,96 ± 0,13	0,69 ± 0,01	0,71 ± 0,16	0,76 ± 0,04
150-160 min	0,89 ± 0,14	0,67 ± 0,05	0,77 ± 0,26	0,8 ± 0,02
160-170 min	0,9 ± 0,13	0,65 ± 0,08	0,82 ± 0,25	0,76 ± 0,13
170-180 min	0,93 ± 0,17	0,65 ± 0,05	0,78 ± 0,25	0,78 ± 0,03

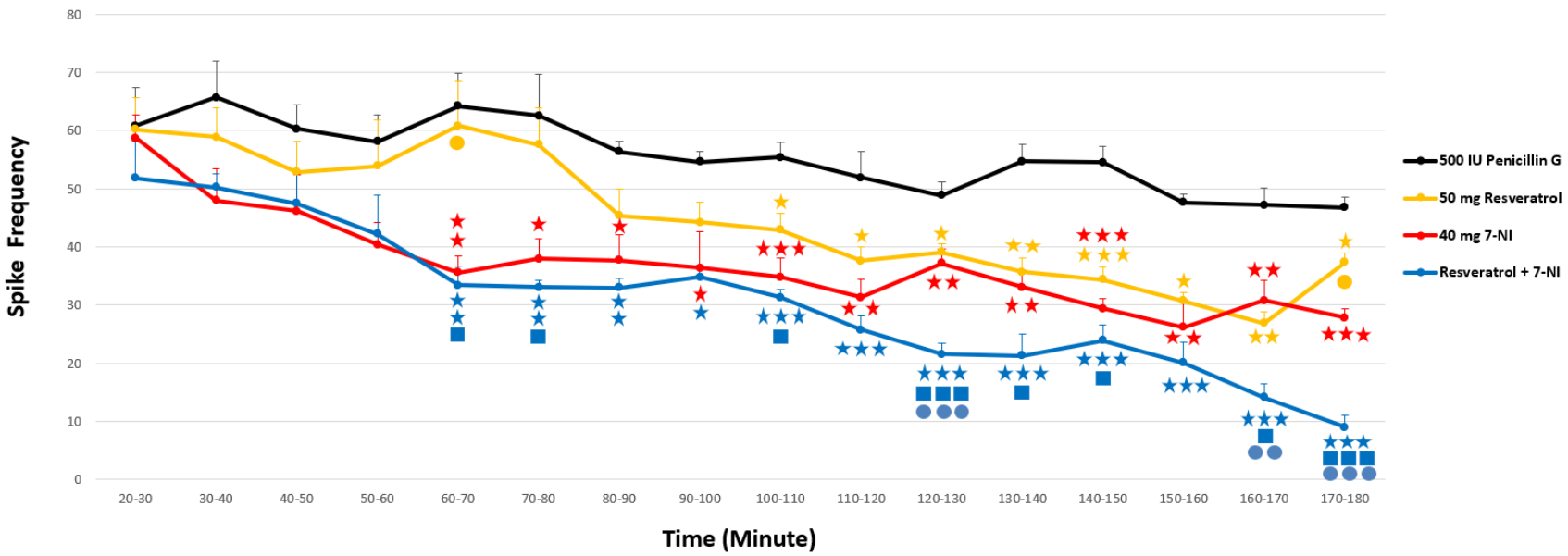


Figure 4.8. Effects of effective dose of resveratrol 50 mg/kg and 7 Nitroindazole 40 mg/kg on spike frequency after 30 min after Penicillin injection. Mean spike number in 10 min interval ± SEM. Comparison between penicillin G 5OO IU with other groups (RSV and 7-NI) (★=p<0.05, ★★=p<0.01, ★★★=p<0.001), (★) comprison between resveratrol to penicillin group, (★) shows comparison between 7-NI to penicillin group, (★) shows comparison between resveratrol + 7-NI to penicillin group . Comparison between resveratrol +7-NI with the resveratrol (50 mg / kg) group (▪=p<0.05, ▪▪ =p<0.01, ▪▪▪=p<0.001). Comparison between resveratrol +7-NI with the 7-NI group (●= p <0.05). ●●=p<0.01, ●●● =p<0.001).Comparison between resveratrol with the 7-NI group (● = p <0.05)

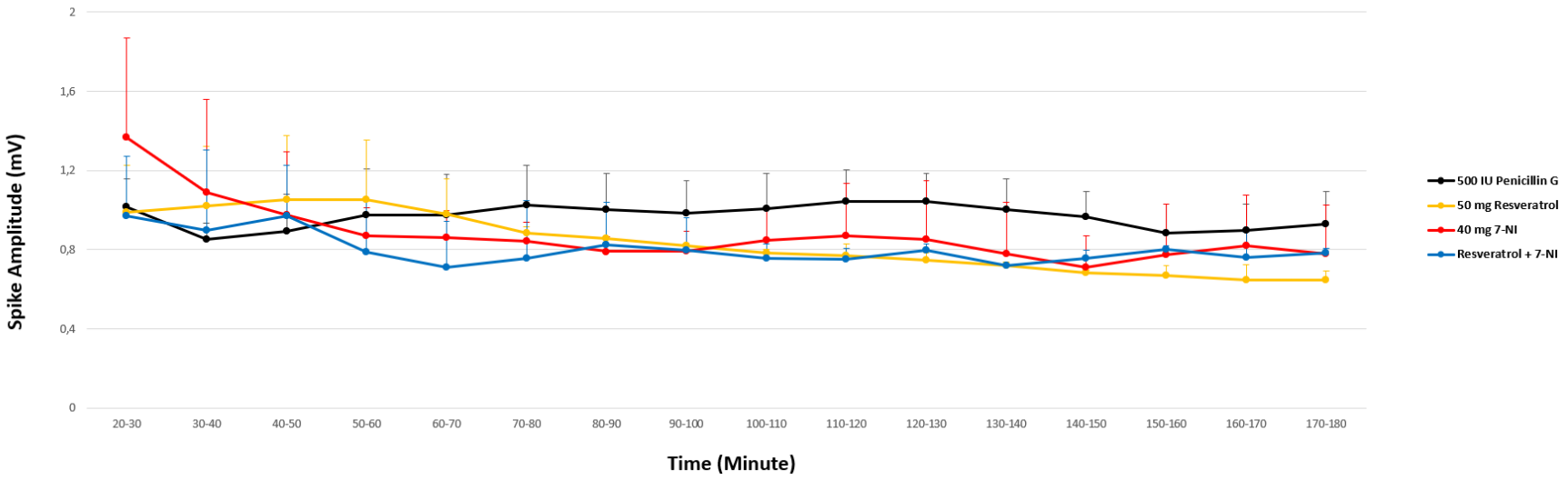


Figure 4.9. Effects of effective doses of Resveratrol 50 mg/kg and 7 Nitroindazole 40 mg/kg on spike amplitude 30 min after Penicillin injection (mean spike amplitude ± SEM).

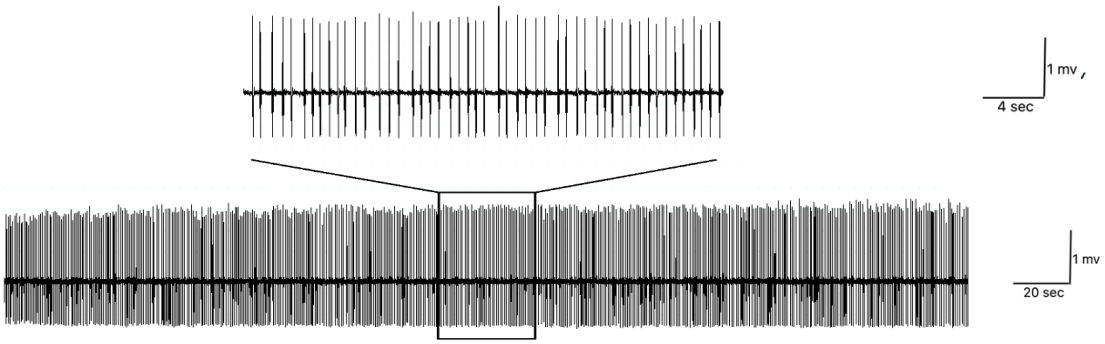
4.1.3.3. Effect of Aminoguanidine on epileptiform activity

Aminoguanidine 100 mg/kg were applied 30 min after Penicillin G 500 IU injection intraperitoneally. The spike frequency decreased significantly between 30-80 minutes ($p<0.05$). There was no significant difference in spike amplitude compared to penicillin group ($p>0.05$) (Figure 4.10-4.12, Table 4.7, 4.8).

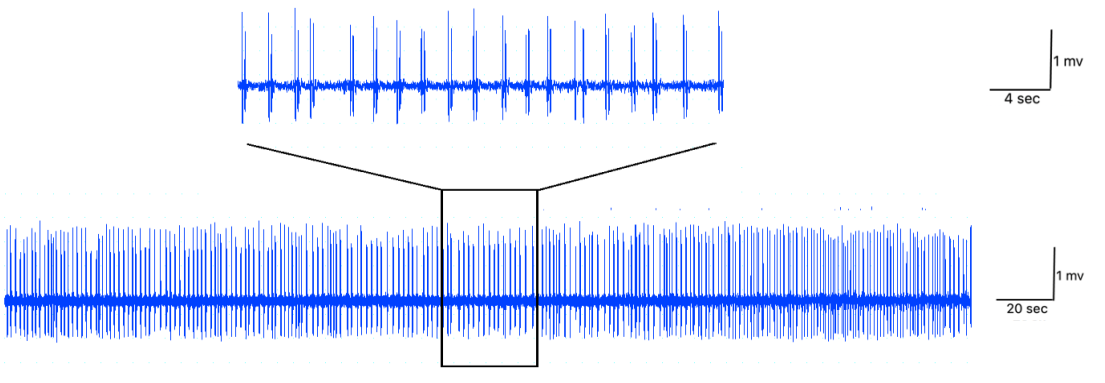
4.1.3.4. Effect of Aminoguanidine and Resveratrol on epileptiform activity

Resveratrol 50 mg/kg and Aminoguanidine 100 mg/kg were applied together intraperitoneally 30 minutes after Penicillin G 500 IU intracortical injection. The spike frequency decreased between 30-180 minutes ($p<0.05$). This decrease was significant between 60-70 and 100-120 minutes ($p<0.01$) and high significant between 120-180 minutes ($p<0.001$). When Resveratrol and Aminoguanidine was combined there was no significant change in spike amplitude (Figure 4.10-4.12, Table 4.7, 4.8).

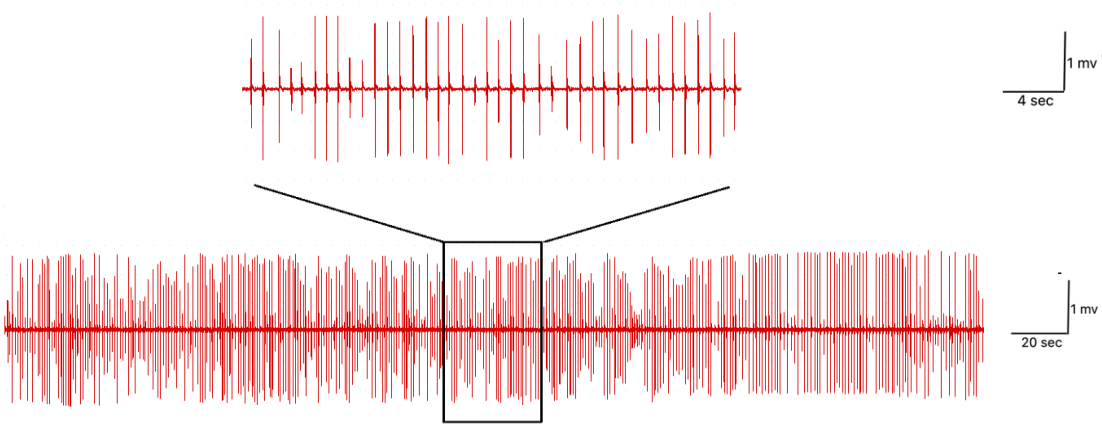
A) Penicillin G 500 IU



B) Resveratrol 50 mg/kg



C) Aminoguanidine 100 mg/kg



D) Aminoguanidine 100 mg/kg + Resveratrol 50 mg/kg

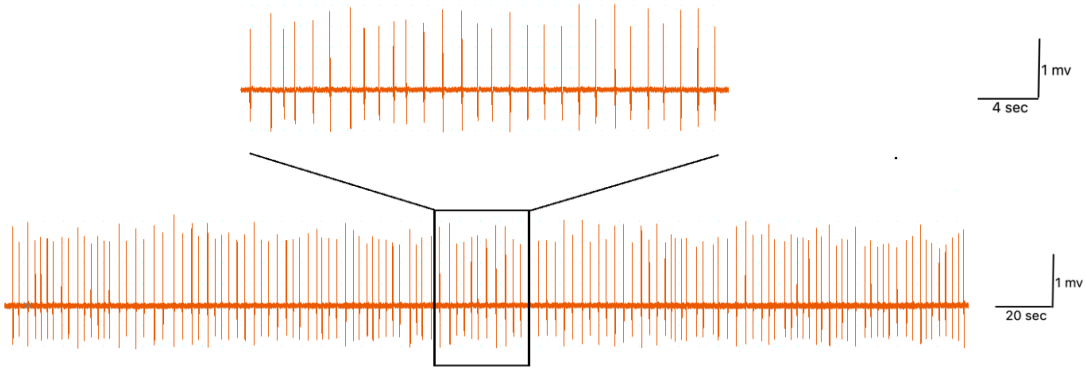


Figure 4.10. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU B) Resveratrol 50 mg/kg + Penicillin G 500 IU C) Penicillin G 500 IU + Aminoguanidine 100 mg/kg D) Penicillin G 500 IU + Aminoguanidine 100 mg/kg + Resveratrol 50 mg/kg.

Table 4.7. Mean values of spike frequencies obtained from Penicillin G 500 IU, Resveratrol 50 mg/kg and Aminoguanidine 100 mg/kg groups in every 10 minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	100 mg Aminoguanidine (Mean ± SEM)	Resveratrol + Aminoguanidine (Mean ± SEM)
20-30 min	60,8 ± 6,6	60,2 ± 5,4	57,2 ± 2,4	54,4 ± 2
30-40 min	65,7 ± 6,3	58,9 ± 5,1	46,2 ± 1,8	44,1 ± 1,5
40-50 min	60,3 ± 4,2	52,9 ± 5,3	43,2 ± 1,5	40,3 ± 4
50-60 min	58,1 ± 4,7	54 ± 7,9	36,2 ± 2	31 ± 2,5
60-70 min	64,2 ± 5,6	60,8 ± 7,6	36,1 ± 2,1	31,8 ± 4,3
70-80 min	62,6 ± 7,2	57,6 ± 6,3	38,5 ± 4,2	32,3 ± 3,4
80-90 min	56,4 ± 1,7	45,4 ± 4,6	44 ± 8,4	31,9 ± 3,9
90-100 min	54,6 ± 1,8	44,2 ± 3,5	42,3 ± 8,1	30,7 ± 3,2
100-110 min	55,4 ± 2,5	43 ± 2,8	49,3 ± 3,5	31,3 ± 3
110-120 min	51,9 ± 4,5	37,6 ± 2,3	47,9 ± 2,9	30 ± 3,6
120-130 min	48,8 ± 2,4	39,1 ± 1,4	46,6 ± 2,4	28,2 ± 2,4
130-140 min	54,7 ± 2,9	35,7 ± 2,5	46,3 ± 3	29,4 ± 3,2
140-150 min	54,6 ± 2,7	34,4 ± 2,1	45,9 ± 3,2	28,8 ± 2,6
150-160 min	47,6 ± 1,4	30,7 ± 1,5	41,8 ± 3,1	28,4 ± 2,3
160-170 min	47,2 ± 2,9	26,9 ± 2	38,6 ± 3	23,7 ± 1,3
170-180 min	46,7 ± 1,8	37,3 ± 1,7	34,4 ± 5,8	18 ± 2,8

Table 4.8. Mean values of amplitude obtained from Penicillin G 500 IU, Resveratrol 50 mg/kg and Aminoguanidine 100 mg/kg groups in every 10 minutes interval (mean spike amplitude ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	100 mg Aminoguanidine (Mean ± SEM)	Resveratrol + Aminoguanidine (Mean ± SEM)
20-30 min	1,02 ± 0,14	0,99 ± 0,24	1,28 ± 0,23	0,94 ± 0,06
30-40 min	0,85 ± 0,08	1,02 ± 0,3	1,19 ± 0,27	0,82 ± 0,07
40-50 min	0,89 ± 0,19	1,05 ± 0,32	1,11 ± 0,24	0,74 ± 0,06
50-60 min	0,98 ± 0,23	1,05 ± 0,3	1,11 ± 0,14	0,74 ± 0,06
60-70 min	0,98 ± 0,2	0,98 ± 0,18	1,13 ± 0,04	0,82 ± 0,06
70-80 min	1,03 ± 0,2	0,88 ± 0,03	1,16 ± 0,02	0,84 ± 0,04
80-90 min	1 ± 0,18	0,86 ± 0,01	0,97 ± 0,18	0,81 ± 0,04
90-100 min	0,98 ± 0,17	0,82 ± 0,02	0,92 ± 0,02	0,83 ± 0,003
100-110 min	1,01 ± 0,18	0,78 ± 0,02	0,95 ± 0,03	0,92 ± 0,14
110-120 min	1,05 ± 0,16	0,77 ± 0,06	0,94 ± 0,09	0,88 ± 0,28
120-130 min	1,04 ± 0,14	0,75 ± 0,04	0,95 ± 0,08	0,91 ± 0,25
130-140 min	1 ± 0,16	0,72 ± 0,02	0,94 ± 0,1	0,93 ± 0,24
140-150 min	0,96 ± 0,13	0,69 ± 0,01	0,9 ± 0,08	0,96 ± 0,2
150-160 min	0,89 ± 0,14	0,67 ± 0,05	0,93 ± 0,05	0,89 ± 0,25
160-170 min	0,9 ± 0,13	0,65 ± 0,08	0,84 ± 0,1	0,89 ± 0,25
170-180 min	0,93 ± 0,17	0,65 ± 0,05	0,76 ± 0,2	0,94 ± 0,2

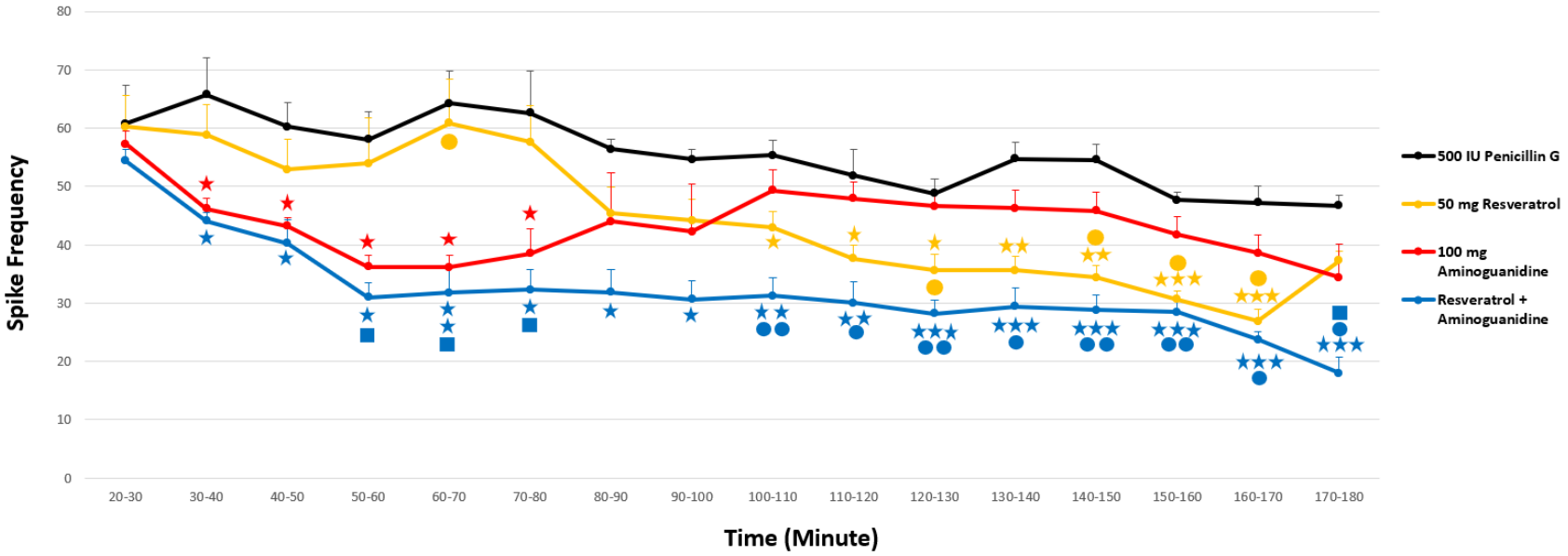


Figure 4.11.Effects of effective dose of Resveratrol 50 mg/kg and Aminoguanidine 100 mg/kg on spike frequency after 30 min after Penicillin injection. Mean spike number in 10 min interval ± SEM. Comparison between penicillin G 5OO IU with other groups (RSV and 7-NI) (★=p<0.05, ★★=p<0.01, ★★★ =p<0.001), (★) shows comparison between resveratrol to penicillin group, (★) shows comparison between aminoguanidine to penicllin group, (★) shows comparison between resveratrol + aminoguanidine to penicillin group. Comparison between resveratrol + aminoguanidine with the resveratrol (50 mg / kg) group (■ =p<0.05) . Comparison between resveratrol +aminoguanidine with the aminoguanidine group (● = p <0.05, ● ● = p< 0.01). Shows comparison between resveratrol and aminoguanidine groups (● = p< 0.01).

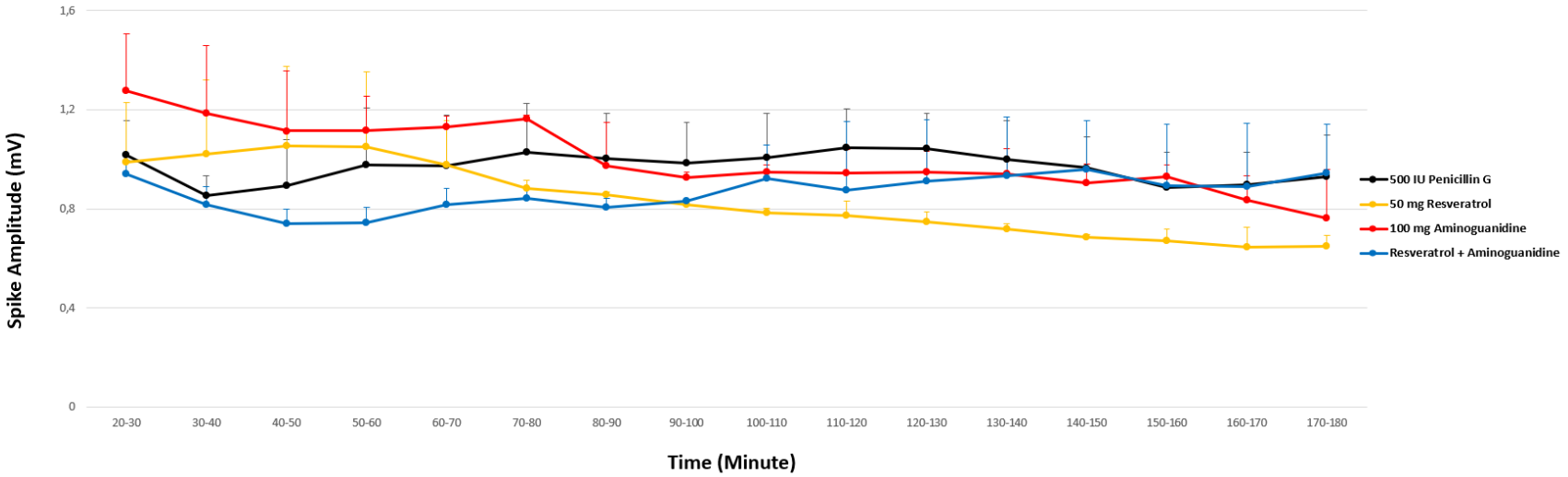


Figure 4.12. Effects of effective dose of Resveratrol 50 mg/kg and Aminoguanidine 100 mg/kg on spike amplitude 30 min after Penicillin injection (mean spike amplitude ± SEM).

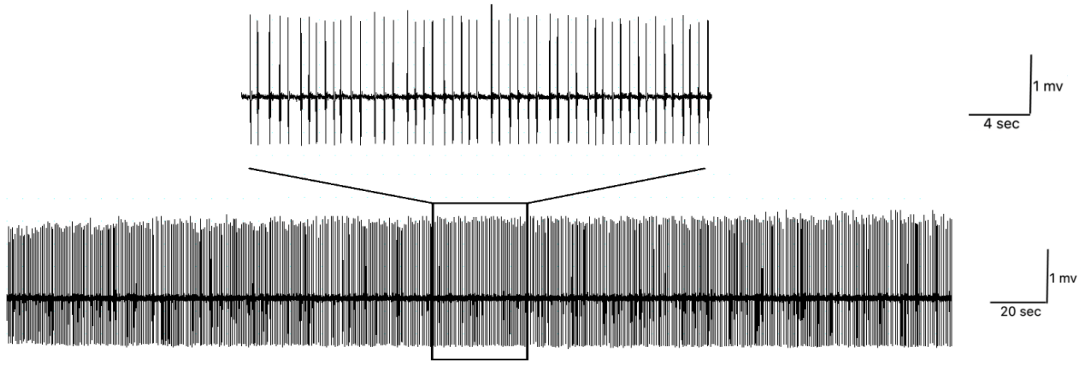
4.1.3.5. Effect of L-NAME on epileptiform activity

Thirty minutes after Penicillin G 500 IU intracortical injection, L-NAME 60 mg was administered intraperitoneally and there was no significant change in both spike frequency and amplitude ($p>0.05$) (Figure 4.13-4.15, Table 4.9, 4.10).

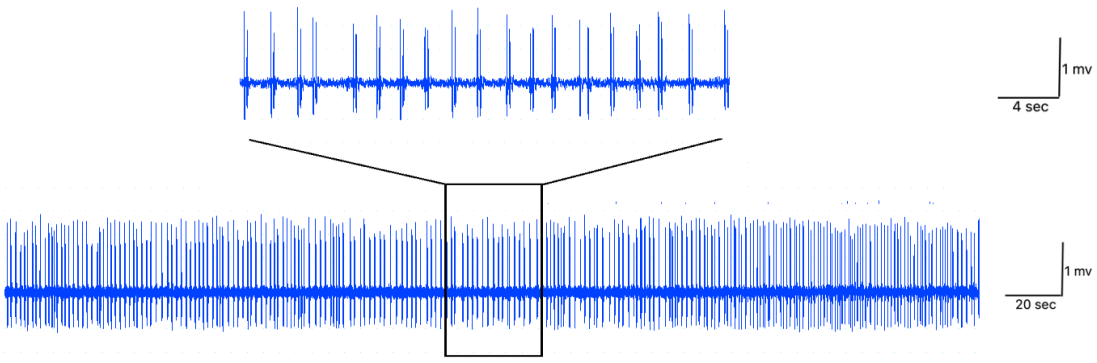
4.1.3.6. Effect of Resveratrol and L-NAME on epileptiform activity

Resveratrol 50 mg/kg and L-NAME 60 mg/kg were applied together intraperitoneally 30 minutes after 500 IU Penicillin injection. There was significant decrease in spike frequency between 160-180 ($p<0.05$) minutes when combined and there was no significant change in spike amplitude till the end of the study (Figure 4.13-4.15, Table 4.9, 4.10).

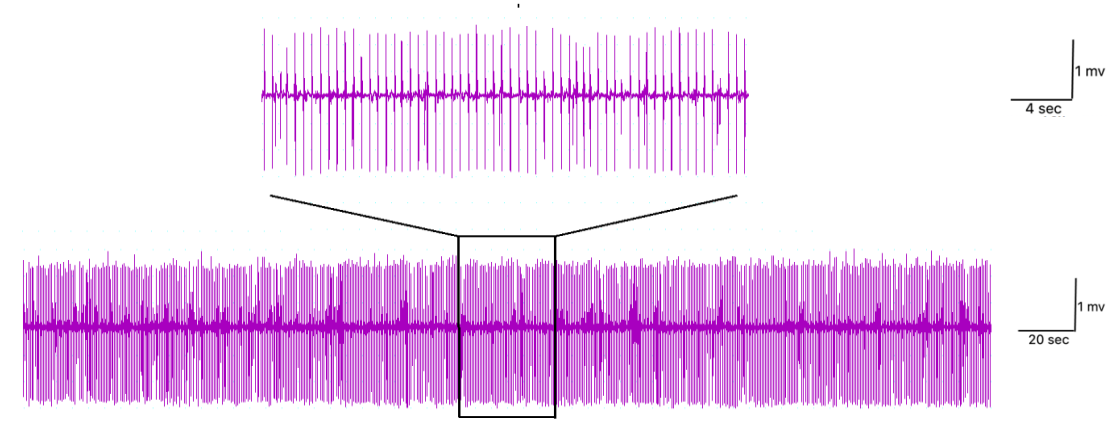
A) Penicillin G 500 IU



B) Resveratrol 50 mg/kg



C) L-NAME 60 mg/kg



D) Resveratrol 50 mg/kg + L- NAME 60 mg/kg

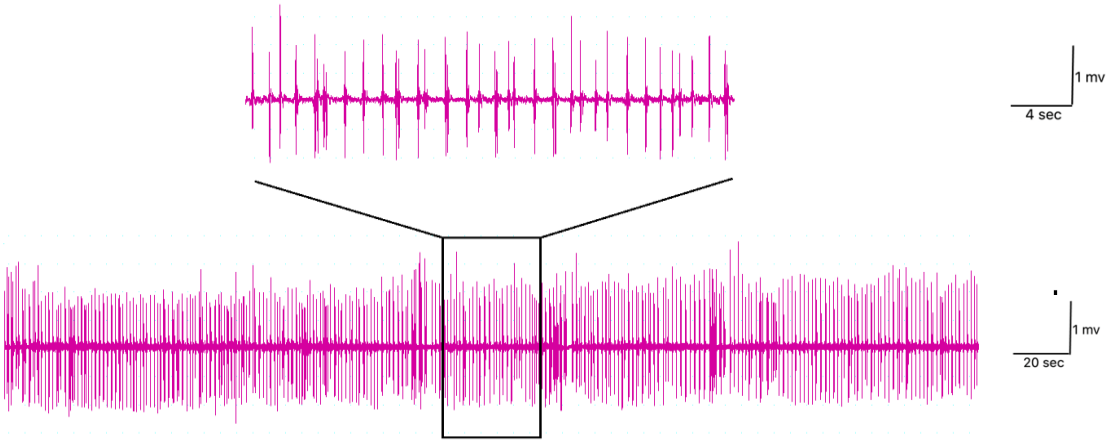


Figure 4.13. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU B) Resveratrol 50 mg/kg + Penicillin G 500 IU C) Penicillin G 500 IU + L-NAME 60 mg/kg D) Penicillin G 500 IU + Resveratrol 50 mg/kg + L-NAME 60 mg/kg.

Table 4.9. Mean values of spike frequencies obtained from penicillin 500 IU, Resveratrol 50 mg/kg and L-NAME 60 mg/kg groups in every 10 minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	60 mg L-NAME (Mean ± SEM)	Resveratrol + L-NAME (Mean ± SEM)
20-30 min	60,8 ± 6,6	60,2 ± 5,4	62,8 ± 3,3	64,9 ± 7,4
30-40 min	65,7 ± 6,3	58,9 ± 5,1	59,2 ± 8,6	58,9 ± 5,6
40-50 min	60,3 ± 4,2	52,9 ± 5,3	56,5 ± 8,4	56,3 ± 6,2
50-60 min	58,1 ± 4,7	54 ± 7,9	60,1 ± 6,1	60,2 ± 5,8
60-70 min	64,2 ± 5,6	60,8 ± 7,6	57 ± 5,3	60,1 ± 1,1
70-80 min	62,6 ± 7,2	57,6 ± 6,3	52,3 ± 4,3	55,7 ± 2,3
80-90 min	56,4 ± 1,7	45,4 ± 4,6	49,5 ± 2,9	55,3 ± 4,4
90-100 min	54,6 ± 1,8	44,2 ± 3,5	51,7 ± 3,9	55,2 ± 3,7
100-110 min	55,4 ± 2,5	43 ± 2,8	49,5 ± 2,9	56,1 ± 1,5
110-120 min	51,9 ± 4,5	37,6 ± 2,3	47,6 ± 3,7	53,2 ± 2,6
120-130 min	48,8 ± 2,4	39,1 ± 1,4	47,7 ± 4	44,1 ± 2,7
130-140 min	54,7 ± 2,9	35,7 ± 2,5	48,6 ± 5,5	44,5 ± 4,2
140-150 min	54,6 ± 2,7	34,4 ± 2,1	48,9 ± 8,8	43,9 ± 3,4
150-160 min	47,6 ± 1,4	30,7 ± 1,5	46,1 ± 5,6	44,5 ± 3,6
160-170 min	47,2 ± 2,9	26,9 ± 2	46,3 ± 2,3	34,4 ± 2,3
170-180 min	46,7 ± 1,8	37,3 ± 1,7	47,2 ± 3,1	36,5 ± 1,5

Table 4.10. Mean values of amplitude obtained from penicillin G 500 IU, Resveratrol 50 mg/kg and L-NAME 60 mg/kg groups in every 10 minutes interval (mean spike amplitude ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	60 mg L-NAME (Mean ± SEM)	Resveratrol + L-NAME (Mean ± SEM)
20-30 min	1,02 ± 0,14	0,99 ± 0,24	1,26 ± 0,27	1,27 ± 0,02
30-40 min	0,85 ± 0,08	1,02 ± 0,3	1,23 ± 0,33	1,15 ± 0,14
40-50 min	0,89 ± 0,19	1,05 ± 0,32	1,15 ± 0,31	1,02 ± 0,3
50-60 min	0,98 ± 0,23	1,05 ± 0,3	1,03 ± 0,27	0,91 ± 0,16
60-70 min	0,98 ± 0,2	0,98 ± 0,18	0,92 ± 0,21	1,1 ± 0,41
70-80 min	1,03 ± 0,2	0,88 ± 0,03	0,94 ± 0,3	1,09 ± 0,45
80-90 min	1 ± 0,18	0,86 ± 0,01	0,95 ± 0,24	1,08 ± 0,46
90-100 min	0,98 ± 0,17	0,82 ± 0,02	0,98 ± 0,28	1,08 ± 0,41
100-110 min	1,01 ± 0,18	0,78 ± 0,02	0,96 ± 0,29	1,03 ± 0,36
110-120 min	1,05 ± 0,16	0,77 ± 0,06	1,02 ± 0,37	1 ± 0,28
120-130 min	1,04 ± 0,14	0,75 ± 0,04	0,95 ± 0,25	0,99 ± 0,2
130-140 min	1 ± 0,16	0,72 ± 0,02	0,93 ± 0,26	0,99 ± 0,13
140-150 min	0,96 ± 0,13	0,69 ± 0,01	0,89 ± 0,25	0,98 ± 0,19
150-160 min	0,89 ± 0,14	0,67 ± 0,05	0,86 ± 0,21	1,02 ± 0,13
160-170 min	0,9 ± 0,13	0,65 ± 0,08	0,79 ± 0,12	0,77 ± 0,07
170-180 min	0,93 ± 0,17	0,65 ± 0,05	0,7 ± 0,06	0,77 ± 0,13

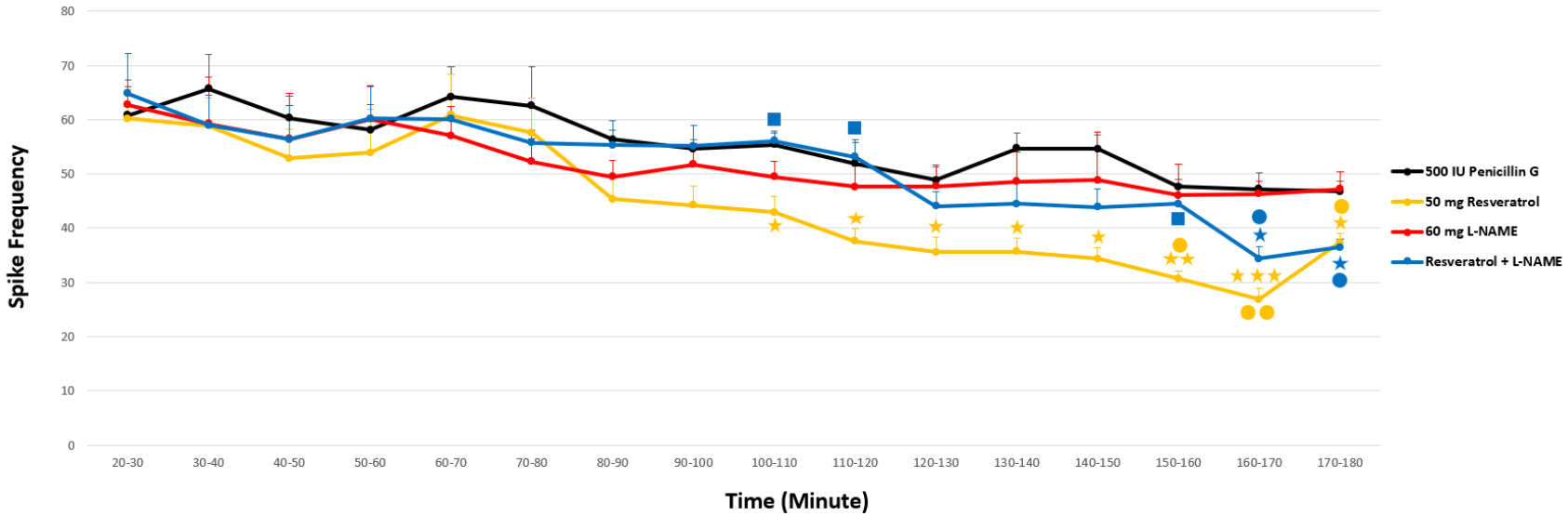


Figure 4.14. Effects of effective dose of Resveratrol 50 mg/kg and L-NAME 60 mg/kg on spike frequency 30 min after penicillin injection. Mean spike number in 10 min interval ± SEM. Comparison between penicillin G 5OO IU with other groups (RSV and L-NAME) (★=p<0.05, ★★=p<0.01, ★★★ =p<0.001), (★) shows comparison between resveratrol to penicillin group, (★) shows comparison between resveratrol + L-NAME to penicillin group. Comparison between resveratrol with resveratrol + L- NAME groups (■=p<0.05). Comparison of resveratrol + L-NAME with L-NAME (effective dose) group (●= p <0.05). Comparison between resveratrol with L-NAME (● = p <0.05, ●● =p<0.01).

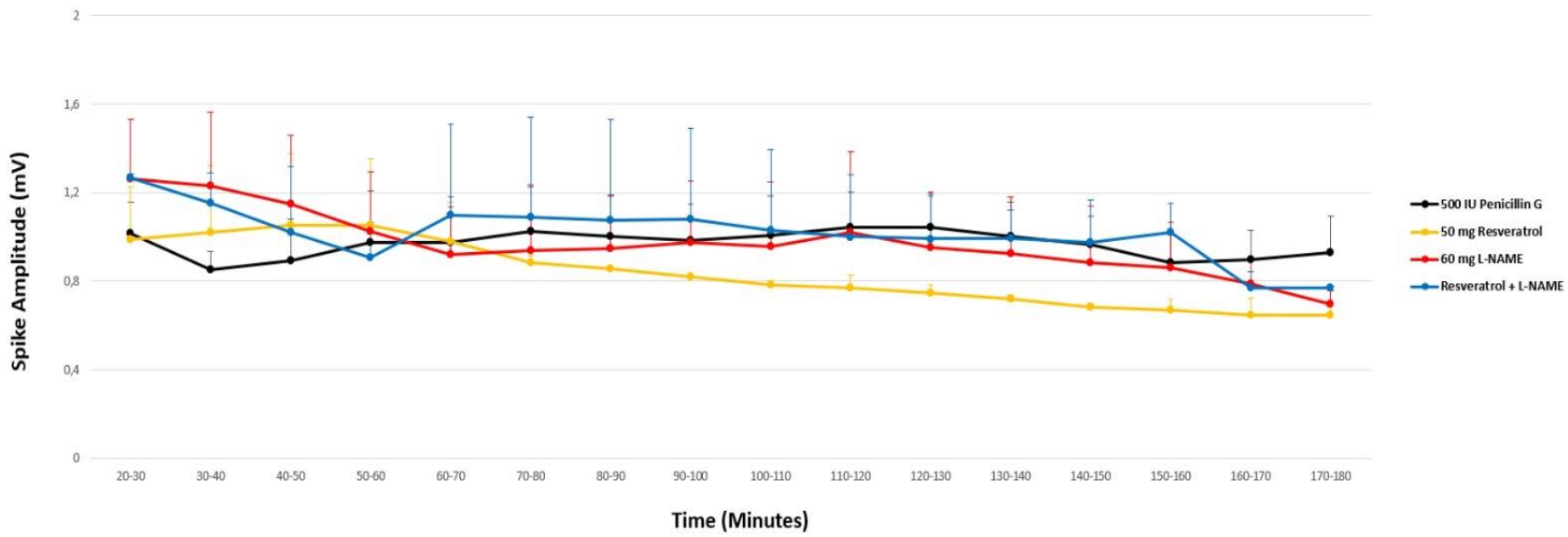


Figure 4.15. Effects of effective dose of Resveratrol 50 mg/kg and L-NAME 60 mg/kg on spike amplitude 30 min after injection of Penicillin (mean spike amplitude ± SEM).

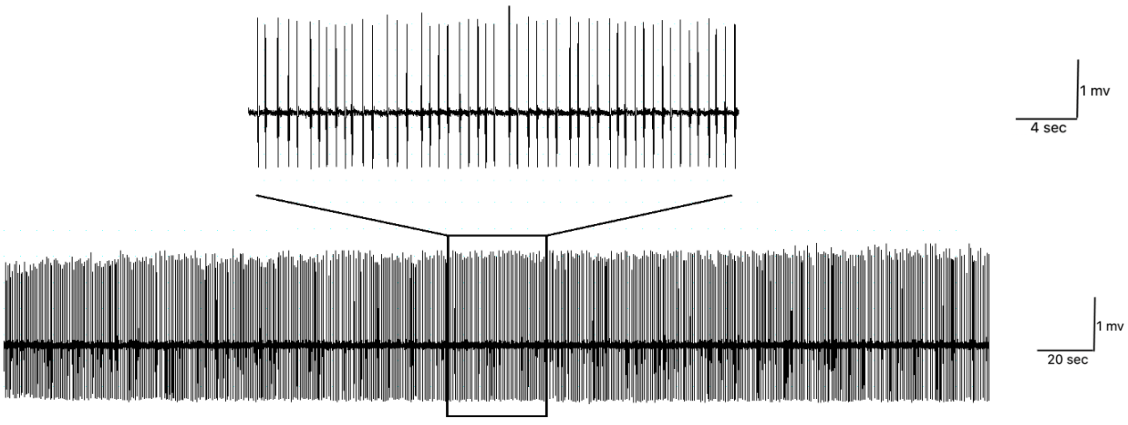
4.1.3.7. Effect of L-Arginine on epileptiform activity

L-Arginine 500 mg/kg was applied intraperitoneally 30 min after Penicillin G 500 IU intracortical injection and the spike frequency reduced significantly at 100 minutes till the of the end of the recording period (180 minutes) ($p<0.05$). This decrease was significant between 100-110, 120-140 and 160-170 minutes ($p<0.01$); and was highly significant in between 140 -160 and 170-180 minutes ($p<0.001$). There was no significant difference in spike amplitude compared to penicillin group ($p>0.05$) (Figure 4.16-4.18, Table 4.11, 4.12).

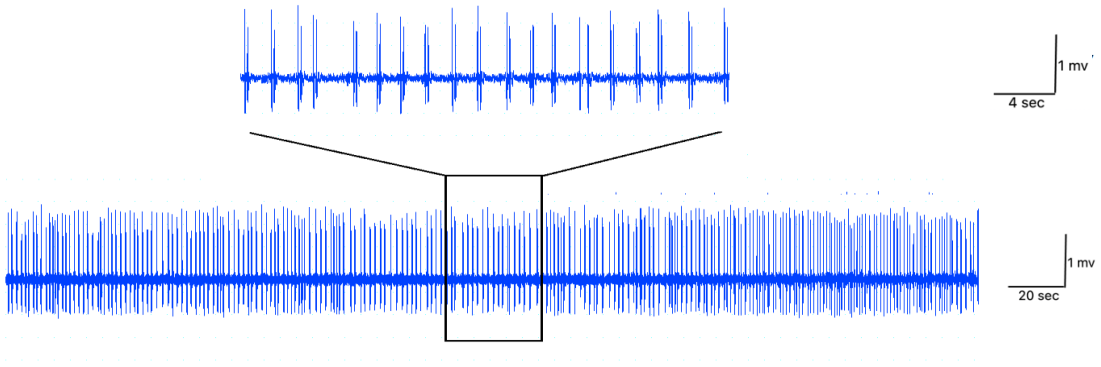
4.1.3.8. Effect of Resveratrol and L-Arginine on epileptiform activity

Thirty minutes after Penicillin G 500 IU intracortical application, the effective dose of Resveratrol 50 mg/kg and 500 mg/kg L-Arginine were administered intraperitoneally. The spike frequency reduced significantly at 50 minutes till the of the end of the recording period ($p<0.05$). This decrease was significant between in 70-80 and 110-120 minutes ($p<0.01$); and was highly significant between 80-110 and 120-180 minutes ($p<0.001$) (Figure 4.16-4.18, Table 4.11, 4.12).

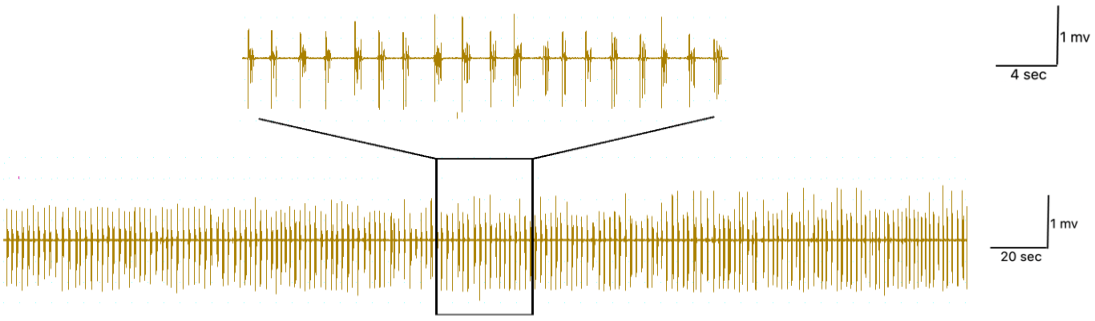
A) Penicillin G 500 IU



B) Resveratrol 50 mg /kg



C) L- Arginine 500 mg /kg



D) Resveratrol 50 mg /kg + L- arginine 500 mg /kg

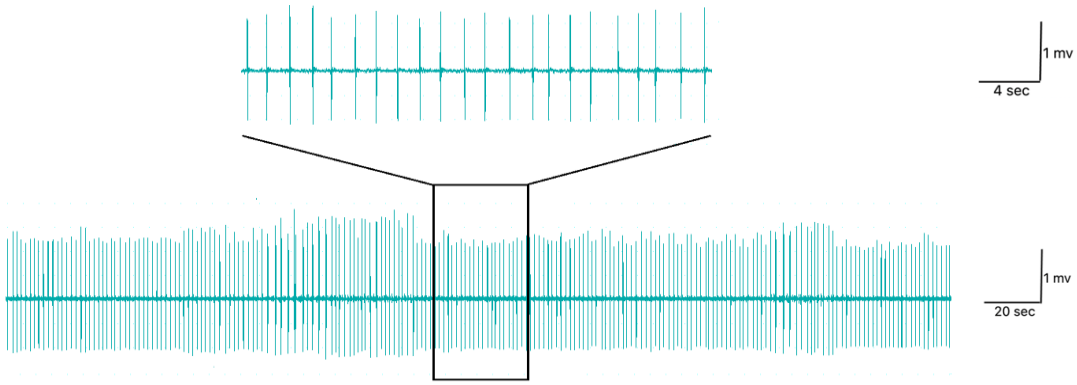


Figure 4.16. Sample images from ECoG records obtained from groups. A) Penicillin G 500 IU B) Resveratrol 50 mg /kg + Penicillin G 500 IU C) Penicillin G 500 IU + L-Arginine 500 mg /kg D) Penicillin G 500 IU + Resveratrol 50 mg /kg + L-Arginine 500mg /kg.

Table 4.11. Mean values of spike frequencies obtained from penicillin G 500 IU, Resveratrol 50mg/kg and L- Arginine 500 mg/kg groups in every 10minutes interval (mean spike number ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	500 mg L-Arginine (Mean ± SEM)	Resveratrol + L-Arginine (Mean ± SEM)
20-30 min	60,8 ± 6,6	60,2 ± 5,4	60,7 ± 4,3	66,9 ± 2,4
30-40 min	65,7 ± 6,3	58,9 ± 5,1	54 ± 2,6	57,6 ± 6,5
40-50 min	60,3 ± 4,2	52,9 ± 5,3	51,4 ± 2,9	47,1 ± 7,2
50-60 min	58,1 ± 4,7	54 ± 7,9	51 ± 0,9	33,9 ± 2,2
60-70 min	64,2 ± 5,6	60,8 ± 7,6	51,7 ± 0,9	36,1 ± 0,6
70-80 min	62,6 ± 7,2	57,6 ± 6,3	55,8 ± 2,1	28,7 ± 2,6
80-90 min	56,4 ± 1,7	45,4 ± 4,6	47,2 ± 2,6	28,7 ± 2,9
90-100 min	54,6 ± 1,8	44,2 ± 3,5	44,1 ± 4,2	28,9 ± 2,6
100-110 min	55,4 ± 2,5	43 ± 2,8	36,9 ± 1,5	27,7 ± 3,6
110-120 min	51,9 ± 4,5	37,6 ± 2,3	35,8 ± 1,9	28,2 ± 2,5
120-130 min	48,8 ± 2,4	39,1 ± 1,4	33,8 ± 3,1	26,6 ± 2,2
130-140 min	54,7 ± 2,9	35,7 ± 2,5	30,7 ± 5,3	27,9 ± 2,2
140-150 min	54,6 ± 2,7	34,4 ± 2,1	28,9 ± 4,9	20 ± 1,7
150-160 min	47,6 ± 1,4	30,7 ± 1,5	27,8 ± 4,3	25 ± 2,1
160-170 min	47,2 ± 2,9	26,9 ± 2	27,9 ± 3,7	21,5 ± 3,5
170-180 min	46,7 ± 1,8	37,3 ± 1,7	22,6 ± 1,6	23,5 ± 3,6

Table 4.12. Mean values of amplitude obtained from penicillin G 500 IU, Resveratrol 50 mg/kg and L-Arginine 500 mg/kg groups in every 10minutes interval (mean spike amplitude ± SEM).

Time	500 IU Penicillin G (Mean ± SEM)	50 mg Resveratrol (Mean ± SEM)	500 mg L-Arginine (Mean ± SEM)	Resveratrol + L-Arginine (Mean ± SEM)
20-30 min	1,02 ± 0,14	0,99 ± 0,24	1,02 ± 0,03	0,85 ± 0,06
30-40 min	0,85 ± 0,08	1,02 ± 0,3	0,91 ± 0,01	0,75 ± 0,14
40-50 min	0,89 ± 0,19	1,05 ± 0,32	0,85 ± 0,02	0,8 ± 0,01
50-60 min	0,98 ± 0,23	1,05 ± 0,3	0,87 ± 0,11	0,7 ± 0,09
60-70 min	0,98 ± 0,2	0,98 ± 0,18	0,94 ± 0,23	0,75 ± 0,14
70-80 min	1,03 ± 0,2	0,88 ± 0,03	0,9 ± 0,25	0,79 ± 0,12
80-90 min	1 ± 0,18	0,86 ± 0,01	0,75 ± 0,05	0,75 ± 0,1
90-100 min	0,98 ± 0,17	0,82 ± 0,02	0,8 ± 0,1	0,7 ± 0,1
100-110 min	1,01 ± 0,18	0,78 ± 0,02	0,82 ± 0,16	0,71 ± 0,09
110-120 min	1,05 ± 0,16	0,77 ± 0,06	0,85 ± 0,19	0,7 ± 0,1
120-130 min	1,04 ± 0,14	0,75 ± 0,04	0,87 ± 0,17	0,69 ± 0,04
130-140 min	1 ± 0,16	0,72 ± 0,02	0,86 ± 0,19	0,71 ± 0,06
140-150 min	0,96 ± 0,13	0,69 ± 0,01	0,81 ± 0,17	0,68 ± 0,05
150-160 min	0,89 ± 0,14	0,67 ± 0,05	0,82 ± 0,16	0,63 ± 0,06
160-170 min	0,9 ± 0,13	0,65 ± 0,08	0,7 ± 0,03	0,61 ± 0,03
170-180 min	0,93 ± 0,17	0,65 ± 0,05	0,6 ± 0,04	0,63 ± 0,07

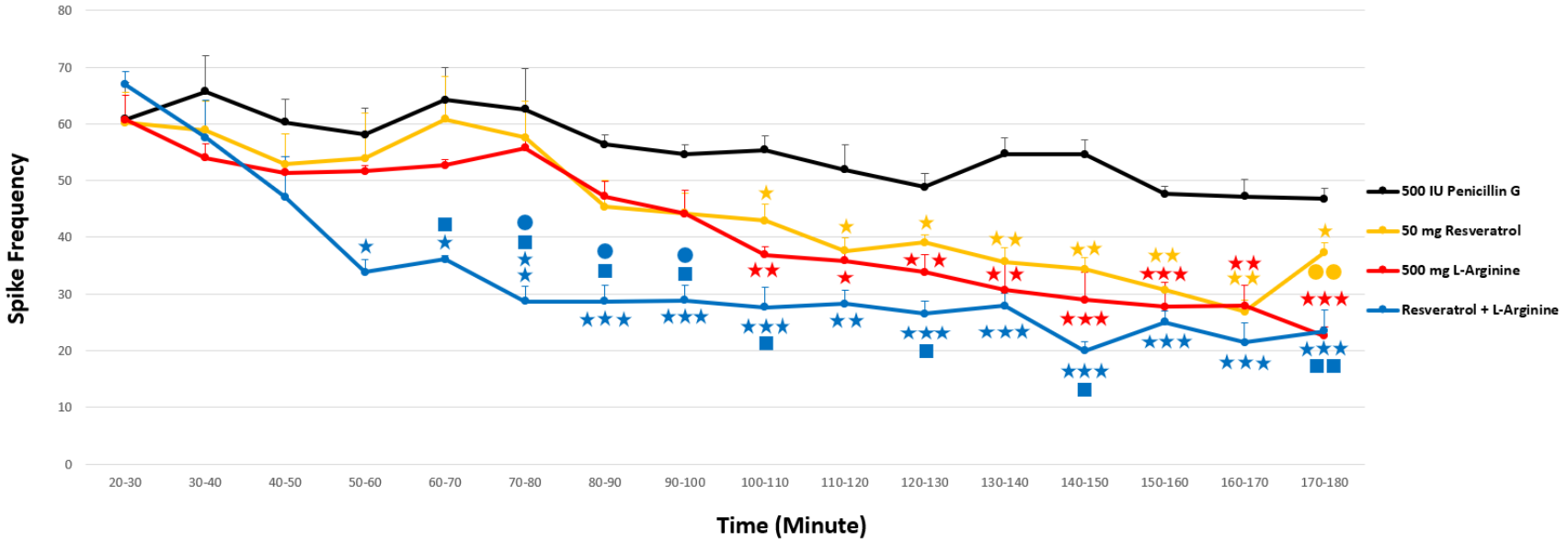


Figure 4.17. Effects of effective dose of resveratrol 50 mg/kg and L-Arginine 500 mg /kg on spike frequency 30 min after penicillin injection. Mean spike number in 10 min interval ± SEM. Comparison between penicillin G 5OO IU with other groups (RSV and L-Arg) (★=p<0.05, ★★=p<0.01, ★★★ =p<0.001), (★) shows comprison between resveratrol to penicillin group, (★) shows comparism between L-Arginine to penicllin group, (★) shows comparison between resveratrol + L-Arginine to penicillin group. Comparison of between resveratrol with resveratrol + L- Arginine group (■=p<0.05, ■■ =p<0.01). Comparison of resveratrol + L-Arginine with the L-Arginine (effective dose) group (●= p <0.05). Comparison of resveratrol with L- Arginine groups (● =p<0.05, ●● =p<0.01).

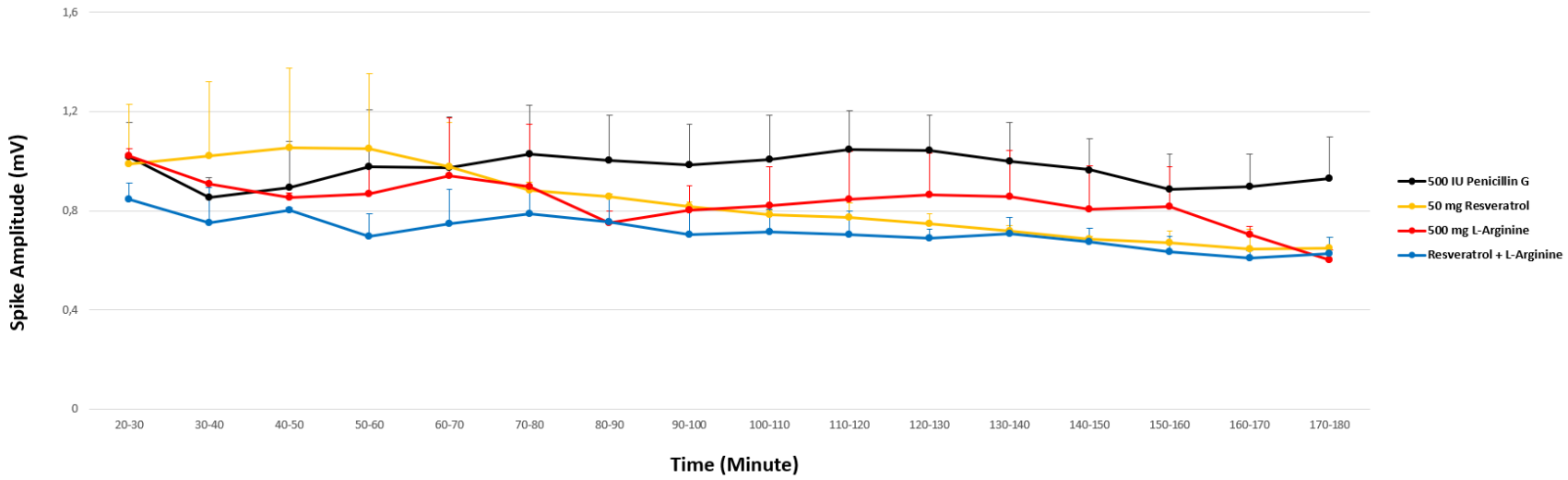


Figure 4.18. Effects of effective dose of resveratrol 50 mg/kg and L-Arginine 500 mg/kg on spike amplitude after injection of Penicillin in 30 min (mean spike amplitude ± SEM).

4.2. DISCUSION

In the present study; the effect of Resveratrol and nitric oxide interaction on Penicillin induced epileptiform activity was investigated.

Epilepsy, one of the most common disorders in the brain and is characterized by seizures but not all seizures are due to epilepsy, for example, febrile seizures or seizures that are caused by drugs (Manford, 2017). About 80% of people with epilepsy live in countries with low incomes and middle incomes. Epilepsy is stigmatized in many parts of the world and individuals do not seek treatment. More than 75% of people with active epilepsy are untreated and this indicates a significant disparity in healthcare, particularly in low- and middle-income countries (Thijs et al., 2019).

Diagnosis is challenging as, in practice, the electrical diagnostic hallmark of epilepsy may be interictally absent, particularly in adults, or when seizures are uncommon and interictal epileptiform discharges may occur in those without seizures (Manford, 2017). In our understanding of the underlying physiological and behavioral changes associated with human epilepsy, animal models for seizures and epilepsy have played a crucial role to develop more effective antiepileptic drugs (Yildirim Mehmet et al., 2006).

4.2.1. Penicillin Acute Focal Seizures

In this study, epileptic spikes and spike-wave complexes started to appear within 2-5 minutes after intracortical Penicillin injection in experimental animals. Within 30 minutes, epileptic activity stabilized and lasted longer than 3 hours. During the time from Penicillin injection to epilepsy formation no statistically significant difference was observed between the experimental groups ($p>0.05$). Also, no significant difference was observed in terms of the spike frequency or amplitude of epileptic activity recorded between the experimental groups within 30 minutes after Penicillin injection ($p>0.05$). Injections of other substances were made during the 30th minute after penicillin injection.

In this research, it was favored the Penicillin model of epilepsy. The reasons for this choice can be summarized as follows: Multiple in *vivo* and in *vitro* findings indicate epileptiform activity by antibiotics (Gutnick et al., 1982). Penicillin's epileptogenic effect was documented as far back as 1945 when Walker and Johnson announced that

epileptiform activity occurred when Penicillin was applied to the cortex of the dog, cat and monkey (Walker et al., 1945).

Among experimental epilepsy models, the most commonly used one is the Penicillin epilepsy and has been used by many researchers. Penicillin administration to the neocortex results in synchronous neuronal discharge, which is electrophysiologically close to human focal interictal epileptic discharges (Marangoz et al., 2012). Even though the experimental Penicillin epilepsy model, that began focally then spreads, causing generalized epilepsy, isn't the same as the cases of clinical epilepsy found in humans, it has correlations with respect to the basic mechanism (Walden et al., 1992).

Penicillin applied to the cortex causes GABA receptor inhibition by acting similarly to bicuculline depending on its molecular structure and therefore suppresses GABA activity causing focal epileptiform activity (Tsuda et al., 1994). Administration of Penicillin into the neocortex has also been reported to increase glutamate release which induce *in vivo* or *in vitro* epileptiform activity (De Boer et al., 1982).

4.2.2. Effect of Resveratrol on epileptiform activity

In this research, the effect of Resveratrol on epileptiform activity was evaluated by electrophysiology ECoG analysis and it was found that Resveratrol decreases the spike frequency and does not change spike amplitude compared to control group, after creating epileptiform activity of intracortical injection of 500 IU Penicillin G in experimental animals. In these animal experiments, when Resveratrol 25mg/kg and 100 mg/kg were applied there was no significant difference in both spike frequency and amplitude ($p>0.05$). Resveratrol 50mg/kg dose caused considerable decrease in spike frequency ($p<0.01$) and 50 mg/kg Resveratrol is considered as effective dose.

Ethemoglu et al. (2017) found that when administered resveratrol (RSV) in three different doses (2, 10, 20 mg/kg) in Penicillin induced epilepsy, there no difference in RSV and control group ($p>0.05$). On the other hand, they coated RES with liposomal vesicle (RSV+ LIP) and it resulted in decrease in spike frequency ($p<0.05$) and exhibited greater anticonvulsant and antioxidant effects. In the current study, 25 mg/kg Resveratrol did not affect spike frequency but 50 mg/kg Resveratrol exhibited anticonvulsant effect.

Resveratrol is an effective neuroprotective compound which exhibits antiepileptic activity in many other animal epilepsy models. Thus, Resveratrol promises to manage acute seizures, to prevent acute seizure or epileptic status, to reduce chronic epilepsy, and cognitive impairment (Wang Y.-J. et al., 2019).

The rate of generalized tonic–clonic seizures decreased in a dose-dependent fashion. Resveratrol (40 mg/kg) augment the effectiveness of sodium valproate and diazepam in the treatment of PTZ-induced seizures. Adenosine was given in conjunction with Resveratrol, and the rate of generalized tonic-clonic convulsions was reduced.

Lu et al. (2015) stated that intraperitoneally administered (i.p.), trans Resveratrol played a neuroprotective function in seizures induced by pentylenetretazol (PTZ), providing an adenosinergic mechanism as a key factor in its anticonvulsant activity. In the same research, the authors have shown that the effect of diazepam (2 mg / kg) and sodium valproate (150 mg / kg) against PTZ-induced seizures was potentiated by Resveratrol (40 mg / kg). Major decrease in the percentage of occurrence of generalized tonic-clonic convulsions was observed when given with a sub-anticonvulsant dose of adenosine (500 mg/kg).

Resveratrol reduces the number of spontaneous seizures dramatically and inhibits the occurrence of epileptiform discharges. It could protect neurons from kainate-induced neuronal cell death in regions of CA1, CA3 and sprouting depressed mossy fiber. It also reduced the level of expression of kainate receptors in hippocampus (Wu et al., 2009).

In the absence of RSV treatment, $FeCl_3$-treated mice had epileptiform EEG discharges significantly and higher levels of the oxidative stress marker MDA in the brain tissue. However, the onset of the epileptiform EEG discharges was delayed in animals receiving RSV (20 or 40 mg / kg i.p.) 30 min prior to $FeCl_3$ treatment and MDA levels were reduced (Wang Q. et al., 2004).

Resveratrol increased the seizure levels caused by NMDA and reduced the frequency of action potential and Evoked Field Potentials (EFPs) evoked by NMDA/glycine. Resveratrol, however, decreased the 4-AP-induced thresholds for myoclonic twitch and face and forelimb clonus, but raised the thresholds at the higher dose (50 mg/kg) for running and bouncing clonus and tonic hindlimb extension. In the

frequency of EFPs and action potential firings evoked by 4-AP, a similar biphasic response to Resveratrol was observed, with enhancement at lower doses but with repression at higher doses (Wang Y.-J. et al., 2019).

4.2.3. Effect of nitric oxide on epileptiform activity

Nitric oxide (NO) is a very important regulatory molecule used as second messenger and transmitter in the nervous, digestive, immune, cardiovascular, and urogenital systems. It is also closely related to pathophysiological disorders such as septic shock, hypertension, stroke, epilepsy and other neurodegenerative diseases, in addition to normal physiological functions (Ayyildiz Mustafa et al., 2007).

In this research, it was examined the relationship between NO and epilepsy using the experimental epilepsy model produced with penicillin. Thirty minutes after penicillin administration, L-NAME 60mg /kg, L-Arginine 500mg /kg, Aminoguanidine 100mg /kg and 7-Nitroindazole 40mg /kg were applied intraperitoneally in each with different experimental groups.

According to the findings of this study, L-NAME 60 mg caused no statistically significant difference in both spike frequency and amplitude ($p>0.05$). L-NAME, a general NOS inhibitor, was given 30 minutes after Penicillin and had no effect on either amplitude nor the frequency in penicillin-induced epileptiform activity in rats (Yildirim M. et al., 2010) . Inhibition of NO production prior to NMDA receptor activation reduced epileptiform activity, but prevention of NO production after the onset of epileptic activity was ineffective. Exogenic NO donor application appears to have a short-term influence on spike frequency, and therefore blocking endogen NO production via L-NAME has no effect (Marangoz et al., 2012).

Marangoz et al. (2012) stated that L-NAME significantly increased the frequency of epileptiform activity in just two time points. The spike amplitude on one timing point was also significantly higher in the L-NAME injection group comparison to the control group. L-NAME had virtually no effect on the frequency and amplitude of epileptiform activity (except these points).

Byun et al. (2009) found in KA-induced iNOS expression, CA3 neuronal death, and activation of microglia were significantly increased by pretreatment with L-NAME.

However, both the KA- and L-NAME induced hippocampal CA3 neuron death with associated decreases in microglial activation and iNOS expression were significantly suppressed by pretreatment with Aminoguanidine. For up to 2 weeks, the protective effect of Aminoguanidine was preserved.

Hrnčić et al. (2015) found that administration of L-NAME increased significantly the number of spike-wave discharges (SWDs) per rat induced by homocysteine thiolactone (HcT) but didn't affect its duration and 7-nitroindazole (75 mg/kg, i.p.) increased duration, but not the number SWDs whereas Aminoguanidine (100 mg/kg, i.p.) increased both parameters significantly.

L-NAME (10 mg/kg, i.p.) had no effect on the seizure threshold modification. The anticonvulsant effect has been significantly reversed once applied 15 minutes prior to onopordia (10 mg/kg) (Hassanzadeh et al., 2019). In vehicle-treated animals, the latency of status epilepticus onset was 43.7 minutes. L-NAME pretreatment seemed to have no effect on the overall EEG power, behavioral seizure score after status epilepticus onset or status epilepticus latency onset (Lee D.-S. et al., 2021).

The pro-convulsant effect of leptin has not been influenced by administering L-NAME (60 mg/kg, i.p.), 30 min before the effective leptin dose (1 μg, i.c.v.) injection (Fig. 2). There was no significant difference between the Penicillin + leptin and Penicillin + L-NAME + leptin groups (Aslan et al., 2010).

In the Penicillin model of rat, 7-NI, at doses of 25 and 50 mg/kg, and L-arginine (500 mg/kg, i.p.), reduced significantly the mean frequency without altering the amplitude. 7-NI and L-arginine both demonstrated anticonvulsant activity in the 40th and 70th minutes after administration, respectively. At doses of 60 and 100 mg/kg, Aminoguanidine decreased the mean frequency of epileptiform activity (Per et al., 2013)

Paul V. et al. (2003) stated that L-NAME, a non-specific NOS inhibitor, did not prevent seizures induced by picrotoxin in rats, whereas L-arginine, a precursor to NO, potentiates the therapeutic potential of antiepileptic drugs such as phenobarbital and diazepam. Additionally, it has been shown that phenobarbital, an antiepileptic drug, does not prevent picrotoxin-induced seizures in animals treated with L-NAME. Non-specific NOS inhibitor was reported to cause deterioration in anticonvulsant effects of antiepileptic

drugs by enhancing GABA activity in the brain according to the recorded findings. In the focal epilepsy model produced by topical administration of bicuculline to the brain cortex in anesthetized rats, NO has shown a vasodilating and anticonvulsant effect (Pereira De Vasconcelos et al., 1995).

7-Nitroindazole 40mg; a selective neuronal NOS inhibitor decreased spike frequency compared to control group ($p<0.01$) and this significant decrease was observed at the 60th min till the end of the study in 180 min.

When compared to the Penicillin group, the administration of 7-NI (40 mg/ kg, i.p.) significantly reduced the frequency of epileptiform activity in the 100 minutes after injection of leptin without changing amplitude (Aslan et al., 2010). In administration of 7-NI (40 mg/kg i.p.), the frequency of epileptiform activity was dramatically reduced in 60 minutes after injecting ghrelin without altering the magnitude compared to Penicillin groups (Aslan et al., 2009).

Zhu et al. (2015) showed that the administration of a 7-NI, has significantly increased the anti-epileptic action of curcumin while the use of a selective iNOS inhibitor. Results show that the anticonvulsant activity of OXC (oxcarbazepine) was significantly potentiated by 7NI (50 mg/kg; i.p.) but not by FBM (Felbamate), LTG (lamotrigine) and TPM (Topiramate) against MES-induced seizures and, at the same time, increased the acute neurotoxic effects of TPM, but not those of FBM, LTG and OXC in the chimney test in mice. 7NI seemed to have no effect on antiseizure activity and acute neurotoxic profiles of all examined AEDs at the lower dose of 25 mg/kg (Luszczki et al., 2006).

The study indicates that 7-NI (50 mg/kg) and CBZ or PB combinations, administered at sub-protective doses, contribute to a substantial reduction in amygdala-kindled seizures in rats. Any pharmacokinetic level association is not likely because 7-NI did not influence either drug's free plasma levels. The protective effects of 7-NI combined with PB or CBZ do not appear to be contingent on NO-mediated processes, as L-arginine (500 mg/kg) was not reversed. The co- administration of 7-NI (50 mg/kg) and CBZ (10-20 mg/kg) substantially decreased seizures and the duration of discharge (Borowicz et al., 2000).

In animals treated with 100 mg/kg of 7-NI, a significant delay in a decrease in the frequency of seizures ($p<0.05$) and the onset of clonic movement ($p<0.05$) were observed

(Paul V. & Ekambaram, 2003). Mülsch et al. (1994) found that pretreatment of rats with 7-Nitroindazole (40 mg/kg, i.p.) during the first 60 minutes, attenuates kainite-induced nitric oxide production and substantially decreased epileptiform activity.

In this study, Aminoguanidine 100 mg decrease spike frequency significant at 100 min till the end studying period compared to control group ($p<0.05$).

Regular treatment of Aminoguanidine (50 or 100 mg/kg/day, i.p.) significantly and dose-dependently attenuated over a 30-day period ($p<0.01$) for the production of spontaneous recurrent seizures as expressed in terms of marked reduction of the observed progressive increase in the spontaneous recurring severity score relative to that observed in the pilocarpine control mice (Rehni et al., 2009) .

Pretreatment effect of AG (100 mg/kg) on lithium-pilocarpine status epilepticus (5 mg/kg) on the anticonvulsant trait of licofelone. AG is injected 15 minutes before to injecting to licofelone and increased licofelone's anticonvulsant effect (5 mg/kg) (Eslami et al., 2016).

The administration of Aminoguanidine (100 mg/kg) reduced to 60 and 40 percent atorvastatin (10 mg/kg)-induced protection against seizure and death respectively. The findings showed that Aminoguanidine has a significant effect in atorvastatin group on the incidence of tone seizure and death following electroshock (10 mg/kg) (P,0.05) (Shafaroodi et al., 2012).

Daily therapy with Aminoguanidine (50 or 100 mg kg/day i.p.) for 15 days significantly and dose-dependently reduced ($p<0.01$) the occurrence of kindled seizures as assessed in terms of substantial progressive suppression compared to that observed in mice belonging to the control group of the PTZ group (Homayoun et al., 2002)

Treatment with Aminoguanidine alone did not alter the number of left hippocampal neurons significantly with regard to control values ($p>0.05$). A comparison of iron and iron + Aminoguanidine rats showed that Aminoguanidine substantially decreases the loss of iron-induced neurons from 43.6% to 19.2% and protects hippocampal neurons from iron toxicity ($p< 0.001$) (Bostanci et al., 2008).

The finding of the current study, L-Arginine 500 mg decrease significant in epileptiform activity at 80 minutes till the end of the study ($p<0.001$). Yildirim M. et al. (2010) showed when L-arginine (500 mg/kg) is applied After 90 minutes, the frequency of epileptiform ECoG activity was reduced and significant effects were observed 120 minutes after L-arginine administration and seemed to last for 60 minutes. The mean amplitude of epileptiform ECoG activity did not change after L-arginine is applied

30 minutes before the effective dose of Ghrelin (1 lg, i.c.) L-arginine (1000 mg/kg, i.p.) significantly decreased the frequency of epileptiform activity within 70 minutes of ghrelin injection without changing amplitude compared with Penicillin group. Ghrelin anti-epileptic activity was reversed by administration of the L-NAME (60 mg/kg, in i.p.), given 30 min prior to ghrelin effective dose injection (1 microg, in i.c.v) (Aslan et al., 2009).

The anticonvulsant effect of pioglitazone, which was given 4 h before PTZ induction, was greatly potentiated by L-arginine (100, 200mg/kg) when co-administered with subeffective pioglitazone (20 mg/kg) (Adabi Mohazab et al., 2012).

When compared to the Penicillin group, the administration of L-arginine (1000 mg/kg, i.p.) 30 minutes prior to the effective dose of leptin (1 g, i.c.v.) significantly reduced the frequency of epileptiform activity in the 70 minutes after injection of leptin without changing amplitude (Aslan et al., 2010).

L-arginine 150, 500, and 750 mg / kg pretreatment demonstrated dose-dependent protection against seizures induced by insulin. At doses of 500 and 750 mg / kg the onset of seizures was substantially delayed. As the dose of L-arginine increased, the duration of seizures and also the number of seizures gradually decreased. The mortality also decreased with increased doses of L-arginine (Bhargave et al., 1999).

Pretreatment of L-Arginine 1000 mg on picrotoxin (PCT) convulsion prolonged the onset time of convulsion, diminished the convulsion frequency, and increased NOS activity and NO in brain regions relative to PCT alone. Whereas L-Arginine post-treatment with PCT convulsion has the maximum effect of increasing NO and NOS activity in brain regions relative to pre-treatment and prolonging the onset time and frequency seizure. Pre- and post-treatment L-Arginine on PCT lowered convulsion

threshold thus increased NO concentration after L-Arginine decreased the intensity of the convulsion and diminished the number of convulsions (Jayakumar et al., 1999).

The anticonvulsant effects of the combined low doses of morphine (0.25 mg/kg) and thalidomide (5 mg/kg) were substantially decreased by pre-treatment with a non-effective dose of NO precursor L-arginine (60 mg/kg, i.p.) ($P < 0.01$). In comparison, pretreatment either with non-selective (L-NAME, 5 mg/kg, i.p.) or selective neuronal (7-nitroindazole, 30 mg/kg, i.p.) NOS synthase (NOS) inhibitors substantially improved the anticonvulsant effects of low thalidomide and morphine combination doses, whereas the inducible NOS Aminoguanidine inhibitor (100 mg/kg, i.p.) did not (Pourshadi Nastaran et al., 2020).

4.2.4. Effect of Resveratrol and nitric oxide interaction on Penicillin induced epileptiform activity.

In the present study, we also investigated the interaction between Resveratrol and nitric oxide in Penicillin induced epileptiform activity. When co-administered effective dose of Resveratrol (50mg/kg) and 7-Nitroindazole (40mg/kg) 30 min after intracortical Penicillin G administration, there was statistically significant decrease in spike frequency ($p<0.001$) compared control group ($p>0.05$) and both potentiated their anticonvulsant effect as compared to when used alone. When Resveratrol 50 mg and Aminoguanidine 100mg were applied intraperitoneally, the spike frequency decreased considerably ($p<0.001$) and there was no significant change in spike amplitude compared to control group. Aminoguanidine increased the anticonvulsant activity of Resveratrol compared with when Resveratrol used alone ($p<0.01$). There was a statistically significant decrease in spike frequency ($p<0.05$) when effective dose Resveratrol 50 mg /kg and LNAME 60 mg/kg were co-administrated compared with control group ($p>0.05$). L-NAME decreased anticonvulsant effect of Resveratrol compared when Resveratrol 50 mg /kg used alone. When both Resveratrol 50 mg and L-Arginine 500mg/kg were applied together there was a significant reduction in spike frequency ($p<0.001$) compared to control group ($p>0.05$).

There had been no previous research on the effects of Resveratrol and nitric oxide on epilepsy, and this study is the first to evaluate this relationship to our knowledge.

However, there are a few studies in the literature dealing with this interaction apart from epilepsy. NO synthase (NOS) activity in synaptosomes isolated from rabbit brain increased approximately three-fold by Resveratrol and the NO released was converted to ONOO (-). Resveratrol increased the lipid fluidity of synaptosomal plasma membranes. These changes suggest that the incorporation of Resveratrol into synaptosomal plasma membranes causes an up-regulation of NO synthase (Fotiou et al., 2010).

Bi et al. (2005) showed that the expression of lipopolysaccharide induced iNOS in microglia was significantly inhibited by Resveratrol and provided a direct basis for Resveratrol mechanism of decreased production of NO.

In chromaffin cells, Resveratrol increased the synthesis of nitrites and NO, and L-NAME decreased both reactions; however, SNAP-induced nitrite and NO augmentation was unaffected by ODQ (oxadiazol quinoxaline) and only slightly inhibited by L-NAME (Padín et al., 2012).

Within those experiments, NO bioavailability in comparison with the vehicle increased significantly only in 100 mmol/l Resveratrol. Systemic Resveratrol treatment increased urinary cGMP and renal blood flow and reduced renal vascular resistance. Each of these actions have been mediated by NO since they were blocked by co-administration of the L-NAME (Gonzalez-Vicente et al., 2014)

Accumulation of methylglyoxal adduct was associated in in vitro with a significant mesenchymal phenotype and increased migration/invasion in anaplastic thyroid cancer cells which were reversed by RSV and AG (Antognelli et al., 2019).

Chen H. E. et al. (2019) they discovered that in male adult offspring, the high-fat (HF) postnatal diet amplifies maternal L-NAME treatment-induced programmed hypertension, which was reduced by Resveratrol. Increased oxidative stress, inhibition of the peroxisome proliferator-activated receptor co-activator 1 (PGC-1) / AMP-activated protein kinase (AMPK) pathway and changed gut microbiota compositions are associated with combined L-LAME and HF diet-induced hypertension.

5. CONCLUSION

1. Penicillin (500 IU, i.c.) was given, and after Penicillin injection, epileptic spikes and spike-wave complexes started to appear within 2-5 minutes. Epileptiform activity stabilized within 30 minutes and continued the recording for 180 minutes.
2. Resveratrol 25, 100 mg /kg was administered intraperitoneally 30 min after intracortical Penicillin injection. There was no statistically significant change in both spike frequency and spike amplitude in comparison to the penicillin group ($p>0.05$).
3. When Resveratrol 50 mg /kg was intraperitoneally administered 30 min after intracortical Penicillin injection, the spike frequency decreased significantly starting from the 100th minute till the end of the study ($p<0.01$), and we considered as affective dose. No statistically significant difference in spike amplitude was observed over the 180 minutes' period in comparison to the penicillin group.
4. 7- Nitroindazole 40 mg /kg was administered intraperitoneally 30 min after intracortical of injection of Penicillin G 500 IU, the spike frequency decreased significantly at the 60th minute until the end of the study period in 180 minutes ($p<0.05$) and there was no difference in spike amplitude relative to penicillin group ($p>0.05$).
5. The effective dose of Resveratrol 50 mg /kg and 40 mg /kg 7-NI were administered intraperitoneally after 30 minutes of 500 IU Penicillin G being added intracortically. The spike frequency decreased significantly at the 60th minute until the end of the study period in 180 minutes ($p<0.01$) and there was no significant change in spike amplitude in comparison to a penicillin group ($p>0.05$). The effect of the combination of Resveratrol with 7-NI was stronger than the anticonvulsant effect of each substance given alone. According to this result, Resveratrol and 7-NI potentialized each other's effects.
6. Aminoguanidine 100 mg was intraperitoneally applied 30 min after injection of Penicillin G 500 IU. The spike frequency decreased at 30-80 minutes ($p<0.05$) and there was no significant change in spike amplitude in comparison to the penicillin group ($p>0.05$).

7. The effective dose of Resveratrol 50 mg and Aminoguanidine 100 mg were intraperitoneally administered together 30 minutes after intracortical injection of Penicillin G 500 IU. The spike frequency reduced ($p<0.01$) relative to the penicillin group and there was no significant change in spike amplitude ($p>0.05$) When Resveratrol and Aminoguanidine were applied together they caused more anticonvulsant effects.
8. Thirty min after Penicillin G 500 IU injected intracortically, L-NAME 60 mg was administered intraperitoneally, and there were no notable differences in both spike frequency and amplitude in comparison to the penicillin group ($p>0.05$).
9. Thirty min after 500 IU Penicillin injection, effective dose of Resveratrol 50 mg and L-NAME 60 mg were applied together intraperitoneally. There was a significant reduction in spike frequency ($p<0.05$) when combined, in comparison to the penicillin group, there were no notable differences in spike amplitude ($p>0.05$). L-NAME decreases anti-convulsant effect of Resveratrol when they combined together.
10. Thirty min after intracortically injected Penicillin G 500 IU, L-Arginine 500 mg were administered intraperitoneally and there was statistically significant change in spike frequency ($p<0.01$) and there was no notable difference in spike amplitude until the end of the study ($p>0.05$).
11. The effective dose of Resveratrol 50 mg and 500 mg L-Arginine were administered intraperitoneally after 30 minutes of 500 IU Penicillin G being applied intracortically. The spike frequency fell significantly between 80-110 and 120-180 minutes ($p<0.001$) and there was no notable difference in spike amplitude ($p>0.05$).

In this study, Resveratrol was determined to have an anticonvulsant effect on Penicillin induced epileptiform activity. The effect of Resveratrol on the epileptiform activity was via NO production that involves nNOS and iNOS. The interaction of NO and Resveratrol potentiated each other and more anticonvulsant effects emerged. Further studies are needed to fully understand the molecular mechanism of NO and Resveratol interaction.

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