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Study of the potential neuroprotective effect of Rifampicin on lithium-pilocarpine induced seizures in rats

A Thesis

submitted in partial fulfillment of the requirements for
Master Degree in ***Pharmaceutical Sciences***
(Pharmacology & Toxicology)

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B.Sc. of Pharmaceutical Sciences, Ain Shams University, May 2015

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2019

Acknowledgement

*First and foremost, I feel always indebted to **ALLAH**, whose blessing I will never be able to thank him for.*

*I would never have been able to finish my thesis without the guidance of **Dr. Samar Azab**, Assistant Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University. Dr. Samar's positive attitude, caring, endless patience, valuable suggestions and continuous encouragement cannot be described by words. Trillion to the power infinity thanks to you, Dr.Samar is not enough.*

*It is my pleasure to express my gratitude to **Dr. Doaa Ahmed**, Lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, to whom I owe much for her keen supervision, generous support, continuous guidance and valuable scientific discussion. Dr.Doaa, I simply could not wish for a better or friendlier supervisor.*

*I want to express my deepest thanks and gratitude to **Dr. Heba Mahdy,** Lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for her enthusiastic help, encouragement, generous attitude, profound understanding and endless support. The excellent friendship of you, Dr Heba is invaluable.*

It is difficult to overstate my gratitude to my colleagues, co-workers and all the department of Pharmacology and Toxicology in the faculty of pharmacy, Ain Shams University, for their continuous support, generous attitude and indispensable help.

Finally my deepest everlasting thanks and appreciation are directed to my beloved husband, parents, grandmother, parents-in–law ,brothers, all my family and friends for their understanding, endless patience and encouragement when it was most required, to them I dedicate this thesis .

To everyone who made this work possible, I am indeed grateful.

Alaa Emam

ABSTRACT

Epilepsy is one of the serious neurological sequelae of bacterial meningitis. Rifampicin, the well-known broad spectrum antibiotic, is clinically used for chemoprophylaxis of meningitis. Besides its antibiotic effects, rifampicin has been proven to be an effective neuroprotective candidate in various experimental models of neurological diseases. In addition, rifampicin was found to have promising antioxidant, anti-inflammatory and anti-apoptotic effects. Herein, we investigated the anticonvulsant effect of rifampicin at experimental meningitis dose (20mg/kg, i.p.) using lithium-pilocarpine model of status epilepticus (SE) in rats. Additionally, we studied the effect of rifampicin on seizure induced histopathological, neurochemical and behavioral abnormalities. Our study showed that rifampicin pretreatment attenuated seizure activity and the resulting hippocampal insults marked by hematoxylin and eosin. Markers of oxidative stress, neuroinflammation and apoptosis were evaluated, in the hippocampus, 24 hr after SE induction. We found that rifampicin pretreatment suppressed oxidative stress as indicated by normalized malondialdehyde and glutathione levels. Rifampicin pretreatment attenuated SE-induced neuroinflammation and decreased the hippocampal expression of interleukin-1β, tumor necrosis factor-α, nuclear factor kappa-B, and cyclooxygenase-2. Moreover, rifampicin mitigated SE-induced neuronal apoptosis as indicated by fewer positive cytochrome c immunostained cells and prevent the elevation of caspase-3 activity in the hippocampus. Furthermore, Morris water maze testing at 7 days after SE induction showed that rifampicin pretreatment can improve cognitive dysfunction. Therefore, rifampicin, currently used in the management of meningitis, has a potential additional advantage of ameliorating its epileptic sequelae.

Keywords: seizure, meningitis, lithium-pilocarpine, Rifampicin, hippocampus

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I. LIST OF ABBREVIATION

Abbreviation	*Term*
°C	Degree Celsius
µl	Micro liter
µg	Micro gram
µmol	Micro mole
A_{sample}	Sample absorbance
$A_{standard}$	Standard absorbance
AAP	4-aminoantipyrene
AED	Anti epileptic drug
Ag	Antigen
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
ATP	Adenosine-5'-triphosphate
AUC	Area under the curve
Aβ	Beta-amyloid
Aβ	Amyloid beta protein
BBB	Blood–brain barrier
BSA	Bovine serum albumin
Ca^{++}	Calcium
CA	Cornu Ammonis
CAT	Catalase
CBZ	Carbamazepine
cm	Centimeter
CNS	Central nervous system
COX-2	Cyclooxygenase- 2
CPSs	Complex partial seizures
CSF	Cerebrospinal fluid
Cu	Cupper
DHBS	3, 5-Dichloro-2-hydroxybenzene sulfonic acid
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
DNA	Deoxyribonucleic acid
EAA	Excitatory amino acid
EAAT	Excitatory amino acid transporter
ECS	Extra cellular space
EEG	Electroencephalogram
ELISA	Enzyme linked immunosorbent assay

Abbreviation	*Term*
FDA	Food and drug administration
Fe	Ferrous
g/gm	Gram
GABA	Gamma-aminobutyric acid
GSH	Glutathione reduced
hr	Hour
H&E	Hematoxylin and Eosin
H_2O	Water
H_2O_2	Hydrogen peroxide
HRP	horseradish peroxidase
i.p.	Intraperitoneal injection
IHC	Immunohistochemical
ILAE	International league against epilepsy
IL-6	Interleukin- 6
IL-1β	Interleukin- 1 Beta
K^+	Potassium,
Kg	Kilogram
LiCl	Lithium chloride
LTA	Lipoteichoic acid
M	Molar
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mmol	Millimole
MPP+	1-methyl-4-phenylpyridinium
mTLE	Mesial temporal lobe epilepsy
MWM	Morris water maze
Na^+	Sodium
Na^+-K^+-ATPase	Sodium potassium ATP pump
NF-κB	Nuclear factor-kappa b
ng	nanogram
nm	Nanometer
nmol	Nanomole
NMDA	N-methyl-D-aspartate
nTLE	Neocortical temporal lobe epilepsy
O.D	Optical density
p.o.	Per oral

Abbreviation	*Term*
pNA	Para -nitro aniline
PC12	Rat pheochromocytoma cells
PD	Parkinson disease
PEG	Polyethylene glycol
R.T	Room temperature
RIF	Rifampicin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
s/sec	Second
SE	Status epilepticus
SEM	Standard error of mean
SOD	Superoxide dismutase
SRS	Spontaneous recurrent seizure
TBA	Thiobarbaturic acid
TBS	Tris buffered saline
TGF-β	Transforming growth factor-beta
TLE	Temporal lobe epilepsy
T_m	Transport maximum
TNF-α	Tumor necrosis factor-alpha
UV	Ultra-violet light
VNS	Vagal nerve stimulation

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1. INTRODUCTION

1.1 Epilepsy:

Epilepsy is a chronic neurological disease of the brain which is characterized by the occurrence of recurrent spontaneous seizures. It contributes 1% of the global burden of disease affecting the lives of nearly 50 million people globally, out of which around 80% reside in developing countries **(Birbeck, 2010; Megiddo et al., 2016)**.

1.1.1 Definition of seizure and epilepsy:

An epileptic seizure is defined as: a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy occurs when someone has an epileptic seizure and their brain shows a pathologic and enduring tendency to have recurrent seizures **(Fisher *et al*., 2014).**

Epilepsy is diagnosed when an individual has **(Fisher *et al*., 2014; Falco-Walter *et al*., 2018)**:

1) At least two unprovoked or reflex seizures>24 h apart;

2) One unprovoked or reflex seizure and a probability of having another seizure similar to the general recurrence risk after two unprovoked seizures (≥60%) over the next 10 years;

Examples of evidence that increases the probability of having additional seizures include:

A) Epileptiform activity on EEG (Electroencephalogram) or

B) A potential epileptogenic abnormality on brain imaging.

3) An epilepsy syndrome; epilepsy syndromes refer to clusters of features (seizure type(s), EEG findings, imaging findings, age-dependent features, triggers and sometimes prognosis) that occur together.

1.1.2 Classification of seizures and epilepsies:

1.1.2.1 The classification of seizure types according to the International League against Epilepsy (ILAE) 2017 is summarized by Figure 1:

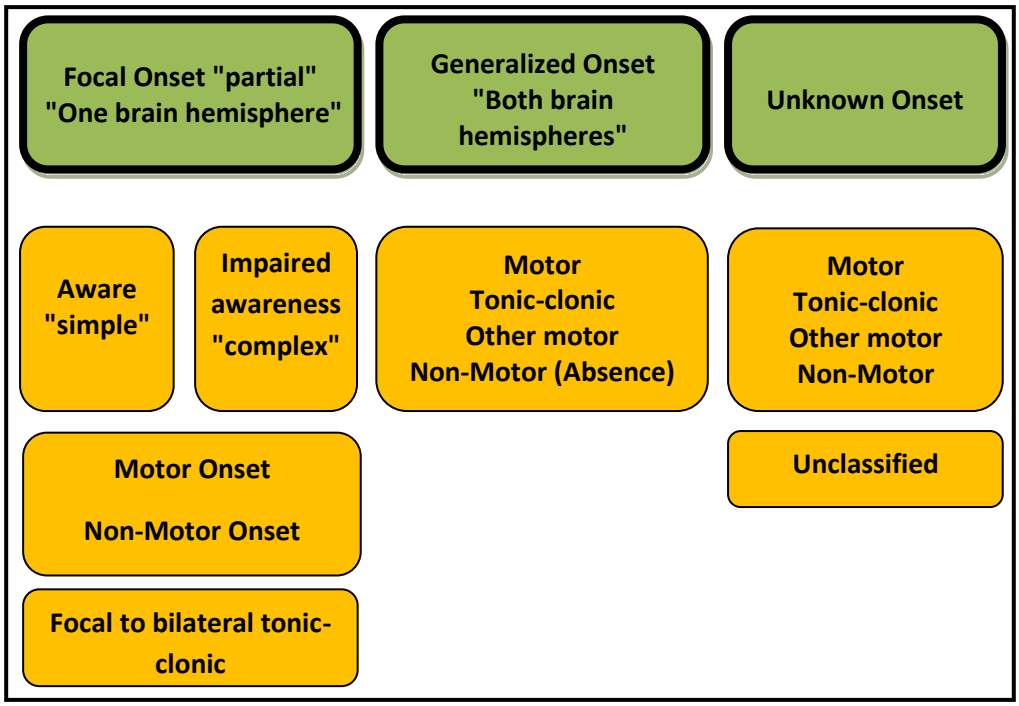

Figure 1: ILAE 2017 classification of seizure types **(Falco-Walter *et al.*, 2018)**.

1.1.2.2 Classification of epilepsy:

After classification of seizure type, the clinician should aim to identify the patient's epilepsy type. To classify an epilepsy type, a patient must have met the definition and the criteria for epilepsy, as was mentioned before. The epilepsy type classification is broader in scope than is the seizure classification as it considers the possibility of having multiple seizure types, and includes information about the overall clinical picture, genetics, imaging, laboratory tests, prognoses and co morbidities **(Falco-Walter *et al.*, 2018)**.

Epilepsy types are classified as:

1) Focal **2) Generalized**

3) Combined Generalized and Focal **4) Unknown**.

The patient is placed into one of these categories after classifying all types of seizures he has and then mapping them in aggregate to one of these four categories. **(Scheffer *et al.*, 2017)**

1.1.3- Epilepsy etiology:

Primary epilepsy (50%) is idiopathic ("unknown cause"). In secondary epilepsy (50%), referred as acquired epilepsy **(Reddy, 2014)**, seizures may result from a variety of conditions including trauma, metabolic imbalances, anoxia, cerebrovascular disease, infections of the central nervous system (CNS), tumors, drug withdrawal seizures, or neurotoxicity **(Loscher and Brandt, 2010)**.

The clinician should consider the etiology of seizures due to its critical impact on epilepsy management and prognostic counseling. The ILAE has defined six etiologic categories, focusing on those etiologies with management implications **(Belousova *et al.*, 2017).**

These categories are:

- **Structural etiology:**

The presence of a finding on neuro-imaging which reasonably can be concluded to be the cause of patient's seizures **(Lapalme-Remis and Cascino, 2016)**

- **Genetic etiology:**

The presence of a specific disease-causing variant in a gene, which is believed to be pathogenic for epilepsy, would lead to a genetic classification. Moreover, having a relevant family history and typical features (EEG, seizure semiology) is sufficient for a genetic etiology classification **(Hildebrand *et al.*, 2013)**.

- **Infectious etiology:**

CNS Infections and infestations are among the most common risk factor for seizures and acquired epilepsy and are probably the most common preventable risk factor for epilepsy **(Vezzani *et al*., 2016; Ramantani and Holthausen, 2017)**. People of any age may develop seizures due to infections. It is important to differentiate between early seizures (also termed acute symptomatic, provoked, or insult-associated seizures), which may occur within the first 1–2 weeks after infection **(Beghi *et al*., 2010)** and late unprovoked seizures, which occur later (often months to years) after infection and are therefore defined as acquired epilepsy **(Lowenstein, 2009).**

Early seizures which occur at or soon after the infection occur in up to 30 % of all CNS infections **(Singhi, 2011)**. and are not considered spontaneous seizures; they are thought to be mechanistically different from any subsequent consequential chronic epilepsy **(Shorvon and Guerrini, 2010).** Early seizures are a risk factor for the later development of epilepsy. However, not all people with early seizures will develop epilepsy **(Vezzani *et al.*, 2016).**

Status epilepticus (SE), defined as continuous seizure activity for more than 5 min or consecutive seizures without recovery of consciousness, can be a serious consequence of CNS infections **(Trinka *et al.*, 2012)**. Status epilepticus is a life-threatening neurological and medical emergency with high mortality,