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ANTICONVULSANT AND PROCOGNITIVE EFFECT OF NONIMIDAZOLE HISTAMINE H3R RECEPTOR ANTAGONISTS /INVERSE AGONISTS IN EXPERIMENTAL ANIMAL MODELS

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ANTICONVULSANT AND PROCOGNITIVE EFFECT OF NON-IMIDAZOLE HISTAMINE H3R RECEPTOR ANTAGONISTS /INVERSE AGONISTS IN EXPERIMENTAL ANIMAL MODELS

Alaa Imad Alachkar

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Under the Supervision of Dr. Bassem Shaban Sadek

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Declaration of Original Work

I Alaa Imad Alachkar, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this dissertation entitled "*Anticonvulsant and Procognitive Effect of Non-imidazole Histamine H3R Receptor Antagonists /Inverse Agonists in Experimental Animal Models*", hereby, solemnly declare that this dissertation is my own original research work that has been done and prepared by me under the supervision of Dr. Bassem Sadek, in the College of Medicine and Health Sciences at UAEU. This work has not previously been presented or published or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my dissertation have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this dissertation.

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Abstract

Epilepsy is a common chronic neurological disorder accompanied by cognitive impairment. Available antiepileptic drugs (AEDs) have not been reported to have ameliorative effects on epilepsy-associated memory impairment. The potential of histamine H3 receptors (H3R) in several neuropsychiatric diseases, including epilepsy and Alzheimer's disease, is well recognized. In this study, a series of H3R antagonists (1-16) were screened for their in vivo anticonvulsant effect in several acute-induced seizures in rats. Moreover, the procognitive effect of the most promising H3R antagonist was investigated in dizocilpine (DIZ)-induced amnesic effect applying several behavioral memory tests. Furthermore, the most promising H3R antagonist was assessed for its simultaneous anticonvulsant and procognitive effect and its modulatory effect on levels of oxidative stress markers, several hippocampal neurotransmitters, and c-fos protein expression in PTZ model. Finally, the promising H3R antagonist was examined for its anticonvulsant effect in PLC-induced SE and its ability to mitigate SE incidence.

The Observed results indicated that H3R antagonist 4 (10 mg/kg i.p.) significantly exhibited high protection in maximum electroshock (MES)-induced seizures facilitated through histaminergic neurotransmission and activation of post-synaptically located H1R and full protection in the PTZ-acute induced seizures, . Moreover, H3R antagonist 4 (5 mg/kg i.p.) showed a procognitive effect that was abrogated with RAM co-injection in all behavioral memory tests. Additionally, treatment with H3R antagonist 4 showed a simultaneous anticonvulsant and procognitive effect in addition to antioxidant effect in PTZ- acute and -chronic models. Furthermore, chronic treatment with H3R antagonist 4 (5 mg/kg i.p.) modified histamine, acetylcholine, and glutamate release, and reduced hippocampal c-fos activation. In addition, RAM administration reversed the protective effects provided by H3R antagonist 4 in PTZ-chronic model. Moreover, and in PLC-induced SE, systemic administration of H3R antagonist 4 (10 mg/kg i.p.) mitigated severity of SE and exhibited antioxidant effect in the hippocampus of the treated rats, facilitated through the histaminergic neurotransmission. The observed findings recommend that the newly developed H3R antagonist 4 provides antiepileptic, memory-enhancing, and antioxidant properties in

a PTZ-induced kindling model of epilepsy and provides neuroprotection in a preclinical PLC-induced SE in rats, highlighting the histaminergic system as a potential therapeutic target for the management of epilepsy with accompanied memory deficits.

Keywords: Histamine H3 receptor antagonist, Epilepsy, Hippocampus, Pentylenetetrazol, Pilocarpine, Oxidative stress, c-fos, Memory impairment, Status epilepticus.

Title and Abstract (in Arabic)

دراسة الفعالية المضادة للصرع والمحسنة للذاكرة لعدد من مثبطات مستقبلات الهستامين-H3 المستخلقة حديثًا في نماذج فئران بحثية مختلفة

الملخص

مرض الصرع هو مرض عصبي مزمن شائع يصاحبه ضعف الإدراك. لم يتم الإبلاغ عن العقاقير المتاحة المضادة للصرع ليكون لها آثار تحسين على ضعف الذاكرة المرتبطة بالصرع. إن إمكانية استخدام مستقبلات الهستامين (H3R) في العديد من الأمراض العصبية والنفسية ، بما في ذلك مرضيِ الصرع والزهايمر ، معروفة جيدًا. في هذه الدراسة تم فحص التأثير المضاد للتشنج لعدد من المركبات الجديدة من مثبطات مستقبلات الهيستامين3(H3R) باستخدام نماذج بحثية مختلفة للصرع. علاوة على ذلك ، تم التحقق من التأثير المقوي للذاكرة لمثبط مستقبلات الهستامين الأكثر فعالية في فقدان الذاكرة الناتج عن استخدام DIZ من خلال تطبيق اختبارات الذاكرة السلوكية. إضافة إلى ذلك، تم التحقيق من تأثيره المضاد للتشنج والمقوي للذاكرة في وقت واحد بالإضافة إلى تأثيره المضاد للأكسدة في النماذج الحادة والمزمنة من PTZ و نموذج PLC الذي يسبب الحالة الصرعية SE. أيضا، تم تقييم تأثير المركب في الحصين على مستويات الناقلات العصبية وعلى مستوى c-fos من خلال تقنية اللمعان المناعي في نموذج PTZ.

أظهرت النتائج أن بعض المركبات كانت فعالة في تخفيف نوبات الصرع الناتجة عن الصدمات الكهربائية MES، و فعالة أيضا في الحماية من نوبات الصرع الناتجة عن استخدام (PTZ) مثل مثبط مستقبلات الهيستامينH3 و رقمه 4، وقد تم إبطال هذا المفعول باستخدام منشط مستقبلات الهيستامين H3 (RAM) وهذا فقط في نموذج نوبات الصرع الناتجة عن الصدمات الكهربائية. علاوة على ذلك ، أظهر مثبط مستقبلات الهيستامين H3 رقم 4 (10 ملغ/ كغ) تأثيرًا مقوي للذاكرة تم إلغاؤه باستخدام حقن RAM المشترك في جميع اختبارات الذاكرة السلوكية. بالإضافة إلى ذلك

، أظهر مثبط مستقبلات الهيستامين H3 رقم 4 تأثيرًا مضادًا للتشنج و لفقدان الذاكرة في وقت واحد وتأثيرًا مضادًا للأكسدة في نماذج PTZ- الحادة والمزمنة. من ناحية أخرى، فإن العلاج المزمن بمثبط مستقبلات الهيستامين 3 رقم 4 قد عدل تحرير الهيستامين والأستيل كولين والغلوتامات ، وقلل من تنشيط c-fos في الحصين. وأدى حقن RAM إلى عكس الآثار الوقائية التي يوفرها مثبط H3R رقم 4 (5 ملغ / كغ) في نموذج PTZ المزمن. إلى جانب ذلك ، فإن العلاج المضاد ل H3R الحاد (10 ملغ/ كغ) قد خفّض الحالة الصرعية SE التي يسببها PLC وأظهر تأثيراً مضاداً للأكسدة في الحصين، وأدى استخدام RAM إلى عكس التأثير الوقائي.

توصي نتائجنا بأن مضادات H3R المشيدة حديثًا قادرة على توفير خصائص مضادة للصرع ، معززة للذاكرة ، ومضادة للأكسدة في نموذج تأجيج الصرع الناجم عن PTZ وتوفر الحماية العصبية في فئران الحالة العصبية التي يسببها PLC في الفئران ، مما يسلط الضوء على نظام الهيستامين كعلاج محتمل للإدارة العلاجية للصرع.

مفاهيم البحث الرئيسية: الصرع ، نماذج الفئران ، مثبطات مستقبلات الهستامين H3 ، ضعف الذاكرة ، الالتهاب العصبي ، الإجهاد التأكسدي ، الحصين.

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Dedication

To my beloved parents and family

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Arachidonic acid
ACh	Acetylcholine
AChE	Acetylcholine esterase
AD	Alzheimer's disease
ADHD	Attention-deficit hyperactivity disorder
AEDs	Antiepileptic drugs
BSA	Bovien serum albumin
BZ_S	Benzodiazepines
cAMP/PKA	Cyclic adenosine monophosphate/ protein kinase A
CAT	Catalase
CBZ	Carbamazepine
CHO-K1	Chinese hamster ovary cell
CNS	Central nervous system
DI	Discrimination index
DIZ	Dizocilpine
DOZ	Donepezil hydrochloride
DZP	Diazepam
EEG	Electroencephalogram
ELISA	Enzyme linked-immunosorbent assay
EPM	Elevated plus maze
ETX	Ethosuximide
FPVD	French pharmacovigilance database

GABA	Gamma-aminobutyric acid
GLU	Glutamate
GPCR	G- protein-coupled receptor
GPx	Glutathione peroxidase
GSH	Glutathione
GTCS	Generalized tonic-clonic seizures
HA	Histamine
HDC	Histidine decarboxylase
hH1R	Human histamine 1 receptors
hH3R	Human histamine 3 receptors
hH4R	Human histamine 4 receptors
HNMT	Histamine-N-methyl transferase
H1R	Histamine H1 receptor
H2R	Histamine H2 receptor
H3R	Histamine H3 receptor
H4R	Histamine H4 receptor
HS	Hippocampal sclerosis
IAP	Inhibitory avoidance paradigm
I.C.V	Intracerebroventricular
IEG	Immediate early gene
IHC	Immunohistochemistry
ILAE	International League against Epilepsy
i.p.	Intraperitoneal
IQ	Intelligence quotient

KA	Kainic acid
Ki	Inhibitor constant
KO	Knockout
LEDs	Light-emitting diodes
LTD	Long-term depression
LTM	Long term memory
LTP	Long-term potentiation
MDA	Malondialdehyde
MES	Maximal electroshock
MFS	Mossy fiber sprouting
MRI	Magnetic resonance imaging
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NO	Nitric oxide
NOR	Novel object recognition
OFT	Open Field Test
PB	Phenobarbital
PHT	Phenytoin
PLC	Pilocarpine
PTZ	Pentylenetetrazol
PYR	Pyrilamine
RAM	(*R*)-α-methylhistamine
ROS	Reactive oxygen species
RT	Room temperature

SAL	Saline
SCO	Scopolamine hydrobromide
SE	Status Epilepticus
SEM	Standard error for the mean
SOD	Superoxide dismutase
SPSS	Statistical package for the social science
STL	Step-through latency
STM	Short term memory
STR	Strychnine
TBA	Thiobarbituric acid
THLE	Tonic hind limb extension
TLE	Temporal lobe epilepsy
TLT	Transfer latency time
TM	tuberomammillary
TMD	Trimethadione
TMN	Tuberomammillary nucleus
VMAT-2	Vesicular monoamine transporter-2
VPA	Valproic acid
ZOL	Zolantidine dimaleate
α-FMH	α-fluoromethyl histidine

Chapter 1: Introduction

1.1 Epilepsy

1.1.1 Epilepsy Definition

Epilepsy is considered one of the most common neurologic diseases, affecting around 65 million people worldwide[1], and it is a complicated cluster of neurological disorders, characterized mainly by chronic persistent and unprovoked interruptions of the normal brain function, known as epileptic seizures. An epileptic seizure is the momentary incidence of signs caused by abnormal excessive or synchronous neuronal activity in the brain[2]. Epilepsy affects around 5-8 per 1000 inhabitants in high-income countries[3], whereas the prevalence is 10 per 1000 in low-income countries, and an even higher percentage has been documented in rural areas[4]. Epilepsy is not a singular disorder existence but a group of disorders manifesting underlying brain dysfunction that might result from various triggers.

The conceptual description of epilepsy, as proposed by the International League Against Epilepsy (ILAE) in 2005, is defined as a brain disorder characterized by an enduring predisposition to generate epileptic seizures, which necessitates at least two unprovoked seizures >24 h apart[2]. However, in 2014 ILAE formulated a practical definition with the addition of time limitation, which is more useful in both diagnosis and treatment of epilepsy. Epilepsy is a brain disorder that meets any of the following conditions that include experience a minimum of two unprovoked seizures taking place >24 h apart, experience of one unprovoked seizure with probability of experiencing additional seizures comparable to general recurrence risk (at least 60%) after two unprovoked seizures, and taking place over the next ten years, and diagnosis of the epilepsy syndrome[5]. Furthermore, patients are considered to be free of epilepsy

if they have not experienced any seizures during the last ten years with no antiepileptic drugs (AEDs) administered over the last five years, or for age-dependent patients that have now passed applicable epilepsy.

1.1.2 Epilepsy Classification

ILAE Commission on classification and terminology in 2010 has revised the attempts previously proposed for the classification of epileptic seizures in 1981 and 1989[6-8]. ILAE identified two types of epileptic seizures, namely generalized and focal seizure. Accordingly, generalized epileptic seizures are seizures originating from large parts of the cortex in both hemispheres (not necessarily the entire cortex), which can involve cortical and subcortical structures. These seizures include several subtypes, e.g. absence, generalized tonic-clonic (GTC), myoclonic, and atonic seizures. On the other hand, focal (partial) epileptic seizures originate from a small specific part of the cortex in one hemisphere[8].

Another ILAE classification of epilepsy is based according to the underlying cause, which are as follows:

1) Genetic: genetic defect(s), where seizures are the main symptom of the disease examples: childhood absence epilepsy and Dravet syndrome,

2) Structural/metabolic: structural or metabolic condition or disease which has been demonstrated to be associated with an increased risk of developing epilepsy in appropriately designed studies. Examples: trauma, stroke, and some infections, and

3) Unknown: nature of the underlying cause is yet not known[9].

1.1.3 Pathophysiology of Epilepsy

An epileptic seizure is due to temporary irregular synchronization of brain neurons that interrupts the usual patterns of neuronal interaction and leads to waning electrical discharges in the Electroencephalogram (EEG), which causes several signs depending on the site of seizure's origin. Within the seizure origin, convulsions are frequently believed to originate from an imbalance between excitation and inhibition either by increased excitation or decreased inhibition[10,11]. The interconnectivity among neuronal networks permits for harmonization of various missions and behaviors may be the cause for the wide broad range of comorbidities associated with epilepsy, including depression, learning disabilities, and autistic features[12]. Several mechanisms can result in neuronal excitation; neurotransmitters can be released from synaptic vesicles and go through the synaptic membrane into the synaptic cleft and immediately activate the receptors located post-synaptically. In addition, extrasynaptic receptors can be activated by neurotransmitters leaking directly into the extrasynaptic space. Moreover, presynaptic neurotransmitters release can be regulated through presynaptic receptors. On the other hand, inhibitory networks are positioned inside the similar or among distinct brain regions and contain several cell types with complicated intrinsic connections.

Under typical physiological environments, the networks are regulated by inhibitory neurons, granting typical functions to arise, but if these neurons are further inhibited, the network may become disinhibited, resulting in a seizure. Consequently, multifactorial inter-neuronal connections among inhibitory or excitatory neurons may cause a pathological neuronal hypersynchrony and seizures[13-15]. Also, and as previously shown by Engel et al., an epileptic seizure is a multifactorial condition

(Figure 1)[16]. The seizure threshold is increased by current AEDs, thus decreasing the tendency of seizure to happen. Epileptogenic abnormalities can be genetic, structural, or metabolic. Finally, the precipitating factors can contribute to the seizure initiation, e.g., exposed to light in photosensitive epileptic patients. None of the previous factors are static and can interchange from one time to another.

From all the above-mentioned indications, epilepsy is considered a condition with persisting cerebral dysfunction that produces persistent epileptic convulsions without the need for a direct stimulus to provoke each seizure[17].

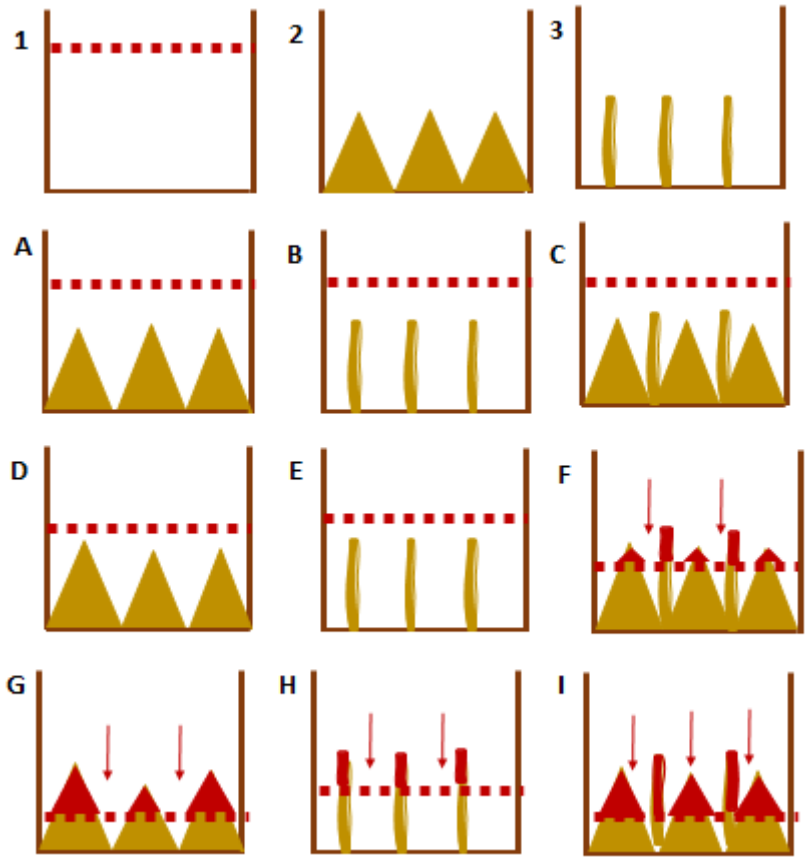


Figure 1: Multifactorial basis of epilepsy.

Multifactorial basis of epilepsy. (1) dashed line indicates seizure threshold. (2) represents epileptogenic abnormalities (3) represents different transit factors (precipitating factors). The other panels illustrate how these three factors interact. Modified from[16].

1.1.4 Antiepileptic Drugs (AEDs)

The precipitous rise in the understanding of neurotransmitters' mode of action and synaptic neurophysiology resulted in improvements in epilepsy treatment due to the development of innovative AEDs. The gained knowledge permitted the development of wisely targeted treatments to compete with traditional practical approaches. Earlier, the long-standing clinically valuable anticonvulsant drugs, including phenobarbital (PB), potassium bromide, and trimethadione were accidentally discovered by investigating their anticonvulsant effect based on their sedative or hypnotic properties, as it was known that sedation will result in damping effect of the neuronal activity[18]. Afterward, by applying a wide range of animal models, several considerable AEDs were clinically released into the market before the 1990s and were categorized as first-generation AEDs.

Phenobarbital (PB):

PB is one of the oldest AEDs. In 1912, PB was launched as a sedative drug, and then its antiepileptic effects were accidentally discovered. PB exerts its antiepileptic effect by enhancing the inhibitory neurotransmission γ-aminobutyric acid (GABA) through binding to $GABA_A$ receptor[19]. PB is still widely used in status epilepticus (SE), although it is effective against almost all types of seizures, excluding absence seizures[20].

Phenytoin (PHT):

PHT was discovered in 1983 by Merritt and Putnam applying electroshock- acute seizure model in cats[21]. PHT showed higher efficiency and less sedating effect than PB[22], and it is still one of the most widely used drugs against generalized and partial

seizures[23]. PHT was found to mainly block sodium channels, resulting in an inhibition of neuronal depolarization [24].

Trimethadione (TMD):

TMD was identified in 1944 by Everett and Richard using acute pentylenetetrazol (PTZ)-induced seizure model. TMD, unlike PHT, is effective against absence seizures[25]. However, TMD is no longer used due to its high risk of adverse effects.

Ethosuximide (ETX):

ETX was discovered in 1958, and nowadays it is only indicated for treating typical childhood absence epilepsy[26]. ETX was found to block T-calcium channels, resulting in an inhibition of neuronal depolarization[24].

Valproic Acid (VPA):

VPA was introduced to the market in 1960s[27]. It is one of the most widely used AEDs around the world not only in epilepsy but also as s mood stabilizer drug, and it is effective against all types of seizures[19]. VPA inhibits voltage-gated sodium channels resulting in an inhibition of neuronal depolarization, increasing GABA neurotransmission not through direct interaction with the GABA receptors, but probably by improving glutamate decarboxylase or inhibiting GABA transaminase enzyme. Also, VPA was found to alter dopaminergic and serotoninergic transmissions[24,27-29].

Benzodiazepines (BZs):

Leo Sternbach was the first to discover the BZs in the USA; however, the first clinically launched BZ (chlordiazepoxide) was in the UK in 1960, and diazepam (DZP) followed in 1963. After that, carbamazepine (CBZ) was introduced to the

market in the 1970s in the USA, and it is effective against generalized seizures[30]. CBZ was found to inhibit voltage-gated sodium channels resulting in an inhibition neuronal depolarization. Moreover, BZs were revealed in their stimulating effects on GABA-mediated inhibition by binding to the $GABA_A$ receptor binding site.

During the 1990s and the 21st century, additional AEDs were discovered and released into the market. These AEDs were categorized as second-generation AEDs (new AEDs). Second-generation AEDs have a broader therapeutic index, fewer side effects, and fewer drug-drug interactions compared with the traditional first-generation AEDs. Several new AEDs were, also, approved for epilepsy treatment, including topiramate, levetiracetam, vigabatrin, lamotrigine, felbamate, gabapentin, tiagabine, oxcarbazepine, zonisamide, pregabalin, rufinamide, and lacosamide[31]. Eslicarbazepine, stiripentol, pregabalin, rufinamide, lacosamide, and perampanel were only licensed for adjunctive use[32].

Overall, the general mechanisms of the available AEDs are related to reducing neuronal excitation and raising postsynaptic inhibition or modifying synchronization of neural networks to reduce the superfluous neuronal excitability associated with seizure development. Moreover, several AEDs inhibit the neuronal depolarization through voltage-dependent ion sodium and calcium channels blockade, e.g., PHT, CBZ, oxcarbazepine, lamotrigine, topiramate, and VPA; or by enhancing potassium channel function, e.g., retigabine[24]. Furthermore, the neuronal depolarization can be blocked due to glutamate-induced inhibition of excitation, e.g., felbamate. Other AEDs

work by stimulating GABA-mediated inhibition, e.g., vigabatrin, tiagabine, clobazam, and PB[24] (Figure 2).

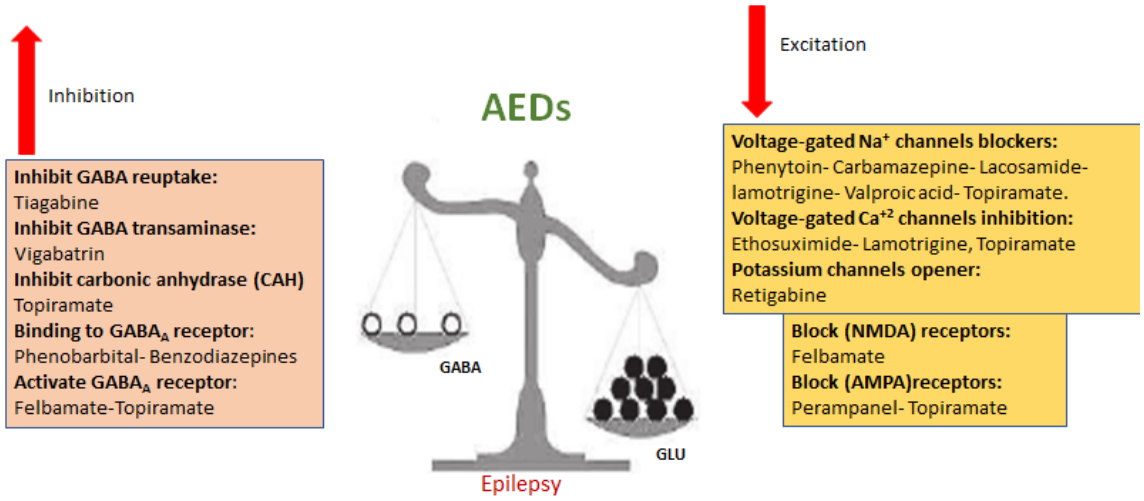


Figure 2: Mechanisms of action of selected available AEDs.

NMDA: *N*-methyl-D-aspartate; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid.

1.1.4.1 Memory deficits associated with clinical use of AEDs

Epilepsy is a chronic disorder with devastating neurological results on the patients, necessitating long time therapy with AEDs. Currently available (AEDs) are not capable of providing the sufficient. seizure control in almost one-third of the patients and also do not stop the underlying epileptogenic alterations[33]. Moreover, the consistent use of one or more AEDs will augment the epilepsy-associated comorbidities, such as cognitive impairment[34]. It is stated that AEDs decrease neuronal irritability; however, it also can damage neuronal excitability, neurotransmitter levels, several genes, different enzymes, and factors that are required in memory and learning processing[35]. It is well documented that apart from the cognitive impairment that occurs in epileptic patients as epilepsy comorbidities, the traditional AEDs can additionally produce cognitive disabilities[36]. Predictably, AEDs should advance

patients' cognitive functions by regulating the number and severity of epileptic activities as well as by enhancing the psychosocial environment that affords additional benefit to cognition. However, it is noted that AEDs treatment induced cognitive deficits in epileptic patients and healthy individuals as well[37]. PB is one of the most AEDs that negatively impacts memory function in epileptic patients, as it was reported that PB treatment evokes behavioral and cognitive impairment, including modification in attention vigilance and short-term memory[38]. Moreover, CBZ treatment also was reported to be associated with learning and memory deficits[39]. Furthermore, the French pharmacovigilance database (FPVD) revealed that VPA, lamotrigine, levetiracetam, gabapentin, pregabalin, and topiramate treatments were associated with memory deficits[40]. On the other hand, earlier studies documented that VPA treatment did not alter memory function[41]. Additionally, clinical studies revealed that PB and primidone were shown to have more cognitive effects than CBZ and PHT in epileptic patients[42,43]. Among the new AEDs, topiramate exhibited a strong correlation with memory impairment, especially short-term memory impairment[44,45].

Moreover, several animal experiments also documented the amnesic effect associated with several AEDs treatments, since in utero exposure of rodents to several AEDs resulted in anatomical and behavioral alternations[46,47]. Also, it was shown that PHT potentiates the memory deficits induced by PTZ-kindling model, while VPA displayed less memory alternations effect when compared with PHT[48]. Interestingly, PHT, PB, and CBZ failed to alter memory deficits associated with PTZ-kindling model, though VPA attenuates the memory impairment induced by chronic PTZ in male Wistar rats[49].

Several interconnected factors produce complex contributions to the associated cognitive deficits. Oxidative stress is considered as one of the main elements that may

contribute to AEDs associated with memory impairment. It has been reported that several AEDs induced a considerable rise in oxidative stress index with chronic treatment in epileptic patients, which was related to the associated memory impairment[50]. Moreover, CBZ and PB treatment induced memory deficits and alterations of oxidative stress in experimental animal models[51]. In addition, AED's chronic treatment was found to affect cognitive functions by suppressing neuronal excitability or enhancing inhibitory neurotransmission. Therefore, the perfect AED would decrease neuronal irritability by avoiding influencing the neuronal excitability and cognitive functions.

1.1.5 Epilepsy Comorbidities

Nowadays, epilepsy is considered to be a spectrum disorder more than periodic spontaneous seizures. For most of the patients, the burden of epilepsy is mainly caused by the associated comorbid conditions. Several psychiatric and behavioral features were documented as epilepsy comorbidities, including depression, memory deficits, anxiety, autism, attention-deficit hyperactivity disorders (ADHD), and learning disabilites[24]. In addition, it was revealed that even one seizure was capable of modifying the neurodevelopment by altering the receptor distribution and expression[52]. Previously, the associated comorbidities were counted as side effects resulting from AEDs treatment or as a secondary outcome due to uncontrolled seizures. However, recently the comorbid conditions are considered as a fundamental part of the epilepsy disorder, and are even usually attributable to an underlying disorder of neuronal networks[53]. Likewise, some psychiatric comorbidities were considered as risk factors for epilepsy development. On the other hand, epilepsy may raise the

possibilities for psychiatric comorbidities, indicating mutual causes and mechanisms[54,55].

One or more neurobehavioral comorbidities are regularly associated with chronic epileptic patients. Cognitive comorbidity is verified in clinical experimental and psychological studies[56-58]. Epileptic patients experience several cognitive problems, including decreased intelligence, attention, language, and memory disabilities[59]. Among them all, learning and memory disabilities are considered as one of the most unbearable neurobehavioral comorbidities of chronic epilepsy[60,61], affecting approximately 30% of epileptic patients[62]. Cognitive and memory impairments are usually correlated with poorly controlled seizures, and with a long period of the disease[63]; also, they are highly associated with epileptic patients during adult life[64]. Besides, memory deficits are highly associated with Temporal Lobe Epilepsy (TLE) patients[65]. Moreover, through applying an Intelligence Quotient (IQ) achievement discrepancy definition, around 50% of epileptic children were classified with memory disabilities in at least one academic field, while 40% to 60% were classified by using the low achievement definition[66]. In addition to the academically recognized defects, epileptic children suffer from learning and attention disabilities[67]. Furthermore, both epileptic adults and children regularly complain of memory disabilites[68].

On the other hand, numerous acute and chronic animal models had indicated that the memory impairment is comorbid with seizures, since acute chemically- and electrically-induced seizure models were found to induce memory impairment in both rats and mice[69,70]. Notably, PTZ kindled rodents were, also, found to exhibit memory dificits[71-73]. Moreover, Pilocarpine (PLC)-induced SE was found to be associated with memory impairments in rodents[74,75].

According to the aforementioned preclinical outcomes in different animal models, it was stated that overtraining may attenuate the seizures-induced memory impairment[76]. Also, it was found that environmental enrichment post-SE seizures might mitigate the apoptosis and the associated memory deficits[77]. Interestingly, the cognitive comorbidity was treated through nonpharmacologic interventions, including cognitive rehabilitation in adults and specific education in children. However, based on the last decade's studies, which demonstrated that the nonpharmacologic treatments were not sufficient in decreasing the associated cognitive comorbidities, pharmacological treatments were required. Therefore, identification of epilepsy comorbidities and its underlying mechanisms may suggest several therapeutic choices, especially by designing opportunities to treat two conditions with a single drug (seizures and memory deficits).

1.1.5.1 Role of oxidative stress in epilepsy and cognitive function

Reactive oxygen species (ROS), including hydroxyl radical (·OH), superoxide radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are produced through regular cellular metabolism[78,79]. Scavenging the physiological ROS can be done through enzymatic antioxidant defense methods, including glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) or non-enzymatic antioxidant defense methods, including vitamin E, vitamin C, and a reduced form of glutathione (GSH). Nevertheless, elevated ROS generation, reduced antioxidant defense system, or both will cause excessive ROS levels resulting in oxidative stress[80]. ROS free radicals will attack several proteins, cell lipids, and also the DNA, especially in the brain, causing lipids peroxidation[81], as the brain consumes the highest amounts of oxygen compared to other organs, and is full of polyunsaturated

fatty acids that are susceptible to lipid peroxidation[82]. Oxidative stress causes functional cellular interruption and cellular destruction and may result in successive cell death through oxidation of lipids, proteins, and nucleotides. Moreover, protein oxidation causes functional alternation and several enzymes deactivation[83]. Furthermore, lipid peroxidation results in membrane structure modification, which disturbs membrane permeability and membrane protein activity[84]. Therefore, the assumed function of oxygen radicals and radical-derived ROS in neurodegeneration disorders (e.g., epilepsy) and cognitive impairment has been well recognized[85-87].

Previous experimental results have shown that oxidative stress is strongly implicated in the pathogenesis of different neurodegenerative diseases and neurologic disorders like Parkinson's disease, epilepsy, stroke, and Alzheimer's disease[88,89]. It has, also, been revealed that oxidative stress plays a significant part in the over-excitation status of the neurons, consequently leading to the death of numerous neuronal cells through various mechanisms like oxidative damage to membrane proteins, including proteins for several receptors and/or ion channels[83]. In epileptic children, high oxidative stress was documented[90], and high MDA levels (an indicator of lipid peroxidation) were observed in patients diagnosed with refractory TLE [91]. Similarly, an elevated oxidative stress level was documented in epileptic patients when compared with healthy individuals[92]. Moreover, preclinical studies in several animal models revealed the significant role of oxidative stress in the initiation and progression of seizures. Importantly, hippocampal high oxidative stress was documented post PLC-, Kainic acid (KA)-, and PTZ-induced seizure models, indicating a neuronal damage[81,93,94]. Likewise, high oxidative stress was noted following PTZ-kindling model in the hippocampus and other brain regions, and it was also noted that treatment with

antioxidant compounds had a mitigating effect on oxidative stress levels and kindling procedure[95-97].

Moreover and based on previous preclinical observations in rodent models, oxidative stress was found to originate from repeated seizures, resulting in exacerbation of the convulsive consequences like the loss of the neuronal cells and cognitive impairment[98]. In addition, it was reported that oxidative stress modulated the synaptic plasticity and cognitive functions[86]. Furthermore, memory-impairment, in elderly patients was associated with reduced antioxidant levels in plasma and brain[99,100]. Likewise, it was noted that oxidative damage in cortical and hippocampal rat synapse during aging could be implicated in the deficit of cognitive functions[101]. Remarkably, the antioxidant effect of numerous compounds originating from different classes was associated with a memory-enhancing effect in several animal models[75,102-104]. Therefore, oxidative stress is considered as one of the causes of memory impairment[105]. Additionally, it was indicated that AEDs treatment elevates the oxidative stress index, which at least could be partially responsible for the associated memory impairment[105]. Accordingly, monotherapy with either PB or CBZ was found to cause an imbalance in serum oxidant and anti-oxidant status in epileptic children. However, this imbalance was regulated with the use of VPA monotherapy[50]. Moreover, chronic treatment with PB and CBZ in rats resulted in elevated brain oxidative stress concomitant with memory impairment; however, treatment with potential anti-oxidant compounds (e.g., curcumin) attenuated both the oxidative stress and associated memory deficits[51]. Similarly, chronic PHT treatment worsened memory deficits and oxidative stress induced by PTZ chronic treatment in mice; nevertheless, treatment with flavonoid with prospective anti-oxidant effect was found to alleviate the associated comorbidities [106]. Contrary, VPA was revealed to induce less side

effects on cognitive function and to attenuate the oxidative stress in both epileptic patients and several animal models[107-109].

1.1.5.2 Role of brain neurotransmitters in epilepsy and associated comorbidities

Simultaneous changes in brain circuitry and chemical signaling are supposed to play an essential part in epileptogenesis and the learning and memory processes. The vital role of neurotransmitters in providing normal brain functions is well documented, and the alternation of several neurotransmitters during a brain insult was noted. The modification of a wide range of neurotransmitters has been noticed to be closely correlated with epilepsy[110]. Numerous central neurotransmitters are known to perform a significant role in epilepsy and memory, including GABA, glutamate (GLU), acetylcholine (ACh), and histamine (HA).

1.1.5.2.1 GABA/GLU

Reduced GABAergic and/or improved glutamatergic transmission resulting in an imbalance between inhibitory and excitatory tone has been recognized as pathological aspects for the production of seizures, both in humans and in animals models[111,112]. Elevated GLU/GABA ratio toward excitation indicates a reduced seizure threshold, which is considered one of the most critical factors leading to epileptogenesis[113].

Several previous findings support the role of GABA in epileptogenesis and treatment of epilepsy, as GABA is considered the primary inhibitory neurotransmitter in the brain. Deformities of GABAergic function have been detected in both genetic and developed animal models of epilepsy[114-116]. Moreover, the hippocampal GABA-induced inhibition system was appreciated in AEDs development, which led to the production of clinically useful compounds (e.g., vigabatrin and tiagabine)[117]. Likewise, the traditional AEDs (BZs) stimulate GABA-mediated inhibition by binding

to the $GABA_A$ receptor. On the other hand, GABA antagonists' compounds characterized by proconvulsant properties, including PTZ and picrotoxin, show the vital role of GABA in epilepsy[118].

GLU is the prevalent excitatory neurotransmitter in the mammalian brain. In epileptic patients, hippocampal GLU levels were higher than in nonepileptic patients, as found by using the microdialysis technique. In addition, it was noted that elevated GLU hippocampal levels were noticed before seizure onset, implying GLU transmission role in triggering the seizure[119]. Moreover, Statues epilepticus (SE)-stimulated GLU release causes overstimulation of non- N-methyl-D-aspartate (NMDA)-type glutamate receptors , resulting in persistent long-term seizure activity, and brain damage[120]. Therefore, the adjustment of glutamatergic neurotransmission at the level of N-methyl-D-aspartate receptors (NMDARs) plays a crucial role in alleviating SE-evoked brain damage. Several available AEDs work through modulating the excitation system, e.g., topiramate inhibits GLU neurotransmission by mitigating non-NMDA-type glutamate receptors via blockade of kainate-evoked currents[117].

Furthermore, with the massive innervation of GABAergic neurons toward cholinergic and glutamatergic neurons, it can be suggested that dysfunction of the GABAergic system may play a role in cognitive impairment in humans. Besides, considerable declines in GABA concentration were observed in severe cases of Alzheimer's and elderly people[121].

Furthermore, GLU was noted to be involved in synaptic plasticity underlying learning and memory, through long term potentiation along with metabotropic and ionotropic glutamate receptors. On the other hand, NMDA receptors antagonist administration exhibited a memory impairment effect in rodents[122,123]. Nevertheless, in pathological

conditions, e.g., epilepsy or Alzheimer's disease (AD), elevated extrasynaptic GLU levels act as a neurotoxic mediator causing depolarization of the neural membranes and cell death through extrasynaptic NMDA receptors[124,125]. Consequently, epilepsy-induced elevated brain GLU levels are considered as a causative factor for both the vulnerability to memory deficit and uncontrolled seizures. In previous preclinical studies, high brain GLU levels were associated with memory impairment in the PTZ-kindling model; however, the memory-enhancement effect was facilitated at least partially through GLU levels normalization[126].

1.1.5.2.2 Acetylcholine (ACh)

ACh is a signaling molecule that provokes numerous activities in the Central Nervous System (CNS) and at the neuromuscular junction. ACh performs a critical role in alternating brain GLU release and sustaining memory formation[127] as low ACh levels in chronic epilepsy were associated with high GLU levels[128]. The role of cholinergic neurons in the incidence and initiation of epilepsy and the interaction with other nervous systems is well documented. On the other hand, PLC, a cholinergic M receptor agonist, is extensively used as PLC-induced SE model[129].

Moreover, in acute PTZ- and PLC-induced seizure models, elevated ACh levels were documented in the hippocampus and other brain regions[130-132]. Also, Maximal electroshock (MES)-induced seizure model increased brain ACh levels in rodents[133]. The elevated ACh levels evoked by seizures provide a convincing indication that activation of ACh receptors aggravates seizure activity[134]. In addition, it was demonstrated that high ACh levels would accumulate and trigger certain neurobehavioral signs such as convulsion[135].

AChE (acetylcholinesterase) is an essential enzyme that hydrolyzes the ACh, terminates the cholinergic transmission, leads to normal nervous system function, and is extensively expressed in the tissues that accept the cholinergic innervations, e.g., muscles cells and neurons[136]. AChE was also documented to be reduced following electrically and chemically- induced seizure models[133,137]. Memory impairment associated with the PLC- and PTZ-induced seizure model has formerly been associated with alterations in the functionality of the brain cholinergic system[133,138]. It is also well documented that the compromised function of AChE is associated with disturbed cholinergic functions, including learning and memory[135,139]. AChE plays a significant role in cholinergic neurotransmission, and reduced levels of AChE generally cause irregular high levels of ACh in the cholinergic synapses, which result in extreme motivation and stimulation of muscarinic and nicotinic receptors causing behavior alternation[107,140].

Oppositely, low ACh levels and elevated AChE were noted in chronic epilepsy in animal models[132,141,142]. It was thought that the enhancement of GLU-mediated excitation via kindling could cause overstimulation in NMDA receptors[143]. Thus, over stimulation will result in reduced viability and eventually death in cholinergic neurons[144]. The AChE overexpression had been remarked to be accompanied by memory disabilities[145,146]. Also, it was claimed that elevated AChE concentrations might be explained as compensatory to cholinergic transmission modulation induced by PTZ-chronic treatment[147]. AChE is considered being an important therapeutic target, as reversible inhibitors of AChE are being used as a memory enhancer in epileptic patients and other neurodegenerative diseases[148].

Finally, accumulating behavioral and molecular data implies the key role for cholinergic neurotransmission in the healthy brain function and several models of seizure generation.

1.1.5.2.3 Brain histamine (HA)

HA as a brain neurotransmitter plays a pivotal role in epilepsy, learning, and memory [149]. It was previously documented that CNS histaminergic system was implicated in a wide range of physiological and behavioral tasks, including locomotor activity, sleep-wake cycle, cognitive functions, appetite control, and stress behavior[150]. The participation of brain HA in the regulation of seizure susceptibility is satisfactorily demonstrated in both clinical and experimental studies that consider HA as an anticonvulsant neurotransmitter[150]. Remarkably, children with febrile seizures exhibited considerably lower histamine concentrations in the cerebrospinal fluid when compared with febrile children without seizures[151]. Moreover, several previous clinical data detected recorded proconvulsant effects of several histamine 1 receptor (H1R) antagonists administered in clinically relevant doses[152-156].

In earlier preclinical studies, intracerebroventricular (i.c.v). HA injection prevented different types of seizures. In addition, treatment with metoprine, an inhibitor of histamine *N*-methyl transferase, a HA catabolizing enzyme, prevented MES-induced seizures[157]. Moreover, low HA concentration was associated with both PTZ- and amygdaloid-kindling seizure models. Furthermore, exogenous HA administration to kindled animals and endogenous histamine elicited by activation of H3Rs provoked a kindling mitigating effect[158-160]. Likewise, histidine and metoprine administration inhibited the kindling procedure, and both therapies were accompanied by increased HA levels in the cerebral cortex and hippocampus[159,161,162]. On the other hand, α-

fluoromethyl histidine (α-FMH), a selective histidine decarboxylase (HDC) inhibitor, diminished HA synthesis and increased seizure activity[163]. Another point is that HDC knockout (KO) mice were more susceptible to seizures[164].

Low levels of HA were observed in the hypothalamus, hippocampus, and temporal cortex in AD patients[165]. Also, numerous preclinical studies indicated the role of the histaminergic system in learning and memory. It was shown that i.c.v. injection of histamine or histidine had improved the social memory in rats; however, α-FMH and the H3R agonist had the opposite effect[166,167]. Moreover, HA or histidine administration had mitigated scopolamine (SCO)- and dizocilpine (DIZ)-induced spatial memory impairment[168,169]. In addition, increasing endogenous HA release by histidine administration had mitigated seizures and the associated memory impairment in kindled rats[170]. On the other hand, decreasing brain HA levels by α-FMH administration significantly caused spatial memory impairment in rats[171]. Furthermore, histamine H1- or H2Rs knockout mice, exhibited impairment of performance in numerous memory experiments[172,173]. However, H3Rs knockout mice showed place recognition enhancement in the Barnes maze test and resistance to SCO-induced memory impairment[174,175].

1.1.6 Temporal Lobe Epilepsy (TLE)

TLE is the most predominant type of refractory symptomatic epilepsy[176]. Hippocampal sclerosis (HS) and aberrant mossy fiber sprouting (MFS) are the main characteristics of TLE. HS is a sever unilateral hippocampal neuronal atrophy in the hippocampus and parahippocampal gyrus. HS is histopathologically recognized by segmental neuronal cell loss in the CA1 and CA4 subfields, astrogliosis, granule cell dispersion, and axonal reorganization[177-179]. Along with the main feature of seizure

foci localization in the limbic system, especially in the hippocampus, entorhinal cortex, and amygdala, TLE has more special features[180]. A frequent discovery of "early precipitating damage" leads to the presence of TLE[181]. Besides, a seizure-free period after the precipitating damage is known as a "latent period" with a high prevalence of mesial sclerosis[182]. Most of these features can be mimicked in TLE chronic animal models, especially SE animal models or kindling, that are close by being the perfect homologous of the aforementioned mode[113].

1.1.6.1 Animal models for TLE or like-TLE conditions

The potential anticonvulsant activity of the old and new AEDs, except for bromides and PB, was initially evaluated in acute animal models, e.g. MES- and PTZ-acute induced seizure models in rodents, indicating that the clinical effect can be predicted by such straightforward laboratory models[183,184]. The fact that preclinical tests utilized for the development of new AEDs, have been formally validated by 'old' AEDs may clarify that none of the new AEDs owns noteworthy features in antiepileptic efficiency against the old treatments; thus, antiepileptic activity has not been modified to any considerable extent by the development of new AEDs[185]. It is not surprising that any of the AEDs that were detected applying MES or PTZ tests appear to be efficient in inhibiting or altering epilepsy since MES and PTZ are acute seizure tests instead of epilepsy models. Thus, models imitating the chronic brain dysfunctions resulting in epilepsy should be utilized in the search for novel, more effective compounds. In the following, the acute- seizure model is for models in which a seizure is evoked by electrical or chemical motivation in naïve non-epileptic healthy rodents. On the other hand, the chronic model is turning the rodents into epileptic animals by electrical or chemical kindling or by using the animals with inborn epilepsy. The phrases

‘anticonvulsant’ and ‘antiepileptic’ are utilized synonymously to imply anti-seizure compound activity; however, the term ‘antiepileptogenic’ is applied to reveal inhibiting or modifying epilepsy as epileptogenesis signifies the gradual conversion of the natural brain to a hyperexcitable epileptic brain[149].

Acquired epilepsy models are epilepsy or epilepsy-like conditions, which are stimulated by electrical or chemical methods in healthy naïve non-epileptic rodents, mostly in rats. Likewise, TLE or TLE-like conditions can be stimulated by electrical or chemical kindling, or by models in which recurring unprovoked seizures developed following SE that is provoked by persistent electrical stimulation of the hippocampus or by chemical compounds applications.

1.1.6.1.1 PLC-induced SE model

Continuous electrical induction of the hippocampus or amygdala evokes (SE). This model is categorized by neuropathological changes similar to mesiotemporal sclerosis (exhibited in several patients with TLE) and by recurring spontaneous seizures developing after the SE. Comparable to electrical models of TLE, SE can also be evoked by chemical convulsant application, e.g., PLC and KA[186,187]. SE is a life-threatening medical situation and neurologic emergency necessitating immediate recognition and treatment. SE is generally defined as a prolonged seizure or several seizures with an incomplete return to consciousness among them[188]. SE is associated with high morbidity and mortality[189].

PLC is a muscarinic agonist either systemically administered or microinjected focally into the hippocampus. PLC induces SE by activation of the M1 muscarinic receptor subtype since M1 receptor knockout rodents lack sensitivity to PLC-induced SE[190]. In addition, PLC-induced SE is inhibited by systemic treatment of atropine, a muscarinic

antagonist[191]. *In-vitro* experiments have revealed that PLC produces an imbalance among excitatory and inhibitory neurotransmission via muscarinic receptors causing the production of SE[192]. Moreover, *in vivo* experiments have shown that PLC provokes an increase in hippocampal GLU levels after the occurrence of convulsion[193], suggesting that M1 receptors induce the origination of seizures and that they are maintained via NMDA receptor motivation[193,194].

Systemic administration of PLC is associated with high mortality rate. PLC can instigate SE, and post-SE treated rodents demonstrate neuronal loss in the hippocampus, particularly in the dentate gyrus. Following a typical latent period, almost 90% of post-SE rodents exhibit unprovoked, frequent convulsions of Racine class 3 or more that persist for life[195], indicating that PLC-induced SE model is considered as a commonly accepted model of TLE[129].

PLC can trigger both acute and chronic seizures, as PLC-systemic administration evokes SE in a high percentage of the treated animals, and almost all of the SE animals exhibited spontaneous recurrent seizures following a latent period of 2–30 days[196-199]. Thus, the PLC-model is nowadays widely utilized in several ways depending on the aims of the experiments, as both the electrical- and chemical-induced SE model may be utilized as experimental models of SE (TLE-like epilepsy model), to assess potential compounds for execution of SE, or to generate chronic epilepsy with persistent unprovoked seizures.

1.1.6.1.1.2 PTZ-kindling model

Kindling is a procedure in which recurrent sub-threshold application of electrical or chemical agents can cause prolonged seizures, progressively raised in length, and intensity of the behavioral disorder[200,201]. The electrical kindling phenomenon was first

described by Goddard[202], where the application of recurrent low-intensity electrical incentives into the brain structures produced myoclonic jerks and tonic-clonic seizures that were generated even 8 weeks after the last stimulation. The PTZ-kindling model was first described by Mason and Cooper[203]. It was noticed that an outcome equivalent to the electrical kindling occurred following sub-convulsant chronic intraperitoneal (i.p.) injection of the PTZ. Also, it was found that PTZ-induce kindling is a long-term phenomenon, as once a behavioral symptom of PTZ administration was reached, a major seizure reaction was displayed on the renewed administration even after months without utilizing a convulsive stimulus. Behavioral manifestation stated as a bilateral forelimb clonus or a more severe stage in two or more consecutive applications is taken as a full kindling state. Ultimately, shifts in the brain's network in the fully kindled state resulted in amplified severity of the seizure and the associated behavioral deficits in reply to a sub-threshold dose of PTZ.

PTZ, a bicyclic tetrazole derivative, was used for the first time as CNS stimulant by Von Meduna in 1934[204]. PTZ can stimulate the epileptogenic effect by inhibiting (GABA)-mediated transmission[205]. PTZ acts as a potent non-competitive antagonist of the $GABA_A$ receptor by binding to the benzodiazepine (BZ) site of $GABA_A$ complex receptor, which was found to consist of a mixture of allosteric binding sites[206-208]. The PTZ-kindling model has been applied dynamically for a quick examination of the epileptogenic processes instead of other expensive and time-consuming chronic epilepsy models. The model allows for accurate recording of seizures time of start. In addition, while the first period of the convulsions requires at least 15 days to develop, dissimilar from acute models, kindling can be employed for recognizing the key actions triggering the epileptogenesis. In such chemical kindling models, beginning from early absence to tonic-clonic seizures, a long-lasting development interval

occurs, which authorizes to explore the detailed activation of seizure activity distribution[203]. PTZ-kindling model is characterized by a continual accretion in seizure liability, neuronal damage, and lack of unprompted repeated convulsions.

Several brain structures were implicated in PTZ-kindling model as described by an earlier study, in which c-fos, an immediate-early gene, was assessed to visualize the brain structures involved in each specific stage of the PTZ-kindling model[209]. Thalamus and hypothalamus were the first brain structures to be engaged in the early absence-like phase of kindling. However, with the incidence of clonic and tonic-clonic seizures post-recurrent sub-convulsive administration of PTZ, sequel engagement in epileptogenesis of the midbrain, brainstem regions, and almost all of the hippocampal subfield were observed. Also, in fully kindled rats, c-fos labeling was enhanced in the dentate gyrus. Moreover, oxidative stress was documented in the hippocampus at the end of the chemical kindling[210].

It was documented that the hippocampus performed a crucial role in the initiation and propagation of convulsions in humans. Also, (HS) and aberrant (MFS) are frequent structural irregularities in refractory TLE patients[211-213], following 2 to 3 systemic applications of PTZ hippocampal MFS in the CA3 region and neurogenesis in the dentate gyrus were evoked[214,215]. Moreover, a decline in the number of functionally active neurons in the hippocampal CA1 area was noted following the sixth PTZ applications[216]. At the end of the kindling, the susceptibility of the brain to PTZ is elevated, causing significant destruction of the antiepileptic systems and hyperactivation of neurons, which triggers chronic epileptization of the brain. The morphological modifications, evoked by kindling, involve hippocampal progressive neuronal cell damage.

It is still debatable whether the post-kindling period of PTZ kindling is considered as a model of the latent period of epileptogenesis of TLE. In the PTZ model, kindling was used for the initiation of seizures, which does not reestablish the etiological aspects for TLE human induction. Precipitating damages in human TLE includes head trauma, toxins, hypoxia/ischemia, infections (e.g., bacterial meningitis), SE, and birth trauma. However, the neuropathological alterations seen in the PTZ kindling model are similar to those reported in PLC-treated animals. Previous morphological experiments on the PTZ-kindling model offered crucial data concerning the reduction in the density and quantity of hippocampal neurons. Oppositely, there is a typical impression that HS does not accompany kindling. Nevertheless, *in-vivo* Magnetic Resonance Imaging (MRI) spectroscopy studies had documented that HS was detected in the PTZ-kindling model via T2-weighted signal intensity of the PTZ treated rodents[210,217]. A few earlier studies documented aberrant MFS following PTZ treatment[218]. In addition, comparably to PLC and KA, recurrent PTZ injections may increase the permeability of the blood-brain barrier, resulting in increased release of hippocampal GLU that would ultimately facilitate convulsion incidence[219,220]. All of the above-mentioned neuropathological features of PTZ-kindled animals imitate a portion of the phenotypic characterization of the human TLE condition. One of the advantages of PTZ-kindling model is that PTZ provokes low mortality, and most of the animals are implicated in convulsion activity. Contrary to KA and PLC models, in which the initial clonic-tonic convulsion happens after a few hours, in the PTZ-kindling model, this procedure necessitates quite a few weeks, making it feasible to examine subtle mechanisms of epileptogenesis[210]. Models of acquired epilepsy, including kindling or the evoked SE- epilepsy model, are generally utilized to investigate the pathophysiology of epilepsy in the pursuit of targets to inhibit epilepsy.

1.2 Histamine as a neurotransmitter in the central nervous system (CNS)

1.2.1 Synthesis, storage, and release of brain histamine

Brain HA, [2-(4-imidazolyl)-ethylamine), has been widely discussed as a medicinal substance in the medical literature (Figure 3). It controls a broad spectrum of activities, including its neurotransmission function[221]. HA has first detected in ergot fungi extracts as uterine stimulants in 1910, from which it is subsequently isolated and extracted from human tissues in 1927[222,223]. HA was identified before introducing the first-generation antihistamine, and even prior to the knowledge of H1 or H2 receptors. The main side effect of antihistamine was the sedation, which implies the central effect of HA. It was isolated the first time from cortical brain human in 1943, following several electrophysiological and behavioral studies with (i.c.v.) infusion of HA. It has been shown as a potential new neurotransmitter to be added to the existing neurotransmitter systems[224].

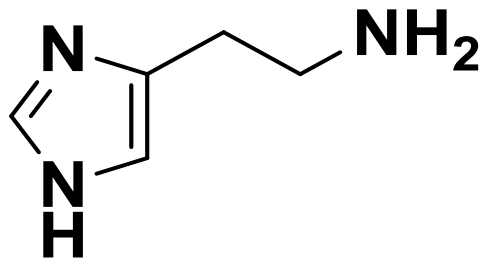


Figure 3: The chemical structure of histamine [2-(1*H*-imidazol-4-yl)ethanamine.

Histamine joins the family of biogenic amines (contain one or more amine), which comprise neurotransmitters, including serotonin and dopamine. Biogenic amines are mainly produced by decarbo.xylation of amino acids. HA is a monoamine that is not able to pass the blood-brain barrier; therefore, it is synthesized in a constricted body

of neurons, sited in the tuberomammillary nucleus (TMN) of the posterior hypothalamus. (HDC) is an enzyme responsible for catalyzing the amino acid 1-histidine to histamine (Figure 4)[225]. As a result, l-histidine is transferred to histamine via the oxidative elimination of the carboxyl group by HDC. HA biosynthesis, therefore, relies on the existence of histidine. At elevated concentrations, histidine may be a substrate for nonspecific decarboxylase. However, these nonspecific synthesis routes can be disclosed with alpha-fluoromethyl histidine (α-FMH), a specific HDC inhibitor[226]. Once synthesized and via vesicular monoamine transporter-2 (VMAT-2), the HA is stored in both soma and varicosities vesicles. The release of HA is calcium-dependent, and it happens post depolarization of the histaminergic neurons[227].

In the brain, HA is stored in two separate compartments, the first one is in vesicles of neuronal endplates, and the second in the mast cells[227]. Moreover, it has been documented that the two compartments are implicated in the given levels of brain HA. Since 50% of brain HA was found to be lacking in mast cells knockout mice $W/W^{v,}$ compared to normal mice[228]. It is noteworthy that central HA biosynthesis and release is modified through the histamine H3 auto-receptors (H3Rs), and it is Cyclic adenosine monophosphate/ protein kinase A (cAMP/PKA-dependent)[229]. Furthermore, other systems are involved in regulating the activation of central histaminergic neurons and the release of HA, via its inputs and receptors such as acetylcholine, norepinephrine, and serotonin[177]. The re-uptake mechanism is not known for HA, which is different from the usual aminergic neurotransmitters[177]. Nevertheless, the central activity of HA is ceased through the histamine N-methyltransferase (HNMT) in the extracellular space. Nι-methylhistamine is produced by methylation via HNMT, then degraded by monoamine oxidase B to Nι-methylimidazole acetic acid (Figure 4)[221,224,226].

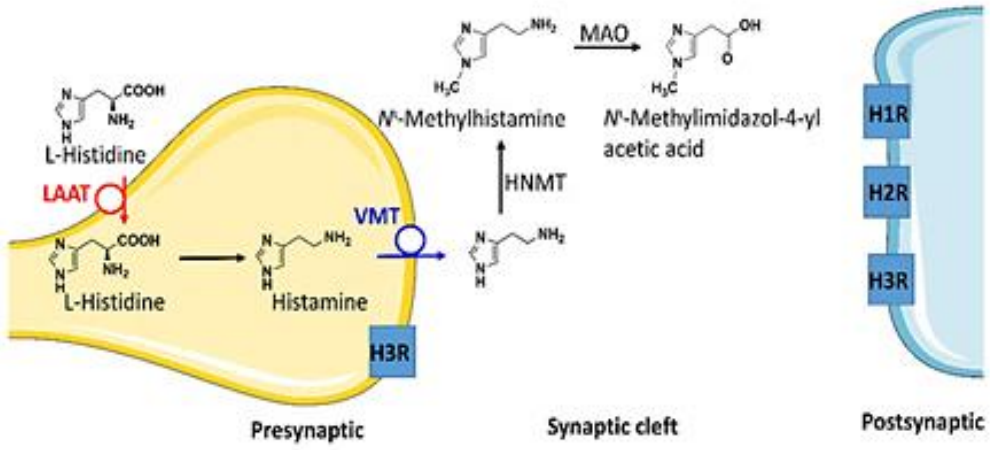


Figure 4: Neuronal histamine biosynthesis, release and metabolism in the CNS.

LAAT, L-amino acid transporter; VMT, vesicle monoamine transporter; HNMT, histamine-*N*-methyl transferase; MAO, monoamine oxidase. Adopted from [225].

1.2.2 Localization of the histaminergic neurons

Neuronal histaminergic cell bodies are sited only in the tuberomammillary nucleus (TMN) of the posterior hypothalamus, and TMN is located among the mammillary bodies and the optic chiasm of the tuber cinereum (Figure 5). Cell bodies of histamine neurons forward projections to various brain regions without a structural outline of their projections, as verified by retrograde tracers. One perikaryal may send out descending and ascending extensions[221,224]. The heterogeneous histaminergic neurons have been described, with distinct mechanisms for regulation and innervation of distinct areas of the brain[230,231]. The histaminergic neurons provide widespread and diffuse projections that spread across the basal forebrain practically to the entire brain, including the thalamus, cortex, hippocampus, striatum, hypothalamus, locus coeruleus, and spinal cord via its ascending and descending pathways[232,233]. Through their expansions, histaminergic neurons seldom create traditional synaptic interaction with their prospective targets. However, HA is released from varicosities and

propagates to the nearby regions as a regional hormone that influences neuronal and non-neuronal components[221,224]. Two different ascending routes involved ventral sending projections to hypothalamus and septum, while dorsal sending to the thalamus, hippocampus, amygdala, and forebrain. The dentate gyrus accepts a considerable amount of histaminergic fibers via the dorsal pathway. Generally, the descending pathway to the brain stem, cerebellum, and spinal cord has a minimum fiber density[221] (Figure 5) [221].

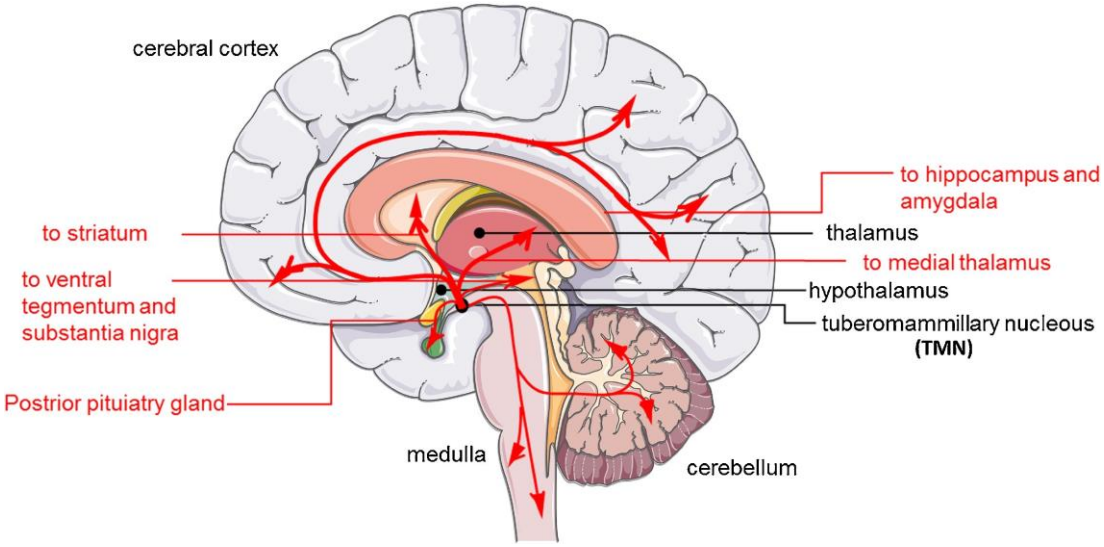


Figure 5: Projections of TMN histamine neurons.

Two ascending pathways: 1) ventral part to the hypothalamus and septum, 2) dorsal part to the thalamus, hippocampus, amygdala, and forebrain. One descending pathway to the brain stem, cerebellum and spinal cord. Adopted from[225].

1.2.3 Histamine Receptors

The morphology of the histaminergic neurons, as already mentioned, recognizes the HA as a neurotransmitter and neuromodulator of a board range of central physiological functions and behaviors via activation of distinct histamine receptors, including neuroendocrine, cardiovascular regulator, locomotion, catalepsy, sexual behavior,

energy homeostasis, drinking and feeding, and learning and memory[221,224,234,235]. Histamine neuroreceptor is regarded as one of the main aminergic structures exhibiting key neurological functions across pharmacologically diverse receptor subtypes. HA receptors, namely H1-H4R, fit into a board superfamily of G-protein coupled receptors (GPCRs), recognized by the presence of seven putative transmembrane spanning domains[236-238]. H1-3Rs are extremely present in the brain; however, H4Rs in the CNS have minimal expression [239,240]. Remarkably, all HA receptors with diverging levels display an active state of confirmation irrespective of any ligand binding[241].

1.2.3.1 Histamine H1 receptors (H1R)

H1R is encoded via chromosome 3 sited gene, which encodes a member of the large, 7-transmembrane-spanning, G-protein-associated receptor family[242]. H1R is activated, when histamine binds to the receptor, through Gq/11 signaling pathway[243]. Moreover, H1R activation may also result in the formation of arachidonic acid (AA) and nitric oxide (NO)[244]. Thus, the activation of H1R is probably capable of modifying the release of the presynaptic transmitter, since AA and NO are considered retrograde messengers[221].

H1Rs are widely distributed in the CNS and are primarily responsible for antihistamine sedative effects[245]. Elevated levels of H1Rs are distrusted mainly in brain areas related to arousal, including thalamus, cortex, aminergic nuclei in the locus corelus and raphe nuclei, septal nuclei, hippocampus, amygdala, and cholinergic nuclei in the mesopontine tegmentum and basal forebrain. In addition, H1Rs present in high density in the nuclei of the cranial nerves, area postrema, nucleus accumbens, molecular layer of the cerebellum, and nucleus tractus solitaries[246].

Furthermore, H1Rs improve cortical glutamate NMDA-receptor mediated currents[247]. Moreover, H1R activation was indicated to result in enhanced expression of the immediate early-gene (IEG) c-fos in the suprachiasmatic neurons[248]. H1R knock out mice normally developed; however, irregular locomotive function, circadian rhythm, and decreased exploratory behavior were observed in a novel environment[249].

1.2.3.2 Histamine H2 receptors (H2Rs)

H2R is encoded via chromosome 5 located gene encoding a member of the large a 7-transmembrane domain, G-protein coupled receptor[250]. H2R was first discovered by Black ae el as peer the histamine receptor's distinct pharmacological report, which is responsible for the promotion of gastric acid secretion[251]. H2R is Gs-coupled protein, and is therefore involved in the cAMP/PKA/cAMP response element-binding protein (CREB) signaling route supplying excitatory impacts[252].

H2R, Similar to H1R, is existed in a high density in the brain and spinal cord; however, as a contrast to H1R, H2R has a low expression in thalamic nuclei, septal areas, and hypothalamic. H1 and H2 receptors are colocalized in hippocampal pyramidal, granule cells, and aminergic nuclei (raphe nuclei, substantia nigra, locus coeruleus, and ventral tegmental area), where they can act synergistically[253]. H2R knock out mice suffered from cognitive dysfunction in specific tests associated with corrupted long-term potentiation in hippocampus[172].

1.2.3.3 Histamine H3 receptors

H3R was first described in 1983 as an auto-receptor regulating the release and synthesis of histamine[254]. Recently it was cloned, and H3R appeared to be a G-coupled protein linked to Gi or Go, a member of the GPCR family[251,255]. As a GPCR family, H3R transduces via α-subunit of the Gi/o protein (Gi/oα) that is adversely linked to

adenylate cyclase[229,256]. Activation of tuberomammillary (TM) located H3Rs causes inhibition of various high-threshold calcium channels, [257]. Nevertheless, unlike other autoreceptors, there is a lack of evidence of H3Rs to be linked to inwardly-rectifying potassium channels.

Remarkably, the H3 receptor gene demonstrates a minimal overall homology to all other biogenic amine receptors[255]. Several H3R isoforms were acquired via alternative splicing of the human H3R gene alongside with other species by applying RT-PCR and genetic information technology of human H3Rs. The obtained isoforms exhibited heterogeneous pharmacology, signal transduction, and distribution in the CNS[256,258]. Interestingly, despite the very low homology observed for the human H3R (hH3R) protein sequence, (21–22%) in comparison to hH1- and hH2Rs, it showed high homology compared to hH4R [38–58%)[259,260]. GPCRs displayed in-vitro constitutive activity; however, H3Rs showed in-vivo constitutive activity at normal physiological levels, unlike the other GPCRs[261,262]. In addition, GPCRs are well known to be present in equilibrium among their active and inactive conformations, and the level of constitutive activity of the respective receptor is determined by the amount of the active conformations and how efficiently they combine to G-protein. Inverse agonists are compounds that stabilize the inactive status, leading to inhibiting the constitutive activity.

H3Rs has a predominant, heterogeneous expression in the CNS, despite its disappearance in the periphery[255,263,264]. Rats' H3Rs are highly expressed in the rostral part of the cerebral cortex, olfactory tubercles, striatum, hippocampus, amygdala, substantia nigra, nucleus accumbens, cerebellum, and brain stem. However, rat's H3Rs are moderately expressed in the hypothalamus and the cell bodies of histamine

neurons[224]. On the other hand, humans' H3Rs are presented primarily in the basal ganglia, hippocampus, globus pallidus, and cortex[246]. This aforementioned distribution is in harmony with their prospective occupation in regulating the synthesis and release of histamine alongside with other the neurotransmitters, including glutamate, dopamine, norepinephrine, serotonin, acetylcholine, and GABA[265-270]. Consequently, the Gi/oα-facilitated decrease in calcium influx appears to clarify the detected histamine-induced inhibition of its own, besides other neurotransmitter release[221].

H3R knockout mice exhibited an overall decrease of locomotor activity, sleep restriction, and lack of sensitivity to waking activity of H3R antagonists and to SCO-induced memory deficits[175,271].

1.2.3.4 Histamine H4 receptors

H4Rs are receptors coupled to Gi/o protein and are mainly expressed in the periphery, unlike H3Rs[259]. According to pharmacological homology among H3 and H4 receptors, pharmacological similarities were observed among the two receptors, primarily with imidazole-containing compounds[272]. H4Rs are mostly involved in immunomodulation, and they are predominantly expressed in cells and tissues of the immune system[273].

1.3 H3Rs as potential targets in neuropsychiatric diseases

Consistent with the extensive projection of the histaminergic neurons and the numerous signaling pathways linked to its receptors, neuronal histamine is involved in multiple CNS actions. Brain histaminergic system alternation has been explained in numerous brain disorders and could have a considerable part in their pathophysiology[240,274].

1.3.1 Epilepsy

The connection among the histaminergic system and the pathogenesis of epilepsy, which is now the matter of extensive evaluation, is still in its early stages, due to the histamine complex brain neurophysiology[224]. The histaminergic system implication in epilepsy pathophysiology and treatment has been described previously in section 1.1.5.2.3. The traditional understanding of epilepsy pathophysiology is regularly restricted to the conservative theory of imbalance among inhibitory GABAergic and excitatory glutamatergic neurotransmission in excitotoxic neuronal death[275].

The imbalance could be modified by several additional neurotransmitter systems, including the histaminergic system. H3Rs have been an attractive target in treating epilepsy by releasing the HA via H3 autoreceptors and, via H3 hetero-receptors, the release of a broad spectrum of dynamic neurotransmitters, e.g., GABA, glutamate, acetylcholine, and others in a pathway-dependent manner[235]. Histamine release is not only controlled by its H3 auto-receptor system but also by GABA through $GABA_A$ and $GABA_B$ receptors and by glutamate via NMDA receptors[276-278]. Moreover, bicuculline, a $GABA_A$ receptor antagonist, drastically reduced clobenpropit-evoked mitigation of amygdaloid kindling[279]. Besides, clobenpropit and iodophenpropit were noted to exert noncompetitive antagonism in NR1/NR2B subunits of NMDA receptors[280]. Furthermore, clobenpropit in cultured cortical neurons blocked NMDA-induced neurotoxicity via enhancing GABA release facilitated by cAMP/PKA[281], which further endorsed the implication of other neurotransmitters in the anticonvulsant provided effect.

The potential anti-seizure protective effect of pitolisant, a non-imidazole H3R inverse agonist, was assessed in 14 photosensitive epileptic patients utilizing the standard

model of photosensitivity and applying 20/40/60 mg dose. Considerable inhibition of generalized epileptiform discharges was documented in most of the patients[282,283]. Unfortunately, despite the present promising experimental animal finding on the potential effect of pitolisant, the latest phase II trial documented that there were no clinical effects of pitolisant in human epilepsy[284]. Furthermore, mounting preclinical, experimental data from both acute and chronic models of epilepsy signify the efficacy of H3 receptor antagonists in epilepsy (Table 1).

Several H3R antagonists were observed to protect against experimental convulsions by boosting brain histamine release, which in turn activates H1 receptors located post-synapticlly[162,279,285,286]. H1Rs appears to be the key mediators of histamine-induced increase of seizure threshold besides the clinical evidence mentioned above of the possible proconvulsant effect of H1R antagonist. Several centrally-acting H1R antagonists were also shown to induce a proconvulsant effect in several models[163,287]. In addition, the H1 receptor antagonist was documented to partially abrogate the anticonvulsant effect provided by several H3R antagonists in numerous animals models, indicating histamine participation[288,289,158,161]. Moreover, the proconvulsant action of H1R antagonists was mitigated by histidine, not by physostigmine, signifying the contribution of histaminergic transmission[290]. Another point is that H1R KO mice demonstrated quicker reactions to amygdaloid and PTZ-induced kindling[291].

From all aforementioned data, it is suggested that the central histaminergic system applies a potent inhibitory function during epileptic seizure episodes, evidently via an H3-dependent mechanism. In agreement with this, H3 receptor antagonists may have a therapeutic value in epilepsy or at least be a feasible approach that can be combined with AEDs, particularly in patients who are non-responsive to conventional therapies.

Table 1: H3R antagonists tested in several animal models of epilepsy.

	Acute	Chronic
Thioperamide	↓MES- induced seizure model[285,292] ↓PTZ- indced seizure model [248,293]	↓PTZ- kindling seizures[162,294] ↓Amygdaloid kindled seizures[286,295].
Clobenpropit	↓MES- induced seizure model[296,297]	↓Amygdaloid kindled seizures[279,286,298] PTZ- kindling seizures[163,299]
AQ 0145	↓MES- induced seizure model [300]	↓Amygdaloid kindled seizures [295]
Iodophenpropit		↓Amygdaloid kindled seizures[301]
ABT-239	↓KA seizure in mice[302]	NA
ST-1394	↓MES- and PTZ- induced seizure model[303]	NA
Pitolisant	↓PTZ- and MES- induced seizure model[304]	NA
DL77	↓MES- induced seizure model[305]	NA
E159	↓PTZ- and MES- induced seizure model[289]	NA

NA, not assessed; ↓, anticonvulsant effect.

1.3.2 Cognitive impairment

Cognition is not only about learning and memory; it involves numerous higher brain functions, such as decision making, attention, and problem-solving. The cognitive process involves complicated connections between cortical and subcortical neuronal pathways. Cognitive deficits include one or more aspects of cognition. Memory impairment is one of the main characteristics of AD disease, and attention is deteriorated in (ADHD). Likewise, schizophrenia patients experienced deficiency in executive function, i.e., cognitive inflexibility[306]. For evaluating the cognitive function in different brain disorders, several behavioral tests have been developed to investigate domains of cognition in animals and clinical trials.

According to the relation of the histaminergic system with several cognitive functions in section 1.1.5.2.3, H3Rs are nowadays an attractive drug target, as H3Rs modify several neurotransmitter releases and have unique localization and neurochemistry. These features make this system uniquely poised to play a role in aspects of learning and memory. Several preclinical experiments in cognition research have focused on tests that evaluate the distinct learning and memory domains believed to be the most altered in diseases such as AD and epilepsy. Other areas, such as attention and impulsivity, are likely to be of importance in ADHD. H3R antagonists may enhance HA synthesis and release, and HA is associated with memory improvement. Additionally, H3R antagonists may enhance the release of other neurotransmitters implicated in cognition and memory, such as ACh in the prefrontal cortex and hippocampus[307-309] (Figure 6). The mechanism by which H3R antagonists modified another neurotransmitter release is complicated. It has been hypothesized that excitation of the neurotransmitter neuron through H1R/H2R, excitation/inhibition of a "third-party" excitatory or inhibitory neuron which influences other neurotransmitter releases, or straight rise of the neurotransmitter release via blockade of H3R hetero-receptors might be conceivable mechanistic reasons for the detected modifying result on neurotransmitter release (Figure 6)[230].

Several promising H3R antagonists have been investigated in clinical trials for their prospective cognitive enhancement effect. Interestingly, GSK-239512, with verified procognitive effect in several memory experiments in animals, has completed phase 1 clinical trial, and the outcomes indicate a moderate efficacy in mild-moderate AD patients (ClinicalTrials.gov trial registration number: NCT00675090)[310]. However, further grander scale testing is required to clarify its effectiveness[311]. Moreover, SAR110894 with documented procognitive effect in rodents[312,313], has accomplished

a phase 2 clinical study for mild-moderate AD, that was tested as an additive to the standard drug donepezil without disclosing the clinical outcome (ClinicalTrials.gov trial registration number: NCT01266525). Despite the observed procognitive effect of ABT-288 in rodents and its outstanding drug-like properties, a clinical phase 2 study evaluating procognitive effect of ABT-288 in patients with mild-moderate AD (ClinicalTrials.gov trial registration number: NCT01018875) and schizophrenia patients with cognitive deficits (ClinicalTrials.gov trial registration number: NCT01077700) has been accomplished without revealing the clinical results of the study. Moreover, CEP-26401 is another effective H3R antagonist that has been demonstrated to enhance social recognition memory in rats[314]. Remarkably, CEP-26401 finished phase 1 of a clinical study focusing on its pharmacokinetics and pharmacodynamics in healthy subjects for cognition indication with no disclosure of the results for the clinical trial (ClinicalTrials.gov trial registration number: NCT01903824). In conclusion, several preclinical and clinical returns have been remarked for various H3R antagonists in experimental memory models involving their disease altering properties and capability to attenuate cognitive deterioration associated with epilepsy in the main.

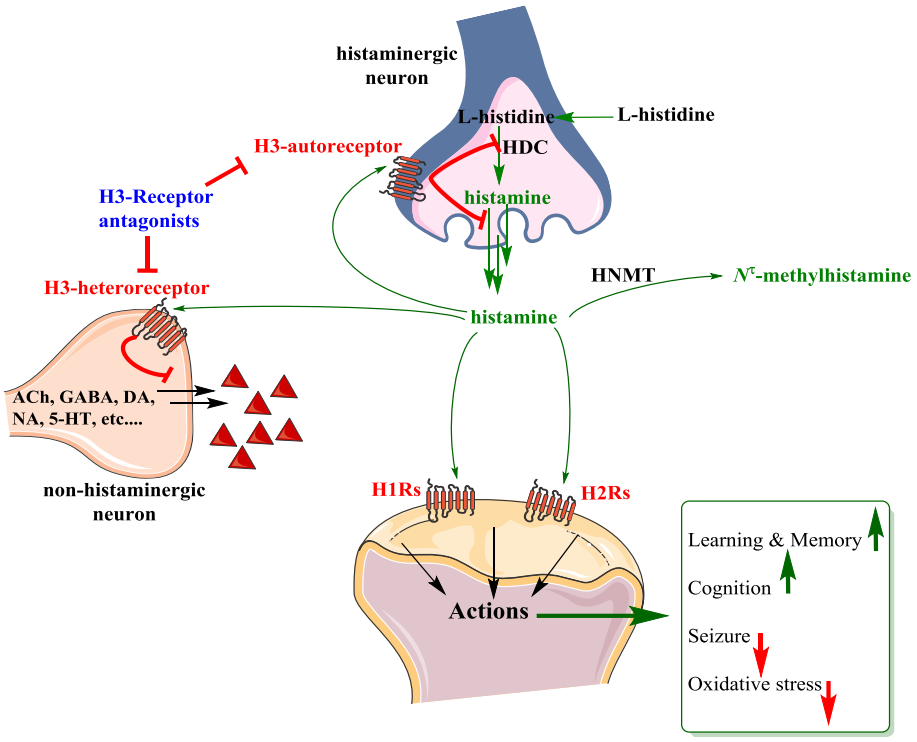


Figure 6: Illustration of possible mechanisms by which histamine H3 receptor antagonists provide various pharmacological effects.

HDC, Histidine decarboxylase; HNMT, Histamine-*N*-methyltransferase. Modified after[230].

1.3.2.1 Role of H3Rs in Long-term memory (LTM)

One essential behavioral test that has been frequently utilized to evaluate long-term memory (LTM) is the inhibitory avoidance paradigm (IAP). IAP is a fear-motivated test that depends on the time of treatment and can assess different aspects of memory. Drugs were administered 30 minutes before the training session (time point 1), directly after the training (time point 2) or before the test session (time point 3). These three separate experiments were used to investigate their effect on memory acquisition, consolidation, or retrieval, respectively. It was indicated that H3R knockout mice were insensitive to SCO-induced LTM impairment in IAP[175]. Also, thioperamide mitigated SCO-induced LTM memory impairment in IAP[315], implying that H3R may be acting through an alternative of cholinergic function in this cognitive domain. Moreover,

E159 and DL77 attenuated DIZ- induced memory impairment in IAP. Furthermore, LTM impairment applying IAP was noted in PTZ-kindled rats associated with hippocampal neuronal death; however, treatment with histidine ameliorated the memory deficit and decreased neuronal hippocampal damage[159]. The enhancement effects of the H3R antagonists could signify an enhanced release of histamine and other transmitters, e.g., ACh[316,317].

1.3.2.2 Role of H3Rs in Recognition memory

Social memory (a type of LTM recognition memory) was one of the earliest domains investigated with H3Rs. Social recognition memory depends on rodent's memory retention, in which an adult rodent utilizes the olfactory cues to recall social contact with a nonspecific adolescent. Thioperamide enhanced social memory in rats[167].

Another type of recognition memory is object recognition. The Novel Object Recognition (NOR) test was used to calculate both long and short-term recognition memory in rodents. In this task, rodents are evaluated for their capability to remember a familiar vs. an unfamiliar object. It was observed that kindled rats were associated with recognition memory impairment[318,319]. Also, GSK207040 and BF2.649 enhanced recognition memory in naïve rats[320,321], while BF2.649 and E159 attenuated SCO- and DIZ-induced recognition memory deficits, respectively[320,322]. According to the previous indication about the role of HA and ACh in recognition memory, it was suggested that the memory improvement observed with H3R antagonists in the earlier experiments was via enhanced brain ACh and HA[167,323].

1.3.2.3 Role of H3Rs in Spatial memory

Several behavioral animal models were developed for assessing spatial memory function in rodents, e.g., water maze, elevated plus maze (EPM), barnes maze, and

radial-arm maze. All of the previous tasks required the rodents to use local information in navigating around their environment. Spatial memory impairment was documented in AD patients and epilepsy animal models[72,133]. Thioperamide and ciproxifan mitigated SCO-induced spatial memory impairment in rats[316,324], but H2R antagonist administration reversed the thioperamide procognitive effect observed in EPM[325]. Most of spatial memory assessment tasks rely on the hippocampus and the septal-hippocampal pathway. Moreover, it is suggested that the enhancement in spatial memory observed with H3R antagonists is via HA and ACh release. H2Rs are also implicated in the procognitive provided effect[306].

1.3.2.4 Role of H3Rs in synaptic plasticity

During memory formation, synaptic plasticity, which is a specific molecular and cellular change that ensues to compel synapses, is crucial for learning and memory. Synaptic plasticity encompasses stimulation or blockade of receptors, ion channels, and proteins, with subsequent modification in the size and quantity of synapses[326]. Long-term potentiation (LTP) and long-term depression (LTD) are types of synaptic plasticity. Among the other brain regions, the hippocampus is a brain area that is extremely implicated in memory formation.

Interestingly, H1- and H2R knockout mice demonstrated *in vitro* significant hippocampal impairment of electrically-induced LTP in CA1 region[172]. In addition, HA, lacking any exterior tetanus motivation that is typically utilized in such trials, induced *in vitro* hippocampal LTP in CA1 region[327].

1.3.2.5 Histamine in the hippocampus

Various brain areas are probably implicated in the memory modulating actions of histamine. Many of the tasks for memory assessing are partially or predominantly dependent on the hippocampal formation. Moreover, HA injection directly into the ventral hippocampus was found to prevent performance in an active avoidance task by acting on H1Rs[328,329]. Furthermore, *in vitro* experiments on hippocampal neurons tend to indicate a memory-facilitating role for histamine, which is increased excitability and facilitation of NMDA receptors and LTP[330].

Even though the hippocampal formation accepts only low to moderate histamine innervation, various well-known effects of histamine have been demonstrated *in vitro* and *in vivo*[331]. HA affects only one of the main excitatory synaptic paths, which is a perforant pathway input to the dentate gyrus in rats[332-334]. At this point, HA inhibits synaptic transmission by around 30% via presynaptic H3R located on the perforant path terminals. This effect can also be observed *in vivo* following i.c.v administration of an H3R agonist[335] or after the motivation of the TM through exploratory behavior[336]. Moreover, HA has a dominant impact on hippocampal excitability, as pyramidal and granule cells are deeply motivated by histamine acting on histamine H2R[337-339]. H1R administration raises the incidence of sharp wave-related ripples recorded in the CA1 region, in freely moving rats[340]. Sharp waves are likely to be essential in the generation of synaptic plasticity and memory consolidation, suggesting the possible implication of the histaminergic system in learning and memory formation[341,342].

Additionally, it was documented that the hippocampus in humans performs an essential role in the creation and distribution of seizures, as TLE in humans is characterized by structural defects like HS[211-213]. Furthermore, several earlier studies

have documented the vital role of the hippocampus in memory process in humans[343], where hippocampus removal resulted in an inability to gain new memories in affected patients[344] also patients with bilateral lesions, including the entire CA1 field of the hippocampus suffered from amnesic effects[345]. Besides, O'keefe et al. documented the importance of the hippocampus for the creation and use of spatial memory in rodenst[346], and this was also supported by a recent study that confirmed that the destruction of the hippocampus or its connections resulted in spatial memory severe deficit[347].

1.3.3 Role of H3Rs in locomotion

Locomotion is a central element of animal activity that can be influenced by several external and internal inducements such as introduction to a novel atmosphere or circadian rhythm, respectively[348,349]. Various disorders are associated with locomotor deficits, including Parkinson's disease and hyperactivity syndrome. Centrally acting compounds may also disturb locomotion that modifies further behavioral parameters, including memory.

Brain HA is involved in locomotor activity, as HDC knockout mice exhibited low locomotor activity. In addition, H1R knockout mice displayed locomotion, exploration, and rearing deficit in a novel atmosphere[249]. Moreover, in numerous earlier experiments, brain HA was observed with locomotor activity enhancement, as α-FMH administration caused brain HA depletion that resulted in spontaneous locomotor activity reduction[350,351]. Furthermore, it was noted that high brain HA levels were accompanied by enhanced locomotion[352]. Lately, it has been demonstrated that selective stimulation of HA neurons enhances locomotion[353]. Several H3R antagonists were reported to increase HA release and ability to improve locomotion[354,355].

However, opposing data were documented, with H3R knockout mice exhibiting lowered spontaneous locomotor activity[356]. In addition, α-FMH administration at a higher dose (100 mg/kg) in ICR mice enhanced locomotor activity at nighttime associated with an 85% reduction in brain GABA levels[357]. Moreover, thioperamide was found to reduce amphetamine-apomorphine-cocaine-evoked hyperactivity, and the effect was reversed by RAM administration[358]. Furthermore, H1R antagonists improved locomotor activity with the contribution of an opioid-dependent mechanism[359]. Interestingly, HA i.c.v. injection showed a biphasic action in rats: a temporary augmentation then a decline in locomotion activity[224].

Chapter 2: Aims and objectives

Based on the aforementioned evidences on the potential anticonvulsant and procognitive effects of several H3R antagonists in animal models, and the observed findings in the lab regarding targeting H3Rs in numerous neuropsychiatric disorders, objectives of this research is to target the histaminergic system with novel H3R antagonists to assess their anticonvulsant and procognitive effect applying different seizure models in rats.

Aims:

1- Evaluate different novel H3Rs antagonists, namely 1-16 of high *in-vitro* affinities for hH3Rs, on their *in vivo* anticonvulsant effect in MES-, PTZ-, and STR-induced acute seizure models.
2- Study the dose-dependent protective effect of the most promising H3R antagonist to validate the specification of the observed effect, and abrogate the protection provided effect by the most promising H3R antagonist by co-administration with the CNS-penetrant H3R agonist, H1R antagonist, and H2R antagonist to explain the mechanism behind the observed protective effect.
3- Assess the procognitive effect of the most promising H3R antagonist in different behavioral memory models, namely IAP and NOR, in the same animal species.
4- Evaluate the simultaneous anticonvulsant and procognitive effect of the promising H3R antagonist in both PTZ acute-induced seizure model and PTZ chronic-kindling model.

5- Examine the modulating effects of the most promising H3R antagonist on several hippocampal oxidative stress markers, AChE, levels of several brain neurotransmitters, and expression of c-fos protein, all of which are associated with both PTZ acute and chronic models.

6- Assess the anticonvulsant effect of the promising H3R antagonist in the PLC-induce SE model and examine the modulating effects of the most promising H3R antagonist on hippocampal oxidative stress markers and AChE following PLC- induced SE model.

Chapter 3: Materials and Methods

3.1 *In vitro* screening of H3R antagonists namely from 1-16

All *in vitro* experiments were conducted in the Department of Technology and Biotechnology of Drugs, Kraków, Poland.

3.1.1 Human histamine H3 receptor (*h*H3R) Binding Affinity for tested Compounds 1–16

The affinity for the human histamine H3R was examined applying radioligand displacement assays with [^{3}H) N^{α}-methylhistamine at membrane preparations from human embryonic kidney (HEK)-293 cells, sturdily expressing the receptor. Experiments were competed in triplicates with seven different concentrations of the test compounds 1-16 (Figure 7). The assessment of the data obtained was performed by the software GraphPad Prism 7 (San Diego, CA, USA), using the "one-site competition" equation. K_i values were calculated from the 50 percent of inhibitory concentration (IC_{50}) values according to the Cheng-Prusoff equation[360] (Table 2).

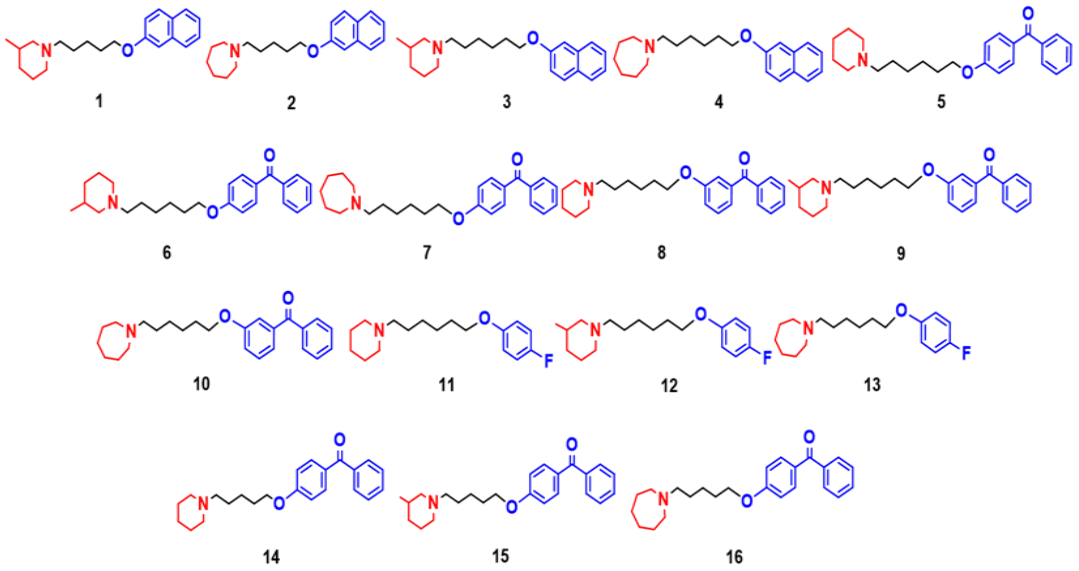


Figure 7: Chemical structures of potential H3R antagonists used in this study.

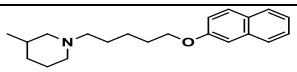
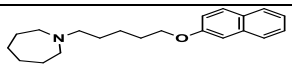
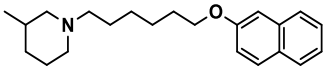
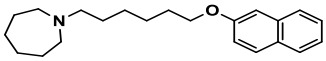
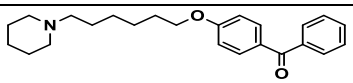
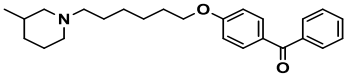
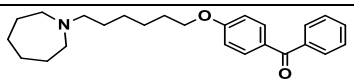
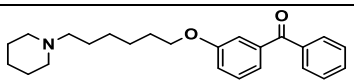
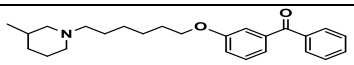
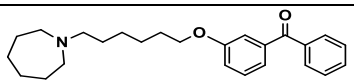
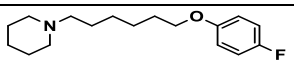
3.1.2 Human histamine H1 receptor (*h*H1R) Binding Affinity for Selected Compounds 4, 7 and 13

Radioligand binding was conducted as mentioned earlier utilizing membranes from CHO-K1 cells sturdily transfected with the human H1 receptor (PerkinElmer., Waltham, MA, USA)[361,362]. Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software, city, state, country) and Ki values were estimated from the Cheng–Prusoff equation[360].

3.1.3 Human histamine H4 receptor (*h*H4R) Binding Affinity for Selected Compounds 4, 7 and 13

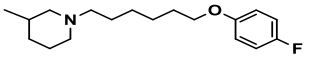
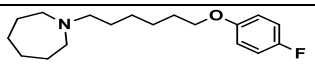
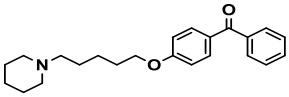
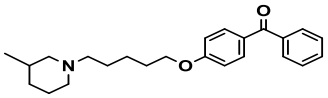
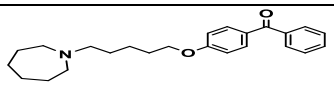
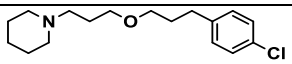
H4R radioligand displacement tests were conducted with Sf9 cell membrane preparations, expressing the hH4R, as described previously[305,362,363]. Tests were competed in triplicates with four suitable concentrations of the test compound. Data were analyzed by GraphPad Prism 7, using the "one-site competition" equation.

Table 2: *In vitro* hH3R affinities of ligands 1-16.

Ligand	Structure	*In-vitro* affinity K_i (hH$_3$R)[a] in nM [CI)
1		42.3[b] [18.3; 97.4)
2		57.3[b] [47.2; 69.7)
3		55.9[b] [44.8; 69.7)
4		69.3[b] [59.2; 81.1)
5		41.1 [25.6; 66.2)
6		52.8 [31.4; 88.8)
7		40.5 [32.9; 50.0)
8		76.1 [53.5; 108.3)
9		110.2 [61.8; 196.4)
10		69.5 [44.4; 108.8)
11		115.2 [78.4; 169.5)

a[3H]N-Methylhistamine binding assay performed with cell membrane preparations of human embryonic kidney (HEK) cells stably expressing the human histamine H3R. CI-confidence interval; b data from[364]; c data from the literature.

Table 2: *In vitro hH3R* affinities of ligands 1-16 (continued).

Ligand	Structure	*In-vitro* affinity K_i (hH_3R)[a] in nM [CI)
12		83.6 [65.8; 106.4)
13		137.2 [60.0; 313.9)
14		36.2 [10.0; 130.3)
15		40.2 [13.5; 119.4)
16		38.5 [10.5; 141.6)
Pitolisant (PIT)		11.69[c]

a[3H]N-Methylhistamine binding assay performed with cell membrane preparations of human embryonic kidney (HEK) cells stably expressing the human histamine H3R. CI-confidence interval; b data from[364]; c data from the literature.

3.2 *In vivo* screening of the H3R antagonists

The *in vivo* screening in this project consisted of two separate sections. The first section was for screening the anticonvulsant effect of all the compounds using electrically- and chemically- induced seizure models. The second section was for assessing the procognitive effect of the promising H3R antagonist applying different behavioral tests.

3.2.1 Animals

Experimental inbred male Wistar rats (Central Animal Facility of UAE University) were used in this study. All animals were maintained in an air-conditioned animal facility room with controlled temperature (24°C±2°C) and in humidity (55%±15%) under a-12 light/dark cycle; all animals had free access to food and water. All experiments of this study were done between 9:00 am to 1:00 pm, and all procedures were performed according to the guidelines of the European Communities Council Directive of November 24, 1986 (86/609/EEC) and was approved for the epilepsy study by the College of Medicine and Health Sciences/United Arab Emirates University (Institutional Animal Ethics Committee, approval number: ERA_2017_5676). All efforts were made to minimize animal suffering and to diminish the number of animals used. In addition, all animal procedures about convulsion studies were carried out by the same experimenter and in a blind manner. For the *in vivo* anticonvulsant and procognitive screening tests, PTZ- acute seizure model and PLC- induced seizure model Wistar rats weighing between 180-220 g (6-8 weeks old) were used in this study, while Wistar rats weighing between 140-160 g (4-6 weeks old) were used for PTZ- chronic model.

3.2.2 Drugs

The H3R antagonists, namely 1-16, that were assessed in this project are:

(1) 3-methyl-1-(5-(naphthalen-2-yloxy) pentyl)piperidine hydrogen oxalate; (2) 1-(5-(naphthalen-2-yloxy)pentyl) azepane hydrogen oxalate; (3) 3-methyl-1-(6-(naphthalen-2-yloxy)hexyl) piperidine hydrogen oxalate; (4) 1-(6-(naphthalen-2-yloxy)hexyl) azepane hydrogen oxalate; (5) phenyl(4-(6-(piperidin-1-yl) hexyloxy) phenyl)methanone hydrogen oxalate; (6) (4-(6-(3-methylpiperidin-1-

yl)hexyloxy)phenyl)(phenyl)methanone hydrogen oxalate; (7) (4-(6-(azepan-1-yl)hexyloxy)phenyl)(phenyl)methanone hydrogen oxalate; (8) phenyl(3-(6(piperidin-1-yl)hexyloxy)phenyl)methanone hydrogen oxalate; **(**9) (3-(6-(3methylpiperidin-1-yl)hexyloxy)phenyl)(phenyl)methanone hydrogen oxalate; (10) (3-(6-(azepan-1-yl) hexyloxy)phenyl) (phenyl)methanone hydrogen oxalate; (11) 1-(6-(4-fluorophenoxy) hexyl)piperidine hydrogen oxalate; (12) 1-(6-(4-fluorophenoxy)hexyl)-3-methylpiperidine hydrogen oxalate; (13)1-(6-(4-fluorophenoxy)hexyl) azepane hydrogen oxalate; (14) phenyl(4-(5-(piperidin-1-yl)pentyloxy) phenyl) methanone hydrogen oxalate; (15) (4-(5-(3-methylpiperidin-1-yl)pentyloxy) phenyl) (phenyl) methanone hydrogen oxalate; and (16) (4-(5-(azepan-1-yl)pentyloxy) phenyl) (phenyl) methanone hydrogen oxalate[364]. All these compounds were synthesized in the Department of Technology and Biotechnology of Drugs (Kraków, Poland), according to the methods described previously[304,365]. Pentylenetetrazol (PTZ, 60 or 40 mg/kg, intraperitoneal (i.p.), strychnine (STR, 3.5 mg/kg i.p.), valproic acid (VPA, 300 mg/kg, i.p.), (PHT, 10 mg/kg, i.p.), (*R*)-α-methylhistamine dihydrochloride (RAM, 10 mg/kg, i.p.), pyrilamine (PYR, 10 mg/kg, i.p.), zolantadine dimaleate (ZOL, 10 mg/kg, i.p.), donepezil hydrochloride (DOZ), dizocilpine hydrogen maleate (DIZ), scopolamine hydrobromide (SCO), pilocarpine hydrochloride (PLC), and scopolamine methyl nitrate were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). (DZP) was manufactured by Gulf Pharmaceutical Industries (Ras Al Khaimah, United Arab Emirates) and was obtained from Dr. Ameen Al Amaydah (Department of Emergency Medicine, Emirates International Hospital, Al Ain, United Arab Emirates). All compounds were dissolved in isotonic saline and administered intraperitoneal (i.p.) at a volume of 1 mL/kg. All doses of test compounds are expressed in terms of the free base.

3.2.3 Screening of the *in vivo* anticonvulsant effect of all the novel H3R antagonists (1-16) in different acute-induced seizure models

3.2.3.1 Maximal Electroshock (MES)-Induced Seizure model

MES test was developed by Toman at el in 1946[366] and still one of the best-validated preclinical used models against generalized tonic-clonic seizures (GTCS). Seizures in the MES test arise from the brain stem upon exposure to auricular excitation through ear electrodes. MES used to screen the protective effect of the potential compounds, and their capability to prevent seizures extends through neuronal tissue[367]. Seizures were induced as described previously[361,289,368], by applying 50 Hz alternating current of 120 mA intensity for 1 second through ear electrodes to each rat, which provokes maximal convulsions of their hind limbs, with tonic extension as the endpoint of the test. Abolition or reduction in the tonic hind limb extension (THLE) time was defined as protection from MES-induced seizure model.

For the initial screening of the anticonvulsant effect of the H3R antagonists (1-16) applying MES-induced seizure model, 18 groups were included in this study, and each group consisted of (n=6-8) rats matched for age and weight (180-220 g) as follows; group (1): control group injected with (SAL 0.9% i.p.), group (2): positive control group injected with PHT (10 mg/kg, i.p.) (the dose of PHT as used before is the minimal dose that protected animals against the spread of MES-induced seizures without mortality), and groups (3-18): treatment groups injected with the novel H3R antagonists 1–16 at a dose of (10 mg/kg, i.p.). All H3R antagonists and PHT were injected 30-45 min before applying the electroshock, and after that, the time of THLE was recorded.

Furthermore, other MES experiments were done for the dose-dependency study for the most promising protective compound, in three separate groups of (n=6-8) rats matched

for age and weight (180-220 g) in each group. The compound with the most promising protection in MES model was administered at doses of 2.5, 5, or 15 mg/kg, i.p. 30–45 min before the MES challenge.

In addition, abrogative studies were conducted, as the most effective dose of the promising H3R antagonist was designated for further analysis, in three separate groups of (n=6-8) rats matched for age and weight, the selected dose of the compound was co-injected with pyrilamine (PYR) (10 mg/kg, i.p.) a centrally acting H1R antagonist, zolantadine (ZOL) (10 mg/kg, i.p.) a centrally acting H2R antagonist 30-45 min prior to MES, or (R)-α-methylhistamine (RAM) (10 mg/kg, i.p.) a centrally acting H3R agonist 20 min prior to MES to ensure its presence in the CNS, as RAM was described to show fast metabolism. An additional three groups of (n=6) rats were tested as follows; SAL was co-injected with PYR, ZOL, or RAM alone (10 mg/kg, i.p.) before MES to exclude any effect of PYR, ZOL, or RAM on THLE time.

3.2.3.2 Chemically- induced seizure models

In this study and according to earlier protocols, two convulsant chemical agents had been used for inducing acute seizures. Namely (PTZ 60 mg/kg, i.p.) and (STR 3.5 mg/kg, i.p.)[369].

3.2.3.2.1 PTZ- induced seizures model

PTZ is a non-competitive $GABA_A$ receptor antagonist, was first synthesized in 1924[370]. PTZ-induced acute seizure model is widely used as a preclinical chemical model against GTCS. According to the previous protocols[371,372]. PTZ (60 mg/kg, i.p.) was systemically administered to all rats and immediately observed for 30 min. Average seizure score and percentage of protection against GTCS were recorded to

assess the protection provided by the potential test compound against seizures. Seizure score was graded according to Racine scale (stage 0, no change in behavior; stage 1, stereotype mouthing, eye blinking, and/or mild facial clonus; stage 2, head nodding and/or severe facial clonus; stage 3, myoclonic jerk in forelimbs; stage 4, clonic convulsions in the forelimbs with rearing; stage 5, generalized clonic convulsions associated with loss of balance)[373].

For the initial screening of the anticonvulsant effect of the H3R antagonists (1-16) applying PTZ-induced seizure model, 18 groups were included in this test, and each group consisted of (n=6-8) rats matched for age and weight (180-220) as follows; group (1): control group injected with (SAL 0.9% i.p.) + PTZ (60 mg/kg, i.p.), group (2): positive control group injected with VPA (300 mg/kg, i.p.) + PTZ (60 mg/kg, i.p.) (the dose of VPA as used before is the minimal dose that protected animals against the PTZ-induced seizures without mortality)[374,375], and groups (3-18): treatment groups injected with the novel H3R antagonists 1–16 at a dose of (10 mg/kg, i.p.) + PTZ (60 mg/kg, i.p.). All H3R antagonists and VPA were injected 30-45 min before PTZ injection.

Moreover, other PTZ experiments were done for dose-dependency study for the most promising protective compound, in three separate groups of (n=6-8) rats, the compound with the most promising protection in PTZ model was administered at doses of 2.5, 5, or 15 mg/kg, i.p. 30–45 min before the PTZ injection.

In addition, abrogative studies were conducted, as the most effective dose of the promising H3R antagonist was designated for further analysis, in three separate groups of (n=6-8) rats, the selected dose of the compound was co-injected with PYR (10 mg/kg, i.p.) a centrally acting H1R antagonist, ZOL (10 mg/kg, i.p.) a centrally acting

H2R antagonist 30-45 min before PTZ, or RAM (10 mg/kg, i.p.) a centrally acting H3R agonist 20 min prior to PTZ to ensure its presence in the CNS, as RAM was described to show fast metabolism. An additional three groups of (n=6) rats were tested as follows; PYR, ZOL, or RAM (10 mg/kg, i.p.) were co-injected with SAL + PTZ to exclude any effect of PYR, ZOL, or RAM on average seizure score.

3.2.3.2.2 STR-induced seizure model

STR is a glycine receptor antagonist, and it is used as a preclinical chemical model against GTCS. According to the previous protocols[371,305] STR (3.5 mg/kg, i.p.) was systemically administered to all rats and immediately observed for 30 min. Average seizure score and percentage of protection against GTCS were recorded to assess the protection provided by the potential test compound against seizures. Seizure score was graded according to Racine scale (stage 0, no change in behavior; stage 1, stereotype mouthing, eye blinking, and/or mild facial clonus; stage 2, head nodding and/or severe facial clonus; stage 3, myoclonic jerk in forelimbs; stage 4, clonic convulsions in the forelimbs with rearing; stage 5, generalized colonic convulsions associated with loss of balance)[373].

For the initial screening of the anticonvulsant effect of the H3R antagonists (1-16) applying STR-induced seizure model, 18 groups were included in this test, and each group consisted of (n=6-8) rats matched for age and weight (180-220) as follows; group (1): control group injected with (SAL 0.9% i.p.) + STR (3.5 mg/kg, i.p.), group (2): positive control group injected with VPA (300 mg/kg, i.p.) + STR (3.5 mg/kg, i.p.) (the dose of VPA as used before is the minimal dose that protected animals against the PTZ-induced seizures without mortality)[374,375], and groups (3-18): treatment groups injected with the novel H3R antagonists 1–16 at a dose of (10 mg/kg, i.p.) + STR (3.5

mg/kg, i.p.). All H3R antagonists and VPA were injected 30-45 min before STR injection.

Furthermore, another STR experiments were done for dose-dependency study for the most promising protective compound, in three separate groups of (n=6-8) rats, the compound with the most promising protection in STR model was administered at doses of 2.5, 5, or 15 mg/kg, i.p. 30–45 min before the STR injection. In addition, abrogative studies were conducted, as the most effective dose of the promising H3R antagonist was designated for further analysis, in three separate groups of (n=6-8) rats, the selected dose of the compound was co-injected with PYR (10 mg/kg, i.p.) a centrally acting H1R antagonist, ZOL (10 mg/kg, i.p.) a centrally acting H2R antagonist 30-45 min prior to STR, or RAM (10 mg/kg, i.p.) a centrally acting H3R agonist 20 min prior to STR to ensure its presence in the CNS, as RAM was described to show fast metabolism. An additional three groups of (n=6) rats were tested as follows; PYR, ZOL, or RAM (10 mg/kg, i.p.) were co-injected with SAL + STR to exclude any effect of PYR, ZOL, or RAM on average seizure score.

3.2.4 Screening of the procognitive effect of the promising H3R antagonist applying different behavioral tests

In vivo screening of the procognitive effect for the most promising H3R antagonist (compound 4) was assessed applying four different behavioral tests.

3.2.4.1 Inhibitory Avoidance Paradigm (IAP)

IAP test is a fear-motivated test mainly used to evaluate the effect of chemical compounds on different types of learning and memory. In this study, retrieval long term memory was assessed post DIZ- or SCO- induced amnesic effect in male Wistar rats applying IAP. IAP test was conducted utilizing Passive Avoidance Apparatus

(Step-through Cage, 7550, Ugo Basile, Comerio, Italy). The apparatus consists of two similar compartments (51 x 25 x 24 cm each) white and black with a grid floor, separated by an automated guillotine door (Figure 8). The IAP test was conducted as previously described[376,377,305,378,379,380], to assess the procognitive effect of the promising compound (compound 4) applying DIZ- and SCO- induced amnesic effect. The experiment consisted of two trails, two consecutive training days, and the test to be performed on the third day with 24 hours' interval. In the first training day, each rat was placed in the white compartment facing the door after 30 seconds of orientation time; the door was automatically raised up. Once the rat entered the dark compartment with his head, and all his four paws the door was lowered, and a foot shock of 0.4 mA (20 Hz, 8.3 ms) was delivered to the grid floor for a duration of 3 seconds, and the step-through latency (STL) time was measured. Cut off time of 60 seconds was given for the rat to cross the door; otherwise, the rat was excluded from the experiment. The intensity of the foot shock was chosen to minimize pain and reactions, such as jumping and screaming, in the examined animals. After the foot shock, the respective rat was immediately transferred to its home cage, and the chambers were cleaned with ethanol 70% in order to eliminate any confounding olfactory cues.

In the second training day (24 hours later), the same protocol was applied except that the cut off time was increased to 300 seconds, and no foot shock was delivered to the rat. On both training days, the rats received an injection of saline 30-45 minutes before starting the experiment. In the test day (24 hours later) same protocol of the second training day was applied, but animals turned amnesic by the administration of DIZ (0.1 mg/kg, i.p.) or SCO (1 mg/kg, i.p.) 30-45 minutes prior starting the test. The IAP task was conducted in the morning between 8:00 am and 12:00 noon. In this experiment, the animals were divided into 7 experimental groups of (n=6-8) rats matched for age

and weight in each group: All groups in the test day were treated with i.p. injections 30–45 minutes before the test as follows; group (1): control group injected with (SAL 0.9% i.p.), groups (2-3): amnesic groups treated with DIZ (0.1 mg/kg, i.p.) or SCO (1 mg/kg, i.p.) + (SAL 0.9% i.p.), groups (4-5): positive control groups injected with DOZ (1 mg/kg, i.p.) + DIZ or SCO, and groups (6-7): treatment groups injected with test compound 4 (10 mg/kg, i.p.) + DIZ or SCO. Moreover, other IAP experiments were done for a dose-dependency study applying the most promising amnesic compound (DIZ or SCO) to induced memory impairment in rats. Three separate groups of (n=6-8) rats were tested as follows; groups 1-3: received DIZ (0.1 mg/kg, i.p.) + test compound 4 (1.25, 2.5, or 5 mg/kg, i.p.) 30-45 minutes before the test.

Furthermore, the procognitive effect of H3R antagonist 4 was further investigated with abrogation tests by co-administration of the most effective dose of H3R antagonist 4, in three separate groups of (n=6-8) rats, the selected dose of the test compound 4 + DIZ was co-injected with PYR (10 mg/kg, i.p.) a centrally acting H1R antagonist, ZOL (10 mg/kg, i.p.) a centrally acting H2R antagonist 30-45 min prior the test, or RAM (10 mg/kg, i.p.) a centrally acting H3R agonist 20 min prior the test to ensure its presence in the CNS, as RAM was described to show fast metabolism. An additional three groups of (n=6-8) rats were tested as follows: DIZ + SAL were co-injected with PYR, ZOL, or RAM (10 mg/kg, i.p.) to excludes any effect of PYR, ZOL, or RAM on STL time. Doses of PYR, ZOL, and RAM were selected according to the previous publications[305,325,304].

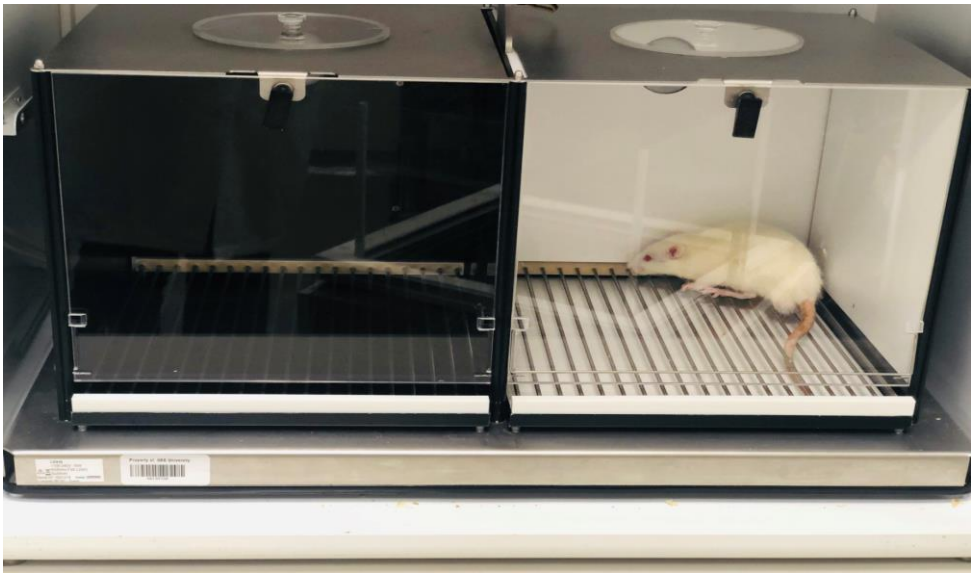


Figure 8: Passive avoidance apparatus utilized in this study.

In test day all rats were treated with DIZ 30 minutes before the test to induce amnesic effect, test compound 4 was injected 30-45 minutes, before DIZ treatment. STL time was measured to assess long-term memory.

3.2.4.2 Novel Object Recognition (NOR) test

NOR test is a behavioral model for testing both short- and long-term recognition memory. Interestingly the test depends on rodents' natural behavior, as there is no reward or penalty for the animals in the NOR test. NOR test was conducted according to the previous protocols[322,381] in an open field box 50 cm × 35 cm × 50 cm (Figure 9). The NOR task was conducted in the morning between 8:00 am and 12:00 noon.

On the first day, two habituation sessions with an interval of 1 hour, were conducted. Each rat was permitted to stay in the box for 3 min habituation time, after that the rat was returned to its home cage and the box was cleaned with ethanol 70% to eliminate any confounding olfactory clues. The experimental session on the second day (24 hours later) consisted of T1 (training session) and T2 (test session), which was conducted after 120 minutes for STM assessment and 24 hours for LTM assessment. In T1, each rat was introduced to two identical objects (9 cm × 5 cm × 9 cm) wood

blocks with the same size, shape, and color, which were placed in opposite corners of the box (30 cm apart) for 3 minutes. The time each rat spent exploring each object was calculated (exploration of an object defined as touching or snuffing the object, other behavioral observations, e.g., turning around or sitting on the object was not considered an experimental behavior). Immediately after training, each rat was returned to its home cage, and the box and the objects were cleaned with ethanol 70% in order to remove any confounding olfactory cues. In T2, an identical familiar object to eliminate any olfactory clues and a novel object (different in shape, size, and color) were presented to each rat, and the time spent exploring both the old and the novel objects was calculated. The animals which explored the objects for less than 10 seconds were excluded from the experiment. All rats were treated with DIZ to induce an amnesic effect to investigate the procognitive effect of the promising compound (4). In this experiment, the animals were divided into 6 experimental groups of (n=6-8) rats matched for age and weight in each group: All groups following T1 were treated with i.p. injections as follows; group (1): control group injected with (SAL 0.9% i.p.), group (2): amnesic group treated with DIZ (0.1 mg/kg, i.p.) + (SAL 0.9% i.p.), group (3): positive control group injected with DOZ (1 mg/kg, i.p.) + DIZ (0.1 mg/kg, i.p.), and groups (4-6): treatment groups were treated with test compound 4 (2.5, 5, and 10 mg/kg, i.p.) + DIZ (0.1 mg/kg, i.p.). In LTM assessment, test compound 4 was re-injected 30-45 min before T2. Besides, the procognitive effect of the test compound 4 was further investigated with abrogation tests by co-administration of the most effective dose of the test compound 4 + DIZ + PYR, ZOL, or RAM (10 mg/kg, i.p.) following T1, also 30-45 minutes for test compound 4, PYR, and ZOL and 20 minutes for RAM before T2 in LTM assessment.

PYR, ZOL, and RAM were co-injected with DIZ to eliminate any possible effects of PYR, ZOL, or RAM on time spent exploring the objects. Doses of the compounds and the treatment schedule were selected in accordance with previously published studies that have reported the memory-enhancing effects of H3R antagonists in the NOR task[322,381]. The discrimination index (DI) was calculated from the variables N–F/N+F (where N is the time the rat spent exploring the novel object and F is the time the rat spent exploring the familiar object in T2).

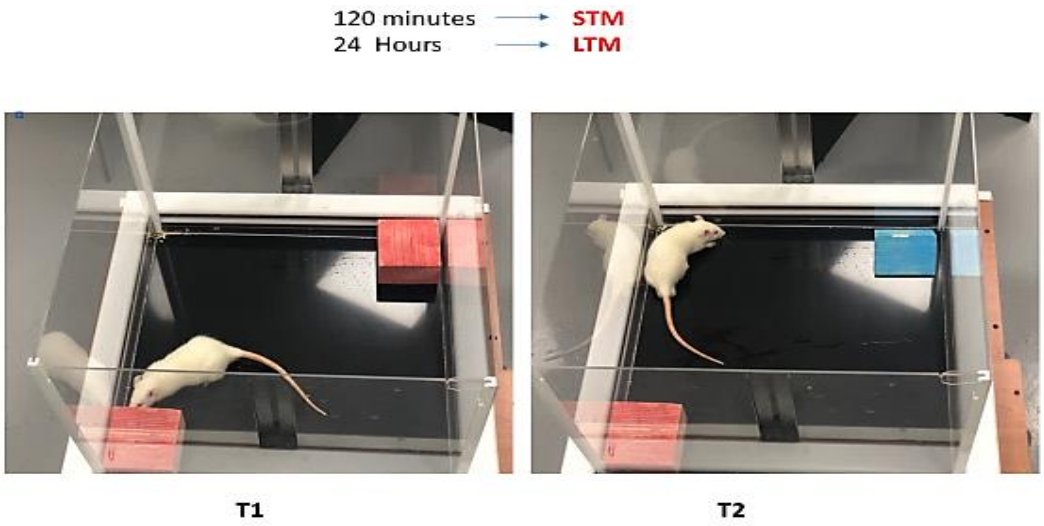


Figure 9: NOR apparatus utilized in this study.

T1: training session, T2: test session. In T1 all rats were introduced to two identical objects. Novel object was replaced one of the identical objects in T2 (2 hours or 24 hours) later for STM or LTM assessment, respectively. Exploration time for each object was measured and DI was calculated. DIZ and compound 4 were injected post T1, and compound was 4 re-injected 30-45 minutes before T2 in LTM assessment.

3.2.4.3 Elevated Plus Maze (EPM) test

EPM test is a behavioral model for estimating anxiety-like behaviors in animals. The EPM apparatus consists of several parts including one central part (8 cm × 8 cm), two opposing open and closed arms (30 cm × 8 cm), and non-transparent walls (30 cm in height) illuminated with four 60 V light-emitting diodes (LEDs) (Figure 10). EMP test

was conducted in the morning between 08:00 am and 12:00 noon, as previously described[322,382]. Each rat was placed in the center of the maze (50 cm above the floor) facing one of the open arms for 5 minutes, the total numbers of entries to both open and closed arms (with head and forepaws) and the time spent in both open and closed arms was manually measured. EPM apparatus was cleaned after each rat with 70% ethanol to remove any confounding olfactory cues. In this experiment, the animals were divided into 4 experimental groups of (6-8 rats matched for age and weight in each group): All animals received i.p. injections 30–45 minutes before the test to assess the anxiety-like parameters and locomotor activity as follows; group (1): control group injected with (SAL 0.9 % i.p.), group (2): positive control group treated with DZP (10 mg/kg, i.p.), and groups (3-4): treatment groups treated with the test compound 4 **(**5 and 10 mg/kg, i.p.)**.** The total number of closed arm entries was an indication of locomotor activity, while the total number of entries and total time spent in open arms were considered to be an indicator of anxiety level.

Figure 10: EPM apparatus used in this study.

All rats were placed in the center, total numbers of entries and the total time spent in both open and closed arms were calculated. Compound 4 was injected 30-45 minutes before the test.

3.2.4.4 Open Field Test (OFT)

Locomotor activity was measured using open field square box 56 × 56 × 56 cm3 (length/width/height) as previously described[382,380,379]. The test box is open from above with black polyvinyl chloride walls and was illuminated with four 60 V LEDs. The floor of the box is divided into 16 equal squares (14 cm in diameter), and the 4 central squares are regarded as the "center" of the field (Figure 11). The test was conducted in the morning between 8.00 am to 12.00 noon. In the test day, each rat was placed in the center of the box and allowed to move freely for 3 minutes, as habituation time, then the rat was returned to its home cage, and the box was cleaned with ethanol 70% to avoid any confounding olfactory clues. 30 minutes later, all the animals received i.p. injection of the tested compound 30-45 minutes before the test session. In the test session, each rat was placed in the center by its tail and allowed to move freely for 3 minutes, animal activity (distance traveled, and time spent in locomotion) was recorded by a digital camera placed above the arena. When a rat moved from one

square to another with its four paws, the one-line crossing is noted, and a distance of 14 cm is recorded. As previously described, the following parameters were manually scored: the number of lines crossed (defined as at least three paws in a square) and time spent in the center of the arena and in the periphery during a 3 min. test. Less time spent in the central area is usually taken as a measure of a higher level of anxiety, while less distance traveled indicates a deficit in locomotor activity. In this experiment, the animals were divided into 4 experimental groups (6-8 rats matched for age and weight in each group): All animals received i.p. injection 30–45 minutes before the test session to assess the anxiety-like parameters and locomotor activity as follows; group (1): control group injected with (SAL 0.9% i.p.), group (2): positive control group injected with DOZ (1 mg/kg, i.p.), and groups (3-4): treatment groups treated with test compound 4 **(**5 and 10 mg/kg, i.p.).

Figure 11: OFT apparatus utilized in the study.

All rats were placed in the center, total distant traveled and total time spent in both the center and the periphery were calculated. Compound 4 was injected 30-45 minutes before the test.

3.2.5 PTZ model for assessing the simultaneous memory impairment associated with PTZ- acute and chronic models

3.2.5.1 PTZ- induced acute model

The PTZ- induced seizure model for assessing the simultaneous memory impairment associated with PTZ- induced seizures protocol was applied as previously described[130,131,133] (Figure 12). On day 1, all animals were injected with PTZ (60 mg/kg, i.p.) after the training session in both IAP and EPM tests. The test session was conducted 24 hours later to assess the memory impairment associated with PTZ-induced seizures. In set of experiments the animals were divided to five experimental groups (12-16) rats matched for age and weight (180-220 g) in each group) as follows; group (1): naïve group injected with (SAL 0.9% i.p.), group (2): PTZ group injected with PTZ (60 mg/kg, i.p.), group (3): positive control group treated with VPA (300 mg/kg, i.p.) + PTZ (60 mg/kg, i.p.), and groups (4-5): treatment groups injected with test compound 4 (5 and 10 mg/kg, i.p.) + PTZ (60 mg/kg, i.p.). VPA and compound 4 were injected 30-45 minutes before the PTZ injection.

3.2.5.2 PTZ- kindling model

The PTZ- kindling protocol was applied as previously described with slight modifications[383,71,384,318,72,385,141]. All animals were injected with a sub-convulsant dose of PTZ (40 mg/kg, i.p.) three times a week for 12 injections (26 days) (Figure 12). The PTZ injections were stopped at the end of the 12 injections or when animals are fully kindled upon the development of seizure score 4 or 5 in three consecutive injections. All animals were observed for 30 minutes after each PTZ injection, and the average seizure score for each rat was recorded according to the following Racine scale: (stage 0, no change in behavior; stage 1, stereotype mouthing, eye blinking, and/or mild facial clonus; stage 2, head nodding and/or severe facial clonus; stage 3, myoclonic jerk in

forelimbs; stage 4, clonic convulsions in the forelimbs with rearing; stage 5, generalized clonic convulsions associated with loss of balance[373]). The kindling percentage and the survival rates for each group were calculated. Different doses of PTZ were applied to induce kindling (35, 37.5, and 40 mg/kg), 40 was the most optimal dose to induce kindling in Wistar rats with less mortality. Inset of experiments the animals were divided to five experimental groups (n=12-16) rats matched for age and weight (140-160 g) in each group as follows; group (1): naïve group injected with (SAL 0.9% i.p.) three times a week, group (2): PTZ group injected with PTZ (40 mg/kg, i.p.) three times a week, group (3): positive control group injected with VPA (300 mg/kg, i.p.) + PTZ (40 mg/kg, i.p.) three times a week, and groups (4-5): treatment groups treated with test compound 4 (5 and 10 mg/kg, i.p.) + PTZ (40 mg/kg, i.p.) three times a week. SAL and PTZ were injected 3 times a week for 12 injections (26 days), while VPA and test compound 4 were injected 30-45 minutes before each PTZ injection. The training session in IAP and EPM was performed 24 hours post the last PTZ injection, followed by the test session (24 hours later). The anticonvulsant and procognitive effects of H3R antagonist 4, were further investigated with abrogation tests by co-administration of the most effective dose of H3R antagonist 4 + (PYR, ZOL, or RAM (10 mg/kg, i.p.) 30-45 minutes before each PTZ injection, only for RAM 20 minutes before each PTZ injection to ensure its presence in the CNS, as RAM was described to show fast metabolism. PYR, ZOL, and RAM were co-injected alone with PTZ or SAL to eliminate any possible effects of PYR, ZOL, or RAM on the kindling procedure and the memory function.

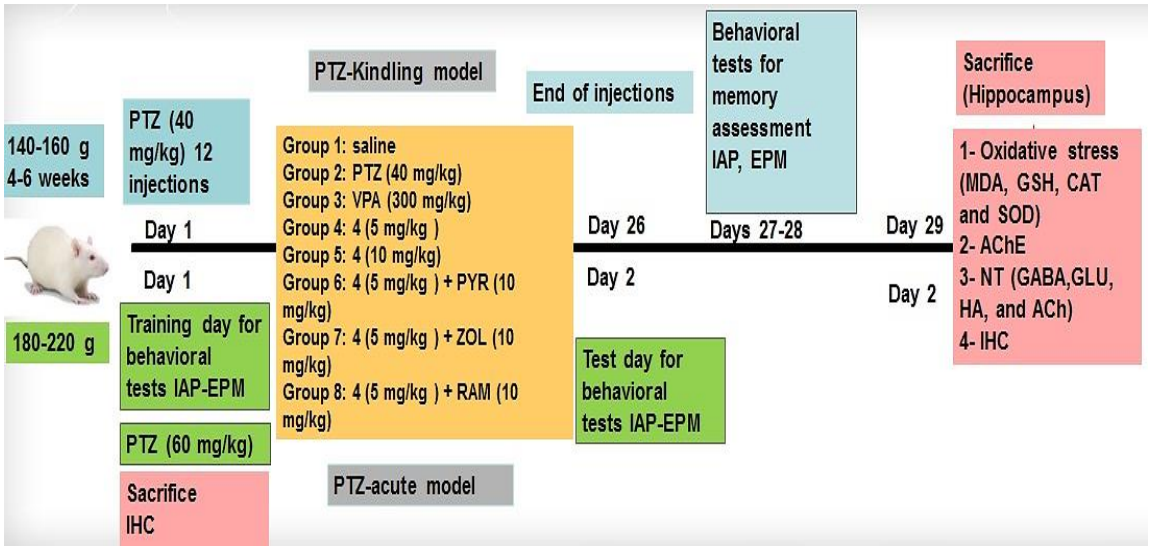


Figure 12: Schematic illustration of both PTZ- acute and PTZ- kindling models, experimental protocol, drug treatments, behavioral studies, and biochemical assessments.

Male Wistar rats were injected with PTZ (60 mg/kg) in acute- PTZ model, while PTZ (40 mg/kg) was injected 3 times a week for 12 injections in PTZ-kindling model. Behavioral studies were done (days 1-2 and days 27-28 for PTZ- acute and chronic models, respectively). All animals were sacrificed in day 2 in PTZ- acute model and 24 hours after the last behavioral assessment (day 29) in PTZ- kindling model for both biochemical assessments and IHC. PTZ: Pentylenetetrazol, IAP: Inhibitory avoidance paradigm, EPM: Elevated plus maze, IHC: Immunohistochemistry, AChE: Acetylcholinesterase, NT: Neurotransmitters, GLU: Glutamate, ACh: Acetylcholine, HA: Histamine.

3.2.5.3 Behavioral tests utilized in both PTZ- acute and chronic models to assess memory impairment associated with PTZ- induced seizures

The memory impairment associated with PTZ seizures was assessed applying (IAP) and (EPM). In PTZ- kindling model the tests were conducted 24 hours after the last PTZ injection. While in PTZ- acute model, the training session was conducted 120 min. before PTZ injection and the test session 24 hours later. Each group consists of (n= 6-8 animals) and different animals were used in each behavioral test.

3.2.5.3.1 Inhibitory Avoidance Paradigm (IAP)

IAP test is a fear-motivated test mainly used to evaluate the effect of chemical compounds on learning and memory, and it is the most widely used behavioral test to investigate memory impairments associated with PTZ acute as well as chronic seizures

as previously described[71,386,387]. IAP was conducted as previously described[388,389,390], as it consists of two trails (training and test). In the training day, each rat was placed in the white compartment, and after 30 seconds habituation time, the door was automatically raised up. Once the rat with all its four paws and head entered the dark compartment, the door was closed, and a foot shock of 0.4 mA (20 Hz, 8.3 ms) was delivered to the grid floor for 3 seconds, and STL time was measured. Immediately after the foot shock, the rat was returned to its home cage, and the compartments were cleaned up with ethanol 70%. Cut off time of 60 seconds was given for the rat to cross the door; otherwise, the rat was excluded from the experiment. In the test session (24 hours later), the same protocol was applied except that the cut off time was increased to 300 seconds, and no foot shock was delivered to the rat. The reduction of STL time is used as an indicator of memory impairment.

3.2.5.3.2 EPM

EPM test is usually used to assess anxiety-related behaviors in rodents. However, and based on the way of experimental conduct, EPM was shown to provide convincing experimental face validity for the assessment of spatial memory impairments associated with PTZ seizures in rodents[69,391,72,392,393]. EPM test for assessing the memory impairment associated with both PTZ acute and chronic models was conducted as previously described[69,391,72,392] (Figure 13). During the acquisition trial on the first day, each rat was placed at the distal ending of the open arm facing away from the central platform. Transfer latency time (TLT), which is the time required for the rat to enter any of the closed arms with its four paws, was measured. After 10 seconds spent in the closed arm, the animal was returned to its home cage, and the apparatus was cleaned with ethanol 70%. Cut off time of 60 seconds was given for the

rat to enter any of the closed arms. Otherwise, the rat was excluded from the experiment. Subsequently, memory retention was examined 24 hours later, applying the same protocol of the first day. Prolonged TLT is an indication of memory impairment of PTZ treated animals[131,49].

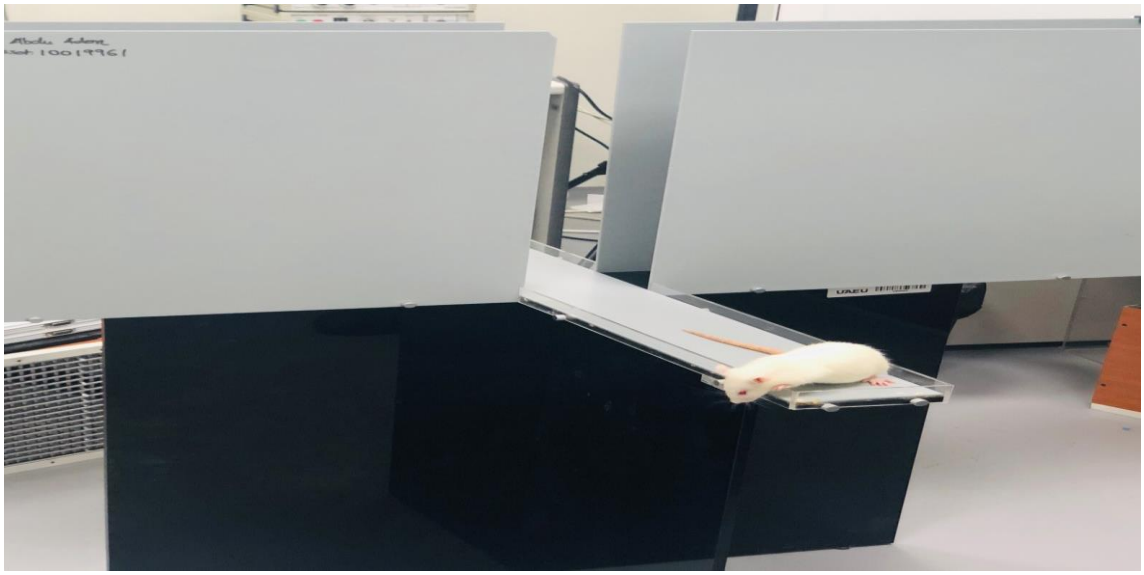


Figure 13: EPM apparatus used in both PTZ-acute and chronic models for memory assessment.

All rats were placed in the distal ending of one of the open arms, TLT was measured as an indicator for memory impairment. TLT: Transfer latency time.

3.2.5.4 Brain collection and tissue preparation for biochemical studies and immunohistochemistry (IHC)

All the rats were divided into two groups, and each group contains (n = 6-8) rats. On day 2 one hour after completing the behavioral assessments in PTZ- acute model, and 24 hours after the last behavioral test in PTZ- kindling model, all the rats in the first half of the animals (n= 6-8 rats) were anesthetized with pentobarbital (40 mg/kg body weight). Cardiac perfusion was carried out using 0.01 M phosphate-buffered saline (PBS) at pH 7.4 in order to flush the blood out in rats. Then brains were quickly

removed and placed on an ice-plate where each brain was cut, and the hippocampus was removed from the brain and immediately snap-frozen in liquid nitrogen for further use. For tissue preparation, the hippocampus was weighed and divided into two equal sections. The first part was homogenized in KCL buffer (Tris–HCl, 10 mM NaCl, 140 mM KCl, 300 mM EDTA, 1 mM Triton-X-100 0.5 %) at pH 8.0 supplemented with protease and phosphatase inhibitor. The homogenate was centrifuged at 10,000×g for 30 min at 4°C. The clear supernatant was collected for estimation of oxidative stress markers: MDA, GSH, CAT, and SOD. The second part was homogenized in ice-cold (0.05 M, pH 8.0) phosphate buffer PB. The homogenate was then centrifuged at 10,000×g for 30 min at 4°C. The clear supernatant was collected for estimation of AChE and neurochemical estimations of HA, ACh, GLU, and GABA.

For IHC method, the other half of the animals were anesthetized with pentobarbital (40 mg/kg body weight), 24 hours after the last behavioral test in PTZ- kindling model, and 2 hours post PTZ injection in PTZ-acute model. Cardiac perfusion was carried out using 0.01 M phosphate-buffered saline (PBS) at pH 7.4 in order to flush the blood out, and all the rats were further perfused with Zamboni solution (buffered picric acid-paraformaldehyde), and the brains were fixed overnight in Zamboni solution at 4°C The day after, the brains were dehydrated with graded concentrations of ethanol, then the ethanol residue was removed by xylene, and lastly, embedded in liquid paraffin for further use in IHC analysis[394]. All experimenters who performed the behavioral, biochemical tests, and IHC analysis were blinded to the experimental groups.

3.2.5.4.1 Oxidative stress markers

3.2.5.4.1.1 MDA

The levels of lipid peroxidation product, MDA, were measured using the MDA detection kit (North West Life Science, Vancouver, WA, USA). The protocol of the assay was followed, as described in the kit datasheet. All the samples and standards (150 μL) were incubated with thiobarbituric acid TBA (150 μL), in the presence of acid reagent to expedite the hydrolysis of MDA for 60 min at 60 °C to form MDA-TBA_2 adduct that absorbs significantly at 532 nm. After that, all the samples and standards were centrifuged for 2 min at 10,000×g. Then all the reaction solutions were transferred to 96 well plate reader to record the absorbance at 532 nm. The assay used a standard curve of MDA to calculate the amount of MDA in the samples. The results are expressed as μM MDA/mg protein.

3.2.5.4.1.2 GSH

The levels of GSH were measured using a commercially available GSH kit (Sigma-Aldrich Co. St Louis, MO, USA). First, all the samples were deproteinized with 5% 5-sulfosalicylic acid solution; after that, the samples were centrifuged at 10,000×g for 10 min to order to eliminate ant precipitated protein, then the supernatant was used in this GSH measurement. Incubation of all the samples and standards (10 μL) in 96 well plates with (150 μL) working reagent (assay buffer + 5, 5′-dithiobis (2-nitrobenzoic acid) DTNB + GSH reductase) for 5 minutes at room temperature RT, then it was diluted with NADPH solution (50 μL). The absorbance of the samples and standards were recorded at 412 nm with the kinetics for 5 minutes by using the microplate reader. The assay used a standard curve of GSH to calculate the amount of GSH in the samples. The results are expressed as μM GSH/mg protein.

3.2.5.4.1.3 CAT

The activity of the antioxidant enzyme, CAT, was estimated using a commercially available assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Briefly, CAT was estimated by adding (20 µl) samples or standards of different concentrations with (100 µl) assay buffer and (30 µl) methanol in a 96-well plate. The reaction was initiated by adding H_2O_2 (20 µl) to each well to produced formaldehyde, and the 96-well plate was covered and incubated on a shaker for 20 min at RT. Potassium hydroxide (30 µl) was used to terminate the reaction, and subsequently, (30 µl) CAT purpald was added to react with formaldehyde and produce bicyclic heterocycle, and then the 96-well plate was covered and incubated on a shaker for 10 min at RT. After that, (10 µl) CAT potassium periodate was added to each well to oxidize the bicyclic heterocycle and change the color to purple. The plate was covered and incubated for 5 min on a shaker at RT. Finally, absorbance was read at 540 nm using a microplate reader. The assay used a standard curve of CAT to calculate the amount of CAT in the samples. The CAT activity was expressed as the amount of enzyme the cause the formation of 1 nmol of formaldehyde per min (nmol/min/mg).

3.2.5.4.1.4 SOD

The activity of the antioxidant enzyme SOD was estimated by using a commercially available assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Briefly, SOD was measured by adding (10 µl) diluted samples or standards of different concentrations with (200 µl) radical detector in a 96-well plate. The reaction was initiated by adding (20 µl) xanthine oxidase to each well; then, the 96-well plate was covered and incubated on a shaker at RT for 30 min. Finally, absorbance was read at 450 nm using a microplate reader. The assay used a standard curve of SOD to calculate

the amount of SOD in the samples. The SOD activity was expressed as the amount of the enzyme required to reveal a 50% dismutation of the superoxide radical (U/ml).

3.2.5.4.2 AChE activity

The activity of AChE was estimated using a colorimetric assay kit (Biovision company, Milpitas, USA). All samples (5 µl) were diluted with (45 µl) AChE assay buffer in 96-well plate; then the reaction was initiated by adding (50 µl) reaction mix (AChE assay buffer, AChE probe, AChE substrate, and choline oxidase enzyme mix) to each well, after that the 96-well plate was incubated in 37°C for 20-30 min. Finally, absorbance was read at 570 nm in a kinetic model using a microplate reader. The assay used a standard curve of AChE to calculate the amount of choline in the samples. AChE activity was expressed as the amount of the enzyme that produces 1.0 nmol of choline per min (nmol/min/mg).

3.2.5.4.3 Neurochemical estimation

3.2.5.4.3.1 Estimation of hippocampus GABA concentration in both PTZ- acute and chronic models

The hippocampal concentration of GABA was estimated using a quantitative sandwich enzyme linked-immunosorbent assay (Elisa) kit (My BioSource company, Milpitas, USA). The protocol of the assay was followed, as described in the kit datasheet. All the samples and standards (50 µL) were incubated with HPR-conjugated reagent (100 µL) for 60 minutes at 37°C. After that, all the wells were automatically washed for 4 times using a wash solution. Later, chromogen A (50 µL) and chromogen B (50 µL) were added to each well, and the plate was incubated for 15 minutes at 37°C. Afterword, the reaction was stopped by adding (50 µL) of stop solution, and the absorbance was recorded at 570 nm 15 minutes later. The assay used a standard curve

of GABA to calculate the amount of GABA in the samples. GABA concentration was expressed as μmol/mg.

3.2.5.4.3.2 Estimation of hippocampus (GLU) concentration in both PTZ- acute and chronic models

The hippocampal concentration of GLU was estimated using a colorimetric assay kit (Biovision company, Milpitas, USA). The protocol of the assay was followed, as described in the kit datasheet. All samples and standards with different concentrations were added to the wells in 96-well plate (10 μL), and each well was brought to (50 μL) by assay buffer. The reaction started by adding (100 μL) of the working reagent (GLU assay buffer, GLU developer, and GLU enzyme mix), and the plate was incubated for 30 minutes at 37°C. After that, the absorbance was recorded at 450 nm. The assay used a standard curve of GLU to calculate the amount of GLU in the samples. GLU concentration was expressed as nmol/mg.

3.2.5.4.3.3 Estimation of hippocampus histamine (HA) concentration in both PTZ- acute and chronic models

The hippocampal HA concentration was estimated using a colorimetric assay kit (Abecam company, Cambridge, United Kingdom). The protocol of the assay was followed, as described in the kit datasheet. All samples and standards with different concentrations were added to the wells in 96-well plate (10 μL), and each well was brought to (50 μL) by HA assay buffer. The reaction started by adding (50 μL) of the working reagent (HA assay buffer, HA probe, and HA enzyme mix), and the plate was incubated for 30 minutes at 37°C. After that, the absorbance was recorded at 450 nm. The assay used a standard curve of HA to calculate the amount of HA in the samples. HA concentration was expressed as nmol/mg.

3.2.5.4.3.4 Estimation of hippocampus (ACh) concentration in both PTZ- acute and chronic models

The hippocampal ACh concentration was estimated using a colorimetric assay kit (Abecam company, Cambridge, United Kingdom). The protocol of the assay was followed, as described in the kit datasheet. All samples in duplicate and standards with different concentrations were added to the wells in 96-well plate (10 μL), and each well was brought to (50 μL) by choline assay buffer. The reaction started by adding (50 μL) of the working reagent (choline assay buffer, choline probe, and choline enzyme mix) in the first group of the samples to measure the free choline, while in the other group same working reagent was added with addition of acetylcholinesterase to measure the total choline, afterword the plate was incubated for 30 minutes at RT. Finally, the absorbance was recorded at 570 nm. The assay used a standard curve of choline to calculate the amount of choline in the samples. ACh concentration was calculated using the equation: total choline- free choline. ACh concentration was expressed as nmol/mg.

3.2.5.4.4 c-fos immunohistochemistry and quantification of c-fos positive cells

Tissue samples embedded in paraffin blocks were sectioned using a microtome (Thermo Scientific™, England) to 5-10 μm sections. Then the sections were collected on gelatin-coated slides and left for 2 hours to dry up at 45^{o}C. The paraffin-embedded sections were deparaffinized and hydrated momentarily in a series of xylene and alcohol of different concentrations then treated with sodium citrate buffer after that washed three times in phosphate buffered saline (PBS). The sections were treated with a blocking agent [% of bovien serum albumin (BSA) in phosphate buffer (PB) and Tween 20] for 45- 60 min, before adding the c-fos mouse monoclonal primary antibody (1:100, Santa Cruz Biotechnology) for overnight incubation at 4°C in a

humid chamber. The primary antibodies against c-fos were labeled with FITC donkey anti-mouse IgG (Jackson ImmunoResearch Inc.). The sections were washed three times in PBS before and after applying the secondary antibody. Then, the sections were covered with fluoromount aqueous mounting medium (Sigma-Aldrich, St Louis, MI, USA) and cover slipped. Two control groups (n=6); sections were processed according to the same protocol explained earlier, but PBS was used to replace the primary or secondary antibodies. The sections were examined using a Nikon fluorescent microscope (Nikon, Japan) equipped with appropriate filters to reveal Alexa 488 (green fluorescent) to visualize the expression of the c-fos protein. The images were taken using a 40× objective lens with a digital DS-Ri2 high-definition color camera connected to the microscope (Nikon, Japan).

3.2.5.4.5 Morphometric analysis

All the images were examined using ImageJ software (NIH, USA), which facilities several imaging system data comparisons, with density (densitometry) taken into consideration, and the left overexpressed nuclei green particles were considered to represent c-fos expression[395]. The number of the hippocampal c-fos positive cells were obtained from 4 sections for each rat (n=6 rats per group). Location in the brain was confirmed using the delineations in the Paxinos Atlas[396]. The number of c-fos immunoreactive nuclei were counted in each field (at 40x) across the hippocampus area over the layer extending from subregions CA1-CA3, then the values were presented as means ± S.E.M of the positive c-fos cells per filed in each rat.

3.2.6 Pilocarpine (PLC)-Induced Status Epilepticus (SE) model

3.2.6.1 Experimental Procedure of PLC-Induced SE

The PLC- induced SE model was applied as previously described[397-399] (Figure 14). PLC (400 mg/kg, i.p.) was systemically administered to all rats and immediately observed for 30 min in separate plastic cages. Latency to first seizure (any epileptic behavioral observed after PLC administration, e.g., wild running, clonus, tonus, and tonic-clonic seizures), number of rats experienced SE, and the survival rates for all the animals at 1 hour after PLC injection were recorded. Earlier findings indicated that seizures and deaths detected within 1 and 24 hours following PLC administration continually pursued in a comparable manner[398,400]. The PLC group consists of the rats, which experienced SE and survived within 1 h. Inset of experiments the animals were divided to seven experimental groups (n=10-12) rats matched for age and weight (180-220 g) in each group as follows; group (1): naïve group injected with (SAL 0.9% i.p.), group (2): PLC group injected with PLC (400 mg/kg, i.p.), group (3): positive control group injected with DZP (10 mg/kg, i.p.) + PLC (400 mg/kg, i.p.) and groups (4-7): treatment groups injected with test compound 4 (2.5, 5, 10, and 15 mg/kg, i.p.) + PLC (400 mg/kg, i.p.).

All groups were treated with scopolamine methyl nitrate (1 mg/kg, i.p.) 30 min before PLC injection to reduce the peripheral cholinergic effects of PLC. Moreover, the protective effect of compound 4 was further investigated with abrogation tests by co-administration of the most effective dose of compound 4 + (PYR, ZOL, or RAM (10 mg/kg, i.p.) 30-45 minutes before the PLC injection, only for RAM 20 minutes before the PLC injection to ensure its presence in the CNS, as RAM was described to show

fast metabolism. PYR, ZOL, and RAM were co-injected alone with PLC or SAL to eliminate any possible effects of PYR, ZOL, or RAM on SE incidence.

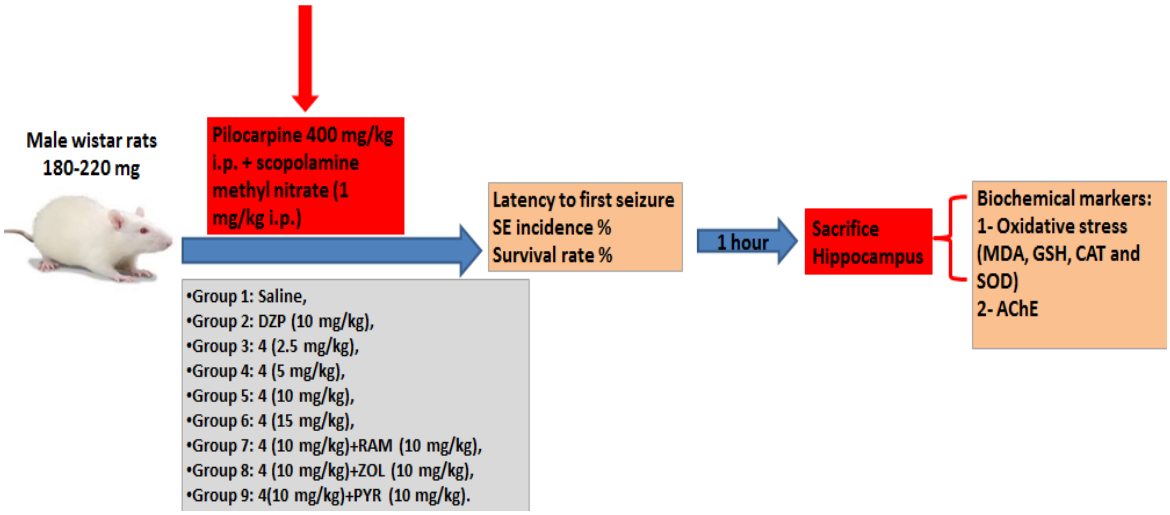


Figure 14: Schematic illustration of PLC- induced SE, experimental protocol, drug treatments, and biochemical assessments.

Male Wistar rats were injected with PLC (400 mg/kg) for PLC- induced SE model. Compound 4 (5 and 10 mg/kg, i.p.) was injected 30-45 minutes before PLC injection. All animals were sacrificed in 1 hour after PLC injection for biochemical assessments.

3.2.6.2 Brain collection and tissue preparation for biochemical assessment

All animals that survived within 1 hour after PLC injection were anesthetized with pentobarbital (40 mg/kg body weight). Cardiac perfusion was carried out using 0.01 M phosphate-buffered saline (PBS) at pH 7.4 for all the animals. Brains were quickly removed and placed on an ice-plate where each brain was cut, and the hippocampus was removed from the brain and immediately snap-frozen in liquid nitrogen for further use. For tissue preparation, the hippocampus was weighed and divided into two equal sections. The first part was homogenized in KCL buffer (Tris–HCl, 10 mM NaCl, 140 mM KCl, 300 mM EDTA, 1 mM Triton-X-100 0.5 %) at pH 8.0 supplemented with protease and phosphatase inhibitor. The homogenate was centrifuged at 10,000×g for

30 min at 4°C. The clear supernatant was collected for estimation of oxidative stress markers; MDA, GSH, CAT, and SOD. The second part was homogenized in ice-cold (0.05 M, pH 8.0) phosphate buffer PB. The homogenate was then centrifuged at 10,000×g for 30 min at 4°C. The clear supernatant was collected for the estimation of AChE activity.

3.2.6.2.1 oxidative stress marker

3.2.6.2.1.1 MDA

Same protocol was explained in 3.2.5.4.1.1.

3.2.6.2.1.2 GSH

Same protocol was explained in 3.2.5.4.1.2.

3.2.6.2.1.3 CAT

Same protocol was explained in 3.2.5.4.1.3.

3.2.6.2.1.4 SOD

Same protocol was explained in 3.2.5.4.1.4.

3.2.6.2.2 AChE

Same protocol as explained in section 3.2.5.4.2. was applied.

3.2.7 Statistical Analysis

For statistical comparisons, the software package Statistical Package for the Social Sciences (SPSS) 25.0 (IBM Middle East, Dubai, UAE) was used. All data are expressed as the means ± standard error of mean (SEM). K_i values at the H3R are provided as means with the 95% confidence interval. All protective effects observed

for H3R antagonists in acute- and chronic- induced seizure models were analyzed using one -way analysis of variance, followed by the Bonferroni or Tukey post hoc tests for multiple comparisons. The results observed for percentage seizures and survival rates in PTZ- kindling model and PLC- induced seizure model is expressed as percentages of the number of animals from each experimental group and were analyzed using a nonparametric test (X2). The criterion for statistical significance was set at $P < 0.05$.

Chapter 4: Results

4.1 *In vivo* screening of anticonvulsant effect of H3R antagonists namely 1-16

4.1.1 Anticonvulsant effect screening of H3R antagonists 1-16 applying MES-induced seizure model

The initial screening for the anticonvulsant effect of acute systemic pretreatment with H3R ligands 1-16 on MES-induced seizure model in male Wistar rats, was completed, and the obtained results were compared with the protective effect of the reference antiepileptic drug PHT in MES-induced seizure in rats (Figure 15). The observed results revealed that acute systemic administration of PHT (10 mg/kg, i.p.) and H3R ligands 1-16 (10 mg/kg, i.p.) demonstrated a significant protection against MES-induced seizures as confirmed by one-way analysis of variance ($F_{(17,108)} = 8.352$; $P < 0.001$).

Following post hoc analysis, the obtained results indicated that acute systemic administration of PHT (10 mg/kg, i.p.) and H3R ligands 3, 4, 5, 6, 7, and 14 (10 mg/kg, i.p.) exhibited a significant protection against MES-induced seizures (significantly decreased THLE time) ($F_{(1,12)} = 31.86$; $P < 0.001$), ($F_{(1,12)} = 23.85$; $P < 0.001$), ($F_{(1,12)} = 34.6$; $P < 0.0001$), ($F_{(1,12)} = 21.48$; $P < 0.05$), ($F_{(1,12)} = 7.34$; $P < 0.05$), ($F_{(1,12)} = 13.5$; $P < 0.05$), and ($F_{(1,12)} = 10.08$; $P < 0.05$), respectively when compared to SAL-treated group. Moreover, amongst the H3R antagonists tested and following post hoc analyses, compound 4 at a dose of 10 mg/kg significantly displayed the most promising protective effect in MES-induced seizure model when compared with the SAL-treated group ($F_{(1,12)} = 34.6$; $P < 0.0001$), and provided comparable protection to that of PHT ($F_{(1,12)} = 1.14$; $p = 0.31$) (Figure 15, Tables 3 & 4). Furthermore, the protective effect

stated with H3R antagonist 4 at a dose of 10 mg/kg, i.p. was significantly higher than that noticed for H3R antagonists 3, 5, 6, 7, and 14 ($F_{(1,12)} = 5.88$; $P < 0.05$), ($F_{(1,12)} = 9.72$; $P < 0.05$), ($F_{(1,12)} = 8.20$; $P < 0.05$), ($F_{(1,12)} = 6.03$; $P < 0.05$), and ($F_{(1,12)} = 7.11$; $P < 0.05$), respectively (Figure 15). However, acute pretreatment with the remaining H3R antagonists 1, 2, 8, 9, 10, 11, 12, 13, 15, and 16 failed to exhibit a protective effect in MES- induced seizure model ($F_{(1,12)} = 0.94$; p = 0.35), ($F_{(1,12)} = 0.03$; p = 0.85), ($F_{(1,12)} = 1.52$; p = 0.24), ($F_{(1,12)} = 0.004$; p = 0.94), ($F_{(1,12)} = 0.38$; p = 0.55), ($F_{(1,12)} = 0$; p = 1), ($F_{(1,12)} = 0.04$; p = 0.83), ($F_{(1,12)} = 0.94$; p = 0.35), ($F_{(1,12)} = 0.44$; p = 0.5), and ($F_{(1,12)} = 0.73$; p = 0.51), respectively when compared with SAL-treated group.

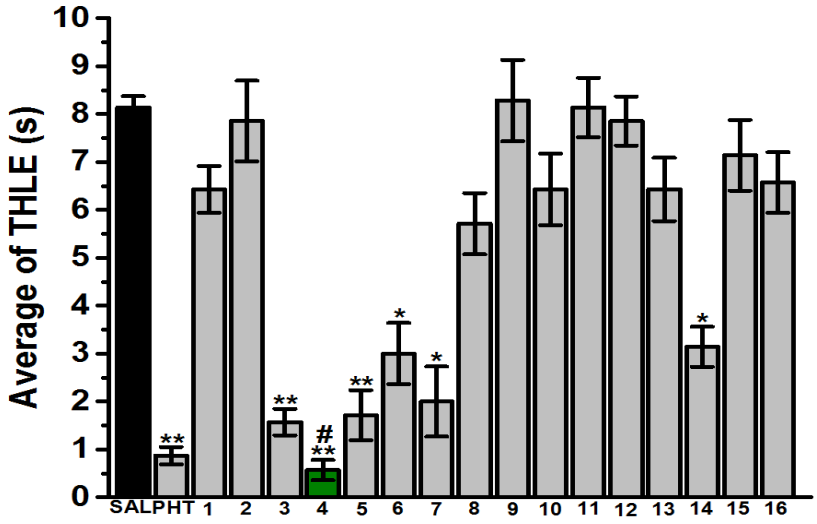


Figure 15: Protective effect of acute systemic administration of H3R antagonists 1-16 (E171-E241) on MES-induced seizure model in male Wistar rats.

The figure shows the protective effect of (PHT, 10 mg/kg, i.p.) and test compounds1-16 (E171-E241) (10 mg/kg, i.p.) on the duration of tonic hind limb extension (THLE) induced in the maximal electroshock (MES) model in male Wistar rats. PHT and H3R antagonists 1-16 were injected 30-45 minutes before applying the MES. Values are represented as mean ± SEM (n=7). $^{*}P < 0.05$ vs. the SAL-treated group. $^{**}P < 0.001$ vs. SAL-treated group. $^{\#}P < 0.05$ vs. the (3, 5, 6, 7, or 14)-treated group.

Furthermore, the results demonstrated that rats pretreated with H3R antagonist 4 (2.5 mg/kg, i.p.) 30-45 minutes before MES test failed to significantly decreased THLE time ($F_{(1,12)}$ = 3.77; p = 0.07) when compared to SAL-treated group. While, pretreatment with H3R antagonist 4 (5 and 15 mg/kg, i.p.) 30-45 minutes before MES test exhibited a significant protective effect against MES-induced seizures ($F_{(1,12)}$ = 42.13; $P < 0.001$) and ($F_{(1,12)}$ = 15.93; $P < 0.05$), respectively when compared with SAL- treated group (Figure 16, Tables 3 & 4). However, post hoc analysis revealed that acute pretreatment with H3R antagonist 4 (5 and 15 mg/kg, i.p.) were protected to a significantly lesser extent when compared with the H3R antagonist 4 (10 mg)-treated group ($F_{(1,12)}$ = 6.09; $P < 0.05$) and ($F_{(1,12)}$ = 8.61; $P < 0.05$), respectively (Figure 16, Tables 3 & 4).

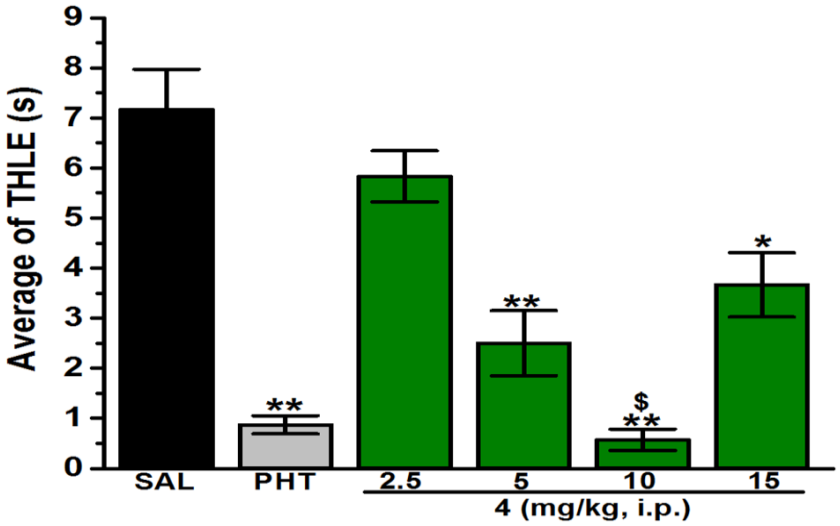


Figure 16: Dose-dependent protective effect of H3R antagonist 4 against MES-induced seizure model.

Dose-dependent effect of H_3R ligand 4 (2.5, 5, 10, and 15 mg/kg, i.p., respectively) on duration of tonic hind limb extension (THLE) induced in MES model in male Wistar rats. Each value represents mean ± SEM (n=7). $^{*}P < 0.05$ vs. SAL-treated group. $^{**}P < 0.001$ vs. SAL-treated group. $^{\$}P < 0.05$ vs. (5, or 15 mg)-treated groups.

Additionally, the abrogation of H3R antagonist 4-provided protection was evaluated by acute systemic co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.), the H1R antagonist PYR, and the H2R antagonist ZOL (Figure 17). As confirmed with post hoc analysis, the results indicated that acute co-administration with RAM and PYR (10 mg/kg, i.p.) significantly abrogated the H3R antagonist 4 (10 mg)-provided protection ($F_{(1,10)} = 24.5$; $P < 0.001$) and ($F_{(1,10)} = 8.91$; $P < 0.05$) when compared with the H3R antagonist 4 (10 mg)-treated group, respectively (Figure 17). However, acute co-injection with ZOL (10 mg/kg, i.p.) failed to provide any significant abrogative effect ($F_{(1,10)} = 1.25$; $p = 0.33$) when compared with the H3R antagonist 4 (10 mg)-treated group (Figure 17). Notably, RAM and PYR acute administration alone with SAL did not affect MES-induced seizures ($F_{(1,10)} = 0.06$; $p = 0.8$) and ($F_{(1,10)} = 2.16$; $p = 0.16$) for SAL– vs. SAL-RAM and for SAL vs. SAL-PYR, respectively (Figure 17).

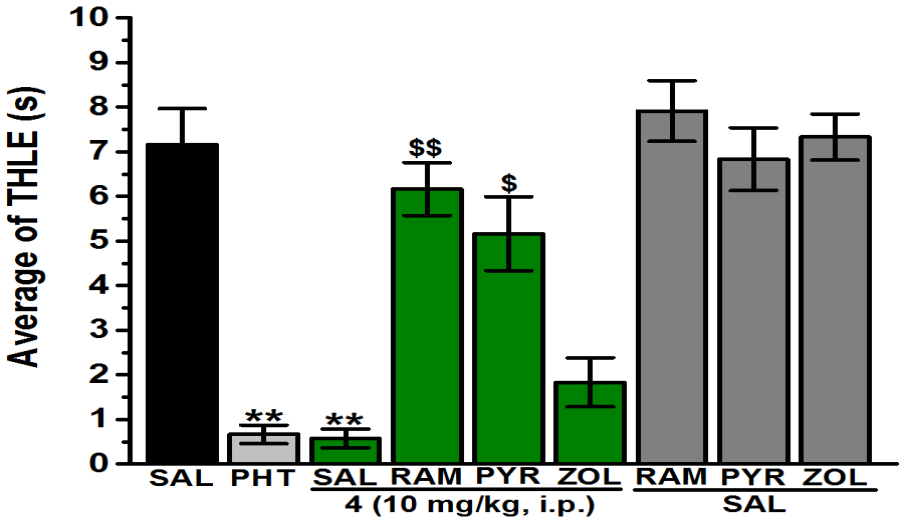


Figure 17: Effect of RAM, PYR, and ZOL pretreatment on the H3R antagonist 4-provided protection against MES-induced convulsions.

Effect of RAM (10 mg/kg, i.p.), PYR (10 mg/kg, i.p.), and ZOL (10 mg/kg) co-injection on the protection provided by H3R antagonist 4 (10 mg/kg, i.p.) against MES-induced convulsions. Each value represents mean ± SEM (n=6). $^{**}P < 0.001$ vs. SAL-treated group. $^{\$}P < 0.05$ vs. H3R antagonist 4 (10 mg)-provided protection against MES-induced convulsions. $^{\$\$}P < 0.001$ vs. H3R antagonist 4 (10 mg)-provided protection against MES-induced convulsions.

4.1.2 Anticonvulsant effect screening of H3R antagonists 1-16 applying PTZ-induced seizure model

The initial screening for anticonvulsant effect of acute systemic pretreatment with H3R ligands 1-16 on PTZ- induced seizure model in male Wistar rats was completed and the obtained results were compared with the protective effect of the reference antiepileptic drug VPA in PTZ- induced seizure in rats (Figure 18). The observed results indicated that acute systemic pretreatment with VPA (300 mg/kg, i.p.) and H3R ligands 1-16 delivered a significant protective action against PTZ-induced seizures as confirmed by applying one-way analysis of variance ($F_{(17,108)} = 23.92$; $P < 0.001$). Following post hoc analysis, the obtained results indicated that acute systemic administration of VPA (300 mg/kg, i.p.) and H3R ligands 4, 7 and 11 (10 mg/kg, i.p.) exhibited full protective actions when compared with the SAL-treated group ($F_{(1,12)} = 653.4$; $P < 0.001$) for all (Figure 18). Moreover, H3R antagonists 12 and 13 provided a significant protective effect against PTZ-induced seizures when compared with SAL-treated group ($F_{(1,12)} = 7.14$; $P < 0.05$) and ($F_{(1,12)} = 22.60$; $P < 0.001$), respectively. Nevertheless, compounds 12 and 13 were less significantly protective when compared with H3R antagonist 4 (10 mg)-treated group ($F_{(1,12)} = 18.18$; $P < 0.05$) and ($F_{(1,12)} = 17.76$; $P < 0.05$), respectively (Figure 18). However, the remaining H3R antagonists 1, 2, 3, 5, 6, 8, 9, 10, 14, 15, and 16 failed to deliver any significant protective effect against PTZ-induced seizures ($F_{(1,12)} = 2.88$; $p = 0.11$), ($F_{(1,12)} = 0.67$; $p = 0.43$), ($F_{(1,12)} = 2.7$; $p = 0.12$), ($F_{(1,12)} = 0.48$; $p = 0.50$), ($F_{(1,12)} = 3.2$; $p = 0.09$), ($F_{(1,12)} = 1.125$; $p = 0.30$), ($F_{(1,12)} = 0.61$; $p = 0.69$), ($F_{(1,12)} = 1.09$; $p = 0.31$), ($F_{(1,12)} = 2.49$; $p = 0.14$), ($F_{(1,12)} = 2.16$; $p = 0.16$), and ($F_{(1,12)} = 1.38$; $p = 0.26$) when compared to SAL-treated group, respectively (Figure 18).

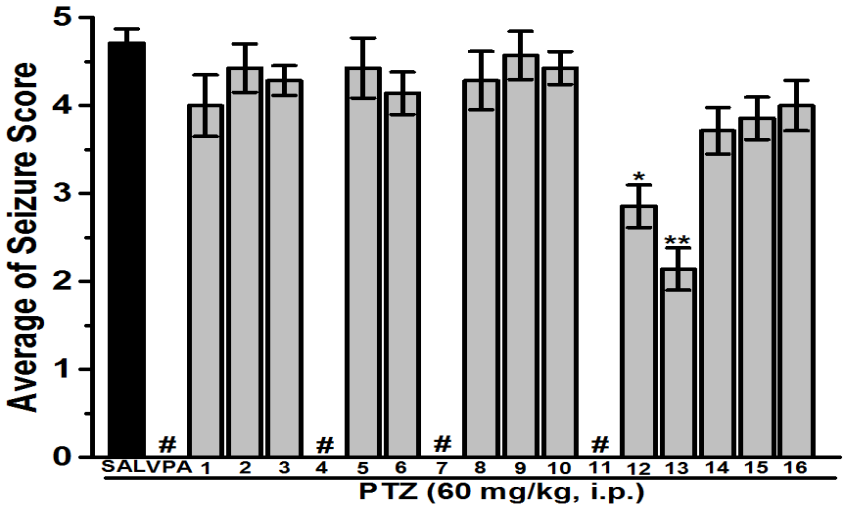


Figure 18: Protective effect of H3R ligands 1-16 pretreatment on PTZ-induced seizure in rats.

The figure shows the protective effects of VPA (10 mg/kg, i.p.) and test compounds1-16 (E171-E241) (10 mg/kg, i.p.) on the average seizure score in the PTZ- induced seizure model in male Wistar rats. VPA and H3R antagonists 1-16 were injected 30-45 minutes before systemic injection of PTZ. Values are represented as mean ± SEM (n=7). $^{*}P < 0.05$ vs. the SAL-treated group. $^{**}P < 0.001$ vs. SAL-treated group. $^{\#}$Full protection.

Furthermore, further statistical analysis indicated that full protection was provided following acute systemic administration with 10 or 15 mg/kg of H3R antagonist 4 ($F_{(1,12)}$ = 653.4; P<0.001) when compared with SAL-treated group (Figure 19). Nevertheless, acute pretreatment with H3R antagonist 4 (2.5 or 5 mg/kg, i.p.) exhibited significantly lesser protection effect when compared with that provided protection of (10 or 15 mg)-treated group of the same compound ($F_{(1,12)}$ = 23.27; $P < 0.001$) and ($F_{(1,12)}$ = 15; $P < 0.001$), respectively and ($F_{(1,12)}$ = 22.81; $P < 0.001$) and ($F_{(1,12)}$ = 235.2; $P < 0.001$), respectively when compared with SAL-treated group (Figure 19).

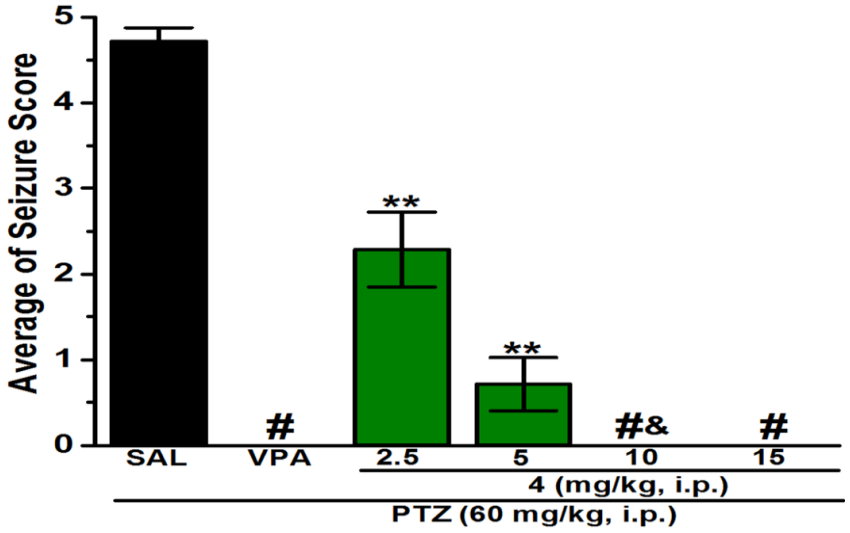


Figure 19: Dose-dependent protective effect of H3R antagonist 4 against PTZ-induced seizure model.

(VPA, 300 mg/kg, i.p.) and test compound 4 (2.5, 5, 10, and 15 mg/kg, i.p.) were injected 30-45 min before PTZ (60 mg/kg, i.p.) treatments. Values are represented as the mean ± SEM (n = 7). $^{**}P<0.001$ vs. SAL-treated group. $^{\&}P < 0.001$ vs. (2.5 and 5 mg)-treated group. $^{\#}$Full protection.

Additionally, the abrogation of H3R antagonist 4-provided protection was evaluated by acute systemic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) (Figure 20). As confirmed with further statistical analysis, the results indicated that RAM, PYR, and ZOL acute co-administration failed to reverse the H3R antagonist 4-provided protection in PTZ-induced seizure model ($F_{(1,10)} = 2.40$; $p = 0.15$), ($F_{(1,10)} = 4.20$; $p = 0.06$), and ($F_{(1,10)} = 4.5$; $p = 0.05$) when compared with H3R antagonist 4 (10 mg)-treated group (Figure 20). Notably, RAM, PYR, and ZOL acute administration alone with PTZ did not affect PTZ-induced seizures ($F_{(1,10)} = 1.58$; $p = 0.23$), ($F_{(1,10)} = 0.16$; $p = 0.69$) and ($F_{(1,10)} = 0.375$; $p = 0.55$), respectively when compared with PTZ- treated group (Figure 20).

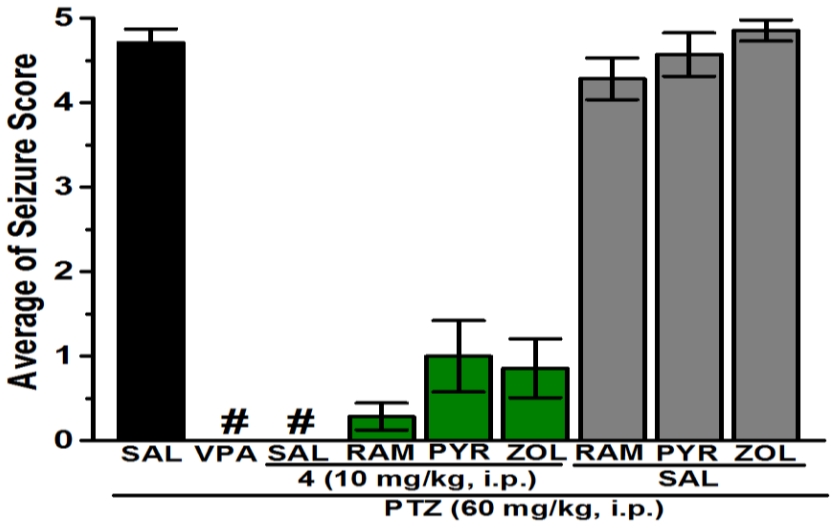


Figure 20: Effect of RAM, PYR, and ZOL pretreatment on the H3R antagonist 4-provided protection against PTZ-induced convulsions.

(VPA, 300 mg/kg, i.p.) and test compound 4 (10 mg/kg, i.p.) were injected 30-45 min before PTZ (60 mg/kg, i.p.) treatments. RAM (10 mg/kg, i.p.), PYR (10 mg/kg, i.p.), and ZOL (10 mg/kg, i.p.) were co-administered 30-45 for PYR and ZOL or 20 minutes for RAM before PTZ injection. Values are represented as the mean ± SEM (n = 6). #Full protection.

4.1.3 Anticonvulsant effect screening of H3R antagonists 1-16 applying STR-induced seizure model

The initial screening for anticonvulsant effect of acute systemic pretreatment with H3R ligands 1-16 on STR- induced seizure model in male Wistar rats was completed and the obtained results were compared with the protective effect of the reference antiepileptic drug VPA in PTZ- induced seizure in rats (Figure 21). The observed results indicated that acute systemic administration of VPA (300 mg/kg, i.p.) and H3R ligand 13 with no appreciable protection in MES- and with a low protection in PTZ-induced seizure, provided reasonable protective effect in STR-induced seizure when compared to the SAL-treated group ($F_{(1,12)} = 63.00$; $P < 0.001$) for compound 13 and ($F_{(1,12)} = 236.31$; $P < 0.001$) for VPA (Figure 21). However, H3R antagonist 4 with the most promising effect in MES-and PTZ-induced seizure models failed to provide any appreciable protection in STR-induced seizure when compared to the SAL-treated

group after 30 min of observation time ($F_{(1,12)}$ = 4.50; p = 0.06) (Figure 21, Tables 3 & 4). On the other hand, all the remaining H3R ligands failed to provide any significant protective effect against STR-induced seizure ($F_{(1,12)}$= 4.5; p = 0.06) for compounds 2, 5, 9, and 14**,** ($F_{(1,12)}$ = 3.69; p = 0.07) for compounds 1, 6, 8, 12, and 16, ($F_{(1,12)}$ = 2.88; p = 0.11) for compounds 3 and 11, ($F_{(1,12)}$ =1; p = 0.33) for compound 7**,** ($F_{(1,12)}$ = 2.4; p = 0.14) for compound 10, and ($F_{(1,12)}$ = 5.25; p = 0.052) for compound 15, when compared to the SAL- treated group (Figure 21, Tables 3 & 4).

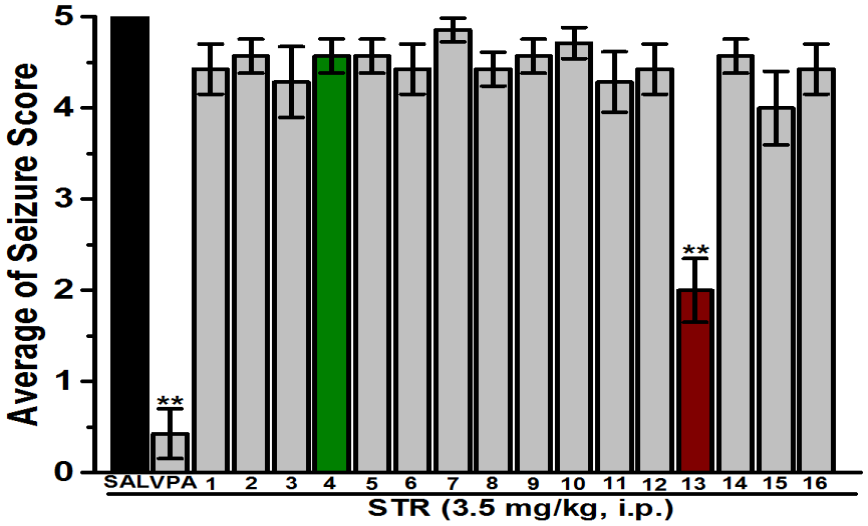


Figure 21:Anticonvulsant effect of H3R antagonists 1-16 pretreatment on strychnine (STR)-induced seizure in rats.

(VPA, 300 mg/kg, i.p.) and test compounds 1-16 (10 mg/kg, i.p.) were injected 30-45 min before STR (3.5 mg/kg, i.p.) treatments. Values are represented as the mean ± SEM (n = 7). $^{**}P < 0.001$ vs. SAL-treated group.

Furthermore, the outcomes indicated that acute systemic pretreatment with H3R antagonist 13 at lower dose (2.5 mg/kg, i.p.) failed to exhibit any protective effect against STR-induced seizures when compared with the SAL-treated group ($F_{(1,12)}$ = 1.00; p = 0.34) (Figure 22, Tables 3 & 4). Moreover, acute pretreatment with compound 13 (5 or 15 mg/kg, i.p.) provided a significant protective effect against STR-

induced seizures when compared with SAL-treated group ($F_{(1,12)} = 39.70$; $P < 0.001$) and ($F_{(1,12)} = 70.59$; $P < 0.001$), respectively (Figure 22).

Additionally, no significant differences in the anticonvulsant effect provided by H3R antagonist 13 (10 mg/kg, i.p.) were obtained when different doses of the same compound were administered (5 or 15 mg/kg, i.p.) ($F_{(1,12)} = 2.84$; $p = 0.12$) and ($F_{(1,12)} = 0.08$; $p = 0.78$), respectively (Figure 22, Tables 3 & 4). Also, the abrogation of H3R antagonist 13-provided protection was evaluated by acute systemic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) (Figure 22). As confirmed with statistical analysis, the results indicated that RAM, PYR, and ZOL acute co-administration failed to reverse the H3R antagonist 13-provided protection in STR-induced seizure model ($F_{(1,10)} = 0.07$; $p = 0.80$), ($F_{(1,10)} = 0.22$; $p = 0.64$), and ($F_{(1,10)} = 0$; $p = 1$) when compared to 13 (10 mg)-treated group, respectively (Figure 22). Notably, RAM, PYR, and ZOL when administrated alone did not affect STR-induced seizure when compared to STR-treated group ($F_{(1,10)} = 2.4$; $p = 0.15$), ($F_{(1,10)} = 3.69$; $p = 0.08$), and ($F_{(1,10)} = 2.08$; $p = 0.17$), respectively (Figure 22).

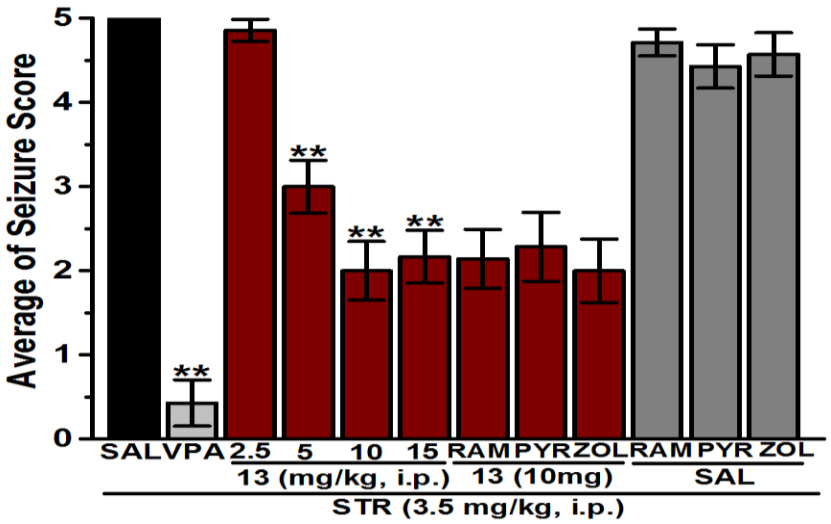


Figure 22: Dose-dependent protective effect of H3R antagonist 13 and effect of RAM, PYR, and ZOL pretreatment on the protection provided by H3R ligand 13 against PTZ-induced convulsions.

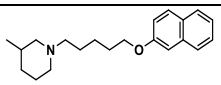
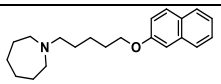
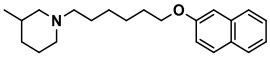
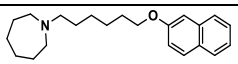
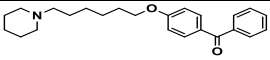
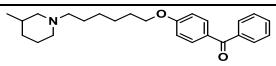
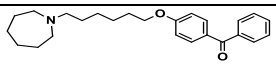
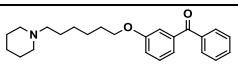
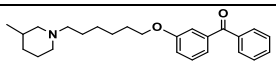
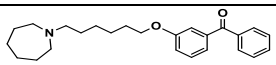
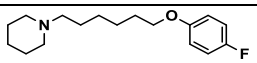
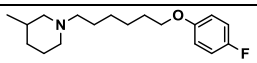
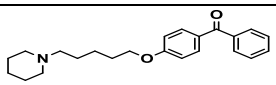
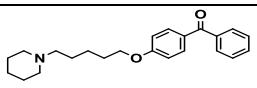
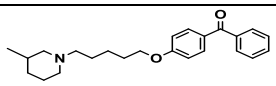
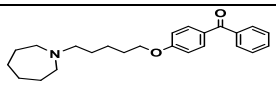
(VPA, 300 mg/kg, i.p.) and test compound 13 (2.5, 5, 10, and 15 mg/kg, i.p.) were injected 30-45 min before STR (3.5 mg/kg, i.p.) treatment. RAM (10 mg/kg, i.p.), PYR (10 mg/kg, i.p.), and ZOL (10 mg/kg, i.p.) were co-administered with compound 13 (10 mg/kg, i.p.) 30-45 before STR injection. Values are represented as the mean ± SEM (n = 6-7). $^{**}P < 0.001$ vs. SAL-treated group.

Table 3: Anticonvulsant effect of H3R antagonists 1-16 in MES-, PTZ-, and STR-induced convulsion models.

Group	MES-induced seizure	PTZ[a]-induced seizure		Group	STR[b]-induced seizure	
	Average THLE (s)	Average seizure score	% Protection against GTCS		Average seizure score	% Protection against GTCS
SAL	8.14±1.17	4.71±0.16	28.57	SAL	5.00±0.00	NP
PHT[c]/ VPA[h]	0.90±0.19**	0.00±0.00**	100	VPA[h]	0.43±0.27**	100
1[d]	6.43±1.14	4.00±0.35	57.14	1[d]	4.43±0.27	42.86
2[d]	7.86±0.84	4.43±0.27	42.86	2[d]	4.57±0.19	28.57
3[d]	1.29±0.17**	4.29±0.17	71.43	3[d]	4.29±0.39	42.86
4[d]	0.57±0.21**	0.00±0.00**	100	4[d]	4.57±0.19	28.57
5[d]	1.71±0.26**	4.43±0.34	28.57	5[d]	4.57±0.19	42.86
6[d]	3.00±0.75*	4.14±0.24	71.43	6[d]	4.43±0.27	42.86
7[d]	2.00±0.49*	0.00±0.00**	100	7[d]	4.86±0.13	14.29
8[d]	5.71±1.39	4.29±0.33	57.14	8[d]	4.43±0.19	42.86
9[d]	8.29±1.57	4.57±0.27	28.57	9[d]	4.57±0.19	42.86
10[d]	6.43±0.75	4.43±0.19	57.14	10[d]	4.71±0.17	28.57
11[d]	8.14±0.62	0.00±0.00**	100	11[d]	4.29±0.33	42.86
12[d]	7.86±0.51	2.86±0.62*	71.43	12[d]	4.43±0.27	42.86
13[d]	6.43±1.14	2.14±0.47**	85.71	13[d]	2.00±0.35**	100
14[d]	3.14±0.59*	3.71±0.56	42.86	14[d]	4.57±0.19	28.57
15[d]	7.14±0.74	3.86±0.51	57.14	15[d]	4.00±0.40	57.14
16[d]	6.57±1.22	4.00±0.53	42.86	16[d]	4.43±0.27	42.86
4[e]	5.83±1.01#	2.29±0.44**#	100	13[e]	4.86±0.13	14.29
4[f]	2.50±1.21**#	0.71±0.31**#	100	13[f]	3.00±0.31**	85.71
4[g]	3.67±0.94*#	0.00±0.00**	100	13[g]	2.17±0.31**	100
4[d]+RAM[d]	6.17±0.59$$	0.29±0.16	100	13[d]+RAM[d]	2.14±0.35	100
4[d]+PYR[d]	5.17±0.83$	1.00±0.42	100	13[d]+PYR[d]	2.29±0.44	100
4[d]+ZOL[d]	1.83±0.55	0.86±0.35	100	13[d]+ZOL[d]	2.00±0.40	100
SAL+RAM[d]	7.92±0.6	4.29±0.25	14.29	SAL+RAM[d]	4.71±0.16	28.57
SAL+PYR[d]	6.83±0.7	4.57±0.26	14.29	SAL+PYR[d]	4.43±0.27	42.86
SAL+ZOL[d]	7.33±0.52	4.86±0.12	14.29	SAL+ZOL[d]	4.57±0.27	28.57

[a]60 mg/kg, [b]3.5 mg/kg, [c]PHT (10 mg/kg, i.p.), [d]10 mg/kg, [e]2.5 mg/kg, [f]5 mg/kg, [g]15 mg/kg, [h]VPA (300 mg/kg, i.p.). H3R antagonists 1-16 (10 mg/kg, i.p.) were injected 30-45 min before MES, PTZ (60 mg/kg, i.p), or STR (3.5 mg/kg, i.p.) challenge. The table shows the protective effects of phenytoin (PHT, 10 mg/kg, i.p.), VPA (300 mg/kg, i.p.), and H3R antagonists 1-16 (10 mg/kg, i.p.) on the duration of tonic hind limb extension (THLE) induced in the maximal electroshock (MES) model in rats and average seizure score in PTZ- and STR- induced seizure models. Protective effects in PTZ- and STR-induced convulsion model are expressed as score of seizures for 30 min observation time after PTZ or STR injection. Dose-dependent effect of H_3R antagonist 4 and 13 (2.5, 5, 10, and 15 mg/kg, i.p.) on duration of THLE induced in MES-, and average seizure score in PTZ- and STR- model in rats. Effect of RAM, PYR, and ZOL (10 mg/kg, i.p.) pretreatment on the protection provided by H3R antagonist 4 (10 mg/kg, i.p.) against MES- and PTZ-, and H3R antagonist 13 against STR-induced convulsions. Each value represents mean ± SEM (n=6-7). $^{*}P < 0.05$ vs. (saline)-treated group. $^{**}P < 0.001$ vs. (saline)-treated group. $^{\#}P < 0.05$ vs. 4(10 mg/kg)-treated group. $^{\$}P < 0.05$ vs. 4(10 mg/kg)-treated group. $^{\$\$}P < 0.001$ vs. 4(10 mg/kg)-treated group.

Table 4: Screening of *in vivo* anticonvulsant effect for H3R antagonists 1-16.

Ligand	Structure	MES[a]-induced seizure	PTZ[b]-induced seizure	STR[c]-induced seizure
1		-	-	-
2		-	-	-
3		++	-	-
4		++	++	-
5		++	-	-
6		+	-	-
7		+	++	-
8		-	-	-
9		-	-	-
10		-	-	-
11		-	++	-
12		-	+	-
13		-	+	+
14		+	-	-
15		-	-	-
16		-	-	-

[a]50-Hz alternating current of 120 mA intensity applied through ear electrodes for a duration of 1 s. [b]60 mg/kg, [c]3.5 mg/kg. –, No protection; +, moderate protective effect; ++, high protective effect.

4.2 *In vivo* screening of the procognitive effect of H3R antagonists 4 applying different behavioral test

4.2.1 Effect of H3R antagonist 4 in both DIZ- and SCO- induced memory impairment in IAP

The procognitive effect of compound 4 (10 mg/kg, i.p.) on DIZ- and SCO-induced memory deficits in IAP in adults male Wistar rats were completed and compared with the standard drug DOZ (1 mg/kg) (Figure 23). Statistical analysis on the average of the STL times indicated, that H3R antagonist 4 (10 mg/kg) and DOZ (1 mg/kg) acute pretreatment 30–45 minutes before the test conferred a significant memory-enhancing effects in both DIZ- and SCO- induced memory deficits in rats ($F_{(6,42)} = 31.74$; $P < 0.001$). Further statistical analysis revealed that DIZ and SCO systemic administration 30-45 min before the test caused memory impairment when compared with SAL-treated group ($F_{(1,12)} = 991.64$; $P < 0.001$) and ($F_{(1,12)} = 486.84.57$; $P < 0.001$), respectively. Moreover, the results revealed that DIZ-treated group significantly reduced STL time when compared to SCO-treated group ($F_{(1,12)} = 99.1$; $P < 0.001$) (Figure 23). Furthermore, it was observed that acute systemic administration of H3R antagonist 4 (10 mg/kg, i.p.) 30-45 min before the test exhibited a stronger significant memory-enhancing effect in DIZ- when compared to SCO- induced memory deficits ($F_{(1,12)} = 325.10$; $P < 0.001$) and ($F_{(1,12)} = 12.48$; $P < 0.05$), for DIZ-treated group vs DIZ+4 (10 mg/kg) group and (SCO)-treated group vs SCO+4 (10 mg/kg) group, respectively and ($F_{(1,12)} = 8.53$; $P < 0.05$)when compared 4+DIZ group vs 4+SCO group. Furthermore, DOZ (1 mg/kg) resulted in a significant enhancement effect of STL times when compared with DIZ- and SCO-induced amnesia groups($F_{(1,12)} = 424.55$; $P < 0.001$) and ($F_{(1,12)} = 145.62$; $P < 0.001$), respectively (Figure 23).

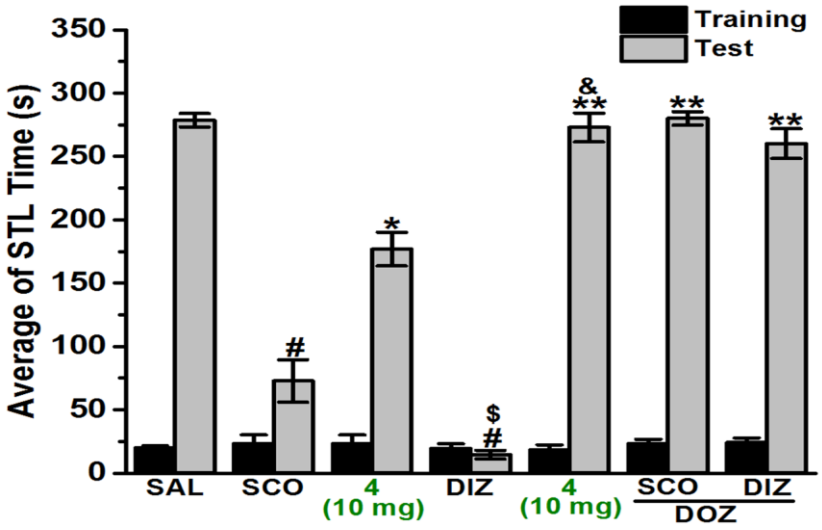


Figure 23: H3R antagonist 4 attenuated SCO- and DIZ-induced memory deficits in the IAP.

Average STL time measured on the first training day before the delivery of foot shock (black columns), and average STL time measured on the test day (gray columns). Acute systemic administration of H3R antagonist 4 (10 mg/kg, i.p.) or DOZ (1 mg/kg, i.p.) 30-45 minutes before the test session. $^{\#}P < 0.001$ for average STL time compared with that in the SAL-treated group. $^{\$}P < 0.001$ for average STLs compared with the SCO-induced amnesic group. $^{*}P < 0.05$ compared with the SCO-or DIZ-treated group. $^{**}P < 0.001$ compared with the SCO-or DIZ-treated group. $^{\&}P < 0.05$ for average STLs compared with the effect of H3R antagonist 4 on SCO-induced amnesic group. The data are expressed as the mean ± SEM (n=7).

Moreover, the procognitive effect of different doses of H3R antagonist 4 (1.25, 2.5, and 5 mg/kg) on DIZ-induced memory deficits in IAP in adult male rats are shown in (Figure 24). Furthermore, statistical analysis of STL times, indicated that acute systemic administration of H3R antagonist 4 in different doses 30–45 minutes before the test conferred a significant memory-enhancing effects ($F_{(4,49)} = 66.81$; $P < 0.001$). Further statistical analysis demonstrated that all other doses of H3R antagonist 4 (1.25, 2.5, and 5 mg/kg) resulted in a significant enhancement of STL times compared with the DIZ-induced amnesia group ($F_{(1,12)} = 104.75$; $P < 0.001$), ($F_{(1,12)} = 269.73$; $P < 0.001$), and ($F_{(1,12)} = 1272.53$; $P < 0.001$), respectively (Figure 26). Besides, more significant memory-enhancing effect was observed when compared 5 (mg/kg) to 2.5 and 1.25 (mg/kg) doses ($F_{(1,12)} = 11.9$; $P < 0.05$)and ($F_{(1,12)} = 47.6$; $P < 0.001$), respectively. However, no significant increase was observed between compound 4 (5

and 10 mg/kg) doses ($F_{(1,12)} = 1.21$; $p = 0.94$) (Figure 24). Moreover, the procognitive effect of H3R antagonist 4 (5 and 10 mg/kg, i.p.) was comparable to the effect provided with the reference drug DOZ (1 mg/kg) ($F_{(1,12)} = 2.62$; $p = 0.13$) and ($F_{(1,12)} = 0.02$; $p = 0.88$), respectively.

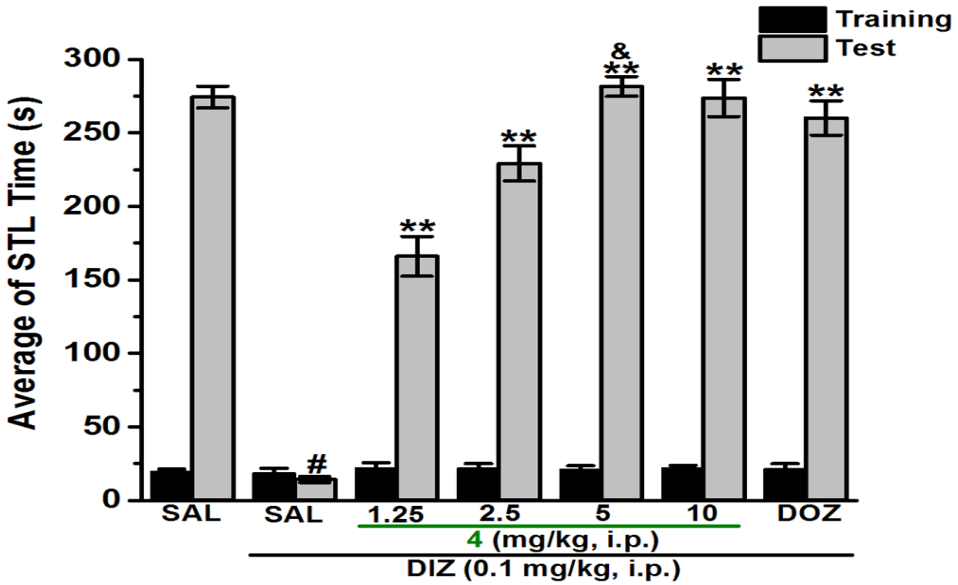


Figure 24: H3R antagonist 4 attenuated DIZ-induced memory deficits in a dose-dependent manner in the IAP**.**

Average STL time measured on the first training day before the delivery of foot shock (black columns), and average STL time measured on the test day (gray columns). Acute systemic administration of H3R antagonist **4** at different doses (1.25, 2.5, 5, and 10 mg/kg, i.p.) or DOZ (1 mg/kg, i.p.) 30-45 minutes before the test session. $^{\#}P < 0.001$ for average STL time compared with that in the SAL-treated group. $^{**}P < 0.001$ for average STLs compared with the (DIZ)-treated group. $^{\&}P < 0.05$ compared with the 1.25 and 2.5 (mg/kg)-treated group. The data are expressed as the mean ± SEM (n=7).

Furthermore, the abrogation of H3R antagonist 4 procognitive provided protection was evaluated by acute systemic co-injection of H3R antagonist 4 most promising dose (5 mg/kg) with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) (Figure 25). As confirmed with statistical analysis, compound 4 (5 mg/kg) procognitive effect was reversed following acute systemic co-administration with RAM 20 min before the test and ZOL 30-45 min before the test ($F_{(1,12)} = 659.00$; $P < 0.001$) and ($F_{(1,12)} = 5.60$; $P < 0.05$),

respectively when compared to compound 4 (5 mg/kg)-treated group. Notably, acute systemic administration of DIZ (0.1 mg)- treated animals with RAM (10 mg/kg) or ZOL (10 mg/kg) failed to significantly alter the STL times observed in the group ($F_{(1,12)}$= 1.31; p = 0.27) and ($F_{(1,12)}$ = 2.01; p = 0.13), respectively when compared with DIZ-treated group (Figure 25). Remarkably, PYR co-injection with compound 4 (5 mg/kg) 30-45 min before the test, failed to reverse the procognitive effect provided by compound 4 (5 mg/kg) ($F_{(1,12)}$ = 0.52; p = 0.48) when compared with compound 4 (5 mg/kg) group (Figure 25). Notably, acute systemic administration of DIZ (0.1 mg)-treated animals with PYR (10 mg/kg) failed to significantly alter the STL times observed in the group ($F_{(1,12)}$= 0.86; p=0.31) (Figure 25).

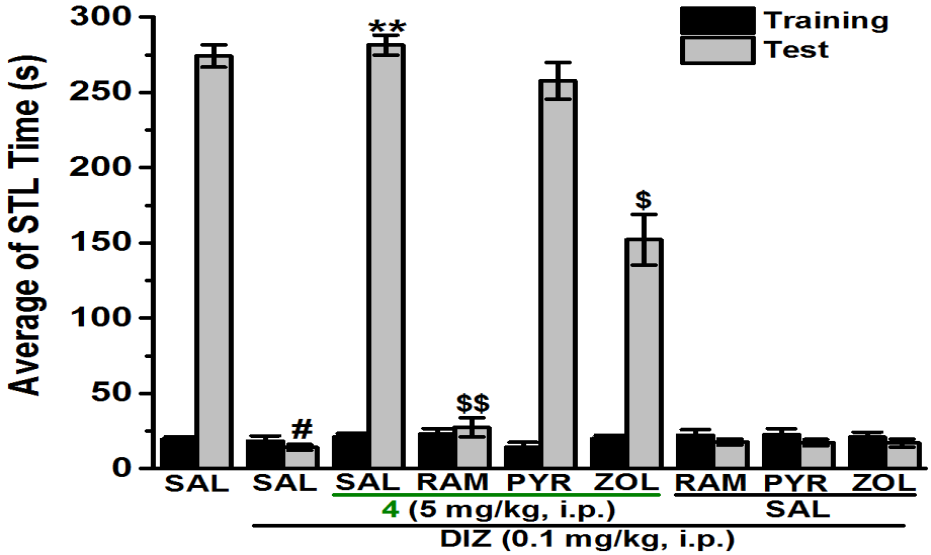


Figure 25: Effect of H3R antagonist 4, RAM, PYR, and ZOL on DIZ-induced memory deficit in IAP.

Gray columns represent the mean STLs measured during the retention test (test latencies) and black columns the mean STLs measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with H3R antagonist 4 (5 mg/kg, i.p.), RAM (10 mg/kg, i.p.), PYR (10 mg/kg, i.p.) or ZOL (10 mg/kg, i.p.) 30-45 min before the test session. $^{\#}P < 0.001$ for mean STLs vs. the value of the SAL-treated group. $^{**}P < 0.001$ for mean STLs vs. the value of (DIZ +SAL)-treated group. $^{\$}P < 0.05$ for mean STLs vs. the value of (DIZ + H3R antagonist 4 **(**5 mg/kg)-treated group. $^{\$\$}P < 0.001$ for mean STLs vs. the value of (DIZ + H3R antagonist 4 **(**5 mg/kg)-treated group. Data are expressed as mean ± SEM (n = 7).

4.2.2 Effect of H3R antagonist 4 in DIZ-induced STM deficits in NOR

The procognitive effect of H3R antagonist 4 on DIZ-induced STM memory deficits in NOR in adult male Wistar rats were completed and discussed in (Figure 26). Statistical analysis on the average of the DIs times indicated that post-T1, acute systemic administration of H3R antagonist 4, and DOZ significantly counteracted the time spent exploring the objects in the T2 session ($F_{(8,54)} = 17.58$; $P < 0.001$). Further statistical analysis on the average of the DIs times showed that DIZ (0.1 mg/kg, i.p.) systemic administration post-T1 significantly impaired the novel object memory in T2 (120 minutes later) when compared with the SAL-treated group ($F_{(1,10)} = 52.54$; $P < 0.001$) (Figure 26). Moreover, further analysis of the DI results showed that acute systemic administration of H3R antagonist 4 (5 mg/kg) and DOZ (1 mg/kg) post T1, significantly counteracted the time spent exploring the objects in the T2 session ($F_{(1,10)} = 6.13$; $P < 0.05$) and ($F_{(1,10)} = 22.13$; $P < 0.001$), respectively when compared with DIZ-treated group (Figure 26). Furthermore, acute systemic administration of H3R antagonist 4 (10 mg/kg) post T1, also significantly counteracted the STM impairment induced by DIZ ($F_{(1,10)} = 6.19$; $P < 0.05$). In contrast, lower dose of E177 (2.5 mg/kg) post T1, failed to reverse the DIZ-induced memory impairment ($F_{(1,10)} = 0.31$; $p = 0.59$) (Figure 26). Notably, no significant difference in the protective effects was observed for either dose of H3R antagonist 4, namely, 5 and 10 mg/kg ($F_{(1,10)} = 0.02$; $p = 0.88$) (Figure 26). Additionally, the abrogation of H3R antagonist 4 procognitive provided protection was evaluated by acute systemic co-injection of H3R antagonist 4 most promising dose (5 mg/kg) with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) (Figure 26). As confirmed with post hoc analysis, that the compound 4 (5 mg/kg) procognitive effect was abrogated with H3R agonist RAM (10 mg/kg, i.p.) co-injection post T1 ($F_{(1,10)} =$

10.11; $P < 0.05$) when compared with compound 4 (5 mg/kg)-treated group (Figure 26). In addition, further statistical analysis of DI revealed that RAM (10 mg/kg, i.p.) alone did not modulate STM in T2 compared with the saline- and DIZ-treated groups ($F_{(1,10)} = 0.92$; $p = 0.35$) and ($F_{(1,10)} = 0.54$; $p = 0.47$), respectively (Figure 26). Nevertheless, acute co-injection of both PYR and ZOL (10 mg/kg) with H3R antagonist 4 (5 mg/kg, i.p.) failed to abrogate the procognitive effect of H3R antagonist 4 (5 mg) with ($P > 0.05$) for both. Importantly, the time spent exploring both objects through T1 and T2 was not significantly different in the DIZ-treated group and the saline-treated group (Table 5). This is essential to confirm the elimination of any confounding factors, such as the failure of DIZ post-training treatment in T1 to alter the spontaneous locomotion and/or motivations of tested animals.

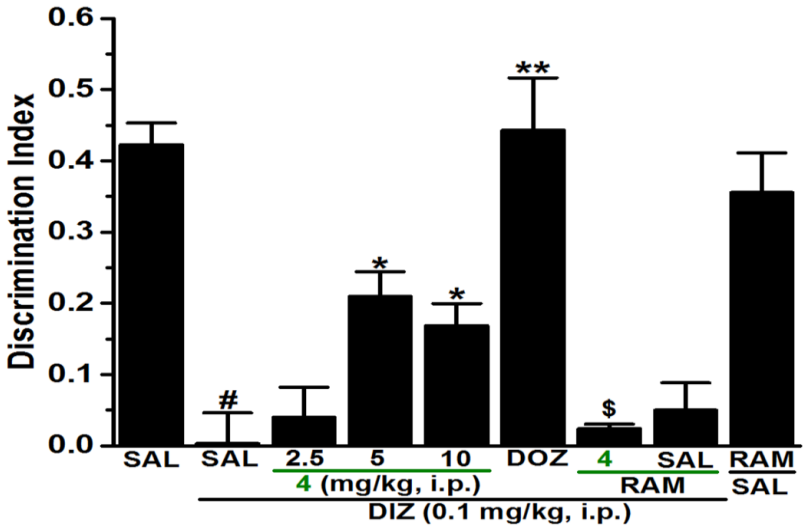


Figure 26: H3R antagonist 4 mitigated DIZ-induced short-term memory deficits in NOR paradigm

H3R antagonist 4 (2.5, 5, or 10 mg/kg, i.p.) or DOZ (1 mg/kg, i.p.) were injected after T1 (training session) with DIZ (0.1 mg/kg, i.p.). The T2 (test session) was conducted 120 minutes (for short-term memory) after T1 (training session). The results are expressed as Discrimination indices for the time spent exploring both objects (familiar and novel). $^{\#}P < 0.001$ compared with the SAL-treated group. $^{*}P < 0.05$ compared with the (DIZ)-treated group. $^{**}P < 0.001$ compared with the (DIZ)-treated group. $^{\$}P < 0.05$ compared with the **4** (5 mg)-treated group. The data are expressed as the mean ± SEM (n=6).

4.2.3 Effect of H3R antagonist 4 in DIZ-induced LTM deficits in NOR

The procognitive effects for H3R antagonist 4 (2.5, 5, and 10 mg/kg) and DOZ (1 mg/kg, i.p.) on time spent exploring both objects in T2 when the compounds were injected in the post-training session T1 and 30-45 minutes before T2 on the following day in DIZ-induced LTM impairment in the NOR task are discussed in (Figure 27). Statistical analysis on the average of the DIs times indicated significant modulatory effects of compound 4 and DOZ on time spent exploring both objects in T2 ($F_{(8,54)}$ = 5.071; $P < 0.001$). Further statistical analysis demonstrated that DIZ (0.1 mg/kg, i.p.) systemic administration post-T1, significantly impaired the novel object memory in T2 (24 hours later) when compared with the SAL-treated group ($F_{(1,10)}$ = 9.77; $P < 0.05$) (Figure 27). Moreover, further analysis of the DI results indicated that acute systemic administration of DOZ (1 mg/kg) when injected post T1 and 30-45 min before T2 (24 h later), significantly improved the DI between the two objects ($F_{(1,10)}$ = 16.34; $P < 0.05$) when compared with the DIZ-treated group (Figure 29). Nevertheless, H3R antagonist 4 (2.5, 5, and 10 mg/kg) acute administration post T1 and 30-45 min before T2, failed to mitigate the DI between the two objects with ($F_{(1,10)}$ = 1.21; p = 0.29), ($F_{(1,10)}$ = 5.67; p = 0.06), and ($F_{(1,10)}$ = 4.25; p = 0.07), respectively when compared with DIZ-treated group (Figure 27). Importantly, the time spent exploring both objects through T1 and T2 was not significantly different in the DIZ-treated group and the saline-treated group (Table 5). This is essential to confirm the elimination of any confounding factors, such as the failure of DIZ post-training treatment in T1 to alter the spontaneous locomotion and/or motivations of tested animals.

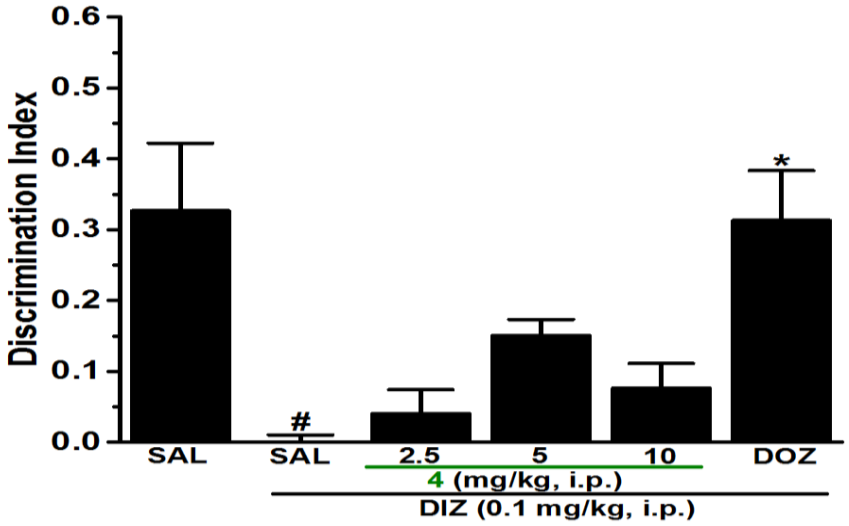


Figure 27: H3R antagonist 4 failed to mitigate DIZ-induced long-term memory deficits in NOR paradigm**.**

H3R antagonist 4 (2.5, 5, or 10 mg/kg, i.p.) or DOZ (1 mg/kg, i.p.) were injected after T1 (training session) with DIZ (0.1 mg/kg, i.p.) and 30-45 minutes before T2. The T2 (test session) was conducted 24h (for long-term memory) after T1 (training session). The results are expressed as Discrimination indices for the time spent exploring both objects (familiar and novel). $^{\#}P < 0.05$ compared with the SAL-treated group. $^{*}P < 0.05$ compared with the DIZ-treated group. The data are expressed as the mean ± SEM (n=6).

Table 5: Effect of H3R antagonist 4 on DIZ-induced total exploratory time spent with both objects during training and test session in NOR paradigm.

		Time exploring objects (s)			
Group	*N*	Training session STM	Test session STM	Training session LTM	Test session LTM
Saline	6	36.50 ± 2.69	40.33 ± 2.75	36.67 ± 2.93	38.17 ± 3.86
DIZ + Saline	6	37.00 ± 2.49	33.75 ±3.09	37.63 ±2.30	38.14 ± 6.47
DIZ + 4 (2.5 mg/kg)	6	38.50 ± 2.37	40.08 ±1.57	36.83 ±1.99	36.17 ± 1.66
DIZ + 4 (5 mg/kg)	6	38.67 ± 2.52	38.63 ± 2.48	37.67 ± 2.55	33.17 ± 2.19
DIZ + 4 (10 mg/kg)	6	38.17 ± 2.17	36.08 ± 0.72	37.50 ± 2.63	37.83 ± 3.16
DIZ + DOZ(1 mg/kg)	6	37.33 ± 4.41	37.75 ± 2.17	38.50 ± 1.16	38.29 ± 2.29
DIZ + 4 (5 mg/kg) + RAM(10 mg/kg)	6	39.17 ± 1.34	39.25 ± 2.39	39.88 ± 2.57	37.96 ± 1.56
DIZ + RAM(10 mg/kg)	6	38.00 ± 4.22	37.42 ± 1.86	39.33 ± 1.56	37.46 ± 1.30
Saline + RAM(10 mg/kg)	6	38.25 ± 2.88	37.42 ± 2.20	38.75 ± 2.14	37.83 ± 4.29

No significant changes in total exploratory times between treated groups was observed. Data are expressed as mean ±SEM (n=6).

4.2.4 Effect of H3R antagonist 4 on animal performance in the EPM paradigm

The modulating effects for H3R antagonist 4 (5 and 10 mg/kg) and DZP (10 mg/kg, i.p.) acute treatment 30-45 minutes before the test, on the anxiety-like behaviors and locomotion activity applying EMP are discussed in (Figure 28). Statistical analysis indicated that, no change was observed in the time spent in the open arms for the H3R antagonist 4 (5 and 10 mg)-treated groups ($F_{(1,10)} = 0.05$; $p = 0.83$) and ($F_{(1,10)} = 0.11$; $p = 0.75$), respectively when compared with SAL-treated group (Figure 28A). Moreover, no significant alternation in the total numbers of entries into the open arms for compound 4 (5 and 10 mg/kg) ($F_{(1,10)} = 0.03$; $p = 0.88$) and ($F_{(1,10)} = 0.008$; $p=0.93$), respectively when compared with the SAL-treated group (Figure 28B). However, Statistical analysis shown that acute pretreatment with DZP (10 mg/kg) significantly altered both the time spent in the open arms and the total number of entries into the open arms ($F_{(1,10)} = 8.60$; $P < 0.05$) and ($F_{(1,10)} = 6.13$; $P < 0.05$), respectively when compared with SAL-treated group (Figure 28A and B). Remarkably, no significant change was observed in the total number of entries into the closed arms after acute systemic administration of H3R antagonist 4 (5 and 10 mg/kg) or DZP (10 mg/kg), ($F_{(1,10)} = 0.005$; $p = 0.94$), ($F_{(1,10)} = 0.59$; $p = 0.46$), and ($F_{(1,10)} = 0.004$; $p = 0.95$), respectively when compared with SAL-treated group (Figure 28C).

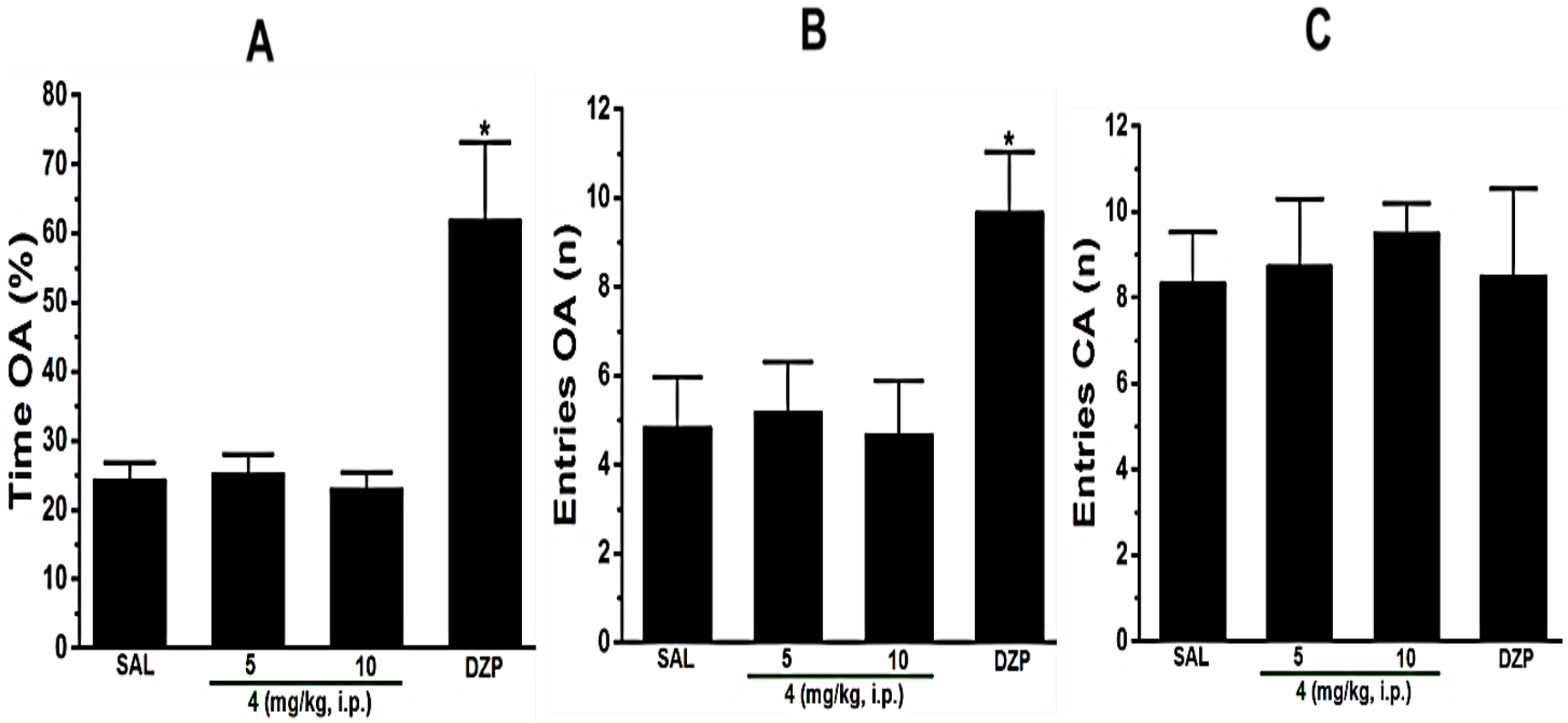


Figure 28: H3R antagonist 4 failed to alter anxiety-like behavior or locomotion in the EPM test.

Effect of H3R antagonist 4 (5 or 10 mg/kg, i.p.) and DZP (10 mg/kg, i.p.) injections on the percentage of time spent in open arms (A), total number of open arm entries (B), and total number of closed arms entries (C). $^{*}P < 0.05$ for the value of the DZP-treated group compared with the SAL-treated groups. The data are expressed as the mean ± SEM (n=6).

4.2.5 Effect of H3R antagonist 4 on animal performance in the OFT paradigm

The modulating effects for H3R antagonist 4 (5 and 10 mg/kg) and DZP (10 mg/kg, i.p.) acute treatment 30-45 minutes before the test, on the anxiety-like behaviors and locomotion activity applying OFT are discussed in (Table 6). Statistical analysis indicated that, no change was observed on the time spent in the center and periphery and on the total time spent for locomotion after acute treatment with H3R antagonist 4 (5 and 10 mg/kg) and DOZ (1 mg/kg) ($F_{(1,10)} = 0.38$; $p = 0.55$), ($F_{(1,10)} = 0.06$; $p = 0.81$), and ($F_{(1,10)} = 0.02$; $p = 0.88$), respectively, for 4 (5 mg/kg), ($F_{(1,10)} = 0.02$; $p=0.88$), ($F_{(1,10)} = 0.02$; $p = 0.89$), and ($F_{(1,10)} = 0.01$; $p = 0.92$), respectively, for 4 (10 mg/kg) and ($F_{(1,10)} = 0.29$; $p = 0.6$), ($F_{(1,10)} = 0.53$; $p = 0.48$) and ($F_{(1,10)} = 1.23$; $p = 0.29$), respectively, for DOZ (1 mg), as shown in (Table 6). Statistical analysis of the results of the total distance traveled, the number of rearing events, and the number of grooming events for H3R antagonist 4 (5 and 10 mg/kg) and DOZ (1 mg/kg) provided essentially the same result, no significant alternation (all $P < 0.05$) Table 6.

Table 6: Effect of acute systemic administration of H3R antagonist 4 on anxiety levels and locomotor activity in open field test.

	Time for locomotion (s)	Distance Travelled (cm)	Time in Center (s)	Time in periphery (s)	No. of Rearing	No. of Grooming
Saline	99.67±6.47	1003.33±51.34	1.67±0.19	78.67±6.40	18.17±2.41	2.00±0.58
4 (5 mg/kg)	98.33±4.78	1085.67±33.95	1.83±0.15	76.67±3.93	16.17±1.79	1.50±0.39
4 (10 mg/kg)	100.86±8.16	999.86±52.44	1.71±0.26	77.14±7.87	15.14±2.54	1.86±0.31
DOZ (1 mg/kg)	109.83±5.31	1022.67±44.65	1.50±0.20	71.33±6.63	16.67±2.55	0.67±0.45

H3R antagonist 4 (5 and 10 mg/kg, i.p.) failed to alter time spent in center arena, time spent in periphery, total time spent for locomotion, total distance travelled, number of rearing, and number of grooming. Data are expressed as mean ± SEM (n=6).

4.3 PTZ- acute model results, behavioral assessment, biochemical measurements and IHC

The anticonvulsant effect of acute pretreatment of H3R antagonist 4 (5 and 10 mg/kg, i.p.) and VPA (300 mg/kg, i.p.) 30-45 minutes before PTZ (60 mg/kg, i.p.) are discussed in section 4.1.2 and described in (Figure 19).

4.3.1 Effect of acute treatment of H3R antagonist 4 on memory impairment induced by PTZ systemic administration applying IAP

The procognitive effect of compound 4 (5 and 10 mg/kg, i.p.) after PTZ-induced memory deficits in IAP in adult male Wistar rats were completed and discussed in (Figure 29). Statistical analysis on the average of the STL times revealed that acute pretreatment with H3R antagonist 4 and VPA 30-45 minutes before PTZ injection conferred a significant memory-enhancing effect in IAP ($F_{(4,25)} = 21.6$; $P < 0.001$). Further statistical analysis showed that PTZ systemic administration post-training session in day 1 caused memory impairment when compared with SAL-treated group ($F_{(1,10)} = 23.97$; $P < 0.05$). Moreover, results of acute pretreatment with VPA (300 mg/kg, i.p.) 30-45 minutes before PTZ injection, indicated a significant improvement in STL time ($F_{(1,10)} = 184.93$; $P < 0.001$) when compared with PTZ- treated group. Further statistical analysis results exhibited, that acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) significantly counteracted the memory impairment induced by PTZ- acute injection ($F_{(1,10)} = 73.93$; $P < 0.05$)and ($F_{(1,10)} = 46.3$; $P < 0.05$) when compared with PTZ-treated group (Figure 29). Remarkably, no significant difference was observed in the procognitive effect provided by H3R antagonist 4 (5 and 10 mg/kg) ($F_{(1,10)} = 0.48$; $p = 0.52$). Notably, no significant difference was indicated between all the groups in the training day, indicating that no modulating

factors, e.g., locomotion or anxiety, have confounded that modulatory effect in the test day.

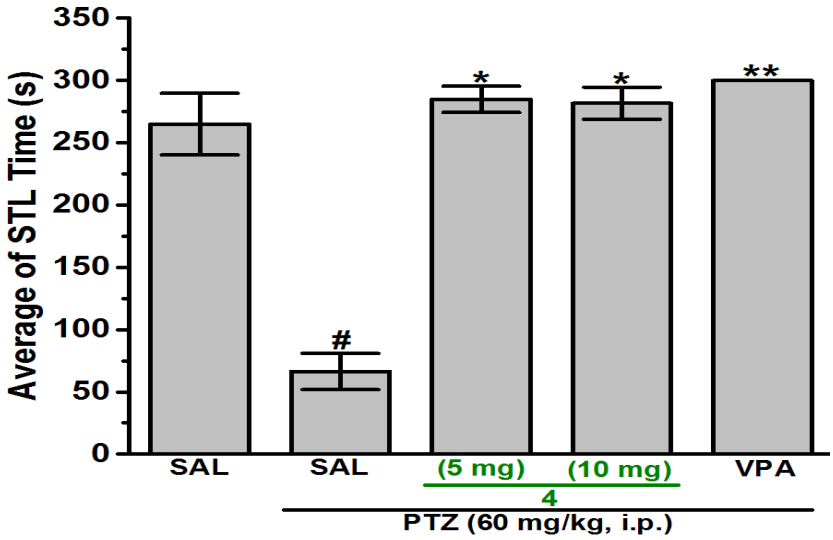


Figure 29: H3R antagonist 4 attenuated PTZ-induced memory deficits in the IAP.

Average of STL time measured on the test day (gray columns). Acute systemic administration of H3R antagonist 4 (5 and 10 mg/kg, i.p.) or VPA (300 mg/kg, i.p.) 30-45 minutes before PTZ- acute administration. $^{\#}P < 0.05$ for average STL time compared with that in the SAL-treated group. $^{*}P < 0.05$ for average STL time compared with the PTZ-treated group. $^{**}P < 0.001$ for average STL time compared with the PTZ-treated group. The data are expressed as the mean ± SEM (n=6).

4.3.2 Effect of H3R antagonist 4 on memory impairment induced by PTZ- acute systemic administration applying EPM

The procognitive effect of compound 4 (5 and 10 mg/kg, i.p.) after PTZ-induced memory deficits in EPM in adult male Wistar rats were completed and discussed in (Figure 30). Statistical analysis on the average of the TLT revealed that acute pretreatment with H3R antagonist 4 and VPA 30-45 minutes before PTZ injection conferred significant memory-enhancing effect in EPM ($F_{(4,25)} = 44.3$; $P < 0.05$). Further statistical analysis showed that PTZ systemic administration post training session in day 1 caused memory impairment when compared with SAL-treated group ($F_{(1,10)} = 18.64$; $P < 0.05$). Moreover, results of acute pretreatment with VPA (300

mg/kg, i.p.) 30-45 minutes before PTZ injection, indicated a significant improvement in TLT time ($F_{(1,10)}$ = 12.34; $P < 0.05$) when compared with PTZ- exposed group. Further statistical analysis on the observed results exhibited, that acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) significantly counteracted the memory impairment induced by PTZ- acute injection ($F_{(1,10)}$ = 18.6; $P < 0.05$) and ($F_{(1,10)}$ = 12.91; $P < 0.05$) when compared with PTZ-treated group (Figure 30). Remarkably, no significant difference was observed in the procognitive effect provided by H3R antagonist 4 (5 and 10 mg/kg) ($F_{(1,10)}$ = 0.48; p = 0.52). Notably, no significant difference was indicated between all the groups in the training day, indicating that no modulating factors e.g., locomotion or anxiety have confounded that modulatory effect in the test day.

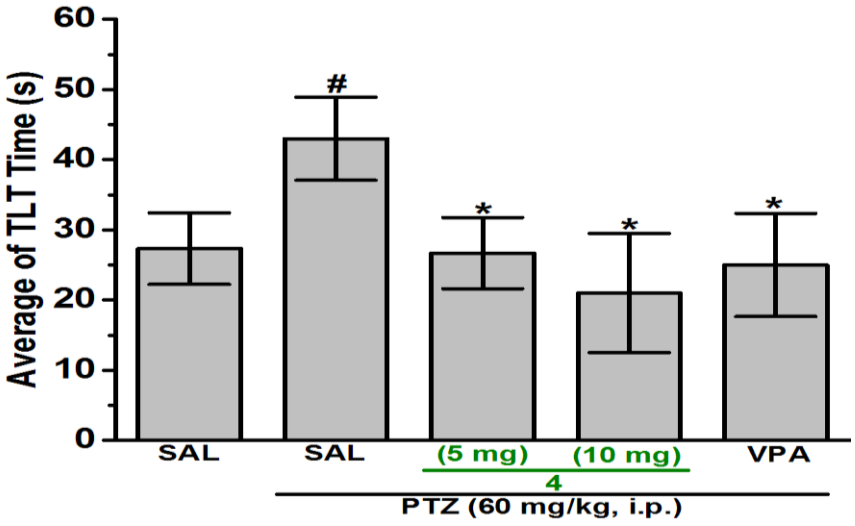


Figure 30: H3R antagonist 4 attenuated PTZ-induced memory deficits in the EPM.

Average of TLT measured on the test day (gray columns). Acute systemic administration of H3R antagonist 4 (5 and 10 mg/kg, i.p.) or VPA (300 mg/kg, i.p.) 30-45 minutes before PTZ- acute administration. $^{\#}P < 0.05$ for average TLT compared with that in the SAL-treated group. $^{*}P < 0.05$ for average TLT compared with the PTZ-treated group. The data are expressed as the mean ± SEM (n=6).

4.3.3 Effect of H3R antagonist 4 on oxidative stress levels in hippocampal brain tissue of PTZ- acute treated rats

The capability of H3R antagonist 4 to attenuate oxidative stress was evaluated in hippocampus tissue of PTZ- treated male Wistar rats and discussed in (Figure 31). Statistical analysis of the observed results demonstrated that acute pretreatment with H3R antagonist 4 and VPA 30-45 minutes before PTZ injection modulated the statues of oxidative stress markers (MDA and GSH) ($F_{(4,25)} = 3.32$; $P < 0.05$) and ($F_{(4,25)} = 6.19$; $P < 0.05$), respectively. Further statistical analysis revealed that MDA levels were markedly increased in PTZ-treated group ($F_{(1,10)} = 16.03$; $P < 0.05$) when compared with SAL-treated group, while GSH levels were significantly decreased in PTZ-treated group ($F_{(1,10)} = 21.56$; $P < 0.05$) when compared with SAL-treated group (Figure 31A and B). Moreover, the results exhibited no significance difference in CAT and SOD levels between PTZ-treated group and SAL-treated group ($F_{(1,10)} = 0.004$; $p = 0.95$) and ($F_{(1,10)} = 1.23$; $p = 0.3$), respectively (Figure 31C and D). Further statistical analysis indicated that acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg) significantly decreased MDA levels ($F_{(1,10)} = 13.82$; $P < 0.05$) and ($F_{(1,10)} = 25.02$; $P < 0.05$), respectively when compared with PTZ-treated group. Also, pretreatment with H3R antagonist 4 (5 and 10 mg/kg) significantly increased GSH levels ($F_{(1,10)} = 32.38$; $P < 0.05$) and ($F_{(1,10)} = 79.81$; $P < 0.001$), respectively when compared with PTZ-treated group. Notably, no significance difference was detected in the antioxidant effect provided by H3R antagonist 4 (5 and 10 mg/kg) ($F_{(1,10)} = 2.43$; $p = 0.18$) and ($F_{(1,10)} = 0.93$; $p = 0.39$) for both MDA and GSH, respectively (Figures 31A & B). Moreover, acute pretreatment with VPA (300 mg/kg) significantly attenuated the oxidative stress by decreasing the MDA levels and increasing GSH levels ($F_{(1,10)} =$

8.75; $P < 0.05$) and ($F_{(1,10)} = 79.72$; $P < 0.001$) when compared with PTZ-treated group (Figures 31A & B).

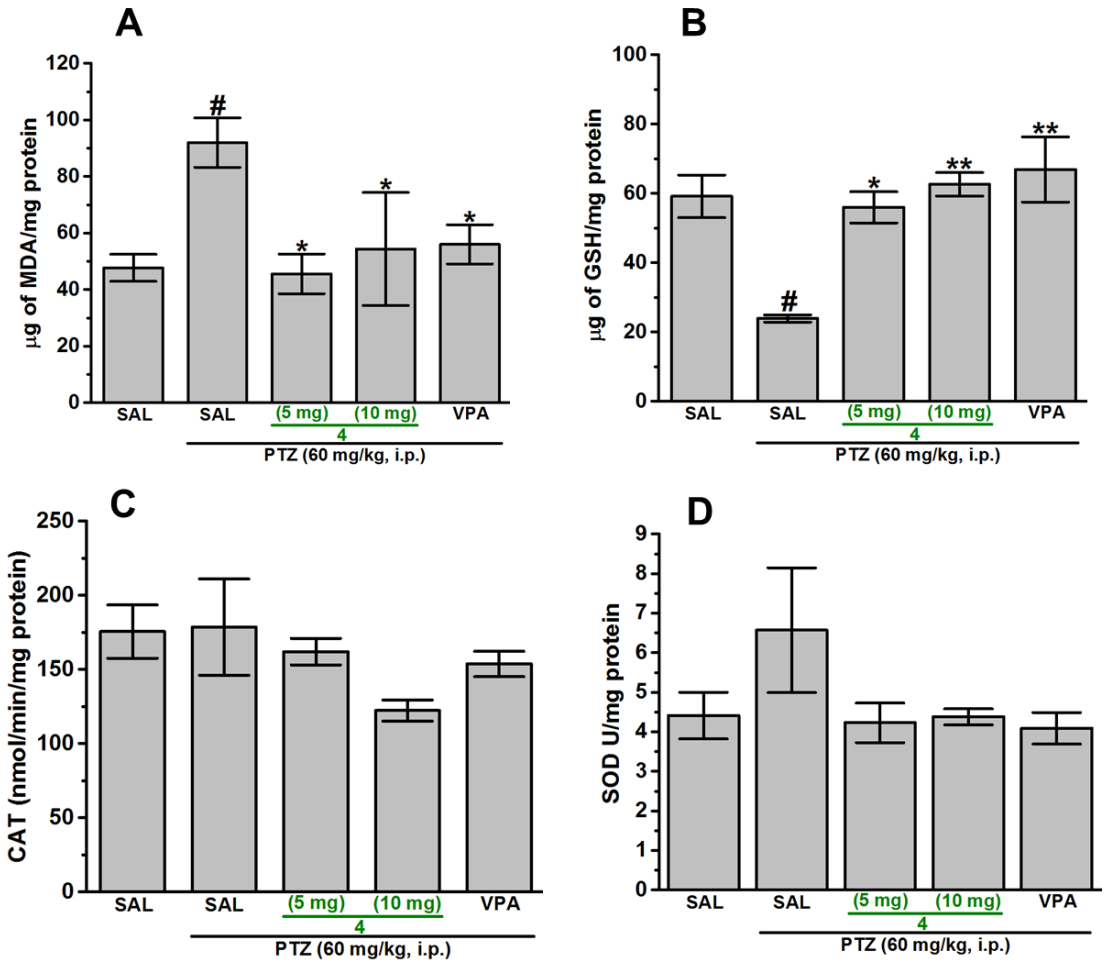


Figure 31: H3R antagonist 4 attenuated oxidative stress levels in hippocampal brain tissue of PTZ- acute treated rats.

Modulated levels of malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were assessed. PTZ-(60 mg/kg)-treated mice showed a significant increase in MDA (A) and significant decrease in GSH levels (B) but failed to alter CAT (C) or and SOD (D) levels compared with SAL-treated rats. H3R antagonist 4 (5 and 10 mg/kg, i.p.) or VPA (300 mg/kg, i.p.) were administered 30-45 min prior to PTZ-injection. H3R antagonist 4 (5 and 10 mg/kg, i.p.) and VPA (300 mg/kg, i.p.) significantly mitigated hippocampal MDA levels (A) and GSH levels (B). Data are expressed as the mean ± SEM (n=6). $^{\#}P < 0.05$ vs. SAL-treated rats. $^{*}P < 0.05$ vs. PTZ-treated rats. $^{**}P < 0.001$ vs. PTZ-treated rats.

4.3.4 Effect of H3R antagonist 4 on AChE activity in hippocampal brain tissue of PTZ- acute treated rats

The effect of H3R antagonist 4 on AChE activity was evaluated in hippocampal tissue of PTZ- treated male Wistar rats and discussed in (Figure 32). Statistical analysis of the observed results indicated that PTZ- acute systemic administration failed to exhibit any significant difference in hippocampal AChE activity when compared with SAL-treated group ($F_{(1,10)}$ = 0.41; p=0.54) (Figure 32).

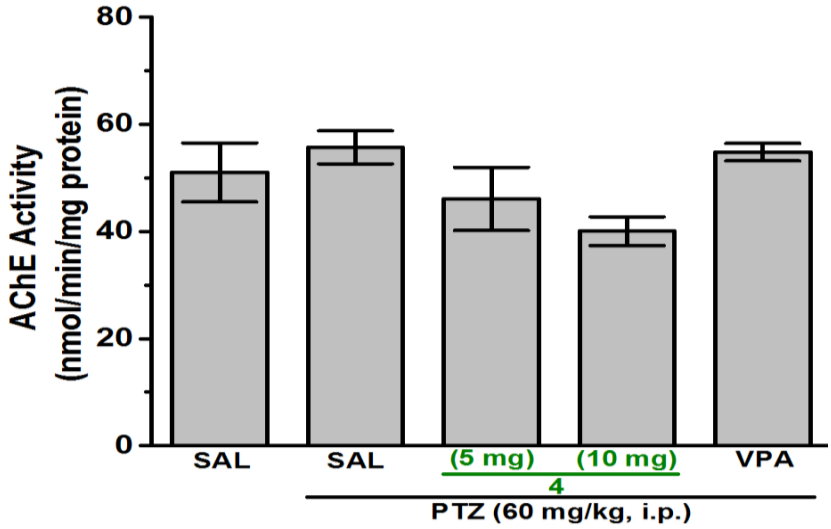


Figure 32: Effect of H3R antagonist 4 on AChE activity on hippocampal brain tissue of PTZ- acute treated rats.

Activity of (AChE) were assessed post PTZ-(60 mg/kg) acute systemic administration. PTZ (60 mg/kg), VPA (300 mg/kg), and H3R antagonist 4 (5 and 10 mg/kg) treatment failed to exhibit any significant change. Data are expressed as the mean ± SEM (n=6).

4.3.5 Effect of acute treatment of H3R antagonist 4 on neurotransmitters' levels in hippocampal brain tissue of PTZ treated rats

The levels of hippocampal neurotransmitters levels in brain tissue of PTZ- acute treated rats were assessed and discussed in (Figure 33). Statistical analysis of the results indicated, that PTZ (60 mg/kg, i.p.) systemic administration failed to exhibit any significant change in brain neurotransmitters (GABA, GLU, HA, and ACh) levels

($F_{(1,10)}$ = 0.19; p = 0.67), ($F_{(1,10)}$ = 0.54; p = 0.82), ($F_{(1,10)}$ = 1.76; p = 0.23), and ($F_{(1,10)}$ = 1.7; p = 0.24) when compared with SAL-treated group.

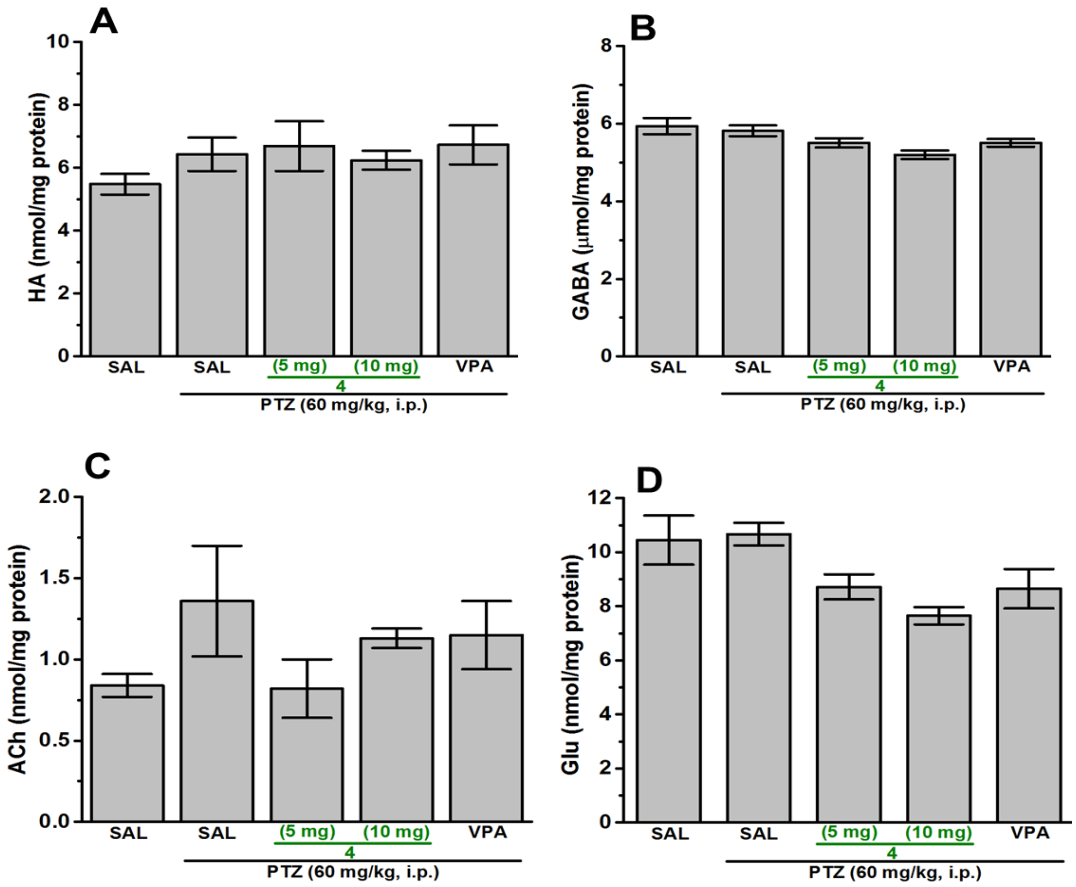


Figure 33: Effect of H3R antagonist 4 on neurotransmitters levels in hippocampal brain tissue of PTZ treated rats.

The levels of hippocampus neurotransmitters levels were assessed post PTZ-(60 mg/kg) acute systemic administration. PTZ (60 mg/kg), VPA (300 mg/kg) and H3R antagonist 4 (5 and 10 mg/kg) treatment failed to exhibit any significant change. Data are expressed as the mean ± SEM (n=6).

4.3.6 Effect of H3R antagonist 4 on c-fos expression in rat's hippocampus after PTZ- acute model

The expression of c-fos in the rat hippocampus after acute systemic administration of PTZ (60 mg/kg, i.p.) is shown in (Figures 34 & 35). Statistical analysis on hippocampal average c-fos positive cells per field, showed that acute pretreatment with H3R antagonist 4 and VPA 30-45 minutes before PTZ injection presented a significant decreasing effect ($F_{(4,25)} = 23.56$; $P < 0.001$). Further statistical analysis revealed that PTZ (60 mg/kg, i.p.) systemic administration significantly raised the average of c-fos positive cells in rat's hippocampus ($F_{(1,10)} = 57.33$; $P < 0.001$) when compared with SAL-tretaed rats. Moreover, acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg) and VPA (300 mg/kg) 30-45 minutes before PTZ injection significantly reduced c-fos positive cells ($F_{(1,10)} = 16.81$; $P < 0.05$), ($F_{(1,10)} = 24.45$; $P < 0.05$), and ($F_{(1,10)} = 53.24$; $P < 0.001$), respectively when compared with PTZ- exposed rats. Notably so significant difference was observed in protective effect provided by H3R antagonist 4 (5 and 10) mg/kg ($F_{(1,10)} = 0.78$; $p = 0.4$) (Figures 34 & 35).

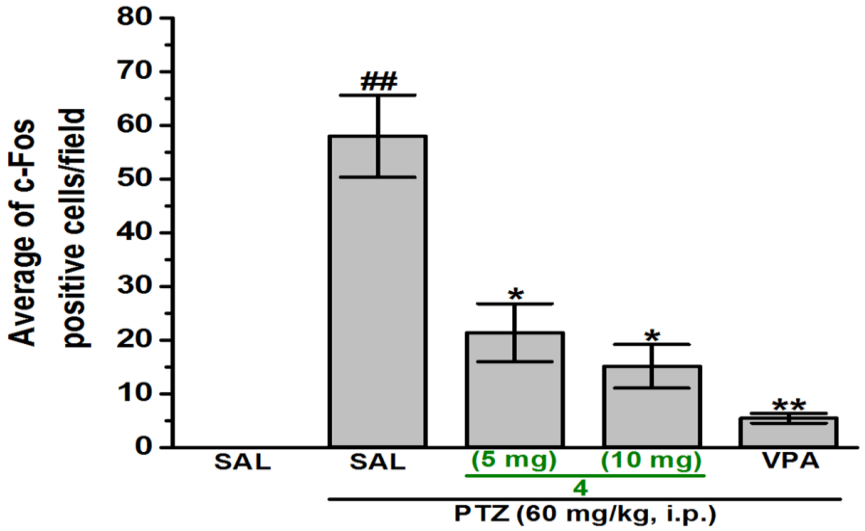


Figure 34: Effect of H3R antagonist 4 on hippocampal c-fos expression in PTZ-acute treated rats.

Quantitative analysis of hippocampal c-fos expression was assessed post PTZ-(60 mg/kg) acute systemic administration. PTZ (60 mg/kg), VPA (300 mg/kg), and H3R antagonist 4 (5 and 10 mg/kg) treatment exhibited a significant modulatory change. $^{\#\#}P < 0.001$ for average c-fos positive cells compared with that in the SAL-treated group. $^{*}P < 0.05$ for average c-fos positive cells compared with the PTZ-treated group. $^{**}P < 0.001$ for average c-fos positive cells compared with the PTZ-treated group. Data are expressed as the mean ± SEM (n=6).

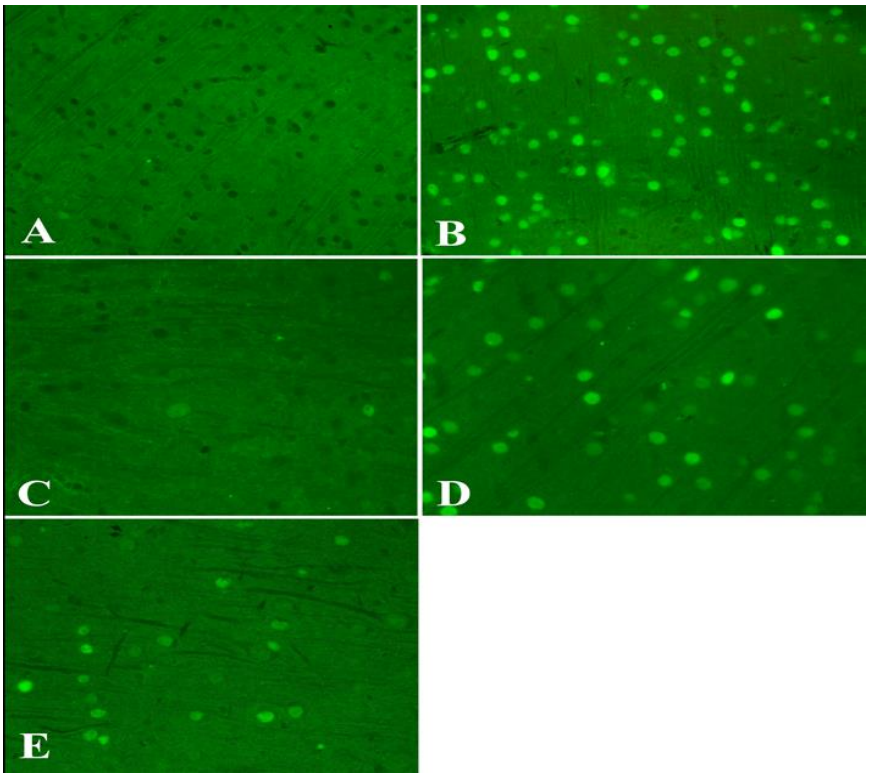


Figure 35: H3R antagonsit 4 reduced c-fos expression in hippocampal tissues of PTZ-exposed rats.

A Profound expression of c-fos positive cells was observed in the acute PTZ-exposed rats compared to SAL-treated rats. In contrast, acute treatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) showed significantly reduced activation of c-fos compared to the PTZ-treated rats. A SAL, B PTZ, C VPA, D 4 (5 mg/kg), E 4 (10 mg/kg).

4.4. PTZ- chronic model results, behavioral assessment, biochemical estimations, and IHC

4.4.1 Effect of chronic administration of H3R antagonist 4 on the average seizure score in PTZ-kindling model

The average seizure score upon each PTZ (40 mg/kg, i.p.) injection was scored and discussed in (Figure 36). The results indicated that repeated administration of a sub-convulsant dose of PTZ (40 mg/kg, i.p.) three times a week led to a significant increase of the average seizure score to seizure 4 or 5 on three consecutive injections starting from the 8th injection. The statistical analysis of the obtained results revealed that average seizure score of PTZ- treated rats increased remarkably from 1st injection to

12th injection ($F_{(1,18)}$ = 47.27; P < 0.001). Furthermore, further statistical analysis indicated that the average seizure score for each PTZ injection was significantly higher ($F_{(1,22)}$ = 19.28; P < 0.05), ($F_{(1,22)}$ = 30; P < 0.001), ($F_{(1,22)}$ = 160; P < 0.001) for 1st to 3th injections, respectively, ($F_{(1,22)}$ = 65535; P < 0.001) for 4th to 7th injections, ($F_{(1,22)}$ = 361; P < 0.001), ($F_{(1,22)}$ = 302.5; P < 0.001), ($F_{(1,21)}$ = 235.2; P < 0.001) for 8th to 10th injections, respectively and ($F_{(1,18)}$ = 65535; P < 0.001) for 11th and 12th injections when compared with SAL- treated rats (Figure 36). However, the results showed that chronic pretreatment with H3R antagonist 4 (5 mg/kg, i.p.) 30-45 minutes before each PTZ injection, caused a significant reduction of the average seizure score starting from the 3rd to 12th injections ($F_{(1,22)}$ = 20; P < 0.05), ($F_{(1,22)}$ = 169; P < 0.001), ($F_{(1,22)}$ = 5; P < 0.05), ($F_{(1,22)}$ = 7.5; P < 0.05), ($F_{(1,22)}$ = 7.5; P < 0.05), ($F_{(1,22)}$ = 6; P < 0.05), ($F_{(1,22)}$ = 14.41; P < 0.05), ($F_{(1,21)}$ = 7.68; P < 0.05), ($F_{(1,18)}$ = 12; P < 0.05), and ($F_{(1,18)}$ = 12; P < 0.05), respectively. While, chronic pretreatment of H3R antagonist 4 (10 mg/kg, i.p.) caused only a significant decrease in the average seizure score in the 2nd, 3rd, 6th, 11th, and 12th injection ($F_{(1,22)}$ = 15.62; P < 0.05), ($F_{(1,22)}$ = 15.62; P < 0.05), ($F_{(1,22)}$ = 5; P < 0.05), ($F_{(1,18)}$ = 12; P < 0.05), and ($F_{(1,22)}$ = 14.7; P < 0.05), respectively when compared with PTZ- treated rats (Figure 38). Moreover, chronic pretreatment with the positive drug VPA (300 mg/kg, i.p.) 30-45 minutes before each PTZ injection led to a significant decrease of the average seizure score among all the injections ($F_{(1,22)}$ = 19.28; P < 0.05), ($F_{(1,22)}$ = 20.86; P < 0.05), ($F_{(1,22)}$ = 289; P < 0.001), ($F_{(1,22)}$ = 49; P < 0.001), ($F_{(1,22)}$ = 15.94; P < 0.05), ($F_{(1,22)}$ = 30.62; P < 0.001), ($F_{(1,22)}$ = 53.57; P < 0.001), ($F_{(1,22)}$ = 20.6; P < 0.05), ($F_{(1,22)}$ = 14.41; P < 0.001), ($F_{(1,21)}$ = 35.56; P < 0.001), ($F_{(1,18)}$ = 14.94; P < 0.05), and ($F_{(1,18)}$ = 7.5; P < 0.05) when compared with PTZ- treated group. Notably, no significant difference was observed

between the average seizure score of H3R antagonist 4 (5 and 10 mg/kg)- treated rats among all the 12^{th} injections (all $P > 0.05$).

Additionally, the abrogation of H3R antagonist 4-provided protection was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before each PTZ injection (Figure 36). As confirmed with statistical analysis, the results indicated that RAM chronic co-injection led to a significant increase of the average seizure score in the 3^{rd}, 4^{th}, 8^{th}, 9^{th}, 10^{th} and 12^{th} when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats ($F_{(1,22)} = 7.1$; $P < 0.05$), ($F_{(1,22)} = 9.41$; $P < 0.05$), ($F_{(1,22)} = 7.27$; $P < 0.05$), ($F_{(1,22)} = 5$; $P < 0.05$), ($F_{(1,21)} = 5.71$; $P < 0.05$), and ($F_{(1,18)} = 5$; $P < 0.05$), respectively (Figure 38). However, chronic co-injection with PYR and ZOL failed to change the average seizure score among all the injections (all $P > 0.05$) when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. Notably, chronic administration of RAM, PYR, and ZOL with PTZ alone, failed to exhibit any significant change on the average seizure score when compared with PTZ- treated rats (all $P > 0.05$).

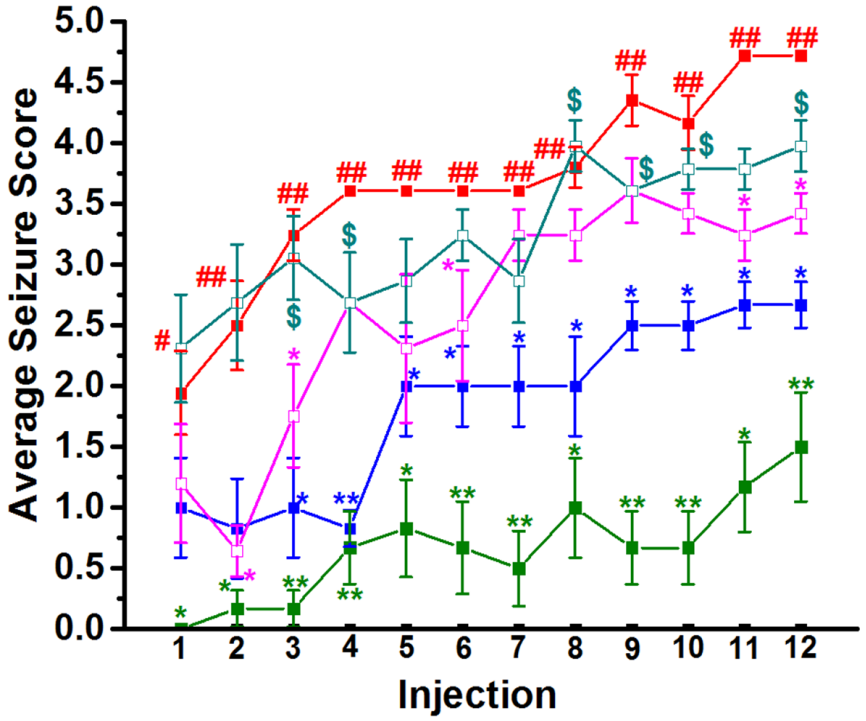


Figure 36: Chronic administration of H3R antagonist 4 attenuated the average seizure score in PTZ-kindling model.

Effects shown are expressed as average of seizure score for 30 min observation time for each PTZ injection. Effect of PTZ-(40 mg/kg), H3R antagonist 4 (5 and 10 mg/kg; i.p.), and VPA (300 mg/kg, i.p.) on average seizure score was assessed. H3R antagonist 4 (5 and 10 mg/kg; i.p.) and VPA (300 mg/kg, i.p.) were administered 30-45 min prior each PTZ-injection. Effects of systemic co-injection with RAM (10 mg/kg, i.p.) on H3R antagonist 4 (5 mg/kg)-provided anticonvulsant effect was evaluated. Values are expressed as the average + SEM (n=12). $^{*}P < 0.05$ vs PTZ-treated group. $^{**}P < 0.001$ vs PTZ-treated group. $^{\#}P < 0.05$ vs saline-treated group. $^{\#\#}P<0.001$ vs saline-treated group. $^{\$}P < 0.05$ vs H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. Black lines represent SAL-treated group and the RAM-treated control group. Green line represents VPA (300 mg/kg)-treated group. Blue line represents H3R antagonist 4 (5 mg/kg)-treated group. Pink line represents H3R antagonist 4 (10 mg/kg)-treated group. Dark-yellow line represents RAM (10 mg/kg) + 4 (5 mg/kg)-treated group. Red represents PTZ (40 mg/kg)-treated group. Violet line represents RAM-treated PTZ-kindled group.

4.4.2 Effect of chronic administration of H3R antagonist 4 on kindling and mortality rate

The kindling and mortality rate were calculated as percentages of the number of animals from each experimental group and were discussed in (Table 7). The observed results revealed that 83.33 % of the PTZ- treated rats (40 mg/kg, i.p.) 3 times a week for 12 injections showed GTCS convulsions (seizure score 4 or 5) in two consecutive injections and 16.67% of the animals died during PTZ- kindling model. Following

statistical analysis, the obtained results indicated that PTZ chronic treatment significantly raised the kindling rate with ($P < 0.001$) when compared with SAL-treated rats, but no significant change was observed in mortality rate (p = 14) when compared with SAL-treated rats (Table 7). However, the kindling rate had significantly decreased to 0.00 %, when rats were chronically pre-treated with H3R antagonist 4 (5 and 10 mg/kg) and VPA (300 mg/kg) 30-45 minutes before each PTZ injection with ($P < 0.001$) when compared with PTZ-exposed rats (Table 7). Also, the mortality rate had reduced to 0.00% with chronic pre-treatment with H3R antagonist 4 (5 and 10 mg/kg) and VPA (p = 0.14) (Table 7).

Additionally, the abrogation of H3R antagonist 4-provided protection was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before each PTZ injection (Table 7). As confirmed with statistical analysis, chronic co-injection with RAM (10 mg/kg) 20 minutes before each PTZ injection significantly raised the kindling rate to 75.00% ($P < 0.001$) when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. In contrast, chronic co-injection with ZOL (10 mg/kg) 30-45 minutes before each PTZ injection markedly elevated the kindling rate to 50.00% ($P < 0.05$) when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats (Table 7). However, chronic co-injection with PYR (10 mg/kg) 30-45 minutes before each PTZ injection failed to significantly alter the kindling rate (p = 1) when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. Furthermore, chronic co-injection with RAM, PYR, and ZOL failed to significantly modify the mortality rate (p = 307, p = 1 and p = 0.307), respectively. when compared with H3R antagonist 4 (5 mg)-treated PTZ-exposed rats (Table 7). Notably, chronic co-injection with RAM,

PYR, and ZOL with PTZ alone failed to alter both kindling and mortality rate (P > 0.05) when compared with PTZ-treated rats.

Table 7: Effect of chronic administration of H3R antagonist 4 on kindling and mortality rates.

Group	Kindling rate	Mortality Rate
SAL	0.00%	0.00%
PTZ	83.33%$^{\#\#}$	16.67%
4 (5 mg)	0.00%**	0.00%
4 (10 mg)	0.00%**	0.00%
VPA	0.00%**	0.00%
4 (5 mg) + RAM	75.00%$^{\$\$}$	8.33%
4 (5 mg) + PYR	0.00%	0.00%
4 (5 mg) + ZOL	50.00%$^{\$}$	8.33%
PTZ + RAM	75.00%	16.67%
PTZ + PYR	83.33%	16.67%
PTZ + ZOL	83.33%	8.33%

H3R antagonist 4 (5 and 10 mg/kg) and VPA (300 mg/kg) were injected 30-45 min before each PTZ injection. Effects shown are expressed as average of seizure score for 30 min observation time for each PTZ injection. Values are expressed as percentages of the number of animals from each experimental group(n=12-16). $^{\#\#}P < 0.001$vs SAL-treated group. $^{**}P < 0.001$vs PTZ-treated group. $^{\$}P < 0.05$ vs H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. $^{\$\$}P < 0.001$vs H3R antagonist 4 (5 mg)-treated PTZ-exposed rats.

4.4.3 Effect of chronic administration of H3R antagonist 4 on PTZ-induced memory deficits in IAP

The procognitive effect of compound 4 (5 and 10 mg/kg, i.p.) after chronic PTZ-induced memory deficits in IAP in male Wistar rats were completed and discussed in (Figure 37). Statistical analysis on the average of the STL times revealed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection conferred a significant memory-enhancing effect in IAP with ($F_{(4,25)} = 11.3$; $P < 0.001$). Further statistical analysis indicated, that PTZ chronic administration significantly decreased STL time when compared with SAL-treated rats ($F_{(1,10)} = 22.06$; $P < 0.001$). Moreover, results of chronic pretreatment with VPA (300 mg/kg, i.p.) 30-45 minutes

before each PTZ injection, indicated a significant improvement in STL time ($F_{(1,10)}$ = 22.06; $P < 0.001$) when compared with PTZ-treated rats. Moreover, further statistical analysis exhibited, that chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) significantly counteracted the memory impairment induced by PTZ- chronic injections ($F_{(1,10)}$ = 14.24; $P < 0.05$) and ($F_{(1,10)}$ = 6.87; $P < 0.05$) when compared with PTZ-treated rats (Figure 37). Remarkably, no significant difference was observed in the procognitive effect provided by H3R antagonist 4 (5 and 10 mg/kg) ($F_{(1,10)}$ = 1.4; p = 0.26). Notably, no significant difference was indicated between all the groups in the training day, indicating that no modulating factors, e.g., locomotion or anxiety, have confounded that modulatory effect in the test day.

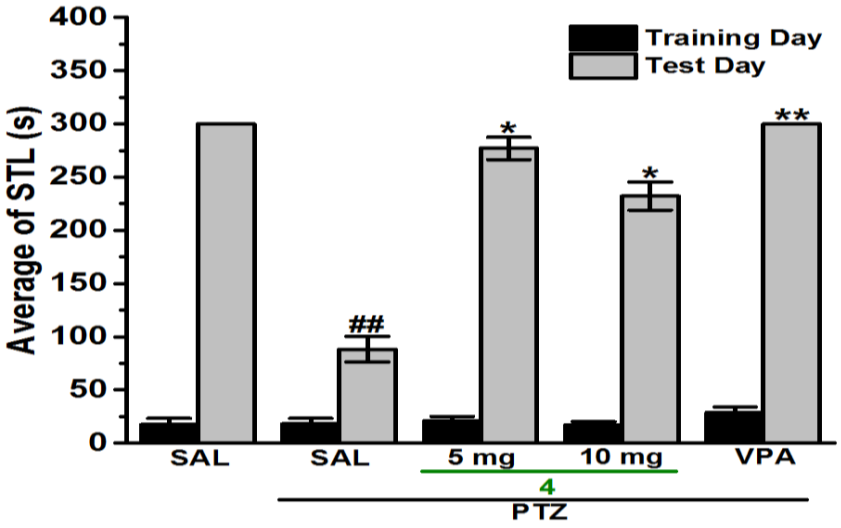


Figure 37: H3R antagonist 4 (5 and 10 mg/kg) chronic treatment counteracted PTZ-induced memory deficits in IAP.

Average of STL time measured on the first training day before the delivery of foot shock (black columns), and average of STL time measured on the test day (gray columns). Effect of chronic systemic administration of PTZ (40 mg/kg, i.p.) for 12 injections, H3R antagonist 4 (5 and 10 mg/kg, i.p.), or VPA (300 mg/kg, i.p.) 30-45 minutes before each PTZ injection on the average of STL time. $^{\#\#}P < 0.001$ for average STL time compared with that in the SAL-treated group. $^{*}P < 0.05$ for average STL time compared with the PTZ-treated group. $^{**}P < 0.001$ for average STL time compared with the PTZ-treated group. The data are expressed as the mean ± SEM (n=6).

Additionally, the abrogation of H3R antagonist 4-provided procognitive effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before each PTZ injection (Figure 40). As confirmed with statistical analysis, chronic co-injection with RAM (10 mg/kg, i.p.) 20 minutes before each PTZ injection significantly decreased STL time ($F_{(1,10)} = 5.42$; $P < 0.05$) when compared with H3R antagonist 4 (5 mg)- treated rats. However, chronic co-injection of PYR and ZOL (10 mg/kg, i.p.) 30-45 minutes before each PTZ injection failed to exhibit any change on STL time ($F_{(1,10)} = 0.21$; $p = 0.65$) and ($F_{(1,10)} = 0.34$; $p = 0.56$), respectively when compared with H3R antagonist 4 (5 mg)- treated rats. Notably, RAM chronic administration with PTZ or SAL alone did not significantly alter STL time when compared with PTZ- treated rats ($F_{(1,10)} = 0.02$; $p = 0.87$) and ($F_{(1,10)} = 3.6$; $p = 0.08$), respectively (Figure 38).

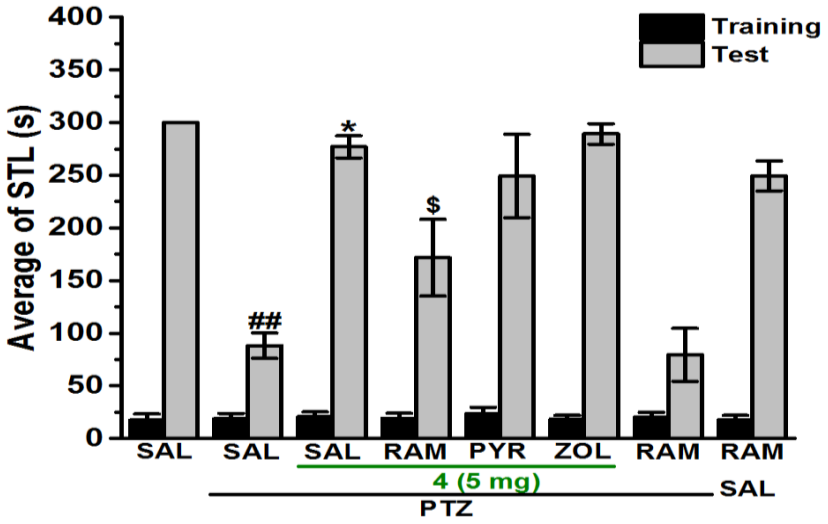


Figure 38: Effect of RAM, PYR, and ZOL on PTZ-induced memory deficit in IAP.

Average of STL time measured on the first training day before the delivery of foot shock (black columns) and the average of STL time measured on the test day (gray columns). Effect of chronic co-injection of RAM, PYR, and ZOL before each PTZ injection on the average of STL time. $^{\#\#}P < 0.001$ for average STL time compared with that in the SAL-treated group. $^{*}P < 0.05$ for average STL time compared with the PTZ-treated group. $^{\$}P < 0.05$ for average STL time compared with the H3R antagonist 4 (5 mg)-treated rats. The data are expressed as the mean ± SEM (n=6).

4.4.4 Effect of chronic administration of H3R antagonist 4 on PTZ-induced memory deficits in EPM

The procognitive effect of compound 4 (5 and 10 mg/kg, i.p.) after chronic PTZ-induced memory deficits in EPM in male Wistar rats were completed and discussed in (Figure 39). Statistical analysis on the average of the TLT revealed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection conferred a significant memory-enhancing effect in EPM ($F_{(4,25)} = 11.91$; $P < 0.001$). Further statistical analysis indicated, that PTZ chronic administration significantly increased TLT time when compared with SAL-treated rats with ($F_{(1,10)} = 5.16$; $P < 0.05$). Moreover, results of chronic pretreatment with VPA (300 mg/kg, i.p.) 30-45 minutes before each PTZ injection, indicated a significant decrease in TLT time ($F_{(1,10)} = 7$; $P < 0.05$) when compared with PTZ-treated rats. Further statistical analysis exhibited, that chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.)

significantly counteracted the memory impairment induced by PTZ- chronic injections by decreasing TLT ($F_{(1,10)}$ = 14.20; $P < 0.05$) and ($F_{(1,10)}$ = 10.82; $P < 0.05$) when compared with PTZ-treated rats (Figure 39). Remarkably, no significant difference was observed in the procognitive effect provided by H3R antagonist 4 (5 and 10 mg/kg) ($F_{(1,10)}$ = 1.47; p = 0.25). Notably, no significant difference was indicated between all the groups in the training day, indicating that no modulating factors, e.g., locomotion or anxiety, have confounded that modulatory effect in the test day.

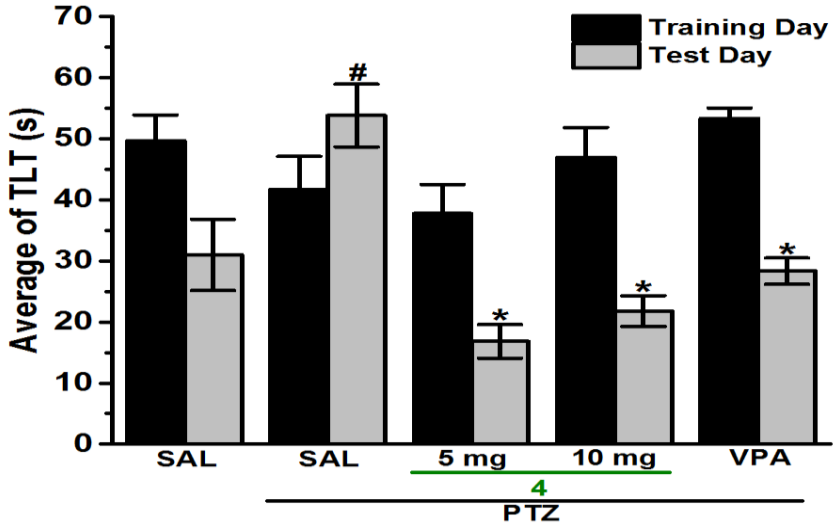


Figure 39: H3R antagonist 4 (5 and 10 mg/kg) chronic treatment counteracted PTZ-induced memory deficits in EPM.

Average of TLT time measured on the first training day before the delivery of foot shock (black columns) and the average of TLT time measured on the test day (gray columns). Effect of chronic systemic administration of PTZ (40 mg/kg, i.p.) for 12 injections, H3R antagonist 4 (5 and 10 mg/kg, i.p.), or VPA (300 mg/kg, i.p.) 30-45 minutes before each PTZ injection on the average of TLT time. $^{\#}P < 0.05$ for average TLT compared with that in the SAL-treated group. $^{*}P < 0.05$ for average TLT compared with the PTZ-treated group. The data are expressed as the mean ± SEM (n=6).

Additionally, the abrogation of H3R antagonist 4-provided procognitive effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before each PTZ injection (Figure 40). As confirmed with statistical analysis, chronic co-

injection with RAM (10 mg/kg, i.p.) 20 minutes before each PTZ injection significantly increased TLT ($F_{(1,10)}$ = 23.93; P < 0.001) when compared with H3R antagonist 4 (5 mg)- treated rats. However, chronic co-injection of PYR and ZOL (10 mg/kg, i.p.) 30-45 minutes before each PTZ injection failed to exhibit any change on TLT ($F_{(1,10)}$ = 3.26; p = 0.1) and ($F_{(1,10)}$ = 4.68; p = 0.06), respectively when compared with H3R antagonist 4 (5 mg)- treated rats. Notably, RAM chronic administration with PTZ or SAL alone did not significantly alter TLT time when compared with PTZ- or SAL- treated rats ($F_{(1,10)}$ = 0.27; p = 0.61) and ($F_{(1,10)}$ = 0.79; p = 0.39), respectively (Figure 40).

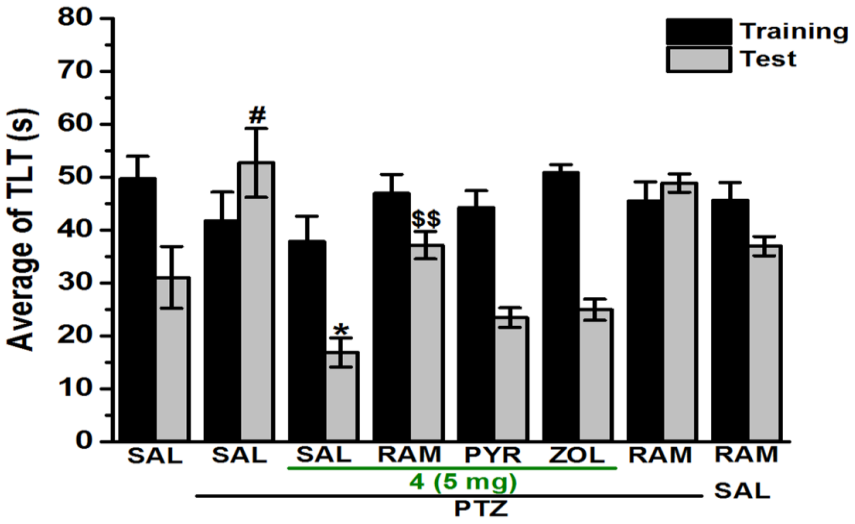


Figure 40: Effect of RAM, PYR, and ZOL on PTZ-induced memory deficit in EPM.

Average of TLT time measured on the first training day before the delivery of foot shock (black columns) and the average of TLT measured on the test day (gray columns). Effect of chronic co-injection of RAM, PYR, and ZOL before each PTZ injection on the average of TLT. $^{\#}P$<0.05 for the average of TLT compared with that in the SAL-treated group. $^{*}P$<0.05 for the average of TLT compared with PTZ-treated group. $^{\$\$}P$<0.001 for the average of TLT compared with the H3R antagonist 4 (5 mg)-treated rats. The data are expressed as the mean ± SEM (n=6).

4.4.5 Effect of chronic administration of H3R antagonist 4 on statues of oxidative stress levels in rat's hippocampus after PTZ-kindling model

The capability of H3R antagonist 4 to attenuate oxidative stress was evaluated in hippocampus tissue of PTZ- treated male Wistar rats and discussed in (Figure 41). Statistical analysis on the average of the MDA, GSH, CAT, and SOD revealed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection presented significant antioxidant effect ($F_{(5,30)} = 10.26$; $P < 0.001$), ($F_{(5,30)} = 28.48$; $P < 0.001$), ($F_{(5,30)} = 10.63$; $P < 0.001$), and ($F_{(5,30)} = 13.86$; $P < 0.001$), respectively. Further statistical analysis of the observed results indicated that MDA levels were markedly increased in PTZ-treated rats ($F_{(1,10)} = 63.96$; $P < 0.001$) when compared with SAL-treated group, while GSH levels were significantly decreased in PTZ-treated rats ($F_{(1,10)} = 16.7$; $P < 0.001$) when compared with SAL-treated rats (Figure 41A and B). Moreover, the statistical analysis of the obtained results exhibited a significance decrease in CAT and SOD levels in PTZ-treated rats when compared with SAL-treated group ($F_{(1,10)} = 25.6$; $P < 0.05$) and ($F_{(1,10)} = 23.71$; $P < 0.05$), respectively (Figure 41C and D). Further statistical analysis indicated that chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg) significantly decreased MDA levels ($F_{(1,10)} = 60.47$; $P < 0.001$) and ($F_{(1,10)} = 18.35$; $P < 0.05$), respectively when compared with PTZ-treated rats. Also, chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg) significantly increased GSH levels ($F_{(1,10)} = 96.74$; $P < 0.001$) and ($F_{(1,10)} = 356.1$; $P < 0.001$), respectively when compared with PTZ- treated rats. In addition, chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg) significantly increased CAT levels ($F_{(1,10)} = 45.06$; $P < 0.001$) and ($F_{(1,10)} = 32.91$; $P < 0.05$), respectively and SOD levels ($F_{(1,10)} = 40.43$; $P < 0.001$) and ($F_{(1,10)} = 37.03$; $P < 0.001$), respectively when compared with PTZ- exposed rats. (Figures 41C & D). Notably, no

significance difference was detected in the antioxidant effect provided by H3R antagonist (5 and 10 mg/kg) ($F_{(1,10)}$ = 0.69; p = 0.42), ($F_{(1,10)}$ = 1.93; p = 0.19), ($F_{(1,10)}$ = 0.01; p = 0.9), and ($F_{(1,10)}$ = 1.6; p = 0.25) for MDA, GSH, CAT, and SOD, respectively (Figure 42). Moreover, chronic pretreatment with VPA (300 mg/kg) significantly attenuated the oxidative stress by decreasing the MDA levels and increasing GSH levels ($F_{(1,10)}$ = 16.18; $P < 0.05$) and ($F_{(1,10)}$ = 8.74; $P < 0.05$)and decreasing both CAT and SOD levels ($F_{(1,10)}$ = 16.06; $P < 0.05$) and ($F_{(1,10)}$ = 7.34; $P < 0.05$) when compared with PTZ- exposed rats (Figures 41C & D).

Additionally, the abrogation of H3R antagonist 4-provided antioxidant effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.) 20 min before each PTZ injection (Figure 41). Statistical analysis of the observed results indicated that chronic co-injection of RAM (10 mg/kg) 20 minutes before each PTZ injection, significantly abrogated the H3R antagonist 4 (5 mg)- antioxidant protective effect (Figure 41). As chronic co-injection of RAM significantly increased MDA levels and decrease GSH levels ($F_{(1,10)}$ = 7; $P < 0.05$) and ($F_{(1,10)}$ = 16.06; $P < 0.05$), respectively when compared with H3R antagonist 4 (5 mg)-treated rats (Figures 41A & B). In addition, chronic co-injection of RAM significantly decreased both CAT and SOD levels ($F_{(1,10)}$ = 6.45; $P < 0.05$) and ($F_{(1,10)}$ = 24.92; $P < 0.05$), respectively when compared with H3R antagonist 4 (5 mg)-treated rats (Figures 41C & D). Notably, chronic pretreatment of RAM with PTZ alone did not significantly alter oxidative stress statues ($F_{(1,10)}$ = 1.28; p = 0.28), ($F_{(1,10)}$ = 0.26; p = 0.62), ($F_{(1,10)}$ = 0.53; p=0.49), and ($F_{(1,10)}$ = 0.42; p=0.54), respectively for MDA, GSH, CAT, and SOD when compared with PTZ-treated rats (Figures 41A & B). Also, chronic pretreatment of RAM with SAL alone did not significantly alter oxidative stress statues ($F_{(1,10)}$ = 1.13; p = 0.31), ($F_{(1,10)}$ = 0.18; p = 0.68), ($F_{(1,10)}$ = 0.02; p =

0.89), and ($F_{(1,10)}$ = 0.42; p = 0.54), respectively for MDA, GSH, CAT, and SOD when compared with SAL-treated rats (Figures 41C & D).

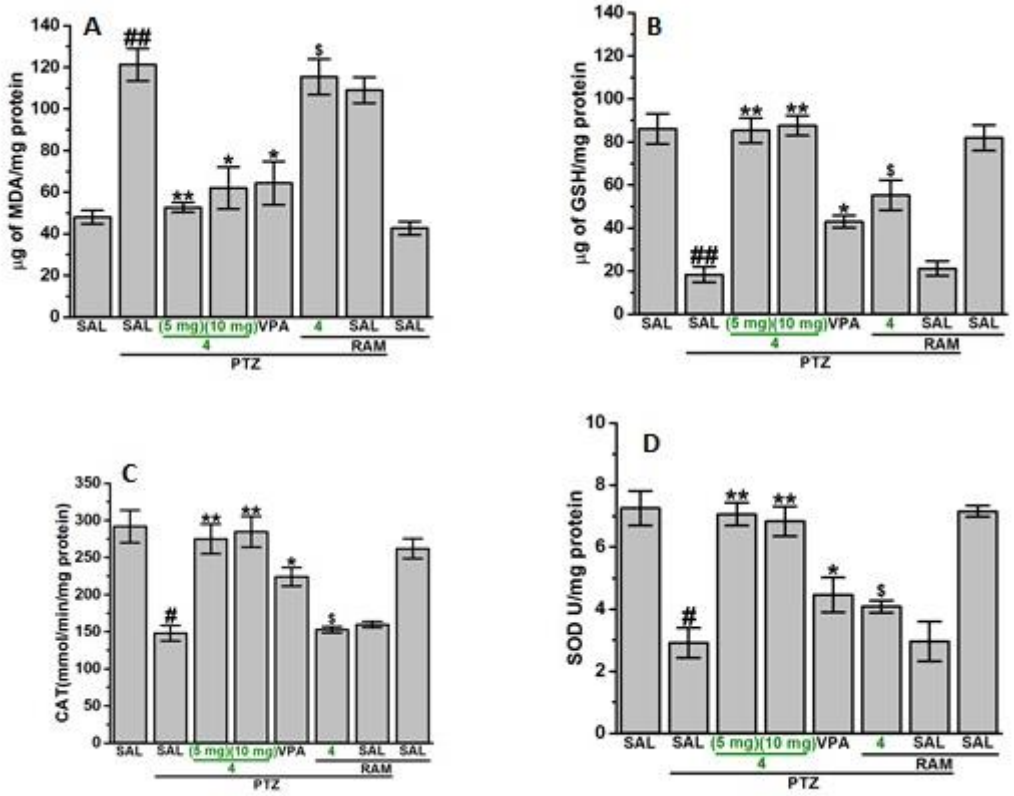


Figure 41: H3R antagonist 4 (5 and 10 mg/kg) mitigated oxidative stress associated with PTZ kindling model.

Levels of malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were assessed in rat hippocampus. Effect of PTZ-(40 mg/kg), H3R antagonist 4 (5 and 10 mg/kg; i.p.) and VPA (300 mg/kg, i.p.) on MDA levels (A), GSH levels (B), CAT levels (C), and SOD levels (D) were assessed. H3R antagonist 4 (5 and 10 mg/kg; i.p.) and VPA (300 mg/kg, i.p.) were administered 30-45 min prior each PTZ-injection. Effects of systemic co-injection with RAM (10 mg/kg, i.p.) on H3R antagonist 4 (5 mg/kg)-provided modulation of oxidative stress levels were assessed. Data are expressed as the mean ± SEM (n=6). $^{\#}P < 0.05$ vs. SAL-treated rats. $^{\#\#}P < 0.001$ vs. SAL-treated rats. $^{*}P < 0.05$ vs. PTZ-treated rats. $^{**}P < 0.001$ vs. PTZ-treated rats. $^{\$}P < 0.05$ vs. H3R antagonist 4 (5 mg)-treated rats.

4.4.6 Effect of chronic administration of H3R antagonist 4 on AChE activity in rat's hippocampus after PTZ-kindling model

The effect of H3R antagonist 4 on AChE levels was evaluated in the hippocampal tissue of PTZ- treated male Wistar rats and discussed in (Figure 42). Statistical analysis on the average of the AChE activity revealed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection presented a significant modulating effect ($F_{(5,30)}$ = 15,05; $P < 0.001$). Further statistical analysis of the observed results indicated that PTZ- chronic systemic administration for 12 injections led to a significant increase of hippocampal AChE activity ($F_{(1,10)}$ = 12.82; $P < 0.05$) when compared to SAL-treated rats (Figure 42). Moreover, chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) 30-45 minutes before each PTZ injection, significantly decreased the elevated hippocampal AChE activity ($F_{(1,10)}$ = 22.67; $P < 0.05$) and ($F_{(1,10)}$ = 15.39; $P < 0.05$), respectively when compared with PTZ- exposed rats (Figure 44). Notably, no significance difference was detected in the protective effect provided by H3R antagonist (5 and 10 mg/kg) ($F_{(1,10)}$ = 1.83; p = 0.21). Furthermore, chronic pretreatment with the positive drug VPA (300 mg/kg, i.p.) 30-45 minutes before each PTZ injection, resulted in a significant decrease of hippocampal AChE activity ($F_{(1,10)}$ = 14.0; $P < 0.05$) (Figure 42). Additionally, the abrogation of H3R antagonist 4-provided effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.) 20 min before each PTZ injection (Figure 44). Statistical analysis of the observed results indicated that chronic co-injection of RAM (10 mg/kg) 20 minutes before each PTZ injection, significantly abrogated the H3R antagonist 4 (5 mg)- protective effect (Figure 42). As chronic co-injection of RAM significantly increased AChE activity ($F_{(1,10)}$ = 47.73; $P < 0.001$) when compared with H3R antagonist 4 (5 mg)-treated rats (Figure 42).

Notably, chronic pretreatment of RAM with PTZ or SAL alone did not significantly alter AChE activity ($F_{(1,10)}$ = 1.8; p = 0.20) and ($F_{(1,10)}$ = 0.3; p = 0.6), respectively when compared with PTZ- or SAL- treated rats (Figure 42).

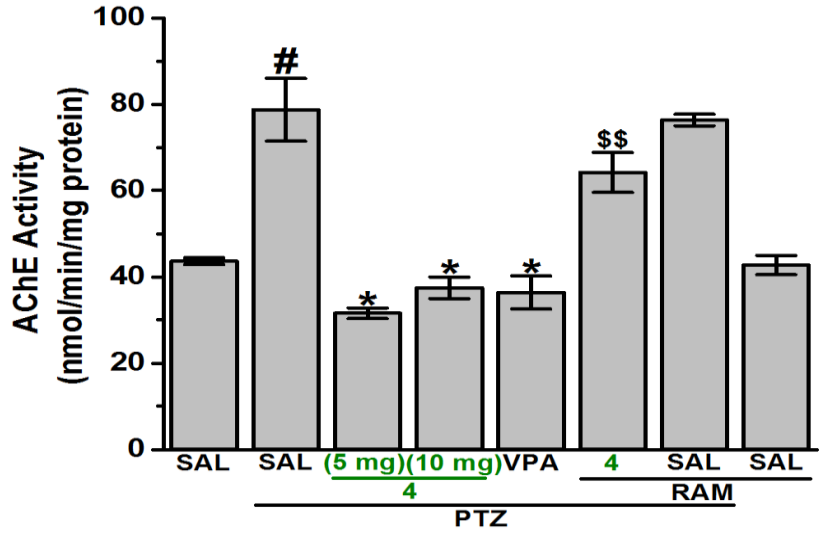


Figure 42: H3R antagonist 4 (5 and 10 mg/kg) chronic treatment modulated AChE activity in rat's hippocampus after PTZ-kindling model.

Levels of AChE activity were assessed in rat's hippocampus. H3R antagonist 4 (5 and 10 mg/kg; i.p.) and VPA (300 mg/kg, i.p.) were administered 30-45 min prior each PTZ-injection. Effect of systemic co-injection with RAM (10 mg/kg, i.p.) on H3R antagonist 4 (5 mg/kg)-provided modulation on AChE activity were assessed. Data are expressed as mean ± SEM (n=6). $^{\#}P < 0.05$ vs. SAL-treated rats. $^{*}P < 0.05$ vs. PTZ-treated rats. $^{\$\$}P < 0.001$ vs. H3R antagonist 4 (5 mg)-treated rats.

4.4.7 Effect of chronic administration of H3R antagonist 4 on neurotransmitters levels in rat's hippocampus after PTZ-kindling model

The capability of H3R antagonist 4 to modulate neurotransmitters concentration was evaluated in hippocampal tissue of PTZ- treated male Wistar rats and discussed in (Figure 43). Statistical analysis on the average of the hippocampal HA, ACh, GABA, and GLU levels revealed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection presented a significant modulating effect ($F_{(5,30)}$ = 7.56; $P < 0.001$), ($F_{(5,30)}$ = 12.62; $P < 0.001$), ($F_{(5,30)}$ = 218.2; $P < 0.001$), and ($F_{(5,30)}$ = 15.76; $P < 0.001$), respectively. Further statistical analysis of the observed

results indicated that PTZ- chronic systemic administration for 12 injections led to a significant decrease in both HA and ACh hippocampal levels ($F_{(1,10)} = 6.69$; $P < 0.05$) and ($F_{(1,10)} = 60.87$; $P < 0.001$), respectively when compared with SAL-treated rats. Furthermore, the obtained results indicated that PTZ chronic administration resulted in a significant increase in GLU hippocampal concentration ($F_{(1,10)} = 53.16$; $P < 0.001$) when compared with SAL-treated rats. However, hippocampal GABA concentration in PTZ-treated rats was not altered ($F_{(1,10)} = 0.09$; p =0.76) when compared with SAL-treated rats (Figure 43). However, further statistical analysis of the observed results revealed, that chronic treatment with H3R antagonist 4 (5 and 10 mg/kg) 30-45 minutes before each PTZ injection significantly increased hippocampal HA concentration ($F_{(1,10)} = 6.67$; $P < 0.05$) and ($F_{(1,10)} = 6.68$; $P < 0.05$), respectively when compared with PTZ exposed rats (Figure 45A). Also, chronic treatment with H3R antagonist 4 (5 and 10 mg/kg) significantly improved ACh levels in the hippocampus ($F_{(1,10)} = 53.51$; $P < 0.001$) and ($F_{(1,10)} = 10.2$; $P < 0.05$), respectively when compared with PTZ-treated rats (Figure 43B). Moreover, chronic treatment of H3R antagonist 4 (5 and 10 mg/kg) markedly decreased GLU hippocampal levels ($F_{(1,10)} = 11.6$; $P < 0.001$) and ($F_{(1,10)} = 5.98$; $P < 0.05$), respectively, but it failed to modify hippocampal GABA concentration ($F_{(1,10)} = 0.49$; p = 0.5) and ($F_{(1,10)} = 0.34$; p = 0.57), respectively when compared with PTZ-treated rats (Figures 43C & D). Notably, no significance difference was observed between H3R antagonist 4 (5 and 10 mg/kg) in the modulating effect of HA, ACh, and GLU levels ($F_{(1,10)} = 0.12$; p = 0.74), ($F_{(1,10)} = 0.66$; p = 0.44), and ($F_{(1,10)} = 0.31$; p = 0.59), respectively. In addition, chronic treatment with VPA (300 mg/kg) 30-45 minutes before each PTZ injection, failed to change hippocampus HA and GABA levels ($F_{(1,10)} = 0.66$; p = 0.44) and ($F_{(1,10)} = 0.66$; p = 0.44), respectively when compared with PTZ exposed rats. However, VPA chronic treatment enhanced

ACh brain levels and decreased GLU brain levels ($F_{(1,10)} = 10.15$; $P < 0.05$) and ($F_{(1,10)} = 6.07$; $P < 0.05$), respectively when compared with PTZ-treated rats (Figure 43). Additionally, the abrogation of H3R antagonist 4-provided effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.) 20 min before each PTZ injection (Figure 43). Statistical analysis of the observed results indicated that chronic co-injection of RAM (10 mg/kg) 20 minutes before each PTZ injection, abrogated the enhancement effect provided with H3R antagonist 4 (5 mg/kg) on hippocampal brain HA and ACh levels ($F_{(1,10)} = 21.18$; $P < 0.05$) and ($F_{(1,10)} = 35.53$; $P < 0.001$), respectively; however, it failed to abrogate brain GLU levels ($F_{(1,10)} = 2.46$; $p = 0.14$) when compared with H3R antagonist 4 (5 mg)-treated rats (Figure 45). Notably, chronic pretreatment of RAM with PTZ or SAL alone did not significantly alter HA levels ($F_{(1,10)} = 0.06$; $p = 0.80$) and ($F_{(1,10)} = 0.6$; $p = 0.47$), respectively when compared with PTZ- or SAL- treated rats, not either ACh levels ($F_{(1,10)} = 1.04$; $p = 0.34$) and ($F_{(1,10)} = 0.84$; $p = 0.39$), respectively when compared with PTZ- or SAL- treated rats, respectively (Figures 43A & B).

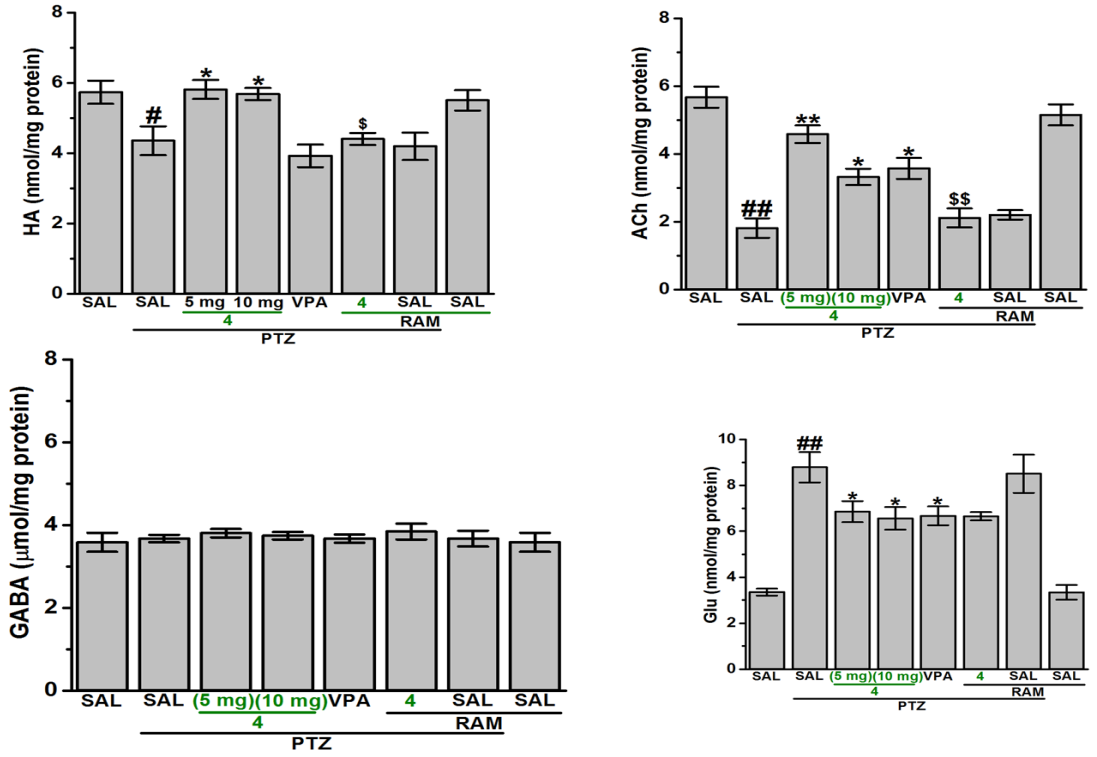


Figure 43: H3R antagonist 4 (5 and 10 mg/kg) chronic treatment modulated HA, ACh, and GLU levels in rat's hippocampus after PTZ-kindling model.

Levels of histamine (HA), acetylcholine (ACh), (GABA), and glutamate (GLU) were assessed in rat's hippocampus. Effect of PTZ-(40 mg/kg), H3R antagonist 4 (5 and 10 mg/kg; i.p.), and VPA (300 mg/kg, i.p.) on HA levels (A), ACh levels (B), GABA levels (C), and GLU levels (D) were assessed. H3R antagonist 4 (5 and 10 mg/kg; i.p.) and VPA (300 mg/kg, i.p.) were administered 30-45 min prior each PTZ-injection. Effects of systemic co-injection with RAM (10 mg/kg, i.p.) on H3R antagonist 4 (5 mg/kg)-provided modulation of brain neurotransmitters levels were assessed. Data are expressed as the mean ± SEM (n=6). $^{\#}P < 0.05$ vs. SAL-treated rats. $^{\#\#}P < 0.001$ vs. SAL-treated rats. $^{*}P < 0.05$ vs. PTZ-treated rats. $^{**}P < 0.001$ vs. PTZ-treated rats. $^{\$}P < 0.05$ vs. H3R antagonist 4 (5 mg)-treated rats.

4.4.8 Effect of chronic administration of H3R antagonist 4 on c-fos positive cell in rat's hippocampus after PTZ- kindling model

The expression of c-fos in rat's hippocampus after chronic systemic administration of PTZ (40 mg/kg, i.p.) three times a week for a total of 12 injections is shown in (Figures 44 & 45). Statistical analysis of hippocampal c-fos positive cells per field, showed that chronic pretreatment with H3R antagonist 4 and VPA 30-45 minutes before each PTZ injection presented a significant decreased effect ($F_{(5,30)} = 11.95$; $P < 0.001$). Further statistical analysis revealed that PTZ (40 mg/kg, i.p.) chronic systemic administration significantly increased the number of c-fos positive cells in rat's hippocampus ($F_{(1,10)}$

= 17.58; $P < 0.05$) when compared with SAL-treated rats. Moreover, chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg) and VPA (300 mg/kg) 30-45 minutes before PTZ injection significantly reduced the number of c-fos positive cells ($F_{(1,10)} = 7.97$; $P < 0.05$), ($F_{(1,10)} = 9.95$; $P < 0.05$) and ($F_{(1,10)} = 11.52$; $P < 0.05$), respectively when compared with PTZ- exposed rats. Notably no significant difference was observed in protective effect provided by H3R antagonist 4 (5 and 10 mg/kg) with ($F_{(1,10)} = 2.55$; p = 0.15) (Figures 44 & 45).

Additionally, the abrogation of the H3R antagonist 4-provided effect was evaluated by chronic co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.) 20 min before each PTZ injection (Figures 44 & 45). Statistical analysis of the observed results indicated that chronic co-injection of RAM (10 mg/kg) 20 minutes before each PTZ injection, abrogated the protective effect provided with H3R antagonist 4 (5 mg/kg) on hippocampal c-fos expression ($F_{(1,10)} = 19.92$; $P < 0.05$) (Figures 44 & 45).

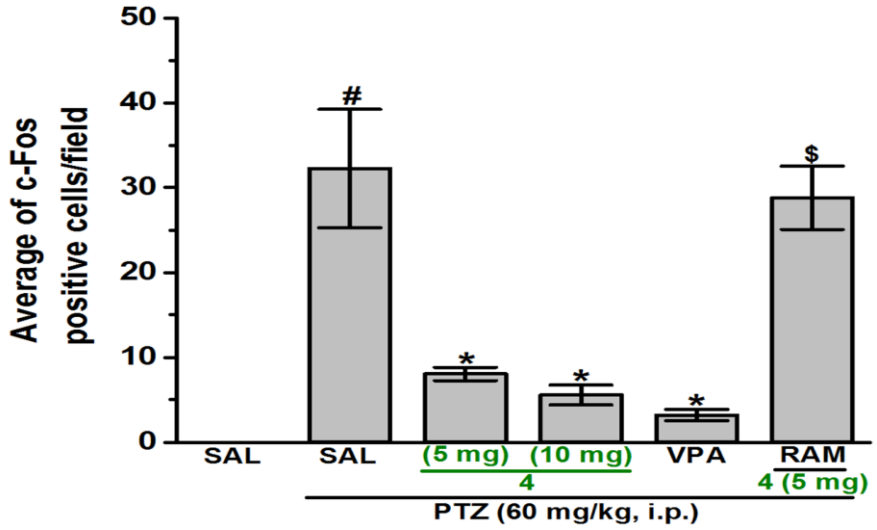


Figure 44: Effect of H3R antagonist 4 on hippocampal c-fos expression in PTZ-kindled rats.

The hippocampal c-fos expression was assessed post PTZ-(40 mg/kg) chronic systemic administration. VPA (300 mg/kg) and H3R antagonist 4 (5 and 10 mg/kg) treatment exhibited significant modulatory change. $^{\#}P < 0.05$ for average c-fos positive cells compared with that in the SAL-treated group. $^{*}P < 0.05$ for average c-fos positive cells compared with the PTZ-treated group. $^{\$}P < 0.05$ for average c-fos positive cells vs. H3R antagonist 4 (5 mg)-treated PTZ-exposed rats. Data are expressed as mean ± SEM (n=6).

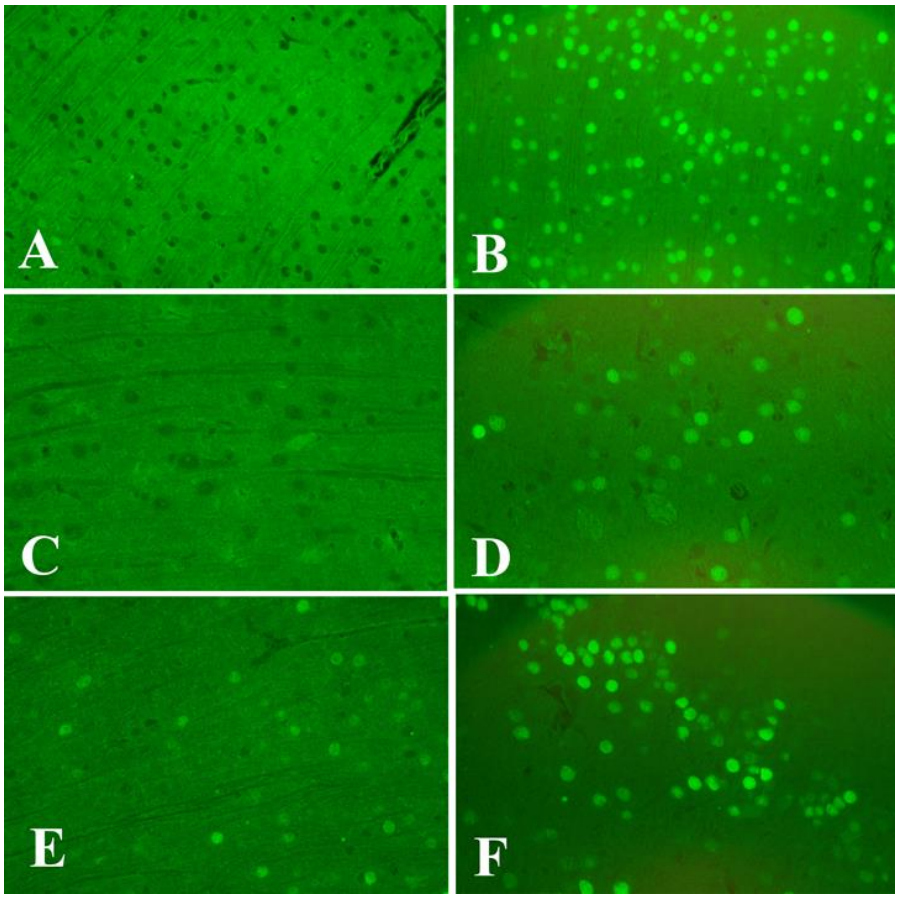


Figure 45: H3R antagonsit 4 decreased c-fos expression in hippocampal tissues of PTZ-exposed rats.

A Profound expression of c-fos positive cells was observed in chronic PTZ-exposed rats compared to SAL-treated rats. In contrast, chronic treatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) showed significantly reduced activation of c-fos compared to the PTZ-treated rats. A SAL, B PTZ, C VPA, D 4 (5 mg/kg), E 4 (10 mg/kg), F RAM + 4 (5 mg/kg).

4.5 Pilocarpine (PLC)-Induced Status Epilepticus (SE)

4.5.1 Effect of H3R antagonist 4 on latency time to first seizure in PLC-induced SE model

The average of the latency time to first seizure for 1 hour following PLC acute systemic administration (400 mg/kg, i.p.) was scored and discussed in (Figure 46). All observed seizures were GTCS with SE. Statistical analysis on the average of the latency time to first seizure in PLC-induced SE model revealed that acute pretreatment with H3R antagonist 4 and VPA 30-45 minutes before PLC injection presented a significant protective effect ($F_{(5,42)} = 7.56$; $P < 0.001$). Further statistical analysis of the obtained results of systemic administration of PLC (400 mg/kg, i.p.) showed that the average of the latency time to the first seizure was significantly higher when compared with SAL-treated rats, as no seizures were observed ($F_{(1,14)} = 49.46$; $P < 0.001$). Moreover, no seizures were observed with acute pretreatment with the reference drug DZP (10 mg/kg, i.p.) and the average of the latency time to the first seizure was significantly altered ($F_{(1,14)} = 49.46$; $P < 0.001$) when compared with PLC-treated rats. Furthermore, further statistical analysis of the obtained results showed that pretreatment with H3R antagonist 4 (2.5 and 15 mg/kg, i.p.) 30-45 minutes before PLC injection failed to prolong the average of the latency time to first seizure ($F_{(1,14)} = 7.2$; $p = 0.06$), and ($F_{(1,14)} = 6.82$; $p = 0.06$), respectively as compared to the PLC-exposed rats (Figure 46). However, acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) 30-45 minutes before PLC injection significantly extended the average of the latency time to the first seizure ($F_{(1,14)} = 13.84$; $P < 0.05$) and ($F_{(1,14)} = 25.44$; $P < 0.001$), respectively when compared to the PLC-exposed rats.

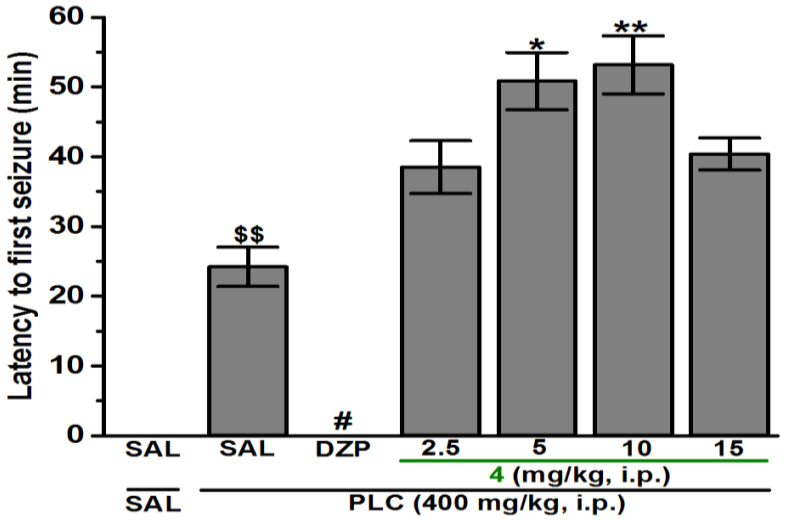


Figure 46: H3R antagonist 4 prolonged the average of the latency time to first seizure in PLC- induced SE model.

Effects of acute systemic injection with H3R antagonist 4 (2.5, 5, 10, and 15 mg/kg, i.p.) or DZP (10 mg/kg, i.p.) on latency time (min) to the first PLC-induced seizure. $^{\$\$}$ P <0.001 vs. SAL treated rats. $^{*}P < 0.05$ vs. PLC-treated rats. $^{**}P < 0.001$ vs. PLC-treated rats. $^{\#}$ Full protection as compared to PLC-treated rats. Values are expressed as the mean ± SEM (n=8).

In addition, the abrogation of H3R antagonist 4-provided protection was evaluated by acute co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before PLC injection (Figure 47). As confirmed with statistical analysis, the results showed that PYR (10 mg/kg, i.p.) co-administration failed to significantly abrogate the average of the latency time to first seizure ($F_{(1,14)} = 1.61$; $p = 0.23$) when compared to H3R antagonist 4 (10 mg)- protective effect. However, co-injection with ZOL (10 mg/kg, i.p.) significantly reversed the protective effects of H3R antagonist 4 (10 mg/kg) on the average of latency time to first seizure ($F_{(1,14)} = 30.71$; $P < 0.001$) when compared to H3R antagonist 4 (10 mg)- protective effect (Figure 47). Moreover, co-injection with RAM (10 mg/kg, i.p.) significantly decreased the average of the latency time to first seizure ($F_{(1,14)} = 4.72$; $P < 0.05$)when compared to H3R antagonist 4 (10 mg)- protective effect (Figure 47).

Notably, systemic administration of RAM, PYR, and ZOL alone did not alter the average of the latency to first seizure ($F_{(1,14)} = 1.76$; $p = 0.22$), ($F_{(1,14)} = 1.73$; $p = 0.21$), and ($F_{(1,14)} = 3.21$; $p = 0.10$), respectively when compared with PLC-treated group.

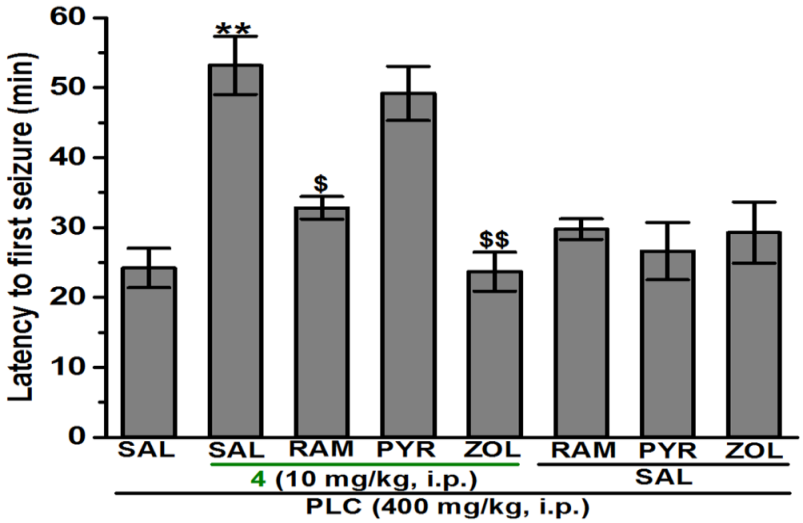


Figure 47: Effect of RAM, PYR, and ZOL on average of latency time to first seizure in PLC- induced SE model.

Effects of acute systemic injection with RAM, PYR and ZOL (10 mg/kg, i.p.) on latency time (min) to the first PLC-induced seizure. $^{**}P < 0.001$ vs. PLC-treated rats. $^{\$}P < 0.05$ vs. H3R antagonist 4 (10 mg)- protective effect. $^{\$\$}P < 0.001$ vs. H3R antagonist 4 (10 mg)- protective effect. Values are expressed as the mean ± SEM (n=8).

4.5.2 Effect of H3R antagonist 4 on SE incidence and survival rate

The SE incidence and survival rate were calculated as percentages of the number of animals from each experimental group and were discussed in (Table 8). Following statistical analysis, the obtained results indicated that all tested animals injected with PLC (400 mg/kg, i.p.) showed convulsions with SE and 66.66% of the animals survived from SE after 1 h from injection with ($P < 0.001$ and $P < 0.05$), respectively when compared to SAL-treated rats (Table 8). However, acute pre-treatment with DZP (10 mg/kg, i.p.) 30–45 min before PLC treatment showed that SE incidence was significantly decreased ($P < 0.001$) and survival rate was markedly increased (P <

0.05) when compared with the PLC-treated rats. In addition, the obtained results revealed that acute pre-treatment with H3R antagonist 4 (2.5, 5, 10, and 15 mg/kg, i.p.) 30-45 minutes before PLC injection significantly reduced SE incidence when compared with the PLC-treated group (all $P < 0.05$) except for H3R antagonist 4 (10 mg/kg) ($P < 0.001$). Furthermore, H3R antagonist 4 (2.5, 5, and 10 mg/kg, i.p.) acute pre-treatment significantly improved survival rate when compared with the PLC-treated group (all $P < 0.05$). However, H3R antagonist 4 (15 mg/kg, i.p.) failed to significantly raise the survival rate when compared with the PLC-treated group (Table 8).

Besides, the abrogation of H3R antagonist 4-provided protection was evaluated by acute co-injection with the CNS penetrant histamine H3R agonist RAM, the H1R antagonist PYR, and the H2R antagonist ZOL (10 mg/kg, i.p.) before PLC injection (Table 8). As confirmed with statistical analysis, the results showed that PYR (10 mg/kg, i.p.) co-administration failed to significantly alter SE incidence and survival rate with ($P > 0.05$) when compared to H3R antagonist 4 (10 mg)- protective provided effect. However, co-injection with ZOL partially reversed the SE incidence average ($P < 0.05$) and reduced the survival rate to ($P < 0.05$) (Table 8). Furthermore, co-injection with RAM raised the SE incidence ($P > 0.05$) and no change was observed on the survival rate. Notably, systemic administration of RAM, PYR, and ZOL alone did not alter SE incidence with (all $P > 0.05$) when compared with H3R antagonist 4 (10 mg)- protective provided effect (Table 8).

Table 8: Effect of H3R antagonist 4 on SE incidence and survival rate in PLC-induced SE model.

Group	Percentage of SE Incidence (%)	Percentage of Survival (%)
SAL	0.00	100.00
PLC (400 mg/kg)	100.00$^{\#}$	66.67
DZP (10 mg/kg)	0.00**	100.00
4 (2.5 mg/kg)	50.00*	100.00
4 (5 mg/kg)	50.00*	100.00
4 (10 mg/kg) [a]	33.33**	100.00
4 (15 mg/kg)	50.00*	83.33
4[a] + RAM[b]	66.67	100.00
4 [a] + PYR[c]	50.00	100.00
4 [a] + ZOL[d]	100.00$^{\$}$	83.33
RAM + PLC	83.33	100.00
PYR + PLC	83.33	83.33
ZOL + PLC	66.67	100.00

[a](10 mg/kg, i.p.), [b](10 mg/kg, i.p.), [c]PYR (10 mg/kg, i.p.), [d]ZOL (10 mg/kg, i.p.). $^{\#}P<0.001$ as compared to SAL-treated rats, $^{*}P < 0.05$, $^{**}P < 0.001$ as compared to PLC-treated rats. $^{\$}P < 0.05$ as compared to H3R antagonist 4 (10 mg/kg)-treated group. Values are expressed as percentages of the number of animals from each experimental group (n = 12).

4.5.3 Effect of H3R antagonist 4 on statues of oxidative stress in rat's hippocampus after PLC- induced SE model

The ability of H3R antagonist 4 to attenuate oxidative stress was evaluated in hippocampal tissue of PLC- treated male Wistar rats and discussed in (Figure 48). Statistical analysis on the average of the hippocampal MDA and GSH levels revealed that acute pretreatment with H3R antagonist 4 and DZP 30-45 minutes before PLC injection presented a significant antioxidant effect ($F_{(6,35)} = 6.48$; $P < 0.001$) and ($F_{(6,35)} = 5.34$; $P < 0.001$). Statistical analysis of the observed results indicated that MDA levels were markedly increased in PLC-treated rats ($F_{(1,10)} = 28.05$; $P < 0.001$) when compared with SAL-treated group, while GSH levels were significantly decreased in PLC-treated rats ($F_{(1,10)} = 69$; $P < 0.001$) when compared with SAL-treated rats (Figures 48A & C). However, no significant difference was observed of both CAT and SOD levels after PLC injection ($F_{(1,10)} = 0.24$; $p = 0.64$) and ($F_{(1,10)} = 1.74$; $p = 0.22$), respectively when compared with SAL-treated group (Figure 48E and F). Moreover,

the obtained results revealed that (GSH) levels were noticeably elevated in the DZP group ($F_{(1,10)}$ = 32.84; $P < 0.001$) as compared to the PLC group, also MDA levels were significantly reduced in the DZP group ($F_{(1,10)}$ = 11.08; $P < 0.05$), respectively when compared with PLC-treated group (Figure 48A and C). Furthermore, the results showed that pre-treatment with H3R antagonist (2.5, 5, 10, and 15 mg/kg, i.p.) 30-45 minutes before PLC injection significantly reduced hippocampal MDA levels ($F_{(1,10)}$ = 9.21; $P < 0.05$), ($F_{(1,10)}$ = 11.74; $P < 0.05$), ($F_{(1,10)}$ = 36.47; $P < 0.001$), and ($F_{(1,10)}$ = 11.76; $P < 0.05$) when compared to PLC-treated group (Figure 48C). In addition, the results demonstrated that pretreatment with H3R antagonist 4 (5, 10 and 15 mg/kg, i.p.) markedly increased GSH levels ($F_{(1,10)}$ = 11.31; $P < 0.05$), ($F_{(1,10)}$ = 12.18; $P < 0.05$), and ($F_{(1,10)}$ = 11.86; $P < 0.05$) when compared with PLC-treated group, while unnoticeable change was observed in animals treated with the lower dose H3R antagonist 4 (2.5 mg/kg, i.p.) ($F_{(1,10)}$ = 1.02; p = 0.34) when compared with PLC-treated group (Figure 48A). In addition, the abrogation of H3R antagonist 4-provided protection was evaluated by acute co-injection with the CNS penetrant histamine H3R agonist RAM (10 mg/kg, i.p.) 20 minutes before PLC injection (Figure 48B and D). As confirmed with statistical analysis, that pretreatment with RAM (10 mg/kg, i.p.) significantly abrogated the protective effect observed by H3R antagonist 4 (10 mg/kg, i.p.) on the levels of MDA and GSH ($F_{(1,10)}$ = 5.89; $P < 0.05$) and ($F_{(1,10)}$ = 9.51; $P < 0.05$), respectively when compared with H3R antagonist 4 (10 mg)-treated PLC-exposed rats (Figure 48B and D). Notably, RAM administered alone with PLC did not modulate the levels of MDA and GSH when compared to PLC-treated group ($F_{(1,10)}$ = 0.98; p = 0.41) and ($F_{(1,10)}$ = 1.91; p = 0.56), respectively, also RAM did not modulate the levels of MDA and GSH when administrated with SAL ($F_{(1,10)}$ = 0.88; p = 0.51) and ($F_{(1,10)}$ = 1.25; p = 0.39), respectively when compared with SAL-treated group.

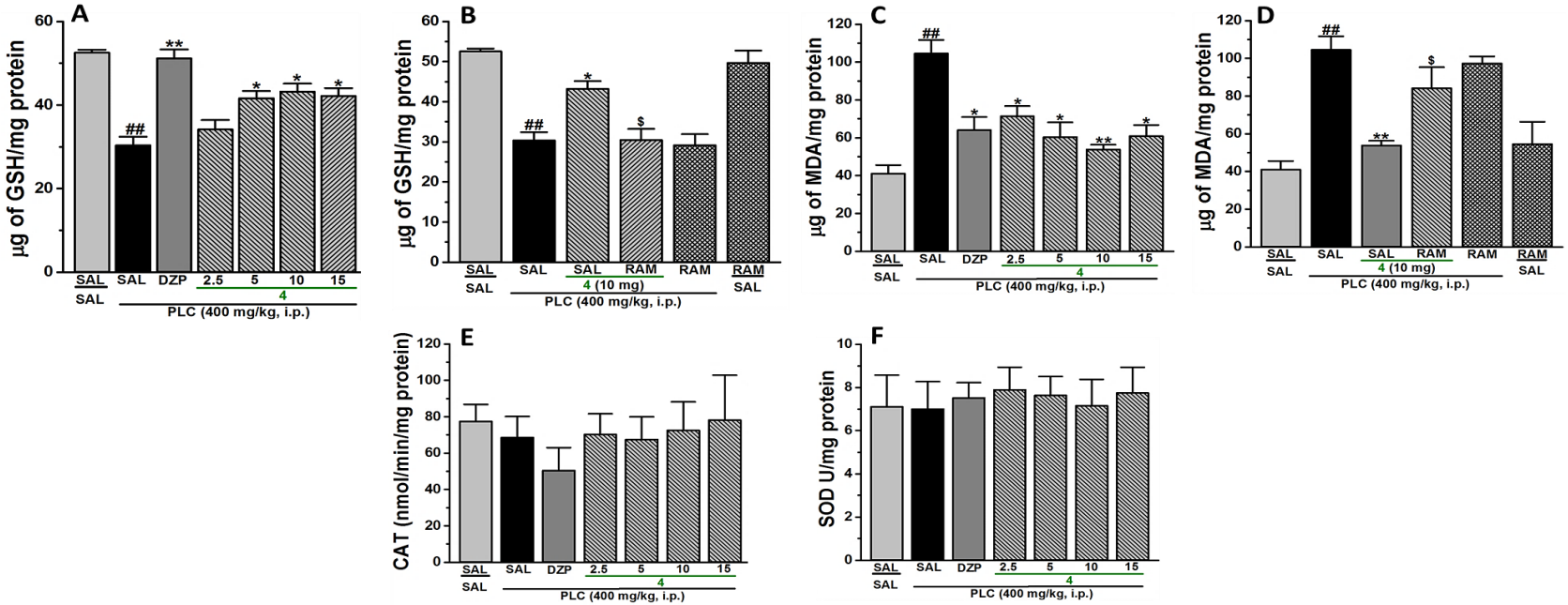


Figure 48: H3R antagonist 4 (5 and 10 mg/kg) mitigated hippocampal oxidative stress associated with PLC-induced SE model.

Levels of malondialdehyde (MDA), glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were assessed in rat hippocampus. Effect of PLC-(400 mg/kg), H3R antagonist 4 (2,5, 5, 10 and 15 mg/kg; i.p.) and DZP (10 mg/kg, i.p.) on GSH levels (A), MDA levels (C), CAT levels (E), SOD levels (F) were assessed. H3R antagonist 4 and DZP were administered 30-45 min prior PLC injection. Effects of systemic co-injection with RAM (10 mg/kg, i.p.) on H3R antagonist 4 (10 mg/kg)-provided modulation of oxidative stress levels were assessed. Data are expressed as the mean ± SEM (n=6). #P < 0.05 vs. SAL-treated rats. ##P < 0.001 vs. SAL-treated rats. *P < 0.05 vs. PLC-treated rats. **P < 0.001 vs. PLC-treated rats. $P < 0.05 vs. H3R antagonist 4 (10 mg)-treated PLC-exposed rats.

4.5.4 Effect of H3R antagonist 4 on AChE activity in rat's hippocampus after PLC-induced SE model

The effect of H3R antagonist 4 on AChE activity was evaluated in hippocampal tissue of PLC-treated male Wistar rats and discussed in (Figure 49). Statistical analysis on the average of the hippocampal AChE activity revealed that acute pretreatment with H3R antagonist 4 and DZP 30-45 minutes before PLC injection presented a significant modulating effect ($F_{(6,35)} = 6.31$; $P < 0.001$). Further statistical analysis of the observed results indicated that PLC- acute systemic administration significantly decreased AChE activity ($F_{(1,10)} = 20.94$; $P < 0.05$) when compared with SAL-treated rats. However, acute pretreatment with H3R antagonist 4 and DZP 30-45 minutes before PLC injection, failed to significantly exhibit any change on hippocampus on AChE activity ($F_{(1,10)} = 0.43$; $p = 0.53$), ($F_{(1,10)} = 0.98$; $p = 0.41$), and ($F_{(1,10)} = 0.97$; $p = 0.10$), respectively (Figure 49).

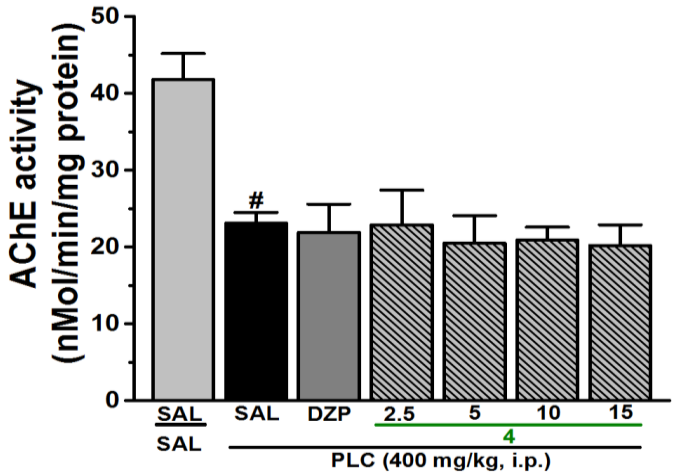


Figure 49: Effect of H3R antagonist 4 on (AChE) activity in rat's hippocampus after PLC-induced SE model.

Effects of acute systemic injection of rats with test compound 4 (2.5, 5, 10, and 15 mg/kg, i.p.) or DZP (10 mg/kg, i.p.) on acetylcholine esterase enzyme activity in the hippocampus of PLC-seizure rats. Quantitative analysis revealed a significant decrease ($^{\#}P < 0.001$) in the AChE activity in the hippocampus of PLC-seizure rats compared to the control rats. Acute systemic treatment with test compound 4 (2.5, 5, 10, and 15 mg/kg, i.p.) or DZP (10 mg/kg, i.p.) to the PLC-rats failed to restore this activity compared to the PLC-treated rats. Values are expressed as the percent mean ± SEM (n = 6).

Chapter 5: Discussion

All novel H3R antagonists in the form of hydrogen oxalate salts were evaluated in radioligand displacement binding assays for affinity at human recombinant histamine H3 receptor (hH3R) stably expressed in human embryonic kidney (HEK-239) cells. Moreover, all examined H3R antagonists, namely 1-16, exhibited affinities at the hH3R in a nanomolar concentration range (K_i: 36-137 nM, Table 2). All the compounds, except for 9, 11, and 13, were comparable with the standard H3R PIT (K_i: 37- 83 nM), while 9,11, and 13 were less potent (K_i: 110-137 nM). Furthermore, as H1R also plays a crucial role in the anticonvulsant activity of the central histamine, selected potential H3R antagonists (4, 7, and 13) were examined in a binding assay at the human histamine H1R and revealed weak affinities ($915 \leq K_i \leq 1338$ nM), being 10-fold lower than at the H3R. In addition, because of the high degree of homology between H3R and H4R, selected potential H3R antagonists (4, 7, and 13) were examined in a binding assay at the human histamine H4R and showed no affinity for hH4R (K_i > 10,000 nM). The potential H3R antagonist 4 displayed high affinity to hH3R (K_i = 69.3 nM), weak affinity to hH1R (K_i = 1273.0 nM) and no affinity to hH4R (K_i > 10,000 nM).

In the present study, the anticonvulsant effect of all the H3R antagonists was screened applying different electrically- (MES) and chemically- (PTZ and STR) acute induced seizure models. All the previous models are preclinical models against GTCS with different mechanisms of action. The H3R antagonists, namely 1-16, varied in their protection level in the three acute seizure models utilized in this present study. In the MES-acute induced seizure model, the results demonstrated that H3R antagonist 4 (10 mg/kg, i.p.) showed the most promising protective effect against GTCS when compared with SAL-treated rats (Figure 15, Table 3). However, H3R antagonist 4

lower doses (2.5 and 5 mg/kg) and the higher dose (15 mg/kg) failed to enhance the (10 mg) H3R antagonist 4-provided protection (Figure 16, Table 3). Consequently, the obtained findings showed a dose-dependence relationship of the protection provided, beside the existence of a maximum effect for H3R antagonist 4 in the MES-induced seizure model reached with a dose of 10 mg/kg, i.p. The previous results are in line with earlier observations that indicated a dose-dependent effect of numerous H3R antagonists examined for their anticonvulsant potential in MES-induced seizure model[303,304,361,371,374]. Markedly, the anticonvulsant effect of H3R antagonist 4 (10 mg/kg, i.p.) was comparable to that noted in rats treated with the reference antiepileptic drug PHT (10 mg/kg, i.p.), and was significantly higher than that noticed for H3R antagonists 3, 5, 6, 7, and 14 (Figure 15, Table 3). PHT is a traditional antiepileptic drug that provided a high protective effect against GTCS induced by MES[401-404]. Additionally, another test in this study revealed that the protective effect of H3R antagonist 4 was abrogated when rats were co-injected with the CNS penetrated H3R agonist RAM and H1R antagonist PYR (10 mg/kg, i.p.) (Figure 17). Notably, RAM and PYR co-injection alone with SAL failed to modify THLE time in the MES test. Suggesting that the protection provided effect by H3R antagonist 4 in the MES-induced seizure model involves, to some extent, to blockade of H3R and interaction with postsynaptically H1R by the released histamine. H3Rs are auto-receptors placed on histaminergic neurons presynaptically, with an inhibitory influence on the biosynthesis and release of histamine[405]. Thus, inhibition of H3Rs by selective H3R antagonists, the same as test compound H3R antagonist 4, would increase the neuronal release of brain histamine, supplying the anticonvulsant protective effect in the MES-induced seizure model. The previous implied mechanism underlying the anticonvulsant effect of H3R antagonist 4 is also in agreement with former preclinical

observations in animal seizure models in which high doses of several centrally acting H1R antagonists used as anti-allergic drugs promoted the development of convulsions of tested animals, indicating the involvement of H1R antagonism, and, consequently, brain histaminergic neurotransmission in the seizure promotion. Moreover, H1 receptor and HDC deficient animals were more susceptible to have convulsions[162,369]. Remarkably, comparable protective effects of imidazole-based (e.g., clobenpropit) and non-imidazole-based H3R antagonists (e.g., DL77 and E159) were previously shown to be abrogated either by H3R agonists or by centrally acting H1R antagonists, signifying an interaction of the H3R antagonism-released histamine with postsynaptically located H1Rs on neurons[289,296,305,361].

In PTZ-induced seizure model, acute pretreatment with H3R antagonists 4, 7, and 11 (10 mg/kg) and VPA (300 mg/kg) 30-45 minutes before PTZ injection exhibited full protection (Figure 18). Moreover, H3R antagonist 4 (15 mg/kg) also showed full protection, whereas lower doses showed a lower protective effect when compared with (10 mg)-protective effect (Figure 19), indicating a dose-dependent effect in PTZ-induced seizure model, which is in line with previous preclinical studies[2,7,13,16,58,65]. Notably, the protective effect provided by H3R antagonist 4 in the PTZ-induced seizure model was comparable to the standard antiepileptic drug VPA (Figure 18). VPA is a traditional antiepileptic drug that provided a high protective effect against GTCS induced by PTZ and STR[289,305,404,406,407], and the dose of VPA (300 mg/kg) used in this study is the minimal dose of VPA that protected animals against PTZ- and STR-induced seizures without mortality. However, the protective effect provided by H3R antagonist 4 was not abrogated with co-injection with RAM, PYR, or ZOL (10 mg/kg) before PTZ injection (Figure 20), suggesting that the protective effect provided by H3R antagonist 4 in the PTZ- induced seizure model is not facilitated through

modulation of histaminergic neurotransmission. The previously obtained outcome can be explained by the difference in the mechanism of seizure induction, triggers, and type of seizures each model represents (MES is considered as a model of GTCS, while PTZ [60 mg/kg) is believed to provoke generalized myoclonic and /or clonic-tonic seizures)[292,408-410]. The failure of RAM to abrogate the protective effect provided by H3R antagonist 4 in the PTZ-induced seizure model, can be explained with H3R antagonist 4 ability to diminish the excitation of glutaminergic or increase the inhibition of GABAergic synaptic transmission via blockade of H3 hetero-receptors function in CNS, thereby demanding further analysis if H3R antagonist 4 modulates the release of GABA or GLU brain concentration.

In the STR- induced seizure model, H3R antagonist 4 failed to exhibit any protective effect against GTCS induced by STR (3.5 mg/kg, i.p.) acute administration (Figure 21), which suggested a lack of effect of the test compound H3R antagonist 4 on the glycine receptors. However, with high antagonist affinity and high *in vitro* selectivity and without any appreciable protective effect against MES- and PTZ-induced seizure models, H3R antagonist 13 exhibited a high protective effect against STR-induced seizure models, indicating that H3R antagonist 13 is protective against convulsion induced through glycine receptors. The failure of H3R antagonist 4 to provide a protective effect in the STR-induced seizure model, despite its anticonvulsant effect in both MES- and PTZ-induced seizure models, can be explained by the variation in mechanisms of action and type of seizures in each model. MES and PTZ are believed to be of the most widely used acute seizure models because seizure initiation is simple, and the predictive value for discovering clinically active anticonvulsant compounds is high. MES is a model for inducing GTCS, and it has frequently been stated that antiepileptic drugs that block MES-induced tonic-clonic extension act via inhibiting

seizure spread[411]. MES- induced seizures are elicited by stimulation of all neuronal pathways in the brain stream, and the abuse only lasts for a short time. Additionally, it was previously indicated that AEDs that prevent MES-induced tonic extension could elicit its action either by compounds that block voltage-dependent Na+ channels, such as PHT and lamotrigine, or by compounds that block glutamatergic excitation mediated by the (NMDA) receptor, such as felbamate[375]. Moreover, the PTZ-induced seizure model represents a model for inhibiting human generalized myoclonic seizures and /or absence seizures[410]. Furthermore, the systemic administration of PTZ is commonly used to induce different types of seizures, depending on the dose applied[412]. Accordingly, low doses of PTZ (20-30 mg/kg) were found to be capable of inducing absence like seizures, medium doses (40-50 mg/kg) resulted in clonic seizures, while clonic-tonic seizures appeared only with the administration of high doses of PTZ (50-60 mg/kg)[413].Electrophysiological studies indicated that PTZ-induced seizures were elicited by decreasing the GABAergic neurotransmission through inhibition of benzodiazepine binding site in $GABA_A$ receptor complex[206,207]. MacDonald and Barker reported that the acute effect of PTZ on cultured mammalian neurons was mediated through blocking GABA-mediated transmission[414]. It was also revealed that AEDs that prevented PTZ-induced generalized seizures could elicit its action either by the modulation of GABAergic neurotransmission or by the reduction of T-type Ca^{2+} currents, such as ETX. Moreover, STR-induced seizures are provoked by the inhibition of glycine-operated chloride ion channels in the brain stem and spinal cord, and STR-induced seizure test is a model of GTCS[415].

Remarkably, a high number of marketed AEDs were not protective in all acute-induced seizure models. For example, PHT and CBZ are protective against THLE in the MES-induced seizure model, but they exhibit no protective effect against GTCS

induced by PTZ or STR. Oppositely, ETX, and tiagabine, which exhibit high protection in chemically-induced seizure models, lack the effectiveness in the MES-induced seizure model. Interestingly, VPA provided a protective effect in both MES- and PTZ-induced seizure models[416,417]. These preclinical indications justify the provided effectiveness of PHT and CBZ, but not ETX and tiagabine, in patients with GTCS, and the opposite in patients with absence seizures, and the protective effect of VPA in patients with both GTCS and absence seizures.

From the previous observations, it was suggested that the compound H3R antagonist 4, namely 1-(6-(naphthalen-2-yloxy)hexyl)azepane hydrogen oxalate, with high antagonist affinity (K_i=69.40 nM) and high *in vitro* selectivity, is promising to be effective against GTCS and absence seizures through the modulation of the histaminergic neurotransmission and consequently of the glutaminergic and/or GABAergic neurotransmissions, since different neurotransmitters, including GABA or GLU, may be implicated in the anticonvulsant provided effect. It can also be postulated that H3R antagonist 4 possibly also blocks histamine H3 hetero-receptors that control the release of other neurotransmitters, including GABA and GLU[224,418]; as previously noted, thioperamide increased the release of HA and GABA from rat hypothalamus[419].

All earlier observations revealed that acute systemic pretreatment with H3R antagonist 4 in electrically- and chemically-induced seizure models exhibited its anticonvulsant effect through modulation of the histaminergic neurotransmission and, at least partially, the post-synaptic H1Rs activation. Besides, mechanisms dependent on GABAenergic and glutaminergic neurotransmission might be involved.

Cognitive impairment accompanied some types of epilepsy at the onset and got worse, particularly with chronic uncontrolled seziures[63]. Memory deficit was also listed as one of the main adverse effects of AEDs[420], as around 70% of epilepsy patients respond well to the mono-therapy treatment. However, the rest require polytherapy, which will further augment the adverse effects[421]. Therefore, more pharmacological effective compounds that control seizures and attenuate memory deficits are required. Remarkably, H3R antagonists/inverse agonists have shown a prospective procognitive effect when compared to other AEDs, as indicated in earlier studies[305,420]. Also, short-term and long-term memory defects associated with DIZ or SCO were attenuated by the use of various H3R antagonists belonging to different chemical classes[305,379,422,423]. PIT has been approved in clinics for increasing wakefulness and fast rhythms, which are features of cognition[282,424]. Furthermore, PIT and numerous H3R antagonists are presently under clinical investigations for their effectiveness in cognitive disorders treatment[425,426]. Besides, the anticonvulsant provided effect of H3R antagonist 4 in both acute MES- and PTZ-induced seizure models was exhibited through modulation of histaminergic neurotransmission and probable involvement of GABAenergic and glutaminergic neurotransmission. The question here is whether this impact on histaminergic neurotransmission can influence DIZ- or SCO-induced memory deficits in male Wistar rats.

The procognitive effect of H3R antagonist 4 was investigated in this study applying IAP in DIZ and SCO-induced amnesic effects in adult male Wistar rats. IAP test is a fear-motivated test mainly used to evaluate the effect of chemical compounds on different types of learning and memory. The obtained results indicated that DIZ (0.1 mg/kg) and SCO (1 mg/kg) acute treatment, 30 minutes before the test, induced memory deficits in IAP with DIZ inducing higher memory impairment effect (Figure

23). The observations are in line with previous studies, which indicate the amnesic effect of both DIZ and SCO in several behavioral tests[123,377,427,428].

DIZ is a noncompetitive NMDA receptor antagonist, and the considerable contribution of NMDA receptors have been proved in the retrieval memory process; also, NMDA receptors were previously indicated to regulate long-term potentiation LTP and memory process including long-term memory[379,429,430]. Similarly, the vital role of GLU has been documented in learning, memory, and neurodegenerative diseases[431]. Numerous studies have also documented the involvement of SCO, a nonselective muscarine antagonist, in retrieval memory process[380,432,433]. Additionally, previous publications had confirmed the essential role of cholinergic neurotransmission in different types of cognitive functions. Likewise, the rise in ACh brain concentration is positively related to high performance during learning[434,435]. Pretreatment with H3R antagonist 4 (10 mg/kg, i.p.), 30-45 minutes before DIZ or SCO injections, mitigated both DIZ- and SCO-induced memory impairment with the higher procognitive effect observed in DIZ-induced amnesic effect (Figure 23). The latter outcome is in line with an earlier study in which clobenpropit attenuated SCO-induced memory impairment applying IAP, and the ameliorative effect was reversed when rodents were co-injected with RAM and PYR[436]. Based on the previous results, it is feasible that H3R antagonist 4 mitigated the memory impairment induced by DIZ or SCO, as a result of antagonizing the H3 auto-receptors resulting in brain histamine release, which is believed to react with and stimulate NMDA and muscarine receptors, respectively. The last assumptions were reinforced by recent publications, which showed that histamine improved the neurotransmission facilitated by NMDA receptors in cultured hippocampal cells and indicated that brain histamine and NMDA receptor collaboration could aid in attenuating the memory impairment induced by DIZ in

IAP[437]. Moreover, i.c.v. injection of histamine and acetylcholine attenuated SCO-induced memory impairment in male Wistar rats[438]. Taken together, it was proposed that other neurotransmitters (e.g., ACh and GLU) released by H3 hetero-receptors might be involved in the provided procognitive effect of H3R antagonist 4. Since H3R antagonist 4 provided a higher procognitive effect in DIZ- rather than SCO induced memory deficits model, the DIZ- induced amnesic model was selected to apply in both IAP and NOR tests.

Furthermore, H3R antagonist 4 (1.25, 2.5, and 5 mg/kg) significantly and dose-dependently attenuated DIZ-induced memory impairment in male Wistar rats. It was observed that H3R antagonist 4 (5 and 10 mg/kg) significantly prolonged STL time when compared with the lower doses (1.25 and 2.5 mg/kg), and there was no significant observed difference in STL time with increasing the dose from 5 to 10 (mg/kg) (Figure 24). H3R antagonist 4 (5 mg/kg, i.p.) was considered the most optimal dose to exhibit a memory-enhancing effect in DIZ-induced amnesic effect applying IAP. The procognitive and dose-dependent effect of various H3R antagonists (e.g., DL77, UW-MD-71, E159, UW-MD-72, GSK189254, and thioperamide) in DIZ-induced memory impairment was previously observed applying IAP[305,307,322,379-381,439]. Remarkably, the memory-enhancing effects of H3R antagonist 4 (5 and 10 mg/kg) were similar to the procognitive effect accomplished with the standard drug DOZ (Figure 24).

Interestingly, H3R antagonist 4 (5 mg/kg) provided procognitive effect in IAP was abolished when rats were co-injected with RAM and ZOL but not with PYR (Figure 25), implying that the procognitive provided effect was facilitated through an alternation of the central histaminergic neurotransmission to some degree with the

interaction between the released histamine and the postsynaptically located H2Rs but not H1Rs. The latter observations for abrogation effects also concurred with those stated for numerous H3R antagonists in previous preclinical studies, as RAM and ZOL co-injection reversed the procognitive effect of DL77 and UW-MD-72, while only co-injection with RAM abolished the protective effect of thioperamide and clobenpropit in IAP[305,315,380]. Notably, acute systemic co-injection of RAM or ZOL with DIZ (0.1 mg) or SAL in IAP failed to significantly alter the STL times observed in each group, indicating that RAM and ZOL have no memory modulating effect when administered alone.

Previously obtained memory-enhancing effects for H3R antagonist 4 in DIZ-induced memory impairment further support that histaminergic pathway through activation of H2Rs is profoundly involved in neuronal pathways essential for modification of retrieval processes.

NOR test is a standard test in rodent to evaluate both STM and LTM recognition memory[440]. Interestingly, NOR test depends on the natural behavioral of the rodent, as there is no incentive or penalty for the animals in NOR test[441,442]. The test evaluates the animal's natural tendency to explore their atmosphere as it is reliant on the animal's natural behavior[440]. The observed results indicated that DIZ treatment following T1 significantly altered the rodent's behavior toward the novel objects in T2, indicating both STM and LTM recognition impairment (Figure 26). The indicated findings were in line with previous studies demonstrating DIZ amnesic effect in NOR test[443]. In this present study, H3R antagonist 4 (5 and 10 mg/kg) significantly attenuated DIZ-induced STM memory deficits in NOR. However, H3R antagonist 4 (2.5 mg/kg) failed to exhibit any protective effect (Figure 26). Notably, no remarkable difference was

observed between the procognitive effects provided by H3R antagonist 4 (5 and 10 mg/kg); also, the observed procognitive effect was comparable to the standard drug DOZ. Then H3R antagonist 4 (5 mg/kg, i.p.) was considered the most optimal dose to exhibit a memory-enhancing effect in DIZ- induced amnesic effect applying NOR. Additionally, the procognitive effect obtained with H3R antagonist 4 was previously documented with several imidazole- based H3R antagonists, e.g., thioperamide and clobenpropit[315] and non-imidazole based H3R antagonists, e.g., E159, pitolisant, ABT-239, GSK189254, and SAR110894 in NOR test. In addition, E159, pitolisant, and ABT-239 exhibited a dose-dependency effect in ameliorating DIZ- induced memory impairments[307,312,320,444,445]. Furthermore, the protective effect of H3R antagonist 4 was abrogated with systemic co-injection with RAM (10 mg/kg), indicating the involvement of the histaminergic system in the procognitive effect provided by the test compound H3R antagonist 4 (Figure 26). It was documented earlier, that RAM co-injection had reversed the protective effect provided by imidazole-based H3R antagonists thioperamide, clobenpropit[315], and ciproxifan[446] as well as the non-imidazole-based H3R antagonist E159[443] in the NOR test. Notably, acute systemic co-injection of RAM with DIZ (0.1 mg) or SAL in NOR failed to significantly alter the time spent exploring the novel object observed in each group, indicating that RAM has no memory modulating effect when administered alone.

In contrast, H3R antagonist 4 systemic administration post-training T1 and 30-45 minutes before T2 (2.5, 5, and 10 mg/kg) failed to exhibit any procognitive effect against DIZ-induced LTM impairment (Figure 27). The earlier result agreed with a former publication in which the non-imidazole based H3R antagonist E159 failed to attenuate DIZ-induced LTM impairment applying the NOR task in rodents[322]. All previously observed results in NOR noticeably designate that histaminergic H3Rs are

deeply participating in neuronal circuits involved in the H3R antagonist 4-provided STM-memory improving effects, but not in LTM-memory ameliorative effects. It is essential to mention that cholinergic and glutaminergic neurotransmission is possibly involved in both IAP and NOR response since both paradigms are impaired by a choline receptor and NMDA receptor antagonists. DOZ, an acetylcholine esterase inhibitor, was selected as a standard drug in both IAP and NOR since DOZ is clinically utilized for memory-enhancing effects in Alzheimer's and dementia patients[447-449]. Numerous previous publications also documented the ameliorative effect of DOZ on the memory impairment induced by DIZ in different behavioral tests[443,445,450-452]. Furthermore, the inconsistencies detected for H3R antagonist 4 in PAP and NOR could be justified with the variation of performs and evaluated elements of both models in rodents. Since NOR measures the natural behavior of animals and benefits from their unique interest in discovering their surroundings, and it does not involve a penalty or compensation, whereas IAP is a fear motivated test which measures retrieval LTM in rodents, and it includes punishment.

The EPM and OFT tests are a necessity for memory tests that require normal rodent movement and regular anxiety levels. Any modulation of the tested compounds on rodent's anxiety levels or locomotion activity might alter or confound their activity in the memory experiment. The EPM test relies on the innate propensity of rodents to favor closed areas across open areas. Acute systemic administration of H3R antagonist 4 with the most procognitive provided effect doses (5 and 10 mg/kg) in IAP and NOR 30-45 minutes before EPM test failed to exhibit any alternation on anxiety parameters, namely, the total time spent in open arms (Figure 28A) and the total number of entries into open arms (Figure 28B). Also, H3R antagonist 4 (5 and 10 mg/kg) failed to display any change in locomotion activity expressed as the total number of entries into closed

arms (Figure 28C). The former results are in agreement with earlier studies, which indicate the failure of several imidazole-based H3R antagonists (e.g., thioperamide and clobenpropit) and non-imidazole-based H3R antagonist (e.g., DL77 and E159) to alter locomotion or anxiety levels in rodents applying EPM[322,453,454]. However, DZP (10 mg/kg) exhibited anxiolytic effect when administered 30-45 minutes before EPM by modifying both the total time spent in open arms and the total number of entries into open arms, but it failed to alter the locomotor activity of the rats (Figure 28). DZP is a benzodiazepine derivate, widely used for the management of human anxiety disorders, and the earlier observation agreed with former publications, which indicated the anxiolytic effect of DZP applying EPM in different rodent species[305,455-457].

Moreover, H3R antagonist 4 at the dose (5 and 10 mg/kg) that showed the most promising procognitive effect in PAP and NOR did not modulate the spontaneous locomotion parameters in OFT; namely, the total distance traveled, the time spent in the central arena as well as in the periphery, and the number of rearing and grooming events during a 3-minute test in the OFT (Table 6). The former results are consistent with previous studies in which imidazole-based H3R antagonists like thioperamide and non-imidazole-based H3R antagonists, e.g., DL77, E159, and A-349821, failed to modify the spontaneous locomotion activity of the tested rodent[305,322,354,381,458]. Moreover, DOZ (1 mg/kg) failed to alter the spontaneous locomotion activity in OFT in male Wistar rats, which was indicated in numerous previous studies as DOZ failed to alter locomotion activity in C57BL/6J mice[450,459] and Wistar rats[305,322]. The total number of grooming is considered to signify the ability of tested animals in making decisions correlated to anxiety-like behaviors[460]. The former observations indicated that H3R antagonist 4 failed to modify anxiety levels or locomotion activity, implying that the procognitive effect provided by H3R antagonist 4 was not correlated to

influence emotional responses (anxiety) or modified spontaneous locomotion activity which counted as confounding features when assessing memory-enhancing effects in PAP and NOR.

Taken together, all the above-mentioned findings demonstrated that the non-imidazole H3R antagonist 4 procognitive provided effects were mediated through histaminergic neurotransmission and – at least partially – H2Rs activation.

Seizures happen due to irregular hypersynchronous uncontrollable brain emissions from neurons, which ultimately result in permanent damage to the neurons and their surroundings. There has been a massive development in epilepsy treatment in the last decades. Though most of the antiepileptic compounds are focused on preventing seizures, they are still unable to prevent the associated comorbidities (e.g., memory impairment). That is why an ideal antiepileptic agent should be able to prevent both the seizures and the associated memory impairments. Based on the previous observed anticonvulsant effect in MES- and PTZ-acute induced seizure models and the procognitive effect of the test compound H3R antagonist 4 applying IAP and NOR behavioral tests, this part of the current study was designed to investigate the ameliorative effect of H3R antagonist 4 on the simultaneous memory impairment associated with PTZ-acute induced seizure and PTZ-kindling models in male Wistar rats. In addition, to explore the probable mechanisms mediating the protective effect of H3R antagonist 4, oxidative stress markers, AChE, neurotransmitters concentration, and c-fos expression were examined.

Even though the temporary seizure lasts typically a minute or less in acute models, the electrographic convulsive activity may stay beyond the behavioral seizures and will consequently influence learning and memory. Here, in this part of the study the effect

of brief seizures lasting 60–70 seconds was investigated by administering a single convulsive dose of (PTZ, 60 mg/kg, i.p.).

The proconvulsant effect of PTZ (60 mg/kg) acute administration and the full protective effect of H3R antagonist 4 (10 mg/kg) in PTZ-acute induced seizure model were reported earlier.

Memory assessment was conducted applying IAP and EPM 24 h following PTZ-induced seizures. IAP is one of the most widely used behavioral tests to investigate memory impairments associated with PTZ acute as well as chronic seizures. On the other hand, the EPM test is usually used to assess anxiety-related behaviors in rodents[461]. However, based on the way of experimental conduct, EPM was shown to provide convincing experimental face validity for the assessment of spatial memory impairments associated with PTZ seizures in rodents[462,463,133,464,131,49]. The observed results of the memory assessment in IAP indicated that PTZ (60 mg/kg) acute administration led to a significant decrease of STL time, which indicates memory impairment in tested;2 rats (Figure 29). The showed result is consistent with earlier documented studies in which seizures associated with PTZ (60 mg/kg) administration resulted in memory impairment applying IAP in male Wistar rats[69,133,465]. Moreover, acute pretreatment with H3R antagonist 4 (5 and 10 mg/kg) mitigated the memory impairment associated with PTZ-acute induced seizures, and no significant difference was observed between the two doses (Figure 29). Furthermore, PTZ (60 mg/kg) administration resulted in spatial memory impairment applying EPM in male Wistar rats (Figure 30). Spatial memory impairment evoked by PTZ acute injection in EPM was previously verified[49,131,386,466]. Furthermore, H3R antagonist 4 (5 and 10 mg/kg) mitigated the memory impairment associated with PTZ-induced seizures, and no

remarkable difference was detected between the two doses (Figure 30). The procognitive effect of H3R antagonists was not documented before in PTZ-acute seizure model. However, the ameliorative effect of thioperamide acute pretreatment on memory impairment associated with SCO- induced learning deficits applying EPM in mice was previously detected[316]. VPA was used as a positive control drug because of its high antiepileptic effect and less harmful effect on memory impairment[375]. VPA (300 mg/kg) acute pretreatment significantly attenuated the memory impairment associated with PTZ-induced seizure model in both IAP and EPM (Figures 29 & 30). The memory-enhancing effect of VPA was previously noted following PTZ-induced seziures[133,386]; also, VPA attenuated memory impairment in a transgenic mouse model of Alzheimer's disease[467]. The procognitive effect of the promising compound H3R antagonist 4 can be confounded in the test day by alternations in locomotion or anxiety levels. However, similar STL and TLT times achieved by rats on the training day between the different groups on IAP and EPM, respectively, demonstrate that these nonspecific factors have been randomized between the groups.

Electrically and chemically acute seizure models allow only for rapid examination of the potential anticonvulsant effect of the respective drugs, whereas PTZ-induced acute model can only investigate the effect of brief seizures on the brain and the associated comorbidities. However, chronic animal models were established to identify the mechanisms underlying epileptogenesis and seizures production and to investigate the emotional, cognitive, and memory impairments associated with epilepsy as well as with the chronic use of several AEDs[468]. It is consensually agreed that the behavioral deficits and memory impairment associated with repetitive seizures increase with the severity and frequency of the convulsions[469]. PTZ-kindling model is an extensively accepted animal model of TLE where repetitive sub-convulsive doses of PTZ reduced

seizure threshold results in behavioral signs of convulsions imitating symptoms of human TLE. In the next part of the study, the antiepileptic and procognitive simultaneous effect of the potent non-imidazole based H3R antagonist 4 was investigated applying PTZ chemical kindling model.

The results indicated that rats chronically treated with PTZ (40 mg/kg, i.p.) three times a week for 12 injections showed decreased seizure threshold as indicated by increased average seizure severity score when compared to naïve animals, and gradually exhibited generalized tonic-clonic convulsions starting from 9th injection which eventually resulted in kindled rats: 83.33% of the rats chronically treated with PTZ were kindled, while 16.67% died (Figure 36). Moreover, the seizure score curve was linear, signifying that PTZ easily penetrated the brain tissue. Therefore, it was considered that repetitive sub-convulsive treatment of PTZ (40 mg/kg, i.p.) three times a week for 12 injections effectively induced a chronic epilepsy-like condition. The obtained results are in harmony with former studies in which PTZ was applied for kindling in male Wistar rats[470,471] and in different species of rodents[388,468,470,472,473]. At the beginning of kindling, an antiepileptic and adaptive system is contrary to neuronal hyperactivity by decreasing the inhibitory neuron activation threshold. Nevertheless, a chronic and repeated administration of the chemical convulsant compound beats these defense methods[210]. At the last stage of the kindling, an increase of the brain sensitivity to PTZ results in significant damage to the antiepileptic mechanisms, leading to chronic epileptization of the brain. Interruptions of balance between inhibitory and excitatory systems cause the progression in the seizure score in PTZ kindling model from 1st to 12th injections, which can begin from a partial numeral of neurons till neuronal hyperactivity becomes satisfactory to the disruption of the balance.

Chronic pretreatment with H3R antagonist 4 (5 and 10 mg/kg, i.p.) 30-45 minutes before each PTZ injection significantly prevented the kindling procedure and reduced the average seizure score in most of the injections (Figure 36). No animals were kindled or died when H3R antagonist 4 (5 and 10 mg/kg) was administered 30-45 minutes before each PTZ injection (Table 7). No significant difference was observed in the average seizure score in each injection, kindling rate, and mortality rate between H3R antagonist 5 and 10 mg/kg; so H3R antagonist 4 (5 mg/kg) was considered the optimal dose in PTZ-chronic model. The protective effect of H3R antagonist 4 (5 mg/kg) was reversed when rats were co-injected with RAM, which indicated that the H3R antagonist 4 provided protective effect was mediated through alternations of the histaminergic neurotransmission (Figure 36). Notably, RAM co-injection alone with PTZ or SAL failed to modify the kindling procedure or the average seizure score. Previous studies had confirmed the earlier result as i.c.v injections of histidine, clobenpropit, and carnosine (a precursor of histidine) before each PTZ injection had significantly and dose-dependably extended the latency to myoclonic jerks and generalized clonic seizures, and the protective effect provided was partially abrogated with co-injection with RAM[160,162,299,474]. Also, thioperamide chronic i.c.v. treatment significantly and dose-dependently delayed the onset of PTZ-kindling and reduced the seizure score in PTZ-chronic model[475]. All the previous observations emphasize the essential role of the histaminergic system in the antiepileptic provided protection against PTZ- kindled model. VPA anticonvulsant effect observed in this study was documented earlier in numerous publications in PTZ-kindling model[103,133,464].

The PTZ kindling procedure was associated with memory impairment in both IAP and EPM tests, as PTZ chronic treatment resulted in a significant decrease of STL time in IAP and significantly prolonged TLT in EPM (Figures 37 & 39). The retrieval memory

impairment accompanied by PTZ chronic treatment was formerly noted applying IAP[71,102,106,388,470,476-478]. Besides, numerous former studies have documented the spatial memory impairment caused by PTZ chronic treatment utilizing EPM[48,72,384,385,477,479]. Moreover, memory impairment was noted in post chronic PTZ treatment applying numerous behavioral tests (e.g., mirror water maze MWM)[318,480,481]. It was documented that memory impairment associated with the PTZ kindling model mimicked human epilepsy[482,483]. The assessment of the procognitive effect of the compound H3R antagonist 4 on the first day can be confounded by several causes, like alternations in locomotion or anxiety levels. However, similar STL and TLT times achieved by rats on the training day between the different groups on EPM demonstrate that these nonspecific factors have been randomized between the groups. These findings are in accordance with previous studies signifying behavioral alternations and memory impairment accompanied by PTZ-induced kindling model, which similarly mimics human epilepsy[484].

Chronic treatment with H3R antagonist 4 (5 and 10 mg/kg) significantly mitigated the memory impairment associated with PTZ- chronic administration (Figures 37 & 39). The concomitant antiepileptic and procognitive effects were indicated in previous studies in which thioperamide i.c.v injection resulted in simultaneous antiepileptic and memory-enhancing effect[475] in male Wistar rats applying MWM. Also, thioperamide and JNJ-5207852 attenuated memory deficits associated with PTZ-kindling model applying the IAP test in weanling mice[485]. No significant difference was observed in the memory-enhancing effect between H3R antagonist 4 (5 and 10 mg/kg). The procognitive effect of H3R antagonist 4 (5 mg/kg) was reversed when rats were co-injected with RAM in IAP and EPM tests, indicating that histaminergic neurotransmission was involved in the provided procognitive effect by the test

compound H3R antagonist 4 in PTZ- kindling model (Figures 38 & 40). Remarkably, rats injected with RAM and PTZ or SAL alone failed to modulate the memory function in each group. Based on the previous results, it is possible that H3R antagonist 4 prevented the kindling effect and mitigated the memory impairment induced by repetitive injections of PTZ, as a result of antagonizing the H3 auto-receptors resulting in brain histamine release, which is believed to react with and stimulate $GABA_A$ receptors. The last assumptions were reinforced by earlier publications, which showed that i.c.v. injection of histidine increased brain histamine concentration and exhibited a memory-enhancing effect in PTZ-kindling model[159,486]. Moreover, histamine administration was documented to potentiate GABA action on $GABA_A$ receptors[487]. VPA (300 mg/kg) chronic treatment also attenuated the memory deficits associated with PTZ-kindling model. The memory-enhancement effect associated with VPA was recently documented in preclinical studies, in which VPA treatment attenuated memory-impairment associated with PTZ-chronic model while PHT failed to provide any effect[48]. Moreover, VPA mitigated SE-induced spatial memory; however, VPA caused spatial memory impairment in normal rats[108].

Oxidative stress is a foundation for several neurological and neurodegenerative disorders, and it has been involved in the pathogenesis of epilepsy. Besides, it was suggested that oxidative stress demonstrates as a result of the initial convulsion, which can initiate the procedure of epileptogenesis and the consequent memory deficit due to brain damage[488]. Oxidative stress has been accompanied by neuronal hyperexcitation produced by different CNS diseases (e.g., epilepsy)[489]. Interestingly, it was observed that antioxidant compounds had mitigated memory impairment in different animal models[490,491]. Therefore, evaluating numerous oxidative stress markers (e.g., MDA, GSH, CAT, and SOD) of tested animals in both PTZ -acute and

-chronic models were required to assess the modifying effects of the prospective novel and centrally acting H3R antagonist 4. Furthermore, and amongst all the brain regions, the hippocampus has gained attention in the PTZ model as it contains several distinct neuronal circuits related to seizure genesis. Moreover, previous publications indicated that PTZ- induced acute seizures resulted in hippocampal morphological changes, which returned to normal one week after[492,493]. Also, it was documented that the hippocampus is involved in the epileptogenesis when tonic-clonic seizures appeared post repetitive sub-convulsive administration of PTZ[210].

In this study, PTZ acute injection led to elevated hippocampal levels of MDA and reduced levels of GSH (Figures 31A & B). The decrease levels of GSH indicated that during seizures, GSH levels were depleted fighting oxidative stress. Consequently, the observed results revealed an elevated MDA level associated with a reduction in GSH levels in the hippocampus 24 h post-PTZ-induced seizures, and these results were in line with prior studies performed on numerous rodents, which indicated oxidative stress in hippocampus and other brain regions[69,131,133]. On the other hand, natural antioxidant enzymes in the brain, e.g., CAT and SOD, are typically used to scavenge the elevated ROS and defend the hippocampus from convulsions. PTZ (60 mg/kg) acute treatment had no significant changes in both hippocampal CAT and SOD levels 24 h following PTZ injection (Figures 31C & D). The aforementioned finding was endorsed previously as SOD levels were noted to be not altered 24 h post-PTZ-induced acute seizures[494,495]. However, another study indicated a decreased level of SOD 15 minutes after PTZ injection[496], which can be explained by the time point difference of brain collection in earlier studies from this study. The obtained observations revealed no change in the hippocampal levels of CAT and SOD activities 24 h post seizures, implying that an enhanced metabolic demand can be showed during the epileptic

seizure, and CAT and SOD activities are not modified in this acute phase of PTZ-induced seizure. Also, the unaffected CAT and SOD activity may be justified by the simultaneous upregulation of CAT and SOD and the enzyme degradation caused by oxidative stress. Elevated oxidative stress by convulsion activity raises the incidence of oxidative brain damage, which might be the major reason of memory deterioration associated with by PTZ seziures[72]. As, it was indicated previously that treatment with antioxidant compounds had ameliorated the memory impairment by decreasing the MDA levels, in traumatic brain injury rats model and Alzheimer's disease[497,498,51]. Additionally, acute pretreatment with numerous antioxidant compounds had attenuated the memory impairment associated with PTZ- induced seizure model[131,133].

Pretreatment with H3R antagonist 4 (5 and 10 mg/kg) 30-45 minutes before PTZ injection had mitigated the hippocampal oxidative stress associated with PTZ-induced seizures (Figure 31). However, the observed antioxidant effect for H3R antagonist 4 was observed to be dose-independent as 5 and 10 mg/kg were statistically comparable in the providing protective effect. The previous observation indicated the potential antioxidant effect of H3R antagonist 4, which participates in the memory-enhancing effect observed with acute treatment. The potential antioxidant dose-independent effect of H3R antagonists was documented earlier as DL77 (10 or 15 mg/kg, i.p.) provided similar antioxidant effects against the cerebellum altered oxidative stress parameters in the VPA-induced autism-like behaviors in mice[454]. Also, it was previously documented that ciproxifan and clobenpropit treatment attenuated oxidative stress in experimental models of schizophrenia[499].

The results indicated that the VPA acute treatment had mitigated the hippocampal oxidative stress induced by PTZ seizures (Figure 31). Also, the antioxidant effect

provided by H3R antagonist 4 was comparable to VPA provided effect. The earlier findings were previously noted as VPA acute pretreatment provided antioxidant and memory-enhancing effect 24 h following PTZ-induced seizures[133,386]. Also, VPA treatment showed an antioxidant effect in children with idiopathic epilepsy[500].

The obtained results in PTZ-kindling model indicated that PTZ repetitive sub-convulsive injections led to elevated hippocampal levels of MDA and reduced levels of GSH, which was previously noted in former publications (Figures 41A & B)[72,384,392,477,501]. Moreover, PTZ repetitive sub-convulsive injections led to decreased hippocampal levels of both CAT and SOD enzymes (Figures 41C & D). The earlier observation was noted previously following PTZ chronic treatment, which indicated elevated hippocampal oxidative stress levels[96,383,502]. Elevated oxidative stress by convulsion activity raises the incidence of oxidative brain damage, which might be the major reason of memory deterioration associated with PTZ kindling[72]. As it was indicated previously that treatment with antioxidant compounds ameliorates the memory impairment by decreasing the oxidative stress levels, in traumatic brain injury rats model and Alzheimer's disease[497,498,51]. The previous results indicated the H3R antagonist 4 (5 and 10 mg/kg) chronic treatment significantly mitigated oxidative stress evoked by PTZ chronic treatment, and the effect was dose-independent (Figure 41).

VPA chronic treatment 30-45 minutes before each PTZ injection had attenuated the hippocampal oxidative stress levels associated with PTZ-repetitive injections (Figure 41). The latter outcome is in harmony with earlier studies in which the VPA antioxidant effect was documented in rodents[133,464,503]. Furthermore, VPA exhibited an antioxidant effect in epileptic children with high oxidative stress level[504,505].

According to all earlier observations, it was suggested that all consequences that occur during oxidative stress are accompanied by the rise in the glutamatergic response that promotes the neuronal hyperexcitability, which triggers epileptic seizures. Therefore, VPA, by positively altering the antioxidant enzymes it regulates hyperexcitability either directly or secondarily to the modulation of the glutamatergic response in resulting epileptic seizures control.

The significant role of the central cholinergic system in memory and cognitive functions is well established[433]. Learning and memory disabilities are associated with changes in the cholinergic system function[138], and it was observed that anticholinergic compounds induced memory deficit in different behavioral tests[506,507,508]. Moreover, various preclinical studies showed notably that H3R antagonists demonstrated an exceptional element by their potential memory-enhancing effect[225,425,509] and by functioning also as hetero-receptors, which can adjust the release of numerous other brain neurotransmitters, e.g., ACh, GABA, serotonin, and dopamine in diverse brain regions[510-512]. AChE is considered an important therapeutic target, as reversible inhibitors of AChE are being used as a memory enhancer in epileptic patients and other neurodegenerative diseases[148,513]. Therefore, assessing the AChE activity of tested animals in both PTZ acute and chronic models was needed to evaluate the modifying effects of the prospective novel and centrally acting H3R antagonist 4 on the cholinergic system.

The observed results indicated that acute PTZ-induced seizures failed to modify hippocampal AChE activity (Figure 32). Nevertheless, previous studies indicated a significant decrease of the brain AChE 24 h after PTZ seizures[131,133], which can be explained by the different brain regions tested. Hippocampus is tested in the current

study while the cerebral hemisphere was tested in the previous studies, suggesting that hippocampal AChE activity was not sensitive to acute PTZ-induced seizure model. However, repetitive seizures induced by chronic PTZ administration resulted in significantly increased of hippocampal AChE activity (Figure 42). Numerous previous studies have endorsed the above finding in which PTZ kindling led to high levels of AChE activity in different brain regions[103,147,383,463]. In accordance with former indications, AChE is a well-recognized enzyme involved in memory impairment in experimental animal models, as inhibition of AChE will cause a hippocampal ACh release that mediates a memory improvement[514]. The AChE overexpression was noted to be associated with memory deterioration[515], and it was postulated that high AChE activity could be explained as compensatory to cholinergic transmission modulation induced by PTZ-chronic treatment[516]. The previous results indicated that H3R antagonist 4 (5 and 10 mg/kg) restored hippocampal AChE activity to normal, and no significant difference was noted in the modulating effect between the two doses (Figure 42). It was also previously noted that AChE inhibitors could be used as memory-enhancer compounds in epileptic patients[517]. Moreover, it was documented that normalizing the abnormal brain of AChE activity in kindled rodents by numerous compounds was associated with memory-enhancing effect in PTZ- kindling model[103,147,383,463]. H3R antagonist 4 was not documented in its *in vitro* profile to have AChE inhibitory effect[368]. However, the modulatory effect of H3R antagonist 4 on AChE activity may be due to the anticonvulsant provided effect, which resulted in an inhibition of the AChE overexpression or a prevention of the notable alternation of the cholinergic transmission. Remarkably, the modulatory provided effect by H3R antagonist 4 (5 mg/kg) was reversed with RAM co-injection, suggesting that the modulatory effect of H3R antagonist 4 on hippocampal AChE activity is related to the

anticonvulsant provided effect facilitated through the released histamine. Notably, RAM injection with PTZ alone failed to modify the hippocampal AChE activity in the PTZ group. But chronic treatment with VPA (300 mg/kg) 30-45 minutes before each PTZ injection reduced the hippocampal elevated levels of AChE activity (Figure 42). This result was previously noted, in which VPA treatment reduced the aberrant AChE activity in different brain regions in PTZ-kindling model, and the modulated effect was accompanied by mitigation of the associated memory impairment[48,147,133]. The modulated effect of VPA on hippocampal AChE activity could be responsible for the provided memory-enhancing effect. The previous finding is contrary to the elevated hippocampal AChE activity observed with PHT treatment associated with memory impairment in PTZ-kindling model[48].

The above-mentioned results had proved that the anticonvulsant and memory-enhancing protective effects of H3R antagonist 4 were related to brain histamine increased synthesis and release from histaminergic nerve terminals. On the other hand, H3 receptors are also recognized as hetero-receptors plus auto-receptors in both the central and peripheral nervous systems, controlling the release of numerous other neurotransmitters. According to all previous observations on the postulated mechanism of action of the test compound H3R antagonist 4 and the suggested involvement of several neurotransmitters (e.g., HA, Ach, and GABA) in the provided anticonvulsant and procognitive effect and for superior comprehension of the connection between epilepsy psychophysiology and the associated memory deficits, evaluating various hippocampal neurotransmitters concentration (e.g., HA, ACh, GABA, and GLU) of tested animals in both PTZ-acute and -chronic models was required to assess the modifying effects of the prospective novel and centrally acting

H3R antagonist 4. Neurotransmitters perform an essential role in controlling neuronal inhibition and excitation and providing natural behavior of memory function[518].

The earlier results indicated that chronic repetitive systemic administration of PTZ (40 mg/kg) 3 times a week for 12 injections resulted in a significant decrease of hippocampal HA concentration 48 h after the last PTZ injection (Figure 43A). However, acute PTZ (60 mg/kg) injection failed to alter hippocampal HA concentration 24 h after injection (Figure 33A). The observed results were consistent with previous studies in which hippocampal HA levels were decreased in fully kindled rats[159,486]. However, hippocampal HA concentration was not changed 10 days after the last PTZ injection in PTZ-kindling model, which can be explained by the time difference of brain collection[519], suggesting that HA brain levels may be normalized 10 days after last PTZ injection. Furthermore, it was earlier noted that PTZ acute treatment decreased hippocampal HA levels 30 minutes after PTZ injection[474], while in this study, HA levels were not changed 24 h after PTZ acute injection[520]. This indicates that HA levels will go back to normal 24 h after PTZ injection, and this implication was endorsed previously as HA levels were normal 8 h after PTZ acute injection[520]. The anticonvulsant effect of the endogenous histamine was recently confirmed, and the function of brain histamine in controlling seizure susceptibility had been recognized[157,158,291,520]. Moreover, the vital role of the histaminergic neurons in the convulsions pathogenesis was noted[521,522]. Likewise, the essential role of brain histamine in learning and memory was recognized[523], as i.c.v. injections of histidine had improved the spatial memory impairment associated with PTZ-kindling model[486]. Furthermore, the increase in HA release attenuated the memory impairment associated with spontaneously hypertensive rat (SHR), an appropriate genetic model for ADHD. Chronic treatment with H3R antagonist 4 (5 and 10 mg/kg) 30-45 minutes before each

PTZ injection significantly restored the depleted hippocampal HA concentration, and the effect was dose-independent (Figure 43A). The previous results are in agreement with the proposed mechanism of action of the centrally acting H3R antagonist 4, which antagonized H3 auto-receptors and enhanced brain HA synthesis and release[298,405]. Previous *in vitro* studies indicated that thioperamide, ciproxifan, clobenpropit, A-304121, and A-317920 application resulted in increased HA release in cortical brain slices[524]. Besides, former *in vivo* experimental preclinical studies indicated the influence of several H3R antagonists in increasing brain HA release (e.g., ciproxifan increased cortical HA release in the SHR model[525]) and HA release in the anterior hypothalamic in urethane-anesthetized rats[526]. Also, GT-2016 increased cortical HA release in freely moving rats[527], while thioperamide increased HA release in the hypothalamus in rats[528].. However, the provided enhancement effect of hippocampal HA release by the H3R antagonist 4 (5 mg/kg) was partially abrogated when rats were co-injected with RAM, confirming that the protective effect provided by H3R antagonist 4 is facilitated through the histaminergic system (Figure 43A). As observed earlier, RAM administration had blocked potassium-induced HA release in cortical brain slices[405]. In addition, RAM administration abrogated both A-304121 and A-317920 enhanced brain HA release effect in cortical brain slices[524].

The results indicated that chronic repetitive systemic administration of PTZ (40 mg/kg) 3 times a week for 12 injections resulted in a significant decrease of hippocampal ACh concentration 48 h after the last PTZ injection (Figure 43B). However, acute PTZ (60 mg/kg) injection failed to alter ACh concentration in the hippocampus 24 h after injection (Figure 33C). ACh plays a vital role in memory, attention, and learning[529], and it was implicated in kindling procedure, as repetitive administration of muscarinic antagonists had decelerated the kindling development[530].

In addition, ACh has been involved in regulating brain GLU release throughout the epileptic action[531]. The indicated results were in line with previous studies which documented that PTZ-kindling model led to decreased concentration of hippocampal ACh in male Wistar rats[141,142,485,532] and zebrafish model[128,533]. In addition, it was noted that hippocampal ACh levels would return to normal 30 days following the last PTZ injection[532]. Nevertheless, other preclinical studies were contrary to the obtained findings; in these studies, elevated ACh levels were noted 60 minutes after PTZ acute administration[130]. However, ACh levels in the hippocampus went back to normal 90 minutes after PTZ acute injection, which can explain the results of unchanged hippocampus ACh levels 24 h after PTZ acute injection. It was postulated that low ACh brain concentration might facilitate the kindling progression[532], and the reduction of hippocampal ACh levels after PTZ chronic systemic administration can be related to cholinergic neurons cell loss induced by chronic repetitive administration of PTZ. This suggestion is further supported by a previous observation in which progressive hippocampal neuronal cell loss was noted following PTZ-kindling model[214,482]. In addition, low brain ACh concentration was associated with memory impairment in PTZ-kindling model[533], and it was documented that donepezil (AChE inhibitor) treatment attenuated the memory impairment induced by PTZ-kindling model[485]. The results indicated that PTZ-chronic model was associated with a reduction in hippocampal cholinergic function, while no change was observed in PTZ-acute model. All aforementioned findings endorsed the contribution of the cholinergic system to both seizure induction and memory function.

Chronic treatment with H3R antagonist 4 (5 and 10 mg/kg) significantly restored the depleted hippocampal ACh concentration. This indicated the modulating effect of H3R antagonists 4 on cholinergic transmission function, and no remarkable difference was

noted between the two doses (Figure 43B). The modulating effect of H3R antagonists on brain ACh levels was previously noted[523]. Initial *in vitro* potassium-stimulated tritium experiments proved the regulatory effect of H3 receptors, where thioperamide increased ACh transmission from entorhinal cortex slices[534]. Then several *in vivo* tests demonstrated the modulatory effect of numerous H3R antagonists on ACh neurotransmission. JNJ-10181457 chronic administration significantly normalized brain ACh levels in translational rat models of cognition[535]. Moreover, ABT-239, GSK189254, and thioperamide increased hippocampal ACh release in Wistar rats[307-309], while BF2.649 enhanced ACh release from prefrontal cortex in Wistar rats[320], and ciproxifan increased hippocampal ACh in freely moving rats[536]. The provided modulating effect of the hippocampal ACh levels by H3R antagonist 4 (5 mg/kg) was abrogated with RAM co-injection (figure 43B). This result is consistent with previous documented observations in which RAM systemic administration prevented potassium-induced cortical ACh release in rats[317] and decreased electrically induced hippocampal ACh release[309]. Likewise, RAM also reversed thioperamide enhanced hippocampal ACh release in freely moving rats[537]. Notably, RAM injection with PTZ alone failed to modify hippocampal ACh levels when compared with PTZ treated rats. The previous results are in agreement with the proposed mechanism of action of the centrally acting H3R antagonist 4, which is antagonizing H3 auto- and hetero-receptors and enhanced brain HA and ACh release[298,405].

The results indicated that chronic repetitive systemic administration of PTZ (40 mg/kg) 3 times a week for 12 injections resulted in a significant increase of hippocampal GLU concentration 48 h after last PTZ injection (Figure 43D). In contrast, acute PTZ (60 mg/kg) injection failed to alter GLU concentration 24 h after injection in the hippocampus (Figure 33D). On the other hand, hippocampal GABA

levels were not changed either 48 h after the last PTZ chronic injection nor 24 h after PTZ acute injection (Figures 43C & 33B). It was indicated that kindling-induced epileptogenesis is a consequence of an imbalance between inhibitory and excitatory activities representing an enhanced glutaminergic or decline in GABAergic transmission in both humans and animals. Also, most of the AEDs working through GABAergic mechanisms effectively control seizures, they were unsuccessful in attenuating the accompanied memory deficits in epileptic patients[538]. Consequently, GABAergic transmission is not the most appropriate contributor to memory deterioration associated with PTZ-kindling model. The finding on unaffected hippocampal GABA levels 48 h after the last PTZ injection was documented earlier in both hippocampus and cortex 10 and 17 days after the last PTZ injection in PTZ-kindling model[519,539]. Additionally, brain GABA levels were not changed 30 minutes after the last PTZ injection[540]. It was also noted that GABA levels in several brain regions, including the hippocampus, were not changed 4 weeks after kindling[541], which may explain the normal GABA levels 48 h after kindling. Hippocampal GABA levels were also previously noted to be unchanged after PTZ acute injection[542]. Nevertheless, the result from this study and the results of previous studies were inconsistent, as GABA levels were decreased 4 h and 1.5 h following the last PTZ chronic injection in the hippocampus and prefrontal cortex, respectively, which can be explained by the time difference of brain collection and different brain region tested[126,542]. Furthermore, it was previously noted that there is a persistent increase of hippocampal GABA release through the kindling epileptogenesis[543], which resulted in GABA decrease few hours following the last PTZ injection, and the previous observations indicated that reduction in GABA levels might go back to normal 48 h after kindling. GLU is the main excitatory neurotransmitter and has been widely involved in synaptic plasticity

underlying memory function[544]. However, high abnormal levels of GLU associated with epilepsy or Alzheimer's diseases serve as a neurotoxic compound. Consequently, high GLU brain levels may be a contributory cause of memory deficits accompanied by uncontrolled seizures[126]. The earlier indication was supported by the observed high GLU levels associated with schizophrenia patients[545,546]. Several former preclinical experiments indicated high GLU levels in different brain regions after PTZ-kindling model[48,383,542,547,548], results which are harmonious with the results indicated in this study. As documented earlier, GLU levels would go back to normal 4 weeks after kindling[541]. However, low hippocampal GLU levels were noted 17 days following the last PTZ injection, which was justified by the upregulation of GLU transporters[519,549]. Also, hippocampal GLU levels were lower 1 h after PTZ acute injection, which can be explained by different brain collecting time, indicating the GLU levels will be normalized 24 h after PTZ acute injection[542]. The previous results indicated a swing in the equilibrium among excitatory and inhibitory actions toward excitation in PTZ-kindling model, suggesting a high neuronal activity, which resulted in decreasing the seizure threshold and inducing memory deficit. The neurotransmitters alternation noted in this study on PTZ-treated rats, which includes the decline in hippocampal HA and ACh levels and increased GLU levels when compared with SAL-treated rats, might be responsible for the observed reduction in seizure threshold of the kindled rats and the observed behavioral changes including memory impairment. Nevertheless, neurotransmitters levels (HA, Ach, GABA, and GLU) were not changed 24 h following PTZ acute injection, indicating that changes will occur during the epileptic seizure, and the alternations will not be noticeable after 24 h. A different technique that detects instant hippocampal neurotransmitters changes, e.g., microdialysis, will be useful in PTZ-acute model.

Chronic pretreatment of H3R antagonist 4 (5 and 10 mg/kg) 30-45 min before each PTZ injection led to a significant dose-independent decrease of the abnormal hippocampal GLU levels associated with PTZ kindling to be comparable to SAL group (Figure 43D). The previous result is contrary to the proposed mechanism of action of H3R antagonists, which usually are supposed to enhance neurotransmitter release, as it was noted that thioperamide increased *in vitro* GLU release after necrosis mediated by NMDA receptors in rat cultured cortical neurons[550]. However, the earlier observation is in harmony with previous experiments in which ciproxifan decreased the hippocampal 4-AP-evoked glutamate release in Sprague Dawley rats[551]. Moreover, it was noted previously that thioperamide administration decreased the potassium-induced GABA release in the prefrontal cortex[552]. Furthermore, it was postulated that H3R antagonists could increase some neurotransmitters release through blockade of H3Rs, a neurotransmitter which could prevent GLU release through its pre- or post-synaptic receptor[552]. The aforementioned result was endorsed with previous *in vitro* experiments, in which HA use inhibited intracellular excitatory post-synaptic potential in rat amygdala and dentate gyrus[265,553]. HA also decreased GLU release in rat dentate gyrus[554], suggesting that the released HA by H3R antagonist 4 can attenuate the abnormal induced hippocampal GLU levels by chronic PTZ administration. However, co-injection with RAM failed to reverse the modulating effect of H3R antagonist 4 (5 mg/kg) at hippocampal high GLU levels (Figure 43D), suggesting that the hippocampal GLU modulating effect provided by H3R antagonist 4 is not only mediated through histaminergic transmission and that another possible mechanism may be contributed. It can be suggested form earlier findings that H3R antagonists may modify neurotransmitters release differently under normal and stimulated conditions[552]. This explains the inhibitory effect of H3R antagonist 4 on the high PTZ-

evoked GLU release, and this modulate effect may be mediated through H3 hetero-receptors. Another point to add is that RAM injection with PTZ alone failed to modify GLU levels in PTZ group. According to the aforementioned observations, it was suggested that reducing the abnormal hippocampal GLU levels is at least partially involved in the antiepileptic and memory-enhancing effect of H3R antagonist 4 in the PTZ kindling model. The results indicated that VPA chronic treatment (300 mg/kg) 30-45 minutes before each PTZ injection significantly restored hippocampal ACh levels and decreased high abnormal hippocampal GLU levels (Figure 43). An earlier study indicated unchanged GABA levels with VPA chronic treatment when compared with PTZ and SAL treated rats in PTZ-chronic model[555], which is consistent with the observed result. Moreover, decreased hippocampal GLU levels were noted with VPA chronic treatment in PTZ-kindled rats[556]. It was also noted that VPA treatment alone caused lower brain GLU levels[557]. The modulation effect of VPA treatment on brain ACh levels was documented previously, wherein VPA administration restored the depleted ACh levels in streptozotocin-induced mice, and the alternation on ACh levels was associated with memory improving effect[558]. The amendment effect of VPA on the primary amino acid neurotransmitters undoubtedly plays an essential part in the antiepileptic and memory-enhancing provided effect. The indicated findings were in agreement with numerous comparable results of chronic VPA treatment with regard to neurotransmitters levels, indicating its contribution to the provided antiepileptic and memory-enhancing effect[557,95,558]. Several other well-recognized AEDs, e.g., as PHT and PB, prevented the progression of kindling but failed to attenuate the accompanied memory impairment in contrast to VPA[48,559]. Nevertheless, VPA prevented kindling associated with an alleviation of memory impairment in PTZ-kindling model.

It was noted that comorbidities accompanied by epilepsy were not only related to a single complication. There is a collective matrix of oxidative stress, neurochemical changes, and histological alterations in the brain that cooperatively provoke comorbidities[59,560,561]. The transcription factor c-fos belongs to the IEG (immediate early gene) family that is rapidly activated after numerous cellular inducements. IEGs are able to detect stimulated neurons of seizure activity and other excitatory provocations. Besides, IEGs is believed to be implicated in the neuronal excitation and to perform an essential role in the kindling process development[562,563]. The basal expression of brain c-fos is low; however, c-fos was noted to be activated rapidly in reaction to many inducements such as seizures induction[564], resulting in c-fos planned use as a valuable indicator of neuronal activity[562,565]. It was documented that c-fos activation was highly expressed in the hippocampus upon the development of tonic-clonic convulsions (seizure 4-5) in both PTZ-acute and -chronic models but not with less seizure scores[209,566,567] implying that only high neuronal induction is needed to boost c-fos expression in the hippocampus; c-fos high expression is a widely stated phenomenon that highlights the molecular alternations occurring during the process of epileptogenesis. Consequently, analysis of the expression of c-fos in the hippocampus 2 h after PTZ (60 mg/kg) acute administration in PTZ-acute model and 48 h after the last PTZ injection in PTZ-chronic model was used to examine hippocampal neuronal activity. The c-fos expression was studied 2 h after PTZ-acute model because it was documented earlier that the expression of c-fos protein reached its highest levels, nearly around 2 h after PTZ injection[567]. The results indicated that c-fos was highly expressed in the hippocampus following both PTZ-acute and -chronic models, indicating hippocampal neuronal excitation (Figures 34, 35, 44, & 45). The previous findings were supported in numerous previous experiments in which c-fos was highly

activated in the hippocampus 2h after PTZ-acute administration in rodents[567-569] and zebrafish[570]. In addition, it was documented that c-fos was highly expressed in the hippocampus in PTZ kindled rats[395,566,568,571]. As noted earlier, PTZ-induced seizures effect is related to activation of excitatory amino acid receptors, which also contribute to the induction of c-fos both *in vitro* and *in vivo*[572-574]. According to the above-mentioned findings of the essential role of the hippocampus in learning and memory process, and the noted memory-enhancement effect associated with decreasing hippocampal c-fos expression in earlier study[568]. The PTZ-induced hippocampal excitability can be correlated to the associated memory deficits. Moreover, seizure-induced c-fos expression has been at least partially related to increased release of GLU in the brain[575,576]. Likewise, the use of NMDA receptor antagonist decreased the seizure-induced c-fos expression when used with the convulsant agent but not alone[577], indicating the correlation between the hippocampal abnormal GLU releases and c-fos expression observed in this study. Furthermore, attenuating of c-fos mRNA expression is related to the drug alleviated effects on seizures, epileptogenesis, and/or epilepsy[563]. It can be suggested the c-fos high expression is correlated with seizures development, kindling procedure, and its associated memory-impairment.

Treatment with H3R antagonist 4 (5 and 10 mg/kg) 30-45 minutes before PTZ injection mitigated hippocampal c-fos expression in both PTZ-acute and -chronic models (Figures 34, 35, 44, & 45). The observed finding was previously noted, showing that ciproxifan alleviated the stress-evoked increase of cortical c-fos expression in mice and attenuated the associated memory deficits[578]. Besides, it was documented that thioperamide mitigated haloperidol-evoked striatum c-fos expression in rats[579]. However, the aforementioned result is contrary to an earlier study which noted that ciproxifan increased c-fos expression when administered alone[536]. This can

be justified by conflicting H3R antagonist effects on c-fos expression under healthy and stimulated conditions similar to the abovementioned finding on GLU. The modulated effect of H3R antagonist 4 (5 mg/kg) in PTZ-kindling model was reversed with RAM co-injection indicating the involvement of the histaminergic system in the provided neuroprotective effect. Based on the earlier observations, it was suggested that the neuroprotective effect of H3R antagonist 4 in PTZ-acute model can be related to the H3R antagonist 4 inhibition of seizures. The anticonvulsant effect of H3R antagonist 4 was indicated earlier in this study, and this is not to be reversed with RAM co-injection. Suggesting the involvement of other neurotransmitters in the provided effect, e.g., GLU, which also was indicated to be related to c-fos expression increase[580]. While in PTZ-chronic models, the neuroprotective effect of H3R antagonist 4 on c-fos expression can be related to its antiepileptic and memory-enhancing effect through antagonizing the H3Rs resulting in increased HA release and lowered the excitatory neurotransmission GLU levels. The results indicated that VPA acute and chronic treatment (300 mg/kg) significantly reduced hippocampal c-fos expression following PTZ systemic administration (Figures 34, 35, 44, & 45). As noted earlier, VPA attenuated flurothyl-induced hippocampal c-fos expression in male Wistar rats[581], indicating the neuroprotective effect of VPA.

Findings from this part of the study showed that chronic treatment with H3R antagonists (H3R antagonist 4) displayed high protection against PTZ-induced kindling, behavioral alternations, oxidative stress, neurochemical modifications, and neuronal activation. All these results highly propose that the anticonvulsant and memory-enhancing effects are owed to oxidative stress mitigation, reduction of AChE activity, enhancement of HA and ACh release, decrease of GLU abnormal levels, and attenuation of hippocampal c-fos expression through auto- and hetero-H3Rs inhibition

(Figure 50). Even though ordinary AEDs inhibited seizures, they usually do not provide memory-enhancing and neuroprotective effects. Numerous treatment approaches have been established for epilepsy therapy; however, they are not protective against epilepsy-induced cognitive impairments[582]. Further investigations are needed in preclinical and clinical developments to provide superior scientific support for developing H3R antagonist 4 as a therapeutic approach for epilepsy treatment and attenuation of epilepsy-associated comorbidities.

SE is a life-threatening condition of continuing seizure activity related to cognitive dysfunction, elevated mortality rate, and comorbidities, necessitating instantaneous involvement with appropriate pharmacological treatments[583-586]. SE is classified as an ongoing seizure for more than 30 min or numerous seizures without complete recuperation of consciousness in any of them[587]. Initial pathophysiological insults of SE in rodents contain severe neuroinflammation, excessive oxidative stress, selective neuronal degeneration, and interruption of the brain-blood barrier, resulting in consequent memory deficiencies[588,589]. In the last part of this study and as a continuation of the above-mentioned investigations, the protective effect of the non-imidazole based H3R antagonist 4 was examined in the PLC-induced SE model. Furthermore, the effect of H3R antagonist 4 on the hippocampal oxidative stress markers as well as AChE activity of tested animals was assessed. PLC-induced seizure model characterized by electroencephalographic waves as well as oral and morphological results that imitate the one observed in TLE. Thus, the PLC-induced seizure model has been accepted as an animal model with resemblance in features to TLE[129,590]. The results indicated that all treated rats with PLC (400 mg/kg) exhibited behavioral modifications and seizures, which were developed within 30 min to SE (Figure 46). These findings were endorsed by earlier studies[591-593]. PLC is a muscarinic

cholinergic agonist that induced seizures through stimulation of the M1 muscarinic receptor subtype. As noted earlier, M1 receptors knocked out rodents that failed to exhibit convulsion in reaction to PLC[190]. Moreover, several cholinomimetics compounds were documented to provoke convulsions and once systemically administered or directly injected into the brain[594,595]. Furthermore, treatment with atropine, a muscarinic antagonist, inhibited PLC-induced SE[191]. Likewise, *invitro* experiments indicated that PLC application resulted in an imbalance between inhibitory and excitatory transmission in cultured hippocampal neurons, causing the initiation of SE[192]. Similarly, *in vivo* experiments supported hippocampal GLU release after PLC administration[193]. Considerable evidence currently endorsed the proposal that seizures are initiated by M1 receptors and are maintained by NMDA receptor stimulation[193,194]. In this study, scopolamine-methyl nitrate an antimuscarinic compound was used to minimize the peripheral cholinergic PLC-induced symptoms, including tremor, salivation, piloerection, and diarrhea. As previously noted, this compound was used to reduce the peripheral cholinergic evoked signs with no effect of seizures induction[596,597]. Treatment with H3R antagonist 4 (5 and 10 mg/kg i.p) 30-45 minutes before PLC injection mitigated PLC-evoked SE (Figure 46). Nevertheless, H3R antagonist 4 (2.5 and 15 mg/kg) failed to provide any protective effect against PLC-induced SE, suggesting a dose-dependent effect of H3R antagonist 4 in PLC-induced seizure model. This was previously remarked in this study in both electrically and chemically induced seizure models. Another important point is that H3R antagonist 4 (5 and 10 mg/kg i.p) anticonvulsant provided effect in the PLC-induced seizure model was considerably higher compared to the upper dose (15 mg/kg, i.p.) or the lower dose (2.5 mg/kg, i.p.), indicating that the optimal protection was detected when the H3R antagonist 4 was applied at the doses 5 or 10 mg/kg: The highest

protection remarkable effect showed at 10 mg/kg, and the effect of H3R antagonist 4 (15 mg/kg) was off target. Nevertheless, unsatisfactory antagonistic of H3R antagonist 4 with H3Rs was noted with the lower dose (2.5 mg/kg). The dose-dependency effect observed for H3R antagonist 4 was indicated earlier, applying different acute seizure models[374]. The aforementioned findings of dose-dependency are in line with former experimental outcomes performed on several rodents[305,443,598,599]. In addition, the anticonvulsant protective effect of H3R antagonist 4 (10 mg/kg) was abolished when rats were co-injected with RAM or ZOL but not with PYR, indicating that the anticonvulsant H3R antagonist 4 provided effect is facilitated through alternation of the central histaminergic neurotransmission and to some level with interaction of the released HA with post-synaptically located H2Rs but not H1Rs (Figure 47). The previous implied mechanism underlying the anticonvulsant effect of H3R antagonist 4 is also in agreement with former preclinical observations in censoriously ill and poly-medicated patient in which high doses of several centrally acting H2R antagonists drugs promoted the development of convulsions[600-602], indicating the involvement of H2R antagonism, and, consequently, brain histaminergic neurotransmission in the seizure promotion in PLC-evoked SE model. It is notable that RAM and ZOL, when administered alone with PLC, did not provide significant changes when compared to PLC-treated rats. According to our information, no previous experiment investigated the effect of H3R antagonists in PLC-induced seizure model; however, the protective effect of H3R antagonists had previously been endorsed in different acute and chronic models[603]. Thus, inhibition of H3Rs by the test compound H3R antagonist 4 would increase the neuronal release of brain histamine and modulate other neurotransmitters releases, including GLU supplying the anticonvulsant protective effect in the PLC-induced seizure model. Interestingly, systemic pretreatment with the traditional

antiepileptic drug DZP (10 mg/kg, i.p.) 30 minutes before PLC administration inhibited the convulsions and SE occurrence evoked by PLC (Figure 46), and this outcome was in agreement with former experimental observations [604-606]. DZP is a traditional antiepileptic drug that mainly produces its antiepileptic effect due to the pharmacological activities of modifying the $GABA_A$–benzodiazepine receptor complex facilitating GABA-mediated inhibitory neurotransmission in the CNS[607-609].

Several preclinical studies have documented that oxidative stress was associated with PLC-induced SE, which resulted in elevated ROS brain levels and neuronal injury [81,399,488,610]. Consequently, and according to the above-mentioned contribution of oxidative stress in the initiation and development of epilepsy, assessment of hippocampal numerous oxidative stress markers (e.g., MDA, GSH, CAT, and SOD) in treated animals was essential to investigate the alleviating effect of the potential and centrally acting compound H3R antagonist 4. Once again, the hippocampus has earned the attention in the PLC-evoked SE model as it contains abundant prominent neuronal circuits connected to seizure genesis, and it is exceptionally vulnerable to PLC-induced neuronal damage. Besides, the hippocampal neuronal damage is considered the hallmark of TLE[611]. The results indicated that PLC acute treatment is associated with hippocampal elevated MDA levels and reduced GSH levels 1 h after PLC-induced SE in tested rats, and these findings are in line with earlier preclinical experiments conducted on different rodents (Figures 48A & C)[398,612-614]. On the other hand, CAT and SOD, the natural antioxidant enzymes levels in the hippocampus, were not altered 1 h after PLC-evoked seizures, and this finding is inconsistent with an earlier observation (Figures 48E & F)[615]. However, several previous outcomes were contrary to the previous results in which CAT levels were reduced 6 h following seizure induction with no change in SOD levels[399,614] . Also, different studies exhibited

reduced levels of CAT and SOD in the hippocampus 24 h following seizures[593,613]. This can be explained by different brain collection time, indicating that an enhanced metabolic requirement can be showed during the epileptic seizure, and CAT and SOD activities are not modified during this acute phase of PLC-induced seizure. It is interesting to note that acute treatment with H3R antagonist 4 (5, 10, and 15 mg/kg) mitigated oxidative stress in the hippocampus of tested animals (Figures 48A & C), demonstrating the antioxidant potential of H3R antagonist 4. Nonetheless, the remarked antioxidant effect of H3R antagonist 4 was dose-independent at 5, 10, and 15 mg/kg provided effects were statistically not different. The latter finding of dose independence is in agreement with an aforementioned indication in which the H3R antagonist DL77 (10 or 15 mg/kg, i.p.) delivered equivalent antioxidant effects in the hippocampus of VPA-induced autism-like behaviors in mice[454]. Surprisingly, H3R antagonist 4 (15 mg/kg) exhibited a protective effect against hippocampal oxidative stress caused by PLC-induced seizures despite its failure in inhibiting PLC-induced SE. But the antioxidant provided effect with H3R antagonist 4 (10 mg/kg) was reversed when rats were co-injected with RAM, implying the participation of H3R histaminergic neurotransmission in the presented alleviating effects on the levels of MDA and GSH (Figures 48B & D). The latter outcome is in line with a recent study in which RAM co-injection abrogated DL77 modulated effect on oxidative stress in VPA-induced autism-like paradigm in mice[454]. However, RAM injection with PLC alone failed to provide any remarkable alternation on MDA and GSH levels in comparison to the PLC group. Another point is that DZP acute pretreatment mitigated PLC-induced oxidative stress in the hippocampus. The latter observation is in agreement with earlier studies in which DZP mitigated stress-induced oxidative stress in male Wistar rats[616] and attenuated PLC-induced oxidative stress in animal model[617].

Thus, a strong relationship was documented among the cholinergic neurotransmission and epilepsy. The contribution of cholinergic neurotransmission to the PLC-induced seizure model has been well recognized[592,618]. Elevated hippocampal cholinergic levels due to neuronal hyperactivity were noted in PLC-induced seizure model[619], which could result in neuronal damage during PLC-induced seizures[620]. Consequently, hippocampal AChE activity levels were investigated in PLC-treated rats following SE. The results indicated low levels of AChE activity in the hippocampus 1 h following PLC-induced SE in treated rats, signifying probable high acetylcholine levels in the hippocampus (Figure 49). The latter observation is in line with several experiments indicating hippocampal reduced levels of AChE activity following PLC administration indicating a critical association of hippocampal AChE activity and pathogenesis of PLC-induced SE[397,621,622]. It was suggested that reduced levels of AChE activity could be due to the regulation of the abnormally elevated levels of the ACh in the cholinergic synapses during SE, which cause extreme stimulation and activation of muscarinic and nicotinic receptors[107,397]. Moreover, brain decreased AChE activity were detected in several chemically and electrically acute-induced seizure models[131,133,623]. However, the elevated ACh levels will be reduced at delayed time points as documented earlier in a previous study, in which DOZ had augmented the neuronal damage and memory deficits associated with PLC-induced SE when administered before PLC injection. Nevertheless, DOZ mitigated the neuronal destruction and cognitive deficiencies when administered 3 weeks after PLC-induced repetitive seziures[624], confirming the critical role of the modulated cholinergic neurotransmission in the initiation and progression of the PLC-evoked SE model.

However, pretreatment with H3R antagonist 4 failed to alter the hippocampal AChE activity in treated rats, indicating the lack of influence of acute treatment of H3R antagonist 4 on the abnormal ACh levels in the hippocampus during PLC-induced SE.

Altogether, the observed results of PLC-induced SE model demonstrate that the novel non-imidazole-based H3R antagonist 4 inhibited PLC-induced convulsions, SE, and mortality in the tested animals which were partially reversed with the CNS-penetrant RAM and ZOL, signifying a connection between the brain histaminergic neurotransmission and the H3R antagonist 4 provided effect. Moreover, the detected finding indicated the significant involvement of oxidative stress in the progression and/or maintenance of seizure activity, and H3R antagonist 4 significantly decreased the susceptibility of tested animals to PLC-induced SE. But a series of further seizure test models with distinct species is still necessitated to further comprehend the present outcomes noted for H3R antagonist 4, and to improve the translational value of its prospective applicability in the therapeutic management of SE.

Chapter 6: Conclusion

In conclusion acute systemic administration of novel and highly affine H3R antagonists (1-16) exhibited various anticonvulsant effects in a range of acute induced seizure models via brain histaminergic neurotransmission. H3R antagonist 4 dose-dependently showed the most promising anticonvulsant activity in MES- and PTZ-induced seizure models. Furthermore, acute systemic administration of H3R antagonist 4 dose-dependently displayed a procognitive effect in DIZ-induced amnesic effect applying both IAP and NOR behavioural tests without modulating locomotion and anxiety-like behaviors of tested animals. These findings enhance further the potential efficacy of H3R antagonists in the treatment of epilepsy and other neuropsychiatric diseases characterized by cognitive deficits.

Acute H3R antagonist 4 significantly provided simultaneous anticonvulsant and procognitive effect by alleviating the memory impairment associated with PTZ acute model, and displayed antioxidant and procognitive effect.

Chronic H3R antagonist 4 significantly mitigated the kindling development and attenuated the memory impairment associated with PTZ chronic treatment. Also, chronic H3R antagonist 4 modulated oxidative stress and neurochemical levels in PTZ-kindled rats. Moreover, chronic H3R antagonist 4 showed neuroprotective effect by reducing c-fos protein expression in PTZ-kindled rats However, further investigations are needed in preclinical and clinical developments to provide superior scientific support for developing H3R antagonist 4 as a therapeutic approach for epilepsy treatment and attenuation of epilepsy-associated comorbidities (Figure 50).

Furthermore, H3R antagonist 4 inhibited PLC-evoked SE, and also exhibited antioxidant effect. However, a series of further seizure test models with distinct species is still necessitated to further comprehend the present outcomes noted for H3R antagonist 4, and to improve the translational value of its prospective applicability in the therapeutic management of SE.

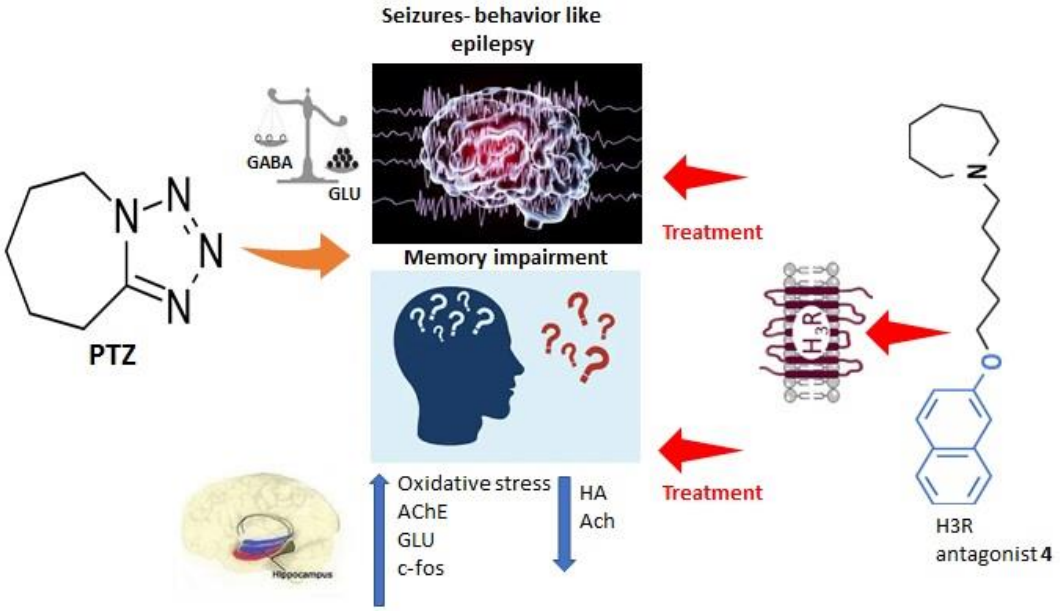


Figure 50: Schematic graph describing the effect of H3R antagonist 4 in PTZ-kindling model.

6.1 Limitation of the study

- Further experiments are essential to clarify the other probable mechanisms by which H3R antagonist 4 elicited its anticonvulsant effect in PTZ-induced seizures.
- Additional abrogative studies are needed to explain the mechanisms behind the provided procognitive, antioxidant, and neuroprotection effects of H3R

antagonist 4 observed in acute PTZ-induced seizure as its anticonvulsant effect was not facilitated through the histaminergic neurotransmission.

- The available ELISA/colorimetric assay was utilized to detect the overall content of hippocampal levels for several neurotransmitters of interest. However, other techniques, e.g. microdialysis technique, should be developed as it measures the instant levels of released neurotransmitters, and therefore, is considered to be a more reliable and accurate technique.
- Memory functions post SE seizures should be studied, as the current study examined only the anticonvulsant effect of H3R antagonist 4 in PLC-induced SE and its ability to mitigate SE incidence.

6.2 Future direction

Future studies are essential to investigate the anticonvulsant protective effect of H3R antagonist 4 when administrated to fully kindled rats or to PLC-epileptic rats, and to evaluate the procognitive effect of H3R antagonist 4 when treated post seizures incidence. Altogether the obtained results opened a novel therapeutic window for managing memory deficits associated with epilepsy. However, the examination of the anticonvulsant effect of H3R antagonist 4 on spontaneous unprovoked seizures occurrence in epileptic transgenic rodents, and the estimation of protective effect of H3R antagonist 4 on other associated comorbidities with epilepsy.

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List of Publications

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