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Research Article

The Neuroprotective Effect of Ursodeoxycholic-acid in Comparison with Carbamazepine in Pilocarpine Induced Status Epilepticus in Experimental Rats

Zainab Adel Omran[1*], Ahmed Hamed Jwied[1]

[1] Department of College of Pharmacy, University of Baghdad, Baghdad, Iraq

* Corresponding author's email: Zainabadilomran94@gmail.com

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ABSTRACT

Background: An epileptic seizure was defined as a transient occurrence of signs and symptoms due to abnormal excessive neuronal activity in the brain. A seizure lasting 30 minutes or longer or a series of seizures in which the patient did not regain normal mental status between convulsions was considered a status epilepticus. Pilocarpine is a drug that acts as an acetylcholine muscarinic agonist. Carbamazepine is a psychotropic medication that is frequently provided to patients with psychological illnesses and epilepsy conditions often treated with anticonvulsant medication carbamazepine. Ursodeoxycholic acid, Ursodiol also known as secondary bile acid, is produced by gut bacteria in most other species including humans. The aim of this study was conducted to evaluate the possible neuroprotective effects of Ursodeoxycholic-acid in comparison to carbamazepine against pilocarpine-induced status epilepticus in experimental rats.

Subjects and Methods: Thirty male Wistar albino rats were utilized in this study, Group I (Control group): Rats received normal saline orally for 3 days. Group II (Lithium chloride + Scopolamine + Pilocarpine): Rats were given lithium chloride followed by pilocarpine administration; scopolamine is also given to the rats 30 minutes before pilocarpine administration. Group III (Carbamazepine): status epilepticus rats received carbamazepine orally for 3 days. Group IV (Small dose of Ursodeoxycholic-acid): status epilepticus rats received Ursodeoxycholic-acid 25 mg/kg/day orally for 3 days. Group V (Large dose of Ursodeoxycholic-acid): pilocarpine-treated rats received UDCA 100 mg/kg/day orally for 3 days.

Results: Based on the measurement of markers: Caspase3, Tumor Necrosis Factor-alpha, and Reduced Glutathione we reported rise in the serum level of Reduced Glutathione, and decrease in the serum level of Caspase3, and Tumor Necrosis Factor-alpha in Ursodeoxycholic-acid treated-group.

Conclusions: It can be concluded that the Ursodeoxycholic-acid has a neuroprotective effect to epileptic rats recognized by its anti-apoptotic, anti-inflammatory, and antioxidant effects

Introduction

An epileptic seizure was defined as a transient occurrence of signs, and/or symptoms due to abnormal excessive or synchronized neuronal activity in the brain. It was believed that epilepsy was a brain disorder marked by an enduring propensity to have epileptic seizures, and by the neurobiologic, cognitive, psychological, and social

repercussions of this condition. The occurrence of at least one epileptic episode is necessary for the diagnosis of epilepsy. In other words, epilepsy meant that a person had experienced at least one seizure and was highly likely to experience others [1]. Status epilepticus (SE): is a neurological emergency that requires immediate treatment to prevent significant morbidity or mortality. Historically, a seizure lasting 30 minutes or longer or a series of seizures in which the patient did not regain normal mental status between convulsions was considered to be in status epilepticus [2]. SE seizures impair GABA-A (Gamma-aminobutyric acid -type A) receptor-mediated inhibition in vital structures. The mechanisms for ending seizures may be compromised by this decreased inhibition. SE can be maintained by transmission that is mediated by potentiated a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic Hippocampus pyramidal neurons that are highly active during seizure SE. N-methyl-D-aspartate receptors (NMDARs) are activated during SE. The activation of these receptors can potentiate AMPARs through GluA1 insertion [3].

Despite the development of newer medications, lithium is still favored in clinical practice for the treatment of bipolar disorder (BD) and is one of the primary maintenance medications [4]. Lithium modifies neuronal activity by reducing excitatory neurotransmission via glutamate and dopamine and enhancing inhibitory neurotransmission via GABA. Lithium has intracellular and molecular effects on the second messenger systems that function in neurons, such as inositol, diacylglycerol (DAG), protein kinase C (PKC), intracellular calcium, and myristoylated alanine-rich C kinase substrate (MARCKS). These changes ultimately affect neurotransmission (sending and receiving signals) and support cellular viability. These procedures are intricate and include numerous proteins [5].

Scopolamine is a nonselective muscarinic antagonist that competitively inhibits G-protein coupled post-ganglionic muscarinic receptors for acetylcholine. It also has central sedative, antiemetic, and amnesic effects in addition to peripheral antimuscarinic effects [6]. This has the effect of relaxing smooth muscles nearby, and reducing gland secretion. While scopolamine, unlike atropine, primarily sedates the central nervous system, over-excitation, and restlessness can develop at higher doses [7].

Since 1875, the alkaloid pilocarpine has been used to treat glaucoma. Many physiological and pharmaceutical researches have shown that pilocarpine has no effect on nicotinic receptors and only stimulates muscarinic receptors. All five muscarinic receptor subtypes can be activated by pilocarpine, although the majority of its therapeutic benefits in people are mediated by the M3receptor [8].

Carbamazepine (CBM) is a psychotropic medication that is frequently provided to patients with psychological illnesses all over the world. Epilepsy, trigeminal neuralgia, and bipolar disorder are all conditions that are often treated with the mood stabilizer, and anticonvulsant medication carbamazepine [9]. Moreover, carbamazepine may inhibit voltage-gated calcium channels, which would prevent neurotransmitter release [10].

Ursodeoxycholic acid (UDCA), Ursodiol also known as secondary bile acid is produced by gut bacteria in most other species including humans. It was first discovered in the bile of bears of the genus Ursus from whence it got its name. It is generated in the liver in some species [11]. A hydrophilic bile acid that mediates its biological effects through a number of different methods. UDCA shields hepatocytes, and cholangiocytes from bile acid-induced damage including inflammation brought on by reactive oxygen species (ROS), and mitochondrial dysfunction. It has been shown that UDCA induces anti-apoptotic pathways and maintains the cell structures of hepatocytes. Moreover, it was shown to reduce oxidative stress in the liver by halting the production of ROS by local macrophages and Kupffer cells [12]. UDCA have been found to have protective properties against a number of brain disorders, including Parkinson's, Alzheimer's, and Huntington's. It would seem that UDCA would improve epileptic models caused by them due to its preventive effect in diseases like Parkinson's, and Huntington's as well as its effect on cell death [13].

Caspase3, A cysteine-aspartic acid protease known as caspase-3, has received a lot of attention recently because of its amazing functions in neuronal development, tissue differentiation, and regeneration. This enzyme, which is a crucial zymogen in cell apoptosis, is not activated until initiator caspases cleave it during the apoptotic influx [14].

Tumor necrosis factor-alpha (TNFa), is a pleiotropic cytokine, meaning that it affects many different kinds of cells. It has been identified as a key regulator of inflammatory reactions, contributing to the emergence of a number of inflammatory and autoimmune diseases [15].

Reduced Glutathione (GSH), one of the natural antioxidants in cells is GSH [16]. The body converts L-cysteine, L-glutamic acid, and glycine into glutathione (GSH), a tri-peptide. In addition to its role as an antioxidant in protecting cells from free radical, and peroxide damage GSH has an important function in various cellular processes, including the synthesis of DNA, proteins, and prostaglandins; the transport of amino acids; the activation of enzymes; and the repair of genomic DNA. More than 90% of the glutathione present in healthy cells and tissues is in the disulfide or oxidized form (GSSG), rather than the reduced form GSH. When the ratio of GSSG to GSH rises, it's a sign of oxidative stress [17].

The present study was designed to evaluate the possible neuroprotective effects of UDCA in comparison with carbamazepine in status epilepticus induced by pilocarpine in experiment rats.

Evaluating the possible anti-apoptotic effect of UDCA on pilocarpine-induced SE by measuring serum caspase3 levels.

Evaluating the possible anti-inflammatory effect of UDCA on pilocarpine-induced SE by measuring serum TNF-α levels.

Evaluating the possible antioxidant effect of UDCA on pilocarpine-induced SE by measuring serum GSH levels.

Subjects and Methods

Both the College of Pharmacy and the University of Baghdad's Ethical and Scientific Review Committees gave their signatures of approval to this study.

The chemicals, drugs and suppliers that used in this study:

Lithium chloride powder (London), Scopolamine ampoule (Julphar, UAE), Pilocarpine Hcl powder (Mack Line, England), Carbamazepine syrup (Novartis, Switzerland), Ursodeoxycholic-acid Suspension (Bio Toscana, Latin America), Normal saline 0.9% (Pharmaceutical solution, industry, Iraq), Diethyl ether liquid (Cambridge, UK), Formaldehyde (Al-Jubail, Saudi Arabia), Tumor

Necrosis Factor ELISA Kit (Zhejiang, china), Serum Reduce Glutathione ELISA Kit (Zhejiang, china), and serum caspase3 ELISA Kit (Zhejiang, China) .

Equipments and Instruments:

The instruments, and other equipment that used in this study: Digital Balance, Sensitive balance, Centrifuge, Disposable syringe 1ml, Gavage size 16 for oral feeding, Single channel micropipette (100-1000) ul, Eppendorf®, refrigerated, and Centrifuge.

On the day of administration, lithium chloride (LiCl) solution freshly prepared by dissolving 127 mg/kg LiCl powder in 1ml of normal saline, which is used as a standard solution for the preparation of doses used in this study [18]. Pilocarpine solution freshly prepared by dissolving each 30 mg/kg of pilocarpine powder in 1 ml of normal saline. Seizure severity was rated by Racine's scale [19] twenty hours before the injection of Pilocarpine, LiCl 127 mg/kg was delivered intraperitoneal (i.p).

Our SE model was produced using LiCl and pilocarpine following the acclimatization period. During 20–24 hours after receiving LiCl (127 mg/kg, i.p) treatment, rats in the SE group were given pilocarpine (30 mg/kg, i.p) 30 minutes before the injection of pilocarpine, rats were administered scopolamine (l mg/kg, i.p.) to treat peripheral symptoms. Rats were then watched for behavioral changes, and seizure grades were assigned based on Racine scores. The experimental group consisted of rats that had seizures that were either grade V or grade VI. When SE did not occur in the SE group of rats, an extra dose of pilocarpine of 10 mg/kg was administered every 30 minutes (the maximum dose was 60 mg/kg) [20]. Pilocarpine sensitivity in animals pretreated with LiCl is increased, with a 20-fold shift in the dose-response curve for the development of seizures. When LiCl is employed, this leads to a large decrease in the amount of pilocarpine required, and a shorter time for the start of SE [21].

Animal selection:

Thirty male Wistar albino rats (4-5 weeks old, weighing 200 ± 20 g), were utilized in this work. These rats were obtained from and housed in the University of Baghdad's College of Pharmacy's animal house. Where they experienced a 12-hour light/dark cycle and stable temperature and humidity levels. Commercial pellets and running water were given to the animals. These rats were handled and kept in the correct circumstances every day for 7 days before receiving the drug to help them become accustomed to their surroundings. The study received approval from the College of Pharmacy at the University of Baghdad's scientific and ethical authorities.

Experimental protocol:

Both the Scientific, and Ethical Committees at the College of Pharmacy/University of Baghdad gave their signatures of permission to this work. In the experiment, thirty rats were employed, and they were distributed equally among five groups of six rats as follows:

Group I (Negative Control): Rats were orally received normal saline 1ml for 3 days.

Group II (Sco+ LiCl +Pilocarpine group): Rats were received LiCl 127 mg/kg, followed by pilocarpine administered in 30 mg/kg 20 h later; Methyl scopolamine (1 mg/kg in rats) is given to the rats thirty minutes before administration of pilocarpine.

Group III (Carbamazepine treatment): SE rats were orally-received 80 mg/kg/day of carbamazepine for 3 days [22].

Group IV (Small dose of UDCA treatment): SE rats were orally received UDCA 25 mg/kg/day for 3 days [23].

Group VI (Large dose of UDCA treatment): SE rats were orally received UDCA 100 mg/kg/day for 3 days [24].

At the end of the experimental period all the animals were sacrificed after euthanizing with diethyl ether anesthesia, blood sample were collected from each rat withdrawn from jugular vein (near to the neck), the blood sample collected and allowed to clot for 30 min at room temperature , then it was centrifuge at 3000 rpm for 20 min to obtain serum.

Biochemical analysis:

Measurement of Serum Caspase-3 Levels:

Caspase3 content in serum was determined using the Nanjing Duly Biotech CO. Ltd. rat caspase-3 ELISA Kit. This procedure is based on transforming the color from blue to yellow by using a stopping solution and a set of calibrations that were received and processed at the same time as the samples, allowing the operator to have a standard curve of optical density vs caspase3 concentration [25].

Measurement of Serum Tumor Necrosis Factor- alpha Levels:

The method used in this ELISA kit is called Sandwich-ELISA. This kit's Micro ELISA –strip plate had been pre-coated with an antibody that was specific to tumor necrosis factor alpha (TNF-alpha). Standards or samples were placed in the relevant Micro ELISA strip plate wells before being linked to the specific antibody. A TNF-alpha specific antibody that was conjugated to Horseradish Peroxidase (HRP) was then added to each Micro ELISA strip plate well. The unattached parts are rinsed away. The tetra-methyl-benzene (TMB) substrate solution was administered to each well. Only the wells with TNF- and an HRP-conjugated TNF-antibody, however, would initially appear blue before changing color to yellow after the stop solution was added. The optical density (OD) is spectrophotometrically measured at 450 nm. The OD value and TNF-concentration have a direct correlation. The amount of TNF-contained in each sample was then calculated by comparing the optical densities of the samples to the standard curve [26].

Measurement of Serum Reduced Glutathione Levels:

The goal of this test is to compare how well the micro ElISA~~sa~~ plate from this kit, which has been pre-coated with GSH, performs during the reaction, reduced glutathione (GSH) can react with dithionitrobenzoic acid (DTNB) to form thio-nitrobenzoic acid and glutathione disulfide. Nitro mercaptobenzoic acid is a yellow product with a maximum absorbance at 420 nm [25].

Statistical analysis:

The data was examined using Graphpad Prism® 7.0, the means of the groups were compared using one-way ANOVA and t-tests (Tukey). all data are shown as mean ± standard deviation (SD) and were deemed significant when ($P>0.05$).

Histopathological Examination:

Histopathological, and neuronal cell number examinations Brains were collected from 6 representative animals in each group, and immediately fixed in 10% phosphate-buffered formaldehyde (Figure 2.1). Subsequently, brains were embedded in paraffin, 5 µm sections were prepared and stained with haematoxylin and eosin (H&E), and examined microscopically. In the hippocampal areas, the number of

cells was counted in a fixed field size (60 000 µm2 for CA1/hilus, and 10 000 µm2for CA3) [27].

Results

Effect of Ursodeoxycholic-acid and Carbamazepine on Serum Caspase 3 Levels:

According to (Table 1), When the group II was compared to the group I, there was a statistically significant rise in the serum caspase3 levels (0.274 .017 vs. 0.190 .015) (P <0.05).

SE rats treated with small dose (S.D) 25 mg/kg/day and large dose (L.D)100 mg/kg/day of UDCA (groups IV and V), showed no difference in the serum level of Caspase-3 as compared to the group I, although there was a significant decrease in the serum level of Caspase-3 as compared to group II.

SE rats treated with CBM (group III), exhibited significant increase in the serum level of Caspase-3 as compared to group I, but yet there was a significant decrease in the serum Caspase-3 levels as compared to group II.

SE rats treated with S.D UDCA and L.D UDCA (groups V and VI) when compared to group IV; there was a considerable decrease in the serum caspase3 levels.

Table 1: Effect of UDCA and CBM on Caspase-3 serum levels.

Treatment Groups	Type of Treatments	Caspase- 3 ng/ml Mean ± SD
I	Normal saline only	0.190 ± 0.015 [a]
II	LiCl+ Sco+ Pilocarpine	0.274 ±0.017 [*]
III	Carbamazepine +Pilocarpine	0.223 ± 0.008 [* a]
IV	S.D UDCA +Pilocarpine	0.195 ± 0.014 [a c]
V	L.D UDCA+Pilocarpine	0.188 ± 0.012 [a c]

-A significant difference between the groups and group I is denoted by the superscript (*). (P< 0.05).

-A significant difference between the groups and group II is indicated by the superscript (a) (P< 0.05).

-A significant difference between groups (IV and V) and group III is indicated by the superscript (c) (P< 0.05).

Effect of Ursodeoxycholic-acid and Carbamazepine on Serum Tumor Necrosis Factor -Alpha Levels

According to (Table 2), When the mean of the group II was compared to the group I, there was a significant rise in serum tumor necrosis factor alpha (TNF-α) levels (34.10 1.64 vs. 26.61 1.68) (P< 0.05).

Treatment SE rats with S.D 25mg/kg/day and L.D 100mg/kg/day of UDCA (groups IV and V) showed non-significant differences in the serum level of TNF-α as compared to group I, but a significant decrease in the serum level of TNFa as compared to group II.

Treatment SE rats with CBM (group III) showed a non-significant difference in the serum level of TNFα- as compared to the group I, but a significant decrease as compared to the group I.

Treatment SE rats with S.D 25mg/kg/day and L.D 100mg/kg/day of UDCA (groups IV and V) exhibited non-significant change in the serum level of TNF-α as compared to group III.

Table 2: Effect of UDCA, and carbamazepine on TNF-α serum levels.

Treatment Groups	Type of Treatments	TNF-α ng/l Mean ± SD
I	Normal saline only	26.61 ± 1.68 [a]
II	LiCl+ Sco+ Pilocarpine	34.10 ± 1.64[*]
III	Carbamazepine +Pilocarpine	28.42 ± 2.21 [a]
IV	S.D UDCA +Pilocarpine	26.49 ± 1.85 [a]
V	L.D UDCA+Pilocarpine	27.26 ± .1.51 [a]

-A significant difference between the groups and group I is denoted by the superscript (*). (P< 0.05).

-A significant difference between the groups and group II is indicated by the superscript (a) (P< 0.05).

Effect of Ursodeoxycholic-acid and Carbamazepine on Reduced Glutathione Levels

According to (Table 3), When the mean of the group II was compared to the group I, (178.5 9.9 vs. 68.5 7.2), there was a significant decrease in the serum GSH levels (P< 0.05).

The GSH serum level in SE rats treated with S.D. 25 mg/kg/day and L.D. 100 mg/kg/day of UDCA (groups IV and V) did not differ significantly from group I, but it increased significantly when compared to group II.

SE rats treated with the CBM (group III) show non-significant changes in GSH serum levels in comparison with the group I, However, there was significant raise in GSH serum levels in comparison with the group II.

SE rats treated with S.D 25 mg/kg/day and L.D 100mg/kg/day of UDCA (groups IV and V) exhibited non-significant change in serum level of GSH in comparison with the group III.

Table 3: Effect of UDCA, and carbamazepine on serum levels of GSH.

Treatment Groups	Type of Treatments	GSH (µmol/l) Mean ± SD
I	Normal saline only	178.5± 9.9 [a]
II	LiCl+ Sco+ Pilocarpine	68.5 ± 7.2*
III	Carbamazepine +Pilocarpine	186.8 ± 17.5 [a]
IV	S.D UDCA +Pilocarpine	169.4 ± 15.7 [a]
V	L.D UDCA+Pilocarpine	169.8 ± 13.7 [a]

-A significant difference between the groups, and group I is denoted by the superscript (*). (P< 0.05).

-A significant difference between the groups, and group II is indicated by the superscript (a) (P< 0.05).

Histopathological Examination of the brain Sections

Negative control group (Group I):

The molecular layer, exterior granular layer, pyramidal layer, inner granular layer, ganglionic layer, and multiform layer in the

cerebral histopathology figures all appeared normal (Figure 1). The putamen of the basal ganglion displayed typical neurons, supporting cells and the nigrostriatal tract (Figure 2). The bundles of myelin sheaths, and normal neurons and supporting cells can be seen in the globus pallidus (Figure 3).

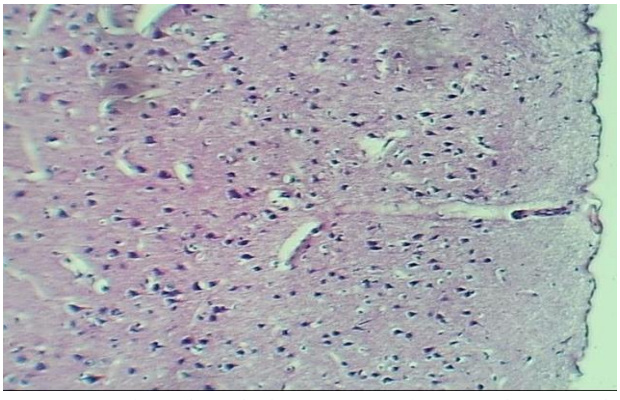

Figure 1: Section of cerebral cortex (negative control group) shows: Molecular layer, outer granular layer, pyramidal layer, inner granular layer, ganglionic layer, and pia mater all seem normally.

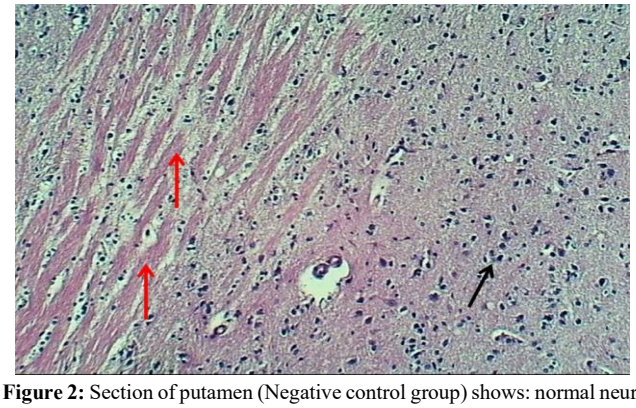

Figure 2: Section of putamen (Negative control group) shows: normal neurons and supporting cells (Arrows), as well as a normal nigrostriatal tract (Red arrow).

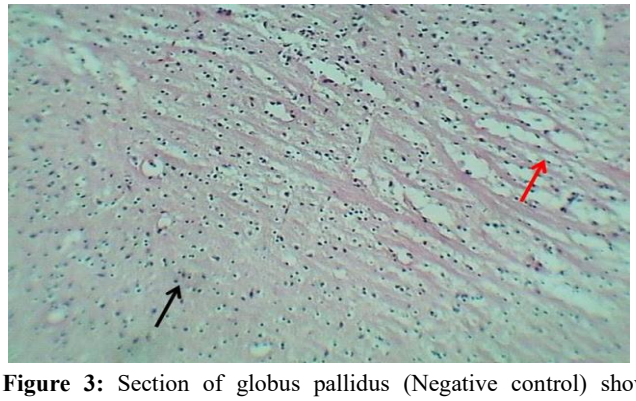

Figure 3: Section of globus pallidus (Negative control) shows: normal appearance of neurons and supporting cells (Black arrows) with shatter of bundles of myelin sheath (Red arrow).

Scopalamine+ LiCl +Pilocarpine group (Group II):

The inner granular layer, and ganglionic layer of the cerebral cortical layers' neurons showed a modest amount of vacuole development in the cerebral layers' histopathological images (Figure 4). Additionally, the putamen of the basal ganglion demonstrated mild vacuolar alterations within the small, and big neurons with normal bundles of the nigrostriatal tract (Figure 5). Figures of the globus pallidus show moderate demyelination of the myelin sheath bundles with supporting cell micro vacuoles. The big neurons, supporting cells and bundles of myelin sheaths in the globus pallidus appear normal (Figure 6).

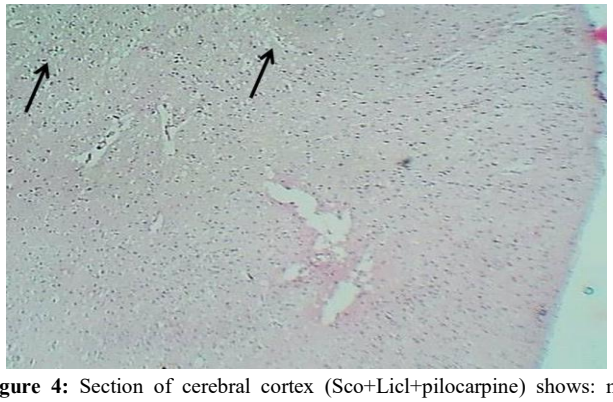

Figure 4: Section of cerebral cortex (Sco+Licl+pilocarpine) shows: mild vacuoles development in the cerebral cortex's (Arrows) inner granular layer and ganglionic layer neurons.

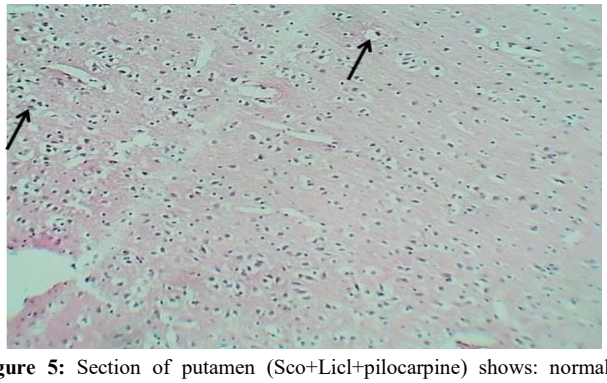

Figure 5: Section of putamen (Sco+Licl+pilocarpine) shows: normal of nigrostriatal tract with mild vacuolar alterations inside supporting cells (arrows).

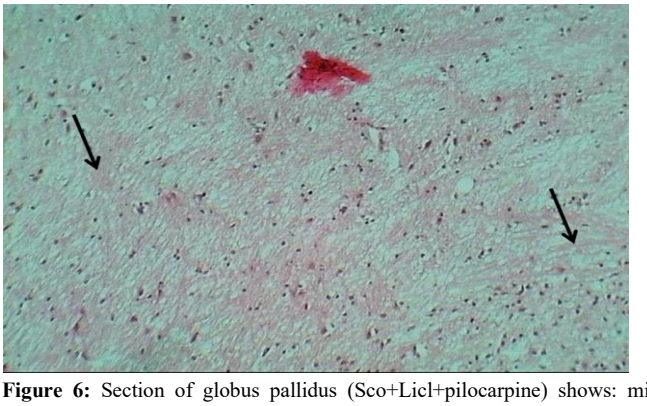

Figure 6: Section of globus pallidus (Sco+Licl+pilocarpine) shows: mild demyelination of the bundles of myelin sheaths with micro/vacuoles of supporting cells (arrows). H&E stain.

Carbamazepine group (Group III):

The histopathological figures of the cerebral layers showed normal appearance of molecular layer, external granular layer, pyramidal layer, inner granular layer, ganglionic layer , and multiform

layer and mild cortical congestion (Figure 7). The subcortical parts of basal ganglion; putamen showed moderate vacuolar changes within the supporting cells around the nigrostriatal tract and within neurons (Figure 8). Globus pallidus also was showed normal appearance of the bundles of myelin sheaths with normal neurons and supporting cells (Figure9).

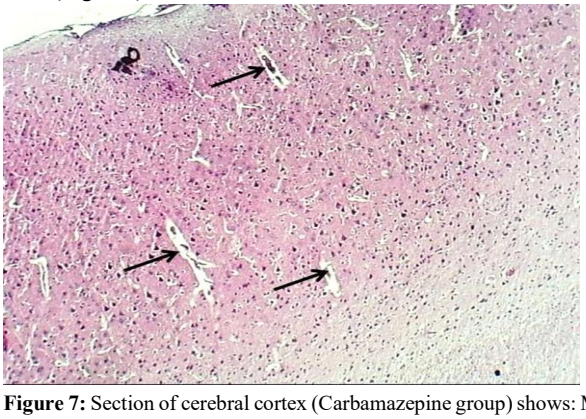

Figure 7: Section of cerebral cortex (Carbamazepine group) shows: Molecular layer, external granular layer, pyramidal layer, inner granular layer, ganglionic layer, and mild congestion (Arrows) are all present in a normal state.

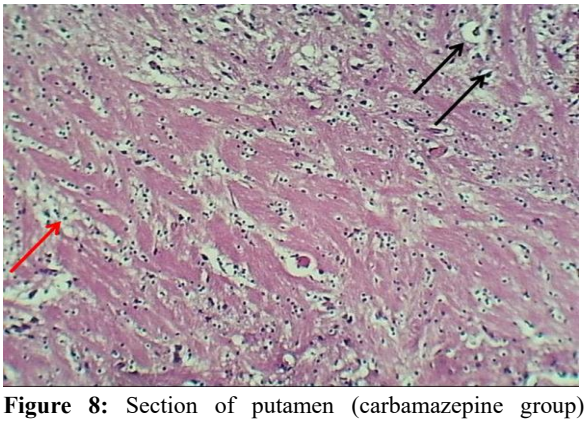

Figure 8: Section of putamen (carbamazepine group) shows: moderate vacuolization of neurons (Black arrows) and supporting cells surrounding the nigrostriatal tract (Red arrow).

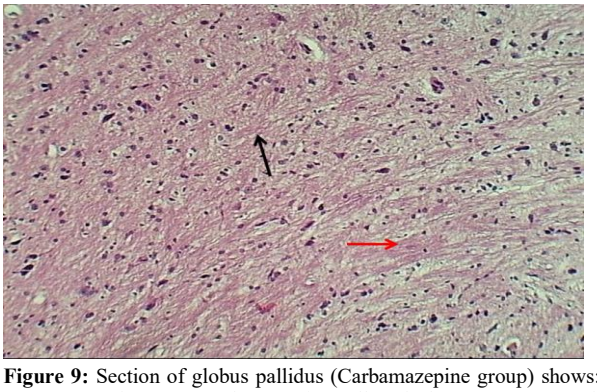

Figure 9: Section of globus pallidus (Carbamazepine group) shows: normal appearance of neurons, and supporting cells (Black arrows) & normal bundles of myelin sheath (Red arrow).

Small dose ursodeoxycholic-acid treatment (Group IV):

The molecular layer, exterior granular layer, pyramidal layer, inner granular layer, ganglionic layer, and multiform layer in the cerebral histopathology figures all appeared normal (Figure 10). Neurons and the nigrostriatal tract appeared normal in the putamen and subcortical regions of the basal ganglia (Figure 11). The bundles of myelin sheaths, and normal neurons, and supporting cells were also seen in the globus pallidus (Figure 12).

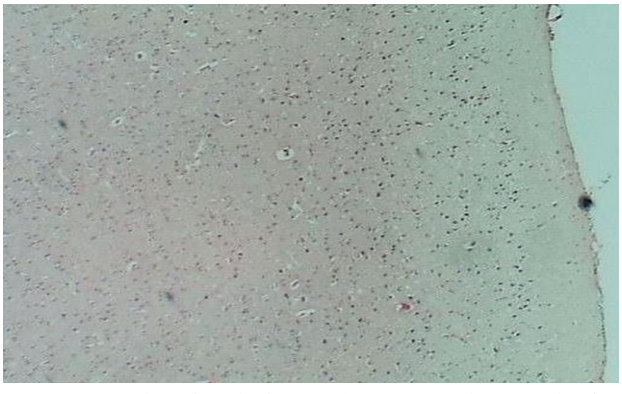

Figure 10: Section of cerebral cortex (S.D UDCA) shows: Molecular layer, external granular layer, pyramidal layer, inner granular layer, ganglionic layer, and pia mater all seem normal.

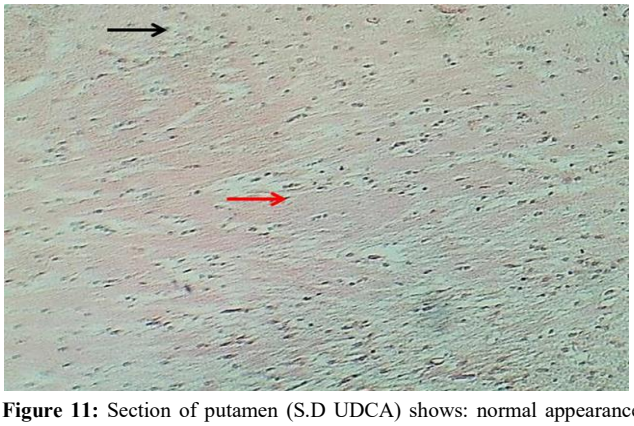

Figure 11: Section of putamen (S.D UDCA) shows: normal appearance of nigrostriatal tract (Red arrow), and neurons (Black arrows).

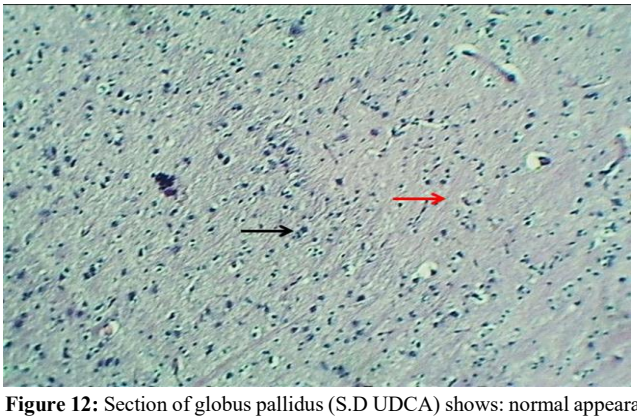

Figure 12: Section of globus pallidus (S.D UDCA) shows: normal appearance of neurons, and supporting cells (Black arrows), and bundles of myelin sheath (Red arrow).

Large dose ursodeoxycholic-acid treatment (Group V):

The molecular layer, exterior granular layer, pyramidal layer, inner granular layer, and ganglionic layer all appeared normally in the cerebral histological figure (Figure 13). The subcortical regions of

basal ganglion, putamen showed mild vacuolar alterations of nigrostriatal tract and neurons with congestion (Figure 14). The globus pallidus also was showed moderate vacular changes within neurons and supporting cells with mild congestion around the bundles of myelin sheath (Figure 15).

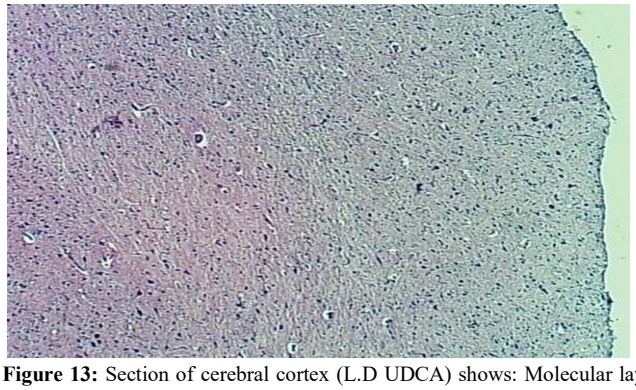

Figure 13: Section of cerebral cortex (L.D UDCA) shows: Molecular layer, outer granular layer, pyramidal layer, inner granular layer, ganglionic layer, and pia mater all seem normal.

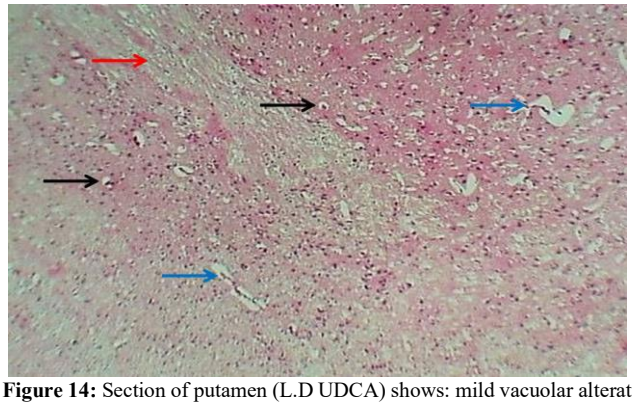

Figure 14: Section of putamen (L.D UDCA) shows: mild vacuolar alterations within nigrostriatal tract (Red arrow), and neurons (Black arrows) and mild congestion.

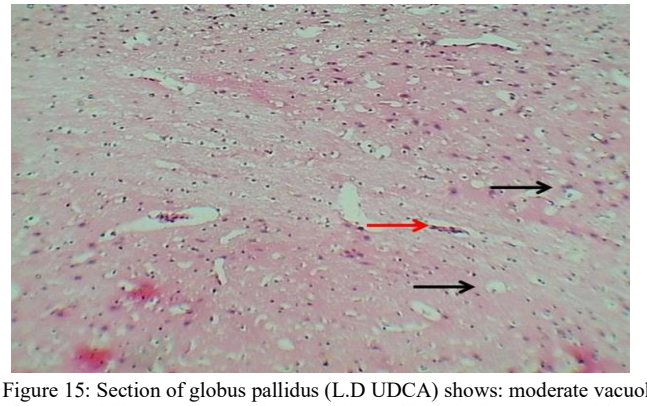

Figure 15: Section of globus pallidus (L.D UDCA) shows: moderate vacuolar changes within neurons, and supporting cells (Black arrows) with mild congestion around the bundles of myelin sheath (Red arrow).

Discussion

During SE brought on by pilocarpine, increased generation of free radicals, there may be a rise in lipid peroxidation, a reduction in GSH levels and after acute (3 days) SE in rats, caspase-3 expression and activation have been shown to increase. TNFa concentrations increased in the entire brain tissue and hippocampal injury brought on by SE has been linked to the production of pro-inflammatory cytokines [28].

Effect of Ursodeoxycholic-acid and Carbamazepine on Serum Caspase 3 levels:

In the current study, intraperitoneal injection (i.p) of pilocarpine induces apoptosis in the rat's brain represented by elevation of the serum Caspase-3 when compared to the negative control group (Table 1). These outcomes are consistent to other previous studies that reported increased Caspase-3 levels following SE [29].

The serum caspase3 levels in SE rats treated with S.D. and L.D. of UDCA considerably decreased when compared to the group II, whereas there was no significant difference when compared to the negative control group (Table 1). These findings supported a prior study's finding that UDCA potently inhibits apoptosis via modulating the Bax/Bcl-xL/cleaved Caspase-3 signaling pathway [30].

The serum Caspase-3 levels of SE rats treated with CBM significantly increased compared to the group II and significantly decreased compared to the negative control group (Table 1). These findings supported prior work that demonstrated no caspase3 activation in the presence of CBM [31].

Effect of ursodeoxycholic-acid and Carbamazepine on Tumor Necrosis Factor-α Levels

After receiving pilocarpine, group II had considerably greater serum TNF-α levels than the negative control group (Table 2). These results corroborated those of a recent study by Han and colleagues, which found that the proinflammatory cytokine TNF-α was substantially expressed in epileptic rats and that an inflammatory reaction played a role in the pathophysiological mechanism of epilepsy [32].

S.D. and L.D. UDCA treatment significantly decreased the serum TNF-α levels after pilocarpine delivery as compared to the group II but had no significant difference when compared to the negative control group (Table 2). These results supported those of an earlier study that showed the UDCA inhibits nuclear factor-B (NF-B) activation to decrease the production of the pro-inflammatory cytokines TNF-α and NO [33].

CBM treatment of SE rats resulted in a significant decrease in the serum TNF-α levels when compared to the group II, but not a significant difference when compared to the negative control group (Table 2). These results, which concurred with earlier research, show that some anti-seizure drugs are capable of reducing inflammation in the brain. The fact that CBM decreased the expression of TNF-α supports this observation [34].

Effect of ursodeoxycholic-acid and carbamazepine on reduced glutathione levels

In the current study, following pilocarpine delivery caused brain damage, as shown by a decreased level of GSH in comparison with the negative control group (Table 3). These results are in agreement with the recent study that discovered decreased antioxidant levels in the hippocampus, cortex, other brain regions, or the total brain, including nitric oxide synthesis (NOS), CAT, SOD, GR, and GSH [35].

Serum GSH levels in SE rats treated with S.D and L.D UDCA were significantly greater than those in group II and showed no significant change when compared to the control group (Table 3) demonstrating a decreased damage of the brain. These results supported those of an earlier study that demonstrated the antioxidant effect of increased SOD, CAT, and GSH-Px activity [36].

Treatment of SE rats with CBM significantly increased the serum GSH levels when compared to the group II and no significant change in the serum GSH levels when compared to the control group (Table 3) demonstrating a decreased damage of the brain. According to these findings, which are in line with a prior study that approved the antioxidant effect of CBM by increase in the activity of antioxidant enzymes such as SOD and GR [37].

Effect of ursodeoxycholic-acid and carbamazepine on Brain Histology

The histological examination of tissue sections from each animal of group II (Figures: 4,5,6), exhibited significant degenerative changes in brain when compared to the negative control group (Figures: 1,2,3), showed a modest amount of vacuole development in the cerebral layers' histopathological images of the inner granular layer and ganglionic layer of the cerebral cortical layers' neurons. Additionally, the putamen of the basal ganglion demonstrated mild vacuolar alterations within the small, and big neurons with normal bundles of the nigrostriatal tract. These neurodegenerative effects of pilocarpine on the brain can be explained by its direct toxicity mediated by its ability to trigger excessive caspase3 expression, TNF-α concentration, free radical production, and decrease in GSH concentration [29,23,35].

Treatment of SE rats with CBM showed improved brain histology although the subcortical parts of basal ganglion; putamen showed moderate vacuolar changes within the supporting cells around the nigrostriatal tract and within neurons (Figure 8). Brain histological improvement changes in rats treated with CBM are due to its anti-inflammatory, antioxidant, and anti-apoptotic effect that act by different mechanisms to protect the brain tissue from ROS, lipid peroxidation, inflammation and apoptosis [31,34,37].

Treatment with S.D and L.D of UDCA showed improved in the brain histology although in L.D UDCA treatment the subcortical regions of basal ganglion, putamen showed mild vascular alterations of nigrostriatal tract and neurons with congestion (Figure 14) and the globus pallidus also was showed moderate vacuolar changes within neurons and supporting cells with mild congestion around the bundles of myelin sheath (Figure 15). Brain histological improvement changes in rats treated with S.D and L.D UDCA are due to its action as anti-inflammatory, antioxidant, and anti-apoptotic effect that act by different mechanisms to protect the brain tissue from ROS, lipid peroxidation, inflammation, and apoptosis [31,33,36].

Recommendations for future studies

Defining the exact molecular mechanism standing for the neuroprotective effect of UDCA on SE patients.

Evaluating the protective effects of UDCA using different doses.

A clinical study to assess the ameliorative effects of UDCA in SE patients.

Conclusion

Our results explore that UDCA at different doses have a beneficial neuroprotective effect in SE rats in comparison with CBM. UDCA neuroprotective effect in SE is recognized by its anti-apoptotic effect (Reduced serum Caspase-3 levels), anti-inflammatory effect (Reduced serum TNF-α levels), and antioxidant effect (Increase serum GSH levels).

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Conflict of Interest

Authors declare no conflict of interest.

Data availability

The datasets produced in this animal experimental study are available from the corresponding author upon reasonable request.

All procedures involving animals were approved by the appropriate institutional ethics committee and conducted in accordance with international standards for animal research.

Author Contributions

All authors contributed equally to the conception of the review, literature search, data interpretation, manuscript drafting, and final approval of the paper.

All authors meet the ICMJE criteria for authorship and agree to be accountable for all aspects of the work.

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