HistoryofMedicine,2023,9(1):382-393
DOI:10.48047/V9.I1.2023.382-393

Neuroprotective effects of Ursodeoxy cholic-acid in comparison to Diazepam against Pilocarpine-induced status epilepticus in experimental rats.

Zainab Adil, Ahmed Hamed

University of Baghdad College of Pharmacy
Zainab.adel1200m@copharm.uobaghdad.edu.iq

Abstract

Background: Epilepsy is the fourth most frequent neurological disorder worldwide. Epilepsy can be diagnosed if someone has two or more spontaneous seizures. Subtypes M1 and M3 of the receptor are affected by pilocarpine, with the M3 receptor having the ability to activate phospholipase C. In this way, the second messenger's inositol triphosphate, diacylglycerol, calcium, and protein kinase are produced. Because of this, M3 cholinergic agonists cause calcium to be upregulated, leading to smooth muscle contraction. Ursodeoxycholic acid is an effective drug for treating cholestatic liver disease. There are a number of interconnected and synergistic ways in which ursodeoxycholic acid affects the liver.

Method: Thirty adult male Wistar rats, weighing 200 ± 20 g were utilized in this study
Group I(negative Control): Rats will be received of N/S(0.5 ml/kg)
Group II(Sco+ LiCa +Pilo): Rats received LiCa; 127 mg/kg, followed by pilocarpine administration (30 mg/kg) .
scopolamine (1 mg/kg in rats) is also given to the rats 30 min before pilocarpine administration.
Group III(Diazepam):Epileptic rats treated with diazepam (20 mg/kg)was started 2 h after the beginning of SE.Group IV (min. dose of UDCA): Epileptic rats will be received UDCA 25 mg/kg/day.Group V (max. dose of UDCA) Epileptic rats will be received UDCA 100 mg/kg/day .

Result: Based on measurement of markers: TNF-α, GSH, we reported increase the level of TNF-α & decrease the level of GSH when given pilocarpine, while we found raise in the GSH level & decrease in the level of TNF-α in UDCA treated-group

Conclusion: It can be concluded that the UDCA improve the biochemical alterations (decrease GSH & increase TNF) that occur after S.E and UDCA have the same effect when comparison with diazepam effect.

Keywords: Status epilepticus(SE), Pilocarpine(Pilo), Diazepam(DZM), Lithium Carbonate(LiCa), Ursodeoxycholic-acid(UDCA), Reduced Glutathione Levels (GSH), Tumor Necrosis Factor-αlpha(TNF-α),Small dose(S.D), Large dose(L.D).

1.Introduction

After headache and stroke, epilepsy is the fourth most common neurological disorder in April 2018. Having two or more seizures that did not have a clear cause could classify a person as epileptic (1). Patient age, concomitant drug use, and the manifestation of adverse effects are all taken into account when designing a treatment plan that is tailored to each patient's specific type of seizure. Instead of being a single ailment, epilepsy is more accurately thought of as a spectrum of diseases with multiple comorbidities (autism, intellectual disability, attention-deficit hyperactivity disorder, learning disabilities, anxiety anddepression) (2). Failure of seizure termination mechanisms and the initiation of processes that prolong seizures beyond their normal duration are now both included in the status epilepticus definition (after time point t1). This state can have long-term (post-t2) repercussions, including as neuronal death, neuronal damage, and alterations to brain networks, depending on the kind and severity of seizures. (3). Seizures in status epilepticus are known to disrupt the inhibition mediated by GABA-A receptors in crucial brain regions. (4). Seizure-ending processes may be jeopardized by this reduced inhibition.

Pilocarpine, or Pilo, is a drug that acts as an acetylcholine muscarinic agonist. The Pilo is useful for treating and managing both acute angle-closure glaucoma and radiation-induced xerostomia.

Xerostomia result in radiation exposure and Sjogren's disease can be treated with Pilo, and it can also be used to treat glaucoma by lowering intraocular pressure (IOP). (5) Additional drugs to treat radiation-induced xerostomia have not been shown to have a positive effect. (6). It acts as a complete and partial agonist at the muscarinic M3 acetylcholine receptor. The excitatory M3 receptor is expressed by gastric and salivary glands as well as smooth muscle cells such as those of the ciliary bodies and pupillary sphincter. Activation of the Gq receptor is triggered by the M3 receptor, which in turn activates phospholipase C. Second messengers such inositol triphosphate, diacylglycerol, calcium, and protein kinase are generated as a result. (7). Not only that, but it can also stimulate the production of more saliva (8).

As a rule, lithium is suggested. Mania and unstable mood are the primary symptoms that lithium is meant to address whether a patient has a history of a manic episode or is on maintenance medication for bipolar disease (9). It is not known how exactly lithium works. It is absorbed rapidly, localized, and excreted unchanged in the urine (there is no metabolism of lithium). Muscle and nerve cells' sodium transport is affected by lithium. It affects the neurotransmitter metabolism, especially of serotonin and catecholamines (10). The inhibition of inositol monophosphate via second messenger systems may have an effect on intracellular signaling. It is through the phosphatidylinositol secondary messenger route that this inhibition ultimately affects neurotransmission. In addition, lithium inhibits protein kinase C, a change in which affects genomic expression associated with neurotransmission. It has been shown that lithium can stimulate neurogenesis, expand gray matter, and appear to increase cytoprotective proteins (11).

Scopolamine can be found in the Hyoscyamusniger (henbane), plants,ScopoliacarniolicaandDaturastramonium (Jimsonweed). These plants' defense strategy involves the production of deadly compounds called belladonna alkaloids. (12). Scopolamine is a nonselective muscarinic antagonist that blocks acetylcholine receptors via competitive binding to G-proteins in the brain's post-ganglionic muscarinic receptors, producing a variety of effects including sedation, amnesia, and suppression of nausea and vomiting in the central nervous system. (13).

The FDA has licensed the benzodiazepine drug diazepam (DZM) for various medical purposes, including non-limited to the management of severe recurring convulsive seizures, the status epilepticus therapy, the anxiety disorders treatment, and the short-term relief of anxiety symptoms. Multiple sites of action of benzodiazepines contribute to their ability to increase gamma-aminobutyric acid (GABA) activity. Benzodiazepines, especially, attached to an allosteric site at the alpha/gamma interdomain junction of the chloride ion channel of the GABA-A receptor. DZM's allosteric interaction with the GABA-A receptor raises the frequency leads to opening the channel ofchloride, allowing for more chloride ion conduction. This change in charge resulting inthe neuronal membrane hyperpolarization and reduces excitability. Cholestatic liver disease is treated and managed with ursodeoxycholic acid (UDCA). Changing the bile acid pool, acting as a city-protectant, immunomodulator, and choleretic, and so on are just a few of the complex and complementary ways in which ursodeoxycholic acid influences the liver. The cholesterol component of biliary lipids is also greatly reduced, showing that UDCA prevents both intestinal absorption and its release into bile (15,16). Ursodeoxycholic acid is a bile acid that has been shown to ameliorate clinical and biochemical markers of liver disease (17,18). Evidence from studies shows it has a significant impact on cellular apoptosis (18,19). It has been proposed that inhibiting FAS ligand-induced apoptosis and mitochondrial release of cytochrome C are two ways to reduce bile acid-induced cell death (20). However, bile acids have been shown to have preventive effects against multiple brain illnesses, such as Huntington's, Alzheimer's and Parkinson's diseases (17, 21,22).

2. Methods

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

2.1 preparation of drugs

On the day of administration, a suspension of Lithium Carbonate(LiCa)(23) freshly prepared by dissolving each 127mg / kg LiCa powder in 1 ml of Normal saline, which is used as a standard solution for the preparation of doses used in this study(24).

Pilocarpine solution Freshly prepared by dissolving each 10mg/kg of Pilo. powder in 1 ml of Normal saline. Seizure severity was rated by Racine’s scale(25). Lithium 127mg/kg was administered intraperitoneal(I.P) 20 h before Pilo administration .pilo was administered in dosesof 3, 10, 30 or 60 mg/kg in order to determine the minimal doserequired to induce continuous electrographic and behavioral seizures(24).

2.2 Animal selection

Thirty adult male Wistar rats, weighing 200 ± 20 g, were utilized in this work. These rats were procured from and kept in the Animal House at the College of Pharmacy / University of Baghdad, 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. Prior to receiving the medicine, these rats were acclimatized to the above settings by being handled and housed there on a daily basis for 7 days.

2.3 Experimental protocol

Both the Scientific and Ethical Committees at the College of Pharmacy/University of Baghdad gave their signatures of approval to this work. Sixty rats were used in the trial, and they were split evenly among five groups of six rates, as follows:

Group I(Negative Control): Rats will be received of N/S(0.5 ml/kg) for 2 days.
Group II (Sco+ LiCa +Pilo group); Rats received LiCa 127 mg/kg, followed by Piloadministered in doses of 3, 10, 30 or 60 mg/kg 20 h later(24),Methylscopolamine (1 mg/kg in rats) is given to the rats thirty minutes before administration of pilo.
Group III(DZM treatment):Epileptic rats treated with DZM (20 mg/kg, I.P was initiated two hours after the SE starting and an additional dose (10 mg/kg, I.P) was given six hours later, hence, rats did not take any treatment(26).
Group IV(minimum dose of UDCA treatment): Epileptic rats will orally-receive UDCA 25 mg/kg/day for 3days(27).
Group VI (maximum dose of UDCA treatment): Epileptic rats will orally-receive UDCA 100 mg/kg/day for 3days(27).

After the experiment was finished, all of the animals were killed by being injected with diethyl ether, which acts as an anesthetic and causes the animal to quickly and painlessly pass away. Serum was extracted by drawing blood from the jugular vein (near the neck) of each rat, letting the samples clot at temperature of room for 30 min, and then centrifuging them at 3000 rpm for 20 min. Supernatant was aliquoted into micro centrifuge tubes at a concentration of 250 l. (Eppendorf). Thereafter, the tubes were stored at 20 degrees Celsius until it was time to calculate the markers.

3.RESULTS

3.1.Effect of ursodeoxycholic-acid and diazepam onTumor Necrosis Factor-α Level :

-According to (Table 3-1) and (Figure 3-1), There was a significant increase in serum Blood TNFa level when the mean of group II (Sco+ LiCa +Pilo-treated group) was compared with the group I (control group) (34.10 ± 1.64vs. 26.61 ± 1.68) (P> 0.05).

-Rats treated with minimum dose (M.D) 25mg/kg/day and maximum dose (Mx.D)100mg/kg/day of UDCA (groups IV and V) exhibited non-significant changes in serum level of TNFaincomparsion with the controls, however, there was significant decrease in serum level TNFa as compared to Sco+ LiCa +Pilo-treated group (group II).

-Rats treated with the DZM-treated group (group III) exhibited non-significant change in serum level of TNFa in comparison with the controls, but yet there was significant decrease in serum level of TNFa as compared to Sco +LiCa+ Pilo-treated group(group II).

-Rats treated with M.D 25mg/kg/day and Mx.D 100mg/kg/day of UDCA (groups IV and V)exhibited non-significant change in serum level of TNFa compared with the DZM-treated group(group III).

Table 3.1: Impacts of UDCA and DZM on TNFa serum levels.

Treatment Groups	Type of Treatments	TNF-αng/l Mean ± SD
I	Normal Saline Only	26.61 ± 1.68 a
II	LiCa+Sco.+ Pilo.	34.10 ± 1.64*
III	DZM+Pilo.	27.25 ± 2.33a
IV	M.D UDCA +Pilo.	26.49 ± 1.85 a
V	Mx.DUDCA+Pilo.	27.26 ± .1.51 a

- Superscript (a) indicates significant difference when groups I, III, IV and V were compared to group II (P<0.05).

-Superscript (*) indicates significant difference when groups II, III, IV and V were compared to the control group (P<0.05).

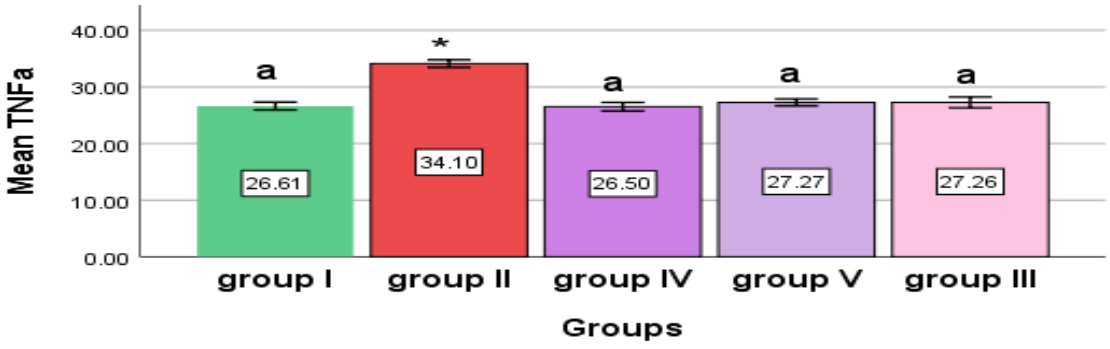

Figure (3-1) :

- Superscript (a) indicates significant difference when groups I, III, IV and V were compared to group II (P<0.05).

-Superscript (*) indicates significant difference when groups II, III, IV and V were compared to the control group (P<0.05).

3.2. Impact of ursodeoxycholic-acid and diazepam on Reduced Glutathione Levels:
According to (Table 3-2) and (Figure 3-2): there was a significant decrease in serum Blood GSH level when the mean of group II Sco +LiCa+ Pilo was compared with the group I (control group) (178.1 ± 16.3 vs. 69.8 ± 10.1) (P> 0.05).

Rats treated (T) with M.D 25mg/kg/day and Mx.D 100mg/kg/day of UDCA (groups IV and V) exhibited non-significant changes in GSH serum level incomparison with the controls (group I), however, there was significant raise in GSH serum level as compared to Sco.+LiCa +Pilo treated group(group II).

Rats treated with the DZM-treated group (group III) show non-significant changes in GSH serum level in comparison with the controls (group I). However, there was significant raise in GSH serum level incomparisonwithSco.+ LiCa + Pilotreated group(group II).

Rats treated with M.D 25mg/kg/day and Mx.D 100mg/kg/day of UDCA (groups IV and V) exhibited non-significant change in serum level of GSH in comparison with the DZM-treated group(group III).

Table 3.2 : Effects of UDCA and DZM 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	LiCa+Sco.+ Pilo.	68.5 ± 7.2*
III	DZM+Pilo.	171.2 ± 17.4 a
IV	M.D UDCA +Pilo.	169.4 ± 15.7 a
V	Mx.DUDCA+Pilo.	169.8 ± 13.7 a

- Superscript (a) indicates significant difference when groups I, III, IV and V were compared to group II (P<0.05).

-Superscript (*) indicates significant difference when groups II, III, IV and V were compared to the control group (P<0.05).

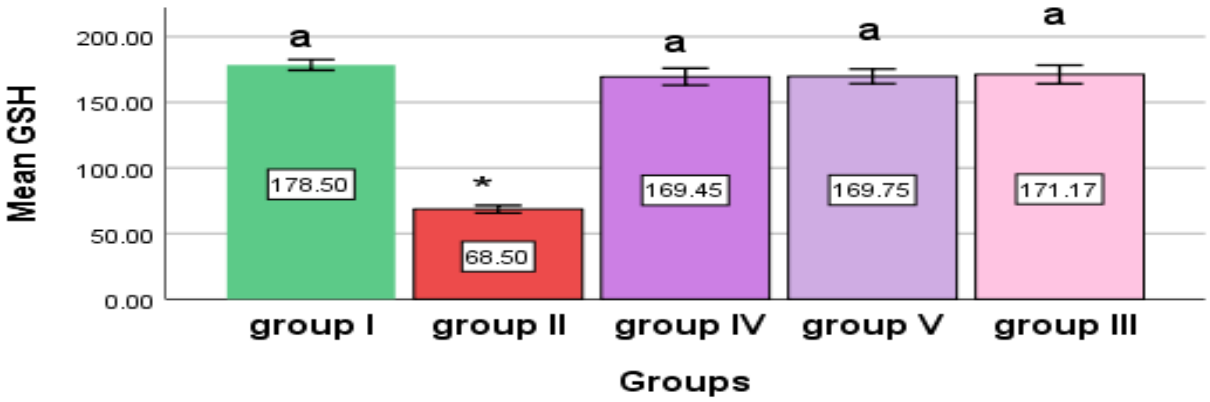

Figure (3-2) :

- Superscript (a) indicates significant difference when groups I, III, IV and V were compared to group II (P<0.05).

-Superscript (*) indicates significant difference when groups II, III, IV and V were compared to the control group (P<0.05).

4.DISCUSSION

4.1 Effect of ursodeoxycholic-acid and diazepam on Tumor Necrosis Factor-α Level:

Tumor necrosis factor alpha (TNF-) is a pleiotropic cytokine, meaning that it affects many different kinds of cells. It plays a role in the development of several inflammatory and autoimmune illnesses and has been recognized as a significant regulator of inflammatory responses. (28) TNF- is a homotrimer protein made up of 157 amino acids, and it is mostly produced by activated macrophages, T-lymphocytes, and natural killer cells. (29) Its primary role is to set off a chain reaction of other inflammatory chemicals, such as cytokines and chemokines. There are two forms of TNF-: soluble and transmembrane.

In comparison to the controls, the Pilo group had significantly higher TNFalevels in the brain after a single I.P dose (table:3-1) (figure : 3-1).

These findings corroborated those of a recent study by Han and colleagues(30), which found that the pro-inflammatory cytokine TNF- was substantially expressed in epileptic rats and that an inflammatory reaction played a role in the pathophysiological mechanism of epilepsy.

After Pilo delivery, M.D and Mx.D UDCA treatment significantly reduced blood TNFa levels compared to the Pilo-treated group, while having no effect on serum TNFa levels compared to the control group or the DZM group (P>0.05), as inFigure 3-1; Table 3-1. These findings corroborated those of a prior study which found that the secondary bile acid UDCA particularly suppresses TNFa-induced release of the proinflammatory cytokine IL-8 from monocytes. The bile acid appears to exert its effects via blocking TRAF-2-mediated NF B activation, which then reduces IL-8 mRNA expression. Mucosal immune responses would be suppressed by UDCA's effects in vivo, so it could offer an alternative to biologics for preventing TNF-a caused inflammation in inflammatory bowel disease (IBD) patients. (31)

Serum levels of tumor necrosis factor alpha (TNFa) were significantly lower in the group treated withDZMafterPilo administration incomparison with the Pilo-treated group and were not significantly different from the control group (Table 3-1) and (Figure 3-1). This indicated less brain injury. To confirm the findings of a prior investigation, we observed that DZM bounds to peripheral benzodiazepine receptorswith high affinity, which are unique from the central benzodiazepine receptors (32). Acute DZM therapy has been demonstrated to reduce interleukin secrete from macrophages and inhibit activity of neutrophil (33). Due to the PBRs existence in the immune and adrenal cells, high doses of DZM (10 and 20 mg/kg) have been demonstrated to decrease inflammation in rats (34). In addition, it has been found that the generation of inflammatory mediators by macrophages, including interleukin-1, tumor necrosis factor-alpha, and interleukin-6, is significantly decreased when mice are treated with the PBRs agonist Ro5-4864 (35).

4.2Effects of ursodeoxycholic-acid and diazepam on Reduced Glutathione Levels:
The body creates glutathione (GSH) from L-cysteine, L-glutamic acid, and glycine to form a tri-peptide. In addition to its role as an antioxidant in protecting cells from free radical and peroxide damage, glutathione (GSH) has an importantfunction 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. (36,37)

In this investigation, a single intraperitoneal injection of Pilo caused brain inflammation, as shown by a higher level of glutathione (GSH) in comparisonwith the controls (table:3-2) (figure : 3-2).

These findings corroborated those of a recent study, which found that Pilo-induced seizure models can be used to learn about the behavioral and neurochemical features of seizures (38,39). Various biochemical systems may undergo lasting alterations during SE, according to other research. During Pilo-induced SE, lipid peroxidation, reduced GSH levels, and free radical overproduction may all occur (40,41).

Serum glutathione (GSH) levels in rats treated with M.D and Mx.D UDCA after Pilo administration were significantly greater than those in the Pilo-treated group and

showed no significant change when compared to the control group and the DZM group (P>0.05; Table 3-2; Figure 3-2). These findings corroborated those of an earlier study showing that UDCA boosted GSH production via activation of the PI3K/Akt/Nrf2 pathway. Therapeutic efficacy in chronic hepatitis may be due in part to this anti-oxidative action of UDCA (42).

Serum glutathione (GSH) levels were significantly higher in DZM-treated rats after Pilo administration compared to the Pilo group, while serum tumor necrosis factor alpha (TNFa) levels were not significantly different from the controls (Table 3-2) and (Figure 3-2). This indicated less brain damage. The findings corroborated those of an earlier study, which proposed using benzodiazepines as antioxidant medicines (43).

CONCLUSION

This study shows that UDCA successfully prevented the S.E. from developing status epilepticus after being triggered by Pilo. in rats. UDCA's protective impact can be attributed, in large part, to the compound's antioxidant and anti-inflammatory properties. As a result, UDCA is a contender for a neuroprotective effect following SE.

References

1. Goldenberg, M.M. Overview of drugs used for epilepsy and seizures: Etiology, diagnosis, and treatment. Pharm. Ther. 2010, 35, 392–415.
2. Stafstrom, C.E.; Carmant, L. Seizures and epilepsy: An overview for neuroscientists. Cold Spring Harb.Perspect. Med. 2015, 5,a022426.
3. Trinka E, Cock H, Hesdorffer D, et al. A definition and classification of status epilepticus: Report of the ILAE Task Force on Classification of Status Epilepticus. Epilepsia. 2015;56:1515–23.
4. Joshi S, Kapur J. GABAA receptor plasticity during status epilepticus. In: Noebels JL, et al., editors. Jasper's Basic Mechanisms of the Epilepsies. Bethesda MD: Oxford University Press; 2012. pp. 545–51.
5. Delli K, Spijkervet FK, Kroese FG, Bootsma H, Vissink A. Xerostomia. Monogr Oral Sci. 2014;24:109-25.
6. Davies AN, Thompson J. Parasympathomimetic drugs for the treatment of salivary gland dysfunction due to radiotherapy. Cochrane Database Syst Rev. 2015 Oct 05;2015(10):CD003782.
7. Carlson AB, Kraus GP. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Aug 22, 2022. Physiology, Cholinergic Receptors.
8. Pronin AN, Wang Q, Slepak VZ. Teaching an Old Drug New Tricks: Agonism, Antagonism, and Biased Signaling of Pilocarpine through M3 Muscarinic Acetylcholine Receptor. MolPharmacol. 2017 Nov;92(5):601-612.
9. Hayes JF, Pitman A, Marston L, Walters K, Geddes JR, King M, Osborn DP. Self-harm, Unintentional Injury, and Suicide in Bipolar Disorder During Maintenance

Mood Stabilizer Treatment: A UK Population-Based Electronic Health Records Study. JAMA Psychiatry. 2016 Jun 01;73(6):630-7.

10. Perveen T, Haider S, Mumtaz W, Razi F, Tabassum S, Haleem DJ. Attenuation of stress-induced behavioral deficits by lithium administration via serotonin metabolism. Pharmacol Rep. 2013;65(2):336-42.
11. Sheng R, Zhang LS, Han R, Gao B, Liu XQ, Qin ZH. Combined prostaglandin E1 and lithium exert potent neuroprotection in a rat model of cerebral ischemia. ActaPharmacol Sin. 2011 Mar;32(3):303-10.
12. Pergolizzi JV, Philip BK, Leslie JB, Taylor R, Raffa RB. Perspectives on transdermal scopolamine for the treatment of postoperative nausea and vomiting. J ClinAnesth. 2012 Jun;24(4):334-45.
13. Zhang XC, Farrell N, Haronian T, Hack J. Postoperative Anticholinergic Poisoning: Concealed Complications of a Commonly Used Medication. J Emerg Med. 2017 Oct;53(4):520-523.
14. Nutt DJ, Malizia AL. New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. Br J Psychiatry. 2001 Nov;179:390-6.
15. Guarino MP, Cocca S, Altomare A, Emerenziani S, Cicala M. Ursodeoxycholic acid therapy in gallbladder disease, a story not yet completed. World J Gastroenterol. 2013 Aug 21;19(31):5029-34.
16. Roma MG, Toledo FD, Boaglio AC, Basiglio CL, Crocenzi FA, Sánchez Pozzi EJ. Ursodeoxycholic acid in cholestasis: linking action mechanisms to therapeutic applications. ClinSci (Lond). 2011 Dec;121(12):523-44.
17. Rodrigues CM, Spellman SR, Sola S, Grande AW, Linehan-Stieers C, Low WC, et al. Neuroprotection by a bileacid in an acute stroke model in the rat. J Cereb Blood Flow Metab. 2002;22(4):463-71.
18. Rodrigues CM, Fan G, Ma X, Kren BT, Steer CJ. A novel role for ursodeoxycholic acid in inhibiting apoptosis bymodulating mitochondrial membrane perturbation. J Clin Invest. 1998;101(12):2790-9.
19. Rodrigues CM, Stieers CL, Keene CD, Ma X, Kren BT, Low WC, et al. Tauroursodeoxycholic acid partiallyprevents apoptosis induced by 3-nitropropionic acid: evidence for a mitochondrial pathway independent of thepermeability transition. J Neurochem. 2000 Dec;75(6):2368-79.
20. Azzaroli F, Mehal W, Soroka CJ, Wang L, Lee J, Crispe IN, et al. Ursodeoxycholic acid diminishes Fas-ligandinducedapoptosis in mouse hepatocytes. Hepatol. 2002;36(1):49-54.
21. Castro-Caldas M, Carvalho AN, Rodrigues E, Henderson CJ, Wolf CR, Rodrigues CM, et al. Tauroursodeoxycholicacid prevents MPTP-induced dopaminergic cell

death in a mouse model of Parkinson's disease. Molecul Neurobiol.2012;46(2):475-86.
22. Keene CD, Rodrigues CM, Eich T, Chhabra MS, Steer CJ, Low WC. Tauroursodeoxycholic acid, a bile acid, isneuroprotective in a transgenic animal model of Huntington's disease. Proc Nat AcadSci U S A.2002;99(16):10671-6.
23. Morrison Jr JM, Pritchard HD, Braude MC, D'Aguanno W. Plasma and brain lithium levels after lithium carbonate and lithium chloride administration by different routes in rats. Proceedings of the Society for Experimental Biology and Medicine. 1971 Jul;137(3):889-92.
24. Hirsch E, Baram TZ, Snead III OC. Ontogenic study of lithium-pilocarpine-induced status epilepticus in rats. Brain research. 1992 Jun 26;583(1-2):120-6.
25. Racine RJ. Modification of seizure activity by electrical stimulation, II: motor seizure. ElectroencephalogrClinNeurophysiol 1972;32:281–94
26. Pitkänen A, Kharatishvili I, Narkilahti S, Lukasiuk K, Nissinen J. Administration of diazepam during status epilepticus reduces development and severity of epilepsy in rat. Epilepsy research. 2005 Jan 1;63(1):27-42.
27. Tschirner A, von Haehling S, Palus S, Doehner W, Anker SD, Springer J. Ursodeoxycholic acid treatment in a rat model of cancer cachexia. Journal of cachexia, sarcopenia and muscle. 2012 Mar;3(1):31-6.
28. Bradley J.R. TNF-Mediated inflammatory disease. J. Pathol. 2008;214:149–160. doi: 10.1002/path.2287. [PubMed] [CrossRef] [Google Scholar]
29. Horiuchi T., Mitoma H., Harashima S., Tsukamoto H., Shimoda T. Transmembrane TNF-alpha: Structure, function and interaction with anti-TNF agents. Rheumatology. 2010;49:1215–1228.
doi: 10.1093/rheumatology/keq031. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
30. Han K, Wang QY, Wang CX, et al. (2018). Ghrelin improves pilocarpine-induced cerebral cortex inflammation in epileptic rats by inhibiting NF-κB and TNF-α. Mol Med Rep, 18(4): 3563–3568.
31. Atreya R, Neurath MF. IBD pathogenesis in 2014: Molecular pathways controlling barrier function in IBD. Nat Rev GastroenterolHepatol12: 67–68, 2015.
32. P. Casellas, S. Galiegue, and A. S. Basile, “Peripheral benzodiazepine receptors and mitochondrial function,” Neurochemistry International, vol. 40, no. 6, pp. 475–486, 2002.
33. M. Schlumpf, R. Parmar, E. E. Bütikofer et al., “Delayed developmental neuro- and immunotoxicity of benzodiazepines,” Archives of Toxicology, Supplement, vol. 17, pp. 261–287, 1995.

34. R. Lazzarini, P. C. Maiorka, J. Liu, V. Papadopoulos, and J. Palermo-Neto, “Diazepam effects on carrageenan-induced inflammatory paw edema in rats: Role of nitric oxide,” Life Sciences, vol. 78, no. 26, pp. 3027–3034, 2006.
35. S. R. Torres, T. S. Fröde, G. M. Nardi et al., “Anti-inflammatory effects of peripheral benzodiazepine receptor ligands in two mouse models of inflammation,” European Journal of Pharmacology, vol. 408, no. 2, pp. 199–211, 2000.
36. Halprin, Kenneth (1967). "The Measurement of Glutathione in Human Epidermis using Glutathione Reductase". Journal of Investigative Dermatology 1967; 48 (2): 149.
37. Scholz RW. Graham KS. Gumpricht E. Reddy CC. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. Ann NY AcadSci 1989:570:514-7. Hughes RE. Reduction of dehydroascorbic acid by animal tissues. Nature 1964; 203: 1068-9.
38. Brozek G, Hort J, Koma´rek V, Langmeier M & Mares P (2000) Interstrain differences in cognitive functions in rats in relation to status epilepticus. Behav Brain Res 112, 77–83.
39. Treiman DM (1955) Electroclinical features of status epilepticus. J ClinNeurophysiol 12, 343–362.
40. Freitas RM, Souza FCF, Vasconcelos SMM, Viana GSB &Fonteles MMF (2003) Acute alterations of neurotransmitters levels in striatum of young rat after pilocarpine-induced status epilepticus. ArqNeuropsiquiatr 61, 430–433.
41. Simonie´ A, Laginja J, Varljen J, Zupan G &Erakovie´ V (2000) Lithium plus pilocarpine induced status epilepticus: biochemical changes. Neurosci Res 36, 157–166.
42. Arisawa S, Ishida K, Kameyama N, Ueyama J, Hattori A, Tatsumi Y, Hayashi H, Yano M, Hayashi K, Katano Y, Goto H. Ursodeoxycholic acid induces glutathione synthesis through activation of PI3K/Akt pathway in HepG2 cells. Biochemical pharmacology. 2009 Mar 1;77(5):858-66.
43. C. G. Neochoritis, C. A. Tsoleridis, J. Stephanidou-Stephanatou, C. A. Kontogiorgis, and D. J. Hadjipavlou-Litina, “1,5-Benzoxazepines vs 1,5-benzodiazepines. One-pot microwave-assisted synthesis and evaluation for antioxidant activity and lipid peroxidation inhibition,” Journal of Medicinal Chemistry, vol. 53, no. 23, pp. 8409–8420, 2010.