IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, 2022

Impact of Coumarin on Brain Tissue ATPases in Wistar Strain Male Albino Rats: Insights into Epilepsy- Umbelliferon, Pentylenetetrazol (PTZ) and Diazepam Induced Toxicity

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Abstract:
Epilepsy represents a complex neurological disorder characterized by abnormal electrical activity in the brain, often leading to detrimental effects on cellular processes. This original research article investigates the toxicity of epilepsy on brain tissue by focusing on the activities of crucial enzymes, including Total ATPases, Na^+ K^+ ATPases, Mg^{2+} ATPases, and Ca^{2+} ATPases in Wistar strain male albino rats. Additionally, the study explores the modulatory effects of coumarin, a naturally occurring compound with potential therapeutic properties. The experimental design involves the induction of epilepsy in the animal model and subsequent evaluation of ATPase activities in the presence and absence of coumarin. The findings reveal alterations in ATPase activities associated with epileptic conditions, shedding light on the biochemical changes occurring in brain tissue. Furthermore, the investigation explores the potential neuroprotective effects of coumarin, providing valuable insights into its role as a modulator of ATPase activities in the context of epilepsy-induced toxicity. This research contributes to our understanding of the molecular mechanisms underlying epilepsy-related alterations in brain tissue and suggests avenues for further exploration of coumarin as a potential therapeutic intervention for mitigating these effects.

Keywords : ATPases, Epileosy, Coumarin, UMB, PTZ, Diazepam, Male Albino Rats .

Introduction:

Epilepsy is a neurological condition characterised by aberrant excessive synchronised neuronal activity in the brain that occurs only briefly **(Thurman *et al.*, 2017).** The major cognitive, psychological, and behavioural aspects of epilepsy exacerbate the vulnerability of seizure incidence, finally leading to a depressed quality of life in people suffering from it **(Beghi *et al.*, 2015).** According to the World Health Organisation, the proportion of the general population having active epilepsy at any given time is between 4 and 10 persons per 1000. Epilepsy is significantly more common in developing countries, with a prevalence of 7-14 per 1000 people **(Camfield and Camfield, 2015).** This condition has been discovered to exist for centuries and currently affects around 50 million people worldwide. Caution signs and symptoms include altered awareness, tingling fingers, convulsive or aberrant movements, and visual or sensory auras **(Kaiboriboon *et al.*, 2013)**. The pathophysiology of epilepsy encompasses both the induction of seizures (ictogenesis) and the transformation of the normal brain into a brain that is prone to seizures (epileptogenesis) **(Fisher *et al.*, 2017)**. There aren't

any antiepileptogenic medications on the market right now that stop epileptogenesis from progressing **(Fisher R.S *et al.*, 2014).** The situation with epilepsy indicates the urgent need for new treatment approaches. When devising a new strategy, it would be logical to take into account chemicals that inhibit both ictogenesis and the advancement of epileptogenesis **(Scheffer *et al.*, 2017)**. The perfect anticonvulsant medication should limit seizures brought on by neurons firing too quickly and excessively.

ATPases:

In addition to the above neurotransmitters, Purinergic transmission has also been implicated in releasing ATP as a co-transmitter in the CNS, along with Glutamate, Noradrenaline, GABA, Acetylcholine and Dopamine **(Gendron *et al.*, 2002).** Evidence suggests that ATPases like Na^+, K^+ - ATPase, Mg^{2+} -ATPase and Ca^{2+} - ATPase play a pivotal role in maintaining ionic gradients coupled with ATP hydrolysis **(Kodama, 1985).**

Signs and Symptoms of Epilepsy:

Temporary confusion, A staring spell, Stiff muscles, Uncontrollable jerking movements of the arms and legs, Loss of consciousness or awareness.

In most cases, a person with epilepsy will tend to have the same kind of seizure each time so that the symptoms will be similar from episode to episode. Seizures are either focal or generalized based on how and where the abnormal brain activity begins **(Fisher *et al.*, 2017).**

Focal seizures:

The two types of seizures that arise from abnormal activity in a single brain area are simple partial seizures, which do not involve a loss of consciousness but may modify emotions or sensory symptoms and focal seizures with impaired awareness, which involve a change in consciousness or loss of awareness and may be mistaken for other neurological conditions such as narcolepsy, migraine or mental illness (**Graus *et al.*, 2016)**.

Generalized seizures:

Clusters of short, five to ten second seizures, called absence seizures or petit mal seizures, are possible. Seizures with tonic properties frequently affect the back, arms, and legs, causing rigid muscles and perhaps impairing consciousness (**Hassel, B., & Bråthen, G. (2020).** Legs are frequently affected by atonic seizures, which result in a loss of muscle control. Repetitive muscle movements characterise clonic seizures, whereas abrupt jerks or twitches are indicative of myoclonic seizures (**He *et al.*, 2020)**. The most dramatic type of seizures are tonic-clonic seizures, sometimes referred to as grand mal seizures because they cause a sudden loss of consciousness and rigidity in the body (**Jia *et al.*, 2019)**.

Causes of Epilepsy: (Source: World Health Organization, 2005).

Seizures resulting from epilepsy can be inherited, as well as from head trauma, anomalies of the brain, infections, injuries sustained during pregnancy, and developmental issues (**Brodie *et al.*, 2017**). The syndrome affects about 40% of people; its aetiology is unknown, however possible explanations include genetics, head trauma, brain abnormalities, infections, prenatal

damage and developmental issues **(John *et al.*, 2015).** A major cause of death for persons over 35 is stroke. Developmental abnormalities, brain damage, and infections are further concerns. **(Wannamaker *et al.*, 2015).**

Diagnosis of Epilepsy:

Epilepsy is a neurological illness marked by irregular brain activity, seizures, odd behaviour, sensations, and loss of consciousness. Seizures and feelings are common diagnostic techniques **Wurm *et al.*, 2005).**

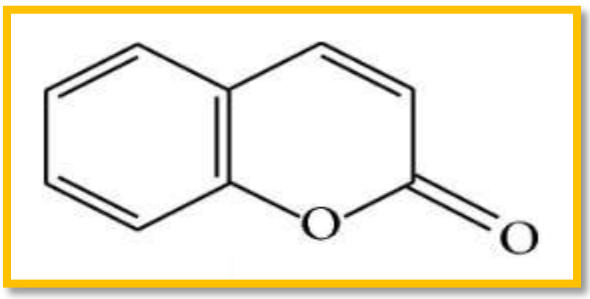
The Treatment Strategies Available for Epilepsy

Many approaches, including as medication, surgery, nutritional therapy, and neuromodulation, can be used to treat epilepsy. Anti-epileptic medications (AEDs) are frequently used to lessen seizures by decreasing electrical activity in the brain. In cases when medication is not working or where the seizures are localised, surgery may be an option. Dietary therapy, which focuses on low-carb, high-fat meals, is a common choice (**Yamamoto *et al.*, 2015)**. One such diet is the ketogenic diet. Neuromodulation stimulates the brain and lessens seizures by means of devices such as deep brain stimulation (DBS) and vagus nerve stimulation (VNS).

About Coumarins:

Coumarins, chemical compounds from the Benzopyrone family, exhibit a wide range of pharmacological effects, including antibacterial, fungal, cancer, antioxidant, diabetic, and sunscreen properties **(Galano *et al.*, 2016).** Plant chemical umbelliferone possesses anti-inflammatory, antioxidant, and antifungal effects. Coumarin derivatives have been demonstrated to inhibit cyclooxygenase 2 (COX-2) and so reduce the generation of free radicals **(Wahy *et al.*, 2017).** They also have antiviral action against viruses such as HRSV and IAV, making them interesting therapeutic candidates. Coumarins have strong anti-diabetic efficacy, with molecules including umbelliferone and herniarin exhibiting neuronal cell death protection. Plant secondary metabolites, such as Umbelliferone, have showed promise in a variety of pharmacological properties **Maleki *et al.* (2020)**.

Coumarin (Source: **Medina *et al*. 2015)**



Materials and Methods:

Procurement of Chemicals:

All the chemicals used in the present investigation were of Analar grade (AR) and Solvents obtained from the following scientific companies: Sigma (USA), Fisher (USA), Merck (India), Himedia (India), TCI (China), Molychem (India) and SRL (India).

Animals

Healthy young adult male albino rats of the Wistar strain (Rattus norvegicus) (3 months old 180±20g) were employed throughout the study period. The rats were kept in a clean rodent room under standard circumstances (28+ 20C), and the animal room was adequately ventilated with a 12h light/dark cycle. Throughout the experiment, the animals were housed in big spacious cages and fed a regular pellet meal and water ad libitum. The experimental animals were handled in accordance with university and institutional animal ethics committee regulations. (Resolution **No: 34/2012-2013/(i)/a/CPCSEA/IAEC/SVU/KY/ dt.01. 07.2012**).

Preparation of Dosage for: Umbelliferon, Pentylenetetrazol and Diazepam.

Umbelliferone (UMB): Umbelliferone, obtained, from Sigma-Aldrich Scientific suppliers, was used for my experiments. I have selected 3 different doses of UMB as described below:

1. 50 mg/kg.b.w; 2. 100 mg/kg.b.w ; 3. 150 mg/k.g.b.w:

All the above doses were prepared with 1% of Tween 80 distilled water and administered to Group III, IV and V of Experimental Rats intraperitoneally (i.p) **(Zagaja *et al.*, 2015).** Convulsions were induced by an intraperitoneal injection of Pentylenetrazole (60 mg/kg.b.w) dissolved in saline **(Gupta *et al.*, 1999).** Diazepam(a standard reference drug) 4 mg/kg.b.w was administered intraperitoneally (i.p) **(Shivakumar *et al.*, 2009)** to the VI group of rats for the purpose of comparison.

Grouping of rats

Total Number of Groups	6 : Each Group with 6 Male Albino Rats (*Rattus norvegicus*)
Group: I	Normal 1% tween 80 Saline Treated Control Rats
Group: II (Epileptic)	Rats Treated with Pentylenetetrazol (PTZ 60 mg/kg body weight) **(Gupta *et al.*, 1999)**
Group: III (Epileptic)	Epileptic rats pretreated with Umbelliferone. (PTZ + UMB 50 mg/kg body weight)
Group: IV (Epileptic)	Epileptic rats pretreated with Umbelliferone (PTZ + UMB 100 mg/kg body weight)
Group: V	Epileptic rats pretreated with Umbelliferone (PTZ + UMB 150 mg/kg body weight)

(Epileptic)	
Group: VI (Epileptic)	Epileptic rats pretreated with Diazepam (reference Drug) (PTZ + DZ 4 mg/kg body weight) **(Shivakumar *et al.*, 2009).**

Biochemical Analysis:

All the below mentioned biochemical estimations were done in 4 selected regions Viz. Cerebral Cortex, Hippocampus, Cerebellum and Pons Medulla of both control and experimental groups of rats on 15th day of Epilepsy induction.

Isolation of Tissues:

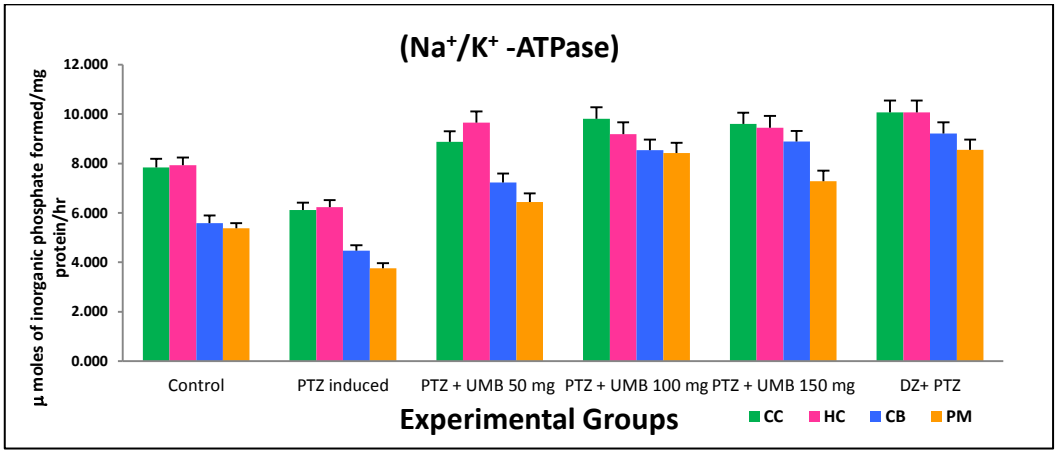
The 15th day saw the cervical dislocation sacrifice of all six groups of rats for biochemical measurements. The isolated brain was put right away on a glass dish that had been cooled. After being divided according to normal anatomical markings (**Glowinski and Iverson, 1966**), four specific brain regions, the cerebral cortex, the hippocampal, the cerebellum and the pons medullaris were frozen in liquid nitrogen (-180^0 C) and kept at -40^0 C until needed. The chosen tissues were used once they had thawed for the biochemical examination. A statistical analysis was performed on the outcomes pertaining to several biochemical markers.

RESULTS:

A. Na^+/K^+ -ATPases:

The study assessed the levels of Na+/K+-ATPase in experimental and control rat brain areas. In the control group, the hippocampal region exhibited the highest level of activity, followed by the cerebral cortex, cerebellum, and pontine medulla. In all of the chosen brain areas, the Na+/K+-ATPase activity was significantly lower in the PTZ-induced group. All brain regions exhibited a significant increase in Na+/K+-ATPase activity in the 50 mg group of the UMB pre-treated groups. Na+/K+-ATPase activity significantly improved in the UMB 100 mg group, with the cerebral cortex exhibiting the highest activity. In comparison to the PTZ-induced group, the DZ-PTZ group displayed increased Na+/K+-ATPase activity in all brain areas. The study found that 100 mg of UMB had a favourable effect on epileptic patients' Na+/K+-ATPases.

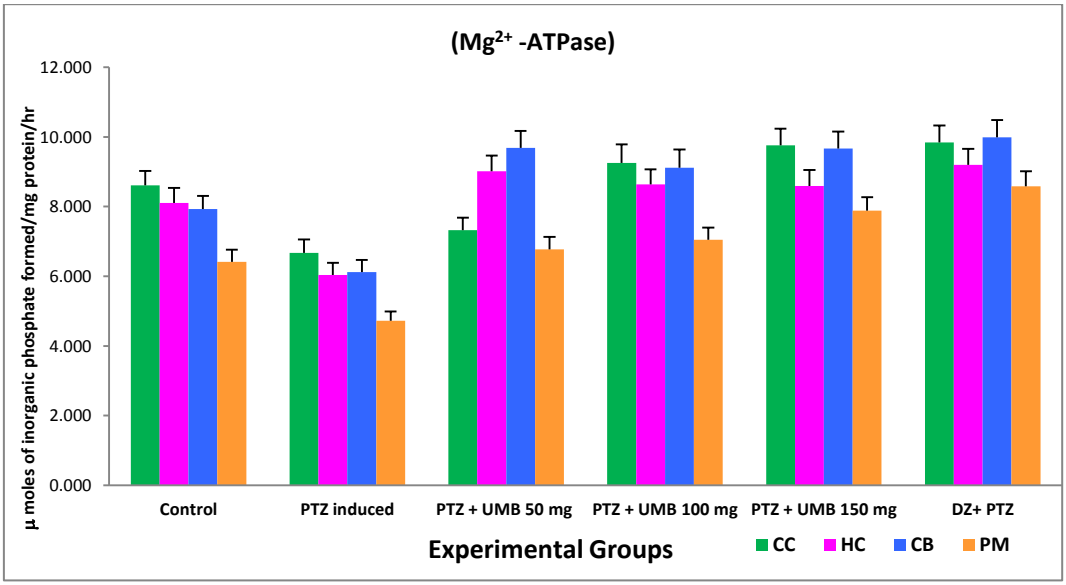
Graph 1: Changes in the activity levels of Na^+/K^+ - ATPase in selected brain regions of Control and Experimental Rats (Values are expressed in μ moles of inorganic phosphate formed/mg protein/hr)



B. Mg^{2+} - ATPase :

The Mg2+ ATPase activity in the brain areas of the experimental and control rats was examined. Cerebral cortex activity was highest in the control group, and then it was in the hippocampal, cerebellar, and pontine medulla. The PTZ + UMB 50 mg group demonstrated a considerable increase in activity compared to the PTZ-induced group which demonstrated a significant decrease in activity. There was a notable increase in activity in the PTZ + UMB 100 mg group, with the cerebral cortex exhibiting the highest activity. There was a notable increase in activity in the PTZ + UMB 150 mg group, with the Cerebellum exhibiting the highest activity. There was a noticeable rise in activity in the DZ + PTZ group. These results imply that UMB might protect brain enzymes and be applied to the management of neurological disorders associated with seizures.

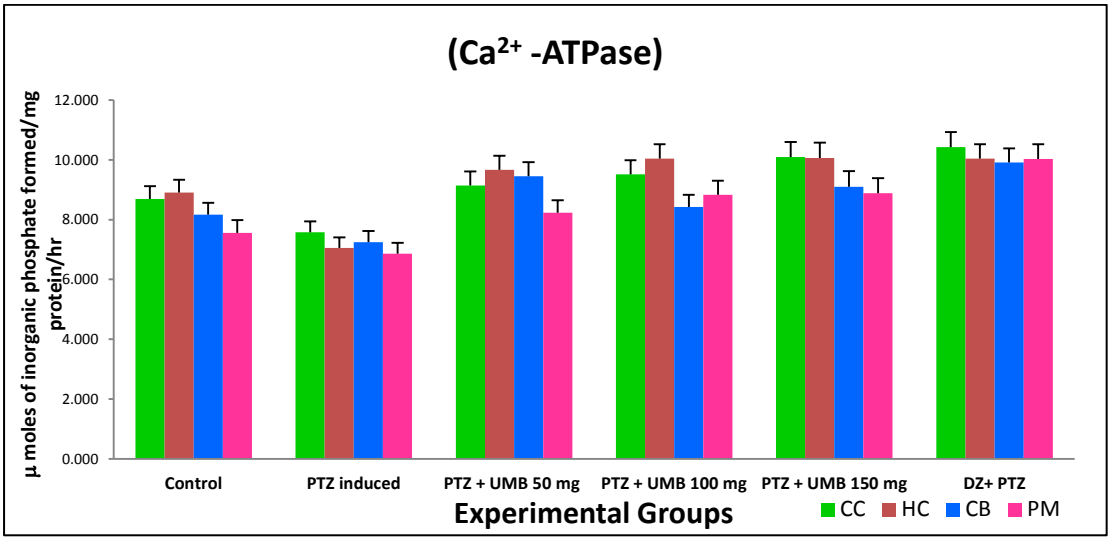
Graph - 2: Changes in the activity levels of Mg^{2+} ATPase in selected brain regions of Control and Experimental Rats (Values are expressed in µ moles of inorganic phosphate formed/mg protein/hr)



C. Ca^{2+}- ATPase :

According to the study, there were notable differences in the levels of Ca2+-ATPase activity in various brain regions between the experimental and control rats. The pons medulla, cerebral cortex, cerebellum, and hippocampal regions all exhibited the highest levels of activity in the control group. The pons medulla experienced the greatest reduction in Ca2+-ATPase activity in the PTZ-induced group. All brain regions exhibited a notable increase in Ca2+-ATPase activity following UMB pretreatment. Improvement was observed in the hippocampus and cerebral cortex of the UMB 100 mg group, but not in the cerebellum or pons medulla. All brain areas exhibited increased Ca2+-ATPase activity in the UMB 150 mg group. Overall, in PTZ-induced epileptic rats, UMB pretreatment can increase Ca2+-ATPase activity.

Graph-3 : Changes in the activity levels of Ca^{2+} - ATPase in selected brain regions of Control and Experimental Rat (Values are expressed in μ moles of inorganic phosphate formed/mg protein/hr)



DISCUSSION:

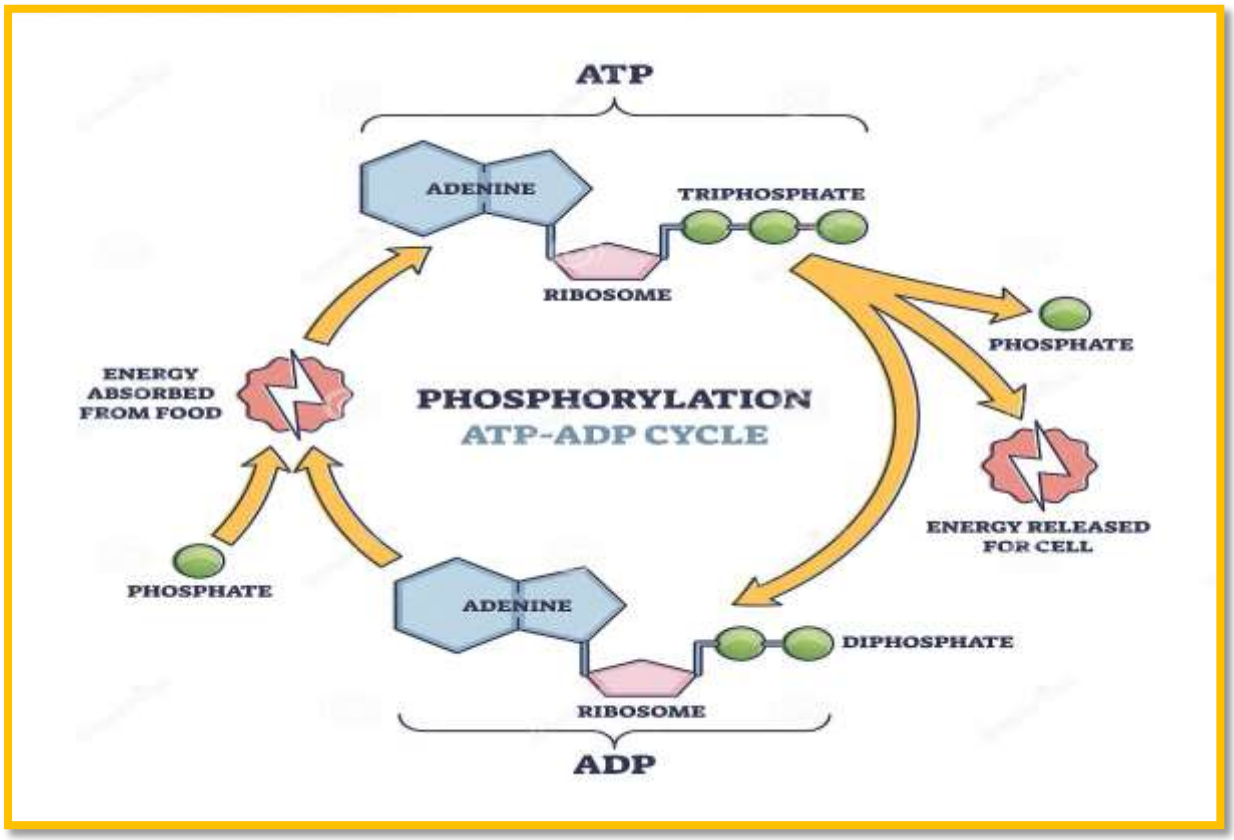
The study analyzed the efficacy of Umbelliferon on the activity levels of the membrane-bound ATPases system in four selected brain regions: Cerebral Cortex, Hippocampus, Cerebellum, and Ponsmedulla **(Zagaja *et al.*, 2022)**. In PTZ-induced epileptic rats, Na+/K+-ATPase activity levels decreased drastically compared to the controls. However, UMB treatment (100mg/kg.b.w.) showed a significant elevation of Na+/K+ -ATPases in all selected brain regions compared to the PTZ group, which were almost close to the controls and also Diazepam treated rats **(Sun *et al.*, 2022).** Na+/K+-ATPase is crucial for the proper functioning of excitatory neurotransmitters, normal cell cycle, and differentiation of the nervous system. Studies have shown that the suppression of Na+/K+-ATPase activity is greater in specific regions of the brain associated with epilepsy than in other areas **(Farhat *et al.*, 2021).** The Na+/K+-ATPase pump plays a crucial role in regulating neuronal

excitability and is impaired in various forms of epilepsy **(Silva *et al.*, 2011).**

Mg2+ ATPases were significantly reduced in the PTZ-induced epileptic rats compared with the control rats. UMB-treated rats exhibited relatively higher levels of Mg2+ ATPases in all brain regions compared to the Epileptic rats, indicating that UMB treatment effectively reversed the Epileptic-induced changes in Mg2+ ATPases **(Albers and Siegel, 2012).** Mg2+ ATPases play multiple roles in different brain regions, and their specific roles may differ depending on the region involved in modulation and control of different functions under different situations **(Ramesh and Pugalendi, 2007).**

The activity levels of Ca2+ ATPases were also significantly reduced in all selected brain regions of the epileptic rats when compared to the control group **(Gall *et al.*, 2005).**

Fig: ATP-ADP CYCLE



Conclusion:

Umbelliferon has shown anti-convulsant and neuroprotective activity along with various anti-epileptic drugs, mitigating the negative impacts of anti-epileptic drugs on neuronal viability. In rats given PTZ to produce epilepsy, the natural bioactive substance umbelliferone demonstrated anti-cholinesterase action. It seems that umbelliferone has a beneficial effect on each of the glutamatergic system's four components. Comparable beneficial effects were noted for the ATPases in rats with epilepsy.

Conflict of interest : None

References :

1. **Albers R.W., and Siegel, G.J.,** (2012). Membrane transport. In: Brady ST, Siegel GJ, Albers RW, Price D (Eds.). Principles of Molecular, Cellular and Medical Neurobiology, 8th edn. Massachusetts, USA: Elsevier Academic Press. *Basic Neurochemistry*: 2: 41-62.
2. **Beghi E, Giussani G, Sander JW.** (2015). The natural history and prognosis of epilepsy. *Epileptic Disord*.17(3): 243–53.
3. **Brodie, M. J., Barry, S. J., Bamagous, G. A., Norrie, J. D., & Kwan, P.** (2017). Patterns of treatment response in newly diagnosed epilepsy. Neurology, 88(17), 1634-1641.
4. **Camfield P, Camfield C.** (2015). Incidence, Prevalence and aetiology of seizures and epilepsy in children. *Epileptic Disord.* 17(2):117–23.
5. **Farhat, E., Devereaux, M. E., Cheng, H., Weber, J., & Pamenter and M. E.** (2021). Na^+/K^+-ATPase activity is regionally regulated by acute hypoxia in naked mole-rat brain. *Neuroscience Letters*, 764, 136244.
6. **Fisher, R. S., Cross, J. H., French, J. A., Higurashi, N., Hirsch, E., Jansen, F. E. and Zuberi, S. M.** (2017). Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia*, 58(4), 522-530.
7. **Fisher, R. S., Cross, J. H., French, J. A., Higurashi, N., Hirsch, E., Jansen, F. E.,...& Scheffer, I. E.** (2014). Operational classification of seizure types by the International League Against Epilepsy: position paper of the ILAE Commission for Classification and Terminology. Epilepsia, 55(4), 522-530.
8. **Galano, A.; Mazzone, G.; Alvarez-Diduk, R.; Marino, T.; Alvarez-Idaboy, J.R.; Russo, N.** (2016). Food Antioxidants: Chemical Insights at the Molecular Level. Annu. Rev. Food Sci. Technol. 7, 335–352.
9. **Gall, D., Roussel, C., Susa, I., Dufour, A. and Stauffer, E.** (2005). Immunohistochemical localization of ATPases, transporters, and CaMKII in the rat cerebellar cortex. *Histochemistry and cell biology*, 124(6), 547-558.
10. **Gendron.F.P. , O. Benrezzak, B.W. Krugh, Q. Kong, G.A. Weisman, A.R.** (2002). Beaudoin Puine signaling and potential new therapeutic approach: possible outcomes of NTPDase inhibition. *Curr Drug Targets*, 3 pp. 229-245
11. **Glowinski, J. and L. Iverson.** (1966). Regional studies of catecholamines in the rat brain. I. The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. Journal of Neurochemistry, 13(7): 655-669.
12. **Graus F, Titulaer MJ, Balu R, *et al.*,** (2016). A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol;15: 391–404.
13. **Gupta, Y.K., Gupta, M., Kohli, K., & Kishore, A.** (1999). Evaluation of antiepileptic potential of Marsilea minuta Linn. in maximal electroshock and

pentylenetetrazole-induced seizures in rats. Journal of Ethnopharmacology, 64(2), 167-172.

14. **Hassel, B., & Bråthen, G.** (2020). Glutamate-mediated excitotoxicity and neurodegeneration in epileptogenesis. Journal of cerebral blood flow and metabolism, 40(4), 729-749.
15. **He, W., Chen, L., Chen, L., Yuan, G., Fang, Y., Chen, W., Wu, D., Zhou, Y., Gao, Y., & Liu, Y.** (2020). A novel coronavirus outbreak of global health concern. The Lancet, 395(10223), 470-473.
16. **Jia C, Zhang J, Yu L, Wang C, Yang Y, Rong X, Xu K and Chu. M.** (2019). Antifungal Activity of Coumarin Against Candida albicans Is Related to Apoptosis. *Front. Cell. Infect. Microbiol.* 8:445.
17. **John, C. C., Carabin, H., Montano, S. M., Bangirana, P., Zunt, J. R., & Peterson, P. K.** (2015). Global research priorities for infections that affect the nervous system. *Nature*, 527(7578), S178-S186.
18. **Kaiboriboon, K., Nagarajan, S. S., Mantle, M., & Kirsch, H. E**. (2013). Brain connectivity aberrancies in an epileptic patient. Journal of clinical neurophysiology, 30(5), 461-463.
19. **Kodama.T.** (1985). Thermodynamic analysis of muscle ATPase mechanisms. *Physiol Rev*, 65 pp. 467-551
20. **Maleki, N., Cheraghi, F., Naji, M., Khoshkrood-Mansoori, B., Khorshidifar, M., & Moosazadeh, M.** (2020). The effect of education on quality of life in patients with epilepsy: a systematic review and meta-analysis. BMC neurology, 20(1), 1-9.
21. **Medina, F. G., Marrero, J. G., Macías-Alonso, M., González, M. C., Córdova-Guerrero, I., García, A. G. T. and Osegueda-Robles, S.** (2015). Coumarin heterocyclic derivatives: chemical synthesis and biological activity. *Natural product reports*, 32(10), 1472-1507.
22. **Ramesh B, Pugalendi K.V. (2007).** Influence of umbelliferone on membrane-bound ATPases in streptozotocin-induced diabetic rats. *Pharmacol Rep*. 59(3):339-348.
23. **Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L.,...& Zuberi, S. M.** (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia, 58(4), 512-521.
24. **Shivakumar, S. I., Suresh, H. M., Hallikeri, C. S., Hatapakki, B. C., Handiganur, J. S., Sankh, K., & Shivakumar, B.** (2009). Anticonvulsant effect of Cyperus rotundus Linn rhizomes in rats. *Journal of Natural Remedies*, 9(2), 192-196.
25. **Silva, L. F. A., Hoffmann, M. S., Rambo, L. M., Ribeiro, L. R., Lima, F. D., Furian, A. F., and Royes, L. F. F.** (2011). The involvement of Na^+, K^+-ATPase activity and free radical generation in the susceptibility to pentylenetetrazol-induced seizures after experimental traumatic brain injury. *Journal of the neurological sciences*, 308(1-2), 35-40.
26. **Sun J, Zheng Y, Chen Z, Wang Y.** (2022). The role of Na^+ -K^+ -ATPase in the epileptic brain. *CNS Neurosci Ther*. 28(9):1294-1302.

27. **Thurman D.J, Logroscino G, Beghi E, Hauser W.A, Hesdorffer D.C, Newton C.R, *et al.*,** (2017). Epidemiology Commission of the International League Against Epilepsy. The burden of premature mortality of epilepsy in high-income countries: A systematic review from the Mortality Task Force of the International League Against Epilepsy. *Epilepsia*. 58(1):17–26.
28. **Wannamaker, B., Morrell, M. J., & Hermann, B. P.** (2015). Epilepsy after stroke. Epilepsy & Behavior, 47, 141-147.
29. **World Health Organization report-2015.**
30. **Wurm, G., Schnizer, M., and Fellner, F. A.** (2005). Cerebral cavernous malformations associated with venous anomalies: surgical considerations. *Operative Neurosurgery*, 57(suppl_1), 42-58.
31. **Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., and Miya, M.** (2017). Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Scientific reports*, 7(1), 1-12.
32. **Zagaja M, Zagaja A, Szala-Rycaj J, Szewczyk A, Lemieszek MK, Raszewski G, Andres-Mach M.** (2022). Influence of Umbelliferone on the Anticonvulsant and Neuroprotective Activity of Selected Antiepileptic Drugs: An In Vivo and In Vitro Study. *Int J Mol Sci.* 23;23(7):3492.
33. **Zagaja, M., Andres-Mach, M., Skalicka-Woźniak, K., Rękas, A. R., Kondrat-Wróbel, M. W., Gleńsk, M., & Łuszczki, J. J.** (2015). Assessment of the combined treatment with umbelliferone and four classical antiepileptic drugs against maximal electroshock-induced seizures in mice. *Pharmacology*, 96(3-4), 175-180.