Title: REPURPOSING OF ANTIEPILEPTIC DRUGS FOR NEPHROPROTECTIVE ACTIVITY: AN ARCHETYPE IN MANAGEMENT OF KIDNEY STONES

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Abstract: WHO Health Status Report (2022) gives a statement: Repurposing of medicines – The underrated champion of sustainable innovation. Around 15% of people globally are affected by kidney stones at some point in their lives. In 2021, 32.1 million cases occurred. This project work discusses the utilization of Anticonvulsant agents that can be used in management of kidney stones. This manuscript discusses the utilization of Anticonvulsant agents that can be used in management of kidney stones. The major objective of this work is to unfold the uses of Anti-Inflammatory property shown by Anti-seizure drugs. A potential moiety that we found could prove to bring about a paradigm shift in management of Inflammation and alleviate the chronic conditions due to Kidney stones is Lacosamide. Despite advances in modern medicine, the development and growth of calculi continues to be a source of concern for mankind, as there is no effective treatment for kidney stones. In the present study we investigated antiurolithiatic activity of different antiepileptic drugs against calcium oxalate crystals in vitro and also performed screening of few drugs insilico. So, the present research aims to give data highlighting the present trends in research of antiepileptic drugs accredited with antiurolithiatic activity. In this experiment we majorly focused on the Repurposing of anti-epileptic drugs for Anti-inflammatory and Urolithiatic activity.

Keywords: Repurposing, Nephrolithiasis,Valproic acid, Maestro, Schrodinger MoE,

Introduction: Kidney stone is a complex that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys. Calcium oxalate is the most frequent urinary stone component; it exists in three different crystalline forms Neuroinflammation is an integral part of epilepsy pathogenesis and other convulsive conditions. Both primary enzymes COX-1 and COX-2, which catalyze the synthesis of inflammatory prostanoids and are main targets for NSAIDs, have been reported as potential

neurotherapeutic targets for epilepsy correction and management [1]. Repurposing is defined as a planned strategic comprehensive technique of finding out new indications for an existing drug.The category of drugs chosen belong to Anti-Epileptic class (AED). The 2 major targets for docking are TNF-and IL-6. Age-standardized prevalence rate (ASPR) of kidney stone was estimated at 21.11%. Also, the ASPR was estimated 24.13% (95% CI 23.7–24.6) in men and 18.7% (95% CI 18.5–18.9) in women[2]. Lacosamide treatment after can mitigate increased levels of IL-1β and TNF-α in the hippocampus.[3]Anticonvulsant drugs such as pregabalin (PGB) and lacosamide (LCM), exhibit potent analgesic effects in diabetic neuropathy[4]. LCM ameliorated the biochemical, histopathological, and immunohistochemical finding via suppression of TNF alpha. LCM can prevent LPS-induced acute liver damage by suppressing both inflammatory and oxidative injuries Lacosamide significantly reduced hippocampal kynurenine levels in LPS and LPS + PILO groups[6]. LCM demonstrated favorable properties against sepsis-induced acute kidney injury reducing pro-inflammatory cytokine release.[7] Generally, newer (second-generation) antiepileptic drugs are associated with fewer systemic side effects and drug–drug interactions, so they tend to be preferred in this population.[8,9]

Literature review:

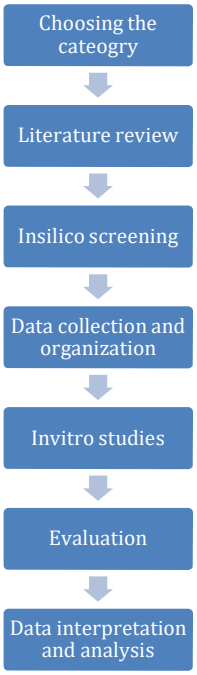
A) General information:
1] Valproic Acid (VPA) significantly inhibited LPS-induced production of TNF-alpha and IL-6 by THP-1 cells, whereas other AEDs did not. The findings are consistent with the idea that VPA suppresses TNF-alpha and IL-6 production via inhibition of NF-kappaB activation [10]

2] Lacosamide: It had antinociceptive effects on both pain readouts assessed in the MIA model for osteoarthritis during different time points of pain development. The data presented in this publication together with recently published animal study data demonstrate lacosamide's ability to reduce arthritic pain behavior induced by multiple mechanisms. This suggests that lacosamide should be evaluated for the treatment for disorders involving inflamamtion [11].

Increased cytokine production possibly due to oxidative stress has repeatedly been shown to play a pivotal role in the pathophysiology of epilepsy and bipolar disorder. Thus few drugs like primidone (PRM), carbamazepine (CBZ), levetiracetam (LEV), lamotrigine (LTG), VPA, oxcarbazepine (OXC), topiramate (TPM), phenobarbital (PB) may exhibit Anti-inflammatory activity[12] After seizure episodes there is an elevated level of IL-6 in the peripheral blood and CSF and Lacosamide along with few other Antiepileptic drugs may reduce the level of Inflammation[13] The results of the study suggest that valproate and levetiracetam led to decrease IL-6 levels[14].Pentoxifylline (PTX) and ethosuximide could potentially alleviate abdominal pain in patients with IBS treated with mebeverine.[15]. An examination of the possible anti-inflammatory mechanisms of gabapentin in the attenuation of neuropathic pain and the interaction between the anti-allodynic effects of gabapentin and interleukin-10 (IL-10) expression in a rat

model of neuropathic pain [16]. Adiponectin exerts anti-inflammatory effects via macrophages, suppressing the production of pro-inflammatory cytokines in response to bacterial lipopolysaccharide (LPS).[17] In vitro and in vivo experiments show that antiepileptic drugs could affect cytokine levels and thus reduce inflammatory condition (e.g., valproate) [18].
Darbufelone may exhibit activity of suppressing IL-6 and thus reduce its serum levels and alleviate inflammation[19]. Rufinamide (RUF) is a uniquely structured anti-epileptic drug (AED) and blocks voltage-gated sodium channels (VGSCs) and shows suprresive activities on TNF alpha and IL-6.

Plan of work:



Preliminary work:

TABLE No 1: Drug Dock scores

SR.NO	DRUGS	DOCK SCORES
1.	Valproic Acid	-4.58
2.	Carbamazepine	-4.61
3.	Clobazam	-4.74
4.	Brivaracetam	-5.16
5.	Clonazepam	-4.99
6.	Ethosuximide	-4.39

7.	Eslicarbamazepine	-4.86
8.	Felbamate	-5.25
9.	**Lacosamide**	**-5.26**
10.	Lamotrigine	-5.16
11.	Leveteracetam	-4.59
12.	Levosime	-5.67
13.	Oxcarbazepine	-4.74
14.	Perampanel	-5.52
15.	Pentobarbital	-5.05
16.	Phenytoin	-5.24
17.	Primidone	-4.65
18.	Progabide	-6.38
19.	Remacemide	-5.42
20.	Retigabin	-6.02
21.	Rufinamide	-4.75
22.	Secobarbital	-5.25
23.	Thalidomide	-4.84
24.	Padsevonil	-5.14
25.	Trimethadone	-4.38
26.	Diazepam	-4.82
27.	Tigabine	-5.21
28	Ethosuximide	-5
29.	Paramethadione	-4.23
30.	Zonisamide	-4.78

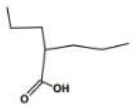
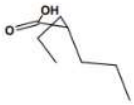
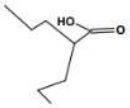
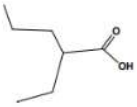
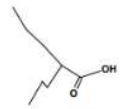
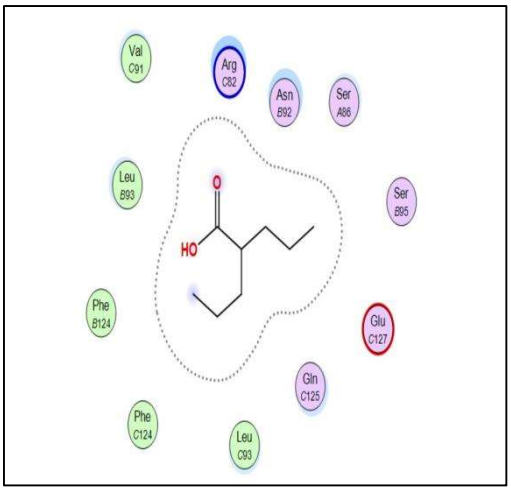
	mol	rseq	mseq	S	rmsd_ref...	E_conf	E_place	E_score1	E_refine
1		1	1	-4.5889	0.5995	-9.1683	-42.6735	-8.2661	-11.7757
2		1	1	-4.4271	1.2171	-7.9916	-45.5551	-8.8709	-17.1083
3		1	1	-4.3715	1.3980	-6.2359	-51.7660	-7.9483	-13.9449
4		1	1	-4.3289	1.1698	-6.9921	-45.6005	-7.3894	-14.0594
5		1	1	-4.3106	2.7915	-9.7947	-45.2818	-7.5707	-15.6977

Figure 1.: Dock score of Valproic acid



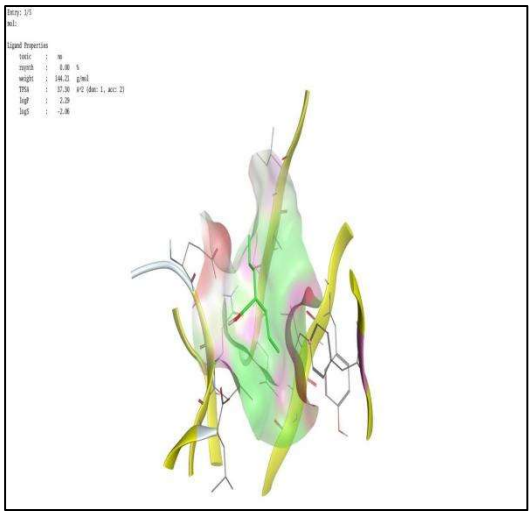


Figure 1.1: Valproic acid ligand interaction Figure 1.2 : Valproic acid surface map

Page 1

	mol	rseq	mseq	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
1		1	1	-5.2685	2.0618	-32.2285	-49.7858	-8.8998	-25.7140	-5.2685
2		1	1	-5.2643	3.0425	-33.0326	-44.8291	-9.0174	-26.1751	-5.2643
3		1	1	-5.1904	1.6671	-31.3039	-74.4827	-8.4346	-24.8786	-5.1904
4		1	1	-5.1768	1.8791	-33.5448	-68.9403	-9.4119	-23.1537	-5.1768
5		1	1	-5.1393	1.3008	-31.8677	-54.2948	-9.1214	-26.8772	-5.1393

Figure 2.1: Dock score of Lacosamide

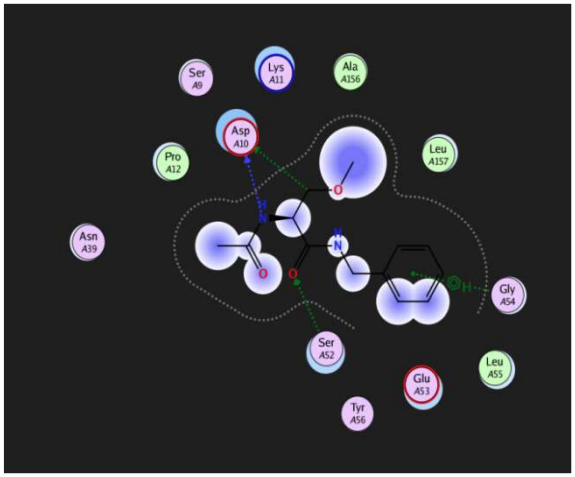


Figure 2.2 : Lacosamide ligand interaction

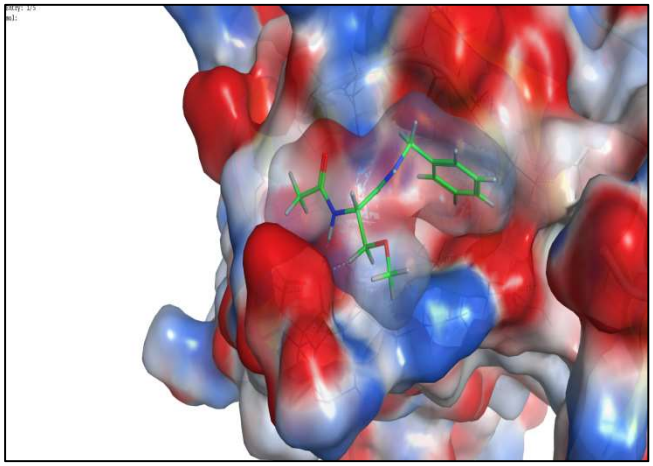
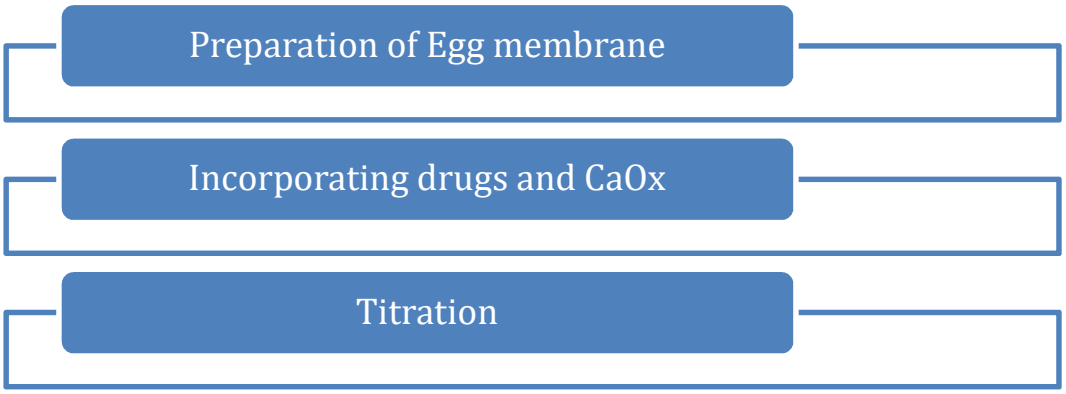


Figure 2.3:lacosamide surface maps

Materials and Methodology:

PROTOCOL FOR NEPROPROTECTIVE ACTVITY



Take 0.1M Hcl, 0.1N NH3, Tris buffer (15gm in 220 mL) and Distilled water

↓

Take a 100ml flask and add Water and HCL ina ratio of 2:8

↓

Dips the eggs in the beaker and let it decalcify until we get a soft membrane

↓

Then Rupture the membrane to remove white part and yolk

↓

After this Clean the membrane in distilled water

↓

Now clean in tris buffer

↓

Then Neutralize in ammonia

↓

After this Put in prepared tris buffer

↓

Refrigerate for 24 hours

↓

Incubate with drug and buffer

↓

Preparation of KmNO4: 33gm in 1000 ml water to make 1 N

Now perform titration and note down the readings

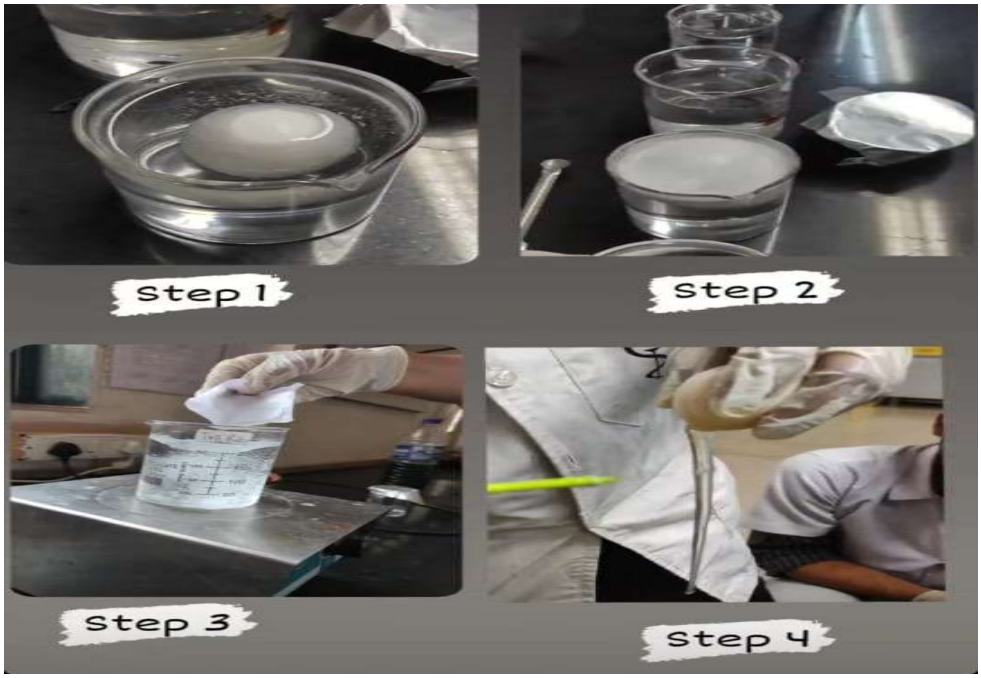
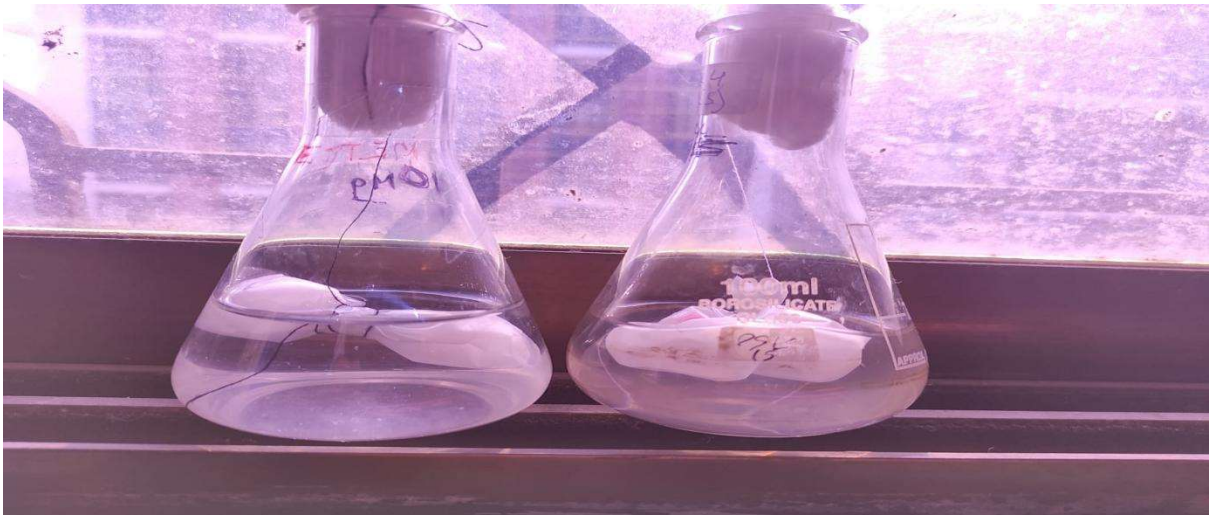


Figure 2.1:Arrangement of conical flask for incubation Figure 2.2: Preparation of cotton plugs for suspending egg membrane

Figure 2.3: Flask ready for incubation

Observations:



Fogure 3.1Before and After incubation

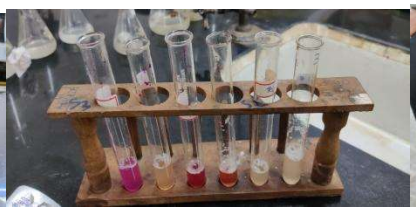
Lacosamide:8 hrs Lacosamide :18 hrs Lacosamide:24 hrs


Valproic acid 8 hrs


Valproic acid:18 hrs


Valproic acid:24 hrs


Cystone:8 hrs


Cystone:18 hrs


Cystone:24 hrs

Figure 4: Isolation of Egg Albumin and slurry preparation


Figure 5.1 Adding all requirements for checking denaturation


Figure 5.2 Heating for protein denaturation

Table No 2 :UV absorbance of Lacosamide ,Ibuprofen and Valproic acid

Concentration (mg/ml)	Ibuprofen	Lacosamide	Valproic acid
1	0.143	0.248	0.183
2	0.292	0.418	0.298
4	0.396	0.571	0.402
6	0.506	0.682	0.606
8	0.689	0.778	0.725
10	0.820	0.980	0.898

Table No 3: %Dissolution of Caox crystals

%Dissolution of Caox crystals			
Drugs	Reading Titrant		% Dissolution
Standard (cystone) 24 Hours Blank (1.7)	1	0.65	61.76%
	2	0.60	64.70%
	4	0.50	70.58%
	6	0.35	79.41%
	8	0.30	82.35%
	10	0.20	88.23%
Cystone 18 Hours Blank (1.4)	1	0.70	50%
	2	0.6	57.14%
	4	0.5	64.28%
	6	0.4	71.42%
	8	0.4	71.42%
	10	0.3	78.57%
Cystone 8 Hours Blank (1.3)	1	0.8	38.46%
	2	0.7	46.15%
	4	0.65	50%
	6	0.60	53.84%
	8	0.50	61.53%
	10	0.40	69.23%
Lacosamide 24 Hours	1	0.75	55.88%

Blank	2	0.70	58.82%
	4	0.60	64.70%
	6	0.55	67.64%
	8	0.40	76.47%
	10	0.35	79.41%
Lacosamide 18 Hours	1	0.9	35.71%
	2	0.8	42.85%
	4	0.7	50%
	6	0.6	57.14%
	8	0.45	67.85%
	10	0.40	71.42%
Lacosamide 8 Hours	1	1	23.07%
	2	0.9	30.76%
	4	0.85	34.61%
	6	0.7	46.15%
	8	0.5	61.53%
	10	0.40	69.23%
Valporoic acid 24 Hours	1	0.48	71.76%
	2	0.45	73.52%
	4	0.40	76.47%
	6	0.40	76.47%
	8	0.3	82.35%
	10	0.2	88.23%
Valporoic acid 18 Hours	1	0.55	60.71%
	2	0.50	64.28%
	4	0.40	71.42%
	6	0.40	71.42%
	8	0.3	78.57%
	10	0.25	82.14%
Valporoic acid 8 Hours	1	0.5	61.53%
	2	0.5	61.53%
	4	0.45	65.38%
	6	0.4	69.23%
	8	0.3	76.92%
	10	0.20	84.61%

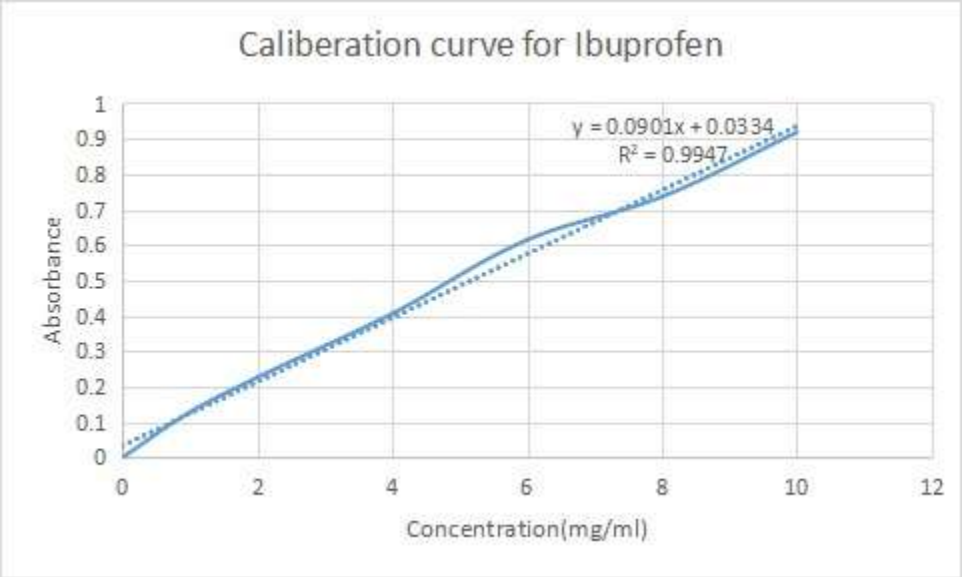


Figure 6.1:Caliberation curve of Ibuprofen

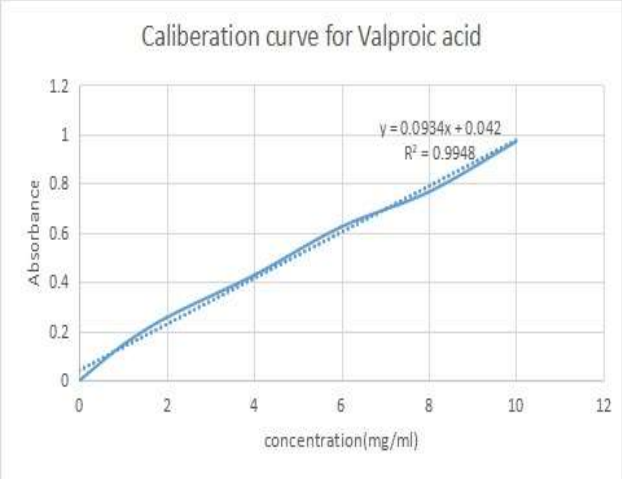


Figure 6.2 Caliberation curve of Valproic acid

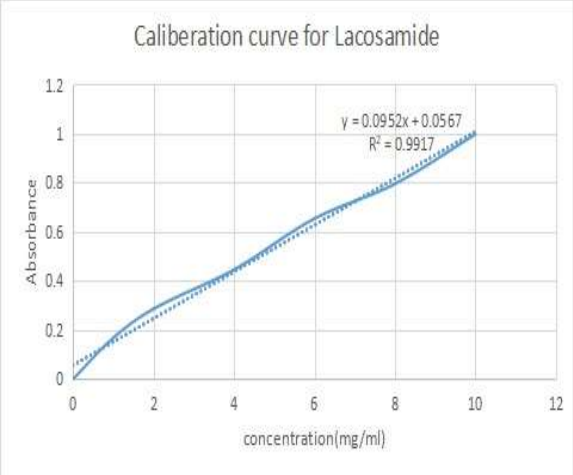


Figure 6.3 Caliberation curve of Lacosamide

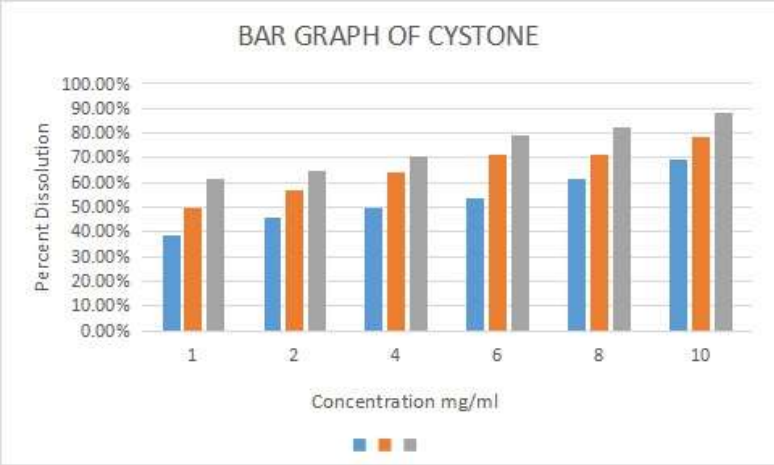


Figure 7.1 Bar graph of Cystone

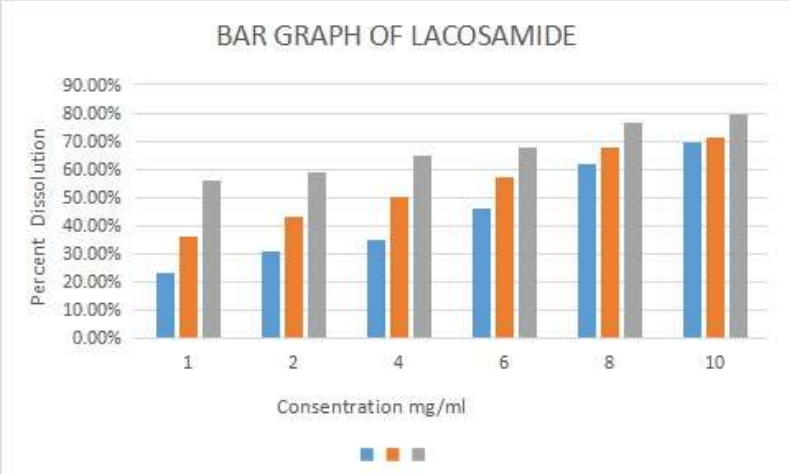


Figure 7.2: Bar graph of Lacosamide

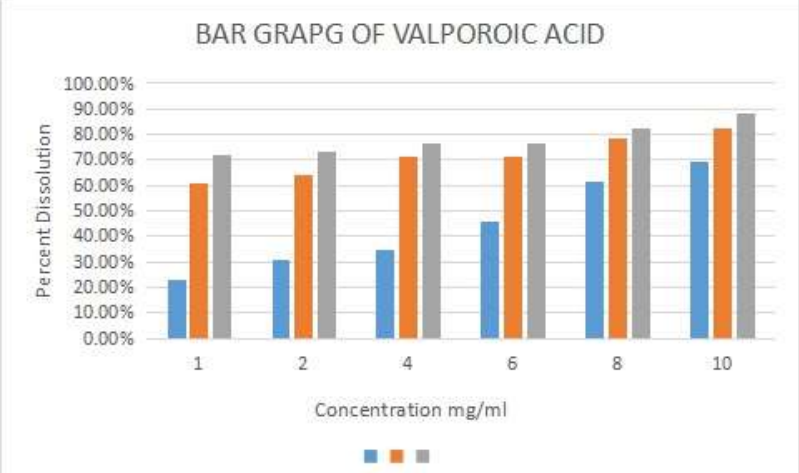


Figure 7.3 :Bar graph of Valproic acid

Results:

1) In the insilico studies the promising candidates found were Progabide, Lacosamide, Lamotrigrine
2) In the Invitro experimental studies Lacosamide showed an inhbitory activity on kidney stones and showed a signifciant result on dissolution of these stones
3) The lacosamide proved to exhibit deriable anti inflammtroy activtity and showed a significant %inhibitory activity on protein denaturation studies.

Discussions:

The results of this study proved that Lacosamide has a significant anti-urolithiatic activity in silico as well as in in-vitro models. 6 different concentrations with 3 different incubation time were calculated and desirable results were obtained for anti-inflammatory and urolithiatic activity.

1) Around 30 moieties were screened belonging to Anti-epileptic drugs out of which 5 most promising candidates were Padsenovil, Retigabine,Lacosamide,Valproic acid,Progabide. The binding affinity and dock score of the candidates were 5.14, 6.01, 5.26,4.98 and 6.3 respectively.

2) 2 candidates were further choosen for invitro studies for anti inflammatory and Urolithaisis which are lacosamide and valporoic acid.These moeities showed prominent %dissolution upto 80% and 85%respectively

3) The lacosamide proved to exhibit desriable anti-inflammatory activity and showed a significant %inhibitory activity on protein denaturation studies.

4) The caliberation curve was ploted in which the r^2 value of lacosamide and valproic acid were found to be 0.9917 and 0.9948 respectively

Conclusion: The objective of this work was to carry out a comprehensive and integrative research of Anti-Epileptic drugs in relation to its clinical uses, structural properties, therapeutic targets, and different molecular, genetic, and systemic action mechanisms in order to consider AED as a candidate for drug repurposing and further explore its scope in Nephrolithiasis .In nutshell and in light of above points it is possible to conclude that Lacosamide could prove to be a potential moiety in management of kidney stones via its anti-inflammatory action.

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