`THERAPEUTIC EFFICACY OF *VISHNU CHAKARA MATHIRAI*- A SIDDHA FORMULATION IN THE MANAGEMENT OF VALIPPU NOI (CONVULSIVE DISORDERS) - A PRE-CLINICAL STUDY

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Doctor of Philosophy

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DECLARATION

I declare that the thesis entitled "**Therapeutic efficacy of *Vishnu Chakara Mathirai*- A Siddha Formulation In The Management of *Valippu Noi* (Convulsive Disorders) - A Preclinical Study"** submitted by me for the degree of Doctor of Philosophy in Siddha medicine is a original record of research work carried out by me during the period of 2011-16 under the guidance of **Prof.Dr.K.Manickavasakam, MD(S),** Former Director, National Institute of Siddha, Tambaram Sanatorium, Chennai-600047 and this work has not been previously formed on the basis, for the award of any degree, diploma, associateship, fellowship in this or any other university (or) similar institution of higher learning.

Place:

Date: **Signature of the Candidate**

(**N.J. MUTHUKUMAR**)

ABBREVIATIONS

AEDs	-	Antiepileptic Drugs
AYUSH	-	Ayurveda Yoga and Naturopathy Unani Siddha Homeopathy
CNS	-	Central Nervous System
EDAX	-	Energy-Dispersive X-Ray Spectroscopy
EO	-	Essential oil
FTIR	-	Fourier Transform Infrared Spectroscope
GABA	-	Gamma Amino Butyric Acid
HCT	-	Hamatocrit
HGB	-	Hemoglobin
HPTLC	-	High Performance Thin Layer Chromatography
ICP-OES	-	Inductively Coupled Plasma Optical Emission Spectra
IR	-	Infra Red
MCH	-	Mean Corpuscular Haemoglobin
MCHC	-	Mean Corpuscular Haemoglobin Concentration
MCV	-	Mean Corpuscular Volume
MES	-	Maximal Electroshock
NMDH	-	N-Methyle-D-Aspartate
NOAEL	-	No Observed Adverse Effect Level
OECD	-	Organization For Economic Co-Operation and Development Guideline
PIS	-	Picrotoxin Induced Seizures
PLIM	-	Pharmacopoeial Laboratory for Indian Medicine
PTZ	-	Pentylene Tetrazol
RBC	-	Red Bloood carpacles
SEM	-	Scanning Electron Microscope
SGPT	-	Serum Glutamyl Pyruvate Aminotransferase
TVC	-	Total Viable Aerobic Count
UV-Vis	-	Ultraviolet-Visible Spectroscopy
VCM	-	Vishnu Chakra Mathirai
XFR	-	X-Ray Fluorescence

INTRODUCTION

AIM & OBJECTIVES

REVIEW OF LITERATURE

PLAN OF WORK

MATERIALS AND METHODS

RESULTS & ANALYSIS

DISCUSSION

SUMMARY

CONCLUSION

RECOMMENDATIONS

BIBILIOGRAPHY

ACKNOWLEDGEMENT

ANNEXURES

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Siddha System of Medicine:

Siddha is one of the ancient holistic system of medicine. It is a science of life, that gives importance to mental and physical well being of human. The word '*Siddha'* comes from the word '*Siddhi'* means heavenly bliss. According to Siddha Perfect health is defined as the state of physical, psychological and social health of a well being of mankind, which has been given in *Thirumanthiram* as:

"One that alleviate bodily ailment is medicine

One that alleviate psychological ailment is medicine

One that prevents ailment is medicine and

One that bestow immortality is medicine"

According to Siddha system of medicine all the objects in this world either living or non-living are composed of five elements (*Panchabootham*) namely:

Earth - *Man*

Water - *Neer*

Fire - *Thee*

Air - *Kaatru*

Ether - *Aahayam*

The entire universe is also made up of *Panchabootham*, so any changes in the universe will reflect in human body. The health is maintained by three basic vital forces (Humours) namely; *Vatham, Pitham*, and *Kabam* called *Uyir thathukkal* (*Mukkutram*) which are activated by the function of *Panchabootham* (Five basic elements)[1.] Whenever the above humours are get affected or not in a balanced state they become *kutrams* and predispose to diseases. In Siddha system of medicine, the diseases of human beings are classified into 4448 types on the basis of *Mukkutram* theory[2].

Line of Treatment in Siddha:

In Siddha system, the treatment is based on *Mukkutram* principles. Treatment is not only for perfect healing but also for the prevention of diseases and rejuvenation of the body. The line of treatment in siddha is as follows[3]:

1. *Kaapu* (Prevention)

2. *Neekam* (Treatment)

3. *Niraivu* (Restoration)

Siddha System, occupies an important place in providing healthcare with its boundless therapeutics and wonderful pharmaceutical preparation of medicine. The management in Siddha system of medicine deals not only as a curative but also as a preventive aspect of diseases, with its internal being - soul, which ensures various therapeutically efficacious and promising drugs which revamps various degenerative disorders and sustains vitality[4,5].

1.1 Epilepsy:

Epilepsy is a condition mentioned in siddha literatures as *Valippu*[6]. It is one of the most frequent neurological disorders of mankind characterized by excessive provisional neuronal discharges ensuing in uninhibited convulsion. Epilepsy is defined as a set of disorders characterized by unusual electrical activity in the brain will result in different behavior, which may manifest as a change in a person's consciousness, movement. Many herbal and herbo mineral formulations and treatment methods were mentioned in siddha literatures for the treatment of Epilepsy. *Vishnu Chakara Mathirai* (*VCM*), a herbo-mineral formulation, is used for many years in Siddha System is taken for the present study.

A seizure is a convulsion is resulting from a paroxysmal discharge of neurons of cerebrum. Epilepsy is a continous tendency to have such seizures, even if a lengthy

interval separate attacks. Epilepsy is a sudden and transient disturbance of functions of cerebrum due to abnormal, recurrent and paroxysymal neuronal discharge in the brain. Epilepsy consisting of group of symptom complexes of which many are static and others are progressive. Each episode of such neurological dysfunction is called a seizure, may be convulsive, when associated with motor manifestations. Non convulsive seizures may manifest as changes in emotional, sensory and cognitive function.

The research for perfect antiepileptic compound and lower toxicity continues to be an area of search in indigenous medicine. Moreover many side effects are reported in many patients treated with present available antiepileptic drugs (AEDs). Hence for long, herbs and traditional systems are also focused, looking for potent and safe anticonvulsives.

Vishnu *Chakara* Mathirai (*VCM*),

Vishnu Chakara Mathirai (*VCM*), a herbo-mineral formulation, is used for many years in Siddha System of Medicine for various ailments especially for *Valippu Noi* (Convulsive disorders). The formulation mentioned in the Siddha Text '*Siddha Vaithyiya Thirattu"* has been indicated for various diseases like *Pakka Vatham* (Paralysis), *Sobai* (Dropsy), *Valippu* (Convulsive disorders) and *Yeppam* (Belching)[7]. Scientific validation of *Vishnu Chakara Mathirai* was not carried out so far. This present study has been done to evaluate the safety and efficacy of *Vishnu Chakara Mathirai (VCM)* against *Valippu Noi* in animal models to support the strong traditional claim.

2. AIM AND OBJECTIVES

AIM

The present study aims to evaluate the preclinical safety and efficacy of *Vishnu Chakara Mathirai* in Convulsive disorder (*Valippu Noi*) in various animal models.

OBJECTIVES

The objectives are to perform the following studies on the trial medicine *Vishnu Chakara Mathirai.*

PHYSICO CHEMICAL STUDIES

This includes

- Phyto chemical analysis
- Physico-chemical analysis of *VCM*
- Analysing *VCM* by UV-Visand FTIR,
- HPTLC finger printing
- Analysing the elemental composition of the drug by ICP-OES and EDAX
- External morphology study of *VCM* by Scanning Electron Microscope
- Microbial load of *VCM* as per the specified protocol given by PLIM, AYUSH

SAFETY STUDIES

Evaluation of the safety of the study drug by conducting

- Acute toxicity of *VCM*,
- Sub-acute toxicity of *VCM*
- Sub-chronic toxicity studies of *VCM* in animal model.

EFFICACY STUDIES

This includes the evaluation of Anticonvulsant activity of *Vishnu Chakara Mathirai* using the following animal models

- Maximal Electroshock Induced Seizures (MES)
- Picrotoxin Induced Seizures (PIS)
- N-Methyl-D-Aspartate (NMDA) induced convulsions
- Pentylenetetrazole (PTZ) induced kindling
- Pentylenetetrazole (PTZ) induced convulsions.

3. REVIEW OF LITERATURE

This chapter deals with the review of literature in various aspects, as disease review, drug review in Siddha and in modern aspects.

3.1.Epilepsy (*Valippu*) in Siddha system[6,8]

3.1.1.Definition

According to siddha system of medicine, Epilepsy (*Valippu*) is characterised by loss of consciousness, decreased intellectual function, violent and jerky movements of limbs and face, deviation of mouth, frothy salivation and Blinking of eyes.

3.1.2. Common Symptoms of *Valippu*

Vallipu Noi mainly affects the children it may continue up to adulthood up to the age of 40, in infants it continues up to the age of 15. The epileptic attack can occurs, when the person is at any set of work or even talking, just before the onset of convulsion the person feels the warming sensation in the form of some hallucination. It continues with presymptamatic stages like anger, giddiness, depression, laziness increased appetite, staring look, sneezing, yawning, twitching of muscles, unconsciousness, anuria, frothy salivation, afterwards the person recovers automatically and becomes normal. Voiding of urine without self control may occur in some persons.They are unaware of their actions which may lead to traumatic injury, fracture of bones, haematemesis, and paralysis in severe conditions. Though the intensity of convulsion in children is not as high compared to adults, continuous fever, flickering of fingers, twitching of upper and lower limb muscles continued by convulsion. In some children convulsion may occur without loss of consciousness, frequency of attack may be even several times in a day. It may also leads to blindness and paralysis

In epilepsy improper medication leads to psychiatric disorder.

In females convulsive attacks are common during menstruation.

3.1.3.Classification:

According to Siddha Literature, *Siddha Maruthuvam Sirappu Valippu Noi* is classified into five types they are:

1. Muppini Vali

2. Kuthirai Vali

3. Kurangu Vali

4. Kakkai Vali

5. Muyal Vali

In the text, *Siddha Maruthuvam Pothu*, *Valippu Noi* is further classified into twenty- one types[8]

1. *Amarakanda Vali*
2. *Kumarakanda Vali*
3. *Brahmakanda Vali*
4. *Kakkai Vali*
5. *Muyal Vali*
6. *Thimir Vali*
7. *Konu Vali*
8. *Sandala Vali*
9. *Marana Vali*
10. *Mano Vali*
11. *Nanju Vali*
12. *Mukkutra Vali*
13. *Iyya Vali*

14. Vil Vali

15. Sura Vali

16. Vikkal Vali

17. Thalai Vali

18. Kozhai Vali

19. Oodu Vali

20. Marbu Vali

21. Thamaraga Vali

3.1.4. Symptoms:

- Vertigo
- Weakness of both Upper And Lower Limb
- Flickering of Finger Tips
- Confusion
- Aphasia (Pretending Like Dumb Person)
- Analgesia
- Increased Thirst
- Increased Sweating
- Excessive salivation with drooling of Saliva
- Sleeplessness

The signs and symptoms of each types of *Valippu* are described in Siddha literatures as mentioned below:

1. Muppini Vali

Though it is one among the disease caused by the derangement of the three humors mostly it occurs due to *Vatham* and *Kabam*. The classical clinical features are deviation of the tongue, face, ears, chin and lips.

2. Kuthirai Vali

Before the appearance of convulsions, symptoms of generalized body pain, weakness of both upper and lower limbs will be present. Giddiness occurs followed by Seizures. Increased sweating in head, shoulders and face is also seen in this type. After an episode of seizure, pain and burning sensation are felt in the throat, shoulder and trunk region.

3. Kurangu Vali

According to Siddha it is an incurable type of *Valippu*, as it is caused by derangement of all the three humors.

4. Kakkai Vali

The subjects stare with eyes rolled up, lower limbs become stiff, tongue and throat dries up. The signs like unaware of passing urine and motion, drenching in sweat will also be seen.

5. Muyal Vali

This type of epilepsy occurs, by the contact of hot water either by looking or pouring the hot water on the head. Convulsion first starts on the facial muscles, with frothing in the mouth and later develops into a generalized one.

6. Thimir Vali

With folded hands, yawn ceaseless and smiles so haughty, dances careless, walk restlessly, nervousness that afflicts the subject and causes them to be awake day and night and hold on to people with all her might.

7. Konu Vali

Papules occur with itching all over the body. Difficulty to smell, choking sensation of the chest, confused intellectual and fever.

8. Kodiya Vali

Tremor and cramping pain occurs, vomiting hiccough, palpitation, nervous weakness and neck pain occurs.

9. Marana Vali

In this type of convulsion the symptoms resembles a patient in his death bed, vomiting, tremor, emaciation, stiffness of limbs and death.

10. Mano Vali

Death of ones spouse loved and loss of material unbound shall cause the patient to lose his head, sheds tears, remains calm like sage or laughs and cries.

11. Nanju Vali

Substances toxic when taken in causes swooning with loss of five sense, intellectual blurs, becomes panic and wanders all around.

12. Mukkutra Vali

Confused state of mind, loss of knowledge, blabbers, dullness of eyes followed by convulsions.

13. Iya Vali

It occurs due to increased *kaba kutram*. Symptoms like coughing, pricking pain in ribs, deviation of eyelids and loss of vision, unable to hear, increased sweating, syncope, intellectual blurring, remains without movements even after recovery of seizure.

14. Vil Vali

Convulsions occur by turning the head and legs either forward or backward or towards lateral like a bow and hence the name *Vil vali*. Excessive frothing with phlegm, numbness of nerves occurs. Muscle spasm in throat and chest causes dyspnea.

15. Sura Vali

As the name indicates, it refers the state of increased fever with convulsion. It is common in childhood. Redness of eyes, yellow or pale face, burning sensation all over the body are present.

16. Vikkal Vali

It is caused by derangement of vital humours, convulsion occurs due to altered *vatham*, excessive appetite due to altered *pitham*, hiccough, throat filled with phlegm, occurs due to altered *Iyam*. Clenching of teeth, redness of eyes, all these symptoms together causes convulsions

17. Thalai Vali

Due to increased *Uthaanan,* it affects the brain which causes cerebral oedema and twitching of nerves due to increased in *kabam* leads to joint pain, stabbing pain, headache.

18. Kozhai Vali

Due to increased *vaatham*, during the onset of disease it causes pain all over the body, accumulation of phlegm in the chest, dyspnoea, vomiting, excessive sweating, yawning occurs along with convulsion.

19. Oodu Vali

In this type of *Valippu*, high temperature accompanied by dryness of mouth, sweating, weakness of both lower limbs, frothy diarrhoea, stiffness of fingers.

20. Marbu Vali

In this type of *Valippu Noi, Pitha Kutram* exceeds from its normal limit and therefore raises *Kabam* along with it, and causing continuous fever, cough, vomiting with phlegm, twitching and convulsions occurs.

21. Thamaraga Vali

Thamaraga valippu shows the symptoms such as headache, chest pain, getting panic often, anger, phlegm accumulates in the chest, the three humours loses it equilibrium and causes seizures.

3.1.5. Treatment in Siddha system of medicines[7,9]:

Purgatives:

Kazharchi Ennai

Merugan Ennai

Siddhathi Ennai

Agasthiyar Kuzhambu

Pulathiyar Kuzhambu

Kumatti Mezhugu

Internal Medicines

Rasa Parpam

Thurusu Parpam

Velli Parpam

Naga Parpam

Vanga Parpam

Sandarasa Parpam

Chandamarutha Chenduram

Ayaveera Chenduram

Mezhugu Types:

Van Mezhugu

Nandi Mei

Chanda Marutha Kuzhambu

Rasagandhi Mezhugu

Rasa Mezhugu

Medicated Oil and Ghee (*Ennai, Nei*) types:

Ganthaga Sudar Nei

Pachonthi Ennai

Veppa Ennai

Aynkootu Thailam

Herbs, minerals and siddha medicines and for convulsions:

Kasthuri Karuppu

Korosani mathirai

Vasambu (Acorus calamus)

Kaantha parpam

Chembu parpam

Sivanaar amirtham

Soodan (Camphor)

Navachara chendhooram

Venkara podi + Karuvapattai

Venkaram (Borax)

Maragathakal Ring

Vairam Ring

Thurusu chendhooram

Perarathai (Alpinia galangal)

Uthamani (Pergularia daemia)

Ilanthai pazham (Ziziphus mauritiana)

Oomathai vithai thalam (Datura seed oil)

Purified Etti (Strychnos nux vomica)

Erukku (Calotropis gigantia)

Kanja (Cannbis sativa)

Kadugu rogini (Scrophulari flora)

Kodiveli root bark (Plumbago zeylanica), Erukku root bark (Calotropis gigantia), Sankan root bark, Kumkumapoo (Crocus sativus)

Sathakuppai

Devadharu

Malai paalai

Pugaiyial

Punnai Nei

External Medicines

Mezhugu Thailam

Muttai Thailam

Sadamanjil Thailam

Alagala Vida Thailam

Vidamutti Thailam

Medicated fumigation

Agasthiyar Kuzhambu

3.2.Epilepsy[10]:

Epilepsy is a group of clinical conditions in which there are recurrent episodes of altered cerebral function. It is associated with hypersynchronous and paroxysmal huge

electrical discharge of neurons of cerebrum. Each episode of neurologic dysfunction is called seizure, which may be convulsive or non-convulsive.

3.2.1 Causes

The cause of Epilepsy is mostly unknown and the condition involves the brain function at neuron level. The conditions which affects the brain may causes epilepsy. In an average, a child of epileptic patient has 1 in 30 chance of being affected. Familial incidence of epilepsy is greater than idiopathic epilepsy.

The causes of epilepsy are

1. Constitutional or Idiopathic:

This type of seizure starts between 5-20 years. No specific cause can be specified and there are no other neurologic abnormalities.

2. Symptomatic seizures:

i. Congenital abnormality like perinatal injuries, Phacoma.
ii. Metabolic disorders like hypoglycemia, hypocalcemia, pyridoxine deficiency, ketoacidosis, lipidosis.
iii. Tumours and space occupying lesions especially in frontal, temporal and parietal lobes. 5 - 10% of cases having cerebellar tumours may have seizures.
iv. Vascular causes like haemorrhage, infarction
v. Degenerative diseases of Central Nervous System.
vi. Infective disease like encephalitis, brain abscess, tuberculoma,
vii. Head injuries :
    - Skull fractures, epidural, subdural haematomas
    - Scars followed by neurosurgical procedures, or head trauma

3.2.2.Classification of Epileptic seizures:

Partial seizures:

i. Simple partial seizure with motor or sensory or autonomic or psychic signs.

ii. Complex partial

iii Partial seizure with secondary generalization

Primarily Generalised seizures:

i. Absence of seizure (Petit mal)

ii. Tonic clonic (Grand mal)

iii. Atonic

iv. Tonic

v. Myoclonic

Special types:

i. Neonatal seizure

ii. Infantile spasms

Status epilepticus:

i. Tonic-clonic

ii. Absence status

iii. Epilepsia partialis continua

Reflexly induced seizures:

i. Specific precipitants

ii. Non-specific precipitants.

3.2.3. Triggering and precipitating factors for seizures:

- Sleep disturbances
- Alcohol particularly withdrawal conditions
- Recreational drug misuse

- Emotional stress
- Physical and mental exhaustion
- Infection and pyrexia
- Hormonal changes associated with menstruation

3.2.4. Mechanisms:

Spread of electrical activity between cortical neurons is normally restricted. In normal brain the synchronous discharge of neurons takes place in restricted groups whose limited discharges are the cause for the normal EEG rhythms. Groups of neurons are activated repetitively and hypersynchromously during convulsion. There is failure of inhibitory synaptic contact between neurons. A partial seizure is epileptic activity confined to one of cortex with a recognizable clinical picture. This activity either remains focal or spreads to generate epileptic activity in both hemisphere and thus a generalized seizure. This spread is called secondary generalization of the partial seizure. The focal onset of a convulsions may not be evident clinically; this means that an apparent tonic-clonic seizure may be either a generalised major convulsion or a tonic-clonic seizure which began as a partial seizure but without clinical evidence of its focal original.

The area of brain is becomes epileptogenic because neurons have a predisposition to be hyper-excitable. Trauma or brain neoplasm are examples of acquired conditions that after the seizure threshold of neurons.

3.2.5. Pathophysiology:

In epileptic seizures there is rhythmical and repetitive hyper-synchronous discharge of many neurons in a localized area of brain reflexed in EEG by low voltage fast activity or high voltage discharges of spikes and wave. The normal balance between the excitatory and inhibitory synaptic influences on normal neurons is lost and the former takes the higher.

3.2.6.Risk factors

Epilepsy is statistically more prevalent in older population. In children, nearly 30 percent of cases are reported in the first 5 years of age. There is no particular group of people who have greater chances of getting epilepsy.

3.2.7. Differential Diagnosis:

Syncope is the commonest systemic disorder confused with epilepsy. With syncopal attacks one may have motor twitches or rarely tonic-clonic convulsions. Since cardiogenic syncope can cause a convulsion while seizure attack can be associated with cardiac arrhythmia, diagnostic monitoring of black out spel must include both ECG and EEG.

Transient global amnesia is more likely to have a vascular rather than epileptic cause. Prodromal symptoms of migraine can be mistaken as ictal events and following headache as post-ictal phenomenon.

3.2.8. Management:

Only half of the patients treated can be expected to become seizure free indefinitely. Monotherapy is best appropriate to the epileptic syndrome. Blood level monitoring helps to adjust drug dosage without producing undesirable side effects and is a must when renal function is changing as during pregnancy. Once a steady state or drug level is known, recurrence of seizure or side effects can be correlated to bioavailability of the drug which in turn may be related to enzyme induction by chronic use of that drug or addition of another drug. A single appropriate drug is started first and the dose is gradually increased over weeks to months until seizure control or side effects occur. The end point varies from patient to patient and the dose level in the table is only an average. When one drug is ineffective, it is replaced with another drug by gradually withdrawing

the first while increasing the second. If seizure activity returns or is exacerbated, it may be transient response to withdrawal of first drug but does not indicate that second drug is ineffective. When multiple seizure types are present concurrent use of two drugs is unavoidable.

3.3. SIDDHA FORMULATIONS FOR CONVULSIVE DISORDERS:

1. *Chanda Rasa Parpam (Sathi Linga Parpam) - The Pharmacopoeia of Siddha Research Medicines.*
2. *Gold Parpam- The Pharmacopoeia of Siddha Research Medicines*
3. *Muthu Parpam- The Pharmacopoeia of Siddha Research Medicines.*
4. *Thamira Parpam- The Pharmacopoeia of Siddha Research Medicines.*
5. *Peranda Parpam- The Pharmacopoeia of Siddha Research Medicines.*
6. *Arithara Parpam - Agathiyar Vaidhyiya Chindamani*
7. *Muthu parpam - Pharmacopoeia of Hospital of Indian medicine*
8. *Peranda parpam - Pharmacopoeia of Hospital of Indian medicine*
9. *Thalaga parpam - Pharmacopoeia of Hospital of Indian medicine*
10. *Thamira parpam- Pharmacopoeia of Hospital of Indian medicine*
11. *Sivanaar Amirtham- Pharmacopoeia of Hospital of Indian medicine*
12. *Veera parpam - Chikicha Rathna Deepam*
13. *Aya chendhooram - The Pharmacopoeia of Siddha Research Medicine.*
14. *Ayakanthanga chendhooram - The Pharmacopoeia of Siddha Research*
15. *Chavvira kattu chendhooram or Shavvira pathangam - The Pharmacopoeia of Siddha Research.*
16. *Kasthuri chendhooram - The Pharmacopoeia of Siddha Research Medicine*
17. *Manosilai Kattu chendhooram - The Pharmacopoeia of Siddha Research Medicine*

18. Pattu karuppu chendhooram - The Pharmacopoeia of Siddha Research Medicine

19. Lingathi Upa chandamarutha chendhooram-Anuboga Vaithya Navaneetham, Part 4

20. Eragu Ramabana Chendhooram - Anuboga Vaithya Navaneetham, Part-4

21. Neela Kanda Vaalai - Anuboga Vaithya Navaneetham, Part-4

22. Poora Mathirai - Anuboga Vaithya Navaneetham, Part-4

23. Asta Karpooram - Anuboga Vaithya Navaneetham, Part-4

24. Chanda Maarutha chendhooram - Anuboga Vaithya Navaneetham, Part-4

25. Veera parpam - Anuboga Vaithya Navaneetham, Part-4

26. Udumbu Rasa Parpam - Anuboga Vaithya Navaneetham, Part-5

27. Gandhaga ennai - Anuboga Vaithya Navaneetham, Part -6

28. Periya Pattu Karuppu - Anuboga Vaithya Navaneetham, Part-7

29. Elagu Vaana Mezhugu - Anuboga Vaithya Navaneetham, Part-7

30. Ramabana Vallathi - Anuboga Vaithya Navaneetham, Part-9

31. Maha Kodasuzhi Anuboga Vaithya Navaneetham, Part-9

32. Sathi Linga Kattu - Pulippani Vaithyam - 500

33. Iynthennai Thailam - Piramma Muni Vaithya Soothiram - Part-I

34. Kadukuennai - The Siddha Formulary of India - Part-II

35. Maal Thevi Chendhooram, Veeramaa Munivar Nasa Kanda Venba.

36. Pethi Mathirai - Agathiyar Paripoornam

37. Kuli surathirku Kiyazham - Agathiyar Paripoornam

38. Kalikkam mai nasiyam - Agathiyar Vaithya Kaandam

39. Sannikku Thailam - Agathiyar Paripoornam – 400

40. Kantha Parpam - Gunapadam (Thathu Jeeva Vaguppu)

41. Onan Sudar Thailam - The Pharmacopoea of Hospital of Indian medicine.

42. Chembu Parpam - Gunapadam (Thathu Jeeva Vaguppu)

43. Pirammi Nei - The Pharmacopoea of Hospital of Indian medicine

44. Mahaaveera Mezhugu - The Pharmacopoea of Hospital of Indian medicine

45. Inji Legium - The Pharmacopoea of Hospital of Indian medicine

46. Agathiyar Kuzhambu - The Pharmacopoea of Hospital of Indian medicine

47. Sara Noi Sankara Kadikaara Chunnan (or) compound Kadikara Mercury Chunnan

48. Thalaga chunnam- Chikicha Rathna Deepam

49. Akkini kumaran - Chikicha Rathna Deepam

50. Linga chendhooram - Chikicha Rathna Deepam

***Kuligai**:*

51. Arutha Mathirai - Chikicha Rathna Deepam

52. Swarna Boopathi Kuligai - Chikicha Rathna Deepam

53. Thamira boopathi kuligai - Chikicha Rathna Deepam

54. Kodasuri kuligai – Siddha Vaithya Thirattu

55. Korasanai kuligai - Siddha Vaithya Thirattu

***Mezhugu**:*

56. Vaan mezhugu - Siddha Vaithya Thirattu

57. Panja Lavana mezhugu - Chikicha Rathna Deepam

Kirutham

58. Maha Mega Kirutham - Piramma muni Karukkidai Soothiram-380.

59. Keezhaneli Kirutham - Theraiyar Maha Karisal

3.4. HERBAL ANTICONVULSANTS:

The pharmacological uses of the plants are primarily attributed to their essential oils and active principles having huge pharmacologic activities in the treatment of various diseases like cancer, cardio-vascular diseases, skin conditions, Gastric and intestinal

disorders, rheumatologic disorders and neurological diseases like convulsive disorders. Some of the herbal products and herbo-mineral products possess certain Central Nervous System properties like including antiepileptic activity and have been traditionally used for a long period in indigenous medicine.

"***Malachra capitata***[11] *L.* (Family: Malvaceae) is a common indigenous medicinal plant which is being used in Indigenous medicine for treating convulsions. The extract of *Malachra capitata L.* (AMC) was studied for the acute toxicity and screened for antiepielptic activity on Maximal Electroshock and Pentylenetetrazole induced convulsion models in albino Wistar rats. Acute toxicity of the extract was non toxic up to the prescribed dose 2000 mg/kg. p.o. Animals were treated with AMC at doses of 250 and 500 mg/kg body weight. Study results showed, the mean period of extensor phase of treated animals reduced extended level than compared to control group. In PTZ induced convulsion model, onset of myoclonic spasm and clonic convulsion onset was delayed in the AMC induced groups. AMC showed anti-epileptic activity against MES and PTZ animal models".

"The acticonvulsant activity of ***Cichorium intybus (C. itybus)***[12] and ***Taraxacum serotinum*** *(T. serotinum)* in the maximal electroshock, pentylenetetrazole and strychnine nitrate (STN) - induced seizure models were done in rats. Anticonvulsant activity was confirmed by the abolition of hind limb tonic extension (HLTE) in MES test and by measuring the latency to PTZ or STN-induced threshold convulsions, and also the length of seizures in the rats. In MES model, 500 mg/kg of *C. intybus* and *T. serotinum* resulted in complete elimination of HLTE in 70 and 50 % of the rats, respectively, compared to 80 % in diazepam-medicated animals. The two extracts at 500 mg/kg prolonged latency to the onset of seizure in PTZ induced model to 144.7 and 114.7 s, respectively (vs 55.2 s in control group; $p < 0.05$). The above two extracts failed to prevent rats against STN

induced seizures. *C. intybus* and *T. serotinum* possess anticonvulsant effect as they both abolish HLTE induced by MES and the latency of seizures delay, produced by PTZ".

"Maximal electroshock seizures in albino rats and pentylene tetarazole treated seizures in albino mice were carried out for antiepileptic activity of ***Vitex-negundo***[13] leaf extract. The ethanolic extract of leaf of *Vitex-negundo* was given orally in different doses (250, 500 and 1000 mg/kg p.o) in MES and PTZ experimental models and the anti epileptic activity were compared with diphenyl hydantoin in MES model as standard and valporic acid in PTZ induced seizures model as control. *Vitex-negundo* given in the doses of 250, 500 and 1000 mg/kg, p.o didn't show any protection against MES, but significant depression in post-ictal was observed in 1000 mg/kg body weight dose in comparison to control. But the below protective dose extract (100 mg/kg, p.o) potential anticonvulsant action of diphenyl hydantoin. The extract in the dose of 1000 mg/kg, po, revealed 50% protection in clonic seizures and mortality within 24-hour against PTZ induced convulsions. It also considerably reduced the duration and number of seizures. The anticonvulsant activity of *Vitex negundo* has not been seen equi-effective with standard drugs. These results reveal that *Vitex negundo* is having antiepileptic activity mainly against PTZ induced convulsions. The potentiation of diphenyl hydantoin and valporic acid with *Vitex-negundo* shows that it may be helpful as an adjuvant therapy used along with standard anticonvulsants".

"The anticonvulsant activity of ***Moringa oleifera***[14] on mice by MES, PTZ and pilocarpine induced seizures in animal models were done by Anu et al. The ethanolic extract of leaves of *Moringa oleifera* at a dose of 200 mg/kg was given to study its anticonvulsant effect on PIS (Pilocarpine induced convulsions), PTZ and MES in swiss albino mice. Reduction of tonic hind limb extension, convulsion period, elimination of seizure was noted respectively for the above models. The ethanolic extracts of leaves of

Moringa oleifera at a dose of 200 mg/ kg significantly ($p<0.001$) reduced the hindlimb extension induced by MES, it also significantly ($p<0.001$) protected the mice from PTZ induced tonic seizures. Status epilepticus state was not reached in the same dose of the plant extract in PIS. This results suggests that *Moringa oleifera* leaves extract may produce anticonvulsant activity, since it prevented the MES induced hind limb extension and reduced the duration of seizures induced by PTZ and abolished status epilepticus in pilocarpine induced seizures".

"The anticonvulsant and anxiolytic activity of the methanolic extract of ***Allium Cepa***[15] *Linn* (MEAC) was carried out by *Gummalla et al*. Anxiolytic activity of methanolic extract of *Allium Cepa* bulbs at 200 and 400 mg/kg doses was observed using open field test (OFT), light and dark transition (L & DT), elevated-plus-maze (EPM). Anticonvulsant activty was studied by INH and MES induced seizures in animal models. MEAC was given orally for seven days considerably increased number of entries and time duration spent in open arms in EPM model; number of squares crossed, latency and time spent in central square in OFT time spent in light zone and number of transitions in LDT model. MEAC in the dose 200 and 400 mg/kg showed significant reduce in the duration of hind limb extension phase in electroshock convulsions; protected the animal against the Isoniazid induced seizures. This showed significant improvement GABA levels in brain after the treaetment with *Allium cepa"*.

"The antiepileptic activity of ***Ocimum basilicum***[16] was studied in pentylenetetrazole induced epilepsy model. In this research, Six equal groups of 48 female mice (for removing gender factor) were studied. The experimental group of animal comprised control, sham, and four treatment groups, they received the extract at different doses of 100, 250, 300 and 350 mg/kg (intraperitoneally; ip), Sixty five minutes before PTZ injection and the factors of symptoms of epilepsy and frequency of seizures

were studied. The results of using various doses (100, 250, 300, 350 mg/kg) of the extract showed that the mice in the dose 100 mg/kg group exhibited the highest incidence of epileptic seizures. The samples received the extract at 100 mg/kg exhibited highest and 250 mg/kg exhibited lowest frequency of myoclonic twitches. The group treated with 250 mg/kg dose, the epileptic symptoms and frequency of seizures were decreased ($p<0.05$). By these results, the hydroalcoholic extract of *O. basilicum* at 250 mg/kg dose could be suggested as an effective supportive drug for preventing epilepsy in the animal model"

"Curcumin"[17] is one of the active biomolecule of Turmeric. The anticonvulsive activity of curcumin was studied in albino Wistar rats in two different methods. First, Curcumin in a dose of 100 mg/kg was tested in comparison to Phenytoin sodium by Maximal Electroshock Seizure (MES) model, the duration of tonic hind limb extension was studied. Secondly, antiepileptic activity of Curcumin was compared to Diazepam in Pentylenetetrazol (PTZ) induced seizure in which onset and severity of convulsions was observed. The obtained results obtained were statistically analyzed. The results reveals that Curcumin considerably decrease the duration of tonic hind-limb extension in MES model with a $p < 0.05$ and it the latency to the onset of seizures was increased with $p< 0.01$ in PTZ model" study reported by *Dr. Kranti Tekulapallan"*

Praveen Kumar Uppala, reported in his studies that "The antiepileptic effect of methanolic extract of ***Brassica nigra***[18] *seeds* on Picrotoxin (PIC) induced convulsion, Pentylene tetrazole (PTZ) and Maximal electroshock induced seizures (MES) in mice were studied. It was observed that the extract at doses of 200 & 400 mg/kg, p.o, significantly extent the tonic seizure onset and duration of incidence of seizures is also reduced in PTZ, PIC and Biccuculine induced seizure models. But in MES model, the extract showed significant effect in reducing tonic hind limb extensions".

"The anti convulsive effect of ***Psidium guajava*** [19] was studied against chemically and electrically induced convulsions by The seizures were induced by pentylene tetratriazole and maximal electroshock methods. The antiepileptic effect of *Psidium guajava* was compared with diazepam a standard anticonvulsive drug. This study results showed that that the extract of *Psidium guajava leaves* at higher and medium doses produce highly significant results. The sustained increases in the onset of convulsion and decreases in the rate of convulsion were seen. This beneficial effect may be due to flavonoids and saponins present in the extract".

"The methanolic extract of of indian medicinal plant ***Celastrus paniculata***[20] was evaluated for the antiepileptic activity using different animal models. Seizure induced animals were treated with *Celastrus paniculatus Wild* whole plant Methonolic extract. (MECP) at the doses 200 mg/kg, 400 mg/kg and 600 mg/kg showed considerable reduction of Pentylenetetrazole and Isoniazid induced epileptic seizures. Onset of seizure was found to increase and the extension period of seizure was found to be reduced in the extract treated animals as compared to control group. The extract of *Celastrus paniculatus* whole plant considerably delayed the epileptic seizure onset induced by INH and significantly reduced the period (in sec) of tonic hind limb extension phase of PTZ-induced epileptic seizure. This suggested that the active principles of *Celastrus paniculatus* contains may possess antiepileptic activity significantly".

"Clausena anisata[21] (Family: Rutaceae) a tropical medicinal plant, have ethnomedical claim in the management of epilepsy. An investigation was carried out to the antiepileptic activity of *Clausena anisata* ethanolic extracts of stem bark, root bark and leaf against PTZ induced convulsions in mice. This study reveals that the bioactive ingredients of root bark of *Clausena anisata* may be beneficial in petit mal epilepsy and

lend pharmacological acceptance to the ethnomedical claim in the management of epilepsy".

"Cicer arietinum[22] (Chickpea) is one of the most important harvests in the world with high nutritional value. The anticonvulsant effect of Chickpeas was carried out by *Soroush Sardari et.al* in animal models of epilepsy. Protective effect of the extract was examined against tonic seizures induced by MES and clonic seizures induced by pentylenetetrazole in mice and electrical kindling model of complex partial seizures in rats. It significantly inhibited clonic seizures induced by PTZ. Phytochemical screening revealed the presence of significant level of alkaloids in the extract and fractions. The anticonvulsant molecule found in the seeds of *C. arietinum* promises an effective and inexpensive source of antiepileptic medication".

"The anticonvulsant effect of methanolic extract stem bark extract of ***Securinega virosa***[23] was carried out in PTZ and 4- amino pyridine induced convusions in mice. The study results showed that the extract of *Securinega virosa* offered protections (80 and 20 %) at the doses 12.5 and 25 mg/kg b w and 20% at a tested 50 mg/kg b w dose in PTZ and 4-aminopyridine induced convulsions, implying that 12.5 mg/kg bw showed a higher protective activity. This study reveals that the plant extract possesses bioactive ingredients that may be beneficial in the treatment of epilepsy and lend credence to the traditional claim in management of epilepsy".

"Hydroalcoholic extracts of ***Mussaenda philippica***[24] (M. Philippica) was carried out for the anticonvulsant activity by maximal electroshock method (MES), pentylenetetrazole (PTZ) induced model, Strychnine (STR) induced seizures at various dose levels. The extract at 100 and 200 mg/kg obtained a significant ($P<0.01$) dose dependent increase in onset of seizure compared to the control in PTZ, MES and strychnine induced seizures. The results obtained indicates that the extracts of *M.*

philippica leaves and sepals may be helpful in controlling the grandmal and petitmal epilepsy".

"The anticonvulsant effect of ***Asparagus racemosus***[25] was studied by the effects on seizures by maximal electric shock, picrotoxin, and strychnine induced convulsive methods in mice. The anticonvulsant activity of ethanolic extract of *A. racemosus* (ETAR) and methanolic extract of *A. racemosus* (MEAR) were studied. In maximal electric shock reduce of the hind limb tonic seizure were analyzed. In picrotoxin induced convulsion, time of onset of seizures and mortality were observed; In strychnine induced convulsion, the time of commencement of tonic convulsions and death were observed and noted. The ETAR (250 and 500 mg/kg, p.o) showed significant ($P<0.001$) activity against acute seizures induced by maximal electric shock (MES), chemical consultants such as strychnine and picrotoxin as compared to MEAR ($P<0.05$) at the same dose compared statistically by ANOVA-Tukey's comparison test. The data obtained reveals that the plant exhibits anti-convulsant property".

"The anticonvulsant activity of ***Butea monosperma***[26] generally used in traditional medicine for the treatment of neuro degenerative diseases, amnesia and mental illness was studies in the paper. The anticonvulsant activity of the crude methanol stem extract of *Butea monosperma* (BMME) and its bioactive compounds at doses of 100, 200 and 300 mg/kg and 20 mg/kg respectively using Pentylenetetrazole induced convulsion, Chemically induced convulsion and Maximal electroshock convulsion were investigated in Swiss albino mice. The onset of Phenobarbital induced sleep was decreased dose dependently, there was a remarkable increase in the duration of sleep in the animals when compared with the control. BMME at doses of 100-300 mg/kg considerably decreased ($p<0.001$) the tonic hind limb extension in MES induced models. There was 66.67% protection against convulsion and 83.33% protection against mortality respectively at the

highest dose of 300 mg/kg. In PTZ induced convulsion, BMME delayed the signs like onset of jerk and clonus, straub tail, and extensor phases in a dose dependent manner. 300 mg/kg of BMME with 45 mg/kg Pentobarbital showed the highest activity in this test. Isolated compound from BMME also produced better effect in PTZ induced seizure model. These findings suggested that the methanol extract of the plant and its isolated bioactive fraction is beneficial in the treatment of epilepsy".

"Cissus quadrangularis[27] Linn. plant is used in the treatment of anorexia, asthma, sickle cells, colds, pains, malaria, asthma. In-vivo animal models of epilepsy (MES, n-methyl-d-aspartate, PTZ, isonicotinic hydrazid acid and strychnine induced convulsions or turning behavior) and insomnia (diazepam induced sleep) were used. The aqueous extract of the stems of *C. quadrangularis* strongly increased the total sleep time induced by diazepam (50 mg/kg i.p.). It also protected mice against MEZ, PTZ, strychnine and n-methyl-d-aspartate-induced convulsion and delayed the seizures onset time induced by iso-nicotinic hydrazid acid. The results reveals that the extract of *C. quadrangularis* possesses anticonvulsant and sedative actions in mice and could evidence in its use in the treatment of insomnia and epilepsy use in Africa".

"The roots of the plant ***Vetiveria zizanioide***[28] belonging to Poaceae family is widely used in traditional medicine since ancient times to treat various medical illnesses including epilepsy. The oil extracted from the roots are indicated in insomnia, depression, anxiety, nervousness, rheumatic diseases, sprain and migrane. To evaluate the antiepileptic activity of *Vetiveria zizanioides* oil in Mice using MES and PTZ induced seizure animal models. Adult male mice 48 nos were selected and 24 mice were allocated to each type of animal model, chemical and electrical. The animals in each model were divided into 4 groups of 6 animals in each control, standard and test groups of two category. Control group received normal saline standard group received sodium valporate

and the two test groups received *Vetiver Oil* (VO) 250 and 500 mg/kg respectively. Antiepileptic activity was studied based on duration of different phases of convulsions and mortality and the results were compared with control and standard. In MES model 250 mg of *Vetiver* oil reduced the duration of hind limb extension (HLE) in 3 out of 6 animals and seizure protection was 50% whereas 500 mg decreased the duration of tonic HLE in 4 animals with the protection of seizure of 66.6%. No animals were died in MES model. In PTZ model both the doses of VO delayed the onset of clonic phase and prevented death in 50% of animals equal to the standard drug ie. sodium valproate. *Vetiver* oil has shown anticonvulsant activity in both PTZ and MES animal models. 500 mg/kg of *Vetiver* oil has higher protection against seizure in MES model and both 250 and 500 mg were found to have same efficacy as in the animals treated with sodium valporate".

"Anticonvulsant potential of ***Bryophyllum pinnatum***[29] was investigated in mice by *Arijit Dutta et al.* They studied the effects of ethanolic extract of the leaves of *Bryophyllum pinnatum* against MES induced convulsions and PTZ seizure model in mice. Parameters observed in MES model were duration of tonic extension of hind-limb, the complete recovery time and percentage protection. In the PTZ model, the parameters observed were the onset of clonic convulsions in the latency period, clonic phase duration, reduction in percentage of clonic phase and mortality percentage. In the MES induced convulsion Phenytoin (25 mg/kg) as standard drug and in PTZ induced model, Diazepam (4 mg/kg) was used as the standard drug. Extract was used in 200, 300 and 400 mg/kg doses. Results obtained in this study validate the anticonvulsant effect of ethanolic extract of *Bryophyllum pinnatum* leaves".

"The anticonvulsant effect of the extract of ***Erythrina variegata***[30] *L* was investigated in mice and rats, in order to verify the traditional use of the plant in the

treatment of epilepsy. The pentyleneterazole (PTZ) and the maximal electroshock seizure (MES) models were used for assessing the anticonvulsant effects of the chloroform extract in mice and rats. The chloroform extract (500 mg/kg p.o) of that showed significant protection (71.4%) against PTZ-induced convulsion and onset of seizures compared with the control group in mice. At 500 mg/kg p.o., the extract also showed significant protection (71.4%) against MES-induced convulsions in rat. The study result indicates that the chloroform extract of *Erythrina variegata L* may be beneficial in both absence and tonic clonic seizures".

In Lork's method the investigation of the anticonvulsant activity of root bark extract of *Carissa edulis* was done "The median lethal dose (LD_{50}) of ***Carissa edulis***[31] extract was determined using *Lork's* method (1983). The anticonvulsant activity of the extract was conducted using PTZ induced convulsion in mice and maximal electroshock test in chicks with benzodiazepine and phenytoin as standards, respectively. While mechanistic studies were conducted using both flumazenil, aGABAA-benzodiazepine receptor complex site antagonist and naloxone a non-specific opioid receptor antagonist. The median lethal dose (LD_{50}) of *Carissa edulis* 282.8 mg/kg and over 5000 mg/kg was administered intra-peritoneal and orally.. *Carissa edulis* produced 40 % and 20 % protection against convulsions at 5 and 20 mg/kg, respectively, when compared with 100 % protection with benzodiazepine. The mean onset and percentage protection against convulsion in mice treated with *Carissa edulis* extract were reduced by flumazenil and naloxone. *Carissa edulis* exhibited dose-dependent inhibition of the seizure induced by MEST with 20 mg/kg provided 90% protection while phenytoin (20 mg/kg) produced 100% protection. These study results shows that *Carissa edulis* possesses bio active constituents having anticonvulsant effect that supports the traditional claims of the use of the plant in the management of epilepsy. Anticonvulsant effect was studied by maximal

electroshock (MES) and Pentylenetetrazol (PTZ) induced convulsions in mice. The extract reduced hind limb tonic extensions (HLTE) produced by MES and also exhibited protective effect in PTZ-induced seizures. It showed that the ethanolic extract of *Cynodon dactylon* has anticonvulsant activity suggesting the depressant action in the central nervous system".

"The anticonvulsant effect of the ethanolic extract of the roots of ***Carissa carandas***[32] (ERCC) on electrically and chemically induced seizures was studied by Karunakar Hegde The anticonvulsant activity of the ethanolic extract of the roots of *C. carandas* (100, 200 and 400 mg/kg, i.p.) was studied on maximal electroshock-induced seizures and pentylene tetrazole-, picrotoxin, bicuculline- and NMDA induced seizures in mice. The latency of tonic convulsions and the number of animals protected from tonic convulsions were noted. ERCC (100-400 mg/kg) significantly reduced the duration of seizures induced by maximal electroshock (MES). But only 200 and 400 mg/kg of the extract conferred protection against convulsion (25 and 50%, respectively) on the mice. The same doses also protected animals from pentylenetetrazole-induced tonic seizures and the onset of tonic seizures produced by picrotoxin and N-methyl-dl-aspartic acid are significantly delayed. The extract had no effect on bicuculline-induced seizures. The data suggested that the ethanolic root extract of *C. carandas* is having anticonvulsant effect via non-specific mechanisms since it reduced the duration of seizures produced by maximal electroshock and latency of seizures were also delayed by inducing pentylenetetrazole and picrotoxin".

"Cymbopogon winterianus[33] (Poaceae) is used for its analgesic, anxiolytic and anticonvulsant properties in Brazilian folk medicine. This report aimed to perform phytochemical screening and to investigate the possible anticonvulsant effects of the essential oil (EO) from fresh leaves of *C. winteri anus* in different models of epilepsy. A

behavioral screening showed that EO (100, 200 and 400 mg/kg; ip) caused depressant activity on CNS. When given concurrently, EO (200 and 400 mg/kg, ip) significantly reduced the number of animals that exhibited PTZ and PIC-induced seizures in 50% of the experimental animals (po0.05). Additionally, EO (100, 200 and 400 mg/kg, ip) significantly increased (po0.05) the latency period of clonic seizures induced by STR. Their results demonstrated a possible activity anticonvulsant of the EO".

The study report of *Shirish d. et al*[34,35] "The anticonvulsant activity of ethanolic extract of roots and rhizomes of *Glycyrrhiza glabra* in mice was assessed by maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) treated albino mice. The lithium-pilocarpine animal model of status epilepticus was also used to assess the anticonvulsant activity in rats. The ethanolic extract *G. glabra* did not reduce the duration of tonic hindlimb extension in the MES test even in the dose of 500 mg/kg. The extract significantly delayed the onset of clonic convulsions induced by pentylenetetrazol. The dose of 100 mg/kg gives protection to all animals. The extract also protected rats against seizures produced by lithium pilocarpine. The ethanolic extract of *G. glabra* inhibits PTZ and lithium-pilocarpine induced convulsions but not MES-induced convulsions".

Table 1: List of Medicinal plants having anticonvulsant activity:

Sl. no.	Plant Name	Part Used	Family	Model Used
1	*Abrus precatorious*	Root	Fabaceae	LIS, SIS
2	*Aegle marmelos*	Fruit	Rutaceae	PTZ, MES
3	*Albizzia lebbeck*	Leaf, Root	Fabaceae	PIC, PTZ, MES
4	*Allium ascalonicum*	Shallot	Liliaceae	LIS
5	*Allium cepa*	Bulb	Liliaceae	LIS, SIS
6	*Allium sativum*	Bulb	Liliaceae	LIS
7	*Alstonia boonei*	Stem bark	Apocynaceae	LIS, MIS

8	*Alstonia scholaris*	Flower	Apocynaceae	MES
9	*Altingia excels*	Entire plant	Hamamelidaceae	ACV
10	*Apium graveolans*	Seed	Apiaceae	MES, MIS
11	*Basella alba*	Leaf, Stem	Basellaceae	LIS, SIS
12	*Basellarubra*	Leaf, Stem	Basellaceae	LIS, SIS
13	*Benincasa hispida*	Fruit	Cucurbitaceae	PTZ, MES, PIC
14	*Berberis lycium*	Root	Berberidaceae	MES
15	*Boerhaavia diffusa*	Root	Nyctaginaceae	PTZ
16	*Borassus flabellifer*	Root	Arecaceae	PTZ
17	*Butea monosperma*	Flower	Fabaceae	MES, PTZ, SIS
18	*Calophyllum apetalum*	Nuts	Clusiaceae	PTZ
19	*Calophyllum tomentosum*	Nuts	Clusiaceae	PTZ
20	*Cannabis indica*	Flowering tops	Cannabaceae	MES
21	*Canscora decussate*	Entire plant	Gentianaceae	MES
22	*Carica papaya*	Leaf	Caricaceae	MES, PTZ
23	*Celastrus paniculatus*	Seed oil	Calastraceae	PIC, SIS, LIS
24	*Centella asiatica*	Entire plant, Leaf	Apiaceae	LIS, PTZ, MES
25	*Chenopodi umblitum*	Aerial part	Chenopodiaceae	MES
26	*Cocculus hirsutus*	Root, Stem	Menispermaceae	MES, MIS
27	*Connarus wightii*	Aerial part	Connaraceae	SIS
28	*Convolvulus pluricaulis*	Entire plant	Convolvulaceae	MES
29	*Crocus sativus*	Stigma and style	Iridaceae	PTZ
30	*Curcuma amada*	Rhizome	Zingiberaceae	MES
31	*Cyathea nilgirensis*	Aerial part	Cyathiaceae	MES
32	*Cylistas cariosa*	Root	Fabaceae	SIS
33	*Delphinium denudatum*	Entire plant	Ranunculaceae	MES
34	*Dillenia indica*	Leaf	Dilleniaceae	MES

35	*Diospyros peregrine*	Entire plant	Ebenacaea -	ACV
36	*Elaeocarpus ganitrus*	Fruit	Elaeocarpaceae	MES, MIS
37	*Elleteria cardamomum*	Seed	Zingiberaceae	ACV
38	*Eryngiumfoetidum*	Entire plant	Apiaceae	SIS
39	*Euphorbia antiquorum*	Entire plant	Euphorbiaceae	MES
40	*Euphorbia tirucalli*	Aerial part	Euphorbiaceae	ACV, MIS
41	*Evolovulus nummularius*	Entire plant	Convolvulaceae	MIS
42	*Grewia hirsuta*	Entire plant	Tiliaceae	SIS
43	*Gymnosporia falconeri*	Aerial part	Celastraceae	MIS
44	*Hibiscus rosasinensis*	Flower	Malvaceae	MES, PTZ
45	*Iris kumaonensis*	Entire plant	Iridaceae	MES
46	*Juniperus marcopoda*	Fruit	Cupressaceae	ACV
47	*Leea indica*	Leaf	Vitaceae	SIS
48	*Leonotis leonurus*	Leaf	Lamiaceae	PTZ, PIC
49	*Lettsomi asetosa*	Aerial part	Convolvulaceae	MES
50	*Luvanga scandens*	Fruit	Rutaceae	MES
51	*Matricaria chamomilla*	Flower	Asteraceae	PTZ
52	*Mikania cordata*	Aerial part	Asteraceae	MES
53	*Mimosa pudica*	Leaf	Mimosoideae	PTZ, SIS
54	*Nardostachys jatamansi*	Rhizome	Valerianaceae	ACV,MES, PTZ
55	*Ocimum sanctum*	Entire plant	Lamiaceae	ACV
56	*Paeonia emodi*	Root	Paconiaceae	MIS, MES
57	*Picrorhiza kurrooa*	Entire plant	Scrophulariaceae	MIS
58	*Pistacia integerrima*	Galls	Anacardiaceae	PTZ
59	*Polypodium vulgare*	Root	Polypodiaceae	MES, PTZ
60	*Pongamia pinnata*	Seed	Fabaceae	MES
61	*Prunus spinosa*	Fruit	Rosaceae	PTZ

62	*Pterocarpussantalinus*	Heartwood, bark	Fabaceae	MES
63	*Rauvolfiatetraphylla*	Entire plant	Apocynaceae	MES
64	*Rhododendron niveum*	Aerial part	Eriacaceae	SIS
65	*Rivea hypocrateriformis*	Fruit	Convolvulaceae	MES
66	*Rubu sellipticus*	Leaf	Rosaceae	MES
67	*Salvia haematodes*	Root	Lamiaceae	MES
68	*Sapindus trifoliatus*	Seed	Sapindaceae	MES
69	*Sepia officinalis*	Shell	Sepiadeae	ACV
70	*Sesbania grandiflora*	Leaf	Fabaceae	MES
71	*Solanum khasianum*	Entire plant	Solanaceae	MES
72	*Stenochlaena palustris*	Entire plant	Polypodiaceae	MES
73	*Swertia purpurascens*	Entire plant	Gentianaceae	MES, SIS
74	*Vanda roxburghii*	Entire plant	Orchidaceae	MES
75	*Vitex negundo*	Leaf	Verbenaceae	LIS, SIS
76	*Withania somnifera*	Entire plant	Solanaceae	MES

3.5. SYNTHETIC- ANTICONVULSANTS

Kinga Sa reported in his study that the "Analgesic and anticonvulsant activity of new derivatives of 2-substituted 4-hydroxy butanamides in mice was reported by Kinga Sa[36] et al The pharmacological activity of four *g*-hydroxybutyric acid (GHB) amide derivatives was investigated. The compounds influence on motor coordination was studied in the chimney test. The compounds at a dose of 25 mg/kg (*ip*) prolonged the nociceptive reaction time latency in the hot plate assay to various degree. Their analgesic efficacy was observed 30 min after their administration (percent of maximal possible effect (% MPE) = 16.93 and 22.72, respectively). In the chimney test, the compounds impaired the animals motor coordination to various degree".

Many compounds, which have different inhibitory potencies towards mGAT1–mGAT4, are either used as anticonvulsant agents like tiagabine or tools served for the research on pharmacological properties of different GAT types[37,38]. Tiagabine, for instance, is highly selective towards mGAT1 (IC50 = 0.8 μM); SNAP-5114 is modestly selective towards mGAT4 (IC50 = 6.6 μM) but has also affinity for mGAT2 (IC50 = 22 μM) and mGAT3 (IC50 = 20 μM), whereas (S)-EF1502 is mGAT2 inhibitor (IC50 = 34 μM) [34]. Another compound, NNC-005 2090 is relatively selective towards mGAT2 (IC50 = 1.4 μM) having some affinity for mGAT3 (IC50 = 41 μM), mGAT1 and mGAT4 (IC50 = 19 μM and IC50 = 15 μM, respectively).

Mentat, a polyherbal preparation, is mostly used for its memory enhancing property[39]. The anticonvulsant profile of mentat in animal models of convulsions, maximal electroshock seizures (MES) and pentylene tetrazole (PTZ). Anticonvulsant activity of mentat was carried out in four groups of animals (rats) (n=6). The animals were given mental for seven days orally (p.o.) with 300 and 600 mg/kg. On 7th day the animals were subjected to MES and PTZ induced convulsions, phenytoin (25 mg/kg) intraperitoneally (i.p.) and sodium valproate (200 mg/kg, i.p.) were used as standards respectively. Mentat exhibits dose related anticonvulsant activity in PTZ and MES induced convulsion models. Mentat in the dose of 300 mg/kg showed significant anticonvulsant effect as compared to control in the two models, while at the dose of 600 mg/kg showed anticonvulsant activity, which was significant as compared to control and reference drugs in MES and PTZ induced models of convulsions. The experimental research suggests that mentat has a broad spectrum antiepileptic activity.

A series of 5, 7-dibromoisatin semicarbazones have been synthesized in good yield, involving aryl urea and aryl semicarbazide formation[40,41]. The structures of the synthesized compounds were confirmed by the spectral datas. All the compounds were

evaluated for anticonvulsant and CNS depressant activities. Anticonvulsant activity was evaluated after intraperitoneal (i.p.) administration to mice by maximal electroshock (MES) induced seizure method and semicarbazide exhibited prominent anticonvulsant activity in the series with little CNS depressant effect as compared to standard drug.

A series of 4-(3-Chlorophenyl)-1-(substituted acetophenone) semicarbazones was synthesized by starting with 3-chloroaniline[42]. This on reaction with sodium cyanate yielded 1-(3'-chlorophenyl) urea followed by reaction withhydrazine hydrate in the presence of ethanol gave 4-(3'-chlorophenyl) semicarbazide which on condensation with acetophenone gets converted in to final compounds. The purity of the newer compounds was checked by m.p. and TLC analysis. The structures of the synthesized compounds were characterized by FTIR, NMR, EIMS-spectral data and elemental analysis. All the synthesized compounds were pharmacologically analsyed for the anticonvulsant activity by Maximal Electroshock method by using phenytoin as standard at a concentration of 30 mg/kg. The anticonvulsant effect compounds were assessed by reduction of hind limb tonic extensor phase. The synthesized derivatives were found to be the most potent anticulvulsive agents.

3.6. VARIOUS ANIMAL MODELS OF EPILEPSY[43]:

Simple Partial Seizure Models.

Using Cortically Implanted Metals

Aluminum Hydroxide Model

Cobalt Model

Zinc Model

Complex Partial Seizure Model.

Kainic Acid Administration (KA)

Repetitive Electrical Stimulation (Kindling animal models)

Administration of Tetanic Toxin

Generalized Tonic Clonic Seizure Models.

Maximum electroshock

Pentilentetrazol induced model

Generalized Partial Seizure Models.

Bicuculline Model

Gaba Abstinence Animal Model

Tetanic Toxin induced Model

Generalized absence seizure models.

Audiogenic seizures in mice.

Status epilepticus.

Pilocarpine

Kainic Acid Model

Kindling Model

Pentylentetrazol Model

3.7. DRUG REVIEW

1. RASAM (MERCURY, HYDRAGYRUM) [44, 45]- Figure 1

Rasam (Mercury (Hg) is a transition metallic element exists as liquid form at room temperature.

Properties of Mercury

Its atomic number is 80, mass 200.6g/mol, melting point -38.83ºC, covalent radius-132+/-5pm, boiling point-356.73˚C, Electronegativity-2.00(pauling scale), heat fusion-2.29 kJ/mol., magnetic ordering-diamagnetic

Synonyms mentioned in Siddha:

Sootham, Punniyam, Karpam, Soorya Virothi, Saamam, Sukilam, Yogam, Eesan, Sivan vinthu, Kanavan, Vasuki Nathan, Baratham, Vinneer

Taste:

Six tastes - dominated by sweet

Potency:

Hot and Cold

Rasam is the chief of all elements. It gives good health, protects the body and cures the disease that affects the body.

General properties:

Proper use of rasam as medicine, cures the diseases of eye, Syphilis, *Gunmam* (eight types of ulcers), *Soolai* (Throbbing pain), *Perm pun* (Chronic ulcer).

Special properties of *Rasam*:

Rasam, unlike other drugs, is useful in the treatment of diseases caused by both heat and cold. But in other drugs, if a drug is useful in diseases caused by heat, it will definitely aggravate the diseases caused by cold.

Rasam is one of the ingredients in the following siddha formulations indicated for various diseases as mentioned below:

Kantha chenduram, Sootha parpam, Muthu chenduram, Siddhivallathi legiyam4, Vanga chenduram, Thamirachenduram6, Kalamega Narayana Chenduram, Rasa Parpam8, Rasa Parpam, Kutta Kuzhi Thylum, Punnuku Thiri, Putru Pugai, Mandoora Parpam, Rasa Parpam, Vellai Seelai, Singi Thylum, Purai mare Patchai Thylum, Vellieya Parpam, Gowri Chinthamani Chenduram, Chandamarutha chenduram, Jeeva Narayana Chenduram, Sootha chenduram, Ointment for wound, Kantha Rasa villai, Uththira Rasa Chenduram, Manmatha Chinthamani Rasa Chenduram, Gowri Chinthamani Ranamugatheera Rasa Chenduram, Sarvanoi Hara Sandamarutha Chenduram, Vithu Rasa Mezhugu, Rathinahaara rasa mezhugu, Gandhaga mezhugu, Padana kulanthagan, Pancha pooth chenduram, Pusanda ramapana mathirai, Rasa vanga pugai.

2. *GANDHAGAM* (SULPHUR) – Figure -2

Sulphur is an chemical element, as according to the periodic desk chemical symbol of *Gandhagam* "S" and atomic number sixteen. Due to the fact it's far 0.0384% of the Earth's crust, Sulphur is the 17th most considerable detail following strontium. Sulphur also takes on many paperwork, which encompass elemental Sulphur, organo-Sulphur compounds in oil and coal, H2S(g) in herbal fuel, and mineral sulfides. Sulphur is extracted by way of using the Frasch manner, a technique where superheated water and compressed air is used to attract liquid Sulphur to the surface. Elemental Sulphur can be produced by lowering H2S, generally discovered in oil and herbal gasoline. For the most part although, Sulphur is used to produce SO2(g) and H2SO4.

Physical Properties of Sulphur

Sulphur is normally found as a light yellow, opaque, brittle stable and orthorhombic crystals. Sulphur have two times the density of water, it is also not soluble in water. then again, Sulphur is particularly soluble in carbon disulfide.

Synonym:

Kaari ilai natham, Parai, Veeryam, Atheetha prakasam, Peejam, Selvi vinthu, Sakthi, Sakthi peejam, Chendthurathu aathi, Naatham, Naatram, Parai natham, Rasa suronitham

General properties:

This is considered useful in the treatment of 18 types of skin diseases, Liver enlargement, abdominal disorders, eye diseases, chronic venereal diseases, chronic diarrhea, gastric ulcer, fever.

Taste:

Bitter and Astringent

Actions:

Laxative, Tonic, Anti septic

It also increases the bile fluids and various secretions of body including skin.

Gandhagam is one of the ingredient in the following Siddha formulations indicated for various diseases as mentioned below:

Kantha chenduram, Sootha parpam, Velieeya chenduram, Pavalavanga chenduram, Muthu chenduram, Siddhivallathi legiyam, Vipuruthi ennai, Thamira chenduram, Kalamega narayana chenduram, Panchpadana chenduram, Rasa parpam, Rasa parpam, Gandhaga parpam, Kuttakuzhithylum, Gandhaga rasayanam, Punnuku thiri, Putru pugai, Mandoora parpam, Rasa parpam, Purai mare patchai thylum, Gowri chinthamani chenduram, Chandamarutha chenduram, Sootha chenduram, Dhanvanthiri

chenduram, Parangi pattai illagam, Vayu mathirai, Neelakanda rasa pathangam, Uththira rasa chenduram, Manmatha chinthamani rasa chenduram, Gowrichinthamani ranamugatheera rasa chenduram, Sarvanoi hara sandamarutha chenduram, Rathinahaara rasa mezhugu, Ganthaga poora parpam, Gandhaga chenduram, Gandhaga mezhugu, Gandhaga chooranam, Gandhaga rasayanam, Pancha padana chenduram, Pancha pooth chenduram, Pusanda ramapana mathirai Guru sanjeevi Mezhugu, Rasa vanga pugai, Linga pathangam, Paal vallathi, Neeradi muthu vallathi, Thengaai nei, Pancha pana chenduram, Narayana chenduram48, Amirtha nandhi mezhugu, Ramapana idi marunthu, Pancha pana chenduram52, Rasagandhi Mezhugu, Maha koda suzhi mathirai, Dhanvanthriya sanda marutha chenduram55, Rasa parpam, Gandhaga parpam, Kirubahara shanmuga chenduram, Pancha padana chenduram, Swarna pushpa rasa chenduram, Gandhaga thylum.

3. *LINGAM* (RED SULPHIDE OF MERCURY) - Figure -3

Cinnabar is generally observed in a big, granular or earthy form and is brilliant brick-crimson in colour. Structure of the cinnabar is a trigonal crystal structure.

Physical properties:

The compostition of *Lingam* is Mercury sulphide (HgS)

Chemical classification is Sulphide

It is bright red to brownish red in colour

Its streak appears as red in colour

Its cleavage is perfect and prismatic

Specific gravity is 8 to 8.2

Synonym:

Inguligam, Kadaivanni karpam, Kalikkam, Kanjanam, Karanam, Karpam, Sandagam, Samarasam, Chenduram, Maniragam, Milecham, Vani, Vanni,

Character:

It is hard, when it is put into fire it becomes smoke, not soluble in water, it has no smell and Taste.

It has hot potency. It has property of tonic. It is effective in the treatment of pyrexia, diarrhoea, delirium, urticaria, dieresis, tuberculosis, syphilis, leprosy, eczema, skin diseases, throbbing pain and vatha diseases.

It has the property of curing the diseases caused by the earth element and cures the disease caused by the water element.

***Lingam* is** is one of the ingredients in the following siddha formulations indicated for various diseases as mentioned below:

Veera mezhugu, Kalamega narayana chenduram, Panchpadana chenduram, Kuttakuzhithylum, Putru pugai, Pilavai kiranth ranathirku pugai, Kantha rasabillai, Linga chenduram, Parangi pattai illagam, Neelakanda rasa pathangam, Uththira rasa chenduram, Manmatha chinthamani rasa chenduram, Pancha padana chenduram, Padana kulanthagan, Pancha pooth chenduram, Pancha pooth chenduram, Guru sanjeevi Mezhugu, Rasa vanga pugai, Neelakanda palai, Guru pathangam, Linga pathangam, Paal vallathi, Thengaai nei, Pancha pana chenduram, Vaalai rasam, Narayana chenduram, Amirtha nandhi mezhugu, Pancha pana chenduram, Maha koda sushi mathirai, Dhanvanthriya sanda marutha chenduram, Kirubahara shanmuga chenduram, Pancha padana chenduram, Soolai kudori, Kantha rasa villai.

4. *THALAGAM* (YELLOW ARSENIC TRISULPHIDE)- Figure -4

Yellow arsenic trisulphide Arsenic(III) sulphide, Orpiment, Sulphuret of arsenic ia a yellow or red crystalline solid or powder. it is combustible and no longer soluble in water. Its density is 3.43 g cm^{-3}, melting point is 310ºC.

Synonym:

Peethagi, Aalembi, Pinjanam, Kalbuththi, Manjal varni, Maaldevi,

General properties:

It is effective in the treatment of skin disease, disease of head and tongue, fever with chills, kabam, urinary tract disease, venereal ulcer in the urethra

Actions:

Expectorant, Antipyretic, Emetic, Convalescent, Tonic

Thalagam is is one of the ingredients in the following siddha formulations indicated for various diseases as mentioned below:

Kalamega narayana chenduram, Panchpadana chenduram, Kuttakuzhithylum, Punnuku thiri, Putru pugai, Vengara podi, Singi thylum, Pilavai kiranth ranathirku pugai, Manmatha chinthamani rasa chenduram, Pusanda ramapana mathirai, Guru sanjeevi Mezhugu, Neelakanda palai, Linga pathangam, Paal vallathi, Thengaai nei, Pancha pana chenduram, Kiranthi araiyappurku ennai, Narayana chenduram, Amirtha nandhi mezhugu, Ramapana idi marunthu, Pancha pana chenduram, Rasagandhi Mezhugu, Maha koda suzhi mathirai, Gandhaga parpam, Kirubahara shanmuga chenduram, Pancha padana chenduram.

5. *MANOSILAI* (ARSENIC DISULPHIDUM-BISULPHURET OF ARSENIC REALGAR-RED ORPIMENT - Figure 5

Physical Characteristics:

It is orange to red in colour, monoclinic in crystal system, luster is resinous, cleavage appears in one direction. Hardness of the red orpiment is 1.5 to 2. specific gravity is 3.5 to 3.6. Streak of the orpiment is orange to orange- yellow in colour.

Synonym:

Silai, Vil, Kunadi, Nanmugan, Devi, Sarajothi, Vani vellachi, Thamarai vasini

Action:

Alterative, Febrifuge, Tonic

General properties:

It is effective in the treatment of skin leprosy, fever with chills, asthma, eye diseases, urinary tract infections, Kaba diseases, cervical adenitis, etc.

Manosilai is one of the ingredients in the following siddha formulations indicated for various diseases as mentioned below:

Vanga chenduram, Kalamega narayana chenduram, Panchpadana chenduram, Neelakanda rasa pathangam, Uththira rasa chenduram, Manmatha chinthamani rasa chenduram, Pancha padana chenduram, Neelakanda palai, Linga pathangam, Pancha pana chenduram, Narayana chenduram, Amirtha nandhi mezhugu, Pancha pana chenduram, Maha koda sushi mathirai, Kirubahara shanmuga chenduram.

6. *KAANTHAM (*MAGNETIC OXIDE OF IRON) – Figure -6

Chemical formula of Iron (III) oxide is Fe_2O_3. It is odourless. Its molar mass is 159.69 mol^{-1}. It is red-brown solid in appearance. Its melting point is 1539 - 1565ºC

Synonym:

Sivaloga sevakan, Tharanikku Nathan, Sootha ankusam, Navaloga thuratty, Kaya siddhiku pathiravan, Murugan puranam,

General properties;

This is very effective in the treatment of swelling, *Gunmum* (ulcer), *Kamalai* (Jaundice), *Megam* (Venereal disease), disease of three humours, *Vellai* (Leucorrhoea), *Kabavatha* disease, *Mantham* (dyspepsia), *Mahodaram* (*anasarca*), *brammiyam* (Gonorrhoea), eye diseases, and splenomegaly. It also increases longevity.

Kaantham is one of the ingredients in the following siddha formulations indicated for various diseases as mentioned below:

Kantha rasa villai, Padana kulanthagan, Pusanda ramapana mathirai, Guru sanjeevi Mezhugu, Pancha pana chenduram, Ramapana idi marunthu6, Rasagandhi Mezhugu, Pancha padana chenduram, Soolai kudori.

7. *KARUNAABI* - Fig -7

Synonyms

Naai, Naabam, Vasanaabi, Vathsanaabi, Vidam, Maarutham,

General properties

Fever, epilepsy, headache, snake bite, internal ulcers, leprosy scorpion poison

Chemical properties

The root contain alkaloids methylveratroylpseudaconine, veratroylbikhasconine, balfourine, veratroylpseudaconine, indaconitine Neoline, chasmanine

Physical propeties

Taste

Bitter

Action

Diaphoretic, diuretic, antiperiodic, anodyne, antiphylogistic, antipyretic, sedative, narcotic.

8. *VEMBU* - Fig 8

Biological activity of Neem compounds

Anti-inflammatory; Antibacterial; Antirheumatic; Hypoglycaemic; Antipyretic; Spermicidal; Antifungal; Diuretic; Antimalarial; Immuno-modulatory etc.

Medicinal Uses

Various parts of the neem tree have been used as indigenous medical system. In folk medicine Neem oil and the bark and leaf extracts have been used to control leprosy, helminthiasis, respiratory disorders, constipation and also as a general health promotin activity. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Neem oil used to control various skin infections. Neek Leaf, Bark, root, flower and fruit all the parts are used treat biliary afflictions, itching, skin ulcers, burning sensations and pthysis.

Taste

Bitter

Action

Antiperiodic, Tonic

9. *PALAGARAI* (CYPRAEA MONETA) Fig - 9

Synonyms

Kaavadi, Soki, Varaadi.

General properties

The White Marine Shell Controls Thirst Diarrhea and Dysentery Toxic Fever, Tuberculosis Indigestion, Jaundice, Splenomegaly, Hepatomegaly, Broncial Asthma, Eye Disease

Physical properties

Palakarai is considered as one of the five wealth of the sea. The marine shell resembles as one of a tamarin seed up to an almond size and is available in white yellow and red colours the white marine shell is considered as superior for medicinal purposes. The marine shell has got bitter mucolytic and hypothermic properties

Taste

Bitter

Actions

Expectorants, Febrifuge, Sedative. Externally it act as Rubefacient,

10. *PAALTHUTHAM* (Zinc sulfate) Fig - 10

Synonyms

Vellai, Thutham, Madalthutham, Nagauppu, Velliauppu.

General Properties

This is considered to Be Useful in treating Ulcer in Genitalia, Piles, Eczema, Eye Diseases, Psychiatric Disease, Epilepsy, Whoping Cough, Bronchial Asthma, Gastric Ulcer, Diahorrea, Metrrogia, Periodic Fever.

Chemical Properties

Zinc sulfate ($ZnSO_4$) is the inorganic compound and was historically known as "white vitriol". The principal commercial preparation of zinc sulfate is the monohydrate granular (36 % of Zinc).

Physical Properties

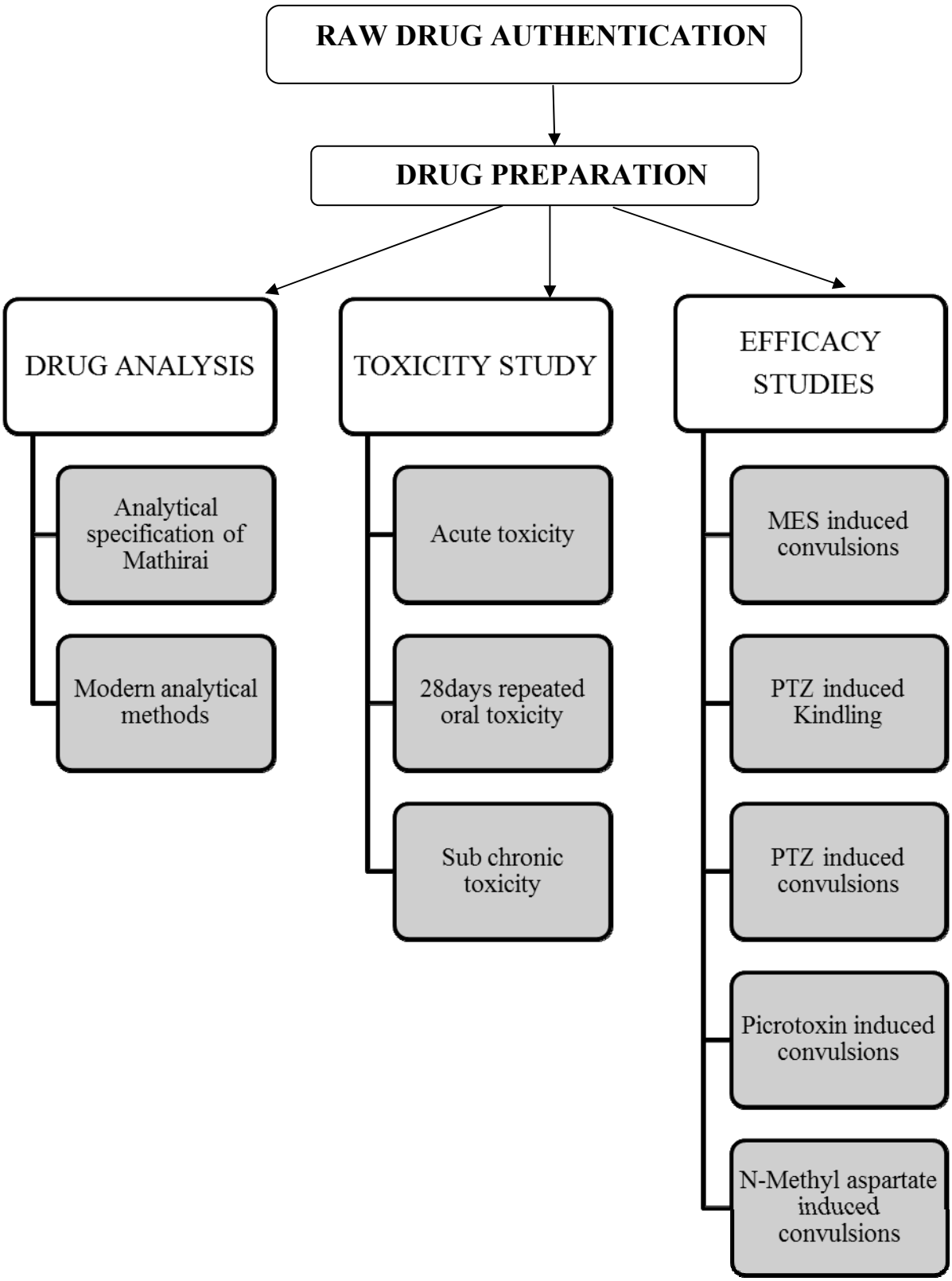
Anhydrous zinc sulfate is a colourless crystaline solid form. All forms are soluble in water and noncombustible.

Action

Tonic, Astringent, Antispasmodic, Emetic

The raw drugs and other ingredients were purchased from country shop in Chennai. The ingredients were authenticated and purified as per the methods prescribed in Siddha literatures.

4. PLAN OF WORK



5. MATERIALS AND METHODS

5.1.1 STANDARD OPERATING PROCEDURE FOR *VISHNU CHAKARA MATHIRAI:*

Ingredients[7]:

i. *Rasam* (Purified Mercury)

ii. *Lingam* (Purified Cinnabar)

iii. *Ganthagam* (Purified Sulphur)

iv. *Karu naabi* (Purified Aconite)

v. *Palagarai* (Yellow orpiment)

vi. *Thalagam* (Purified Calamine)

vii. *Kaantham* (Purified Lode stone)

viii. *Manosilai* (Purified Red Orpiment)

ix. *Veppam Pazha Saru* (Neem Fruit Juice)

5.1.2 PURIFICATION OF INGREDIANTS[45]:

i. Purification of *Rasam*:

Rasam : 35 gm

Thumbai (*Leucus aspera*) juice: 166.25 gm

Rasam was mixed with the juice of whole plant *thumbai* (*Leucas aspera*) and insolated and kept in sunlight (*Suriya Pudam)*. This procedure was repeated for ten days by adding fresh juice everyday. Then it was insolaed without adding the juice. This process was also repeated for one more day. Then the *rasam* and the powder present along with it was placedin a mud pot. The juice of *thumbai* was added and sealed and buried for twenty days. It was then taken out after washing with water.

ii. Purification of *Lingam*:

Lime juice, cow's milk and Adathoda (*Acalypha indica)* juice were mixed in equal proportion and allowed to fuse with cinnabar so as to get it in a consolidated potency state.

iii. Purification of *Ganthagam*:

Ganthagam was placed in an iron spoon. Add small quantity of cow's butter, then the spoon was subjected to heat till the butter and *Ganthagam* melts. This mixture was immersed in inclined position in cow's milk. This procedure was repeated for 30 times to get purified *Gandhagam*. Each time, fresh milk was used.

iv. Purification of *Karunabhi*:

Naabi was soaked in a cow's urine for one day and dried in sunlight.

vi. Purification of *Palagarai*:

Palagarai powder was soaked in *Thamaratham* fruit juice and placed in daylight for a whole day. The process was repeated next day also using fresh juice and continued for 15 days.

vii. Purification of *Thalagam*:

Thalagam piece was buried within limestone. Then palm toddy was poured on the limestone for ten times after that *thalagam* was washed and dried.

viii. Purification of *Kaantham*:

Kaantham was soaked in the root juice of *Ponnavarai* (*Cassia auriculata*) and insolated from morning to evening for ten days. Then it was dried for two days without adding the juice. This process was repeated twice and washed to obtain purified and detoxified *kaantham*.

ix. Purification of *Manosilai*:

Manosilai was triturated with ginger (*Zingiber officinalis*) juice and then dried.

5.1.3 PREPARATION OF VISHNU CHAKARA MATHIRAI:

The purified *Rasam, Lingam, Kandhagam, Nabhi, Palagarai, Thuththam, Thalagam, Kantham* and *Manosilai*. The ingredients were powdered and ground with *Veppam (Neem) Pazha Juice (Azadirachta indica* fruit juice*)* to a rolling consistency. After grinding to a soft consistency of soft pill, it was rolled as pills of 130 mg (*One Kuntri*) and allowed to dry.

Figure: 1 (*RASAM* -MERCURY)

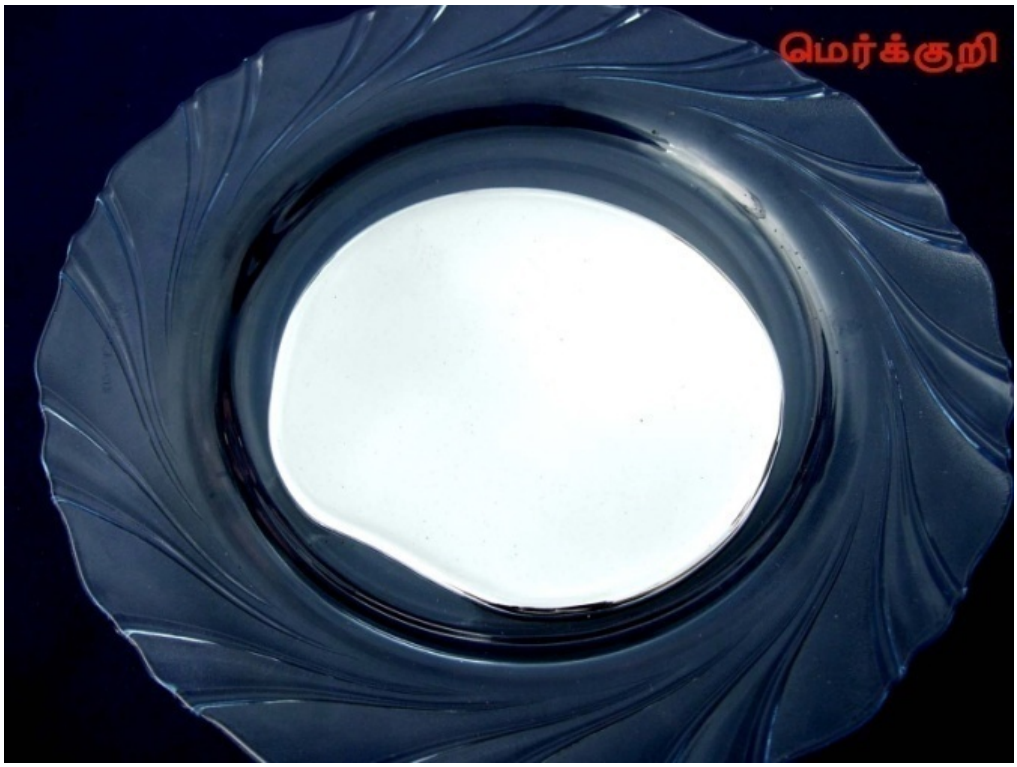


Figure: 2 (*GANDHAGAM - SULPHUR)*

Figure: 3 (*LINGAM*)

Figure: 4 (*THALAGAM*)

Figure: 5 (*MANOSILAI*)

Figure: 6 (MAGNETIC OXIDE OF IRON)

Figure7: *KARUNAABI* (*Aconitum ferox*)

Figure: *8 VEPPAM PAZHAM* (*Fruits of Azadiracta indica*)

Figure: 9 (*PALAGARAI) Cypraea moneata*

Fig.10 *PAAL THUTHAM* (Zinc sulfate)

5.2 CHARACTERISATION OF THE DRUG

5.2.1 PHYTOCHEMICAL SCREENING [47,48]

The prepared tablets (10 gm) were crushed well and dissolved in 100 ml of ethanol to subject phytochemical screening. The list of chemicals used for the phytochemical screening of *VCM*, their grade and supplier are tabulated below.

Table: 2

1. Chemicals used:

Sl.No	CHEMICAL	GRADE	SUPPLIER
1.	Ethanol	AR	ALDRICH
2.	Hexane	LR	MERK
3.	Acetone	LR	SD FINE
4.	Silica Gel	LR	SISCO
5.	Hydrochloric Acid	LR	SD FINE
6.	Methanol	LR	SD FINE
7.	Chloroform	LR	SD FINE
8.	Morquies Reagent	AR	SD FINE
9.	Mayers Reagent	AR	MERK
10.	Dragendorffs Reagent	AR	SD FINE
11.	Picric Acid	AR	ALDRICH
12.	Pottassium Iodide	AR	ALDRICH
13.	Ammonia	AR	ALDRICH
14.	Bromine Water	AR	ALDRICH

Tests for alkaloids in VCM:

a. Morquies test:

"For detecting the alkaloids 2-3 gms of the sample was ground with sufficient chloroform to make slurry. Ammonical chloroform was added and the mixture was mixed for 1minute. Taking out of alkaloids from choloroform was done by shaking the solution with 0.5 ml of 2 N-H_2SO_4 and separation of the acid layer by means of a dropper. A few

drops of drug solution were tested with the following alkaloidal reagents A small quantity of the drug solution was placed in a glass plate and allowed to evaporate to dryness. A drop of water and Morquies reagent ($HgCl_2$ + KCN) was added and the colour was observed. Appearance of Reddish colour which turns blue indicates the presence of alkaloids".

b. Mayers test:

"2 ml of the solution was added with Meyers reagent (1.36 g Mercuric chloride + 3.0 gm Kl in 100 ml of water). Appearance of greyish white precipitate indicates the presence of alkaloids".

c. Dragendorffs test:

"The Dragendorffs reagent was prepared by dissolving 8 gm of bismuth nitrate acid (20 ml) and 27.2 gm of Kl in 50 ml of water separately and mixing the two solutions and making up in to 100 ml with water. 2 ml of drug solution was added to this reagent and the colour was observed". Reddish brown precipitate explained the presence of alkaloids in *VCM*.

d. Hayers test:

"Hayers reagent is a saturated solution of picric acid in water. 2 ml of drug solution was added with the reagent and colour was observed". Reddish brown precipitate explained the presence of alkaloids in *VCM*.

e. Wagners test:

"Wagners reagent is a solution of Kl_3 in water. It was prepared by dissolving 1.3 gm of I_2 in a solution of Kl (2 gm) in water and made in to 100 ml. 2 ml of drug solution was added with the reagent and colour was observed". Red coloured precipitate showed the presence of alkaloids.

Test for Quinine (Bromine - ammonia test) in VCM:

"To about 10 ml of (1 gm in 1000) solution of sample was added with 0.25 ml of Br_2/H_2O and shaken well. Then about 2 ml of dil.NH_3 solution was added". No bright colouration showed the absence of quinine.

Test for Morphine (Iodic acid test)

"Morphine liberates iodine from iodic acid which gives blue colouration. 2 ml of *Vishnu Chakra Mathirai* solution, acidified with sulphuric acid to a solution of KlO_3 containing starch". Absence of deep blue colouration seen.

Test for Terpenoids in *VCM* (Leibermann Buchard test):

"2 ml of medicine solution was dissolved in chloroform and to these 2 drops of acetic anhydride was added and concentrated sulphuric acid was added along the sides of the test tube and the colour was observed". Appearance of red colour pointed out the presence of terpenoids in *VCM*.

Test for Flavanoids in *VCM* (Shinoda's test):

"2 ml of drug solution in alcohol was warmed and to the warmed solution a piece of Magnesium ribbon was added followed by 2 drops of concentrated HCl drop by drop". Absence of orange or yellow colour pointed out the absence of flavanoids in *VCM* .

Test for Methylene dioxy group in *VCM* (Labat test)

"3 ml of drug solution was mixed with 2 gm of gallic acid and 2 drops of con. H_2SO_4 was added. The mixture was heated in boiling water bath for two minutes and the colour was observed". Dark blue colour was not formed which pointed out the absence of methlene dioxy group in *VCM*.

Test Phenols OH group in *VCM* ($FeCl_3$ test):

"2 ml of drug solution was dissolved in alcohol and warmed then 2 drops of neutral ferric chloride was added and the colour was observed". Presence of brown green colour pointed out the occurrence of phenolic hydroxyl group in *VCM*.

5.2.2 PHYSICO - CHEMICAL ANALYSIS OF VCM[49,50]

The analysis was done to determine the physic-chemical characters of *VCM*, as per the protocol for testing of Ayurvedic, Siddha and Unani Medicines, by PLIM, Ghaziabad, New Delhi

1. pH

"0.5 gm of the prepared *VCM* was dissolved in ethanol solution and the pH of the *VCM* was found out by using pH meter".

2. **Loss on drying**

"Accurately 1 gm of sample was weighed and taken in the dish. The dish was covered with lid and dried in the drying chamber till two consecutive weights remain within ± 0.5 mg. After drying was completed, the sample was cooled in desiccators and weighed. From the difference of weights the ash content was calculated".

$$\text{Loss on drying}\,(\%\text{w/w}) = \frac{\text{Loss in weight}\,(\text{g})\text{X}100}{\text{Mass of the sample}\,(\text{g})}$$

3. Total ash

"3 gm of sample was accurately weighed and incinerated in a silica dish at a temperature of 650º C until free from carbon. Then the residue was cooled and weighed. The percentage of ash was calculated".

$$\left[\text{Percentage of total ash}\,(\%\,\text{w/w}) = \frac{\text{Mass of ash}(\text{g})\text{X}100}{\text{Mass of the sample}(\text{g})}\right]$$

4. Acid insoluble ash

"The ash obtained from *VCM* was boiled for 5 minutes with 25 ml of dilute Hcl and the insoluble matter was collected and washed with hot water and set fire to constant weight". The percentage of acid insoluble ash in *VCM* with reference to the air dried.

$$\text{Percentage of acid insoluble ash (\%w/w)} = \frac{\text{Mass of acid insoluble matter (g) X 100}}{\text{Mass of the sample (g)}}$$

5. Water soluble extractive

"5 gm of the drug was powdered with 100 ml of water in a closed flask for 24 hours and permitted to place for eighteen hours. The content was filtered rapidly, then allowed to evaporated and then dried at temperature of 105^{o} C, in an oven for maintaining constant weight". The percentage of water soluble extract of the air dried *VCM* was calculated.

$$\left[\text{Percentage of water soluble extraction (\%w/w)} = \frac{\text{Mass of the residue (g) X 100 X 100}}{\text{Mass of the sample (g) X 25}}\right]$$

6. Alcohol soluble extractive

"5 gm of powdered drug was mixed with 100 ml of ethanol, in a closed flask for 24 hours and allowed to stand for eighteen hours. The content was filtered rapidly and evaporated to dryness over a water bath, and weighed". The percentage of alcohol-soluble extract in *VCM* was calculated.

$$\text{Percentage of alcohol soluble extract (\%w/w)} = \frac{\text{Mass of residue (g) X 100X100}}{\text{Mass of the sample (g) X 25}}$$

7. Estimation of sulphur

"500 mg of *VCM* was weighed and 100 ml of N/10 Iodine solution was added. The mixture was allowed to stand for half an hour. Then 5 ml of Hcl and 5 ml of nitric acid was added and allowed to stand for 1 hour. The content was evaporated on hot plate to dryness. The excess of iodine was removed by adding hydrochloric acid. The residue was dissolved in boiling water and 15 ml of 25 % Ba Cl_2 was added. Then allowed to stand for overnight. The precipitate was filtered through Whatman no 41 filter paper. The filter paper with residue was kept in a pre-weighed crucible and ignited in a muffle furnace". From the weight of the residue, the percentage of sulphur in the drug was calculated.

Amount of Sulphur = Weight of ashless filter paper after ignition X factor of Sulphur (0.1373) X100 / Weight taken

8. Estimation of Mercury in *VCM*

"0.5 gm of Vishnu Chakara Mathirai was weighed and taken in 500 ml Kjeldhal flask with this and 15 ml of conc sulphuric acid, 2 ml of conc. Nitric acid was added and refluxed for 4 hrs. The yellow precipitate obtained was filtered by Whatmann 41 paper in a 250 ml beaker. The precipitate was dissolved in dilute sulphuric acid and made up the volume 100 ml. 50 ml of made up solution was pipetted in a 250 ml standard flask and 0.1M potassium permanganate solution was added drop-wise until mild pink colour persisted. Then 2 ml of ferric ammonium (II) sulphate indicator was added and titrated with 0.1 M ammonium thiocyanate". From the titre value the percentage of mercury in the drug was estimated.

$$\text{Amount of Mercury} = \frac{\text{Titre value X 0.01003X250 X 100}}{\text{weight of sample X volume pipette (50 ml)}}$$

9. Microbiological Contaminant Study[50].

Test for E.coli in *VCM* sample:

The test sample was ground and dissolved in water and diluted with phosphate buffer at pH 7.2. Test for micro organisms like E. *coli, P. aeruginosa, Staphylococcus aureus* and *Aflatoxins* in the test sample were studied.

Total Viable Aerobic Count (TVC)

The total viable aerobic count (TVC) of the sample was analysed using series dilution method. In this method a series of 12 tubes each containing 9-10 ml of digest medium of soybean casein was taken. To each of the tubes 1 ml *VCM* solution was added and the tubes were incubated at 30-35° C for 3 days. The number of microorganisms formed was determined

Plate count for bacteria

To a petri dish a mixture of 1 ml of drug solution and 15 ml of liquefied casein-soybean digest agar was added and incubated at 30-35° C for 72 hours, The number of colonies formed were counted

Plate count for Fungi

To a petri dish a mixture of 1 ml of drug sample and 15 ml of antibiotics was added and incubated at 20-25° C for 5 days. The number of colonies formed was counted

Test for Specific micro organism:

Test for E.Coli:

A small quantity of the homogenized lactose broth was prepared and 1 ml of *VCM* solution in 100 ml of Mac Conkey broth was added and incubated at 43-45° C for 18-24 hours. Formation of red, non-mucoid colonies of Gram-negative rods. This rods were surrounded by a reddish zone of precipitation shows the presence of E.Coli.

Pseudomonas aeruginosa:

The *VCM* solution was mixed with buffered sodium chloride peptone solution (pH 7.0) 100 ml of soybean-caesein digest medium was inoculated with small amount of the above solution and incubated at 35-37° C for 24-48 hours. The appearance greenish fluorescence colour indicates the presence of *Pseudomonas aeruginosa.*

Staphylococcus aureus.

1 ml of *VCM* solution was added with the prepared subculture with Baired-Parker agar and incubated at 35-37° C for 24-48 hours. Black colonies of Gram-positive cocci surrounded by clear zones, indicates the presence of Staphylococcus aureus.

10. Determination of Aflatoxins:

Test for aflatoxins:

These tests are designed to detect the possible presence of aflatoxins-B highly toxic contamination in any material of plant origin. Aflatoxins are deadly secondary metabolites created by Aspergillus fungus group flavus and parasiticus. Aflatoxins are genotoxic and carcinogenic and might cause harm to humans (EFSA, 2007). The VCM was analysed for Aflatoxins.

5.2.3 MORPHOLOGICAL DETERMINATION BY SEM[51-53]

A Scanning Electron Microscope (SEM) is one type of electron microscope that produces images of a test drug by scanning it with a focused beam of electrons. The electrons interact with atom of the drug and producing various signals. These signals contain information about the composition and surface topography of the test drug. The beam position is combined with the detected signal and produces the image. An image displayed the topography of the surface will be formed by scanning the sample and collecting the secondary electrons, which are emitted using a special detector. SEM can measure the resolution better than one nanometer. Samples can be observed in high

vacuum, in low vacuum and in wet conditions (in environmental SEM) at various range of temperatures. The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. By scanning the test drug *VCM* using a special detector, an image displaying the topography of the surface is created.

The *VCM* was subjected to SEM analysis by using Carl Zeiss MA15/EVO 18 Scanning Electron Microscope with the resolution of 3 nm at 30 kv with SE detector and the angle was measured from this angle, the size of the particles was calculated.

5.2.4 . EDAX

Energy-dispersive X-Ray spectroscopy (EDAX) analytical technique used for the elemental analysis of Vishnu chakara mathirai. From this the elemental composition of the specimen can be found out by using Odford Instrument Nano Analysis, INCA Energy Micro Analysis System. The powder of *VCM* was subjected to EDAX analysis and the elemental composition was found out.

5.2.5. INDUCTIVE COUPLED PLASMA OPTICAL EMISSION SPECTRA (ICP-OES) [54-56]

The digestion of VCM was done by adding 2 ml of nitric acid with 3 ml of Hydrochloric acid with 100 mg of *VCM* in ANTONPAAR MULTIWAVE-3000 microwave digester. Assessement of metallic constitution was made by ICP-OES analysis using Perkin-Elmer 5300 DV ICP-OES. The powder of *Vishnu Chakara Mathirai* was subjected to ICP-OES analysis and the elemental composition was found out.

5.2.6. TLC Fingerprinting Profile of Tablet (Based on Alkaloids)

Sample Preparation:

1 g of test drug was weighed and extract with 15 ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20 ml; 1ml of concentrated ammonia was then added. The mixture was shaken with chloroform. The

chloroform layer was collected and evaporated alloed to dry. The dried residue was dissolved in methanol.

Stationary phase:

Silica Gel 60

Mobile phase:

Toluene: Ethyl acetate: Diethylamine (7:2:1)

Procedure:

20, 30 µl test solutions were applied on a precoated silica gel 60 F_{254} HPTLC plate (E. Merck) of uniform thickness 0.2 mm using Linoma t5 sample applicator. The plate was developed in the solvent system to a distance of 8 cm. The plate was observed under UV light at 254 nm and 366 nm using CAMAG REPROSTAR.

5.2.7. SPECTRAL STUDIES[57-59]:

The *Vishnu Chakara Mathirai* was characterized by UV-Visible spectrometry and FT-IR Spectroscopy.

i. ULTRA VIOLET-VISIBLE SPECTRA

Ultra Violet absorption spectroscopy identify the type of compounds which absorbs UV radiation. The compounds with unbonded electrons or those with the conjugated double bonded compounds such as aromatic compounds can be identified by this technique.

The spectrophotometer used for our experiment (UV-260 Shimadzu spectro-photometer) has a range of 340 mm - 960 nm with tungsten halogen lamp as light source and silicon photo diode as detector. About 0.1 g of *VCM* sample was dissolved in 100 ml of ethanol and the optical density was found out from 400 nm - 540 nm and the λ_{max} was seen at 470 nm.

ii. INFRA RED SPECTROSCOPY:

Chemical composition of *VCM* is analysed by IR Spectrospcopy it. The structure of molecule of *VCM* is also identified by IR septrum. The principle of the technique is that a chemical substance shows marked selective absorption in the infrared exposed region. After the absorption of Infra red radiation, the drug molecules vibrate at many rates. It gives rise to close-packed absorption bands. IR spectrum of a test substance is a fingerprint for its identification. Band position in an infrared spectrum may be expressed conveniently by the wave number 'υ' whose units is cm^{-1}.A Nicolet 5700 FTIR USA, instrument was used for recording the IR spectra with 2-3 mg of the sample as KBr pellet. IR spectra of the drug was recorded.

A small quantity of dry KBr was mixed with a little amount the sample and ground for homogenization. An IR lamp was used for drying during mixing. The mixture was then pressed in to a transparent thin pellet at 5 ton/cm^2. These pellets were used for IR spectral recording.

5.3. SAFETY ASSESSMENT OF VCM (TOXICOLOGICAL STUDIES)

Animals for the experiment of *Vishnu Chakara Mathirai*

Adult Wistar albino rats weighing between 200-250 gm were used for this study. Rats were procured for VCM study, from Central Animal House of B.L Baid Mehta. The animals were housed in cages in temperature-regulated rooms with12 hrs light, 12 hrs dark cycle, and had an right to use to food and water ad libitum[60]. The animals were permitted for acclimatization to the laboratory environment for one week. The study was approved by NIS IAEC number 1248/AC(09)CPCSEA-9/Dec 2013/3), and all the experiments were performed as per the CPCSEA guidelines.

Drugs/Chemicals and Equipments

Picrotoxin, Phenytoin Sodium and Sodium Valproate were procured for analysis of Vishnu chakara mathirai from Sigma-Aldrich Chemical Co. Ugo Basile current electroshockmachine (Model 7800 with corneal electrodes) was purchased from J.S.Scientific, Pallavaram, Chennai. The major chemicals were procured from Aldrich Chemical Corporation. All the chemicals used in the grade of analytical purpose.

5.3.1 ACUTE ORAL TOXICITY - OECD GUIDELINES - 423

Acute toxicity study was carried out as per OECD-423 guidelines.

Animals:

Healthy Wistar albino female rat weighing between 200 gm and 220 gm. Study carried out at three female rats under fasting condition. Observed every one hour for signs of toxicity for first 24 hours, then daily for about 14 days**.**

Methodology

Selection of animal species:

The preferred rodent species was healthy young adult strain Wistar female albino rats, age of the animal between 8 and 12 weeks old and its weight fell in an interval

within ± 20 % of the mean weight of the animals, were used which should be nulliparous and non-pregnant.

Housing and feeding[60]:

The temperature maintained in animal experimental room was 22º C (+3º C), relative humidity was at least 30% artificial photoperiod. For feeding, usual laboratory diets were used and limitless of drinking water was supplied.

Preparation of animals:

The animals were randomly selected and marked for the purpose of individual identification, then kept in their cages for seven days prior to study for acclimatization to the laboratory conditions.

Observation:

Observations were made on Body weight changes, Assessments of posture, Signs of Convulsions, Limb paralysis, Lacrimation, Salivation, Change in skin colour, Piloerection, Defecation, Sensitivity response, Muscle gripness, Locomotion, Body tone, Rearing and Urination.

5.3.2. SUB-ACUTE TOXICITY[62]:

`Based on OECD guidelines the minimum period for sub acute toxicity study for a drug is 28 days. This study was carried out as per OECD Guidelines.

Experimental Animals:

28-days repeated oral toxicity study of *VCM* was performed according to OECD test guideline 407, Forty young healthy adult Wistar albino rats weighing between 100-120 gm/ b.wt., were used for the study. Every Animal was kept separate with a well ventilated polypropylene. 12-hrs light and 12-hrs dark environment was maintained.

Room temperature 22°C (±3° C) and relative moisture 50–70% were maintained in the room. Animals had free access to pelleted feed and Reverse osmosis (Rios, USA) purified water *ad libitum*.

Grouping and Treatment

All the animals were acclimatized for seven days to the laboratory conditions prior to experimentation. In this experiment animals were randomly selected into four groups of 10 animals each (5/cage/sex). Animals were grouped as follows,

Dose :

Low dose: 50 mg/kg/day; p.o

Mid dose: 100 mg/kg/day; p.o

High dose: 200 mg/kg/day; p.o

Table: 3 Sub Acute Toxicity Study

Group	Main Groups	No. of animals	
		Male	Female
Group I	Control (5 ml kg. b.wt distilled water)	5	5
Group II	Low dose (50 mg/kg b.wt.)	5	5
Group III	Mid dose (100 mg/kg b.wt.)	5	5
Group IV	High Dose (200 mg/kg b.wt.)	5	5

Drug preparation and adminsitrations:

100 mg of VCM was dissolved in standard volume of distilled water. The solution was given in the required quantity according to the individual body weight of animals. Animals received test drug by oral gavage once daily for a period of 28 days.

The animals of all groups were observed daily for mortality, morbidity and other clinical signs of toxicity till the end of study. Body weight, food and water intake of the

animals were also recorded. At the end of study period, the overnight fasted (water *ad libitum*) animals were anaesthetized with ketamine, blood samples were collected from retro-orbital vessels in tubes containing EDTA for hematological analysis. Plain tubes were used for biochemical analysis for appreciate serum separation.

After blood collection, the animals in group 1 to 4 were sacrificed on 29th day. All euthanized animals were examined for gross pathological changes of major organs followed by the vital organs including brain, heart, lung, liver, spleen, both kidney, stomach, both testicles and ovaries were collected and their weight were measured. The harvested organs were fixed in 10% neutral buffer formalin solution. Histopathological studies were carried out.

Observation

- Body weight was recorded once in a week till completion of the experiment.
- Animals were observed for mortality twice daily till the completion of experiment.
- Following test drug administration, experimental animals were observed daily for clinical signs till completion of the experiment.
- Feed consumption of individual animals was recorded daily till completion of the experiment.
- **Blood Parameters:** Blood samples were collected through retro orbital puncture on day 29. Prior to blood collection, the animals were overnight fasted but had free access to water. Blood samples were taken from experimental animal to analyse following parameters.
    - **Haematology**
    - **Biochemistry**
    - Carbohydrate mechanism: Glucose

- Lipid metabolism : Total cholesterol, triglycerides
- Protein metabolism : Total protein, albumin

  Liver function
- Hepatocellular : Serum Glutamyl pyruvate aminotransferase (SGPT)
- Hepatobiliary : Liver enzymes, Bile acid, Total bilirubin γ-glutamyl transferase
- Renal function : Creatinine and urea

- **Necropsy:** All survived animals were sacrificed using CO_2 euthanasia on the day 29. The following were the observations carried out during necropsy.

  - **Gross Pathology -** All the experimental animals were subjected to detailed gross necropsy which includes gross examination of external orifices, skin with mammary gland, thymus gland, lymph nodes, eyes, brain, trachea, thyroid gland, heart, both lungs, stomach, small and large intestines (with Peyer's patches), spleen, liver, adrenals gland, both kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow, spinal cord and other gross lesions.

  - **Relative Organ Weight -** Absolute weight of brain, heart, liver, paired kidneys, paired adrenals, spleen, paired testes, paired epididymides, male sex glands, uterus with cervix, paired ovaries and thymus was recorded at necropsy. Absolute organ weight was converted into relative organ weight and expressed in percentage as mentioned below

  - Relative organ weight (%) = $\frac{\text{Weight of the organ (g) X 100}}{\text{Final body weight (g) of animal}}$

- **Histopathology -** Histopathology examination was performed for below mentioned organs of animals from control and high dose groups and for the organs from low and mid dose groups that showed no evidence of gross abnormalities. Organs such as skin with mammary gland, lymph nodes, eyes, brain, trachea, thyroid, thymus, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow and spinal cord of all the animals were taken and they were fixed in 10% Neutral buffered formalin for 48 hrs, processed for paraffin embedment, sectioned and stained with H&E for histopathological evaluation.

5.3.3. SUB-CHRONIC TOXICITY OF *VISHNU CHAKARA MATHIRAI*[62-63]:

Sub chronic toxicity study was carried out as per the OECD guidelines-408. In this study the study animals (Rats) were divided into 4 groups of 10 animals each (5 male and 5 female). The *Vishnu Chakara Mathirai* was dissolved in distilled water and given orally with dose of 50 mg, 100 mg and 200 mg/kg.b.wt was given to each group of rats once daily for 90 days, Toxic features such as mortality and the body weight changes and other toxic signs were monitored on First day and on 90th day. At the end of the study, all animals were withdrawn food for 16-18 h and then anesthetized with 1 gm of pentobarbital sodium (intra-peritoneally) injection of on 91st day. Blood samples collected from control, standard and Vishnu chakara mathirai administered groups for hematological and biochemical analyses from common carotid artery. The internal organs and tissues were observed for gross lesions. For histopathological examination, 10 % neutral buffered formaldehyde was used to preserve all tissues.

Subchronic toxicity study (OECD-408)

Objectives

1. To reveal NOAEL in *Vishnu Chakaram Mathirai* treated animals
2. To characterize dose-response relationships
3. To identify and study the specific organs affected after repeated administration

Duration

90 days.

Animal:

Wister albino rats of either sex

Observation

- Body weight was recorded once in a week till completion of the experiment.
- Animals were observed for mortality twice daily till the completion of experiment.
- Following test drug administration, experimental animals were observed daily for clinical signs till completion of the experiment.
- Feed consumption of individual animals was recorded daily till completion of the experiment.
- **Blood Parameters:** Blood samples were collected through retro orbital puncture on day 29. Prior to blood collection, the animals were overnight fasted but had free access to water. Blood samples were taken from experimental animal to analyse following parameters.
    - **Haematology**
    - **Biochemistry**
    - Carbohydrate mechanism: Glucose
    - Lipid metabolism : Total cholesterol, triglycerides
    - Protein metabolism : Total protein, albumin

- Liver function Hepatocellular : Serum Glutamyl pyruvate aminotransferase (SGPT),
- Hepatobiliary : Liver enzymes, Bile acid, Total bilirubin γ-glutamyl transferase
- Renal function : Creatinine and urea

Necropsy: All survived animals were sacrificed using CO_2 euthanasia on the day 29. The following were the observations carried out during necropsy.

- **Gross Pathology -** All the experimental animals were subjected to detailed gross necropsy which includes gross examination of external orifices, skin with mammary gland, thymus gland, lymph nodes, eyes, brain, trachea, thyroid gland, heart, both lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals gland, both kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow, spinal cord and other gross lesions.
- **Relative Organ Weight -** Absolute weight of brain, heart, liver, paired kidneys, paired adrenals, spleen, paired testes, paired epididymides, male sex glands, uterus with cervix, paired ovaries and thymus was recorded at necropsy. Absolute organ weight was converted into relative organ weight and expressed in percentage as mentioned below
- Relative organ weight (%) = $\frac{\text{Weight of the organ (g) X 100}}{\text{Final body weight (g) of animal}}$
- **Histopathology -** Histopathology examination was performed for below mentioned organs of animals from control and high dose groups and for the organs from low and mid dose groups that showed no evidence of

gross abnormalities. Organs such as skin with mammary gland, lymph nodes, eyes, brain, trachea, thyroid, thymus, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymides, male sex glands (as whole), ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow and spinal cord of all the animals were taken and they were fixed in 10% Neutral buffered formalin for 48 hrs, processed for paraffin embedment, sectioned and stained with H & E for histopathological evaluation.

5.4. EFFICACY STUDIES OF VCM (ANTICONVULSANT ACTIVITY OF VCM)

Anti convulsant activity of *VCM* were carriedout by using the following animal models[64,65].

1. Maximal Electroshock Induced Seizures Model (MES)
2. Picrotoxin induced seizures (PIS)
3. N-Methyl-D-Aspartate (NMDA) test
4. PTZ induced kindling in rats
5. PTZ induced convulsion in rats.

Experimental animals.

Male wistar rats and Male swiss mice were used for this study. They were housed in standard Laboratory conditions 22° C, 12 : 12 artificial lighting).. They provided with free acess to food and water. The study protocol for this animal experiment on *VCM* was approved by IAEC, National Institute of Siddha, Chennai.

5.4.1 Maximal Electroshock Induced seizure (MES) [66,67,68]:

After 7 days of acclimatization period, rats weighting between 200 - 250 gms were randomly divided into four groups. Each group consists six animals and they were iovolved in the experiment.

Group allocation and treatment:

Table 4:

Group	Treatment	No. of animals
I	Control (Distilled water 5 ml/kg. b. wt)	6
II	Standard (Phenytoin – 25 mg/kg b.wt)	6
III	*VCM* 100 mg /kg b.wt	6
IV	*VCM* 200 mg / kg b.wt	6

Maximal Electro shock induction was carried out by adopting the method which was mentioned by 'Wolfgang Lescher et.al". "The group-I was kept as vehicle control which received distilled water at the dose of 5 ml/kg b.wt, while the Group-II received Phenytoin 50 mg/kg b.wt and the Group-III and Group-IV received *VCM* 100 mg/kg b.wt and 200 mg/kg b.wt respectively. The treatment was continued for 15 days. In the 15th day, by using a electro convulsiometer, Maximal Electro Shock seizures were induced for 0.2 seconds (60 Hz alternating current of 150 million intensity). A drop of electrotyte solution with lignocaine were applied to the corneal electrodes prior to administration to the rats". Suppression of tonic hind limb extension was taken as a measure of efficacy and all the experimental groups with VCM were compared with the control.

5.4.2 Picrotoxin induced seizure[69]:

Swiss albino mice were used for the picrotoxin induced seizure model for administering the VCM. The mice were divided into four groups and each group contain six animals. The Group-I was served as control received 3 ml/kg b.wt) distilled water. The Group-II received standard drug Phenytoin (25 mg/kg b.wt.), the IIIrd and IVth group received the *VCM* 100 mg/kg b.wt and 200 mg/kg b.wt respectively daily for a period of one week. The animals were kept individually in transparent mice cages for 60 min for acclimitization before starting the experiment.

Group allocation and treatment:

Table 5

Group	Treatment	No. of animals
I	Control (Distilled water 3 ml/kg b.wt)	6
II	Standard (Phenytoin - 25 mg/kg b.wt)	6
III	*VCM* 100 mg /kg b.wt	6
IV	*VCM* 200 mg / kg b.wt	6

"Seizures were induced in animals by the i.p. injection of 7.5 mg/kg b.wt of picrotoxin 1 hour after the administration of their respective treatment.

Animals wer observed for convulsions for a period of 30 min. Hind limb extension was taken at tonic convulsion. The onset of tonic convulsion and the number of animals having convulsion or not convulsing within the observation peiod were noted and recorded. The anticonvulsant activity was determined by calculating the protection percentage".

5.4.3 N-Methyl-D-Aspartate test[70,71]

Swiss albino mice were used for this study. 24 animals were divided into 4 different groups and each group consists of 6 animals (n = 6).

Table 6

Group	Treatment	No. of animals
I	Negative Control (NMDA 75mg/kg b.wt)	6
II	Standard (Phenytoin - 25 mg/kg b.wt)	6
III	*VCM* 100 mg /kg b.wt	6
IV	*VCM* 200 mg / kg b.wt	6

The first group received only NMDA, and the II group served as standard and received Phenytoin 25 mg/kg b.wt. and the III and IV group received *VCM* 100 mg /kg b.wt anb *VCM* 200 mg / kg b.wt respectively except Group-I. The method described by *Stanislaw J et al* was used in the present study. "After one hour of *VCM* administration of subcutaneous injection of NMDA (75 mg/kg) was given to the all the four groups of animals. They were observed for 30 min. The animals did not show turning behavior within this observation period were declared as protected animals aganiste NMDA. Turning behavior was characterized by two consecutive 360° cycles fulfilled by the same animal. Number of turnings for standard (Phenytoin) was fixed as zero and the turnings

were calculated and statistically evaluated". The percentage of protection was calculated with negative control.

5.4.4 PTZ induced kindling model test[72-74]:

This method used for testing the anticonvulsant activity of *VCM* was according to Hitoshi et.al. Twenty four rats having the weight between 200-250 gm were randomly divided into 4 different group.

Grouping and Treatment:

Table 7:

Group	Treatment with VCM	Animals
I	Vehicle Control (5ml/kg b.wt)	6 nos.
II	Standard (Phenytoin - 25 mg/kg b.wt)	6 nos.
III	*VCM* 100 mg /kg b.wt	6 nos.
IV	*VCM* 200 mg / kg b.wt	6 nos.

Chemical kinding was induced in this method. An injection of PTZ (30 mg/kg b.wt) was injected to all groups of animals on alternate days i.e three times a week. The rats were observed for a period of 30 min after PTZ and the seizure activity score was recorded by using a scoring system from (0-5).

0 - No change

1 - Hyperactivity, restlessness, twitching

2 - Head nodding, head clonus, Myoclonus jerks

3 - Unilateral / Bilateral limb clonus

4 - Forelimb clonic seizures

5 - Generalized clonic seizures with loss of lighting reflex

Animals showing five staged continuous seizures were considered to be kindled after which the PTZ treatment was stopped. The increased and persistent sensitivity to

PTZ was ascertained by challenging the rats with 30 mg/kg b.wt PTZ on third and tenth day after the PTZ treatment had ended.

5.4.5 PTZ induced seizures[75]:

This method described by Maresx. C et al was adopted for evaluating PTZ induced seizures in rats. The Wister albino rats of 220 to 250 g weight of either sex were used for this present study. 30 Wister albino rats were randomly divided into 5 different group and each group consist of 6 animals.

Group allocation of animals:

Table 8:

Group	Treatment	No. of animals
I	Vehicle Control (5 ml/kg b.wt)	6
II	Negative Control (PTZ 75 mg/kg b.wt)	6
III	Standard (Phenytoin - 25 mg/kg b.wt)	6
IV	*VCM* 100 mg /kg b.wt	6
V	*VCM* 200 mg / kg b.wt	6

First group served as vehicle control kept as negative control. The group II served as Negative control which received PTZ 70 mg/kg.b.wt. alone and the third group was kept as standard which received Phenytoin at the dose of 25 mg/kg b.wt. Group-IV and Group-V received 100 mg and 200 mg of *VCM* respectively.

Except group II all the other groups received their respective treatment of *VCM* orally for 10 days. On the 10^{th} day after 1 hr of last dosing. PTZ was used as an inducing agent. The animals were observed continuously for 1 hr by placing them in observation cage. The onset of seizure and tonic clonic convulsion timings were recorded

individually. The difference in the onset of convulsions, duration between groups were statistically evaluated.

Statistical Analysis :

Statistitical analysis were performed using statistical package for social science for windows version 18 (SPSS inc. Chicago). To compare characteristics between groups one way ANOVA was used to compare means between two groups. When ANOVA show significan Dunnett's post hock test was used to assess the different between groups. 'p' value of less than 0.05 are was considered as significant.

6. RESULTS & ANALYSIS

The physico-chemical analytical studies, toxicological and pharmacological evaluation of the *Vishnu Chakara Mathirai* were observed at various angles:

6.1 PHYTOCHEMICAL SCREENING:

The trial drug *VCM* 5 gm was dissolved in 100 ml of ethanol and tested for phytochemical components like alkaloids, Terpenoids, Flavanoids and Phenols. The following is the results obtained:

Table 9 (Phyto-chemical screening report of *VCM*):

S.No.	Name of the tests	Result	Inference
1.	Morquies test	+	**Presence of Alkaloids**
2.	Mayers test	+	
3.	Dragendorffs test	+	
4.	Hayers test	+	
5.	Wagners test	+	
6.	Bromine – ammonia test	-	**Absence of Quinine**
7.	Iodic acid test	-	**Absence of Morphine**
8.	Leibermann Buchard	-	**Absence of Terpenoids**
9.	Shinoda's test	-	**Absence of Flavanoids**
10.	Labat test	-	**Absence of Methelene dioxy group**
11.	$FeCl_3$ test	+	**Presence of phenols**

6.2 PHYSICO-CHEMICAL ANALYSIS

6.2.1 PRELIMINARY INVESTIGATION

The various physico-chemical parameters like pH, Loss on drying at 105^{o} C, Total Ash, Acid insoluble ash, Water-soluble extract, Alcohol soluble extract, Percentage of sulphur, Mercury of the prepared pills were determined by the standard AYUSH protocol. The results are presented in table 10

Table 10 (Preliminary Investigation of *VCM*)

Sl. No	Parameters	Percentage composition
1.	pH	6.50
2.	Loss on drying at 105^{o} C	7.31
3.	Total ash	7.70
4.	Acid insoluble ash	2.12
5.	Water soluble extractive	85.32
6.	Alcohol soluble extractive	15.20

6.2.2 HEAVY METAL ANALYSIS- ICP-OES (INDUCTIVE COUPLED PLASMA OPTICAL EMISSION SPECTRA)

The heavy metals analysis of *VCM*, as per the guidelines of Pharmacopeal Laboratory for Indian Medicine, Ministry of AYUSH, Govt. of India, using ICP-OES is tabulated below table 11.

Table 11 (Heavy Metal Analysis of *VCM*)

Parameters	Levels (ppm)
Lead	1.13
Cadmium	Not Detcted (Detection Limit : 0.01)
Arsenic	2.03
Mercury	0.83
Sulphur	8.82

EDAX

Energy Dispersive X Ray Analysis (EDAX) of the *VCM* was carried out and the elements present like, Carbon, Oxygen, Magnesium, Sulphur and Calcium were estimated. The data are tabulated in terms of percentage weight (wt %) and atom percentage (at %). (Fig-11).

Table 12 (Results of EDAX Analysis of *VCM*)

Sl. No	Element	wt %	at %
1	Carbon	24.98	42.25
2	Oxygen	21.88	37.01
3	Magnesium	5.80	9.82
4	Sulphur	4.96	8.39
5	Calcium	1.50	2.54

Table 13 (Physico chemical Analysis of *VCM*)

Physico chemical analysis of *VCM* were carried out as and results were tabulated below:

Sl. No	Parameters	Results	Reference of test methods
1.	Appearance	Light brown colured round shaped tablet	IP Vol-1, 1996, p 7
2.	Average weight of a tablet	0.1058 g	IP Vol-1, 2014, p 7
3.	Uniformity of weight	03.86 to 106.8%	IP Vol-1, 2014, p 7
4.	Disintegration time	58 sec	IP Vol-1, 2014, p 256
5.	Each tablet of average weight contains: Mercury Sulphur	 0.0003792 % w/w 0.001925% w/w	IP Vol-1, 2014, p277

6.2.4. HPTLC Analsysis :

VCM was evaluated for HPTLC analysis and it revealed 11 peaks related to alkaloid components. The peaks are seen at 0.04, 0.11, 0.18, 0.22, 0.29, 0.35, 0.40, 0.60, 0.70 and 0.85. the major peak was seen at 0.85. Table showed the peak values and Rf values of 20µ sample of VCM. The area percentage are; 7.85, 2.42, 5.73, 8.37, 16.13, 5.33, 2.52, 25.67, 4.20 and 21.79 under 254nm of UV. In 366 nm of UV it showed 11 peaks in the Rf value of 0.06, 0.13, 0.20, 0.24, 0.32, 0.37, 0.44, 0.52, 0.63, 0.73 and 0.87. The maximum peak was noted in 0.87. The area percentage are 12.82, 2.61, 6.28, 7.62, 14.98, 5.19, 2.79, 1.36, 24.26, 3.24 ad 18.84.

6.2.5 SPECTRAL STUDIES

The Ultra Violet -Visible and Infra Red spectrum of the *VCM* was recorded (Fig - 12, 13) in sophisticated instruments. The various absorption frequencies are tabulated in Table-14

Table 14 (UV and IR spectral data of *VCM*)

UV Nm		**Infra Red cm^{-1}**
Reflectance	**Absorbance**	3299
1216	224	2918
1044	269	2082
971	338	1615
888	371	1411
		1017
		870

6.2.6 Pesticides Contaminant study.

The presence following pesticide contaminant of *VCM* was examined using series dilution method and their concentration was found below detectable limit.

Table 15 (Pesticides contaminant report of *VCM*) :

Contaminant	**Result**
Aldrin	Below detectable limit
Dieldrin	
Chlordane (cis & trans)	
Cis-Chloride	
Transchlordane	
Chlorothalonil	
DDT (all isomers)	

Contaminant	Result
p.p-DDT, 1	Below detectable limit
Dicofol	
Dieldrin (see Aldrin)	
Endosulphan (all isomers)	
Alpha & Beta –Endosulphan	
Endosulphan Sulphate	
Endrin	
HCH (sum of isomers, except the gamma isomer)	
Alpha, beta & Delta-HCH	
Heptachtor (sum of heptachtor expoxide expressed as heptachlor))	
Heptachlor	
Heptachlor epoxide	
Lindane (gamma-HCH)	
Organophosphorus pesticides	
4-bromo-2-chlorophenol (metabolite of Profenphos)	
Acephate	

Table: 16 - Microbiological contaminent study of *VCM*

The total viable aerobic count (TVC) of *VCM* was examined (QAS/5.131/Rev.1) specification by using series dilution method. The micro organisms detected are presented below:

Sl .No	Parameters	Result
1.	Total Bacterial Count	29,000 CFU /g
2.	Total Fungal Count	70 CFU/g
3.	E.Coli	Absent /g
4.	Staphylococcus aureus	Absent /g
5.	Salmonella	Absent /g
6.	Pseudomoss aeruginosa	Absent /g

6.3 TOXICOLOGICAL STUDY:

6.3.1 Acute toxicity:

The acute toxicity study result of *VCM* was mentioned below in the table 17. The parameters like the Body weight, Assessments of posture, Body tone, Piloerection, Defecation, Sensitivity response, Locomotion, Muscle gripness, Urination Signs of Convulsion Limb paralysis, Lacrimation, Salivation and mild rearing were observed and presented here.

Table 17 (Acute toxic study report of *VCM*)

Parameters
Body weight was normal
Assessments of posture was normal
No Signs of Convulsion Limb paralysis
Body tone was normal
No sign of Lacrimation
No sign of excess Salivation
No significant changes in skin colour
Piloerection was normal
Defecation was normal
Sensitivity response was normal
Locomotion was normal
Muscle gripness was normal
Mild rearing was observed
Urination was normal

PHYSIOLOGICAL OBSERVATIONS

VCM was administerd with a dose of 2000 mg/kg and the physiological characters like Alertness. Touch Response, Respiration, Aggressiveness, Pile erection, Grooming, Gripping, Decreased Motor Activity, Tremors, Convulsions Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis, Analgesia, Lacrimation, Exophthalmos, Diarrhoea, Writhing, Mortality were observed and presented here.

Table 18 (Physiological observations of animals in acute toxic study)

S.No	Parameters	Observation
1	Alertness	Positive
2	Aggressiveness	Negative
3	Pile erection	Negative
4	Grooming	Negative
5	Gripping	Negative
6	Touch Response	Positive
7	Decreased Motor Activity	Negative
8	Tremors	Negative
9	Convulsions	Negative
10	Muscle Spasm	Negative
11	Catatonia	Negative
12	Muscle relaxan	Negative
13	Hypnosis	Negative
14	Analgesia	Negative
15	Lacrimation	Negative
16	Exophthalmos	Negative
17	Diarrhea	Negative
18	Writhing	Negative
19	Respiration	Positive
20	Mortality.	Negative

6.3.2. SUB- ACUTE TOXICITY

The sub-acute toxic study of various doses *VCM* was carried out. Each category 5 animals of male and 5 female animals were observed. The observations are presented below

Table 19 (Clinical observation of individual animals in the Sub-acute study of *VCM*)

Group	Treatment	A. No.	Sex	Test days														
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I	Control	101	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		102	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		103	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		104	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		105	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		106	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		107	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		108	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		109	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		110	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
II	Low dose	201	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		202	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		203	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		204	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		205	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		206	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		207	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		208	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		209	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		210	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
III	Mid dose	301	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		302	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		303	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		304	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		305	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		306	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		307	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		308	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		309	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		310	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
IV	High dose	401	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		402	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		403	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		404	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		405	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		406	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		407	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		408	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		409	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		410	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N- Normal

(Clinical observation of individual animals in the Sub-acute study of *VCM* - Contd.)

Group	Treatment	A. No.	Sex	Test days													
				15	16	17	18	19	20	21	22	23	24	25	26	27	28
I	Control	101	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		102	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		103	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		104	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		105	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		106	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		107	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		108	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		109	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		110	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
II	Low dose	201	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		202	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		203	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		204	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		205	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		206	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		207	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		208	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		209	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		210	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
III	Mid dose	301	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		302	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		303	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		304	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		305	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		306	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		307	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		308	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		309	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		310	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
IV	High dose	401	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		402	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		403	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		404	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		405	M	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		406	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		407	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		408	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		409	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		410	F	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N- Normal

Table 20 (Effect of *VCM* on body weight changes – sub acute study report)

After administration of *VCM* for different groups (control, low dose, mid dose and high dose) the change in body weight on every seventh day for subsequent 28 days (0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th}) were recorded for Male and Female. The observed statistics for Male (M) rat and Female (F) rat and their average (MF) are presented here:

Group	Treatment	Sex	Body weight (kg)				
			Day 0	Day 7	Day 14	Day 21	Day 28
I	Control	M	134.00±5.70	141.60±5.90	149.40±6.12	158.40±5.70	171.40±6.99
		F	121.80±2.91	129.40±2.27	139.00±2.92	148.00±2.43	159.20±2.96
		MF	127.90±3.64	135.50±3.61	144.20±3.64	153.20±3.40	165.30±4.12
II	Low dose	M	136.20±5.00	145.40±6.22	155.00±3.96	161.60±3.88	171.80±4.42
		F	118.40±3.09	126.80±4.26	135.40±3.80	145.00±3.39	153.40±2.04
		MF	127.30±4.06	136.10±4.72	145.20±4.17	153.30±3.68	162.60±3.83
III	Mid dose	M	137.40±3.96	146.80±4.72	158.80±4.29	165.00±3.21	172.20±3.95
		F	119.20±4.44	127.40±4.50	135.40±3.31	143.40±4.76	151.00±4.11
		MF	128.30±4.13	137.10±4.46	147.10±4.66	154.20±4.50	161.60±4.44
IV	High dose	M	135.00±5.16	143.20±6.35	150.60±6.45	161.60±6.65	170.60±5.71
		F	117.60±3.91	127.80±4.37	137.40±4.58	144.60±4.12	151.60±4.28
		MF	126.30±4.21	135.50±4.45	144.00±4.33	153.10±4.65	161.10±4.62

Values expressed in mean ± SEM

Table 21 (Effect of *VCM* on relative organ weight in the sub acute study)

The *VCM* treated animal groups and control groups, the relative organ weights were also studied. Brain, Lungs, Heart, Liver, Spleen, Kidney, Adrenals, Sex glands of male and female rats were measured after administration of control, low dose, mid dose and high dose of *VCM*. The following table gives the measured values for male (M), female (F) and their average (MF).

Group	Treatment	Sex	Brainwt (%)	Lungs wt (%)	Heartwt (%)	Liver wt (%)	Spleen wt (%)	Kidney wt (%)	Adrenals wt (%)	Sex glands wt (%)
I	Control	M	1.20 ±0.06	0.89 ±0.05	0.39 ±0.02	3.84 ±0.20	0.48 ±0.08	0.93 ±0.04	0.02 ±0.00	1.64 ±0.08
		F	1.23 ±0.03	0.85 ±0.02	0.41 ±0.01	4.10 ±0.07	0.51 ±0.04	1.01 ±0.03	0.03 ±0.00	0.04 ±0.00
		MF	1.21 ±0.03	0.87 ±0.03	0.40 ±0.01	3.97 ±0.11	0.49 ±0.04	0.97 ±0.03	0.02 ±0.00	0.84 ±0.27
II	Low dose	M	1.19 ±0.02	0.92 ±0.06	0.48 ±0.03	4.80 ±0.23	0.54 ±0.02	1.08 ±0.03	0.02 ±0.00	1.70 ±0.07
		F	1.36 ±0.03	0.90 ±0.11	0.47 ±0.02	4.61 ±0.23	0.49 ±0.02	1.07 ±0.06	0.03 ±0.00	0.06 ±0.00
		MF	1.28 ±0.03	0.91 ±0.06	0.48 ±0.01	4.71 ±0.16	0.51 ±0.02	1.08 ±0.03	0.03 ±0.00	0.88 ±0.28
III	Mid dose	M	1.18 ±0.03	0.76 ±0.03	0.48 ±0.01	4.35 ±0.24	0.50 ±0.04	1.00 ±0.04	0.02 ±0.00	1.66 ±0.07
		F	1.34 ±0.07	1.08 ±0.16	0.48 ±0.03	4.54 ±0.15	0.49 ±0.02	1.00 ±0.04	0.03 ±0.00	0.06 ±0.01
		MF	1.26 ±0.04	0.92 ±0.09	0.48 ±0.01	4.45 ±0.14	0.50 ±0.02	1.00 ±0.03	0.02 ±0.00	0.86 ±0.27
IV	High dose	M	1.15 ±0.05	0.99 ±0.18	0.47 ±0.03	4.16 ±0.21	0.49 ±0.04	1.00 ±0.06	0.02 ±0.00	1.71 ±0.16
		F	1.28 ±0.04	0.80 ±0.04	0.45 ±0.03	3.99 ±0.28	0.52 ±0.03	0.82 ±0.03	0.03 ±0.00	0.05 ±0.01
		MF	1.21 ±0.04	0.89 ±0.09	0.46 ±0.02	4.07 ±0.17	0.50 ±0.02	0.91 ±0.04	0.03 ±0.00	0.88 ±0.29

Values expressed in mean ± SEM

Table 22 (Effect of *VCM* on feed consumption - sub acute study report)

The feed consumption for different groups (control, low dose, mid dose and high dose) were recorded for Male and Female on every week end for four weeks subsequently (1^{st}, 2^{nd} , 3^{rd}, & 4^{th} week), after administration of *VCM*. The observed data of *VCM* treated animals and Control groups presented here.

Group	Treatment	Sex	Cumulative feed intake (kg)			
			I Week	II Week	III Week	IV Week
I	Control	M	54.86±10.33	52.57±2.58	54.86±4.71	68.71±4.96
		F	46.14±5.91	55.29±3.39	59.57±2.00	55.00±4.95
		MF	50.50±8.12	53.93±2.99	57.21±3.35	61.86±4.95
II	Low dose	M	61.57±7.50	66.57±2.79	65.71±1.71	69.71±7.19
		F	51.86±7.78	59.71±2.45	58.29±2.76	58.57±4.93
		MF	56.71±7.64	63.14±2.62	62.00±2.24	64.14±6.06
III	Mid dose	M	49.14±8.20	63.00±1.45	54.14±3.38	70.29±4.86
		F	44.71±7.09	56.14±6.16	43.57±5.67	60.29±2.36
		MF	46.93±7.64	59.57±3.80	48.86±5.64	65.29±3.61
IV	High dose	M	52.43±10.93	62.00±6.58	59.00±4.35	59.00±2.25
		F	53.29±7.30	58.86±5.38	49.00±3.95	62.00±4.86
		MF	52.86±9.12	60.43±5.98	54.00±4.15	60.50±3.56

Values expressed in mean ± SEM

Table 23 (Effect of *VCM* on haematology in the sub-acute study)

The haematology reports like; WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT values for male and female rats with control, three different doses of *VCM* were observed. The observed different parameters of *VCM* treated animals and Control groups presented here.

Group	Treatment	Sex	WBC (10^3/uL)	RBC (10^6/uL)	HGB (%)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/µL)
I	Control	M	10.38 ±1.42	6.18 ±0.15	14.18 ±0.19	43.88 ±1.09	70.96 ±0.34	23.00 ±0.68	32.40 ±1.01	1448.20 ±70.91
		F	9.02 ±0.92	5.65 ±0.18	14.86 ±0.22	43.10 ±1.20	71.04 ±0.20	24.60 ±1.00	34.50 ±1.41	1641.00 ±90.19
		MF	9.70 ±0.83	5.92 ±0.14	14.52 ±0.18	43.49 ±0.78	71.00 ±0.19	23.80 ±0.63	33.45 ±0.89	1544.60 ±62.91
II	Low dose	M	10.18 ±0.67	5.48 ±0.11	14.36 ±0.27	38.78 ±0.81	70.74 ±0.18	26.22 ±0.39	37.08 ±0.59	1654.80 ±85.01
		F	9.58 ±0.88	6.08 ±0.18	15.20 ±0.49	43.36 ±1.39	71.30 ±0.23	25.02 ±0.43	35.08 ±0.63	1657.00 ±111.62
		MF	9.88 ±0.53	5.78 ±0.14	14.78 ±0.30	41.07 ±1.07	71.02 ±0.17	25.62 ±0.34	36.08 ±0.53	1655.90 ±66.14
III	Mid dose	M	9.82 ±0.61	5.95 ±0.13	14.42 ±0.21	42.38 ±1.03	71.28 ±0.26	24.28 ±0.52	34.08 ±0.82	1473.40 ±103.99
		F	9.12 ±1.61	5.91 ±0.19	14.58 ±0.33	43.20 ±0.81	71.30 ±0.47	24.14 ±0.22	34.14 ±0.14	1501.00 ±82.75
		MF	9.47 ±0.82	5.93 ±0.11	14.50 ±0.19	42.79 ±0.63	71.29 ±0.25	24.21 ±0.26	34.11 ±0.39	1487.20 ±62.82
IV	High dose	M	9.00 ±1.55	6.19 ±0.19	14.58 ±0.21	43.96 ±1.29	70.96 ±0.24	23.64 ±0.84	33.28 ±1.14	1428.40 ±83.78
		F	8.34 ±1.11	5.63 ±0.22	17.81 ±3.34	40.36 ±1.66	71.01 ±0.34	25.06 ±0.47	35.23 ±0.73	1726.00 ±40.88
		MF	8.67 ±0.90	5.91 ±0.17	16.20 ±1.67	42.16 ±1.16	70.98 ±0.20	24.35 ±0.51	34.25 ±0.72	1577.20 ±66.27

Values expressed in mean ± SEM

Table 24 (Effect of *VCM* on Plasma biochemistry - sub acute study report)

The values of *VCM* treated animals blood samples were analysed for of Glucose, Triglycerides, Cholesterol, SGPT, ALP, y-GT , BUN, LDH, T.Protein, Albumin, Creatinine for male and female rats of control and three different doses of *VCM* treated groups were recorded. The values are given below.

Sl. No	Treatment	Sex	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	SGPT (U/L)	ALP (U/L)	γ-GT (U/L)
I	Control	M	93.50 ±5.01	49.85 ±1.51	33.06 ±1.96	42.76 ±2.38	250.82 ±47.09	6.04 ± .23
		F	82.24 ±4.18	63.62 ±5.11	34.16 ±1.40	45.14 ±2.47	238.28 ±38.48	7.28 ±1.39
		MF	87.87 ±3.60	56.74 ±3.40	33.61 ±1.15	43.95 ±1.67	244.55 ±28.74	6.66 ±0.95
II	Low dose	M	85.79 ±1.53	69.62 ±6.37	34.28 ±1.34	47.30 ±8.01	235.47 ±56.80	8.34 ±0.66
		F	92.20 ±8.01	47.45 ±2.61	39.91 ±1.07	53.05 ±4.53	260.42 ±66.18	7.79 ±1.18
		MF	89.00 ±3.99	58.54 ±4.92	37.09 ±1.24	50.17 ±4.44	247.94 ±41.32	8.06 ±0.64
III	Mid dose	M	80.70 ±8.19	71.53 ±10.50	36.79 ±2.02	50.55 ±4.15	182.60 ±46.44	8.97 ±1.20
		F	92.14 ±3.05	77.32 ±10.04	38.47 ±2.49	44.71 ±2.11	235.96 ±30.60	8.43 ±1.63
		MF	86.42 ±4.54	74.42 ±6.91	37.63 ±1.54	47.63 ±2.40	209.28 ±27.69	8.70 ±0.96
IV	High dose	M	97.97 ±5.37	59.20 ±3.45	34.11 ±1.98	49.67 ±4.43	219.16 ±28.54	7.84 ±1.06
		F	91.87 ±3.03	53.46 ±3.34	32.50 ±3.02	44.91 ±5.10	184.86 ±39.59	8.59 ±1.28
		MF	94.92 ±3.08	56.33 ±2.46	33.31 ±1.72	47.29 ±3.28	202.01 ±23.71	8.22 ±0.79

Values expressed in mean ± SEM

(Continuation of Table 24)

Group	Treatment	Sex	BUN (mg/dL)	LDH (U/L)	T. Protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)
I	Control	M	9.96±0.53	219.22±30.61	3.89±0.40	1.44±0.11	0.63±0.13
		F	10.57±1.31	281.48±28.52	4.46±0.30	1.79±0.10	0.58±0.09
		MF	10.27±0.68	250.35±22.29	4.17±0.26	1.62±0.09	0.60±0.07
II	Low dose	M	12.58±0.58	358.52±53.51	3.21±0.23	1.89±0.11	0.41±0.05
		F	11.83±0.97	305.44±22.58	3.78±0.26	1.49±0.16	0.38±0.02
		MF	12.21±0.55	331.98±28.77	3.49±0.19	1.69±0.11	0.40±0.03
III	Mid dose	M	11.82±0.89	321.32±17.80	3.80±0.29	1.76±0.19	0.35±0.03
		F	10.82±0.91	315.62±30.27	3.94±0.30	1.97±0.13	0.46±0.06
		MF	11.32±0.62	318.47±16.58	3.87±0.20	1.86±0.12	0.40±0.03
IV	High dose	M	11.12±1.42	253.08±24.88	4.62±0.54	1.79±0.13	0.46±0.05
		F	10.73±0.47	246.58±49.72	3.98±0.31	1.83±0.15	0.51±0.07
		MF	10.92±0.71	249.83±26.23	4.30±0.31	1.81±0.10	0.49±0.04

Values expressed in mean ± SEM

Table 25 (Effect of *VCM* on gross pathology- sub acute study report)

The various organs and tissues are analysed after administration of control, low dose, mid dose and high dose of *VCM*. The control groups of male are numbered from 101 - 105 and female from 106 - 110. Similarly low dose group male from 201 - 205, female from 206-210 and mid dose male group from 301 - 305 female from 306-310 and high dose male group from 401-405 and female from 406-410. The results are summarised below.

Group	Treatment	A. No.	Sex	Organs/Tissues	Observation
I	Control	101	M	External orifices, skin with mammary gland, various lymph nodes, both eyes, brain, trachea, thyroid gland, thymus gland, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder of *VCM* treated animals, both testes, epididymides, male sex glands (as whole), both ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone and bone marrow and spinal cord	No abnormality detected
		102	M		No abnormality detected
		103	M		No abnormality detected
		104	M		No abnormality detected
		105	M		No abnormality detected
		106	F		No abnormality detected
		107	F		No abnormality detected
		108	F		No abnormality detected
		109	F		No abnormality detected
		110	F		No abnormality detected
II	Low dose	201	M		No abnormality detected
		202	M		No abnormality detected
		203	M		No abnormality detected
		204	M		No abnormality detected
		205	M		No abnormality detected
		206	F		No abnormality detected
		207	F		No abnormality detected
		208	F		No abnormality detected
		209	F		No abnormality detected
		210	F		No abnormality detected
III	Mid dose	301	M		No abnormality detected
		302	M		No abnormality detected
		303	M		No abnormality detected
		304	M		No abnormality detected
		305	M		No abnormality detected
		306	F		No abnormality detected
		307	F		No abnormality detected
		308	F		No abnormality detected
		309	F		No abnormality detected
		310	F		No abnormality detected
IV	High dose	401	M		No abnormality detected
		402	M		No abnormality detected
		403	M		No abnormality detected
		404	M		No abnormality detected
		405	M		No abnormality detected
		406	F		No abnormality detected
		407	F		No abnormality detected
		408	F		No abnormality detected
		409	F		No abnormality detected
		410	F		No abnormality detected

Table 26 (Effect of *VCM* on histopathology- sub acute study report)

Group	Treatment	A. No.	Sex	Organs/Tissues	Observation
I	Control	101	M	External orifices, skin with mammary gland, various lymph nodes, both eyes, brain, trachea, thyroid gland, thymus gland, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder of *VCM* treated animals, both testes, epididymides, male sex glands (as whole), both ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone and bone marrow and spinal cord	No abnormality detected
		102	M		No abnormality detected
		103	M		No abnormality detected
		104	M		No abnormality detected
		105	M		No abnormality detected
		106	F		No abnormality detected
		107	F		No abnormality detected
		108	F		No abnormality detected
		109	F		No abnormality detected
		110	F		No abnormality detected
II	Low dose	201	M		No abnormality detected
		202	M		No abnormality detected
		203	M		No abnormality detected
		204	M		No abnormality detected
		205	M		No abnormality detected
		206	F		No abnormality detected
		207	F		No abnormality detected
		208	F		No abnormality detected
		209	F		No abnormality detected
		210	F		No abnormality detected
III	Mid dose	301	M		No abnormality detected
		302	M		No abnormality detected
		303	M		No abnormality detected
		304	M		No abnormality detected
		305	M		No abnormality detected
		306	F		No abnormality detected
		307	F		No abnormality detected
		308	F		No abnormality detected
		309	F		No abnormality detected
		310	F		No abnormality detected
IV	High dose	401	M		No abnormality detected
		402	M		No abnormality detected
		403	M		No abnormality detected
		404	M		No abnormality detected
		405	M		No abnormality detected
		406	F		No abnormality detected
		407	F		No abnormality detected
		408	F		No abnormality detected
		409	F		No abnormality detected
		410	F		No abnormality detected

6.3.3 Sub-chronic toxicity:

In the sub-chronic toxicity evaluation of *Vishnu Chakara Mathirai*, the body weight changes and other signs of toxicity were monitored and summarized in table 27. Blood samples of experimental animals for hematological and bio-chemical analyses were taken from common carotid artery. The internal organs and some tissues were observed for gross lesions.

Table 27 (Effect of *VCM* on body weight changes in the sub-chronic toxicity study)

Group	Treatment	Sex	Body weight (kg)						
			Day 0	Day 15	Day 30	Day 45	Day 60	Day 75	Day 90
I	Control	M	140.20 ±4.51	148.80 ±5.42	162.00 ±5.10	169.00 ±5.31	177.60 ±4.47	180 ±6.64	184 ±6.98
		F	132.80 ±4.27	142.60 ±3.93	149.40 ±4.61	157.00 ±4.71	169.80 ±4.97	174 ±4.54	180 ±2.22
		MF	136.50 ±3.18	145.70 ±3.32	155.70 ±3.86	163.00 ±3.90	173.70 ±3.41	180 ±5.66	186 ±4.44
II	Low dose	M	140.40 ±5.21	149.40 ±6.42	159.60 ±5.49	168.20 ±5.81	176.80 ±5.21	182 ±6.44	188 ±2.24
		F	130.80 ±5.70	139.60 ±5.24	149.00 ±4.47	158.80 ±5.24	169.40 ±4.92	176 ±6.88	180 ±4.42
		MF	135.60 ±3.98	144.50 ±4.23	154.30 ±3.78	163.50 ±4.01	173.10 ±3.59	182 ±2.44	186 ±3.44
III	Mid dose	M	142.40 ±4.30	149.60 ±4.23	158.40 ±4.23	165.00 ±4.32	175.40 ±3.17	180 ±4.46	186 ±5.66
		F	133.60 ±6.35	142.40 ±5.69	151.60 ±5.47	160.20 ±5.45	168.80 ±5.21	176 ±5.43	182 ±6.54
		MF	138.00 ±3.90	146.00 ±3.55	155.00 ±3.45	162.60 ±3.38	172.10 ±3.08	179 ±6.88	184 ±5.44
IV	High dose	M	140.40 ±4.01	149.80 ±3.28	159.40 ±4.34	167.60 ±3.34	173.60 ±3.54	179 ±5.44	189 ±6.44
		F	132.40 ±3.91	139.80 ±5.05	151.00 ±3.94	159.80 ±5.28	166.40 ±5.33	178 ±4.34	190 ±8.44
		MF	136.40 ±2.96	144.80 ±3.29	155.20 ±3.10	163.70 ±3.22	170.00 ±3.25	178 ±5.54	188 ±9.66

Values expressed in mean ± SEM

Table: 28 (Effect of *VCM* on relative organ weight in sub chronic study)

Group	Treatment	Sex	Brain wt (%)	Lungs wt (%)	Heart wt (%)	Liver wt (%)	Spleen wt (%)	Kidney wt (%)	Adrenal wt (%)	Sex glands wt (%)
I	**Control**	M	1.05 ±0.01	1.14 ±0.01	0.51 ±0.01	3.24 ±0.04	0.49 ±0.09	0.90 ±0.01	0.02 ±0.00	1.50 ±0.07
		F	1.08 ±0.03	0.59 ±0.04	0.5 ±0.02	3.38 ±0.11	0.58 ±0.03	0.85 ±0.02	0.02 ±0.00	0.08 ±0.00
		MF	1.09 ±0.04	0.56 ±0.02	0.53 ±0.01	3.41 ±0.02	0.56 ±0.04	0.88 ±0.01	0.03 ±0.00	0.94 ±0.26
II	**Low dose**	M	1.14 ±0.02	1.09 ±0.06	0.49 ±0.02	3.81 ±0.08	0.58 ±0.04	0.98 ±0.05	0.02 ±0.00	1.45 ±0.09
		F	1.21 ±0.05	0.62 ±0.04	0.46 ±0.01	3.82 ±0.04	0.48 ±0.05	0.88 ±0.04	0.03 ±0.00	0.04 ±0.01
		MF	1.11 ±0.02	0.87 ±0.06	0.45 ±0.01	3.0 ±0.03	0.52 ±0.03	0.98 ±0.04	0.03 ±0.00	0.91 ±0.25
III	**Mid dose**	M	1.13 ±0.02	0.62 ±0.05	0.49 ±0.02	3.26 ±0.14	0.58 ±0.08	0.94 ±0.07	0.03 ±0.00	1.54 ±0.13
		F	1.16 ±0.02	0.68 ±0.06	0.41 ±0.01	3.4 ±0.05	0.49 ±0.02	0.79 ±0.02	0.02 ±0.00	0.06 ±0.01
		MF	1.14 ±0.03	0.84 ±0.05	0.42 ±0.02	3.64 ±0.07	0.52 ±0.05	0.89 ±0.05	0.03 ±0.00	0.69 ±0.26
IV	**High dose**	M	1.12 ±0.02	1.02 ±0.16	0.44 ±0.02	4.11 ±0.08	0.49 ±0.02	0.90 ±0.04	0.02 ±0.00	1.40 ±0.20
		F	1.12 ±0.02	0.85 ±0.02	0.41 ±0.02	3.18 ±0.11	0.52 ±0.05	0.90 ±0.05	0.03 ±0.00	0.04 ±0.00
		MF	1.12 ±0.02	0.98 ±0.06	0.41 ±0.01	4.10 ±0.11	0.46 ±0.02	0.94 ±0.04	0.02 ±0.00	0.68 ±0.24

Values expressed in mean ± SEM

Table: 29 (Effect of *VCM* on feed consumption on *VCM* in the subchronic study)

Group	Treatment	Sex	Cumulative feed intake (kg)		
			1st month	2nd Month	3rd month
I	Control	**M**	228.43±9.42	253.43±2.47	283.43±4.35
		F	218.54±8.84	254.57±3.19	289.14±1.94
		MF	220.50±7.92	254.00±2.83	256.29±3.15
II	Low dose	M	256.86±5.38	265.43±6.88	262.57±3.86
		F	192.29±4.82	181.29±2.56	183.43±3.53
		MF	193.57±5.10	193.36±4.72	198.00±3.70
III	Mid dose	M	168.00±5.37	166.29±5.85	169.43±4.32
		F	181.00±3.69	184.71±3.00	186.14±3.81
		MF	175.00±4.53	173.50±4.42	172.79±4.02
IV	High dose	M	177.00±3.56	176.86±4.07	180.29±3.02
		F	137.86±3.71	138.29±1.46	170.14±3.99
		MF	180.43±3.63	178.57±2.76	175.71±3.51

Values expressed in mean ± SEM

Table: 30 (Effect of *VCM* on Biochemical analysi of Sub chronic study) :

Group	Treatment	Sex	Glucose (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	SGPT (U/L)	ALP (U/L)	γ-GT (U/L)
I	Control	M	96.84 ±10.24	58.44 ±7.92	49.81 ±4.65	70.87 ±6.57	225.09 ±14.48	4.71 ±0.65
		F	100.61 ±09.12	57.80 ±4.42	56.88 ±7.18	55.22 ±4.95	269.72 ±44.30	8.12 ±1.68
		MF	89.68 ±6.26	57.62 ±4.62	52.84 ±3.85	62.34 ±4.47	268.26 ±12.79	7.71 ±0.98
II	Low dose	M	108.63 ±09.24	56.34 ±6.29	52.20 ±6.46	52.22 ±3.39	290.80 ±19.68	7.32 ±1.90
		F	124.41 ±2.66	82.40 ±4.48	62.52 ±4.47	68.92 ±7.92	282.23 ±19.19	6.30 ±0.39
		MF	107.12 ±4.26	64.34 ±5.11	57.36 ±4.08	60.57 ±4.92	286.52 ±13.04	6.81 ±0.93
III	Mid dose	M	86.78 ±4.62	55.68 ±2.60	52.21 ±1.64	66.18 ±6.14	188.81 ±12.70	5.65 ±0.57
		F	86.85 ±1.66	56.52 ±1.12	46.12 ±2.43	58.10 ±4.10	189.05 ±28.46	5.68 ±0.75
		MF	94.12 ±2.20	56.14 ±1.45	50.61 ±1.70	62.18 ±3.18	198.38 ±10.56	5.66 ±0.44
IV	High dose	M	89.64 ±2.11	68.24 ±09.31	49.04 ±2.43	44.11 ±1.24	218.44 ±12.94	5.06 ±1.65
		F	86.58 ±1.43	82.73 ±09.51	49.12 ±4.26	60.18 ±3.42	306.33 ±28.39	5.54 ±0.84
		MF	84.12 ±1.27	78.91 ±07.22	49.15 ±1.88	50.64 ±2.06	252.28 ±18.98	6.10 ±0.97

Values expressed in mean ± SEM

(continuation of Table 30)

Group	Treatment	Sex	BUN (mg/dL)	LDH (U/L)	T. Protein (g/dL)	Albumin (g/dL)	Creatinine (mg/dL)
I	Control	M	11.40±0.40	424.40±18.19	4.24±0.12	1.33±0.19	0.32±0.02
		F	11.24±0.64	468.18±31.05	4.69±0.36	1.36±0.12	0.34±0.02
		MF	10.97±0.23	459.99±16.65	4.32±0.21	1.64±0.19	0.31±0.02
II	Low dose	M	12.66±0.90	600.10±42.03	4.11±0.24	1.85±0.11	0.31±0.04
		F	11.94±0.22	482.52±20.24	4.66±0.12	1.79±0.16	0.32±0.01
		MF	13.02±0.39	424.11±30.41	4.42±0.16	1.76±0.12	0.31±0.01
III	Mid dose	M	12.33±0.55	428.31±41.13	4.14±0.12	1.81±0.14	0.33±0.01
		F	12.11±0.33	365.03±49.67	4.34±0.12	1.76±0.08	0.46±0.05
		MF	12.26±0.58	368.12±40.17	4.16±0.10	1.65±0.04	0.42±0.04
IV	High dose	M	12.04±1.22	368.18±47.15	4.46±0.44	1.70±0.04	0.44±0.04
		F	11.94±0.66	386.66±28.13	4.11±0.21	1.53±0.06	0.42±0.03
		MF	11.00±0.53	397.67±34.40	4.12±0.08	1.54±0.04	0.42±0.01

Values expressed in mean ± SEM

Table: 31 (Effect of *VCM* on Gross Pathology- Sub chronic study)

Group	Treatment	Sex	Organs/Tissues	Observation
I	Control	M	External orifices, skin with mammary gland, various lymph nodes, both eyes, brain, trachea, thyroid gland, thymus gland, heart, lungs, stomach, small and large intestines (with peyer's patches), spleen, liver, adrenals, kidneys, urinary bladder of *VCM* treated animals, both testes, epididymides, male sex glands (as whole), both ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone and bone marrow and spinal cord of *VCM* treated animals	No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
II	Low dose	M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
III	Mid dose	M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
IV	High dose	M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		M		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected
		F		No abnormality detected

6.4 ANTI-CONVULSANT ACTIVITY:

6.4.1 Maximal Electroshock Induced Seizures model (MES)

Wistar rats weighing between 200-250 gms were divided into four groups (n=4). Group I animals served as control and received vehicle Group II served as standard received phenytoin Group III was administered with *VCM*, Group IV was administered with *Vishnu Chakara Mathirai* at higher concentration.

The treatment was continued for 15 days. On the 15th day, seizures were induced to all the groups of animals using electro convulsiometer at 60 Hz alternating current of 150 milliamps intensity elicited Maximal Electro Shock (MES) seizures for 0.2 second were applied. A drop of electrolyte solution with Lignocaine were applied to the corneal electrodes prior to application to the rats. Test drug was administered and standard group received phenytoin. Suppression of tonic hind limb extension was taken as a measure of efficacy of *VCM* and all the experimental groups were compared with the control.

The Phenytoin administrated rats showed Tonic hind limb flexion of 1.24 secs and *VCM* at lower dose showed 2.64 secs and at higher dose it gave 1.86 secs. Similarly Tonic hind limb extension of Phenytoin was found to be 0.00 and that of lower dose of *Vishnu Chakara Mathirai* showed 8.82 secs. Whereas at higher dose it showed 6.42 secs. The clonus of the animals showed 5.44, 11.66, 10.66 secs respectively. Stupor of the animal indicated 0.00, 68.8, 54.8 secs for Phenytoin, *VCM* at lower dose and *VCM* at higher dose respectively. The percentage protection of Phenytoin, *VCM* lower dose, *VCM* higher dose were found to be 70.6, 37.15 and 43.41 respectively.

Table: 32 (*Effect of Vishnu Chakara mathirai on MES Induced Convulsions)*

Sl. no	Groups	Treatment	Tonic hind limb flexion (sec)	Tonic hind limb extension (sec)	Clonus (sec)	Stupor (sec)
1	Group I	Control	4.44±0.38	15.24±0.86	18.54±2.44	110.34±3.44
2	Group II	Phenytoin	1.24±0.22***	0.00	5.44±0.56***	0.00
3	Group III	*VCM* 100 mg	2.64±0.52**	8.82±0.98***	11.66±1.34***	68.88±2.38***
4	Group IV	*VCM 200* mg	1.86±0.68***	6.42±0.24***	10.66±2.33***	54.82±1.04***

Data expressed as mean±SEM. n=6, p <0.05,** p <0.01,*** p <0.001 (compared with control).*

Table: 33 (Percentage protection on MES induced convulsion)

S. no	Groups	Treatment	% protection
1	Group I	Control Distilled water (5ml/kg)	0
2	Group II	Phenytoin (25mg/kg)	70.61
3	Group III	Vishnu chakra mathirai (200mg/kg)	37.15
4	Group IV	Vishnu chakra mathirai (400mg/kg)	43.41

6.4.2 Picrotoxin induced seizures

Mice were kept individually in transparent mice cages for 60 min. Seizure was induced by picrotoxin. Animals were observed for convulsion for a period of 30 min. Hind limb extension was taken as tonic convulsion. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. The ability of extract to prevent or delay the onset of the hind limb extension exhibited by the animals was taken as an indication of anticonvulsant activity. Onset and duration of convulsions in the mice were noted and recorded and protection percentage was determined.

The onset of tonus for Phenytoin, *VCM* lower dose and *VCM* higher dose were found to be 430, 234 and 356 secs respectively. Onset of clonus for Phenytoin, *VCM* lower dose and *VCM* higher dose were found to be 190, 143, and 120 secs respectively. Percentage mortality of control was fixed as 100 and that of standard Phenytoin as zero. The percentage mortality of Phenytoin, *VCM* lower dose and *VCM* higher dose were calculated and it showed below the control level as 14, 75 and 40 respectively.

Table: 34 (Effect of *Vishnu chakara mathirai* on Picrotoxin Induced Seizures):

Group	Dose mg/kg	Onset of tonus in secs	Onset of clonus in secs	% Mortality
Control	3 ml	120±3.4	58±4.2	100
Phenytoin	25	430±2.7	190±6.5	14
Vishnu chakra mathirai	100	243±6.3	143±3.2	75
Vishnu chakra mathirai	200	356±4.3	120±5.4	40

Data expressed as mean±SEM. n=6, p <0.05,** p <0.01,*** p <0.001 (compared with control).*

6.4.3 N-Methyl-D-Aspartate (NMDA) test:

Mice were injected subcutaneously with NMDA, 1 h after administration of *Vishnu Chakara Mathirai* 100 and 200 mg/kg and Phenytoin 25 mg/kg. Animals were observed for 30 min. Animals that did not exhibit turning behaviour within the 30 min of observation period were declared protected. Turning behaviour was characterized by two consecutive 360° cycles fulfilled by the same animal.

Number of turnings for Phenytoin was fixed as zero and that of *Vishnu Chakara Mathirai* lower and higher dose were found to be 36 and 20 respectively. The percentage protection of the above doses were 40 and 68 respectively with respect to Phenytoin (standard).

Table: 35 Effect of *Vishnu Chakara mathirai* in NMDA induced seizures:

Groups	Dose	No of turning	% Protection
Negative control (NMDA)	75mg	60±1.24	0
Phenytoin	25mg	0	100
Vishnu chakra mathirai	100mg	36±1.32	40
Vishnu chakra mathirai	200mg	20±2.1	68

Data expressed as mean±SEM. n=6, p <0.05,** p <0.01,*** p <0.001 (compared with control).*

6.4.4 PTZ Induced Convulsions:

Anti-convulsant activity of *VCM was* carried out by Pentelene tetrazole (PTZ) method. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted. Onset and duration of convulsions in the mice were noted and recorded and protection percentage was determined.

Table: 36 (Effect of *VCM* Drug on PTZ Induced Convulsions):

GROUP	ONSET OF CONVULSIONS	DURATION OF CONVULSIONS
CONTROL	173.4±1.02	74.0±1.3
STANDARD	652.0±2.2***	14.0±0.7***
VCM 100mg	469.0±1.30***	32.80±0.86***
VCM 200mg	557.4±1.07***	25.30±1.15***

Data expressed as mean±SEM. n=6, p <0.05,** p <0.01,*** p <0.001 (compared with control).*

6.4.5 PTZ induced kindling

Anti-convulsant activity of *VCM was* carried out by Pentelene Tetrazole (PTZ) Kindling method. The different parameters like Latency of seizure, Tonic hind limb flexion, Tonic hind limb extension, Clonic seizures and Postictal depression were recorded.

Table: 37 (Mean duration of different parameters in PTZ induced kindling)

Parameters	Control	Phenytoin	VCM 100mg	VCM 200mg
Latency of seziure	330.66 ± 10.8	0	419 ± 8.73***	0
Tonic hind limb flexion	1.5 ± 0.23	0	1.5 ± 0.23^{ns}	0
Tonic hind limb extension	10.66 ± 0.61	0	10.66 ± 0.43^{ns}	0
Clonic seziures	5.5 ± 0.44	0	3.5±0.54*	0
Postictal depression	340.16 ± 3.78	0	189.16 ± 1.15***	0

Data expressed as mean±SEM. n=6, p <0.05,** p <0.01,*** p <0.001 (compared with control).*

Table: 38 (Percentage protection from clonic seziures in PTZ induced kindling)

S.no	Group	% Percentage protection
1	Control	0
2	Phenytoin	100
3	VCM 100mg	30
4	VCM 200mg	90

Vishnu Chakara Mathirai (*VCM)* was prepared as per the guidelines and characterized at various angles.

Table : 9 shows the results of various phyto-chemical analysis of *VCM*. The drug possesses alkaloids and phenols, which have high therapeutic values. It also gives negative test for terpenoids, flavanoids, morphine, and quinine in *VCM*. The absence of morphine in the *VCM* reveals the fact that the tablet can be used without addictive effect.

Table 10 gives result of the physicochemical analysis of *VCM*. It shows *VCM* has slightly acidic pH (pH = 6.5) and it has only 7.3 % loss on drying which indicates low moisture content which is needed for long term preservation[76]. The total ash content was 7.7 % and acid insoluble content was only 2.12 %. These two parameters indicate the presence of high amount of absorption of the *VCM*. The water soluble *Vishnu chakara mathirai* and alcohol soluble *VCM* were found to be 85.32 % and 15.12 % which are essentially required for intestinal absorption[77,78]. This indicates the increased bioavailability of the *VCM*. All the parameters obtained were within permissible limits.

VCM was further tested for the presence of heavy metal and the percentage of heavy metals like lead, cadmium, mercury and arsenic was presented in **Table 11.** The percentage of lead was found to be only 1.13 ppm, which is well below the acceptable limit. Cadmium was not detected and Arsenic was present only 2.03 ppm which is also within permissible limit. Similarly, the concentration of mercury was only 0.83ppm and that of sulphur was only 8.82ppm. These data ensure the safety of the *VCM*.

Energy Dispersive X Ray Analysis (EDAX) of *VCM* was carried out and the elements like, Carbon, Oxygen, Magnesium, Sulphur and Calcium were estimated. From the spectra (Fig 11) the atom percentage of the elements are found to be as follows.

Carbon = 42.25%, Oxygen= 37.01%, Magnesium = 9.82%, Sulphur = 8.39% and Calcium = 2.54% **Table: 12.**

The prepared *VCM* was subjected to ICP-OES and the results of the medicine are given in the **Table 13.** The *VCM* was found to be light brown colour with round shape. Average weight of a tablet = 0.1058 g, Uniformity of weight = 3.86 to 106.8%, Disintegration time = 58 sec, Total Ash= 32.45% w/w, Acid Insoluble Ash = 11.57 % w/w, Loss on Drying at 105 C = 4.742 w/w, Water Soluble Extractive (WSE) = 4.602 % w/w Alcohol Soluble Extractive (ASE) = 2.781 % w/w Mercury = 0.0003792 w/w. Sulphur =0.001925% w/w.

Ultra Violet and Infra Red spectra of the drug were recorded (Fig 12). The absorbance data and reflection presented in **Table 14**. The peak at 224 nm, 269 nm, 338 nm, 371 nm UV spectrum for absorbance and peak at 1216 nm, 1044 nm, 971 nm, 888 nm for reflection of UV spectrum reveals the fact that it absorbs light only in the observable region. The IR spectra (Fig 13) shows peaks at various frequencies. The peak at 3299 cm^{-1} shows the presence of alcoholic functional group and peak at 2918 cm^{-1} indicates the presence of aldehyde group. The peak at 2082 cm^{-1}, and 1615 cm^{-1} are due to carbonyl stretching and peaks at 1411 cm^{-1}, 1017 cm^{-1} and 870 cm^{-1} indicates the C-H stretching.

To study the particle size of *VCM* by Scanning Electron Microscope (SEM) (Fig 14 & 15). The particles of *VCM* are found to be spherical in shapes and sizes are in the range from 7 to 50 microns. Hydrophobicity of *VCM* gives these particles a tendency to aggregate together to form larger particles. *VCM* exhibited larger sizes and agglomeration of the particles. Larger size of the medicine may be due to the agglomeration of the particles of *VCM* by repetitive cycles of calcinations implicated in the process of preparation.

Table 15 shows the *VCM* analysis report of pesticide contaminant and their concentration was found to be lesser than the permissible limit. This support for the applicability of *VCM.*

The microbial study of *VCM* was studied as per the guidelines of W.H.O (QAS/5.131/Rev.1) specification NMT 10^5 CFU/g in **Table 16**. From this study result, it is clear that total bacterial count was found to be 29500 CFU/g, total fungal count was found to be 70 CFU/g, specification NMT 10^5 CFU/g). In the prepared *VCM, E. Coli, Staphyloccus aureus, Salmonella, Pseudomonas aeruginosa* were found to be absent. These data showed that the total bacterial count and total fungal count was found to be within WHO permissible limits.

Table 17 shows the result of acute toxic study. From this table it was found out that after administration of *VCM*, Body weight, Assessments of posture, Body tone, Piloerection, Sensitivity response, Locomotion, Muscle gripness, Defecation, and Urination were found to be normal. Signs of Convulsion Limb paralysis, Lacrimation and Salivation were found to be absent. No significant colour change was observed and mild rearing was noticed in this study.

In the acute toxicity study, the animals were treated with different concentration of *VCM* from the range of 5 mg/kg b.wt to 2000 mg/kg b.wt which did not make signs of toxicity, in the *Vishnu chakara mathirai* treated groups as compared to the controls when observed for 14 days of the acute toxicity experimental time. These results showed that a single oral dose of *VCM* showed no death of *Vishnu chakara mathirai* treated rats even under upper dosage levels indicating the high margin of safety of *VCM*. In acute toxicity test the *VCM* was found to be non toxic at the dose level of 2000 mg/ kg body weight. There were no treatment related deaths, abnormal clinical signs, remarkable body weight changes or differences in feed consumption were observed in test drug administrated rats.

In **Table 18** the physiological characters of animals after administration of *VCM* are depicted. From this it is inferred that Alertness, Touch Response and Respiration shows positive whereas Aggressiveness, Pile erection, Grooming, Gripping, Decreased Motor Activity, Tremors, Convulsions Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis, Diarrhea, Analgesia, Exophthalmos, Lacrimation, Writhing and Mortality were found to be negative.

Sub acute toxicity

The sub-acute toxic study was carried out with control, low dose, mid dose and high dose of *VCM*. Each category 5 numbers of male (numbered from 101-105) and 5 numbers of female (numbered from 106-115) were observed. The continuous clinical observations for 28 days are presented in **Table 19**. From this report it is clear that the animals were found to normal upto 28 days after administration of *VCM*. This clearly proves the *VCM* has no toxicity in animal models.

There was no treatment related deaths, abnormal clinical signs, remarkable body weight changes or differences in feed utilization were observed in Vishnu chakara mathirai administrated rats. Control and Vishnu chakara mathirai treated groups have no notable changes in hematological parameters such as HCT, HGB, RBC, MCH, MCV, MCHC, platelet count and WBC.

No significant difference in glucose, total cholesterol, triglycerides, total protein, albumin, glutamyl pyruvate aminotransferase (GPT), and also no noteworthy difference in alkaline phosphatase, γ-glutamyl transferase, total bilirubin, creatinine, urea were observed between the control and Vishnu chakara mathirai administered animals.

No significant difference in any of the organs weight was observed between the control and test drug administered animals. No gross and histopathological findings were observed in all the experimental animals.

In the sub-acute toxicity study, after administration of *VCM* for different groups (control, low dose, mid dose and high dose) the change in body weight on every seventh day for subsequent 28 days (0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day) were recorded for both Male and Female. The observed data for Male (M) and Female (F) and their average (MF) and presented in **Table 20**. The change in body weight of male rats with control on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days are 134.00±5.70, 141.60±5.90, 149.40±6.12, 158.40±5.70, 171.40±6.99 respectively. The values are expressed in mean ± SEM. For female rats it was found to be 121.80±2.91, 129.40±2.27, 139.00±2.92, 148.00±2.43 and 159.20±2.96 respectively.

The change in body weight of male rats with low dose (50 mg/kg b.wt) on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days are found to 136.20±5.00, 145.40±6.22, 155.00±3.96, 161.60±3.88, 171.80±4.42, respectively For female rats it was 118.40±3.09, 126.80±4.26, 135.40±3.80, 145.00±3.39, 153.40±2.04, respectively. Calculation of average weight of male and female animals gives the the following values (MF) 127.30±4.06, 136.10±4.72, 145.20±4.17, 153.30±3.68, 162.60±3.83 respectively

Similarly the observed values for mid dose (100 mg/kg b.wt) for male rats on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} days are 137.40±3.96, 146.80±4.72, 158.80±4.29, 165.00±3.21, 172.20±3.95 respectively and for female rats 119.20±4.44, 127.40±4.50, 135.40±3.31, 143.40±4.76, 151.00±4.11 respectively, the average yields are 128.30±4.13, 137.10±4.46, 147.10±4.66, 154.20±4.50, 161.60±4.44, respectively

When high dose (200 mg/kg b.wt) of *VCM* was administered, the body weight measurements yield the following results. For male rats the weight measurement on 0^{th} , 7^{th}, 14^{th} , 21^{st} and 28^{th} days are 135.00±5.16, 143.20±6.35, 150.60±6.45, 161.60±6.65, 170.60±5.71, respectively and for female rats 117.60±3.91, 127.80±4.37, 137.40±4.58, 144.60±4.12, 151.60±4.28 respectively the average yields are 126.30±4.21, 135.50±4.45,

144.00±4.33, 153.10±4.65, 161.10±4.62 respectively. These data clearly indicate the change in weight of both male and female rats are normal which support the in-toxicity of the prepared *VCM* up to the concentration of 200 mg/kg.

The weight of internal organs of *VCM* treated animals Brain, Lungs, Heart, Liver, Spleen, Kidney, Adrenals, Sex glands were measured after administration of control, low dose, mid dose and high dose of *VCM* are mentioned in **Table 21. C**ontrol male: 1.20±0.06, 0.89±0.05, 0.39±0.02, 3.84±0.20, 0.48±0.08, 0.93±0.04, 0.02±0.00 and 1.64±0.08. and for female 1.23±0.03, 0.85±0.02, 0.41±0.01, 4.10±0.07, 0.51±0.04, 1.01±0.03, 0.03±0.00, 0.04±0.00 and Male female 1.21±0.03, 0.87±0.03, 0.40±0.01, 3.97±0.11, 0.49±0.04, 0.97±0.03, 0.02±0.00, 0.84±0.27

For low dose (50mg/kg b.wt) male 1.19±0.02, 0.92±0.06, 0.48±0.03, 4.80±0.23, 0.54±0.02, 1.08±0.03, 0.02±0.00, 1.70±0.07, for female 1.36±0.03, 0.90±0.11, 0.47±0.02, 4.61±0.23, 0.49±0.02, 1.07±0.06, 0.03±0.00, 0.06±0.00. For male female, 1.28±0.03, 0.91±0.06, 0.48±0.01, 4.71±0.016, 0.51±0.02, 1.08±0.03,0.03±0.00, 0.88±0.28

For mid dose (100mg/kg b.wt) male 1.18±0.03, 0.76±0.03, 0.8±0.01, 4.35±0.24, 0.50±0.04, 1.00±0.04, 0.02 ±0.00, 1.66±0.07. For female 1.34±0.07, 1.08±0.16, 0.48±0.03, 4.54±0.15, 0.49±0.02, 1.00±0.04, 0.03±0.00, 0.06±0.01 for male female 1.26±0.04, 0.92±0.09, 0.48±0.01, 4.45±0.14, 0.50±0.02, 1.00±0.03, 0.02±0.00, 0.86±0.27.

High dose (200mg/kgb b.wt) male showed 1.15±0.05, 0.99±0.18, 0.47±0.03, 4.16±0.21, 0.49±0.04, 1.00±0.06, 0.02±0.00, 1.71±0.16, for female 1.28±0.04, 0.80±0.04, 0.45±0.03, 3.99±0.28, 0.52±0.03, 0.82±0.03,0.03±0.00, 0.05±0.01. For male female 1.21±0.04, 0.89±0.09, 0.46±0.02, 4.07±0.17, 0.50±0.02, 0.91±0.04, 0.03±0.00, 0.88±0.29.

The feed consumption for control and various dose of *VCM* treated animal groups were recorded for Male and Female on every weekend for four weeks subsequently (I, II, III and IV). The observed data for Male(M) and Female (F) and their average (MF) was presented in **Table 22**. The cumulative feed intake (in Kg) for the control rats at the end of 1st week, 2nd week, 3rd week and 4th week for male rats are 54.86±10.33, 52.57±2.58, 54.86±4.71, 68.71±4.96 respectively and for female rats 46.14±5.91, 55.29±3.39, 59.57±2.00, 55.00±4.95 respectively, All values are expressed in mean ± SEM. Calculation of average of these two gives the following values 50.50±8.12, 53.93±2.99, 57.21±3.35, 61.86±4.95 respectively.

When low dose of *VCM* (100 mg) was administered to rats the cumulative feed intake (in Kg) at the end of 1st week, 2nd week, 3rd week and 4th week for male rats are 61.57±7.50, 66.57±2.79, 65.71±1.71, 69.71±7.19 respectively and for female rats 51.86±7.78, 59.71±2.45, 58.29±2.76, 58.57±4.93 respectively, the average of which yields 56.71±7.64, 63.14±2.62, 62.00±2.24, 64.14±6.06, respectively.

The cumulative feed intake (in kg) at the end of 1st, 2nd, 3rd and 4th week for male rats, when mid dose (200 mg) of *VCM* was administered are 49.14±8.20, 63.00±1.45, 54.14±3.38, 70.29±4.86, respectively and for female rats 44.71±7.09, 56.14±6.16, 43.57±5.67, 60.29±2.36 respectively, the mean of which gives 46.93±7.64, 59.57±3.80, 48.86±5.64, 65.29±3.61 respectively.

When high dose (200 mg) of *VCM* was administered the cumulative feed intake (in Kg) at the end of 1st, 2nd, 3rd and 4th week for male rats are 52.43±10.93, 62.00±6.58, 59.00±4.35, 59.00±2.25 respectively and for female rats 53.29±7.30, 58.86±5.38, 49.00±3.95, 62.00±4.86 respectively the average of which gives 52.86±9.12, 60.43±5.98, 54.00±4.15, 60.50±3.56 respectively.

From the above data it is clear that the cumulative feed in take for the successive four weeks are more or less same with the control. This reveals that there is no significant change in the feed intake after administration of prepared *VCM* up to the concentration of *200* mg/Kg.

The haematological report on sub acute study of animals after administration of *VCM* at different concentrations give the following results. (T**able 23)** The WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT values for male rats with control, are 10.38±1.42, 6.18±0.15, 14.18±0.19, 43.88±1.09, 70.96±0.34, 23.00±0.68, 32.40±1.01, 1448.20±70.91 respectively and for female rats 9.02±0.92, 5.65±0.18, 14.86±0.22, 43.10±1.20, 71.04±0.20, 24.60±1.00, 34.50±1.41, 16.41.00±90.19 respectively. On average it yields 9.70±0.83, 5.92±0.14, 14.52±0.18, 43.49±0.78, 71.00±0.19, 23.80±0.63, 33.45±0.89, 1544.60±62.91 respectively.

For low dose the values are 10.18±0.67, 5.48±0.11, 14.36±0.27, 38.78±0.81, 70.74±0.18, 26.22±0.39, 37.08±0.59, 1654.80±85.01 respectively (for male rats) and 9.58±0.88, 6.08±0.18, 15.20±0.49, 43.36±1.39, 71.30±0.23, 25.02±0.43, 35.08±0.63, 657.00±111.621 respectively (for female rats). Their mean gives 9.88±0.53, 5.78±0.14, 14.78±0.30, 41.07±1.07, 71.02±0.17, 25.62±0.34, 36.08±0.53, 1655.90±66.14 respectively.

Similarly for mid dose 9.82±0.61, 5.95±0.13, 14.42±0.21, 42.38±1.03, 71.28±0.26, 24.28±0.52, 34.08±0.82, 1473.40±103.99 respectively (for male rats) and 9.12±1.61, 5.91±0.19, 14.58±0.33, 43.20±0.81, 71.30±0.47, 24.14±0.22, 34.14±0.14, 1501.00±82.75 respectively (for female rats). Their mean value gives 9.47±0.82, 5.93±0.11, 14.50±0.19, 42.79±0.63, 71.29±0.25, 24.21±0.26, 34.11±0.39, 1487.20±62.82 respectively

When high dose of *VCM* was administered the observed values of male rats are 9.00±1.55, 6.19±0.19, 14.58±0.21, 43.96±1.29, 70. 96±0.24, 23.64±0.84, 33.28±1.14, 1428.40±83.78 respectively and for female rats 8.34±1.11, 5.63±0.22, 17.81±3.34, 40.36±1.66, 71.01±0.34, 25.06±0.47, 35.23±0.73, 1726.00±40.88 respectively. The average of these two gives 8.67±0.90, 5.91±0.17, 16.20±1.67, 42.16±1.16, 70.98±0.20, 24.35±0.51, 34.25±0.72, 1577.20±66.27 respectively. The WBC value decreases from 9.70 to 8.67 but RBC remains constant. HGB increases HCT decreases MCV decreases. MCH increases. MCHC increases PLT increases.

The Plasma biochemistry report is given in **table 24.** The concentration of Glucose, Triglycerides, Cholesterol, SGPT, ALP, y-GT, BUN, LDH, T.Protein, Albumin, Creatinine for male and female rats after administration of control, low dose of *VCM*, Mid dose and High dose of *VCM* were recorded. The values are given below.

For control: 93.50±5.01, 49.85±1.51, 33.06 ±1.96, 42.76±2.38, 250.82±47.09, 6.04± 1.23, 9.96±0.53, 219.22±30.61, 3.89±0.40, 1.44±0.11, 0.63±0.13 (male). 82.2 4±4.18, 63.62±5.11, 34.16±1.40, 45.14±2.47, 238.28±38.48, 7.28 ±1.39, 10.57±1.31, 281.48±28.52, 4.46±0.30, 1.79±0.10, 0.58±0.09 (female). Average 87.87±3.60, 56.74±3.40, 33.61±1.15, 43.95±1.67, 244.55±28.74, 6.66±0.95, 10.27±0.68, 250.35±22.29, 4.17±0.26, 1.62±0.09, 0.60±0.07

For low dose: 85.79±1.53, 69.62±6.37, 34.28±1.34, 47.30±8.01, 235.47±56.80, 8.34±0.66, 2.58±0.58, 358.52±53.51, 3.21±0.23, 1.89±0.11, 0.41±0.05 (male) 92.20±8.01, 47.45±2.61, 39.91±1.07, 53.05±4.53, 260.42±66.18, 7.79±1.18, 11.83±0.97, 305.44±22.58, 3.78±0.26, 1.49±0.16, 0.38±0.02 (female) Average 89.00±3.99, 58.54±4.92, 37.09±1.24, 50.17±4.44, 247.94±41.32, 8.06±0.64, 12.21±0.55, 331.98±28.77, 3.49±0.19, 1.69±0.11, 0.40±0.03

For mid dose: 80.70±8.19, 71.53±10.50, 36.79±2.02, 50.55±4.15, 182.60±46.44, 8.97±1.20, 11.82±0.89, 321.32±17.80, 3.80±0.29, 1.76±0.19, 0.35±0.03 (male) 92.14±3.05, 77.32±10.04, 38.47±2.49, 44.71±2.11, 235.96±30.60, 8.43±1.63, 10.82±0.91, 315.62±30.27, 3.94±0.30, 1.97±0.13, 0.46±0.06 (female) Average 86.42±4.54, 74.42±6.91, 37.63±1.54, 47.63±2.40, 209.28±27.69, 8.70±0.9611.32±0.62, 318.47±16.58, 3.87±0.20, 1.86±0.12, 0.40±0.03,

For high dose: 97.97±5.37, 59.20±3.45, 34.11±1.98, 49.67±4.43, 219.16±28.54, 7.84±1.06, 11.12±1.42, 253.08±24.88, 4.62±0.54, 1.79±0.13, 0.46±0.05 (male) 91.87±3.03, 53.46±3.34, 32.50±3.02, 44.91±5.10, 184.86±39.59, 10.73±0.47, 246.58±49.72, 3.98±0.31, 1.83±0.15 (female) Average: 8.59±1.28, 94.92±3.08, 56.33±2.46, 33.31±1.72, 47.29±3.28, 202.01±23.71, 8.22±0.79, 0.51±0.07, 10.92±0.71, 249.83±26.23, 4.30±0.31, 1.81±0.10, 0.49±0.04

Effect of *VCM* on gross pathology is given in **Table 25.** The various organs /tissues are analysed after administration of control, low dose, mid dose and high dose of *VCM*. The control groups of male or numbered from 101-105 and female from 106-110, similarly low dose group male from 201-205, female from 206-210 and mid dose male group from 301-305 female from 306-310 and high dose male group from 401-405 and female from 406-410. External orifices, mammary gland, lymph nodes, eyes, brain, trachea, thyroid, thymus, heart, lungs, stomach, small and large intestines spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymis, male sex glands, ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow and spinal cord of all the animals control, low dose, mid dose and high dose of *VCM* shows no abnormality.

Table 26 shows the histo-pathological report of sub acute study on *VCM*. The various organs / tissues *Vishnu chakara mathirai* treated group are analysed. The liver has

shown in fig 16, 17 and 18, Spleen in fig 19, 20, and 21, kidney in fig 22, 23 and 24 respectively. The control groups of male or numbered from 101- 105 and female from 106 – 110, similarly low dose group male from 201 – 205, female from 206-210 and mid dose male group from 301 - 305 female from 306-310 and high dose male group from 401-405 and female from 406-410. mammary gland, lymph nodes, eyes, brain, trachea, thyroid, thymus, heart, lungs, stomach, small and large intestines spleen, liver, adrenals, kidneys, urinary bladder, testes, epididymis, male sex glands ovaries, uterus with cervix, vagina, peripheral nerve, skeletal muscle, bone with bone marrow and spinal cord of all the animals control, low dose, mid dose and high dose of *VCM* shows no abnormality. This proves the safety characteristics of *VCM*.

Sub chronic toxicity:

There was no treatment related deaths, abnormal clinical signs, remarkable body weight changes or differences in feed utilization were observed in Vishnu chakara mathirai administrated rats. Control and Vishnu chakara mathirai treated groups have no notable changes in hematological parameters such as HCT, HGB, RBC, MCH, MCV, MCHC, platelet count and WBC.

No significant difference in glucose, total cholesterol, triglycerides, total protein, albumin, glutamyl pyruvate aminotransferase (GPT) and also no noteworthy difference in alkaline phosphatase, γ-glutamyl transferase, total bilirubin creatinine urea were observed between the control and Vishnu chakara mathirai administered animals.

No significant difference in any of the organs weight was observed between the control and test drug administered animals. No gross and histopathological findings were observed in all the experimental animals.

In the subchronic toxicity, every fifteen days observed any change in body weight of *Vishnu chakara mathirai* treated group for 90 days (0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and

90^{th} day) were recorded for Male and Female. The observed data for Male and Female and their average (MF) and presented in **Table 27.** The average change in body weight of the rats with control on 0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} days are 136.50±3.18, 145.70±3.32, 155.70±3.86, 163.00±3.90, 173.70±3.41, 180±5.66 and 186±4.44 respectively. The values are expressed in mean ± SEM. When high dose (*200* mg) of *VCM* was administered, the body weight measurements yield the following results. The average change in body weight of the rats on 0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} days are 136.40±2.96, 144.80±3.29, 155.20±3.10, 163.70±3.22, 170.00±3.25, 178±5.54, 188±9.66 respectively. These data clearly indicate the change in weight of both male and female rats are normal which support the in-toxicity of the prepared *VCM* up to the concentration of *200* mg/kg.

The relative organ weights of brain, lungs, heart, liver, spleen, kidney, adrenals, sex glands were measured after administration of *VCM* (**Table 28)** for control, low dose, mid dose and high dose all were found to be normal. In sub-chronic toxicity showed no abnormal changes in the internal organs of the test animals. No significant difference in hematological parameters such as HCT, HGB, RBC, MCH, MCV, MCHC, platelet count and WBC were observed between the control and test drug administered animals. No significant difference in biochemical analysis were observed between the control and Vishnu chakara mathirai administered animals. No significant differences in any of the organs weight was observed between the control and test drug administered animals. No gross and histopathological findings were observed in any of the experimental animals. NOAEL of *VCM* was found to be > 200 mg/kg when administered for a period of 90 days in rats. No signs of toxicity were observed in animals from different dose groups during the dosing period of 90 days. The values obtained was within normal limits Histopathological examination revealed normal architecture in comparison with control

and treated animals. In Sub-chronic toxicity showed no abnormal change internal organs of the test animals.

The feed consumption for control, Standard and Vishnu chakara mathirai treate groups were recorded for Male and Female on every month end for three months subsequently (I, II, and III), after administration of *VCM* . The observed data for Male and Female and their average (MF) is presented in **Table 29.** The cumulative feed intake (in Kg) for the control rats at the end of 1st, 2nd and 3rd month for male rats are 228.43±9.42, 253.43±2.47, 283.43±4.35 respectively and for female rats 218.54±8.84, 254.57±3.19, 289.14±1.94, respectively, All values are expressed in mean ± SEM. Calculation of average of these two gives the following values 220.50±7.92, 254.00±2.83, 256.29±3.15, respectively.

When low dose(100 mg) of *VCM* was administered to rats the cumulative feed intake (in kg) for male rats are 256.86±5.38, 265.43±6.88, 262.57±3.86, respectively and for female rats 192.29±4.82, 181.29±2.56, 183.43±3.53, respectively, the average of which yields 193.57±5.10, 193.36±4.72, 198.00±3.70, respectively. For mid dose (200 mg) of *VCM* the values of male rates are 168.00±5.37, 166.29±5.85, 169.43±4.32 respectively and for female rats 181.00±3.69, 184.71±3.00, 186.14±3.81 respectively the mean of which gives 175.00±4.53, 173.50±4.42, 172.79±4.02, respectively. When high dose (*200* mg) of *VCM* was administered the cumulative feed intake (in kg) recorded at the end of 1st month, 2nd month and 3rd month are 177.00±3.56, 176.86±4.07, 180.29±3.02 respectively and for female rats 137.86±3.71, 138.29±1.46, 170.14±3.99 respectively the average of which gives 180.43±3.63, 178.57±2.76, 175.71±3.51 respectively.

From the above data it is clear that the collective feed intake for the three successive months is more or less same with the control. This reveals that there is no

significant change in the feed intake after administration of prepared *VCM* up to the concentration of *200* mg/kg.

The biochemical analysis of sub-chronic toxicity study of *VCM* on rats is presented in **Table 30.** After administration dose of *Vishnu chakara mathirai* were recorded. These data reveals all the parameters recorded are in agreement with the standard values. This added a support for the applicability of *VCM* as a safe drug.

Table-31 shows the gross pathological report of *Vishnu chakara mathirai* in subchronic study. The various tissues of internal organs were analysed after administration of control, low dose, mid dose and high dose of *VCM*. The control groups of male are numbered from 101-105 and female from 106-110, similarly low dose group male from 201-205, female from 206-210 and mid dose male group from 301-305 female from 306-310 and high dose male group from 401-405 and female from 406-410. Mammary gland, lymph nodes, eyes, brain, trachea, thyroid gland, thymus, heart, lungs, stomach, intestines, spleen, liver, adrenal gland, both kidneys, urinary bladder, both testes, epididymis, male sex glands, ovaries, uterus vagina, peripheral nerve, skeletal muscle, bone and spinal cord of all the animals in control, low dose, mid dose and high dose of *VCM* shows no abnormality. This further proves the safety of prepared *Vishnu chakara mathirai*.

Anti-convulsant activity of *Vishnu chakara mathirai* was carried out by Maximal electroshock induced seizures, picrotoxin, N-Methyl-D-Aspartate, PTZ induced and PTZ kindling models. The advantage of using the models is the pharmacological profiles were comparable to the human condition. The models were related to GABAergic neurotransmission.

Maximal electroshock induced seizures

MES induced seizures the tonic hind limb flexion was found to be 2.64 sec at low dose level (200 mg/kg) and 1.86 sec at high dose level (*200* mg/kg) and the data tabulated in **Table 32**. It is comparable with that of standard (Phenytoin) 1.24 sec. The tonic hind limb extension was found to be 8.82 sec and 6.42 sec sec for low and high dose respectively. These values were nearer to the standard (Phenytoin). The clonus values are 11.66 sec and 10.66 sec for low and high dose respectively comparable with that of the standard (5.44 sec) and stupor values are 68.88 sec and 54.82 sec for low and high dose respectively comparable with that of standard (Fig 16-20) The percentage protection was calculated which shows that for low dose it is 37.15% and for high dose 43.41 % (**Table 33).** It afforded maximum protection to the mice with 200 mg (fig 20).

Continuous administration of Phenytoin at the dose of 25 mg/kg b.wt showed statistically significant reduction in the Tonic hind limb flexion and stupor when compared to the control ($p<0.001$).

VCM treated at the dose of 100 mg/kg b.wt. for a period of 15 days showed a statistically significant increase in the tonic hind limb extension time ($p < 0.001$). The animals treated with 200 mg/kg b.wt. showed statistically significant reduction in clonus and stupor time when compared to normal vehicle control animal ($p<0.001$). The animals treated with a dose of 200 mg/kg b.wt. showed a statistically increase ($p<0.001$) tonic hind limb flexion and extension. It also showed a statistically significant reduction in ($P<0.001$) clonus and stupor time.

Picrotoxin induced seizures

Picrotoxin induced seizures animal model was carried out with low and high dose of *VCM* with phenytoin as standard. Onset of tonus, Onset of clonus were measured in **Table 34** shows the onset of tonus values 243 and 356 sec for low (100 mg/ Kg) and

high dose (200 mg/Kg) respectively and onset of clonus values 143 and 126 sec for low and high dose respectively (control and standard 58 and 190 respectively) Percentage mortality of control was fixed as 100 and that of standard Phenytoin as zero. The percentage mortality of Phenytoin, *VCM* lower dose and *VCM* higher dose were calculated and it showed below the control level as 75% and 45 % respectively. This shows *VCM* releases the convulsions at the dose of 200 mg/kg effectively (fig 21-23).

The animals treated with 100 mg/kg b.wt of *VCM* delayed the onset of tonus and clonus in mice when compared with control animals. It also reduced the mortality percentage as dependent manner (15 % for 100 mg & 40 % for 200 mg) when compared with standard.

N-Methyl-D-Aspartate model

Showed the observations of N-Methyl-D-Aspartate model for the antiepileptic characteristics of *VCM*. Shown in **Table35** Phenytoin was used as standard. This test proved the turning behaviour of the animals by two consecutive 360° cycles fulfilled the permissible limit. The number of turning values were found to be 36 and 20 for low (100 mg/Kg) and high dose (200 mg/Kg) respectively. It is comparable with the standard. The percentage protection was calculated which shows that for low dose it is 40% and for high dose 68 % This shows *VCM* releases the convulsions at the dose of 200 mg/kg, effectively. (fig 24-25)

PTZ Kindling method

In PTZ Kindling method the different parameters like Latency of seizure, Tonic hind limb flexion, Tonic hind limb extension, Clonic seizures and Postictal depression were recorded in **Table 36**,**37**. For control, standard, *VCM* (100 mg) and *VCM* (200 mg) Latency of seizure are 330.6, 0, 416 and 0 respectively. Tonic hind limb flexion was

found to be 1.5, 0, 1.5 and 0 respectively. Tonic hind limb extension are 10.66, 0, 10.66 and 0 respectively. Clonic seziures are 5.5, 0, 3.5 and 0 respectively, Post ictal depression values are 340.16, 0, 189.16 and 0 respectively.

The animals pretreated with *VCM* showed a significant reduction in turning behavior of mice when compared to control group animals. The percentage protection of *VCM* 100 mg/kg b.wt. and *VCM* 200 mg/kg.b.wt was 68.

The animals administered with *VCM* at the dose of 100 mg/kg/b.wt significantly reduce the latency of seizures ($p<0.001$). It also reduced the clonic seizures($p<0.05$) and postictal depression ($p<0.001$) when compared to control group.

Table 38 explain the percentage of protection from clonics seizures in PTZ method of *Vishnu chakara mathirai* treated group. Control shows 0 %, standard 100 %, *VCM* (100 mg) gives 30 % and *VCM* (200 mg) gives 90 %. These data reveals the fact that *VCM* at higher dose (200 mg) is as good antiepileptic Siddha formulation.

Fact of results that out of five tested models, *VCM* (200 mg/kg) exhibited maximum antiepileptic activity in picrotoxin model. The *VCM* might raised the seizure threshold or act as an agonist of Gama Amino Butric Acid with improvement in GABA ergic neurotransmission by increasing GABA levels in brain.

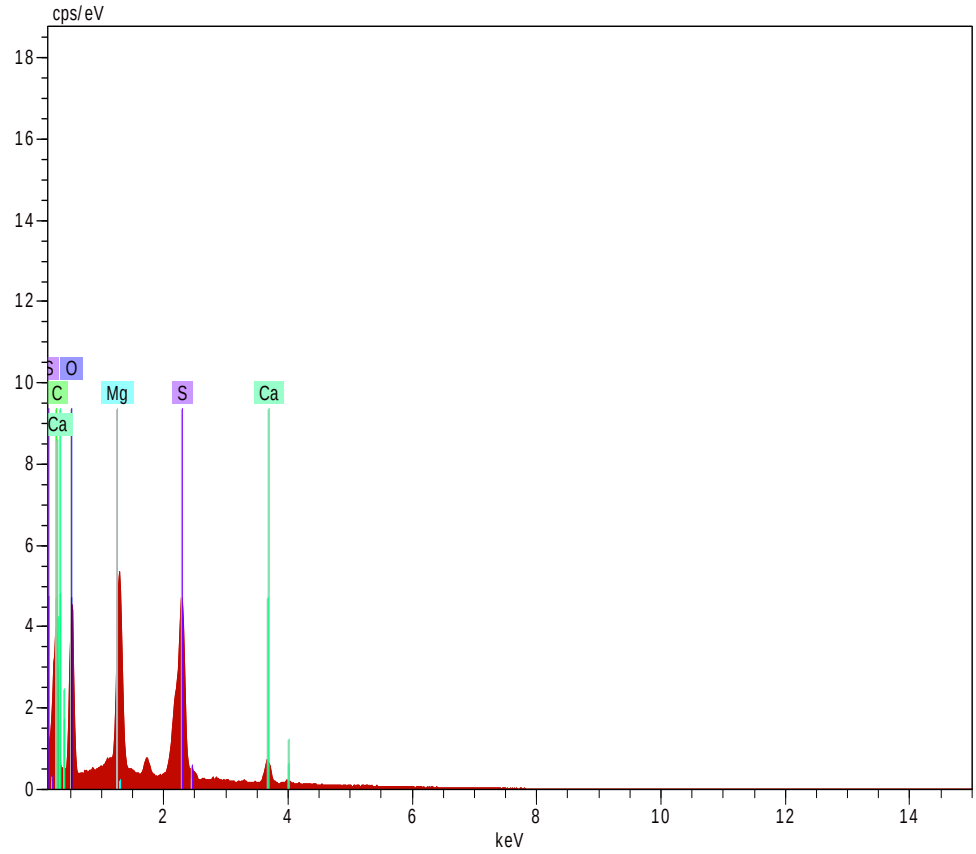
Maximal electroshock induced seizures in animal model was efficacy of Vishnu chakara mathirai is nearer to the standard. The clonus and stupor of the study animals also showed comparable values with the standard Picrotoxin induced seizures animal model revealed the fact that the percentage mortality of *VCM* was below the control level. In MES and picrotoxin models, maximum percentages of inhibition for the drug was found to be 73.11% and 86.62% respectively at the dose of 200 mg/kg, p.o. In NMDA model, maximum delay in latency of convulsions was observed at 200 mg/kg, p.o. N-Methyl-D-

Aspartate test proved the turning behaviour of the animals by two consecutive 360° cycles fulfilled the permissible limit.

The pre treatment of *VCM* at the dose of 100 mg/kg b.wt and 200 mg/kg b.wt statistically ($p<0.001$) reduce the onset of convulsions and reduce the duration of convulsions when compared with the normal control group.

Out of all the five tested models, *VCM* showed maximum antiepileptic activity in picrotoxin model. It may antagonize the action of picrotoxin which is a GABA antagonist. The GABA-agonist action of *VCM* might be responsible for enhanced GABAergic neurotransmission. Phyto-chemical analysis of *Vishnu Chakara Mathirai* revealed the presence of alkaloids and phenols which may be a supporting factors in the treatment of convulsions. Hence the presence of antioxidant principles in *VCM* might partially contribute the antiepileptic activity.

Figure: 11 EDAX SPECTRUM OF *VCM*



Spectrum: Acquisition 4920

El AN Series unn. C norm. C Atom. C Error (1 Sigma) K fact. Z corr. A corr. F corr.

[wt.%] [wt.%] [at.%] [wt.%]

C 6 K-series 24.98 42.25 53.74 3.26 0.495 0.853 1.000 1.000

O 8 K-series 21.88 37.01 35.34 2.81 0.253 1.464 1.000 1.000

S 16 K-series 5.80 9.82 4.68 0.23 0.023 4.276 1.000 1.008

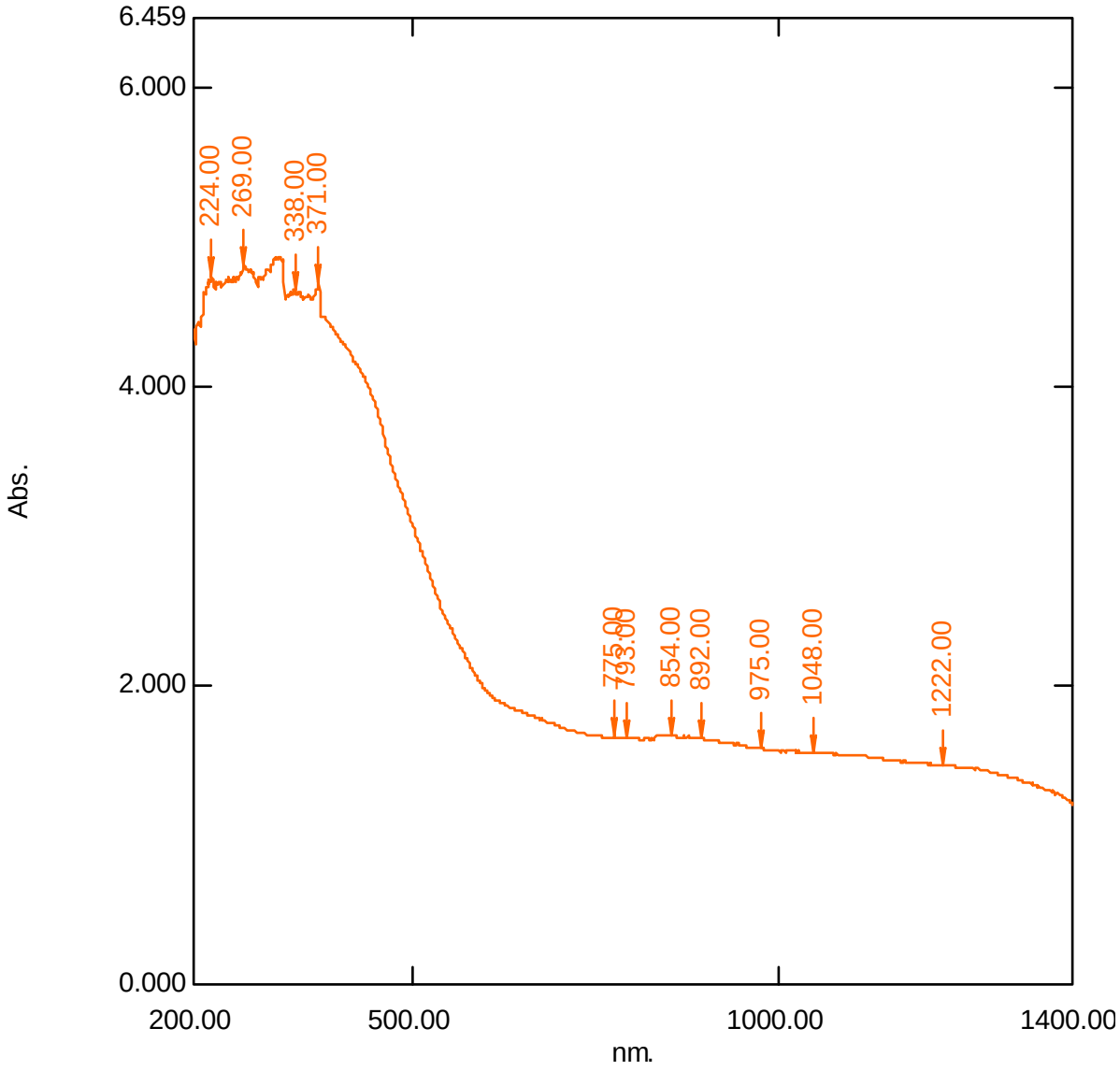
Mg 12 K-series 4.96 8.39 5.27 0.29 0.028 3.003 1.000 1.003

Ca 20 K-series 1.50 2.54 0.97 0.08 0.006 4.408 1.000 1.020

Total: 59.11 100.00 100.00

Figure: 12 ULTRA VIOLET SPECTRUM OF *VCM*

VC-Absorbance



VC-reflectance

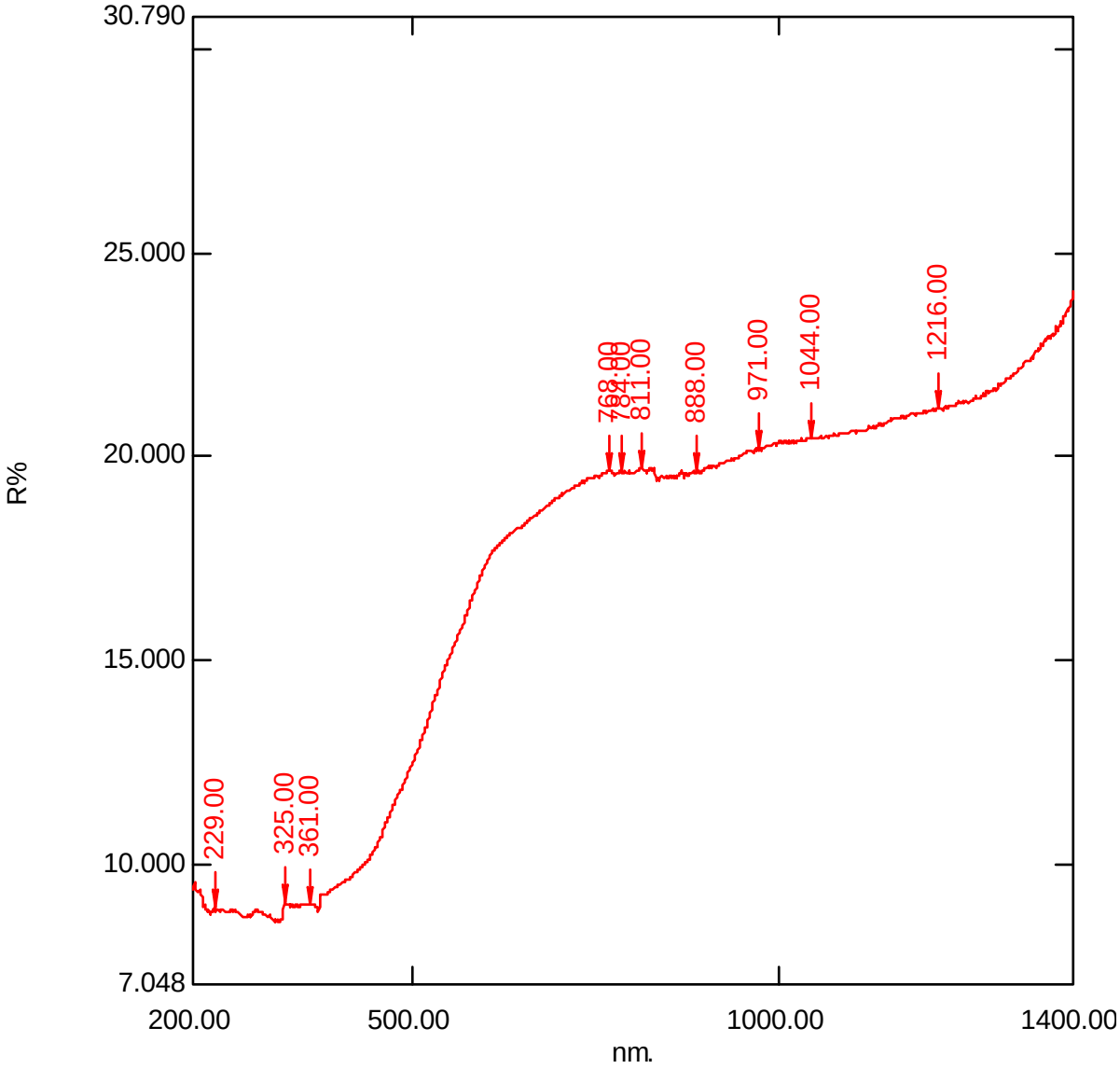


Figure: 13

INFRA RED SPECTRUM OF *VCM*

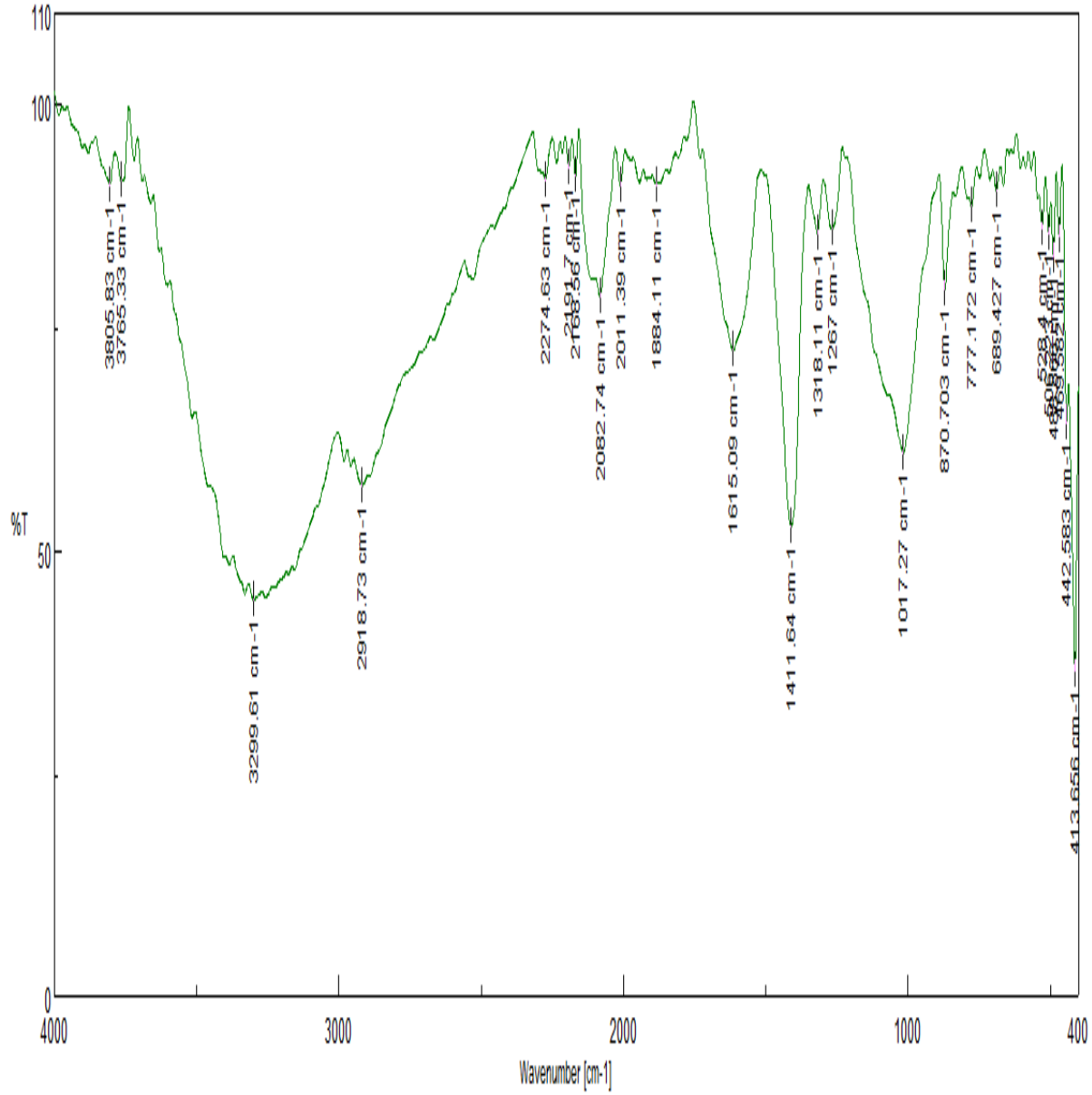


Figure: 14

SCANNING ELECTRON MICROSCOPE PICTURE OF *VCM*
VIEW FIELD: 42.4 μm,MAGNIFICATION : 3

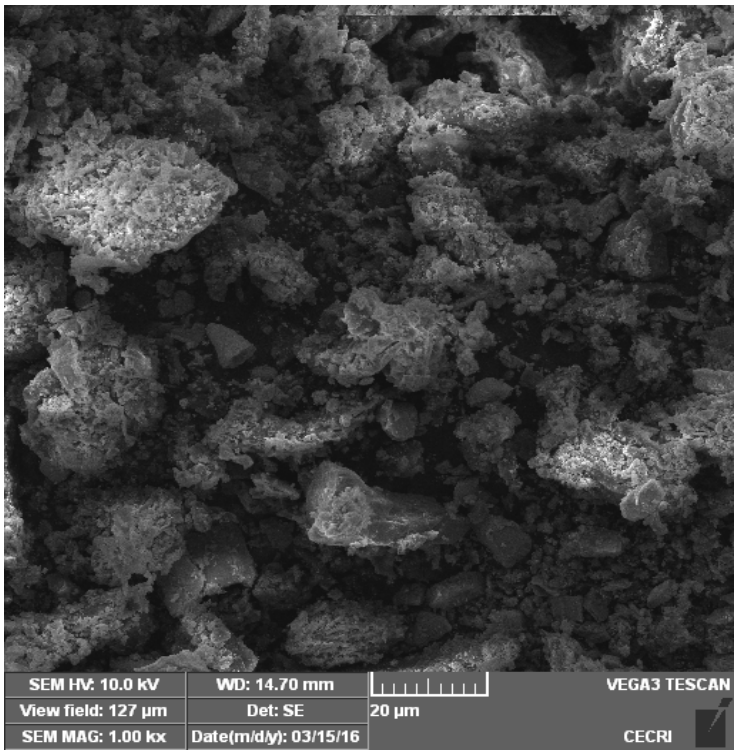


Figure: 15

SCANNING ELECTRON MICROSCOPE PICTURE OF *VCM*
VIEW FIELD: 25.5 μm, MAGNIFICATION : 5

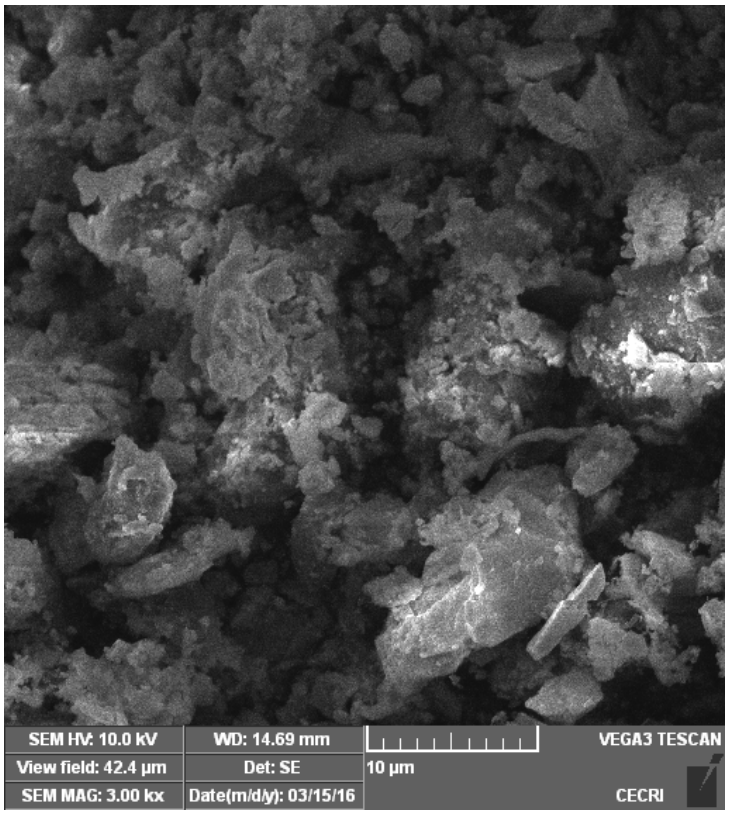


FIGURE16

EFFECT OF *VCM* ON MES INDUCED CONVULSION

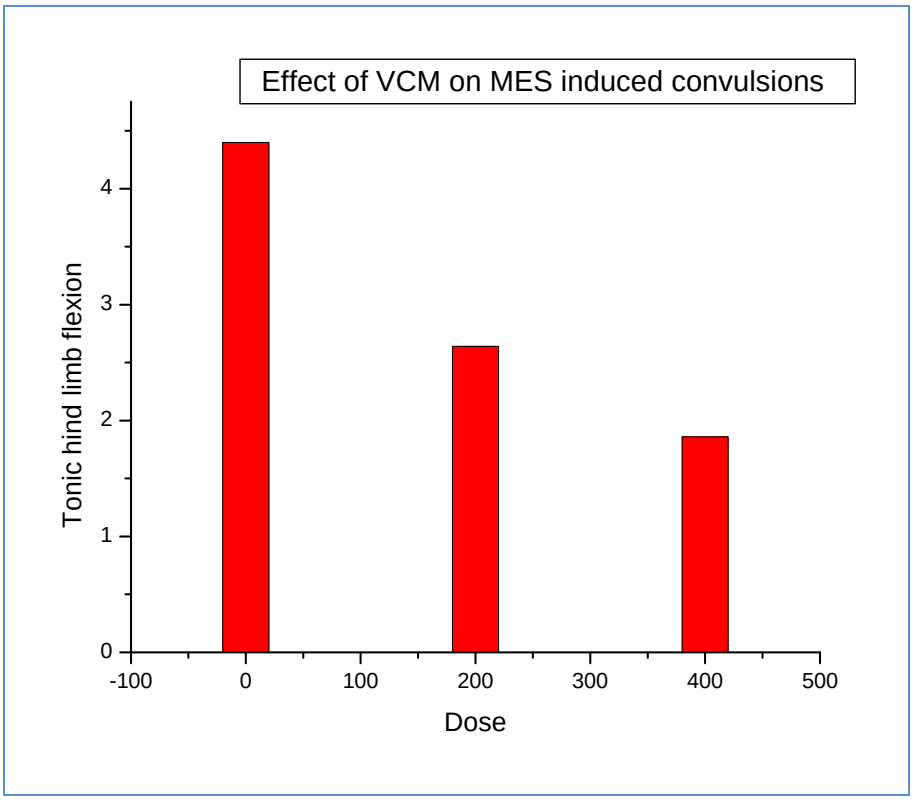


Figure17

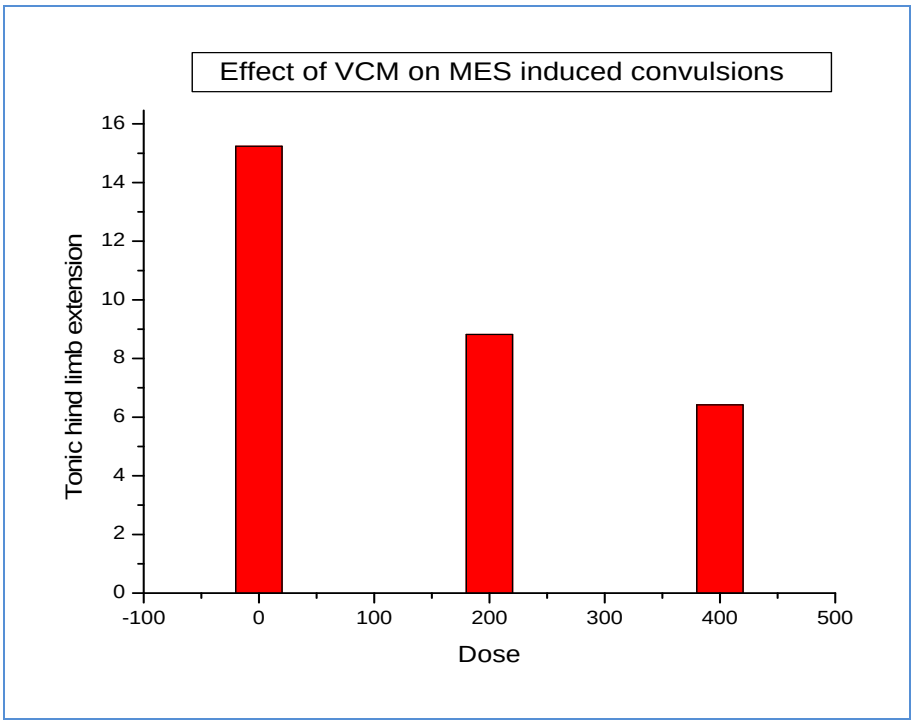


Figure 18

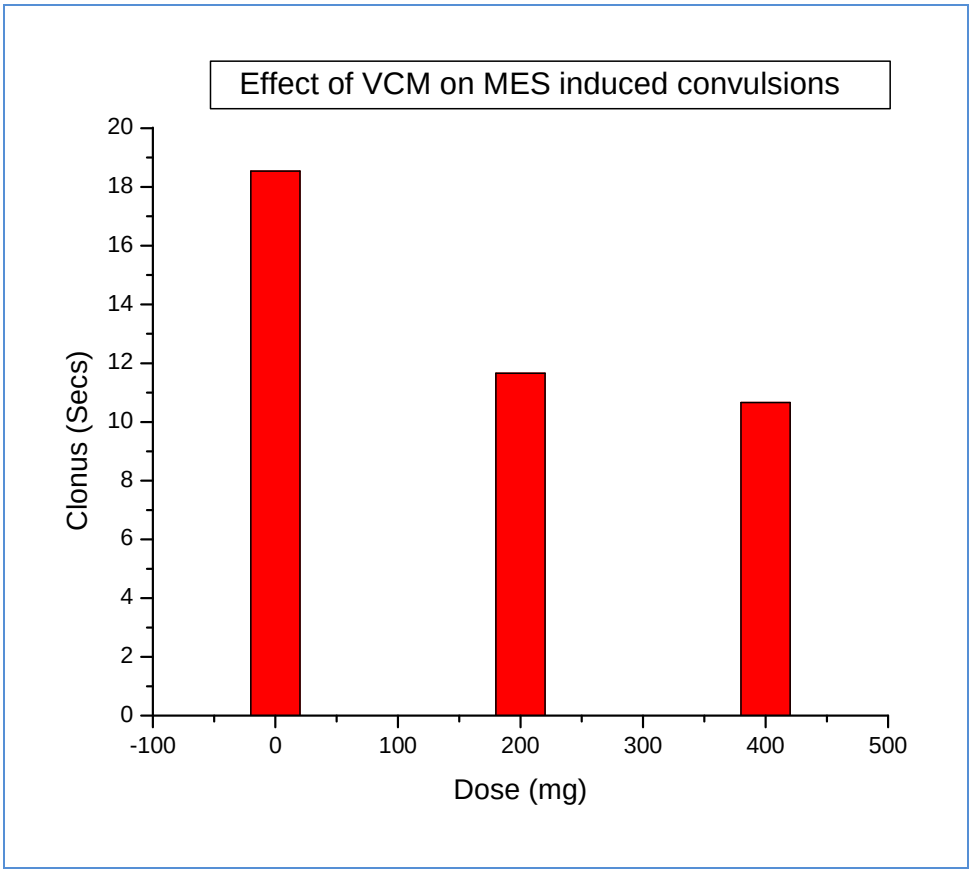


Figure 19

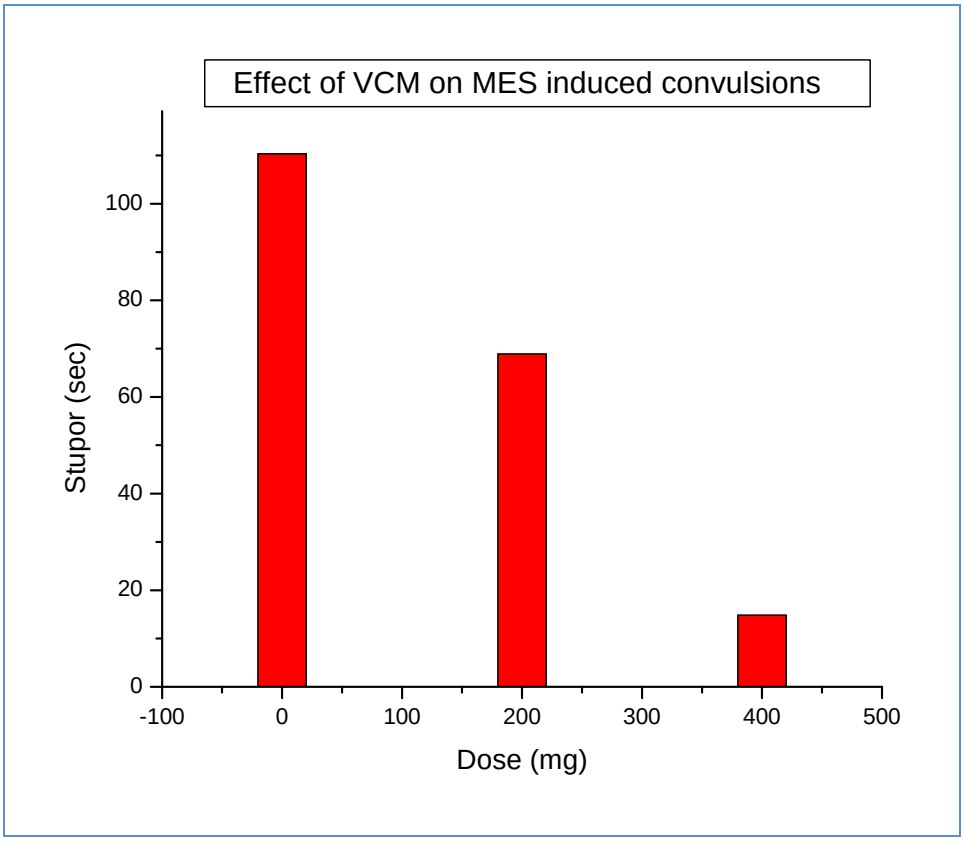
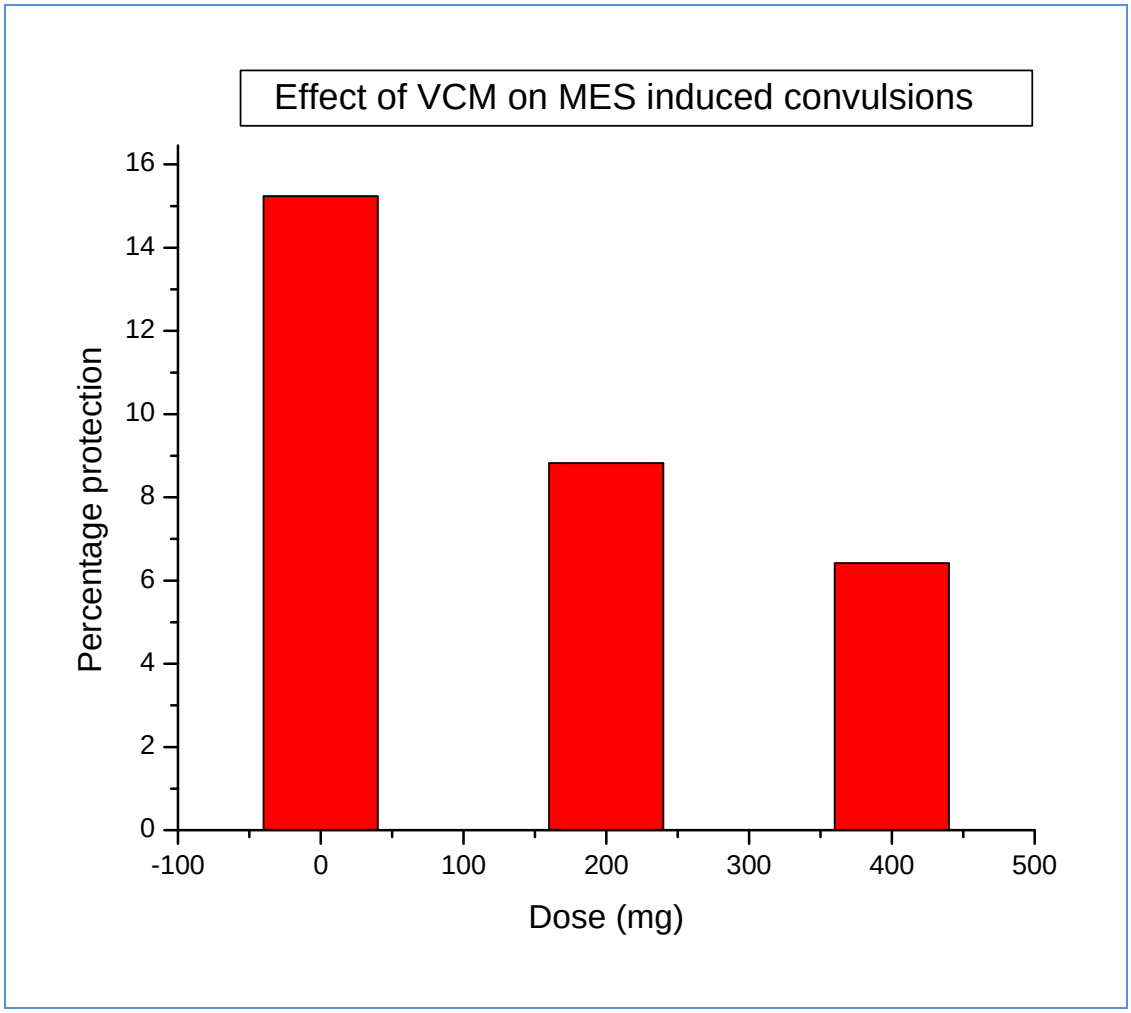
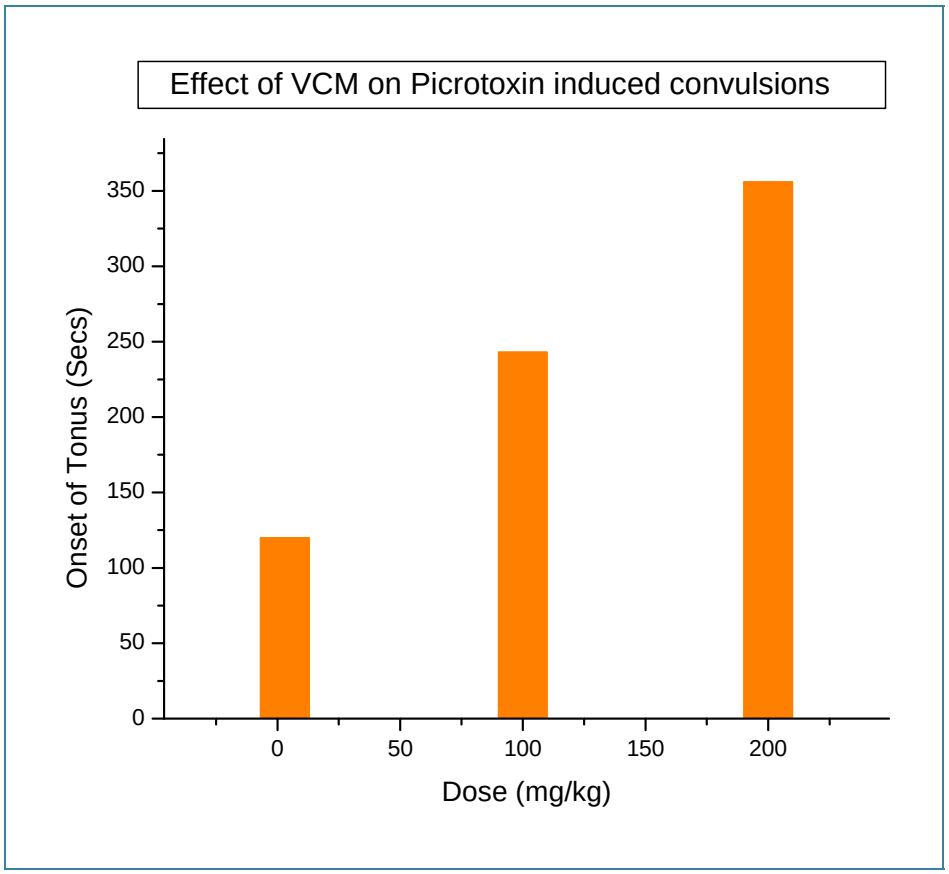


Figure 20


Effect of VCM on MES induced convulsions
Percentage protection
16
14
12
10
8
6
4
2
0
-100
0
100
200
300
400
500
Dose (mg)

EFFECT OF *VCM* ON PICROTOXIN INDUCED MODEL:(Fig 21,22)



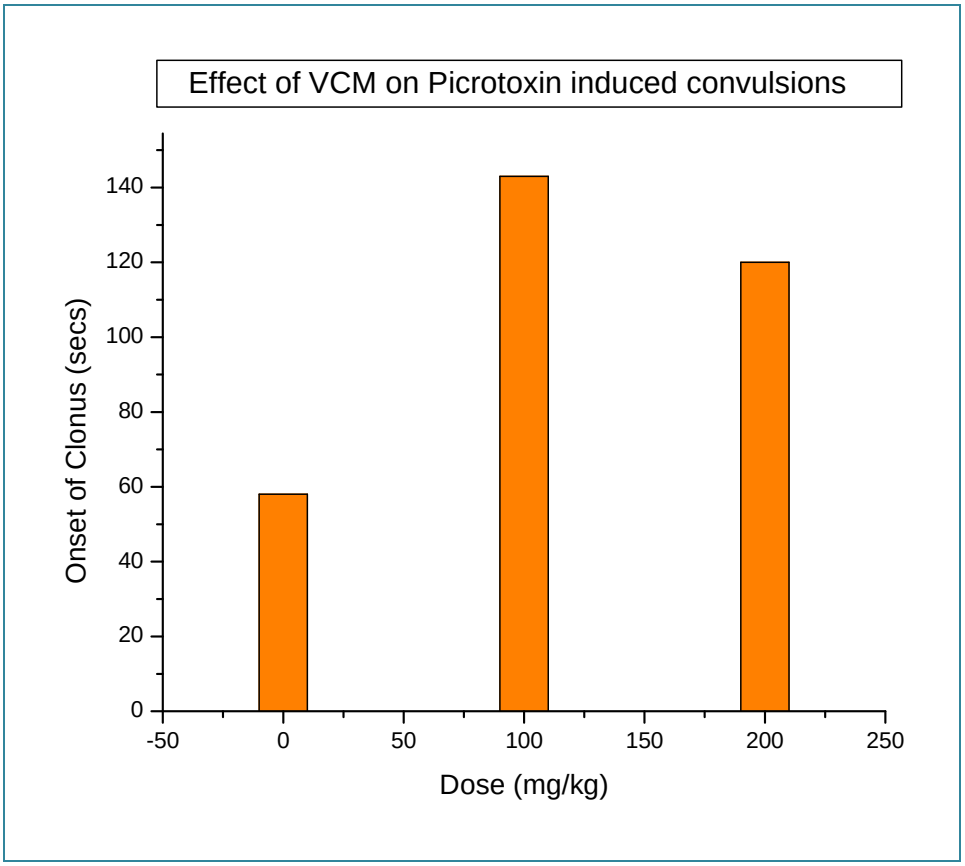


Figure 23

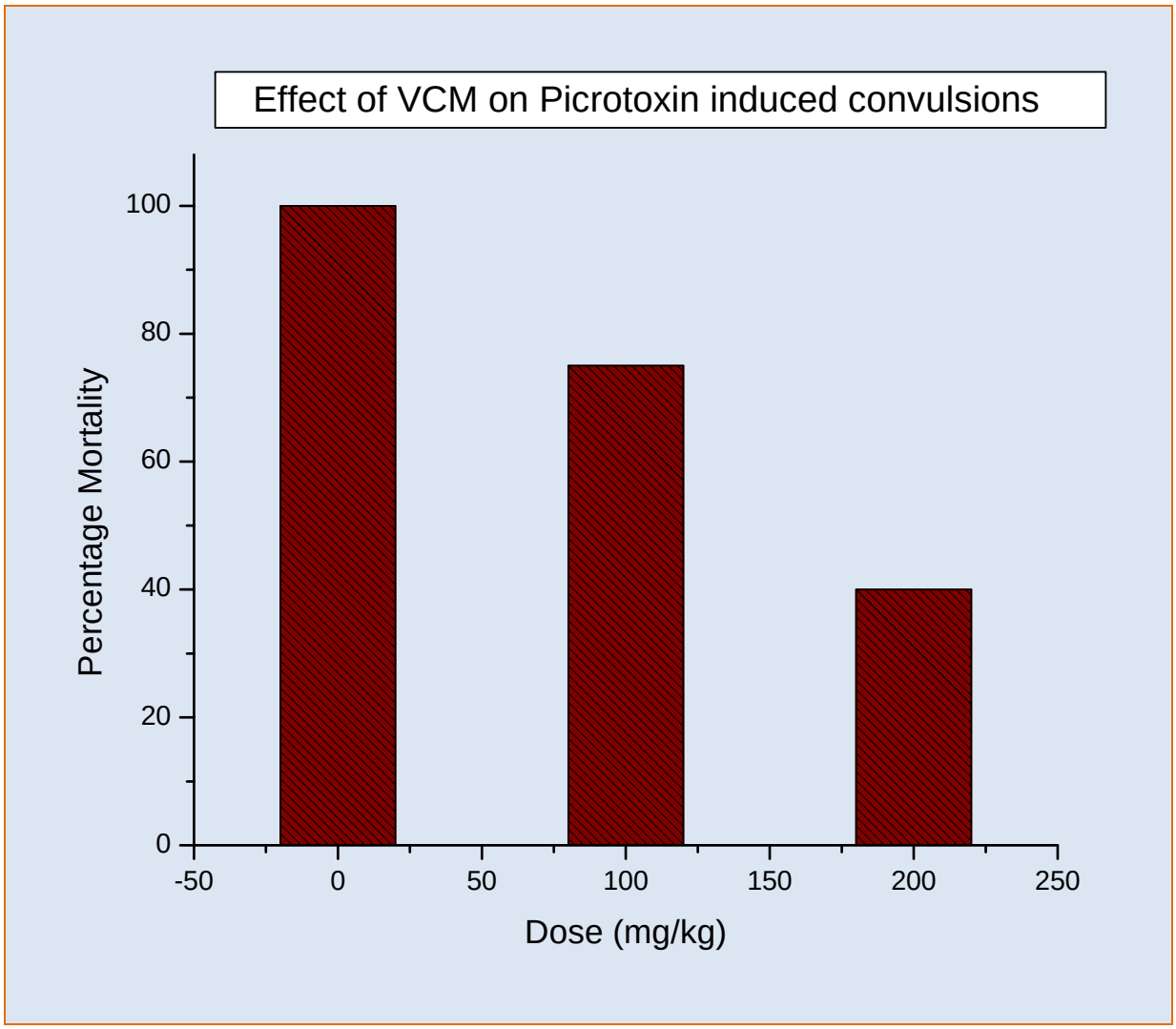

Effect of VCM on Picrotoxin induced convulsions
Percentage Mortality
100
80
60
40
20
0
-50
0
50
100
150
200
250
Dose (mg/kg)

Figure 24 EFFECT OF *VCM* ON NMDA MODEL

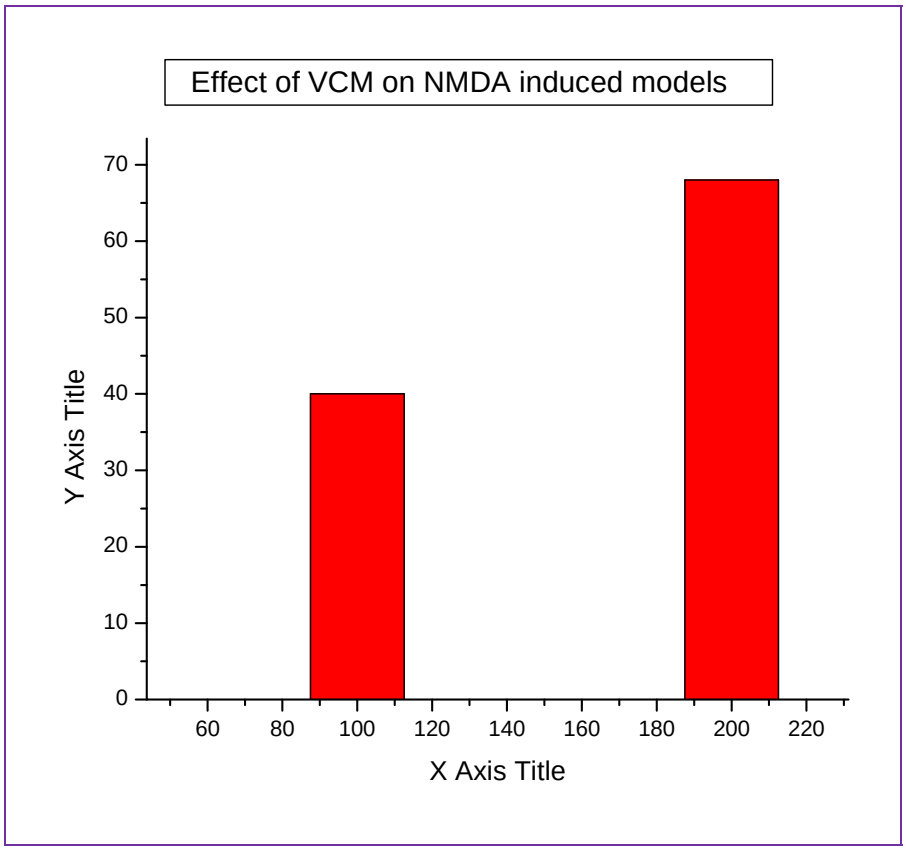


Figure 25 EFFECT OF VCM on NMDA INDUCED CONVULSION

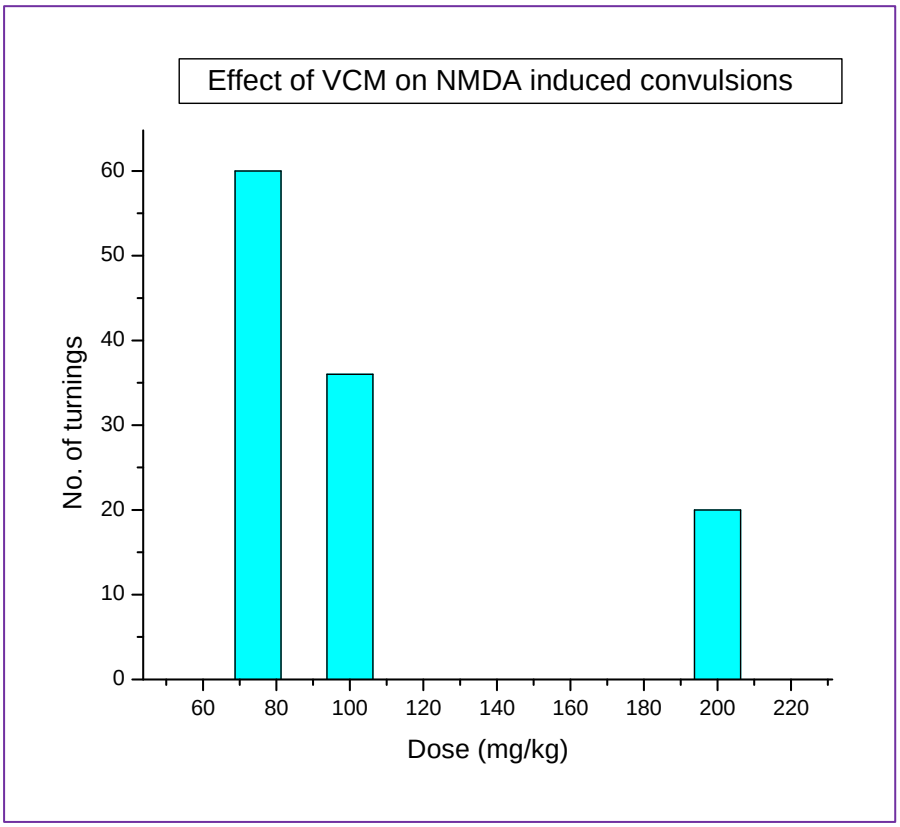
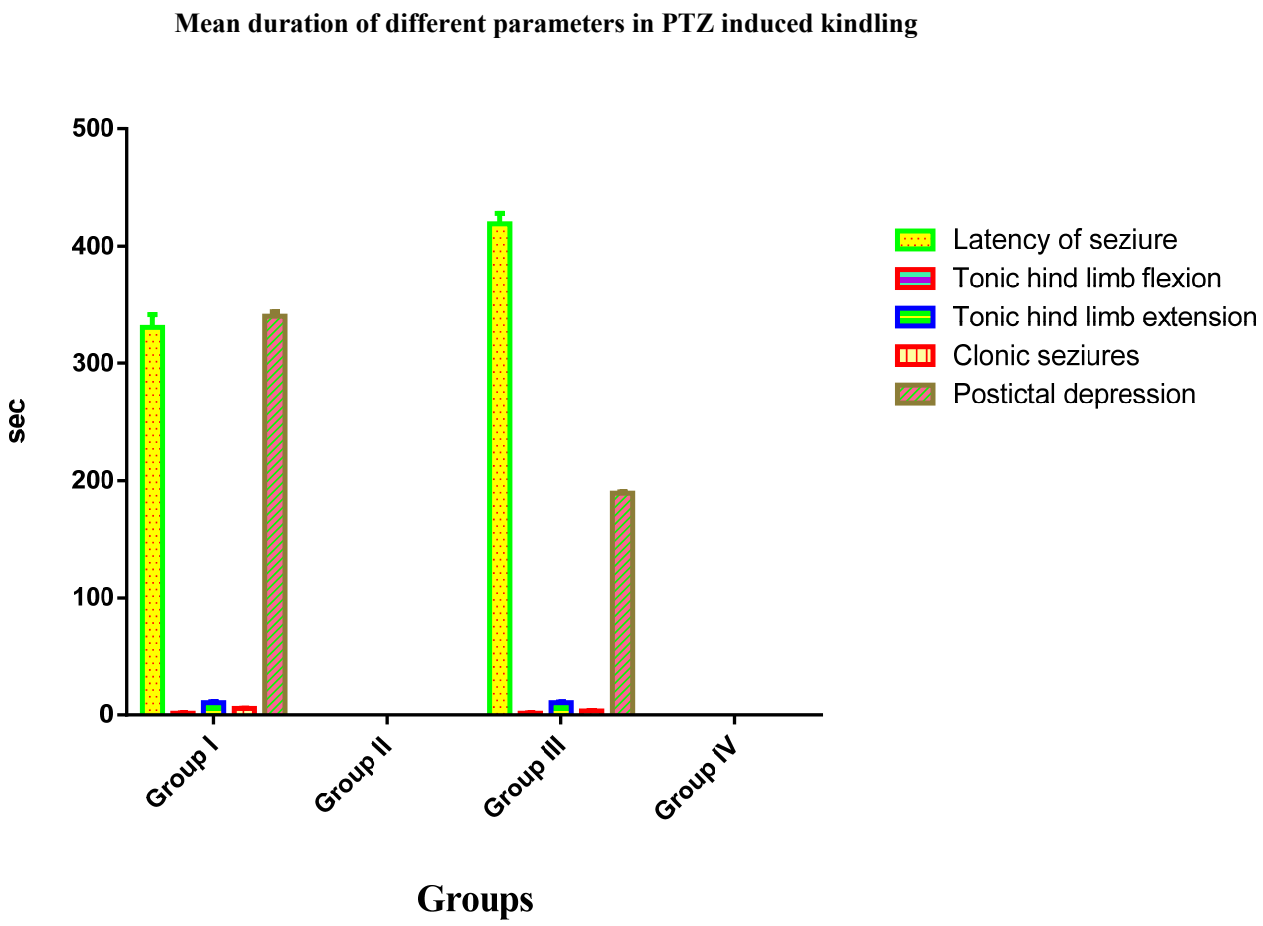


Figure 26

EFFECT OF *VCM* ON PTZ MODEL



EFFECT OF *VCM* ON PTZ KINDLING MODEL

Figure 27

Percentage protection from clonic seziures in PTZ induced kindling

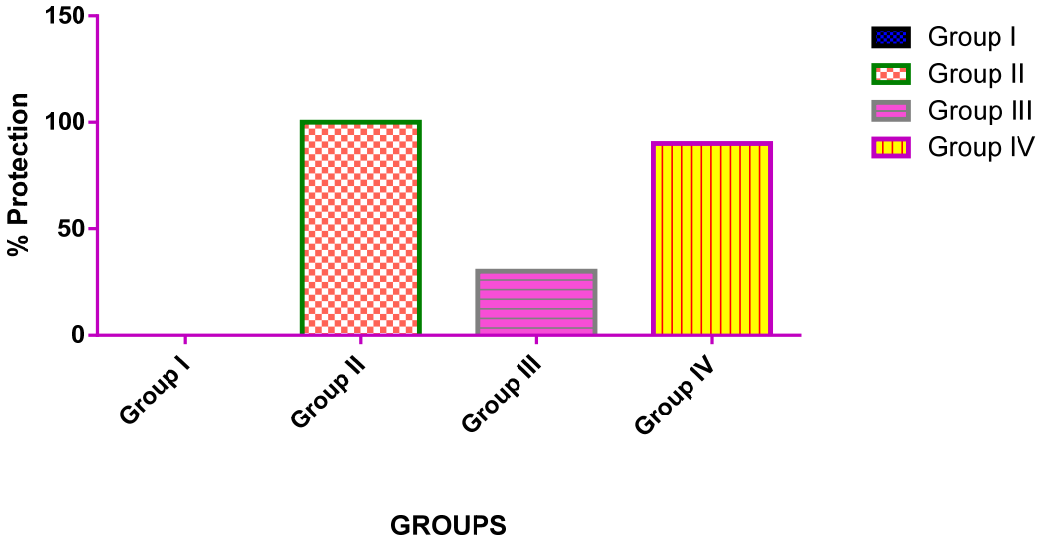


Figure 28

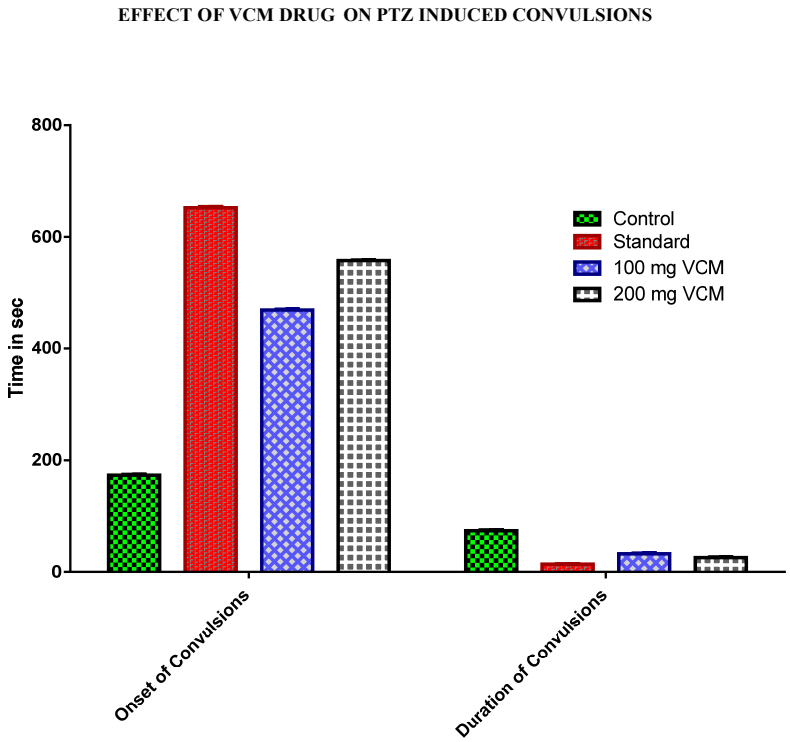


Figure: 29

TLC FINGERPRINTING PROFILE OF Tablet (Based on Alkaloids)

PHOTO DOCUMENTATION UNDER UV

AT 254nm **AT 366nm**

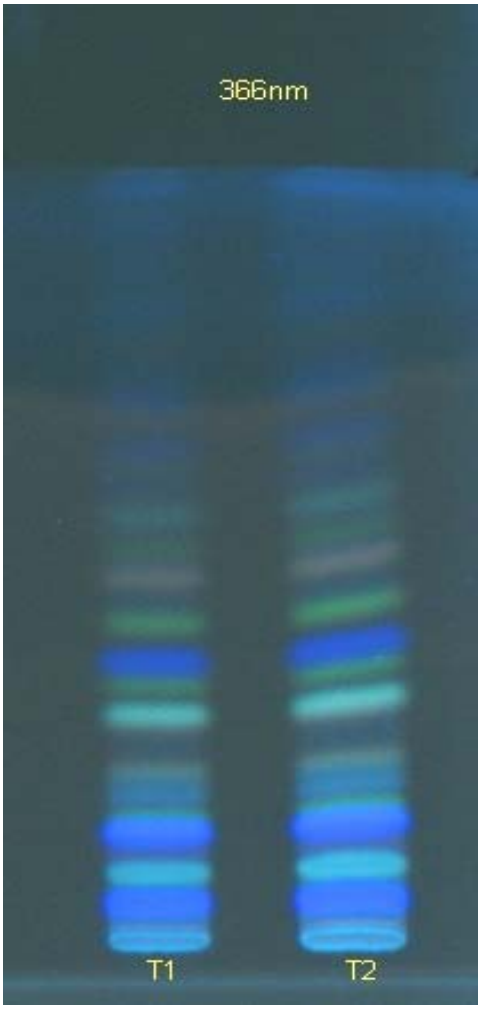


Figure 30

HPTLC 3D DISPLAY @ 254 NM Based on Alkaloid

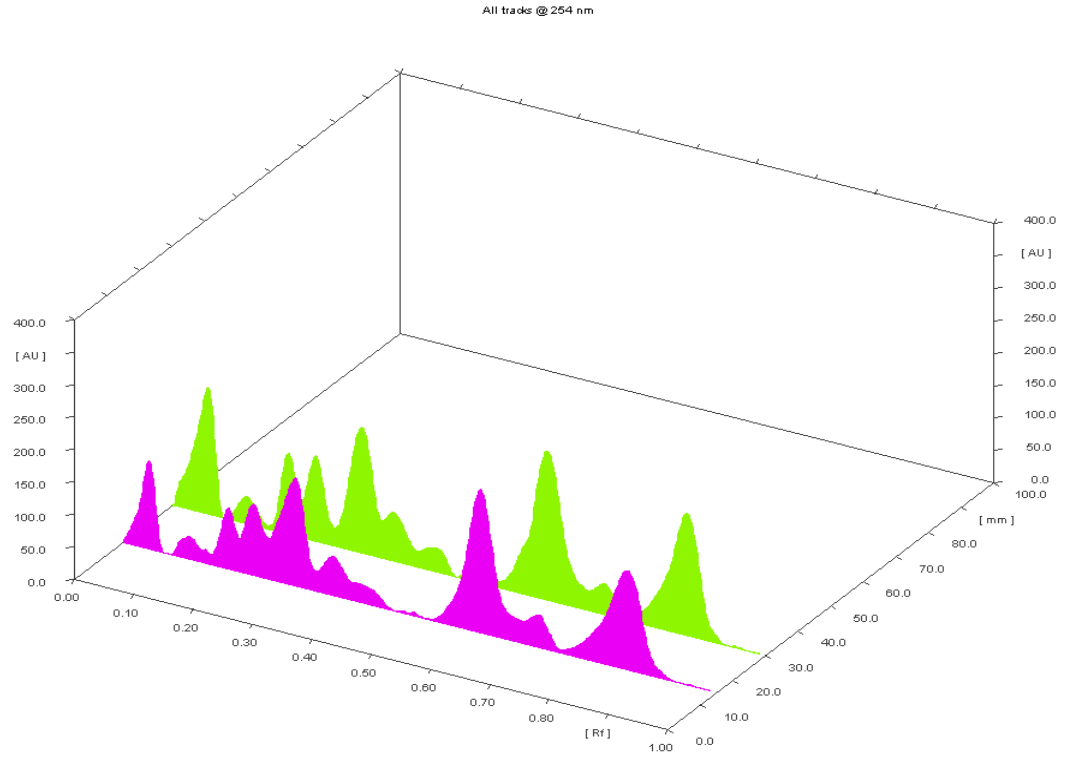


Fig 31PEAK DISPLAY (20µl of Sample)

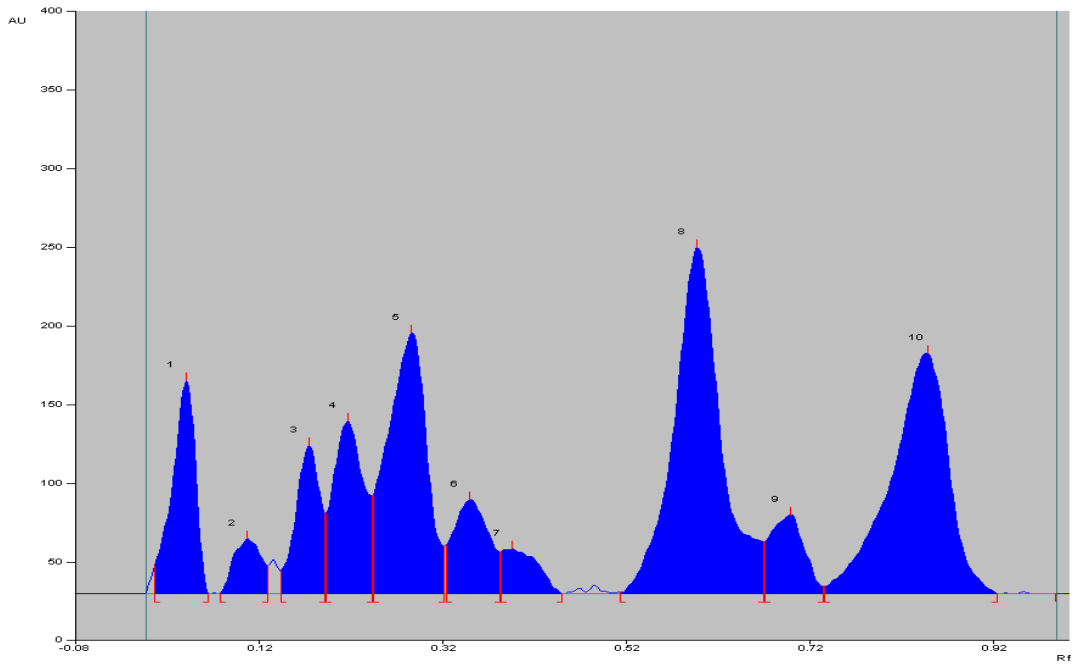


Table :39 (Peak and Area related to Rf value)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.01	17.7	0.04	135.4	12.89	0.07	0.2	2557.3	7.85
2	0.08	0.1	0.11	34.9	3.32	0.13	18.0	790.2	2.42
3	0.15	14.6	0.18	94.0	8.94	0.20	50.7	1867.5	5.73
4	0.20	51.0	0.22	109.6	10.43	0.25	62.3	2726.4	8.37
5	0.25	62.9	0.29	165.6	15.76	0.33	30.2	5256.8	16.13
6	0.33	30.7	0.35	59.9	5.70	0.39	26.6	1736.8	5.33
7	0.39	26.8	0.40	28.3	2.69	0.45	0.2	820.0	2.52
8	0.52	0.8	0.60	220.1	20.95	0.67	33.1	8365.5	25.67
9	0.67	33.6	0.70	50.1	4.77	0.74	4.8	1369.9	4.20
10	0.74	4.9	0.85	152.8	14.54	0.93	0.2	7100.0	21.79

FIG 32 PEAK DISPLAY (30µl of VCM)

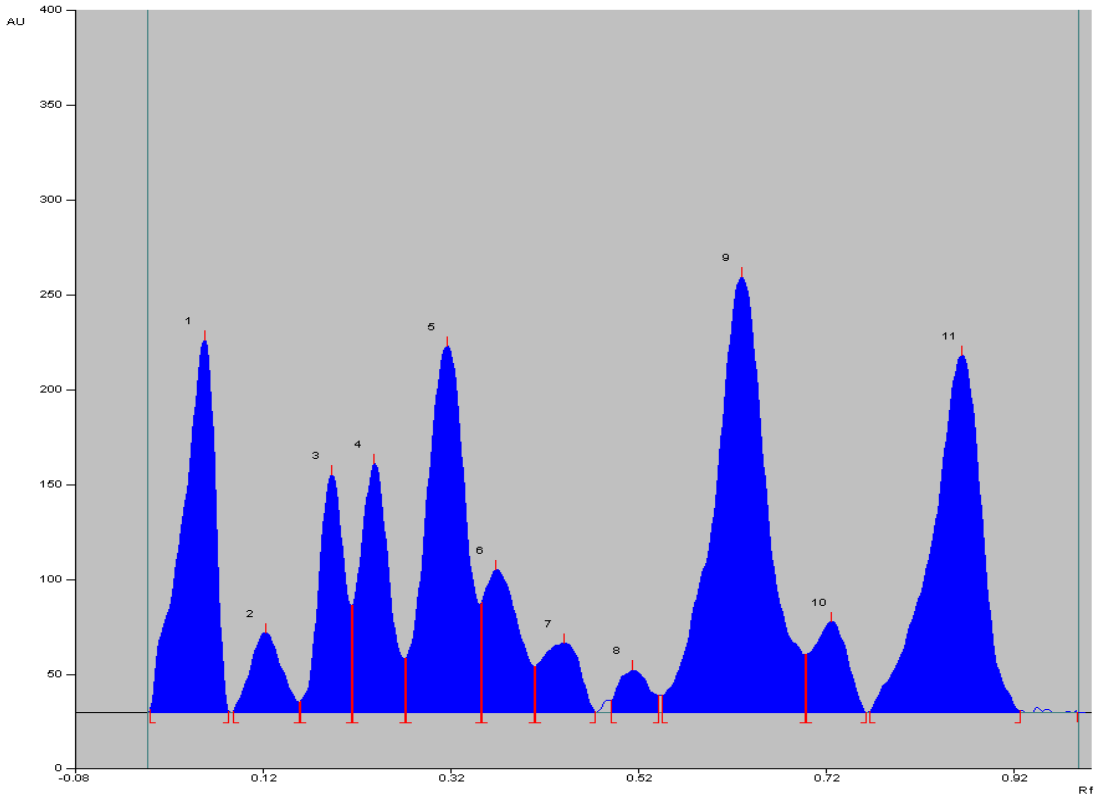
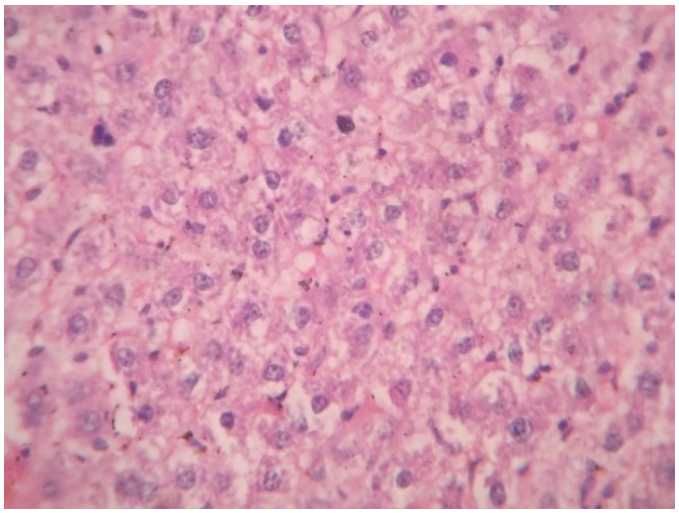


Table :40 (Peak and Area related to Rf value)

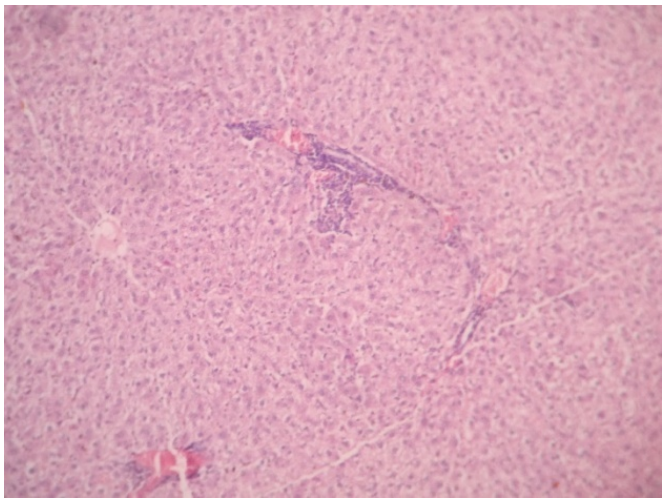
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.00	1.7	0.06	196.7	15.26	0.09	0.5	5072.8	12.82
2	0.09	0.6	0.13	42.2	3.27	0.16	5.6	1034.2	2.61
3	0.16	5.9	0.20	125.2	9.71	0.22	56.4	2484.2	6.28
4	0.22	56.5	0.24	131.5	10.20	0.27	28.3	3016.5	7.62
5	0.28	28.7	0.32	193.3	14.99	0.35	57.0	5928.1	14.98
6	0.36	58.3	0.37	75.5	5.86	0.41	24.2	2053.6	5.19
7	0.41	24.4	0.44	36.7	2.85	0.48	0.3	1104.3	2.79
8	0.49	6.4	0.52	22.4	1.73	0.54	9.3	537.4	1.36
9	0.55	9.1	0.63	229.8	17.82	0.70	30.6	9599.5	24.26
10	0.70	30.7	0.73	47.8	3.71	0.77	0.0	1283.5	3.24
11	0.77	0.3	0.87	188.3	14.60	0.93	1.1	7455.6	18.84

HISTO PATHOLOGY OF LIVER

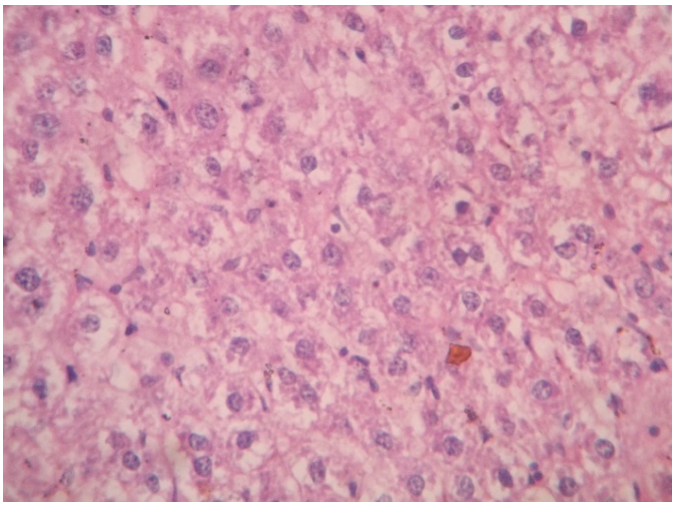
Figure: 33 low dose



Mid dose

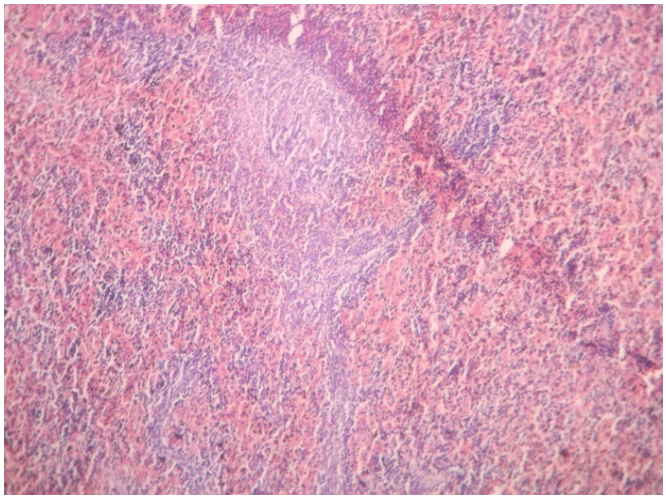


High dose

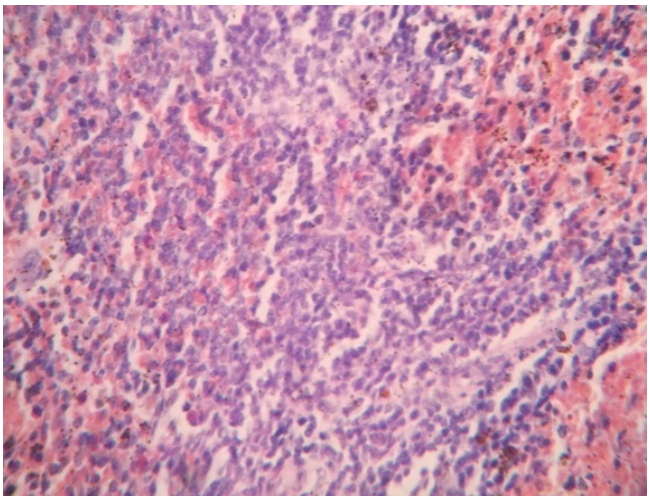


HISTO PATHOLOGY OF SPLEEN

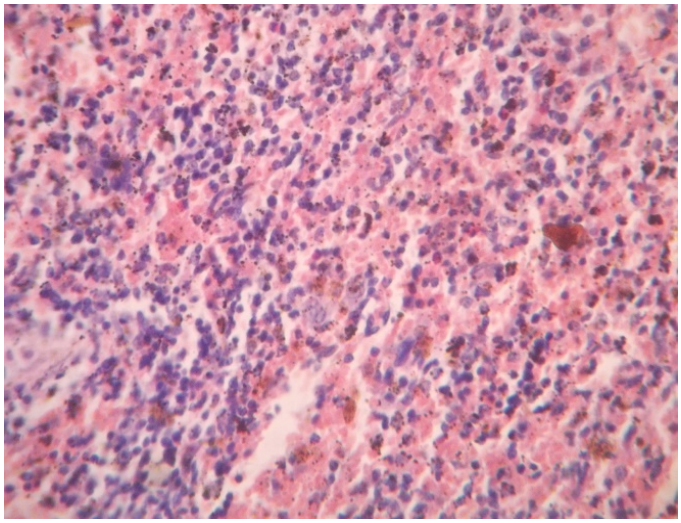
Figure: 34 low dose (50 mg)



Mid dose (100 mg)

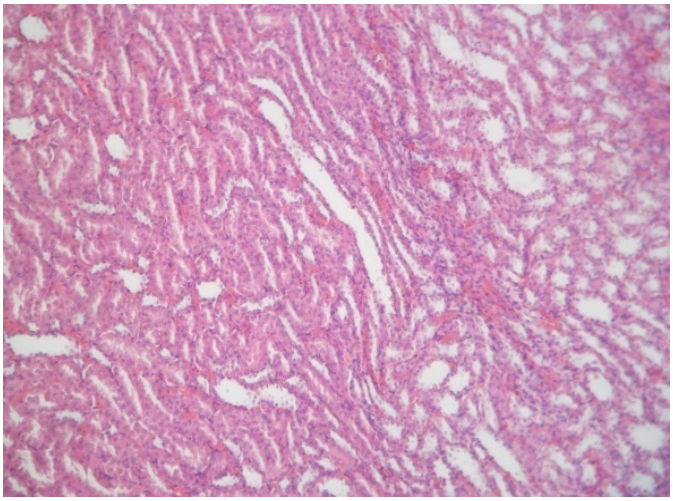


High dose(200 mg/kg.bw)

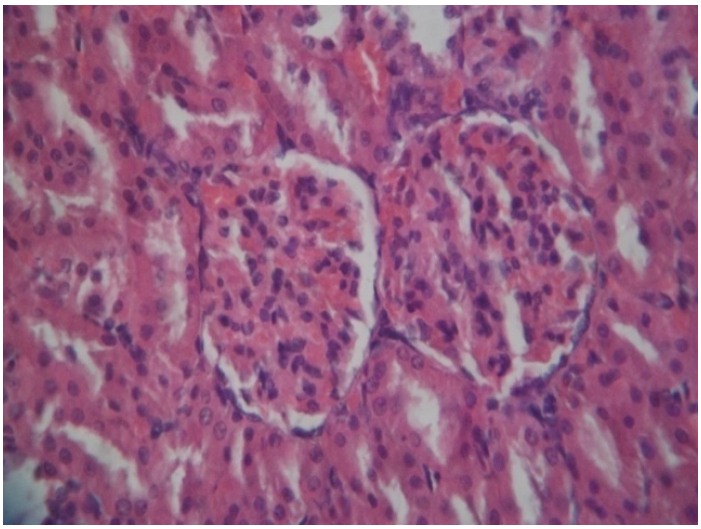


HISTO PATHOLOGY OF KIDNEY

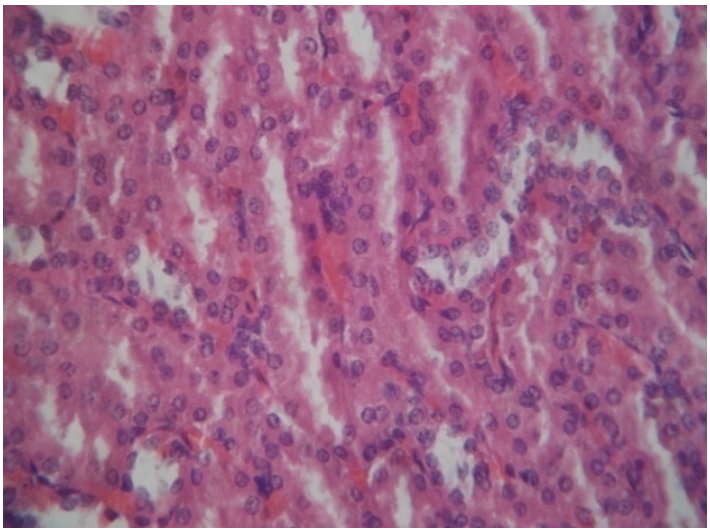
Figure: 35 Kidney low dose (50 mg/kg.b.wt.



Mid dose(100 mg/kg.b.wt.)



High dose (200 mg/kg.b.wt.)



7. DISCUSSION

Epilepsy is one of the most prevalent disorders of nervous system. Despite various drugs available, there are still some patients with resistance to drugs are inquisitive of indigenous medicines. Anticonvulsive medicines are effective in controlling seizures in convulsive disorders is about 70-80% of patients, but their use is often limited by their side effects. The medicinal plant products with confirmed activities in the nervous system are used mainly because of their active constituents in treating diseases. Side effects have been reported in many cases treated with present available antiepileptic drugs. The research for perfect antiepileptic medicine with more discriminating activity and lower toxicity is still a challenge in the medical field. Hence for long, herbs and traditional systems like Siddha are also being looked in for potent and safe anticonvulsives.

Siddha System occupies an important place in providing healthcare with its boundless therapeutics and wonderful pharmaceutical preparation of medicine.The Siddha treatment deals not only as a curative but also as a preventive. It ensures various therapeutically efficacious and promising drugs, which revamps various degenerative disorders and sustains vitality.

Vishnu Chakara Mathirai (*VCM*), a herbo-mineral formulation, is being for many years in Siddha System of Medicine. The formulation mentioned in the Siddha Text '*Siddha Vaithyiya Thirattu'*; has been indicated for various diseases like *Pakka Vatham* (Paralysis), *Sobai* (Dropsy), *Valippu* (Convulsive disorders) and *Yeppam* (belching) No studies were established in anticonvulsant property of *VCM*.

The raw drugs and other ingredients were purified as per the methods prescribed in Siddha literatures. The purified *Rasam, Lingam, Kandhagam, Nabhi, Palagarai, Thuththam, Thalagam, Kantham* and *Manosilai* were powdered and ground with *Vepam*

pazha juice to a rolling consistency. After grinding to a soft consistency, it is made as maathirai of 130 mg (*One Kuntri*) and allowed to dry. The trial drug was subjected to phyto chemical and physico-chemical analysis, morphological analysis, spectral studies, toxicity studies and anticonvulsant studies by MES, picrotoxin and NMDA models.

In preliminary phytochemical studies, it is found out that constituents present in *VCM*. In physico-chemical analysis the aqueous solution of *VCM* shows a pH of 6.5 which is suitable for oral administration. The low percentage (7.31 %) of loss on drying at 105^{o} C reveals the fact that the moisture component present in the sample is very less which is required for preservation of the pills for long duration. Total ash content was found to be only 7.7 %, Acid insoluble ash 21.25 % and 35.32 % of Water soluble *Vishnu Chakara Mathirai* which added an extra support for cost-effective use of the drug. The EDAX and XRF procedures applied on the sample gives the value of trace elements present in it. The percentage of heavy metals like mercury, cadmium and arsenic were in *VCM* were within the WHO permissible limit. This proves *VCM* can be given as safe drug for consumption.

UV spectrum shows peak at 224 nm, 269 nm, 338 nm, 371 nm UV spectrum for absorbance and peak at 1216 nm, 1044 nm, 971 nm, 888 nm for reflection reveals the fact that it absorbs light only in the visible region of the electromagnetic radiation. The IR spectra shows peaks at frequencies. 3299 cm^{-1} 2918 cm^{-1}, 2082 cm^{-1}, 1615 cm^{-1}, 1411 cm^{-1}, 1017 cm^{-1}, 870 cm^{-1} indicates the carbonyl hydroxyl stretching and bending vibrations of the molecules present in the sample.

SEM analysis of the particle shows that the sizes of the particles are 5 nm to 20 nm which helps for better absorption in intestine.

VCM was evaluated for HPTLC analysis and it revealed 11 peaks related to alkaloid components. The peaks are seen at 0.04, 0.11, 0.18, 0.22, 0.29, 0.35, 0.40, 0.60, 0.70 and 0.85. the major peak was seen at 0.85. Table showed the peak values and Rf values of 20µ sample of VCM. The area percentage are 7.85, 2.42, 5.73, 8.37, 16.13, 5.33, 2.52, 25.67, 4.20 and 21.79 under 254nm of UV. In 366 nm of UV it showed 11 peaks in the Rf value of 0.06, 0.13, 0.20, 0.24, 0.32, 0.37, 0.44, 0.52, 0.63, 0.73 and 0.87. The maximum peak was noted in 0.87. The area percentage are 12.82, 2.61, 6.28, 7.62, 14.98, 5.19, 2.79, 1.36, 24.26, 3.24 ad 18.84.

The total viable aerobic count analysis revealed the absence of Aflotoxin B_1, B_2, G_1, G_2, *P. aeruginosa, E. coli, S. aureus and Salmonella*. Total bacterial count was found to be 29500 CFU/g, Total fungal count was found to be 70 CFU/g, which are within permissible limits

In Acute toxicity study, no mortality was observed. Food consumption of all treated animals was found normal. The acute toxicity study also revealed that the drug was found to be safe upto 2000 mg/kg/b.wt. Body weight change in drug treated animals was found normal.

Subacute toxicity study, the haematological and biochemical parameters of the animals treated with *VCM* did not show any difference when compared to the control group. The body weight, feed and water consumption was normal throughout the study period. The histopathological studies are also revealed nil pathology.

In sub chronic toxicity study period ,no mortality was observed throughout the study period. Feed and water intake were also normal for all the groups. The biochemical, haematological and histopathological reports showed statistical variation. Hence 50, 100

and 200 mg/kg, p.o. doses were selected to evaluate the antiepileptic activity of *Vishnu Chakara Mathirai*.

Efficacy studies:

In MES method, the *VCM* significantly reduce the tonic hind limb extension ($p<0.011$, $p<0.001$) and also reduces the clonus and stupor timing. The *VCM* showed a dose dependent protection against MES induced convulsions in rats (37.14 % & 41.41%).

In Picrotoxin induced seizures, the animals treated with100 mg and 200 mg/kg b.wt of *VCM* delayed the onset of tonus and clonus in mice when compared with control. It also reduced the mortality percentage as dose dependent manner (75% and 40&) when compared to standard.

N-Methyl-D-Asparate Test: The animals pretreated with *VCM* in 100 and 200 mg/ kg/b.wt significant reduced the turning behaviour of mice when percentage protection of above doses were 40 and 68 respectively.

PTZ induced kindling in rats: The animals administered with *VCM* at the dose of 100 mg/kg/b.wt significantly reduce the latency of seizures ($p<0.001$). It also reduced the clonic seizures($p<0.05$) and postictal depression ($p<0.001$) when compared to control group.

PTZ induced epilepsy: The pretreatment of *VCM* at the dose of 100 mg and 200 mg /kg/b.wt significantly reduced ($p<0.001$) the onset of convulsions when compared with the control group.

8. SUMMARY

- The *Vishnu Chakara Mathirai* possess the specified charcters as mentioned by the Pharmacopoeial Laboratory for Indian Medicine, Ministry of AYUSH.
- The phytochemical analysis revealed the presence of alkaloids and phenols which may helpful for the therapeutic efficacy.
- The ICP OES analysis revealed that the toxic elements like mercury, arsenic, cadmium, lead and sulphur were found to be WHO permissible limits.
- The spectral studies revealed the fingerprint of *VCM* which can be used for its bulk production.
- The SEM and EXAX analysis showed the basic elements present in *VCM* as well as the external morphology of *Vishnu Chakara Mathirai*.
- The TLC and HPTLC finger printing is much helpful in identifying the active principle in VCM.
- The drug *VCM* was found to the safe as the LD_{50} level was fall above 2000mg/kg.b.wt.
- The NOEAL level was found to be > 200 mg/kg b.wt.
- The subacute and subchronic toxicity study revealed no toxic effects in the animals.
- Efficacy studies revealed good antiepileptic activity as it controls the maximum electro shock in rats, Picrotoxin induced seizures in mice, Picrotoxin induced kindling as well as seizures and N-Methyl-D-Aspartate (NMDA) test in mice.

9. CONCLUSION

The present study was undertaken to establish the literature that *Vishu Chakara Mathirai* (*VCM*), a herbo mineral formulation of Siddha system of medicine, would be effectively used against epilepsy (*Valippu Noi*), through various animal models. The safety as well as efficacy of *VCM* was studied extensively using standard and recommended scientific techniques. The trial drug *VCM* was found to be both safe and effective in epilepsy among animal models. The findings are strongly supportive of the traditional claim and use of *VCM* as an anti-epleptic medicine. From this study findings, it is also recommended that the medicine *VCM* can be taken for further research in the form of pharmacokinetic and pharmacodynamic studies as well clinical trials among human subjects to further establish the finding of this study.

10. RECOMMENDATIONS

As *VCM* shows very good anti epileptic activity in animal models, therefore it is recommended for a pharmacokinetic and pharmacodynamic study. The drug is also be recommended for a clinical trial in epileptic patients.

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(21)

CERTIFICATE

This is certify that the project title..... Therapeutic efficacy of Selected Siddha formulations in the Management of Valippu Noi (Convulsive disorders) – A Preclinical Study .....has been approved by the IAEC.

Prof. Dr. K. MANICKAVASAKAM — Dr. B. JAYACHANDRAN DARE

Name of Chairman/Member Secretary IAEC: Name of CPCSEA nominee:

Signature with date

14/6/11

Chairman/Member Secretary of IAEC: **CPCSEA nominee:**

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DECISION

Opinion of the Institutional Ethics Committee – Please Check one
Approved Approval _____ Modifications required prior to approval (Please specify on space below) _____ Disapproval Date of review: 23-06-2011 Signed: K. Manimekh 23/6/11 (Please print name) Dr.K.MANICKAVASAKAM, Secretary / Dr.V.SUBRAMANIAN, Chairperson (Please delete as appropriate, Chairperson, Secretary)
Modifications needed Nil
The research proponent is hereby informed that the Institutional Ethics Committee will require the following: 1. All adverse drug reactions (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days. 2. The progress report to be submitted to the IEC at least annually 3. Upon completion of the study, a final study status report needs to submitted to the IEC.

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Protocol title: Therapeutic efficacy of selected Siddha formulations in the management of Valippu Noi (Convulsive disorders) - A preclinical study	
Documents filed	**Yes**
Clinical trail Protocol (others – Specify) **Preclinical studies**	**Yes**
Informed consent documents	**Yes**
Any other documents	**IEC, IAEC Approval**
Date of IEC approval	**23.06.11** **NIS/IEC/11/2/25**

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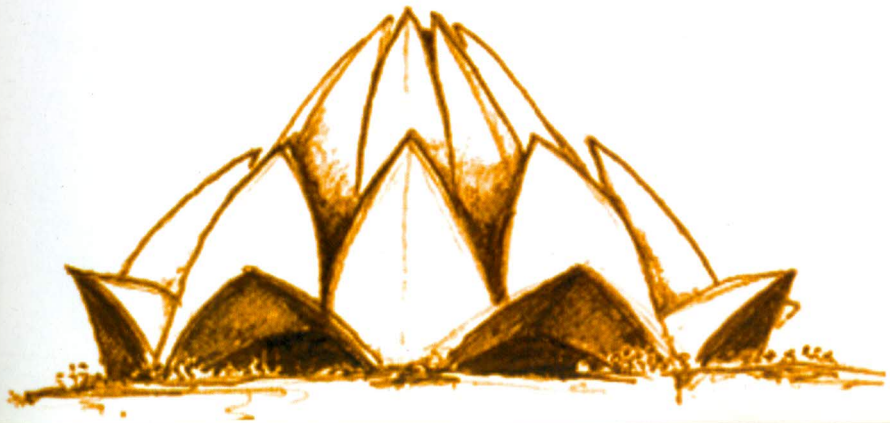

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PHYSICO CHEMICAL AND PHYTOCHEMICAL ANALYSIS OF VISHNU CHAKRA MATHIRAI –A SIDDHA HERBO MINERAL FORMALATION

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ABSTRAC

Standardization of Siddha drugs is a great challenge for the scientific field, particularly for the herbo mineral formulations. Standardization is the need of the hour to explore the values of the siddha drugs for global acceptance. Vishnu Chakra Mathirai (VCM), a herbo-mineral formulation, is being used for many years in Siddha System of Medicine. The formulation mentioned in the Siddha Text 'Siddha Vaithyiya Thirattu'; has been indicated for various diseases like Pakka Vatham (Paralysis), Sobai (Dropsy), Valippu (Convulsive disorders) and Yeppam (Belching). The present study is aimed to standardize the formulation by conducting the physico-chemical analysis, screening studies.

KEYWORDS Siddha system of Medicine, Vishnu Chakra Mathirai, phyto-chemical, Physico-chemical Analysis.

1. Introduction

Siddha system of medicine defines; Health is defined as the state of physical, psychological, social and spiritual component of a human being which has been given in this text Thirumanthiram as:

"One that cures physical ailment is medicine
One that cures psychological ailment is medicine
One that prevents ailment is medicine and
One that bestows immortality is medicine"

The word "Siddha" literally means "Established truth". "Siddhi" means as object to be obtained such as perfection in life or heavenly bliss. Siddha is a Science of life and it is a holistic medical system that gives importance to mental as well as physical well being of suffering humanity. According to Siddha system of Medicine all the objects in this world either living or non-living are composed of five elements (Pancha bootham) namely, Earth - Man, Water - Neer, Fire - Thee, Air - Kaatru, Ether - Aahayam. The universe is also made of up of these above boothams, so any changes in the universe will reflect in human body. According to Siddha the health of human body is maintained by the three vital forces (Uyir Thathukkal) namely; Vatham, Pitham and Kabam which are functioned by the influence of Pancha boothams. In Siddha the diseases of mankind are classified into 4448 types on the basis of Mukkutram. Derangement of these kutrams produces diseases. Epilepsy is a condition mentioned in siddha literatures as valippu. Many herbal and herbo mineral formulations were mentioned in siddha literatures. Epilepsy is one of the most frequent neurological disorders of man characterized by excessive provisional neuronal discharges ensuing in uninhibited convulsion. Epilepsy can be defined as a group of disorders characterized by unusual electrical activity in the brain leading to altered behaviour which may manifest as a change in a person's consciousness, movement. Vishnu Chakra Mathirai (VCM), a herbo-mineral formulation, is used for many years in Siddha System of Medicine is taken for the present study.

2. Materials and Methods

2.1 Ingredients:

The Ingredients of Vishnu Chakra Mathirai are; Rasam (Purified Mercury), Lingam (Purified Cinnabar), Ganthagam (Purified Sulphur), Karu naabi (Purified Aconite), Palagarai (Yellow orpiment), Thalagam (Purified Calamine), Kaantham (Purified Lode stone), Manosilai (Purified Red Orpiment), Veppam Pazha Saru (Neem Fruit Juice) were obtained from raw drug store in Chennai.

2.2 Preparation of Vishnu Chakkara Tablet:

The raw drugs and other ingredients were authenticated and purified as per the methods prescribed in Siddha literatures. The purified Rasam, Lingam, Kandhagam, Nabhi, Palagarai, Thuththam, Thalagam, Kantham and Manosilai were powdered and ground with Vepam pazha juice to a rolling consistency. After grinding to a soft consistency of soft pill, it was rolled as pills of 130 mg (One Kuntri) and allowed to dry.

2.3 Procurement and Authentication of Raw Drugs

Raw drugs were collected from raw drug store in Chennai, identified and authenticated from the department of Gunapadam, National Institute of Siddha, Chennai-47.

3 Physico-chemical analysis:

The physico-chemical analysis was done as per the protocol for testing of Ayurvedic, Siddha and Unani Medicines, by PLIM, Ghaziabad, under the Ministry of AYUSH, Ministry of Health and Family Welfare, New Delhi.

3.1 pH

0.5 gm of the prepared drug was dissolved in ethanol and the pH of the solution was found out using pH meter.

3.2. Loss on drying

Accurately 1 gm of sample was weighed and taken in the dish. The dish was covered with lid and dried in the drying chamber till two consecutive weights remain within ± 0.5 mg. After drying was completed, the sample was cooled in desiccators and weighed. From the difference of weights the ash content was calculated.

$$\text{Loss on drying (\%w/w)} = \frac{\text{Loss in weight (g)} \times 100}{\text{Mass of the sample (g)}}$$

3.3 Total ash

3 gm of sample was accurately weighed and incinerated in a silica dish at a temperature of 650° C until free from carbon. Then the residue was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

$$\left[\text{Percentage of total ash } (\%\,w/w) = \frac{\text{Mass of ash(g)} \times 100}{\text{Mass of the sample(g)}}\right]$$

3.4 Acid insoluble ash

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected and washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was calculated

$$\text{Percentage of acid insoluble ash } (\%w/w) = \frac{\text{Mass of acid insoluble matter (g)} \times 100}{\text{Mass of the sample (g)}}$$

3.5 Water soluble extractive

5 gm of the coarsely powdered drug was macerated with 100 ml of water in a closed flask for 24 hours and allowed to stand for 18 hours. The content was filtered rapidly, evaporated and dried at 105o C, in an oven, to constant weight. The percentage of water soluble extractive with reference to the air-dried drug was calculated.

$$\left[\text{Percentage of water soluble extractive } (\%w/w) = \frac{\text{Mass of the residue (g)} \times 100 \times 100}{\text{Mass of the sample (g)} \times 25}\right]$$

3.7 Estimation of sulphur

500 mg of sample was weighed and 100 ml of N/10 Iodine solution was added. The mixture was allowed to stand for half an hour. Then 5 ml of hydrochloric acid and 5 ml of nitric acid was added and allowed to stand for 1 hour. The content was evaporated on hot plate to dryness. The excess of iodine was removed by adding hydrochloric acid. The residue was dissolved in boiling water and 15 ml of 25 % Ba Cl2 was added. Then allowed to stand for overnight. The precipitate was filtered through Whatman no 41 filter paper. The filter paper with residue was kept in a pre-weighed crucible and ignited in a muffle furnace. From the weight of the residue, the percentage of sulphur in the drug was calculated.

Amount of Sulphur = Weight of ashless filter paper afterignition X factor of Sulphur (0.1373) X100 / Weight taken

3.8 Estimation of Mercury

0.5 gm of sample was weighed in 500 ml Kjeldhal flask, and 15 ml of conc sulphuric acid, 2 ml of conc. Nitric acid was added and refluxed for 4hrs. The yellow precipitate obtained was filtered through Whatmann 41 paper in a 250 ml standard flask. The precipitate was dissolved in dilute sulphuric acid and made up the volume 100 ml. 50 ml of made up solution was pipetted in a 250 ml standard flask and 0.1M potassium permanganate solution was added drop-wise until faint permanent pink colour persisted. Then 2 ml of ferric ammonium (II) sulphate indicator was added and titrated with 0.1M ammonium thiocyanate. From the titre value the percentage of mercury in the drug was estimated.

Amount of Mercuy = Titre value X 0.01003X250 X 100weight of sample X volume pipette (50 ml)

4. Phyto-chemical screening

The prepared tablets (10 gm) were crushed well and dissolved in 100 ml of ethanol to subject phytochemical screening.

4.1Tests for alkaloids

4.11 Morquies test:

For detecting the alkaloids 2-3 gms of the sample was ground with sufficient chloroform to make slurry. Ammonical chloroform was added and the mixture was stirred for one minute. Extraction of alkaloids from choloroform was accomplished by shaking the solution with 0.5 ml of 2 N-H2SO4 and separation of the acid layer by means of a dropper. A few drops of drug solution were tested with the following alkaloidal reagents A small quantity of the drug solution was placed in a glass plate and allowed to evaporate to dryness. A drop of water and Morquies reagent (HgCl2 + KCN) was added and the colour was observed. Appearance of Reddish colour which turns blue indicates the presence of alkaloids.

4.1.2 Mayers test:

2 ml of the solution was added with Meyers reagent (1.36 g Mercuric chloride + 3.0 gm Kl in 100 ml of water). Appearance of greyish white precipitate indicates the presence of alkaloids.

4.1.3 Dragendorffs test:

The Dragendorffs reagent was prepared by dissolving 8 gm of bismuth nitrate acid (20 ml) and 27.2 gm of Kl in 50 ml of water separately and mixing the two solutions and making up in to 100 ml with water. 2 ml of drug solution was added to this reagent and the colour was observed. Appearance of reddish brown precipitate showed the presence of alkaloids.

4.1.4 Hayers test:

Hayers reagent is a saturated solution of picric acid in water. 2 ml of drug solution was added with the reagent and colour was observed. Appearance of reddish brown precipitate showed the presence of alkaloids.

4.1.5 Wagners test:

Wagners reagent is a solution of KI3 in water. It was prepared by dissolving 1.3 gm of I2 in a solution of Kl (2 gm) in water and made in to 100 ml. 2 ml of drug solution was added with the reagent and colour was observed Red coloured precipitate showed the presence of alkaloids.

4.1.6 Test for Quinine (Bromine - ammonia test)

To about 10 ml of (1 gm in 1000) solution of sample was added with 0.25 ml of Br2/H2O and shaken well. Then about 2 ml of dil.NH3 solution was added. No bright colouration showed the absence of quinine.

4.1.7 Test for Morphine (Iodic acid test)

Morphine liberates iodine from iodic acid which gives blue colouration. 2 ml of sample solution, acidified with sulphuric acid was added to a solution of KlO3 containing starch. Absence of deep blue colouration.

4.1.8 Test for Terpenoids (Leibermann Buchard test):

2 ml of drug solution was dissolved in chloroform and to these 2 drops of acetic anhydride was added and concentrated sulphuric acid was added along the sides of the test tube and the colour was observed. Appearance of red colour indicated the presence of terpenoids.

4.1.9 Test for Flavanoids (Shinoda's test):

2 ml of drug solution in alcohol was warmed and a piece of Magnesium ribbon was added followed by 2 drops of concentrated Hcl drop by drop. Absence of organge or yellow colour indicated the absence of flavanoids.

4.1.10 Test for Methylene dioxy group (Labat test)

3 ml of drug solution was mixed with 2 gm of gallic acid and 2 drops of con. H2SO4 was added. The mixture was heated in boiling water bath for two minutes and the colour was observed. Absence of dark blue colour indicated the absence of methlene dioxy group.

4.1.11 Test Phenols OH group (FeCl3 test):

2 ml of drug solution was dissolved in alcohol and warmed then 2 drops of neutral ferric chloride was added and the colour was observed. Presence of brown green colour indicated the presence of phenolic hydroxyl group.

Results :

Table showing the physico-chemical analysis of VCM :

Sl. No	Parameters	Results	Range
1.	Appearance	Light brown coloured round shaped tablet	-
2.	Average weight of a tablet	0.1058 g	-
3.	Uniformity of weight	03.86 to 106.8%	92.5 % to\ 107.5%
4.	Disintegration time	58 sec	NMT 60 min
5.	Total Ash	32.45% w/w	1-25%
6.	Acid Insoluble Ash	11.57 % w/w	0.1-10%
7.	Loss on Drying at 105 C	4.742 w/w	1-20%
8.	Water Soluble Extractive (WSE)	4.602 % w/w	4-85%
9.	Alcohol soluble Extractive (ASE)	2.781 % w/w	4-85%
10.	Each tablet of average weight contains (NS) Mercury Sulphur	3.792 % w/w 1.925% w/w	-

Table showing the Phyto-chemical screening report of VCM) :

Sl. No	Name of the tests	Result	Inference
1	Morquies test	+	Presence of Alkaloids
2	Mayers test	+	
3	Dragendorffs test	+	
4	Hayers test	+	
5	Wagners test	+	
6	Brmine – ammonia test	-	Absence of Quinine
7	Iodic acid test	-	Absence of Morphine
8	Leibermann Buchard test	-	Absence of Terpenoids
9	Shinoda's test	-	Absence of Flavanoids
10	Labat test	-	Absence of Methelene dioxy group
11	$FeCl_3$ test	+	Presence of phenols

Summary and Conclusion:

The physico-chemical analysis results of Vishnu Chakra Mathirai shows, it has slightly acidic (pH = 6.5) and it has only 7.3 % loss on drying which indicates low moisture content, needed for long term preservation. The total ash content is 7.7 % and acid insoluble content is only 2.12 %. These two parameters indicate the presence of high amount of bio availability of the drug. The water soluble extract and alcohol soluble extract were found to be 85.32 and 15.12 which are required for intestinal absorption. This also indicates the high bio-availability of the drug. All the parameters obtained were within permissible limits.

The phyto-chemical results of the Vishnu Chakra Mathirai gives positive test for alkaloids and phenols which shows only these two components are present which are non-toxic .It also gives negative test for terpenoids, flavanoids, quinine and morphine. The absence of morphine reveals the fact that the tablet can be used without addictive effect.

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Characterisation and Elemental analysis of a Siddha Herbo Mineral formulation – Vishnu Chakra Mathirai

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Abstract

Siddha is one of the oldest indigenous system of medicine which is founded by Siddhars. The volumes of literatures in Siddha system of medicine describes about 4448 diseases and their management by herbal, herbo mineral and animal origin drugs. *Vishnu Chakra Mathirai* - a siddha herbo mineral formulation mentioned in Siddha literatures, indicated for various conditions like rheumatic and other neurologic disorders. The elemental analysis and characterization study of *Vishnu Chakra Mathirai* of UV-Vis, FTIR, Particle size analysis by Scanning Electron Microscope and EDAX, XRF study are presented in this paper.

Keywords: Siddha, herbo mineral, UV-Vis, FTIR, SEM, EDAX, XRF.

1. Introduction

According to Siddha system of Medicine all the objects in this world either living or non-living are composed of five elements (*Pancha bootham*) namely, Earth – *Man*, Water – *Neer*, Fire – *Thee*, Air – *Katru*, Ether – *Aahayam*. The universe is also make of up of these above *boothams*, so any changes in the universe will reflect in human body. According to siddha the health of human body is maintained by the three vital forces (*Uyir Thathukkal*) namely; *Vatham*, *Pitham* and *Kabam* which are functioned by the influence of *Panchaboothams*. In Siddha the diseases of mankind are classified are classified into 4448 types on the basis of *Mukkutram*. According to Siddha system of medicine health is defined as the state of physical, psychological, social and spiritual component of a human being which has been given in this text Thirumanthiram as:

"One that cures physical ailment is medicine
One that cures psychological ailment is medicine
One that prevents ailment is medicine and
One that bestows immortality is medicine"

The word "*Siddha*" literally means "Established truth". "*Siddhi*" means as object to be obtained such as perfection in life or heavenly bliss. *Siddha* is a Science of life and it is a holistic medical system that given important to mental as well as physical well being of suffering humanity. Derangement of these *kutrams* produces diseases. Epilepsy is a condition mentioned in siddha literatures as *valippu*. Many herbal and herbo mineral formulations were mentioned in siddha literatures. *Vishnu Chakra Mathirai* (*VCM*), a herbo-mineral formulation, is used for many years in Siddha System of Medicine is taken for the present study. Pshychiatric conditions and other neuropsychiatric disorders were dealt in the Siddha text (*Agathiryar Manida Kirukku Nool*) had a scientific approach to understanding mental diseases and their management by internal and external treatment procedures. Epilepsy is one of the most frequent neurological disorders of man characterized by excessive provisional neuronal discharges ensuing in uninhibited convulsion. Epilepsy can be defined as a group of disorders characterized by

unusual electrical activity in the brain leading to altered behaviour which may manifest as a change in a person's consciousness, movement.

2. Materials and Methods

2.1 Ingredients:

The Ingredients of Vishnu Chakra Mathirai are; *Rasam* (Purified Mercury), Lingam (Purified Cinnabar), *Ganthagam* (Purified Sulphur), *Karu naabi* (Purified Aconite), *Palagarai* (Yellow orpiment), *Thalagam* (Purified Calamine), *Kaantham* (Purified Lode stone), *Manosilai* (Purified Red Orpiment), *Veppam Pazha Saru* (Neem Fruit Juice) were obtained from raw drug store in Chennai.

2.2 Preparation of Vishnu Chakkara Tablet:

The raw drugs and other ingredients were authenticated and purified as per the methods prescribed in Siddha literatures. The purified *Rasam, Lingam, Kandhagam, Nabhi, Palagarai, Thuththam, Thalagam, Kantham* and *Manosilai* were powdered and ground with *Vepam pazha juice* to a rolling consistency. After grinding to a soft consistency of soft pill, it was rolled as pills of 130 mg (*One Kuntri*) and allowed to dry.

2.3 Procurement and Authentication of Raw Drugs

Raw drugs were purchased from Gopalan Asan stores, Nagercoil and the raw drugs were identified and authenticated by the Department of Gunapadam, National Institute of Siddha, Chennai-47.

3 Morphological characterisation studies by SEM:

Morphological characterization study was conducted by Scanning Electron Microscope (SEM). Scanning Electron Microscope (SEM) is one type of electron microscope that produces images of a test drug by scanning it with a focused beam of electrons. The information about the drug surface topography and composition were analyzed by the detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons that can be detected depends on the angle at which the beam meets surface of the sample. SEM can measure the resolution better than 1 nanometer. By scanning the sample and collecting the secondary electrons that are emitted using a special detector, an image displaying the topography of the surface is created. The VCM sample was subjected to SEM analysis and the angle was measured from this angle, the size of the particles was calculated (Figure 1a, b).

Figure: 1(a) Scanning Electron Microscope picture of VCM

View field: 42.4 μm, Magnification : 3

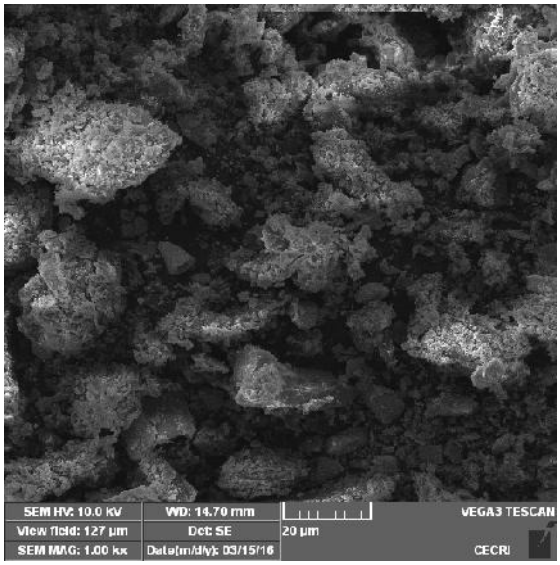
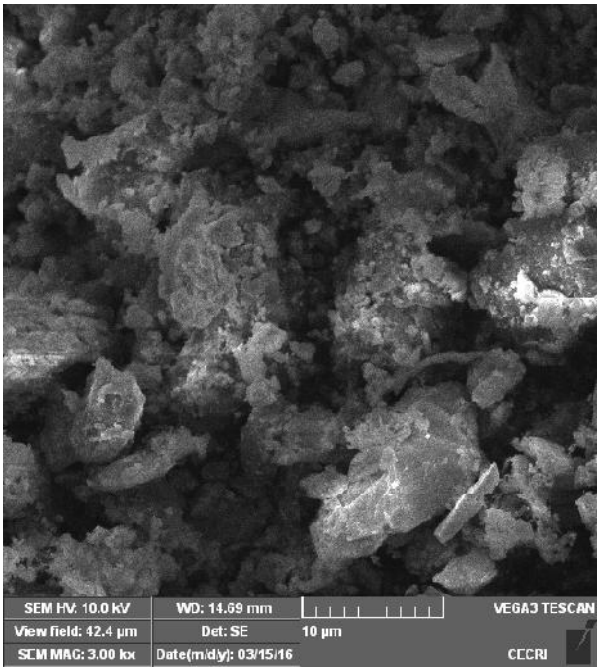


Figure: 1(b) Scanning Electron Microscope picture of VCM

View field : 25.5 μm, Magnification : 5



4 Spectral studies:

The *Vishnu Chakra Mathirai* was characterized by UV - visible spectrometry and FT-IR Spectroscopy.

4.1 Ultra Violet-Visible spectra

UV absorption spectroscopy can characterize those types of compounds which absorb UV radiation. The compounds with unbonded electrons or those with the conjugated double bonded system such as aromatic compounds can be identified by such technique.

Identification is done by comparing the absorption spectrum and λ_{max} with that of known compound. In order to record UV absorption spectrum the usual practice is to measure the amount of radiation absorbed at various wavelengths. Then a curve is plotted between wavelength and absorption.

The spectrophotometer used for our experiment (UV-260 Shimadzu spectro-photometer) has a range of 340 mm - 960 nm with tungsten halogen lamp as light source and silicon photo diode as detector. About 0.1 g of drug sample was dissolved in 100 ml of ethanol and the optical density was found out from 400 nm - 540 nm and the λ_{max} was observed to be at 470 nm.

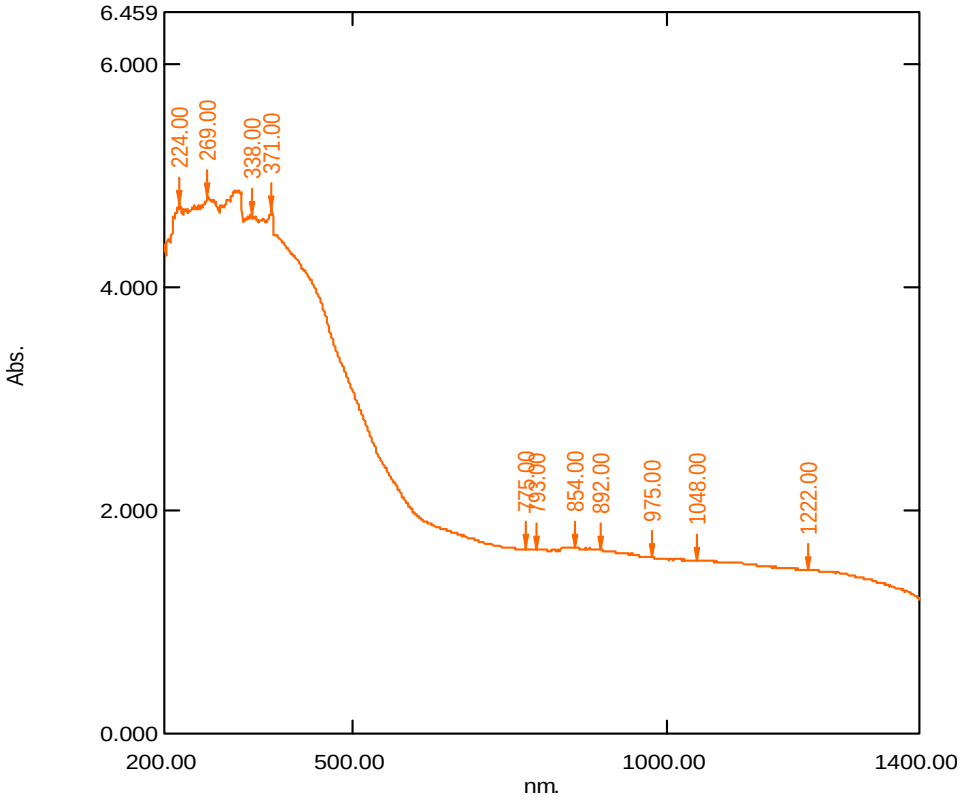
Ultra Violet and Infra Red spectra of the drug were recorded. The absorbance and reflectance data were presented in the below presented table. The peak at 224 nm, 269 nm, 338 nm, 371 nm UV spectrum for absorbance and peak at 1216 nm, 1044 nm, 971 nm, 888 nm for reflection reveals the fact that it absorbs light only in the visible region of the electromagnetic radiation (Figure 2 a, b) . The IR spectra (Fig 3) shows peaks at various frequencies. The peak at 3299 cm^{-1} shows the presence of alcoholic functional group and peak at 2918 cm^{-1} indicates the presence of aldehyde group. The peak at 2082 cm^{-1}, and 1615 cm^{-1} are due to carbonyl stretching and peaks at 1411 cm^{-1}, 1017 cm^{-1} and 870 cm^{-1} indicates the C-H stretching.

Table 1. Showing Ultra Violet and Infra Red spectra:

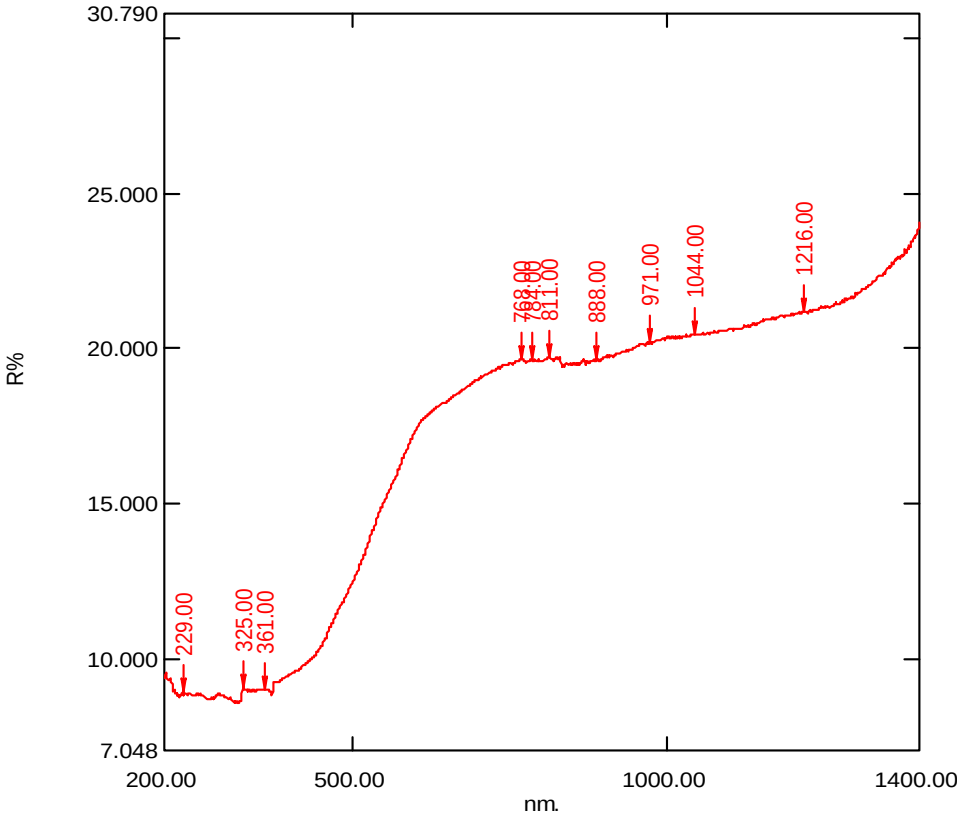
UV nm		Infra Red cm^{-1}
Reflectance	**Absorbance**	3299
1216	224	2918
1044	269	2082
971	338	1615
888	371	1411
		1017
		870

Figure 2 Ultra Violet Spectrum of VCM

a. Absorbance in UV Spectra



b. Reflectance in UV Spectra



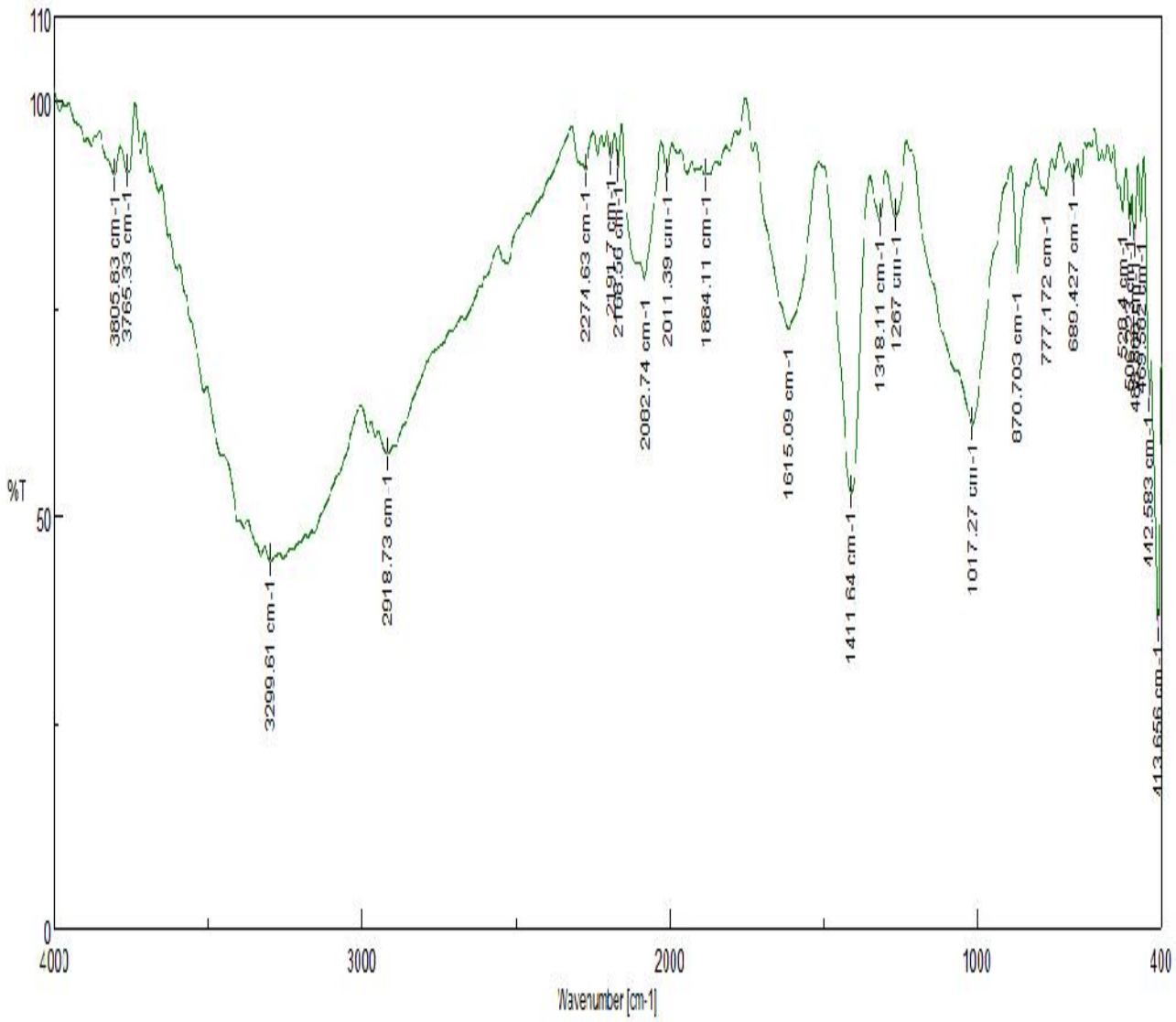
4.2 Infra Red Spectroscopy:

Infrared spectroscopy is one of the most powerful analytical techniques which offer the possibility of chemical identification. It provides useful information about the structure of molecule. The technique is based upon the simple fact that a chemical substance shows marked selective absorption in the infrared region. After absorption of IR radiations, the molecules of a chemical substance vibrate at many rates of vibration, giving rise to close-packed absorption bands, called an IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in IR spectrum which will correspond to the characteristic functional groups and bonds present in a chemical substance. Thus, IR spectrum of a chemical substance is a fingerprint for its identification.

Band position in an infrared spectrum may be expressed conveniently by the wave number 'υ' whose units is cm^{-1}.A Nicolet 5700 FTIR USA, instrument was used for recording the IR spectra with 2 - 3 mg of the sample as KBr pellet. IR spectra of the drug was recorded.

A small quantity of dry KBr was mixed with a little amount the sample and ground for homogenization. An IR lamp was used for drying during mixing. The mixture was then pressed in to a transparent thin pellet at 5 ton/cm^2. These pellets were used for IR spectral recording.

Figure: 3 Infra Red Spectrum of VCM



5. Elemental analysis

5.1 EDAX

Energy-dispersive X-Ray spectroscopy (EDAX) is an analytical technique used for the elemental analysis of a sample. It relies on an interaction of source of X-ray excitation and a sample. Its characterization capabilities are that each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as beam of X-rays, is focused into the sample being studied. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer. From this the elemental composition of the specimen can be found out. The powder of VC Mathirai was subjected to EDAX analysis and the elemental composition was found out (Table 2).

5.2 XRF

X-Ray Fluorescence (XRF) is the emission of characteristic fluorescent X-rays from a material that has been excited by bombarding with high-energy X-rays The phenomenon is widely used for elemental analysis and chemical analysis, particularly in the investigation of metal based research and in geochemistry, forensic science, archaeology. The powder of Vishnu Chakra Mathirai was subjected to XRF analysis and the elemental composition was found out.

Table 2 Elemental analysis of VCM

Sl. No	Element	wt %	at %
1	Carbon	24.98	42.25
2	Oxygen	21.88	37.01
3	Magnesium	5.80	9.82
4	Sulphur	4.96	8.39
5	Calcium	1.50	2.54

Summary

To study the particle size of VCM Scanning Electron Microscope (SEM) analysis was carried out. The particles are found to be spherical in shapes and sizes are in the range from 7 microns to 50 microns. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. This sample exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation.

Ultra Violet and Infra Red spectra of the drug were recorded. In the UV the peak at 224 nm, 269 nm, 338 nm, 371 nm UV spectrum for absorbance and peak at 1216 nm, 1044 nm, 971 nm, 888 nm for reflection reveals the fact that it absorbs light only in the visible region of the electromagnetic radiation.

The IR spectra shows peaks at various frequencies. The peak at 3299 cm^{-1} shows the presence of alcoholic functional group and peak at 2918 cm^{-1} indicates the presence of aldehydic group. The peak at 2082 cm^{-1}, and 1615 cm^{-1} are due to carbonyl stretching and peaks at 1411 cm^{-1}, 1017 cm^{-1} and 870 cm^{-1} indicates the C-H stretching.

Energy Dispersive X-Ray analysis (EDAX) of VCM was carried out and the elements present like, Carbon, Oxygen, Magnesium, Sulphur and Calcium were estimated. From the spectra atom percentage of the elements are found to be as follows. Carbon = 42.25%, Oxygen= 37.01%, Magnesium = 9.82%, Sulphur = 8.39% and Calcium = 2.54%.

Conclusion

Vishnu chakra mathirai is a commonly prescribing siddha herbo-mineral formulation used many years for neurological disorders, rheumatic diseases and other degenerative disorders. The standardization and elemental analysis of the siddha formulation were studied. The encouraging study reports showed that the prepared formulation would be adding evidence for the curative effect of prescribing this medicine since ancient years. This study outcome will be a boon for the young indigenous medical and herbal researchers. It will be initiative step in the field of standardization of siddha drugs for global acceptance.

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