NATIONAL INSTITUTE OF SIDDHA

Tambaram Sanatorium, Chennai – 47

Affiliated to the Tamil Nadu Dr. M.G.R. Medical University

Chennai - 600 032

TOXICITY STUDIES OF *VEERA CHENDURAM*

(DISSERTATION SUBJECT)

For the partial fulfillment of the requirements to the Degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH VI – *NANJU NOOLUM MARUTHUVA NEETHI NOOLUM*

SEPTEMBER – 2007

CERTIFICATE

Certified that I have gone through the dissertation submitted by Dr. **T.Prem kumar** a student of final MD (Siddha) Branch-VI Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram sanatorium, Chennai-47, and the dissertation work of **"Toxicity studies of Veera Chenduram"** has been carried out by individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

Place: Chennai-47.
Date:

HOD& Professor
Dept.of Nanju Noolum Maruthuva Neethi Noolum
National Institute of Siddha
Chennai-47.

AKNOWLEDGEMENT

I am thankful to **Prof. Dr. V.Arunachalam, MD (S)**, Director, National Institute of Siddha, Tambaram sanatorium, Chennai, for providing me this opportunity to carry out this dissertation work.

It is with great pleasure that I expressed my heartful gratitude to **Prof. Dr. S. Boopathi Raj, MD (S)**, HOD, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatorium, Chennai for his valuable guidance, constant advice and suggestion for carrying out this work in the best possible manner.

I gladly acknowledge **Dr. V. Banumathi, MD (S)**, Associate professor, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram sanatorium, Chennai, for his valuable advises and helps during this study.

I am deeply indebted to **Dr. M. Rajasekar, MD (S)**, Lecturer, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram sanatorium, Chennai, who have sent words of encouragement and suggestions for this study.

I express my sincere thanks to **Prof. Dr. S. P. Pandi Perumal, MD (S),** Principal, Govt. Siddha Medical College, Chennai for his help and support to complete this dissertation in good shape.

I acknowledge my thanks to **Dr. S. K. Sasi, MD (S)**, HOD, Department of Noi Naadal, National Institute of Siddha, Tambaram sanatorium, Chennai, for her support in pathological investigation of this study.

I am deeply indebted to **Mr. P. Jeyabal, M.Sc.**, Asst.Professor (Bio statistics), National Institute of Siddha, Tambaram sanatorium, Chennai, for his valuable help throughout this study.

I extend my sincere thanks to each and every **faculty of NIS** for their guidance throughout this dissertation work.

In all humility, I salute with grate thanks to the **Tamil Nadu Dr. M.G.R Medical University** and **Dept. of AYUSH, Ministry of Health & Family Welfare, Govt. of India** for granting permission to take this study.

I render my sincere thanks to **Dr. Kalavathy Kamalakar Rao, M.B.B.S., DCP**, Research officer, Dept. of pathology in Central Research Institute for Siddha, Arumbakkam, Chennai – 106 for her guidance in this study.

I express my sincere thanks to **Dr. Sharad D. Pawar, M.V.Sc**., Research officer, Dept. of pharmacology in Central Research Institute for Siddha, Arumbakkam, Chennai – 106 for his excellent guidance in Toxicology study.

I particularly wish to express my sincere appreciation for the help and cooperation extended to me by **Mr. J. Anbu, M.Pharm**, **Ph.D.,** Lecturer, Vel's College of Pharmacy, Pallavaram, Chennai who have been kind enough to offer suggestions to carry out this work in the best manner.

I express my thanks to **VIMTA Labs,** Adyar, Chennai and **Vaishnavi Histopathology and Cytology Centre,** T.Nagar, Chennai for their help in lab investigations of this study.

I would like to convey my thanks to **Dr. M. Logamanian**, **MD (S), Ph.D** Library in-charge, National Institute of Siddha, Tambaram sanatorium, Chennai, for their kindly help during this study.

I wish to thank **Mrs. A. Vimala and Mr. J. Rathinan** Lib. Assistants, NIS, Chennai for their help to make this dissertation in full form.

I would be remiss if I do not express my gratitude to **my parents** who have inspired me towards grater efforts to achieve excellence in this work.

It is with great pleasure and deep satisfaction that I acknowledge my esteemed **colleagues and friends**, each has been most gracious, diligent and resourceful in their efforts to accomplish.

I express my thanks to **Jan Xerox** for their excellent work to complete this dissertation in good shape.

S.No INDEX

INTRODUCTION

Nature has many remedies for our ailment, nature has created innumerable plants, metals, poisonous substances, minerals, organic substances and products of animal origin.

The science of medicine is of fundamental importance to man's well being and his survival. So it must be originated with man and developed gradually as civilization advanced.

Siddha system is perhaps the earliest medical science that laid stress on positive health, a harmonious blending of physical, mental, social and spiritual welfare of an individual.

Appreciation and appropriate of siddha science and system is sure to give us a happy, healthy and harmonious life.

Siddhars have selected things from the nature which can render relief to innumerable ailments of mankind suffered and its suffering. Siddha system of medicine is ingrained in Tamilians. We grew up with this medical system.

Apart from the usage of herbs it could be asserted that the siddhars were pioneers in the use of metals and minerals in the treatment of diseases.

Siddha system has its own well-developed chemistry with thousands of mineral and metallic preparations. The siddha system is a wonderful treasure house that has immense potentialities to solve any medical problem.

Any effective remedy must be safe in usage, easy to administer, available with easy access and cheap enough to only through the siddha system of medicine since it is already having its roots in the rural areas and people are already practicing home remedies.

Siddha literature is brilliant in its evolution of a rest cycle of original occult knowledge dealing with the essentials of religion and science in consonance with the principle of the theoretical and practical knowledge.

We are now witnessing a renaissance in siddha medicine and indeed in all the natural system of medicine such as Ayurvedha, Unani, Chinese traditional medicine etc. disease which cannot be satisfactorily managed by modern medicine are successfully treated by this natural system of medicine.

However, there successes are readily questioned by the modern science. It is alleged that even if this treatment is successful some days used in the siddha system are toxic and dangerous for use. The fact that the siddha physicians use plants such as Etti, Serankottai etc., and chemical such mercury, arsenic, cinnabar etc., which are toxic in raw farm, add weightage to this argument.

The untrue belief of toxicity posing a major obstacle to the renaissance of siddha medicines. The explanations such as raw forms are purified to remove toxicity and the drugs are used with established safety from time immemorial are not bought by modern science.

The efficacy of many siddha preparations have been established but their safety are not the siddha physicians should embrace ourselves the siddha drugs are indeed safe and we should do this by using well established, modern and scientific methods. ***Veera chenduram*** a widely used siddha medicine, contains perchloride of mercury found in mountains naturally. This is used for the treatment of several ailments.

This toxicity study of ***veera chenduram*** just a small step in the right direction of establishing the safety of siddha drugs. This is dedicated to the glory of siddha system.

AIM & OBJECTIVES

Veeram is one of the *pancha soodham* in Siddha system. It is used for many diseases in Siddha system of medicine.

AIM

The aim of the dissertation is toxicity studies of *Veera Chenduram*.

OBJECTIVES

The toxicity of ***Veera chenduram*** has been evaluated in the following aspects:

1. Collection and Identification of the test material
2. Purification of the raw drug *Veeram*.
3. Preparation of the *Veera chenduram*
4. XRD study of the *Veeram* (impure), purified *Veeram* and *Veera chenduram*

5.Toxicological studies of the *veera chenduram*

REVIEW OF LITERATURE

GUNAPADAM ASPECT

வீரம்

வீரம் வெள்ளை நிறமானதாயும், எவ்வித வாசனையும் இல்லாததாயும், ஒரு விதக் காரமுள்ளதாயும் இருக்கும். இது சிறிய் போலாவது அல்லது பெரிய பளிங்குக் கற்களைப் போலாவது இருக்கும்.

வீரத்தின் வேறுபெயர்கள்:

வீரத்துக்குச் சவ்வீரம், சரக்குச்சுண்ணம், வெள்ளைச்செந்தூரம், வராகத்தின் தலை, பறங்கிப்பாடாணம்

மீனாஷிமைந்தன், கொச்சிவீரம், பூவிந்துசேவகன், சரக்குச்சுண்ணம், பறங்கிப் பாஷாணம், பறிமித்துரு, சாரத்தின்சத்துரு என்று பல்வேறு பெயர்கள் வழங்கப்படுகின்றன.

ஆனைவீரம், பசுவீரம, அத்திவீரம், பசுபதிவீரம், பலவீரம், சிறியானைவீரம்.

போகர் நிகண்டு 1200ல்

"வீரத்தின் பேர்தனையே விளம்பக் கேளு
விளங்கியதோர் திறவு திறனுமாகும்
மாரமாமதி வெள்ளை செந்தூரமாகும்
மாகான வாதிகளுக் கெற்பமாகும்
வாரமாம் வகாரத்தின் தனையனாகும்

மன்னியதோர் பூவிந்து வுத்தம சேவகனாம்

தூரமா மலைகளிலே உற்பவித்த

சுயம்பான வீரத்தின் வேருமாமே”

போகர் கருக்கிடை நிகண்டு - 500ல்

”வீரமென்றால் வெகுவீரன் சுத்த வீரன்

வேதைக்கோ ரணவீரன் மகத்தாம் வீரன்

பாரமென்றால் செயவீரன் விற்பன்ன வீரன்

பாதைக்கோ பலவீரன் பாரவீரன்

வீரத்தின் பொதுக்குணம்:

”குன்மமொடுகுட்டங் கொடியவனி லத்திரட்டு

துன்மாங் கிசப்பெருக்கஞ் சூலைநோய் - வன்மையுறு

காமியபுண் ணாதியநோய் கண்டாற்சவ் வீரனெனுஞ்

சாமிநா மத்தையுச் சரி”

பொழிப்புரை:

சவ்வீரத்தின் நாமத்தை உச்சரித்தால் குன்மம், குறைநோய், தீங்கை விளைவிக்கின்ற மகாவாத ரோகங்களின் கூட்டம், துர்மாமிச வளர்ச்சி, சூலை நோய்கள், வன்மை பொருந்திய பெண் போகத்தினால் விளைகின்ற (கொறுக்கு, அரையாப்பு முதலிய) புண்கள் ஆகிய இவை நீங்கும். இதனைப் பலவகைப்பட்ட கண்நோய்களுக்கும் உபயோகிக்கின்றனர்.

சுவையும் செய்கையும்

சுவை

கார்ப்பு, உப்பு சுவை

வீரியம்

வெப்ப வீரியம்

பிரிவு

கார்ப்பு பிரிவு

செய்கை

உடற்தேற்றி,

கிருமிநாசினி,

அழுகலகற்றி,

புண்ணுண்டாக்கி.

அளவு:

1/32 உளுந்தெடையிலிருந்து (2 மிகி.) 1/16 உளுந்தெடை (4 மி.கி) வரை.

பொதுபண்புகள்:

நெருப்பிலிட்டால் எளிதாய் உருகும். 295^{O} டிகிரிக்கு எரித்தால் பொங்கிப் புகையாகப் பறந்துவிடும். இதைப் பதங்கித்தால் அரைப் பிரகாசமான அல்லது வெள்ளையான பளபளப்புள்ள பொடியாகும். வீரமானது தண்ணீரிலும், சாராயத்திலும், ஈதரிலும் தாராளமாகக் கரையும் தன்மையுடையது.

இரசகலப்புள்ள கனப்பொருள்களில் நீரில் கரையத்தக்கதில் இதுவே மிக முக்கியமானதாகும். ஆகையால் இது செயநீர்களிலும், திராவகங்களிலும் அதிகமாகச் சேர்க்கப்படுகிறது. இக்கட்டியின் மீது கொஞ்சம் சுண்ணாம்பு தடவினால் சிவந்து காண்பிக்கும். வீரப் பொடியைச் சுண்ணாம்பு நீருடன் கலந்தால் மஞ்சள் நிறமான வண்டல் அடியில் படிந்து பின்புறம் சிவந்து போகும். இதை உட்கொண்டால் உடலிலுள்ள தாதுக்கள் அழுகிப் போக ஒட்டாமல் செய்கிற தன்மையுடையது.

இதை மருந்துகளோடு சேர்த்தால் சரக்குகள் மடிந்து மக்கித் தம் குணங்கள் கெட்டுப் போகாமல் காக்குங் குணமுள்ள வீரமானது வைப்புச் சரக்கு ஆகும்.

வீரச்சுத்தி

இப்பொருள் கொடிய நஞ்சாதலின் அளவில் சிறிது அதிகப்படினும் நஞ்சாகிக் கொல்லும் எனவே இதனைத் தூய்மை செய்தே கையாளல் வேண்டும்.

இளநீரில் சிறிது சூடனைக் கலந்து ஒரு பானையிலிட்டு வீரத்தை துலாயந்திரமாக நீரில் படாமல் ½ மணி நேரம் எரித்து எடுத்தல் வேண்டும்.

வீரச் செந்தூரம்

சவ்வீரம், வெங்காரம் இவ்விரண்டையும் சமஎடை எடுத்துப் பொடித்து அகலிலிட்டு நெருப்பில் வாட்டச் செந்தூரமாம்.

தீரும் நோய்கள்

சுரம், சன்னி, பிதற்றல், வலிமையுள்ள வாதநோய்கள், வாந்தி, பேதி, சூலை

அளவு:

1/32 உளுந்தெடையிலிருந்து (2மி.கி) 1/16 உளுந்தெடை (4மி.கி) வரை உபயோகிக்கலாம்.

வெங்காரம்

வேறு பெயர்கள்:

பொரிகாரம், காரம், உருக்கினம், உருக்குமித்திரன், டங்கணம், தூமத்தையடக்கி.

சுவை:

இனிப்புடன் கூடிய துவர்ப்பு.

வீரியம்:

வெப்பம்.

செய்கை:

குளிர்ச்சியுண்டாக்கி,

சிறுநீர்ப்பெருக்கி,

ருதுவுண்டாக்கி,

கற்கரைச்சி,

பிரசவகாரி.

பொது குணம்:

சொறிபுடையெண் குன்மநமை சோரி யாசம்
பறிகிரகணி கல்லூனம் பன்னோய் - நெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடஞ் சந்நி
யிடங்கணங்க லக்கிற்போ மெண்.

பொழிப்புரை:

வெங்காரத்தினால் தவளைச்சொறி, புடை, எண்வகைக் குன்மம், தினவு, இரத்தமூலம், ஒழுக்குக் கிரகணி, அச்மரி, பங்குவாதம், பல் நோய், நாளவழியைத் தடுக்கின்ற மூத்திரக்கிரச்சிரங்கள், கபாதிக்கம், புழு, பாம்பு முதலியவைகளால் உண்டாகும் நஞ்சு, சந்நிபாதம் முதலிய நோய்கள் தீரும்.

சுத்தி முறைகள்:

வெங்காரத்தை நீர்வற்றும்படி பொரித்து எடுக்கச் சுத்தியாம்.

MODERN ASPECT

MERCURIC CHLORIDE

Synonyms

Mercury bichloride; corrosive sublimate; mercury (II) chloride; mercury perchloride

Molecular Weight: 271.52

Chemical Formula: HgCl2

Mercuric chloride exists in the film of heavy, colourless masses of prismatic crystals or as a white crystalline powder. It has a styptic, nauseous, metallic taste. It is soluble in eighteen parts of cold water and three parts of boiling water. It is readily soluble in alcohol (90 percent) ether and glycerin and is very soluble in solutions of alkaline chlorides. On account of its antiseptic properties it is largely used in medicine as well as in taxidermy.

Physical and Chemical Properties

Appearance :

White crystals or powder.

Odour:

Odourless.

Solubility:

7.4g in 100g of water.

Specific Gravity:

5.4

pH:

3.2 (0.2 M solution)

Boiling Point:

302C (576F) Sublimes.

Melting Point:

276C (529F) Sublimes.

Vapor Density (Air=1):

8.7

Vapor Pressure (mm Hg):

1 @ 136.2C (277F)

Stability and Reactivity

Stability

Stable under ordinary conditions of use and storage. Slowly decomposes to metallic Hg in the presence of organic matter and sunlight, and becomes volatile at 300C (572F).

Incompatibilities

Reacts violently with potassium and sodium. Incompatible with many compounds: formates, sulfites, phosphates, albumin, ammonia, gelatin, carbonates, hypophosphites, sulfides, alkalis, alkaloid salts, lime water, antimony and arsenic, bromides, borax, reduced iron, copper, iron, lead, tannic acid and vegetable astringents.

BORAX

Other Names:

Borax decahydrate, Boricin, Dinatrium fetraborat decahydrat, Disodium tefraborate decahydrae, Gerstley borate, sodium biborate decahydrate, solubor.

Borax is an important boron compound, a mineral and a salt of boric acid. It is usually a white powder consisting of soft colorless crystals that dissolve easily in water. Borax is used in detergents and cosmetic as an igredients

Systematic name	:	Sodium tetraborate decahydrate
Molar formula	:	$Na_2B_40_710H_2O$
Molar Mass	:	381.37 g/mol
Appearance	:	White solid

Sources:

It occurs naturally in evaporate deposits produced by the repeated evaporation of seasonal lakes. The most commercially important deposits are found in Turkey, California and other locations in the American South West. The Atacama Desert in chile and in Tibet. Borax can also be produced synthetically from other boron compounds.

Properties:

Density and phase	:	$1.73g/cm^3$, Solid
Solubility in water	:	$5.1g/100ml(20^oC)$
Melting Point	:	75^oC
Boiling Point	:	32^0C
Crystal Structure	:	Monoclonic

TOXICOLOGICAL ASPECT

SIDDHA ASPECT

வீரநஞ்சுக் குறிகுணம்:

இது நீரில் வேகமாகக் கரையும் தன்மையுடையது. இது உட்கொண்டவுடனேயே குருதியோடு கலந்து விடத்தை மிக வேகமாக விளைவித்துவிடும். இது பிறரைக்கொல்லும் பொருட்டும், தற்கொலை புரிந்துகொள்ளவும் பயன்படுத்துவதுண்டு.

குறிகுணம்:

இதை உட்கொண்டால் வாயில் ஒருவகைக் களிம்புச் சுவை உண்டாகும். வுாயில் நீரூறும். வாய் புண்ணாகி வீங்கும். தொண்டையும், ஆமாசயம் யாவும் வீங்கிப் புண்ணாகும். வாந்தியும் , மலபேதியும், குருதிக் கழிச்சலும் உண்டாகும். எச்சிலைக்கூட விழுங்க முடியாதபடி தொண்டையில் வலி ஏற்படும். முகம் வீங்கிவிடும். உடம்பிலுள்ள தோல் முழவதும் பட்டை பட்டையாக வெடித்துச் சிலை நீர வடியும். புக்கம், விலா முதலிய இடங்களில் அதிக நோவும், குடைச்சலுமுண்டாகும். அதிக நீர் வேட்கை, விக்கல் , மூர்ச்சை, மயக்கம், வலி முதலியன உண்டாகும். இதோடு சாவும் உண்டாகும்.

வீரநஞ்சு முறிவு

முறையாகச் சவ்வீர மொய்குழலாய் கொண்டால்
சிறுநெருஞ்சிற் சாறுண்ணத் தீரும் - அறையக்கேள்
நீலிவே ராகுமே நெய்ச்சட்டிச் சாறாமே
பாலி தென்னங் கள்ளும் பகர்

அண்டத்தின் வெண்கருவை யாவின்பா லிற்கலந்(து)

உண்டுவர வீர னுரமகலுங் - கண்டறிவாய்

ஏணற் கொடியே யிளநி ரருந்திடினும்

மாணப்பெருமை வழுத்து.

பொழிப்புரை:

தேவையான பொருட்கள் :

சிறு நெருஞ்சிச்சாறு,

நீலிவேர்ப்பட்டை,

நெய்ச்சட்டிக்கீரை,

தென்னங்கள்.

செய்முறை:

1. 20 மி.லிட் சிறு நெருங்சிக் சாற்றைக் காலையிலும் மாலையிலும் குடித்துவர வேண்டும்.
2. நீலி வேர்ப்பட்டையை வெந்நீர்விட்டு அரைத்து ஒரு சுண்டைக்காய் வீதம் எடுத்து 80 மி.லிட் இள வெந்நீரில் கரைத்துக் காலையிலும் மாலையிலும் குடித்துவரவேண்டும்.
3. 20 மி.லிட் நெய்ச்சட்டிக்கீரைச்சாற்றை காலையிலும் மாலையிலும் குடித்துவர வேண்டும்.
4. தென்னங்கள் அருந்தி வரலாம்.

MODERN ASPECT

Inhalation

Cause irritation to the respiratory tract. Symptoms include sore throat, cough, pain, tightness in chest, breathing difficulties, shortness of breath and headache. Pneumonitis may develop. Can be absorbed through inhalation with symptoms to parallel ingestion. Vapor inhalation can burn the mucous membrane of the nose and throat.

Ingestion

Cause burning of the mouth and pharynx, abdominal pain, vomiting, corrosive ulceration, bloody diarrhoea. May be followed by a rapid and weak pulse, shallow breathing, paleness, exhaustion, central nervous system problems, tremors and collapse. Delayed death may occur from renal failure.

Skin Contact

Cause irritation and burns to skin. Symptoms include redness and pain. May cause skin allergy and sensitization.

Eye Contact

Cause irritation and burns to eyes. Symptoms include redness, pain, and blurred vision; may cause serious and permanent eye damage.

Teratogen

Can damage the developing fetus and decrease fertility in males and females.

Reproductive Toxicity

All forms of mercury can cross the placenta to the foetus, but most of what is known has been learned from experimental animals.

Aggravation of Pre-existing Conditions

Persons with nervous disorders, or impaired kidney or respiratory function, or a history of allergies or a known sensitization to mercury may be more susceptible to the effects of the substance.

veeram Before Purified

veeramAfter purified

Venkaram *Before purified*

***Venkaram* After Purified**

MATERIALS AND METHODS

Procurement and identification of the *Veeram*

Procurement of the *Veeram*

The drug was collected from the local market in Chennai.

Identification of *Veeram*

The *Veeram* was identified as mercuric Chloride by the base of its physical properties in chemical laboratory.

Purification of the *Veeram*

The purification method of *Veeram* was selected in accordance with the reference made in the *Gunapadam Thathu Jeeva Vaguppu*.

1 palam of camper was dissolved in tender coconut and kept in a mud pot. 1 palam was suspended over the contents of pot and heated for ½ hour.

Preparation of *Veera Chenduram*

1 palam of *veeram* and *venkaram* was taken in a mud pot. Fried it up to chenduram consistency

X- RAY POWDER DIFFRACTION (XRD)

The XRD analysis of the drug *Veera chenduram* was done in Department of Nuclear physics, University of Madras.

The powder method of diffraction was devised independently by Debye and Scherrer. Powder diffraction method involves the diffraction of monochromatic X- rays by a powder specimen. Monochromatic usually means a strong Kα characteristic component of the filtered radiation from an X- ray tube operated above the Kα excitation potential of the target material.

Selection of Kα renders the incident beam to be a highly monochromatised one. The focusing monochromatic geometry results in narrower diffracted peaks and low background at low angles. The sample is mounted vertically to the Seemann- Bohlin focusing circle with the scintillation counter tube moving along the circumference of it. It is possible to record the diffracted beam from 2 to 160 degrees. The diffractometer is connected to a computer for data collection and analysis. The scintillation counter tube can be moved in step of 0.01 degree by means of a stepper motor and any diffracted beam can be closely scanned to study the peak profile.

Identification of the material

The powder diffraction of a substance is characteristic of the substance and forms a sort of fingerprint of the substance to be identified. The peaks of the X-ray diffraction pattern can be compared with the standard available data for the conformation of the structure. For the purpose of comparison, many standards are available, some of which are, Willars hand book, Joint Committee on Powder Diffraction Standards (JCPDS) Pepdfwin and National Bureau of Standards.

TOXICITY STUDIES

ACUTE TOXICITY STUDY

EXPERIMENTAL STUDY

VEHICLE USED:

Water was used as vehicle. Starting dose was 5mg/kg. And the subsequent doses are 10, 50, 100, 250, 500, 1000 2000 and 4000mg/kg p.o. used in this study.

ACUTE TOXICITY STUDY

Veera chenduram suspended in water was administered to the groups of wistar rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the butter vehicle. Ten females and ten males were used for each dosage level. The principles of laboratory animal care were followed. Observations were made and recorded systematically 1, 2, 4 and 24 hr after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hr prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hr and these animals were then maintained for a further 13 days and observations made daily. At the conclusion of the experiment, all surviving animals were sacrificed with anesthetic ether and their organs such as liver, lungs, heart, spleen, adrenals, kidneys, testes and ovaries were excised and weighed. The pathological observations of these tissues were performed on gross. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50}.

Statistical analysis

Either the analysis of variance (ANOVA) or Dunnet multiple comparison test (Instat-3 computer program) was employed to analyze the results statistically. A statistical comparison was carried out using the Dunnnet Multiple comparison Test. All values were expressed as back transformed mean ±S.D. Differences below the probability level of 0.01 were considered statistically significant.

SUBACUTE TOXICITY

Three groups of 6 rats received *Veera chenduram* by intra-gastric gavages at the dose of 50mg/kg, 100mg/kg, 200mg/kg body weight every for 28 days. During the period of administration the animals were weighed and food and water intake were monitored. After 28 days all surviving animals were fasted overnight. Animals were sacrificed by decapitation and blood samples were collected into heparinized tube for hematological parameters and non-heparinized centrifuge tubes. The Brain, Lung, Liver, Pancreas, Spleen, Stomach, Testes, Ovary, Heart and Kidney were collected and weighed.

Biochemical estimations

The serum separated was analysed to evaluate the liver enzymes. Total protein, Albumin, Globulin, Blood glucose, Total Cholesterol, HDL, LDL, VLDL, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, SGOT, SGPT, ALP, γ-Glutamyl Transferase, Creatinine, Urea, Uric Acid, Sodium, Potassium, Chloride.

Haematological assay

Blood samples collected in the heparinized tubes were used to investigate TRBC, Hb, PCV, MCV, MCH, RDW, ESR, Platelets, TLC and DLC using the standard method.

Histopathological study

Histopathological investigation of the vital organs were done The organ pieces (3-5 µm thick) were fixed in 10% formalin for 24 hr and washed in running water for 24 hr. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50℃ and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis

Values were expressed as mean ± SD. The statistical analyses of variance were done by ANOVA followed by the dunnet test.

RESULTS AND OBSEVATIONS

XRD study of the *Veeram (impure)*, purified *Veeram* and *Veera chenduram*

The sample of ***Veeram*** is matched with standard graph mercuric chloride.

The sample of ***venkaram*** is matched with standard graph of sodium biborate.

The sample of purified ***venkaram*** is matched with standard graph of tincalconite

Toxicological studies of the *Veera chenduram*

Acute

Death was recorded during the treatment period in treated groups given 1g/kg of *Veera chenduram* orally. The treatment of *Veera chenduram* on rats possess significant changes in general behavioural pattern and produced major signs of toxicity from the dose level of 500mg / kg and above in an acute exposure. The animals showed changes in general behaviour other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, convulsion finally at the dose level of 1g/kg.

Sub acute

In the sub acute toxicity study, after 28 days of alternate day treatment of *veera chenduram* in single oral dose showed insignificant body weight changes (Table-1) during the experimental period. But significant ($P<0.01$) increase in food intake was observed in all the groups after one week of drug treatment (Table-2). There was a gradual decrease in water consumption was observed in all the groups throughout the study period (Table-3).

In the hematological parameters, there was a marked increase in TLC and RDW was observed and also decrease in haematocrit value ($P<0.01$) was noted in the animals given 50mg/kg dose of *veera chenduram* (Table-4).

Similarly, from the biochemical analysis, the ALP, SGOT, SGPT levels were decreased significantly ($P<0.01$) in all the dose treated groups but there was no major modifications in the other biochemical parameters. A fall of blood glucose level was observed in the groups treated with 50mg/kg dose of *veera chenduram* (Table-5).

Table-6 shows that the urea, sodium concentrations are drastically increased ($P<0.01$) in the entire dose treated groups with respect to control.

The results of urine analysis indicate that the urine volume is gradually decreased in the dose dependent manner after *veera chenduram* treatment. In the same manner there was a slight alterations was observed in PH. The colour intensity of the urine is increased on the basis of drug dose range. In the urine collected from the 200mg/kg *veera chenduram* treated group few RBC, Pus and epithelial cells were seen (Table-8).

The isolated vital organ weights were changed significantly after 28 days of *veera chenduram* treatment in experimental animals. Particularly, liver, lung, spleen and stomach weights were decreased (Table-9).

HISTOPATHOLOGICAL CHANGES (200mg dose level)

1. **In brain :**

   oedema,

   microcytic degeneration.

   Asterocytic proliferation

2. **In Lungs:**

   Emphysematous changes

3. **In Liver:**

   Inflammatory cells around portal triad.

   Hepatocytes nuclei shows nuclear damage.

4. **In Stomach:**

   Congestion and erosion superficially.

5. **In Kidney:**

   Marked tubular necrosis

Table-1 **Incremental dose finding experiment and its Signs of Toxicity after oral administration of *Veera Chenduram* in rats.**

No	Treatment	Dose	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	I	5	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	II	10	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	III	50	+	+	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4	IV	100	+	+	-	+	-	+	-	-	-	-	+	-	_	+	-	-	-	-	+	-
5	V	250	+	+	+	+	-	+	-	-	-	-	+	-	_	+	-	-	-	-	+	-
6	VI	500	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+	-	-	+	-
7	VII	1000	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+
8	VIII	2000	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+

1. **Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Increased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Number of Deaths (Mortality**)

Table 1. Body wt (g) of albino rats exposed to *Veera chenduram* for 4 weeks.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	103.33±12.11	103.33±10.32	106.66±12.11	101.66±11.69	119.16±10.20
50	106.66±8.16	109.96±9.17	105±8.2	105±10.48	115±10.48
100	106.66±12.11	112.5±10.84	105±10.48	110.83±10.20	110±12.64
200	110.83±10.2	112.5±10.84	109.16±10.2	116.6±7.52	120±5.47

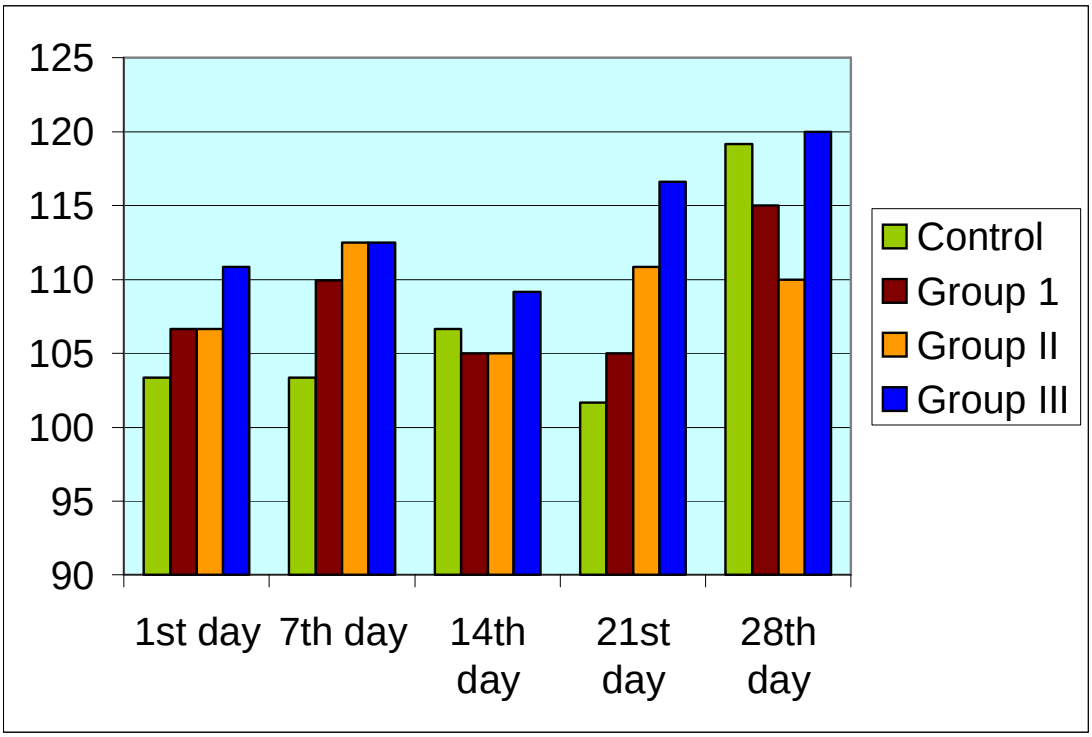


Table 2. Food (g/day) intake of albino rats exposed to *Veera chenduram* for 4 weeks.

Dose (mg/kg/day)	Days(gms/rats)				
	1	7	14	21	28
Control	40.66±2.73	44.16±2.85	45.33±2.94	43.16±2.48	48.33±2.58
50	38.66±2.65	43.16±5.26	38±2.09**	43±2.68	44.83±2.48
100	35.33±2.06	39.5±4.08*	32±2.89**	39.83±2.13	38.66±2.16**
200	30.33±1.86**	41±3.68	31.33±2.42**	39.83±2.40	40.5±3.88**

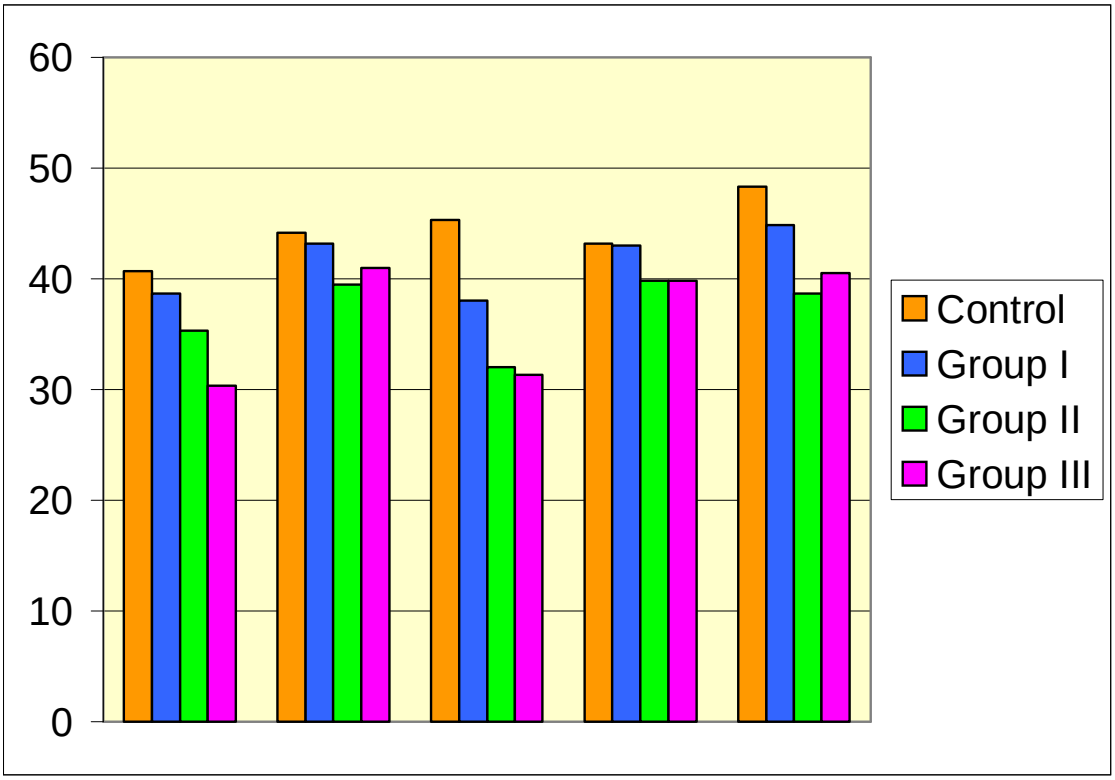


Table 3.Water(ml/day)intake of male and female albino rats exposed to *Veera chenduram* for 4 weeks.

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	80.33±2.42	60.33±2.42	60±2.09	53.10±2.48	50.33±1.86
50	73.66±2.25**	65±1.67**	50.5±1.76**	49.8±2.04NS	45.5±3.14**
100	70±2.09**	68.66±2.33**	49.66±1.36**	40.16±1.32**	44.84±2.04**
200	71.66±2.25**	69.83±2.04**	43.5±2.66**	50.33±2.42NS	45±1.67**

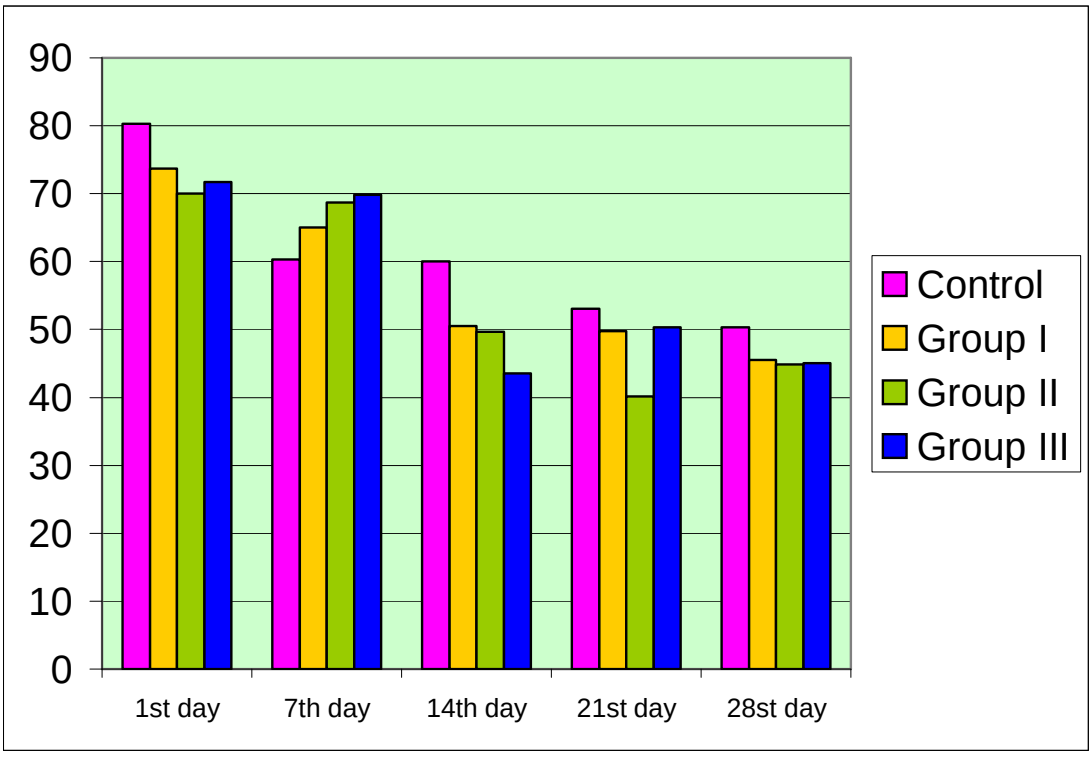


Table 4. Hematological parameters after 4 weeks treatment with the *Veera chenduram*

Parameter	Control	50mg/kg	100 mg/kg	200 mg/kg
Red blood cell (million/cumm)	7.84±0.54	7.98±0.33	7.98±0.26	8.06±0.31
HB gm/dl	50.26±1.09	50.51±0.53	50.03±0.34	50.31±0.36
Leukocyte ($x10^6$/cumm)	10427±601.34	18251±1134**	23566±1398**	27816±1424**
Platelets/µl	1149±148.20	1210±77.03	1139±84.02	1067±59.76
RDW	14.45±0.97	17.36±0.69**	16.05±0.68**	14.01±0.64**
MCV(fl)	61.7±0.36	54.50±0.71	55.65±4.72	56.14±4.74
DLC(%)				
N	6±1.54	7.16±0.98	6.5±1.04	6.5±1.04
L	90.33±1.63	90.66±1.75	90.83±1.94	90.33±1.63
M	2.5±0.54	0.5±0.54	1.66±0.81	1.5±0.54
E	1.160.75	1.16±0.98	1±0.63	1.66±1.03
B	0±0.00	0.5±0.54	0±0.00	0±0.00
ESR(mm/hr)	1.33±0.51	1.33±0.51	1±0.00	1±0.00
PCV(%)	48.42±3.47	43.47±2.48**	44.33±2.39*	45.32±3.57
MCH (pg)	19.45±0.19	19.44±0.74	18.84±0.71	18.98±0.93

Table 5. Effect of treatment with *Veera chenduram* biochemical parameters.

LFT

Parameter	Control	50mg/kg	100 mg/kg	200 mg/kg
Total Bilirubin(mg/dL)	0.21±0.04	0.25±0.05	0.16±0.05	0.18±0.07
Bilirubin Direct(mg/dL)	0.1±0.00	0.1±0.00	0.11±0.04	0.08±0.04
Bilirubin Indirect (mg/dL)	0.11±0.04	0.15±0.05	0.11±0.04	0.08±0.07
ALP (U/L)	230.5±9.75	295.66±12.86**	269±8.60**	244.83±13.36
SGOT (U/L)	178.83±27.6	146.5±11.67**	144.16±14.67**	127.16±10.64**
SGPT(U/L)	59.66±7.86	22.83±6.30**	13.83±1.72**	10.66±2.16**
Total Protein(g/dl)	8.35±0.49	8.01±0.38	6.1±0.60	5.38±0.64
Albumin(g/dl)	3.1±0.32	3.35±0.51	2.85±0.25	2.53±0.33
Globulin(g/dl)	5.25±0.70	4.5±0.84	3.25±0.37	2.85±0.31
GGT(U/L)	7.9±0.35	9.8±0.60	9.30±0.41	8.2±0.28
Blood glucose(mg/dl)	105.16±3.48	72.5±10.61**	109.83±5.77	98.33±9.41

Table-6 RFT

Parameter	Control	50mg/kg	100 mg/kg	200 mg/kg
Urea(mg/Dl)	59.46±2.57	176.85±12.75**	208.16±12.27**	240.16±10.32**
Creatinine (mg/dL)	0.65±0.10	2.53±0.48	2.96±0.14	3.13±0.25
Uric acid (mg/dL)	1.41±0.37	1.8±0.17	1.48±0.22	1.11±0.08
Na (m.mol/Lr)	130.76±11.45	147.16±9.26**	145.5±9.37**	148.66±14.16**
K (m.mol/Lr)	4.6±0.22	7.53±0.85	6.81±0.78	6.03±0.59
Cl (m.mol/Lr)	110.16±9.02	104±5.32	109.5±7.81	114.83±9.04

Table-7 LIPID PROFILE

Dose (mg/kg)	Control	50mg/kg	100 mg/kg	200 mg/kg
Total cholestrol(mg/dL)	37.5±4.08	42.66±3.55	40±1.41	37.33±3.77

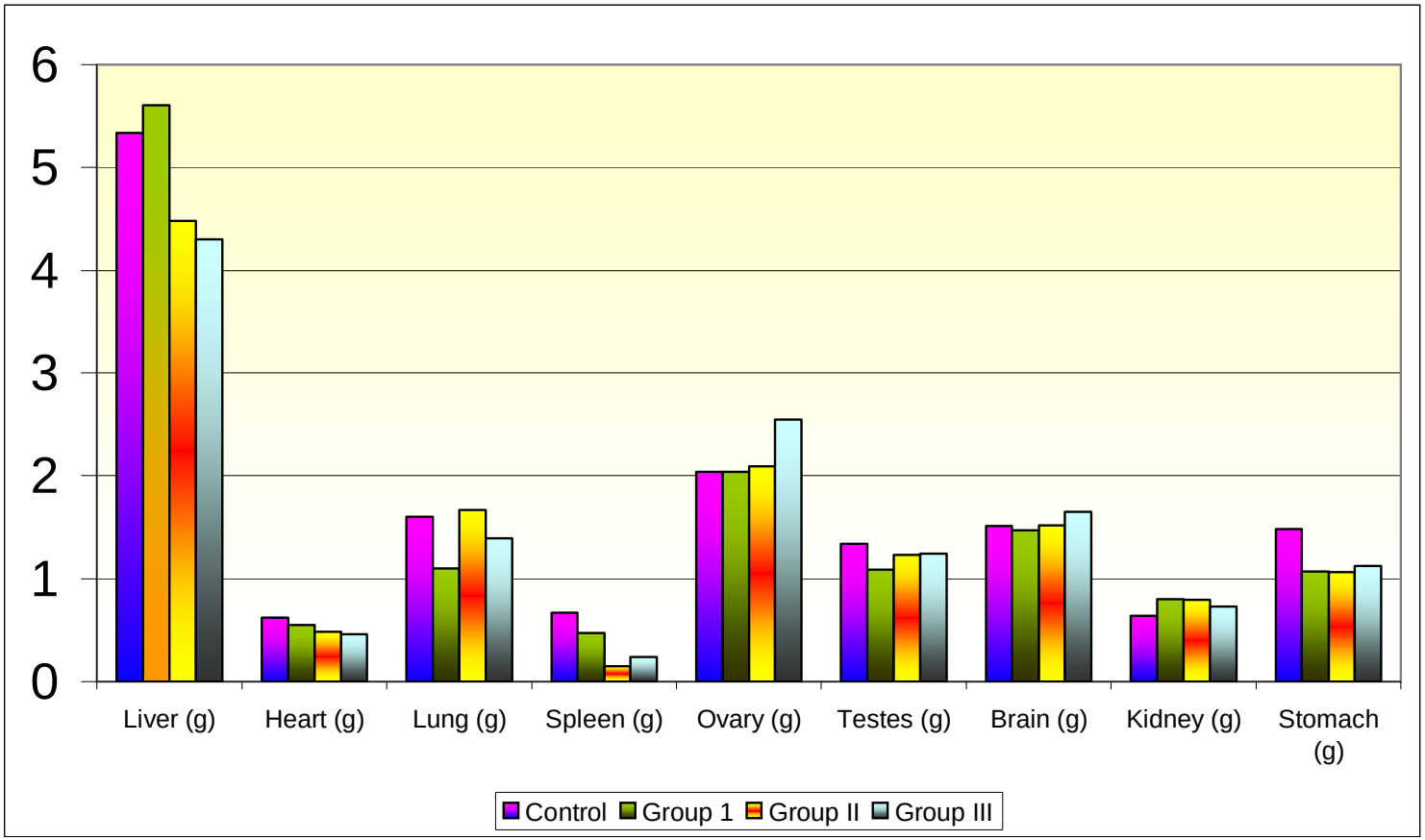
Table-8 URINE ANALYSIS

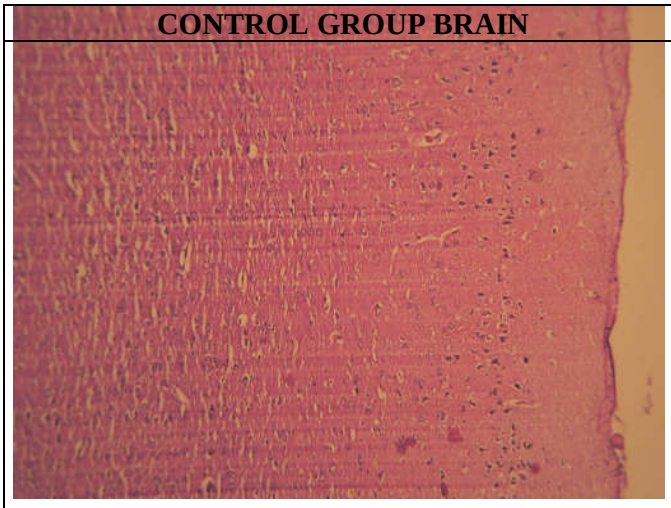
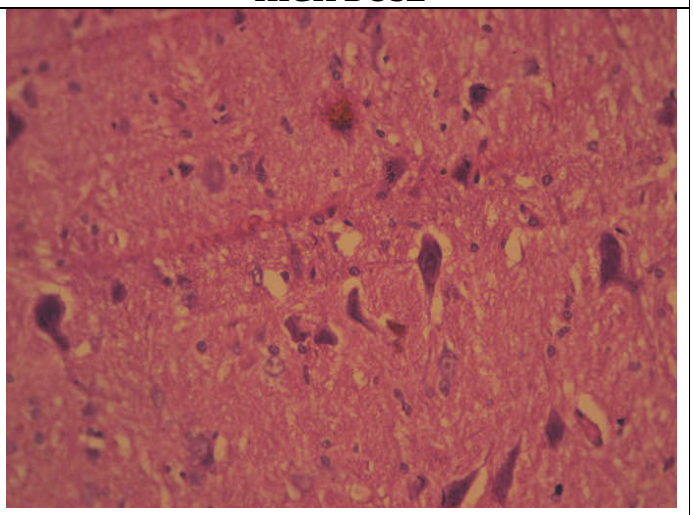
Parameters	Control	50mg/kg	100 mg/kg	200 mg/kg
Volume	2.8ml/24hr	1.8ml/24hr	1.2ml/24hr	1.4ml/24hr
Colour	Straw Yellow	Orange Yellow	Orange	Reddish Orange
Transparency	Clear	Clear	Clear	Clear
Specific gravity	1.010	1.010	1.010	1.010
PH	7.2	>7.8	>8.0	>8.0
Protein	Nil	Nil	Nil	Nil
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	-ve
Blood	Absent	Absent	Absent	Present
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	Nil	3-4cells/HPF	5-6cells/HPF	5-6cells/HPF
RBCs	Nil	Nil	Present	Present
Epithelial cells	1-2cells/HPF	1-2cells/HPF	2-3cells/HPF	2-3cells/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table 9. Effect of oral administration of a *Veera chenduram* on organ weight

Parameter	Control	50mg/kg	100 mg/kg	200 mg/kg
Liver (g)	5.34±0.07	5.61±0.14**	4.48±0.26**	4.3±0.15**
Heart (g)	0.62±0.04	0.55±0.02	0.48±0.08	0.46±0.03
Lung (g)	1.60±0.07	1.10±0.14**	1.67±0.20	1.39±0.09
Spleen (g)	0.67±0.04	0.47±0.02	0.15±0.03**	0.24±0.04**
Ovary (g)	2.04±0.05	2.04±0.10	2.09±0.11	2.55±0.42**
Testes (g)	1.34±0.12	1.09±0.09*	1.23±0.07	1.24±0.07
Brain (g)	1.51±0.08	1.47±0.11	1.52±0.04	1.65±0.11
Kidney (g)	0.64±0.05	0.80±0.08	0.79±0.07	0.73±0.07
Stomach (g)	1.48±0.10	1.07±0.07**	1.06±0.08**	1.12±0.07**

**P<0.01; *P<0.05. N=6 *Values are mean ± S.D. (One way anova followed by Dunnett's test).*


6
5
4
3
2
1
0
Liver (g)
Heart (g)
Lung (g)
Spleen (g)
Ovary (g)
Testes (g)
Brain (g)
Kidney (g)
Stomach (g)
Control
Group 1
Group II
Group III

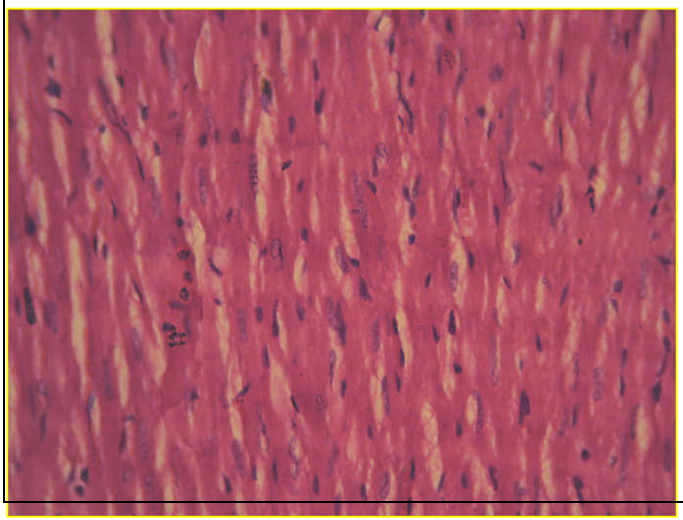
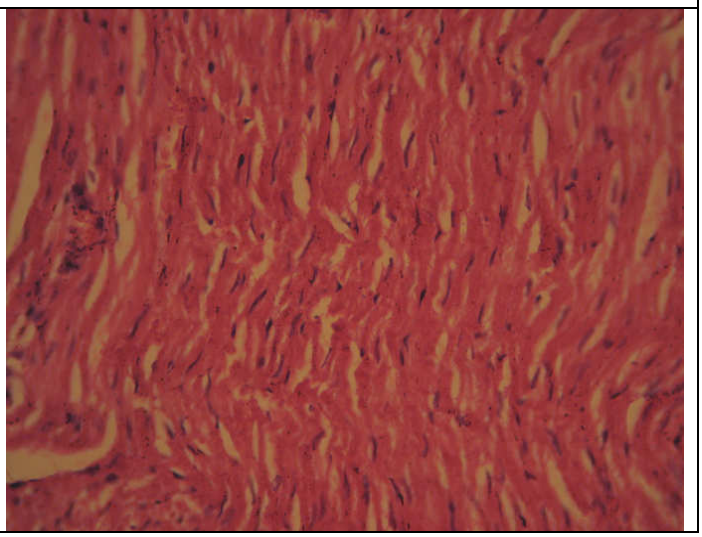
CONTROL GROUP BRAIN	HIGH DOSE
	

Changes in brain

Oedema

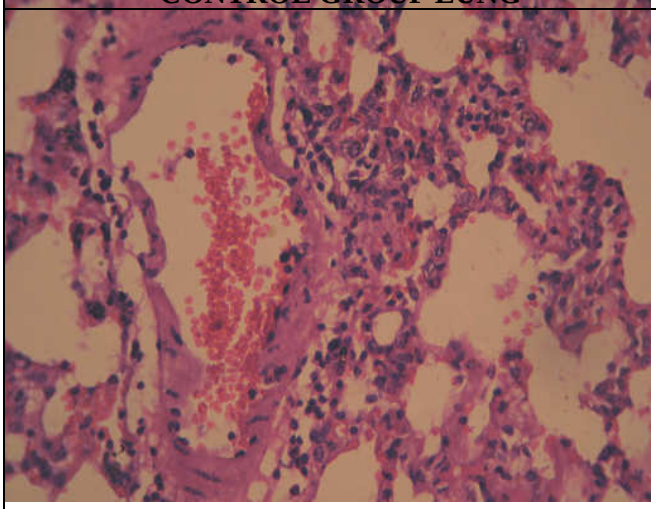
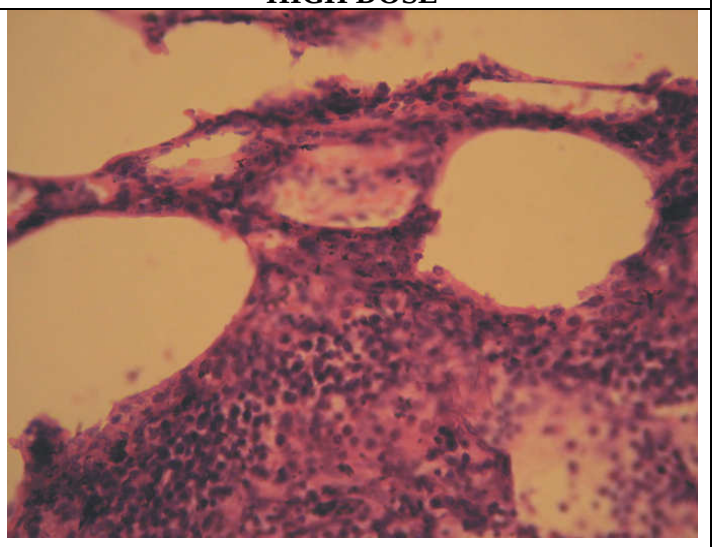
Asterocytic proliferation

Degenerative changes (nural fibre damage)

CONTROL GROUP HEART	HIGH DOSE
	

Changes in Heart

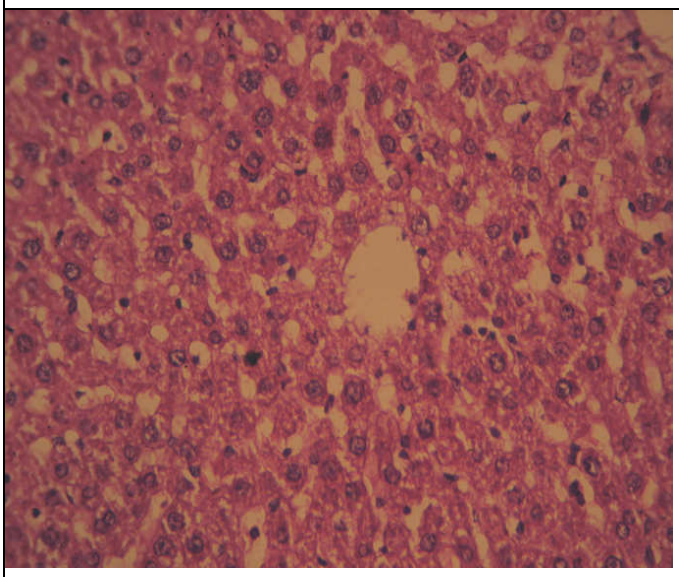
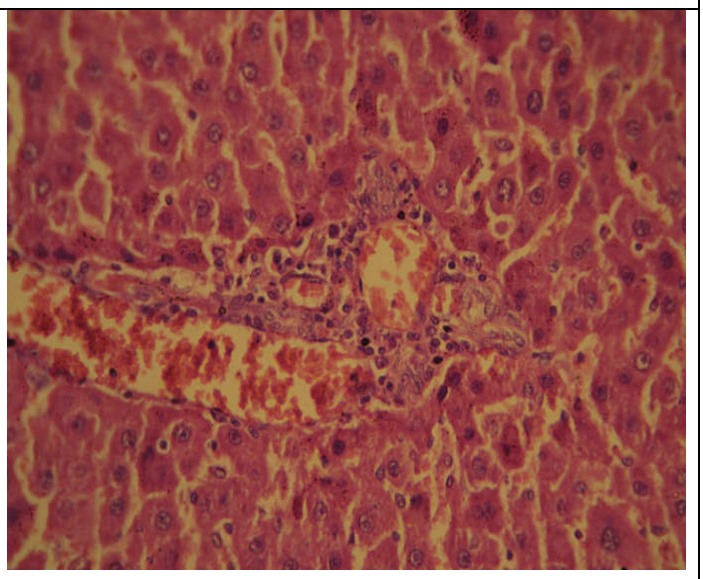
No Changes (Normal)

CONTROL GROUP LUNG	HIGH DOSE
	

Changes in Lung

Interstitial Fibrosis

Emphysematous changes

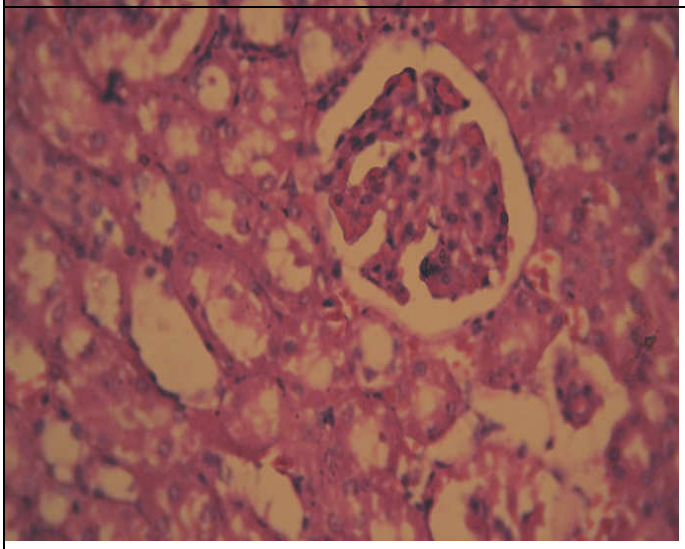
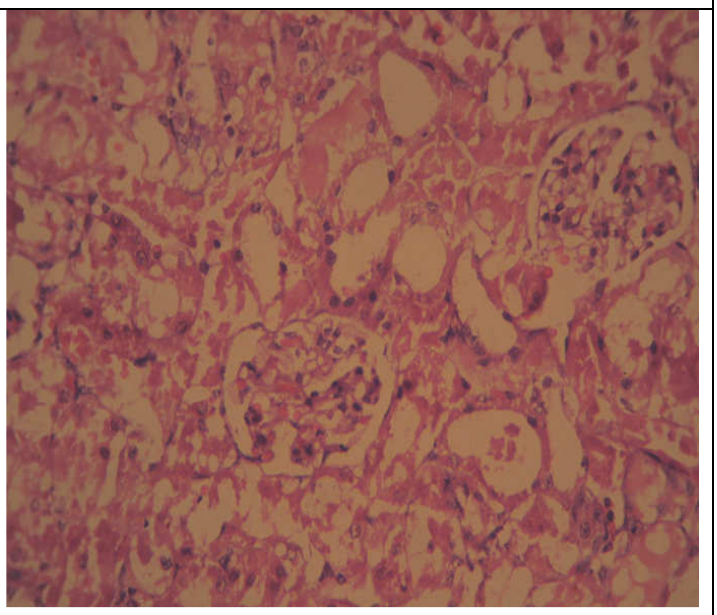
CONTROL GROUP LIVER	HIGH DOSE
	

Changes in Liver

Inflammatory cells around portal triad

Hepatocytes show condensed nucleus

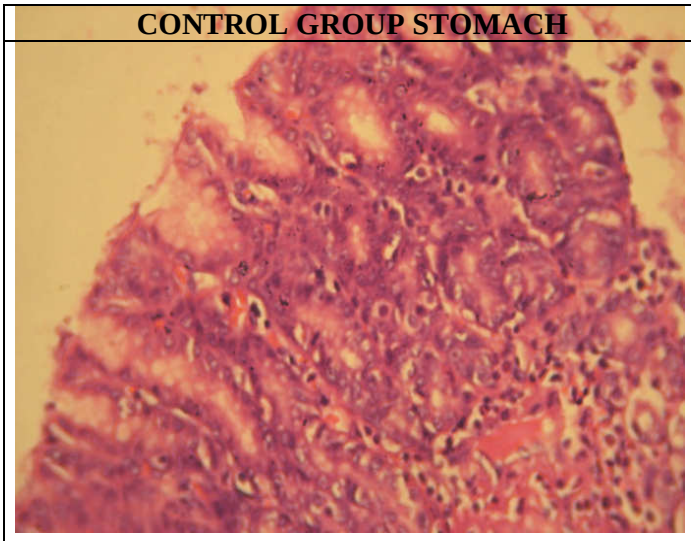
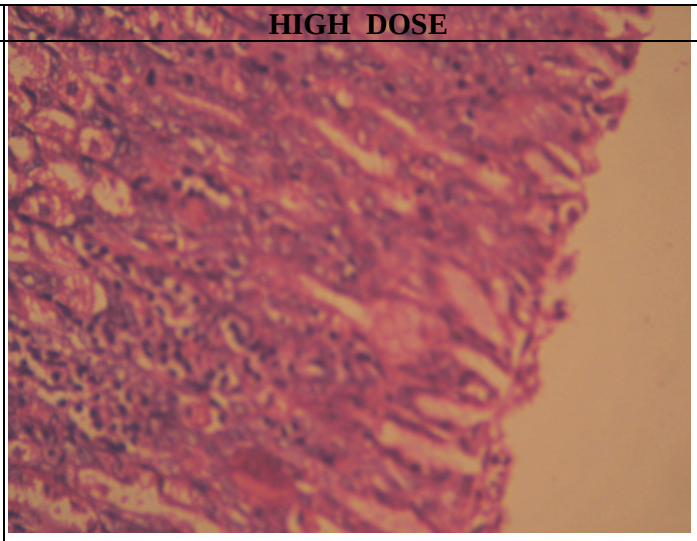
Congested vessels

CONTROL GROUP KIDNEY	HIGH DOSE
	

Changes in Kidney

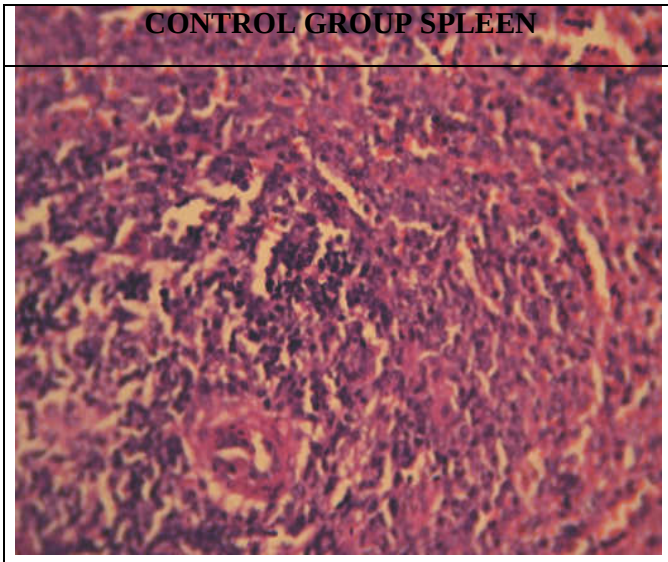
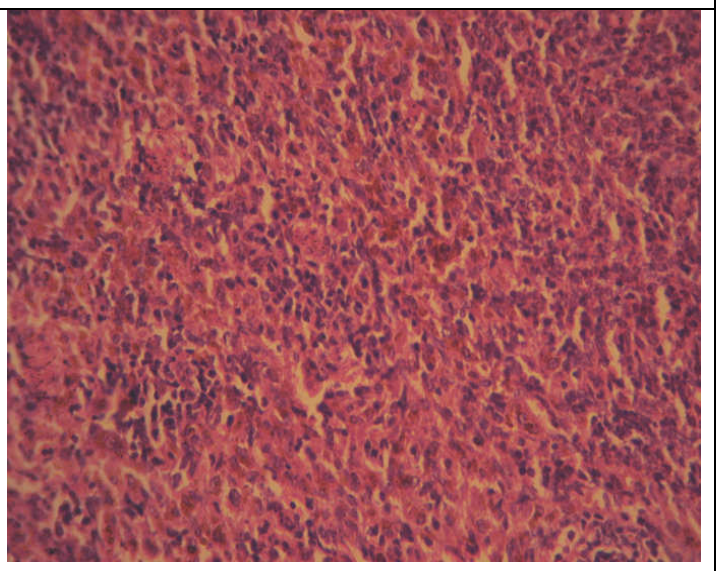
Marked tubular necrosis

Haemorrhage

CONTROL GROUP STOMACH	HIGH DOSE
	

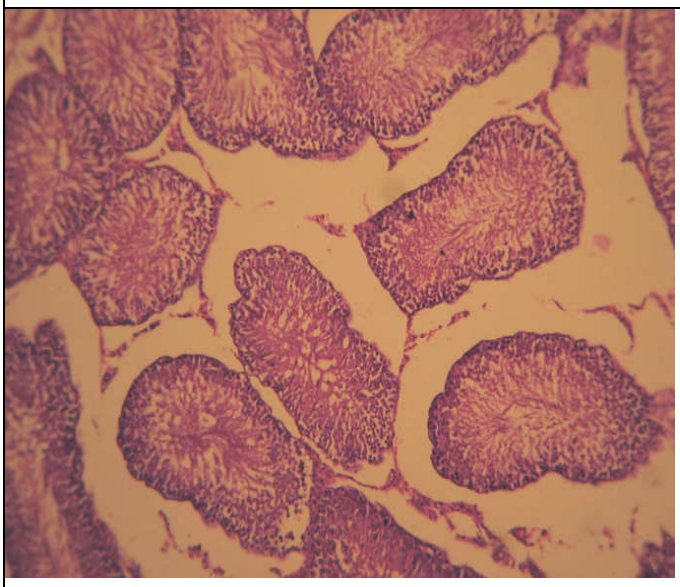
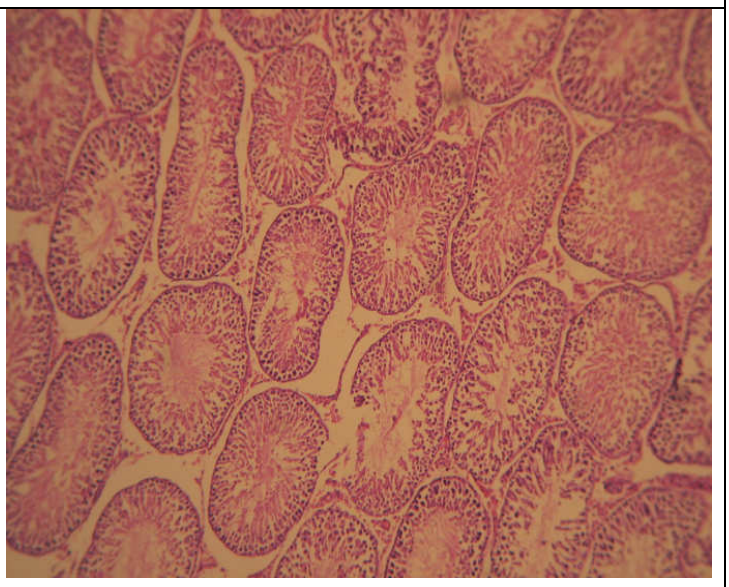
Changes in Stomach

Congesion and erosion superficially.

CONTROL GROUP SPLEEN	HIGH DOSE
	

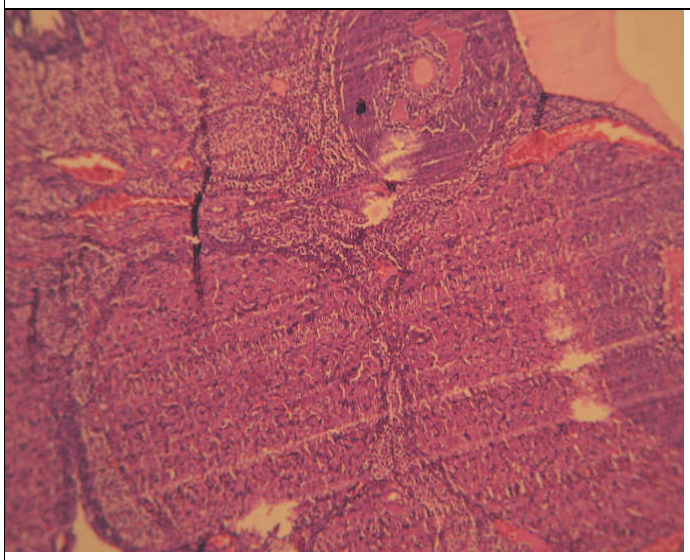
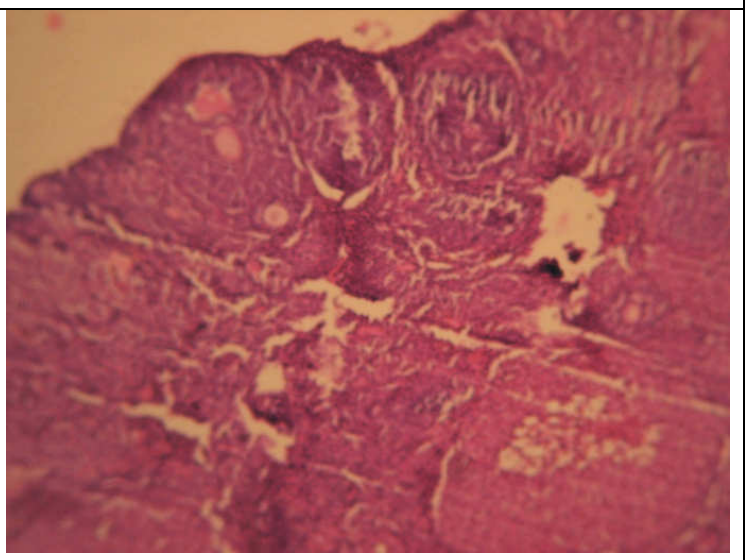
Changes in Spleen

No Changes (Normal).

CONTROL GROUP TESTES	HIGH DOSE
	

Changes in Testes

No Changes (Normal).

CONTROL GROUP OVARY	HIGH DOSE
	

Changes in Ovary

No Changes (Normal).

DISCUSSION

In this study ***Veera Chenduram*** was analyzed in chemical and toxicological aspects.

The results of acute and sub acute toxicity study revealed that the treatment of *veera chenduram* on rats possess significant changes in general behavioural pattern and produced major signs of toxicity from the dose level of 500mg/kg and above in an acute exposure. The animals showed changes in general behaviour other physiological activities like giddiness, sniffing, aggressiveness, tachypnoea, convulsion finally at the dose level of 1g/kg.

In the sub acute toxicity study, after 28 days of alternate day treatment of *veera chenduram* in single oral dose showed insignificant body weight changes (Table-1) during the experimental period. But significant ($P<0.01$) increase in food intake was observed in all the groups after one week of drug treatment (Table-2). There was a gradual decrease in water consumption was observed in all the groups throughout the study period (Table-3).

In the haematological parameters, there was a marked increase in TLC and RDW was observed (Table-4).

Similarly, from the biochemical analysis, the ALP, SGOT, SGPT levels were decreased significantly ($P<0.01$)in all the dose treated groups but there was no major modifications in the other biochemical parameters. A fall of blood glucose level was observed in the groups treated with 50mg/kg dose of *veera chenduram (*Table-5).

Table-6 shows that the urea, sodium concentrations are drastically increased ($P<0.01$) in all the dose treated groups with respect to control. There was no effect on total cholestrol. The results of urine analysis indicates that the urine volume is gradually decreased in the dose dependent manner after *veera chenduram* treatment. In the same manner there was a slight alterations was observed in PH. The colour intensity of the urine is increased on the basis of drug dose range. In the urine collected from the 200mg/kg *veera chenduram* treated group few RBC, Pus and epithelial cells were seen (Table-8). The isolated vital organ weights were changed significantly after 28 days of *veera chenduram* treatment in experimental animals. Particularly, liver, lung, spleen and stomach weights were decreased (Table-9).

In histopathological studies the brain and kidney were mostly affected. Stomach shows superficial erosion.

So, based on the results it can be concluded that the *veera chenduram* falls under the category of drug with high toxicity and it can be suggested that the use of *veera chenduram* clinically for long term therapy may cause severe toxic symptoms like liver damage, respiratory ailments and impotence in females. Therefore in conclusion this study presents strong evidence of the toxic effect of the *Veera chenduram*. These results showed that the use of the Veera chenduram is not safe and suitable for the extensive utilization or for longterm therapy even at the dose level of 50mg/kg clinically.

SUMMARY

Veera Chenduram a widely used Siddha medicine contains mercuric chloride. This is used for the treatment of several ailments.

Veeram purchased from local market. The drug was prepared as per Siddha literature. First the prepared drug was studied electrochemical analysis (XRD) studies.

After the chemical study the test drug were studied for toxicity. During the acute toxicity study the animals fasted overnight were given single oral dose of ***Veera Chenduram*** (5 to 2000mg /kg body wt) suspended in water. Animals of the control group received only water (2ml). Death was recorded during the treatment period in treated groups given 1kg/ kg of ***Veera Chenduram*** orally. The treatment of ***Veera chenduram*** on rats possess significant changes in general behavioral pattern and produced major signs of toxicity from the dose level of 500mg /kg and above in an acute exposure.

The sub acute toxicity was studied in 28 days as per OECD Guidelines 407. During the period of administration the animals were weighed and food and water intake were monitored. After 28 days all surviving animals were fasted overnight and collected 24 hours urine. Animals were sacrificed by decapitation and blood samples were collected for lab investigation. The Brain, Lung, Liver, Pancreas, Spleen, Stomach Testes, Ovary, Heart and Kidney were collected and weighed. Histo pathological examinations of the tissue samples were taken from these organs. The treated rats revealed some pathological lesions. The brain and kidneys were mostly affected.

CONCLUSION

In conclusion, these findings suggest that the ***Veera Chenduram*** is practically toxic or lethal after an acute exposure at the dose level of 1g/kg. The ***Veera Chenduram*** affects almost all the vital organs at the maximum dose range of 200mg/kg. The normal human dose is very minimal to compare the study group doses. If given the test drug as per literature do not produce toxicity. Hence, to avoid the major adverse reactions the duration of treatment is minimized.

Because of an ideal man of 70 k.g. body weight requires 4mg of the drug two times daily. But rats body weight are approximately 100 – 120gms. We have given 50mg /kg, 100mg/kg, 200mg/kg daily. These doses are more than human dose. So we can come to conclusion that the drug was in normal dose it might not produce any pathological changes.

The aim of giving such a high dose was to find out the type of toxicity if the drug was given in abnormal high dose. This toxicity due to overdose could occur in a patient, if proper dose is not prescribed by the physician or followed by the patient.

So the recommended human dose may not produce any ill effects and this must be proved by further animal studies and also by clinical studies by volunteers. This dissertation work is the first step for continuous research in this title.

BIBLIOGRAPHY

1. அகத்தியர் ஏம தத்துவம் என்னும் பஞ்ச காவிய நிகண்டு, 2 ஆம் காண்டம்

   எஸ்.பி.இராமச்சந்திரன், தாமரை நூலகம், சென்னை 26.

2. அகத்தியர் வைத்திய காவியம் 1500

   எஸ்.பி.இராமச்சந்திரன், தாமரை நூலகம், சென்னை 26.

3. அனுபோக வைத்திய நவநீதம் பாகம் 4

   ஹக்கிம்.பா.மு.அப்துல்லா சாய்பு, தாமரை நூலகம், சென்னை 26.

4. குணபாடம் தாது சீவ வகுப்பு

   மரு. இரா.தியாகராஜன் எல்.ஐ.எம்., இந்திய மருத்துவம் மற்றும் ஓமியோபதித் துறை, சென்னை 106.

5. சட்டம் சார்ந்த மருத்துவமும் நஞ்சு மருத்துவமும்

   மரு.ப.அ.முகம்மது இக்பால், எம்.டி(சித்தா)

6. சாம்பசிவம் பிள்ளை அகராதி II

7. சித்த வைத்திய பதார்த்த குண விளக்கம் (தாது மற்றும் சீவ வகுப்பு)

   சி.கண்ணுசாமிப்பிள்ளை, பி.இரத்தின நாயகர் அண்ட் ஸன்ஸ், சென்னை 79.

8. நஞ்சு முறிவு நூல்

   க.ச.முருகேச முதலியார்

9. போகர் நிகண்டு 1200

   எஸ்.பி.இராமச்சந்திரன், தாமரை நூலகம், சென்னை 26.

10. Hand book of Chemistry and Physics

    David R. Lide, 86th edition

11. The Elements,

    John emsley, 3rd edition

12. Medical Toxicology

    Richard c. Dart.

13. Cinical Toxicology

    Ford, Delaney, Ling and Erickson

14. ComprehensiveToxicology

I.Glenn sipes, Charlene A. Mcqueen, A. Jay Gandolfi.

15. Toxicology

Hans Marquardt, Siegfried G Schafer, Roger O Mcclellan.

16. MODY'S Medical Jurisprudence and Toxicology

K.Mathiharan & Amrit K.Patnoik.

17. The Essentials of Forensic Medicine and Toxicology

K.S.N.Reddy.

18.Dacie JV Lewis S (1991). Practical Hematology 7th edition Churchill Livingstone New York. pp. 50-56.

19.Gornall AC Bardawill RJ David MM (1949). Determination of serum proteins by means of buiret reaction. J. BioChem. 177: 751-762.

20.Lamb GM (1981). Manual of veternary techniques in Kenya published by CI A-GEGy. pp.100-100.

21.Lorke D (1983). A new approche to acute toxicity testing . Arch. Toxicol. 54. 275-287

22.Lowry OH Robert NR Wu MI Hixon WS Crawford EF (1954). The quantitative histochemistry of brain. II Enzyme measurement. J. Biol. Chem. 207: 19.

23.Sacher RA Mcpherson RA (1991). Widmann's clinical Interpretation of Laboratory Test. Pennsylvania. USA. pp .416-443

24.Zlatkis A Zak oyle JA (1952). A new method for the Direct determination of serum cholesterol. J. Lab. Clin. Med. 41: 496-492.

25. Witthawasku P Ampai Panthong Kanjanapothi D Taesothikul T Lertprasertsuke (2003). Acute and subacute toxicities of saponin mixture isolated from Schefflera leucantha Viguier. J. thnopharmacol. 89: 115-121.