PRECLINICAL SAFETY EVALUATION OF VISHATHIRKU POORA MATHIRAI

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"PRECLINICAL SAFETY EVALUATION OF VISHATHIRKU POORA MATHIRAI ''** is a bonafide and genuine research work carried out by me under the guidance of **Dr.R.MADHAVAN, M.D(S)**., Head of the Department, Department of Nanju Maruthuvam, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

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Certified that I have gone through the dissertation submitted by **Dr.R.PALRAJ (Reg No:321716201)** a student of final year M.D(s), Branch-VI, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai - 47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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CONTENTS

1. INTRODUCTION:

The Siddha system of medicine is one of the ancient systems. The unique nature of this system is its continuous service to humanity for more than 5000 year is combating diseases and in maintaining its physical, mental and moral health[(1)]. The word "Siddha" comes from the word Siddhi which means "an object to be attained" or "Perfection" or "heavenly bliss". Those who attained or achieved the Siddhi are known as Siddhars. The first Siddhar is considered as Lord Siva. He taught Siddha to his consort Parvathi and in turn she handed it down to Nandhi who had taught the Siddhars such as Agasthiyar, Bogar, etc... The sage Agasthiyar is considered as a father figure of siddha medical system [(2)].

According to their mode of application the Siddha medicine could be categorized into two classes

***Internal medicine**s* - which are classified in to 32 categories

External medicines - It is also classified in to 32 categories [(1)].

The internal medicine in this system is prepared from raw material of herbs, metals, minerals and animal products. In our Siddha system of medicine we had thousands of medicine with the indications for Toxic bites .Among various medicines mentioned to treat Toxic bites in Siddha literature. *"**Vishathirku Poora Mathirai**"* is one of the formulations from **"Ramadevar Ennum Yakobhu eluthiya Vaithya Sindhamani 700"**. The Ingredients of "Vishathirku Poora Mathirai" are Rasam (Mercury), Gandhagam (Sulphur), Pooram (Calomel) and Kuppai meni leaf juice (*Acalypha indica*). It is used to treat Toxic bites [(3)].

According to WHO, worldwide, up to five million people who were bitten by snakes cause considerable morbidity and mortality. There was an estimated

2.4 million envenomations (poisoning from snake bites) and 94000-125000 deaths annually, with an additional 400000 amputations and other severe health consequences such as infection, tetanus, scarring, contractures and psychological sequel. The majority of the snake bites occur in Africa and South East Asia [4].

The uses of scientific tools are essential to validate the traditional claim. Though Siddha drugs are considered to be safe and effective, it is the utmost duty of the physicians to standardizing the Siddha prepared medicine before trying out in human being. So far there is no scientific rationale carried out for its toxicity profile, hence I proposed to take Vishathirku Poora Mathirai for my dissertation study to evaluate the toxicity profile in animal model through acute and 90 days repeated oral toxicity studies as per OECD guideline 423 and 408. Thus this study will help in further extension of the therapeutic potential of Vishathirku Poora Mathirai.

2. AIM & OBJECTIVES

AIM:

To evaluate the safety profile of "**Vishathirku Poora Mathirai"** **– A** Herbo Mineral preparation

OBJECTIVES:

Primary Objectives:

- To evaluate the safety profile of Vishathirku Poora Mathirai through toxicity studies as per OECD guideline 423 & 408.

Secondary Objectives:

- Collect the literature review of ingredients of Vishathirku Poora Mathirai.
- Authentication and Purification of ingredients of Vishathirku Poora Mathirai.
- Preparation of the Medicine as per literature.
- To study the Physico chemical, Biochemical analysis and Phyto chemical analysis of Vishathirku Poora Mathirai,
- To study the Heavy metal analysis of test drug.

3.REVIEW OF LITERATURE – SIDDHA ASPECT:

இரசம்

வேறு பெயர்கள்:

தசாங்க நிகண்டில் கூறியவை:

காரம், சூதம், புண்ணியம், கற்பம், சாமம், சத்து, சூரிய விரோதி, சாதி, சூத்திரன், துள்ளி, ஈசன், வீரியம், சூழ்ச்சி, நீர், விண்மருந்து, இரதம், சுக்கிலம், போகம், மூலம், சிந்தூரம், சிந்து, பக்கிரம், பாரதம் மற்றும் கனல் பூதம்.

சட்டமுனி நிகண்டில் கூறியவை:

இனிமை, சிவசக்தி, தனிமை, பனிமை, கனிமை, சிவன் விந்து, கேசரி, சரக்கில் கலந்திடு சீவன், சங்கரன் விந்து, காவன், பாவன், ஆதி, சுந்தரம், தூமம், மந்தரம், மஞ்சி, மாருதம், விந்தரம், சிலை, கணவன், மலைக்குரவன், கந்தன் மற்றும் காவக்குடியோன்.

இரசத்தின் பிரிவுகள்:

1. இரசம்
2. இரசேந்திரன்
3. சூதம்
4. மிசரகம்
5. பாரதம்

சுவை:

அறுசுவை. சிறப்பாய் இனிப்பு.

வீரியம்:

வெப்ப, சீத வீரியம் இரண்டும் உடையது.

பிரிவு:

எப்பொருளை இதற்கு துணை மருந்தாய் கொடுக்கிறோமோ அப்பொருளின் பிரிவை அடையும்.

செய்கை:

உடல் தேற்றி

உடல் உரமாக்கி

மலம் போக்கி

பித்த நீரகற்றி

வீக்க முறுக்கி

உமிழ் நீர் பெருக்கி

சிறுநீர் பெருக்கி மற்றும்

மேக நாசினி

மகிமை:

இது சீதளத்தால் உண்டாகும் நோய்களின்றி வெப்பத்தல் உண்டாகும் பிணிகளுக்கும் வெளியாட்சி மற்றும் உள்ளாட்சி எல்லாவற்றிற்கும் தலைசிறந்தது.

பாதரசம் பொதுகுணம்:

"விழிநோய் கிரந்திகுன்மம் மெய்ச்சூலை புண்குட்

டழிகாலில் விந்துவினால் அத்தை- வழியாய்

புரியு விதி யாது புரியினோ யெல்லாம்

இரியுவிதி யாது மில்லை"

பொருள்

சிவ வீரியம் என்கின்ற இரசத்தை முறைப்படி மருந்தாக்கிக் கொடுக்க அது கண்ணோய், கிரந்தி, எண்வகை குன்மம், சூலை, பெரும்புண், தொழு நோய், வளிக்குறைவு முதலிய நோய்களை நீக்கும்.

நற்குணம்

குருதியை சுத்தி செய்து துர்நீரை நீக்கல்,

குருதியையும் சுக்கிலத்தையும் பெருக்கல்

பசியை தூண்டல்

கிருமிகளை கொன்று புண் புரைகளை ஆற்றுதல்

முக வசிகரத்தை உண்டாக்குதல்

மறதியை ஒழித்து மூளைக்கு கவன சக்தியை தரல்

நரம்பு கூட்டங்களை வன்மையுறச் செய்தல்

மனதை ஒருநிலையில் நிறுத்தி ஞானத்தை விருத்தி செய்தல்

மூப்பை ஒழித்து ஆயுளை வளர்த்தல்

தீக்குணம்:

இரசத்தை சரியான முறையில் முடித்து உண்ணாததால் வரும் குற்றங்கள் அநேகம் அவை இரச தோடம் எனப்படும். இரச தோடம் இரண்டு வகைப்படும் அவை

1. 8 வகை தோடம் மற்றும்

2. 7 வகை சட்டை ஆகும் இவற்றை தகுந்த முறையில் சுத்தி செய்து தோடங்களை நீக்கி மருந்திற்கு பயன்படுத்த வேண்டும்.

இரச சுத்தி முறைகள்:

1 பலம் இரசத்திற்கு தும்பை சமூலச்சாறு 4 பலம் விட்டு சூரிய புடம் வைக்கவும் 10 நாட்கள் சாறு விட்டும் பிறகு சாறு விடாமல் வெயிலில் வைத்து எடுக்கவும். பின்னர் இத்தூளை 2 படி தும்பை சாறு விட்டு கவசம் செய்து பூமியில் 20 நாள் புதைத்தெடுத்து நீர் விட்டு கழுவி கொள்ளவும். இவ்வாறு சுத்தி செய்த இரசம் பற்பம் செந்தூரம் குருகுளிகை முதலியவற்றிற்காகும்.

1 பலம் இரசத்தை செங்கல் தூளிலும் மஞ்சள் பொடியிலும் ஒவ்வொரு மணி நேரம் ஆட்டி சுத்த நீரில் அலம்பி, 1 படி மேனி சாற்றில் இட்டு அடுப்பேற்றி சாறு சுண்டும்வரை எரித்து எடுக்க சுத்தி ஆகும்.

வேண்டியளவு இரசத்தை தூயகெட்டித் துணியிலிட்டு 1000 முறை பிழிந்தெடுக்கவும்.

விதை நீக்கின மிளகாயில் இரசத்தை விட்டு கோவை இலையை அரைத்து 4 விரற்கடை கனம் கவசித்து சீலைமண் 7 செய்து குக்கிடப்புட மிட்டெடுக்க சுத்தியாகும்.

பத்தியம்:

இரச சம்மந்தமான மருந்துகள் அருந்துங்காலத்து மீன், உப்பு, மிகு சீதம், மிகு வெப்பம், மந்தப்பொருள், எண்ணெய், மதுபானம், கைப்பு, கார்ப்பு. புளிப்பு, பெண்போகம் ஆகாது.

இரச நஞ்சு பொது குறிகுணங்கள்:

தூய்மை செய்யாததும் நன்றாக முடிக்க பெறாததும் அளவுக்கு அதிகமான இரசத்தை உண்பதனால் குற்றம் உண்டாகும். வாயில் அட்சரத்தை போல புண் உண்டாகும். பனங்கள்ளை போல் வாய் குழம்பும். வாய் தொண்டை இவை வெந்து வீங்கும். வயிறு குடல் இவை புண்ணாகும், பக்கத்தில் அடிக்கடி வலித்துக்கொண்டேயிருக்கும், வயிற்றின் மீது பட்டை பட்டையாய் தேமல் படரும், காலடியில் வெடிப்பு கண்டு விட நீர் கசியும். காது செவிடுபடல், கண் பார்வை இழத்தல், சிரங்குப்புண், செம்படை போல் உடலில் படைகள் உண்டாகும், பித்தன்

போல் வாய் பிதற்றும், துணிகளை கிழித்து அவிழ்த்து எறியும், கல்லால் அடிக்கவும் மலையேறி குதிக்கவும் தண்ணீரில் விழுந்து முங்கி முங்கி வெளி வரவும், வியர்வை பெருகும் ஆகிய குறிகுணங்களை காட்டும்.

இரச நஞ்சு முறிவு:

குண்டிக் காயை பிடித்தக்கால் சாயப்பட்டையை பொடித்து வெல்லத்துடனே கலந்து கொடுக்க வேண்டும்.

பல்லுக்கிட்டினால் கோவைத்தண்டுச்சாற்றை நாக்கில் பிழிய தீரும்.

நெஞ்சு வற்றி உடல் உள் வெதும்பி மேல் எரிவு எடுத்து கை கால் மண்டி எரிந்து நினைவில்லாமல் கிடந்தால் அறுகங்கிழங்கை ஆய்ந்து எடுத்து அரைத்து வெள்ளாட்டுப்பால் பசும்பால் பருத்திக் கொட்டைப்பால் மோர் இவைகளில் ஏதாவதொன்றில் கரைத்து வடிகட்டி கொடுக்கவும்.[5]

அவுரி வேர்ப்பட்டையை வெந்நீர் விட்டு அரைத்து சுண்டைக்காயளவு காலை மாலை 3 நாள் கொடுக்க வேண்டும்.

வெள்ளை முட்சங்கன் இலைச்சாறு அல்லது மிதிபாகலிலைச் சாறு இவைகளில் ஏதாவதொன்றை 80 மி.லி வீதம் காலை மாலை 3 நாள் கொடுக்கவும்.

துளசிவேர்ப்பட்டைக் குடிநீர்

கருவேல் கொப்புளிக்குடிநீர்

எருக்கு கொப்புளிக்குடிநீர்

சுரைக்கருப்பு

தயிர்வெல்லம்.[6]

சேரும் மருந்துகள்:

குப்பிச் செந்தூரம்.[7]

சுயமாக்கினி செந்தூரம்.[8]

சுர மாத்திரை.[8]

சந்திரோதய குளிகை.[9]

பாடாண மாத்திரை.[9]

இரச பற்பம்.[10]

இரச செந்தூரம்.[10]

திரிநேத்திர மெழுகு.[10]

சஞ்சீவி குழம்பு.[11]

பரஞ்ஜோதி மாத்திரை.[11]

மலைக்காத்தான் குளிகை.[11]

சகல விடத்திற்கு இரத்தின மை.[12]

விஷக்கடிக்கு நாபிக் குழம்பு.[12]

விஷக்கடிக்கு இரசாதிக் குழம்பு.[12]

கந்தகம்

வேறுபெயர்:

காரிழையின் நாதம், சக்தி, பரைவீரியம், பீஜம், செல்வி விந்து, நாற்றம், பரைநாதம், சக்தி பீஜம், பொன்வர்ணி.

பாடாணங்கள் அறுபத்து நான்கில் பிறப்பு கந்தகம், வைப்பு கந்தகம், கோழித்தலை கெந்தி வைப்பு, வாண கெந்தி வைப்பு என்று நான்கு வகை பாடணங்கள் கூறப்பட்டுள்ளது

கந்தகத்தின் வகைகள்:

வெண்மை நிறம்- எல்லா நோய்களையும் போக்கும்.

கிளி மூக்கு சிவப்பு நிறம்- நவலோகத்தை ஏமமாக்கும்

பொன்மை நிறம்- நெல்லிக்காய் போன்று இருக்கும் சூதகத்துடன் கலந்து உறவாகி சுத்தமாய் இருக்கும்.

காகத்தின் நிறம்- நரை திரை அற்றுப் போகும்.

பதார்த்த குண சிந்தாமணியில் வாண கந்தகம், நெல்லிக்காய் கந்தகம் ஆகியவற்றின் குணம் கூறப்பட்டுள்ளது.மருந்துகளில் கையாளப்படுவது நெல்லிக்காய் கந்தகமாகும்.

நட்புச் சரக்கு:

"கந்தகத்தின முமிரசந்தா னென்றாரே"

கந்தகத்தின் நட்புச் சரக்கு இரசம்

பகைச் சரக்கு:

"கொல்லுமே தாம்பிரத்தை கெந்தி கொல்லும்"

கந்தகத்தின் பகைச் சரக்கு செம்பு

சுவை:

கைப்பு, துவர்ப்பு,

செய்கை:

மலமிளக்கி,

உடல் தேற்றி,

வியர்வை பெருக்கி,

கிருமிநாசினி,

குணம்:

சிறிய அளவில் கந்தகத்தை அருந்த அது உடம்பில் சேர்ந்து வியர்வை, பால், சிறுநீர் இவற்றில் வெளிப்படும், விரேகியில் சிறப்பாக செயல்படும். அதிக அளவில் அருந்த பேதியை உண்டு பண்ணும்.

நெல்லிக்காய்க் கந்தகத்தின் பொது குணம்:

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டமந்தம்

வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா

விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி

திடக்கிரக ணீகபம்போந் தேர்"

நெல்லிக்காய்க் கந்தகத்தினால் பதினெண்குட்டம், மந்தம், கல்லீரல் வீக்கம், பெருவயிறு வகைகளுள் ஒன்றாகிய கவிசை, குன்மம், கொடுமையை செய்கின்ற விடக்கடிகள், நாட்பட்ட மேக நோய்கள், நாட்பட்ட கிராணி முதலியன தீரும்.

வாண கந்தகத்தின் குணம்:

"வாணக் குழாய்க்கந்தி வாசனையைக் கண்டவுடன்

காணக் கிருமி சொறி காணாவாம் - தோணும்

பெருவியா திக்கூட்டம் பேருமத நூலின்

மருவியா ழுங்கொடியே வாழ்த்து"

இரத்த தாதுக்களில் பிறந்தக் கிருமிகள், சொறி, குறைநோய்க் குட்டங்கள், நாட்பட்ட கீல் வாதம், சுவாச காசம், மாரடைப்பு, கண்டமாலை, குத நெகிழ்ச்சி போன்ற நோய்கள் தீரும்.

கந்தகம் தாய் மகவை வளர்ப்பது போல நோய்களின் வெப்பத்தை மாற்றி உடம்பை தேற்றுவிக்கும் என்று தேரன் பொருட்பண்பு நூலில் கூறப்பட்டுள்ளது.

சுத்தி முறைகள்:

புளியம் பழவோட்டை பற்றி இருக்கும் கசிவை ஊறவைத்திறுத்த நீர், காடிநீர், புளித்த மோர், காளான் சாறு இவைகளை தனித்தனி 6 பலம் எடுத்து சட்டியிலிட்டு அதன் மேல் ஒரு பலம் கந்தகத்தை வைத்து மேல் மூடி அடுப்பேற்றித் தீபாக்கினியாய் 2 சாமம் எரிக்க மலினம் மேல் தங்கி கந்தகம் சுத்தி ஆகும்.

மருதோன்றி கல்கத்தை பசுவின் தயிரில் கலந்து சட்டியிலிட்டு அதன் மேல் கந்தகத்தை வைத்து மற்றொரு சட்டியால் மூடி சீலை செய்து குழியில் புதைத்து மேல் சட்டி கொண்டு மூடி 5 வறட்டி புடமிட கந்தகம் சுத்தி ஆகும். இவ்விதம் 7 முறை செய்யவும்.

கந்தகத்தை ஒரு இரும்பு கரண்டியிலிட்டுச் சிறிது பசுவெண்ணெய் இட்டு உருக்கி பசும்பாலில் சாய்க்கவும். இவ்விதம் 30 முறை செய்யக் கந்தகம் சுத்தியாம்.

பாலுக்கு பதில் வாழைக் கட்டை நீரில் கெந்தியை 10 முறை உருக்கி சாய்த்தெடுக்க சுத்தியாம். இம்முறையில் கந்தகத்தில் உள்ள எண்ணெய் நீங்கும் என்று கூறுவர்.

அளவு : 10 முதல் 30 உளுந்தெடை[5]

கந்தக நஞ்சு குறிகுணம்

கொடிய நஞ்சுத் தன்மை உடையது அன்று. சுத்தி மற்றும் மருந்து செய் முறையில் சரியாக கவனம் பெறாத கந்தகத்தை உட்கொண்டால் நாளடைவில் நஞ்சை உண்டாக்கும். கண்கள் மஞ்சள் நிறமாக பூத்து இருக்கும். முகம் வெளுத்து இருக்கும். உடம்பு தன் இயற்கை ஒளி குன்றி பீர்க்கம் பூப்போன்று நிறமடையும். பற்கள் கறுத்து பாசி அடையும். வியர்வை மஞ்சள் சிறுநீர் வெள்ளாட்டு நீரை போலும் மலம் சாமந்தி பூவை போன்று மஞ்சள் நிறமாயிருக்கும். வாயில் புகை நாற்றம் உண்டாகும்.

முறிவு :

1. தாமரை வித்தை இளநீரில் அரைத்து உண்ன தீரும்.

2. மிளகு நீலி வேர் சீரகம் இவற்றை குடிநீர் வைத்து கொடுக்க தீரும்.

3. மணத்தக்காளி வேர்ப்பட்டை 10 கிராம், அரிசித்திப்பிலி 10 கிராம், அதிமதுரம் 10 கிராம் இவைகளை குடிநீரிட்டு ஒரு மண்டலம் அல்லது நோய் நீங்கும் வரை காலையிலும் மாலையிலும் கொடுக்க கந்தக நஞ்சு முறியும்.[6]

சேரும் மருந்துகள்:

கந்தக தைலம் [7]

கந்தக ரசாயனம் [8]

சன்னி பைரவம் [13]

கந்தக செந்தூரம் (14)

சண்ட மாருத செந்தூரம் (15)

மால் தேவி செந்தூரம் (16)

கந்தக மெழுகு (17)

ஆறுமுக செந்தூரம் (18)

நாத குரு தைலம் (19)

பூரம்.

(இரசக்கற்பூரம்)

தொகைச்சரக்குகளில் கூறப்பட்ட 64 பாடாணங்களுள் காணப்படாதிருந்தும், பூரம் மருத்துவர்களால் பாடாண வகைகளுள் ஒன்றாகவே கருதப்படுகின்றது. இ.ந்து இரசம், உப்பு இவைகளின் கூட்டினால் செய்யப்படுகின்ற சரக்காகும்.

சுவை:

உப்பு கார்ப்பு

வீரியம்

வெப்பம்

பிரிவு:

கார்ப்பு

குணம்:

கடுமை நோய்களைச் சமனம் செய்யும்,

பேதியை உண்டு பண்ணும்

பித்தநீரை அதிகப்படுத்தும்,

செய்கை:

உடல்தேற்றி,

உமிழ்நீர்பெருக்கி,

கிருமிநாசினி

பொதுக்குணம்:.

“இடைவாத சூலையெரி சூலை குன்மந்

தொடை வாழைவாத மாஞ்சோணி- யிடையாதோ

வொக்குர சுகர்ப்பூர மொன்றேயள வொடுநல்

இக்கு வெல்லத் தேழு நாளீ.”

நல்ல இரச கர்ப்பூரத்தை அளவுடன் கரும்பு வெல்லத்தில் 7 நாள் கொடுக்க, இடுப்பைப்பற்றிய சூலை, ஆங்காங்கு எரிச்சலைத் தருகின்ற சூலை, வாதகுன்மம், தொடை வாழை, வாதரத்த நோய் முதலியன தீரும். .

பூரத்திலுள்ள மலினங்களை நீக்குதற்கும், விடக் குறிகுணங்களை உண்டு பண்ணாதிருத்தற்கும், அதிக வீரியத்தைக் கொடுத்தற்கும் இதனைச் சுத்தி செய்ய வேண்டும்.

சுத்தி.

கம்மாறு வெற்றிலை, மிளகு ஆகிய இரண்டையும் கால் பலம் (8.75 கிராம்) வீதம் நிறுத்தெடுத்துத் சிறிது நீர் விட்டு அரைத்து, கல்கத்தை ஒரு படி (1.3 லிட்.) நீரில் கலந்து, ஒரு பலம் (35 கிராம்) பூரத்தை சீலையில் முடிந்து துலாயந்திரமாய் நீரில் அமிழும் படி செய்து, சிறிது எரிக்கவேண்டும். நீர் முக்காற் பங்கு சுண்டிய பிறகு, பூரத்தை எடுத்து நீர் விட்டுக் கழுவி வெய்யிலில் உலர்த்தி எடுக்கச் சுத்தியாம்.

வேறு

ஒரு பலம் (35 கிராம்) பூரத்திற்கு முலைப்பாலினால் மூன்று மணி நேரம் சுருக்குக் கொடுத்துப் பிறகு வெள்ளைப்பூண்டுத் தைலத்தினால் 9 மணி நேரம் சுருக்கிட்டு எடுத்தக் கொள்ளவும்.

இலேகியங்களில் சேர்க்க வேண்டிய பூரத்தை, முசுமுசுக்கைக் சாற்றினால் சுருக்கிட்டுக் கழுவவும்.

பூர நஞ்சுக் குறிகுணம்

பூரம் விடமித்தால், முகத்தில் செவ்வாப்புப் போல முகப்பரு, வேர்க்கரு அதிகமாய் உண்டாதல், மார்பின் பள்ளத்தில் பருக்கட்டிப் புண்ணிரணம் காணுதல், இதைப் பக்கத்தில் வலி, வாயில் காரம் படாதபடி புண்ணாதல், பீச வீக்கம், உண்ணாக்கில் வீரணம், பேதி, இரத்தக் கழிச்சல் முதலிய துர்க் குணங்களைக் காண்பிக்கும்.

முறிவு

நிலப்பனைக் கிழங்கு, வல்லாரை வேர், பொன்னாங்காணி வேர் கண்டுபாரங்கி, ஆகிய இவை ஒவ்வொன்றும் தனித்தனிக் கால் பலம் (8.75 கிராம்) எடுத்து, ஒன்று சேர்த்துக் குடிநீரிட்டு, இரு வேளை வீதம் இரண்டு அல்லது மூன்று வாரம் பத்தியத்துடன் அருந்த நீங்கும்.[5]

சேரும் மருந்துகள்:

பூரபற்பம்.[5]

பூரக்கட்டு.[5]

இரசக்கருப்பூரக்குளிகை.[5]

பூரக்களிம்பு.[5]

பூரஎண்ணெய்.[5]

பூரப்பொடி.[5]

குப்பைமேனி

வேறுபெயர்:- அரிமஞ்சரி, பூனைவணங்கி, மேனி

இப்பூண்டு இந்தியா முழுவதிலும் கிடைக்கும் தோட்டங்களிலும் வழியோரங்களிலும், வளர்ந்திருப்பதைக் காணலாம் இ.ந்து ஓர் அடி உயரமுள்ளது வடநூல்கள் இதனைப் பற்றியாதொன்றும் கூறவில்லை என்கிறார்கள் ஆயினும் "மார்ஜலமோகினி" என்கிற பெயர் மட்டும் தொகுத்துள்ளதாகக் காணப்படுகிறது

ப-உ: இலை, வேர், சமூலம்.

சுவை- கைப்பு, கார்ப்பு,

தன்மை- வெப்பம்,

பிரிவு- கார்ப்பு

செய்கை:

துயரடக்கி

புழுக்கொல்லி

பெருமலம்போக்கி

சிறுநீர்பெருக்கி

வாந்தியுண்டாக்கி

கோழையகற்றி

சூதகமுண்டாக்கி

குணம்:

இதன் இலையால், பல்லடிநோய், தீச்சுட்டபுண், பயிர்வகையின்நஞ்சு, வயிற்றுவலி, வளிநோய், மூலம், நமைச்சல், குத்தல், இரைப்பு, மூக்குநீர்பாய்தல், கோழை ஆகியவை நீங்கும்.

“தந்தமூலப் பிணிதீத்தந்திடு புண்சர்வவிடம்

உந்து குன்மம் வாதம் உதிரமூ- லந்தினவு

சூலஞ் சுவாசம் தொடர்பீ சங்கபம்போம்

ஞாலங்கொள் மேனியதனால்”

குப்பைமேனி

“காந்தி மெயிலை கறிகனி வுடனயில மெய்

யாந் திமிர்வாத நோயாதிகள் போய்விடும்”

(பொ-ள்)

குப்பைமேனி இலையை உணவு முறையாய்ப் பிரியமுடன் புசித்துவரின், உடம்பிலுள்ள திமிர்வாதம் முதலிய நோய்கள் நீங்கும்.

வ-கு: இலையை பொடித்தோ, அல்லது குடிநீரிட்டோ, அல்லது இலையைச் சாறு பிழிந்தோ, உள்ளுக்குக் கொடுக்கலாம்.

அளவு: சிறியவர்களுக்கு- இலை ரசம் அல்லது குடிநீர் 1 முதல் 4 தேக்கரண்டி வரை கொடுக்க, வயிற்றைக் கழியச் செய்யும், கோழையை அகற்றும், வயிற்றுப் புழுவைக் கொல்லும்.

இதனைப் பெரியவர்களுக்கு 15 மி.லி. முதல் 30 மி.லி. சிறியவர்களுக்கு 1 தேக்கரண்டி வீதம் கொடுக்க, வாந்தியாகும்.

இலையைப் பொடித்துத் தக்க அளவாகக் குழந்தைகளுக்குக் கொடுக்க, மலப்புழுக்கள் வெளிப்படும். அல்லது இலையையும், சிரிதளவு பூண்டையும் சேர்த்தும் கொடுக்கலாம்.

இலைப் பொடி 950 மி.கி. 1,300 மி.கி வரையில் கொடுக்க, அ.்.து இருமல் முதலிய நுரையீரல் நோய்களைப் போக்கும்.

இலையையும் உப்பையும் சேர்த்து அரைத்துச் சொறி சிரங்குகளுக்குத் தேய்த்துக் குளித்து வர, அவை குணமாகும்.

இலைச்சாற்றை எண்ணெயுடன் சேர்த்துக் காய்ச்சி வலிக்குத் தேய்த்து வரலாம்.

இலையை அரைத்து, புண், நஞ்சுக்கடி. இவைகளுக்குப் போடலாம். அலலது, இலையை மஞ்சளுடன் கூட்டி அரைத்துப் பூசலாம்.

சுண்ணாம்புடன் கலந்து, நோயுடன் கூடிய கீல் வீக்கங்களுக்கும் கட்டிகளுக்கும் பூசலாம். இதையே காது வலிக்குக் காதைச்சுற்றிப்பூச, நோய் தணியும்.

இலையுடன் உப்புச் சேர்த்துச் சாறு பிழிந்து, தினந்தினம் காலையில் இரு மூக்குகளிலும் நசியமிட்டு, குளிர்ந்த நீரில் தலை முழுகிவர, வெறிநோய், நீங்கும்.

இலையை அரைத்துக் கழற்சிப் பிரமாணம் உருண்டை செய்து, எருவாய் வழியாய் உட்செலுத்த, நாட்பட்ட மலக்கட்டு நீங்கும்.

சாறுபிழிந்து இத்துடன் சிறிது வேப்பெண்ணெய் கலந்து, இறகில்தோய்த்துத் தொண்டையில் அல்லது உள்நாக்கில் தடவ, சிறு குழந்தைகளுக்கு வயிற்றில் தங்கியிருக்கும் கோழைக்கட்டு, வாந்தியினால் வெளிப்படும்.

இச்சாற்றையே தலைவலிக்கும் தடவலாம். இலைப் பொடியைப் படுக்கைப் புண்களுக்கு வைத்துக் கட்ட, புழுக்கள் சாகும்.

இலைச் சாற்றைச் சுண்டக்காய்ச்சி மெழுகு பதத்தில் எடுத்து 130மி.கி.-260 மி.கி கொடுக்க, குழந்தைகளுக்கு காணும் இருமல் போகும்.

இலையை அரைத்து மேகப்புண்களுக்கு வைத்துக் கட்டலாம்.

இலையைக் குடிநீரிட்டு, சிறிது உப்புச் சேர்த்துக் குடிக்க, மலத்தைக் கழிக்கும்.

“இலைமேனிய யிறிவிளக்கெண்ணெயின் மெய்யட்டியிலை மேனியையா”.

(பொ-ள்)

குப்பைமேனியின் கீரையை ஆமணக்கு எண்ணெயில் தாளிதம் பண்ணி ஒரு மண்டல கற்பமுறையாய் உண்க. அது வாய்வுடனே சேர்ந்த பொல்லாத சேத்தும பிணிகள் யாவற்றையும் போக்கி, உடல்நலத்தை உண்டுபண்ணும்.

வேர்.

வேரைக் குடிநீரிட்டு அல்லது வெந்நீர் விட்டு இடித்துச் சாறு பிழிந்து தக்கஅளவில் கொடுக்க,கழியச்செய்யும்.

வேரை அரைத்து சுமார் ஒரு கொட்டைப்பாக்களவு நீரிற் கலந்து 3 நாள் கொடுத்து உப்பில்லாப் பத்தியம் வைக்க, எலிவிடம் தீரும். ஆனால், வாந்தியையும் கழிச்சலையும் உண்டாக்கும்.

சமூலம்

இதில் 105 கிராம் எடுத்து இடித்து, ஓர் ஆழாக்குத் திராட்சைச் சாராயத்தில் 7 நாள் ஊற வைத்து, இடையிடையே கிளறிவிட்டு, நன்றாய்ப் பிழிந்து சாறெடுத்து வடிகட்டி வைத்துக் கொண்டு 20 துளி முதல் 1 தேக்கரண்டி வரை தேனிற் கொடுக்க, இது மேற்கூறிய குணங்களைக் கொடுக்கும்.

இதனால் செய்யப்பட்ட மேனித்தைலத்தை 7 கிராம் - 28.35 எடுத்து மணப்பாலில் கலந்து கொடுக்க, கிருமி வெளிப்படும். இத்தைலத்தையே வாதநோய்களுக்கு மேலுக்குப் பூசலாம் [20]

REVIEW OF LITERATURE – MODERN ASPECT:

MERCURY

Mercury is a transition metal. A transition metal is one of the elements found between Groups 2 (IIA) and 13 (IIIA) on the periodic table. The periodic table is a chart that shows how chemical elements are related to one another. Mercury has long been known as quicksilver, because it is a silver liquid. The chemical symbol also reflects this property. The symbol, Hg, comes from the Latin term *Hydragyrum*, meaning "watery silver."

Mercury has been known for thousands of years. In many cultures, people learned to make mercury metal from its most important ore, cinnabar. When heated cinnabar releases mercury as a vapor (gas), the vapor is cooled and captured as liquid mercury.

SYMBOL: Hg

ATOMIC NUMBER & MASS: 80 &200.59

FAMILY: Group 12 (II B), Transition metal

Some mercury compounds are known to be poisonous. For example, mercuric chloride (corrosive sublimate) was often used to kill pests and, sometimes, people. On the other hand, some mercury compounds have been used as medicines. For instance, it was long used as a cure for skin rashes. In the last forty years, the dangers of mercury have become better known. As a result, mercury use is now being phased out.

PHYSICAL PROPERTIES:

solid) at a temperature of –38.85°C (–37.93°F). It can be changed into a gas ("boiled") at 365.6°C (690.1°F). Its density is 13.59 grams per cubic centimeter.

Mercury has two physical properties of special interest. First, it has very high surface tension. Surface tension is a property of liquids that make them act like they are covered with a skin. Second Mercury is also a very good conductor of electricity

CHEMICAL PROPERTIES:

Mercury is moderately active. It does not react with oxygen in the air very readily. It reacts with some acids when they are hot, but not with most cold acids.

OCCURRENCE IN NATURE:

The abundance of mercury in the Earth's crust is estimated to be about 0.5 parts per million. That makes it one of the 20 least common elements. It very rarely occurs as an element. Instead, it is usually found as a compound. Its most common ore is cinnabar, or mercuric sulfide (Hgs). Cinnabar usually occurs as a dark red powder. It is often called by the common name of vermillion or Chinese vermillion.

EXTRACTION:

Mercury is still prepared as it was hundreds of years ago. Cinnabar is heated in air. The compound breaks down to give mercury metal:

The mercury metal is then purified by distillation. Distillation is the process of heating two or more liquids to their boiling points. Different liquids boil at different temperatures. The liquid that is wanted (such as mercury) can be collected at its boiling point. Mercury that is more than 99 percent pure can be collected by distillation.

MERCURIC COMPOUNDS:

- Mercuric arsenate ($hghaso_4$): waterproofing paints
- Mercuric benzoate ($Hg(C_7G_5O_2)_2$): medicine; used to treat syphilis
- Mercuric chloride, or mercury bichloride, or corrosive sublimate ($hgcl_2$): disinfectant, tanning of leather, spray for potato seedlings (to protect from disease), insecticide, preservation of wood, embalming fluid, textile printing, and engraving
- Mercuric cyanide ($Hg(CN)_2$): germicidal soaps (soaps that kill germs), photography
- Mercuric oxide (hgo): red or yellow pigment in paints, disinfectant, fungicide (to kill fungi), perfumes and cosmetics
- Mercuric sulfide (hgs): red or black pigment in paints
- Mercurous chloride, or calomel (Hg_2Cl_2): fungicide, maggot control in agriculture, fireworks
- Mercurous chromate (Hg_2cro_4): green pigment in paints
- Mercurous iodide (Hg_2I_2): kills bacteria on the skin

USES:

- Use of mercury is in the preparation of chlorine.
- Use of mercury in switches and other electrical applications.
- Mercury is also used in dental applications, measuring instruments (such as mercury thermometers and barometers), and coatings for mirrors.

HEALTH EFFECTS:

Effects occur over very long periods of time. People who work with mercury, for example, may take in small amounts of mercury over months or years. Health problems develop very slowly. These problems can include

- Inflammation of the mouth and gums;
- Loosening of the teeth;
- Damage to the kidneys and muscles;
- Shaking of the arms and legs; and
- Depression,
- Nervousness, and
- Personality changes.[21]

SULPHUR:

Sulphur (in British English, sulphur) is a chemical element. It is abundant, multivalent, and nonmetallic. Under normal conditions, Sulphur atoms form cyclic octatomic molecules with a chemical formula S_8. Elemental Sulphur is a bright yellow, crystalline solid at room temperature. Sulphur is an essential element for all life, but almost always in the form of Organo Sulphur compounds or metal sulfides. Three amino acids two vitamins are Organo Sulphur compounds. Sulphur is one of the core chemical elements needed for biochemical functioning and is an elemental macronutrient for all living organisms.

Symbol: S

Atomic mass: 32.065 u

Atomic number: 16

Electron configuration: [Ne] $3s^2 3p^4$

Melting point: 115.2 °C

Electro negativity: 2.58

Physical properties

Sulphur can forms several poly atomic molecules. The best-known allotrope is octa Sulphur, cyclo-S_8. Octa Sulphur is a soft, bright-yellow solid that is odorless, but impure samples have an odor similar to that of matches. It melts at 115.21 °C (239.38 °F), boils at 444.6 °C (832.3 °F) and sublimes easily. The structure of the S_8 ring is virtually unchanged by this phase change, which affects the intermolecular interactions. Between its melting and boiling temperatures, octa Sulphur changes its allotrope again, turning from β-octa Sulphur to γ-Sulphur, again accompanied by a lower density but increased viscosity due to the formation of polymers. At higher temperatures, the viscosity decreases as depolymerization occurs.

Chemical properties

Sulphur burns with a blue flame with formation of Sulphur dioxide, which has a suffocating and irritating odor. Sulphur is insoluble in water but soluble in carbon disulfide and, to a lesser extent, in other non polar organic solvents, such as benzene and toluene. Sulphur reacts with nearly all other elements with the exception of the noble gases, even with the notoriously unreactive metal iridium (yielding iridium disulfide). Some of those reactions need elevated temperatures.

Uses

- The most important form of Sulphur for fertilizer is the mineral calcium sulfate. Sulphur improves the efficiency of other essential plant nutrients, particularly nitrogen and phosphorus.
- Elemental Sulphur is one of the good fungicides and pesticides.
- Sulphur dioxide and various sulfites have been used for their antioxidant antibacterial preservative properties in many other parts of the food industry.
- Sulphur (specifically octa Sulphur, S_8) is used in pharmaceutical skin preparations for the treatment of acne and other conditions. It acts as a catalytic agent and also kills bacteria, fungi, scabies mites, and other parasites.

Health Effects:

Effects occur over very long periods of time. People who work with mercury, for example, may take in small amounts of mercury over months or years. Health problems develop very slowly. These problems can include

- Eye and respiratory disturbances
- Chronic bronchitis
- Chronic sinus effects.
- Irritation to the mucous membranes.
- Asthma.[22]

Mercurous chloride (calomel):

Mercury (I) chloride is the chemical compound with the formula Hgcl. Also known as the mineral calomel or Mercurous chloride, this dense white or yellowish-white, odorless solid is the principal example of a mercury (I) compound. It is a component of reference electrodes in electrochemistry.

Synthesis:

Mercurous chloride forms by the reaction of elemental mercury and mercuric chloride: $Hg + HgCl_2 \rightarrow Hg_2Cl_2$

It can be prepared via metathesis reaction involving aqueous mercury (I) nitrate using various chloride sources including NaCl or HCl.

$$2\ HCl + Hg_2(NO_3)_2 \rightarrow Hg_2Cl_2 + 2\ HNO_3$$

Ammonia causes Hg_2Cl_2 to disproportionate:

$$Hg_2Cl_2 + 2\ NH_3 \rightarrow Hg + Hg(NH_2)Cl + NH_4Cl$$

Properties:

Mercury is unique among the group 12 metals for its ability to form the M–M bond so readily. Hg_2Cl_2 is a linear molecule. The mineral calomel crystallizes in the tetragonal system, with space group I4/m 2/m.

The Hg–Hg bond length of 253 pm (Hg–Hg in the metal is 300 pm) and the Hg–Cl bond length in the linear Hg_2Cl_2 unit is 243 pm. The overall coordination of each Hg atom is octahedral as, in addition to the two nearest neighbors, there are four other Cl atoms at 321 pm. longer mercury polycations exist. Mercurous chloride not soluble in water

Use in Medicine:

Syphilis was frequently treated with Mercurous chloride before the advent of antibiotics. It was inhaled, ingested, injected, and applied topically. Both mercuric-chloride and Mercurous chloride treatment for syphilis and poisoning during the course of treatment were so common that the latter's symptoms were often confused with those of syphilis. It was applied topically to alleviate ulcerative symptoms.

Health Effects:

- It can cause serious internal damage, including ulcers to the stomach, mouth, and throat, and corrosive damage to the intestines.
- Mercurous chloride also tends to accumulate in the kidneys, causing severe corrosive damage which can lead to acute kidney failure.
- Common side effects of acute Mercurous chloride poisoning include burning sensations in the mouth and throat, stomach pain, abdominal discomfort, lethargy, vomiting of blood, corrosive bronchitis, severe irritation to the gastrointestinal tract, and kidney failure.
- Chronic exposure can lead to symptoms more common with mercury poisoning, such as insomnia, delayed reflexes, excessive salivation, bleeding gums, fatigue, tremors, and dental problems.[23]

Kuppaimeni (Acalypha indica):

Kingdom: Plantae

Phylum: Tracheophyta

Class: Equisetopsida

Order: Malphigiales

Family: Euphorbiaceae

Genus: Acalypha

Species: Acalypha indica

Botanical name: Acalypha indica

Vernacular names:

English: Indian acalypha

Tamil: Kuppaimeni

Malayalam: Kuppamani, kuppameni

Kannada: chalmari

DESCRIPTION:

An erect annual herb that can be easily distinguished by the cup-shaped involucres that surrounds the small flowers in the catkin-like inflorescence it can grow up to 1.2 m (3.9 ft) tall in favorable circumstances, but is usually smaller.

The leaves are broad ovate, 1.2 cm–6.5 cm × 1 cm–4 cm (0.47 in–2.56 in × 0.39 in–1.57 in) The leaf base is rounded to shortly attenuate. The leaf margin is

basally 5-nerved and is crenate-serrate with an acute or obtuse apex. The petiole is 1.5–5.5 cm (0.59–2.17 in) long. The flower spikes are axillary, 2.5–6 cm (0.98–2.36 in) long, monoecious, with a rachis terminating in a triradiate hood. The tiny male flowers are white-green, located on the upper part of the flower spikes, and are ebracteate, minute, and clustered with vermiculiform anthers. The pollens are roughly round and approximately 10-12 microns in diameter. The green female flowers are located lower on the spikes, and are subtended by 3–7 mm (0.12–0.28 in) long sub orbicular-cuneiform, many-nerved, toothed bracts that are foliaceous. The ovary is hispid, 3-lobed. Styles are 3, each 2-fid. Capsules are hispid, 3-valved and concealed by a bract. The stem is striate (longitudally ribbed) and pubescent. The fruit is 1.5–2 mm (0.059–0.079 in), 3-lobed, tuberculate and pubescent.

GEOGRAPHICAL DISTRIBUTION:

Acalypha indica occurs widely throughout the old World tropics. In Africa, it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia, Yemen, and Oceania. It has been introduced to the New World Tropics.

Chemical constituents:

The arial parts contain a glycoside as well as flavonoidsKaempferol glycosides ,Clitorin,Nicotiflorinand biorobin.Tannins, βsitosterol, acalyphamide, aurantiamide, S uccinimideand flindersin (pyranoquinolinone alkaloid) have also been isolated. [(24)]

4. MATERIALS AND METHODS

PREPARATION OF THE TEST DRUG:

4.1.1. Drug selection

The study drug *"**Vishathirku Poora Mathirai**"* has been selected from the Siddha literature, **"Ramadevar Ennum Yakobhu eluthiya Vaithya Sindhamani 700"**

4.1.2. Ingredients of "*Vishathirku Poora Mathirai*":

Purified Rasam (Mercury) – 1 *palam (35* gm)

Purified Gandhagam (Sulphur) – 1 *palam* (35 gm)

Purified Pooram (Calomel) – 4 *palam* (140 gm)

Kuppai meni leaf juice (*Acalypha indica*) -Q.S [3]

4.1.3. Procurement of Raw drugs:

All the raw materials were obtained from K.Ramasamy chetty country drug shop, No: 177, Rasappa Chetty Street, Park town, Chennai-600003. Kuppai meni leaves were collected from NIS campus, Tambaram sanatorium, Chennai-600047

4.1.4. Identification and Authentication:

Rasam (Mercury- Hydragyrum), Gandhagam (Sulphur) and Pooram (Calomel) was identified and authenticated by the Dept of Gunapadam and herbal ingredients of *Vishathirku Poora Mathirai* were identified and authenticated by the Dept of Medicinal Botany, National Institute of Siddha, Chennai - 47.

4.1.5. Purification of raw drugs:

Rasam (Mercury- Hydragyrum):

- Mercury *(Rasam)* – 35 gram
- Brick powder *(Sengal thool)* – required quantity

- Turmeric powder *(Curcuma longa) (Manjal)* - required quantity
- Juice of leaves Indian Acalypha *(Acalypha indica)* - 1.3 Litre

Mercury is triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the mercury is boiled with the juice of ***Acalypha indica- kuppai meni*** until it is detoxified.

Pooram (Calomel):

The poultice made of betel leaf **(Piper betle)- *vetrilai* and pepper *(Piper nigrum)* – *milagu*** each 8.75gm is taken and dissolved in 1.3 litre of water calomel 35 gm and tied with a cloth and immersed in the liquid from the cross bar and heated. After the water is reduced to ¾ of its volume the calomel is taken out, washed with water and dried to get it in purified form. [4]

Gandhagam (Sulphur):

Sulphur is melted in the stemp juice of **Plantain tree *(Musa paradisiaca)* – *vaalai kattai charu*** for ten times to get it purified. [4]

4.1.6. METHOD OF PREPARATION:

Take the above mentioned quantity of drug after purification is triturated with Kuppai meni leaf juice (Requirement quantity) until it attend pill rolling make it into the Panavedai alavu (488 mg) of tablet and dried. Then it is dried in shade. [3]

4.1.7. Storage of test drug:

The test drug is stored in an air tight glass container.

4.1.8. Administration of the drug:

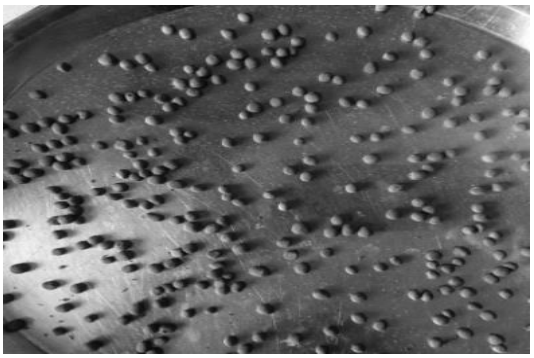
Form of the drug: Mathirai

Route: Enteral *(oral)*

Adjuvant: Hot water.

4.1.9. Indications: Toxic bites (Visha kadigal) [3] Fever (Suram) Gastric ulcer (Gunmam)

Ingredients of Vishathirku Poora Mathirai		
	Before purification	**After purification**
Rasam		
Gandhagam		
Pooram		
Kuppai meni leaf juice		

Preparation of Vishathirku Poora Mathirai	
	
	

4.2. STANDARDIZATION OF VISHATHIRKU POORA MATHIRAI:

The standardization of test drug is essential to exhibit the purity and quality of drug. This is basically done by physicochemical, Phytochemical and biochemical analysis. The physicochemical analysis has been done at Noble Research Solution Chennai. Biochemical analysis was done at National Institute of Siddha. Phytochemical analysis was done at Noble Research Solution, and instrumental analysis was done at Noble Research Solution and Sastra University Tanjore.

4.2.1. ORGANOLEPTIC CHARECTER ANALYSIS:

The organoleptic characters of the sample drug were evaluated.1gm of the test drug was taken and the colour, odour, Taste and solubility were viewed by naked eye under sunlight. Then the result is noted.

4.2.2. PHYSICO CHEMICAL ANALYSIS:

Determination of Moisture Content (Loss on Drying):

5 g of the drug without preliminary drying was weighed accurately in a tared evaporating dish, dried at 105ºC for 5 hours, cooled in dessicator and weighed. Later the drying and weighing process was continued at one hour interval until difference between two successive weighings of sample corresponds to not more than 0.25 percent. When the constant weight was obtained the percentage of moisture content was calculated with reference to the air dried drug.

Determination of Total Ash:

2 to 3 g of drug was weighed in the pre weighed and tared Gooch crucible, kept in the muffle furnace at a temperature not exceeding 450ºC until ash free from carbon was obtained then cooled, eighed and the percentage of the total ash content were calculated with reference to the air dried drug.

Determination of Alcohol soluble extractive:

5g of coarsely powdered air dried drug was macerated with 100ml of absolute alcohol in a closed flask for twenty-four hours, shaken frequently during six hours and allowed to stand for eighteen hours. After filtering the solution 25ml of this filtrate was evaporated in a tarred flat bottomed shallow dish, and dried at 105ºC until a constant weight was obtained. Later the percentage of alcohol-soluble extractive with reference the air-dried drug was calculated.

Determination of Water soluble extractive:

5g of coarsely powdered air dried drug was macerated with 100ml of chloroform water in closed flask for twenty-four hours, shaken frequently during six hours and allowed to stand for eighteen hours. After filtering the solution 25ml of this filtrate was evaporated in a tarred flat bottomed shallow dish, and dried at 105ºC until a constant weight was obtained. Later the percentage of water-soluble extractive with reference to the air-dried drug was calculated percentage of water-soluble extractive with reference to the air-dried drug was calculated.

Determination of Total Sugar:

1mg/ml of concentrated drug to be tested was prepared by dissolving it in distilled water. To this 3ml of 52% per choric acid, 0.1ml of 80% phenol and 5 ml conc. H2SO4 were added, cooled for a few minutes after which the absorbance was measured at 490nm. The same procedure was repeated with sugar solution in place of the drug. After plotting a standard graph with absorbance on y-axis and concentration on x- axis the concentration of sugar present in the drug was calculated.

Determination of ph:

1% of the substance was prepared using distilled water and stored properly. Prior to ph measurement ph electrode was calibrated using buffers of ph 4, 7, 10. After calibration, measurement was taken with a solution of known ph such that the reading should not differ by more than 0.02 from the original value. If the difference is greater than 0.05, the set of measurements was repeated. Later the ph electrode was dipped into the drug to be tested and kept as such until a constant reading was obtained.

Disintegration time:

Methodology:

Tablets is placed basket containing medium of that simulate the gastric content which contains sodium chloride, pepsin and hydrochloric acid. The pH is about 1.2 – 1.4. The flask is cylindrical with a hemispherical bottom. The flask is maintained at $37^{o} \pm 0.5^{o}C$ by a constant temperature bath. The motor is adjusted to turn at the specified speed 100 RPM.

4.2.3 BIO -CHEMICAL ANALYSIS OF VISHATHIRKU POORA MATHIRAI:

The bio–chemical analysis of Vishathirku Poora Mathirai done at Biochemistry Lab, National Institute of Siddha, Chennai, 47

Preparation of Extract:

5gm of ***Vishathirku Poora Mathirai*** is weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

1.TEST FOR ACID RADICALS

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Sulphate:** 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No cloudy appearance present.	Absence of sulphate
2.	**Test For Chloride:** 2ml of the above prepared extracts is added with 2ml of dil-HCl is added until the effervescence ceases off...	No cloudy appearance present.	Absence of chloride
3.	**Test For Phosphate:** 2ml of the extract is treated with 2ml of dil.ammonium molybdate solution and 2ml of con.HNO_3	No yellow precipitate present	Absence of Phosphate
4	**Test For Carbonate:** 2ml of the extract is treated with 2ml dil. magnesium sulphate solution	Cloudy appearance present	Presence of carbonate
5.	**Test For Fluoride & Oxalate:** 2ml of extract is added with 2ml of dil. Acetic acid and 2ml dil. Calcium chloride solution and heated**.**	Absence of Cloudy appearance	Absence of fluoride and oxalate
6.	**Test For Nitrite:** 3drops of the extract is placed on a filter paper, on that-2 drops of dil.Acetic acid and 2 drops of dil. Benzidine solution is placed.	No characteristic changes	Absence of Nitrite

2. TEST FOR BASIC RADICALS

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Lead:** 2ml of the extract is added with 2ml of dil.potassium iodine solution.	No yellow Precipitate is obtained.	Absence of Lead
2.	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide is added in 5 drops to excess.	Yellow colour appearance	Presence of aluminum
3.	**Test For Iron:** To the 2ml of extract add 2ml of dil.ammonium solution To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Mild red colour appear Blood red colour appearance	Iron present
4.	**Test For Zinc:** To 2ml of the extract dil.sodium hydroxide solution is added in 5 drops to excess and dil.ammonium chloride is added.	White precipitate is formed	presence of Zinc
5.	**Test For Calcium:** 2ml of the extract is added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance or white precipitate formation is present	Presence of calcium
6.	**Test For Magnesium:** To 2ml of extract dil.sodium hydroxide solution is added in drops to excess.	White precipitate is obtained	presence of Magnesium
7.	**Test For Mercury**: 2ml of the extract is treated with 2ml of dil.sodium hydroxide	Yellow precipitate is obtained	presence of mercury

	solution.		
8.	**Test For Arsenic:** 2ml of the extract is treated with 2ml of dil.sodium hydroxide solution.	No brownish red precipitate is obtained	Absence of arsenic

OTHER CONSTITUENTS

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Test For Starch :** 2ml of extract is treated with weak dil. Iodine solution	No blue colour formation	Absence of starch
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No brick red colour developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the extract is treated with 2ml of dil.potassium iodide solution. b) 2ml of the extract is treated with 2ml of dil. Picric acid.	Reddish brown precipitation appears Yellow precipitation Appears	Presence of Alkaloid
4.	**Test For Amino Acid:** 2 drops of the extract is placed on a filter paper and dried well. 20ml of Biurette reagent is added**.**	Violet colour is not developed	Absence of amino acids

4.2.4. PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the Vishathirku Poora Mathirai is done at Noble Research Solutions Perambur Chennai had been done by using standard procedures.

Test for alkaloids:

Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for Coumarin:

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of Coumarin is indicated by the formation of yellow color.

Test for Saponins:

To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for glycosides- Borntrager's Test:

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

Test for flavonoids:

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for phenols:

Lead acetate test:

To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for steroids:

To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Triterpenoids:

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of Triterpenoids.

Test for Cyanine:

Anthocyanin:

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100^{o}C. Formation of bluish green colour indicates the presence of Anthocyanin.

Test for Carbohydrates - Benedict's test:

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biurette Test):

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

4.2.5. INSTRUMENTAL ANALYSIS:

HEAVY METAL ANALYSIS BY AAS:

Standard: Hg, As, Pb and Cd – Sigma

Methodology:

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion:

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO3.

Standard preparation:

As & Hg- 100 ppm sample in 1mol/L HCl

Cd & Pb- 100 ppm sample in 1mol/L HNO3

AFLATOXIN

Standard:

- ✓ Aflatoxin B1
- ✓ Aflatoxin B2
- ✓ Aflatoxin G1
- ✓ Aflatoxin G2

Solvent:

Standard samples were dissolved in a mixture of chloroform and acetonitrile (9.8: 0.2) to obtain a solution having concentrations of 0.5 µg per ml each of Aflatoxin B1 and Aflatoxin G1 and 0.1 µg per ml each of Aflatoxin B2 and Aflatoxin G2.

Test solution: Concentration 1 µg per ml

Procedure:

Standard Aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5 µL, 7.5 µL and 10 µL. Similarly the test sample was placed and Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85:10:5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

PESTICIDE RESIDUE:

Extraction:

Test sample were extracted with 100 ml of acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene R and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

TEST FOR SPECIFIC PATHOGENS:

Methodology:

One part of the test sample was dissolved in 9 ml of sterile distilled water and the test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37ºC for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation in each differential media

Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
E-coli	***EC***	***EMB Agar***
Salmonella	***SA***	***Deoxycholate agar***
Staphylococcus Aureus	***ST***	***Mannitol salt agar***
Pseudomonas Aeruginosa	***PS***	***Cetrimide Agar***

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

MICROBIAL CONTAMINATION:

Objective:

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

Methodology:

Test sample was admixed with sterile distilled water and the mixture were been used for the sterility evaluation. About 1ml of the test sample was inoculated in sterile petri dish to which about 15 ml of molten agar 45^{o}C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37^{o} C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

Observation:

No growth was observed after incubation period, reveals the absence of specific pathogen.

4.3. TOXICOLOGICAL STUDIES

ACUTE AND SUBCHRONIC TOXICITY STUDIES OF VISHATHIRKUPOORA MATHIRAI (VPM) ON WISTAR ALBINO RATS

The following in vivo toxicity studies were carried out on VPM. Acute oral toxicity study and 90 days Sub chronic toxicity studies by OECD Test Guidelines (423&408). The toxicity studies were carried out after getting IAEC approval from National Institute of Siddha, Chennai-47. **IAEC Approval Number is NIS/IAEC VII/ 28082018/13** for Acute and Sub chronic toxicity studies.

4.3.1 ACUTE TOXICITY STUDY:

1. Experimental Animals:

Species: Wister albino Rats

Sex: Female

Age / Weight at start of Test: 8- 12 weeks / 140 – 160 g b.wt.

Acclimatization Period: 7 Days prior to dosing.

Housing: Individually in polypropylene cages

Husbandry: 12 hour light/ 12 hour dark cycle.

Room temperature: 22^{o} c ($\pm$ 3^{o})

Relative Humidity: 30 – 70 %

Feed and Water: Rodent pelleted feed, RO purified water *ad libitum*

Identification: Animals will be kept in Individual cages and numbered.

The Female albino rats were obtained from the authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram Chennai and stocked in the animal house at National Institute of Siddha, Chennai – 47. Animals were housed in a cage at

$22^{o}c \pm 3^{o}$ and relative humidity 30 – 70 % and have free access to standard rat pellet diet (Sai meera Feeds Pvt. Ltd, Bangalore).

2. EXPERIMENTAL DETAILS:

a) Animal selection and Identification:

The animals are randomly selected for each group. Each group contains 3 female animals. They were marked on head, body and tail with picric acid solution prepared in water for identification in each group.

Test guideline: OECD guidelines (423)

Length of exposure to test substance: 1 day

No of animals: 3 Female / group

Test groups: Control, **VPM** 300mg, 2000mg/kgb.wt (Treated orally)

Groups No. of Rat:

Group I: Control-Water -3Female

Group II: Test drug (*VPM*)-300mg/kg b.wt 3Female

Group III: Test drug (*VPM*)-2000mg/kg.b.wt 3Female

b) Route of administration:

Oral route was selected because it is the normal route of clinical administration.

c) Administration of Dose:

The animals were fasted (only feed was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each group. A single dose of the test drug solution (300,2000mg/kg) was consecutively administered by oral gavage using intubation cannula. The feed was withheld for another 4hrs after dosing. The toxicological effect was assessed on the basis of morbidity and mortality.

3. Experimental procedure:

Female Wister Albino Rats of weighing 140-160g were used for acute toxicity study. Animals were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet. After seven days of acclimatization the animals are divided into three groups randomly (Group I, II & III). Each group contains 3 female Wister albino rats. Group I control treated with water, Group II treated with 300mg/kg.b.wt and group III were treated with 2000mg/kg.b.wt dosage of Vishathirku Poora Mathirai by oral route after 12 hrs fasting with free from water. After drug administration behavioural parameters are monitored for the first 4 hours continuously (1/2 hr, 1hr, 2 hr, 3 hr, 4 hr) and recorded. Then the animals were observed once daily for further 14 days for any mortality and morbidity. The animals are die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the intra peritoneal injection of Pentothal Sodium on the 15th day of the Study period. The toxicological effect was assessed on the basis of morbidity and mortality.

4. OBSERVATIONS:

Observations were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- ½ hour , 1 hour, 2 hour ,4 hour and up to 24 hour observation
- All rats were observed twice a day on 2 weeks
- Body weight were monitored at weekly once
- Feed intake and water intake were calculated per day

a. Cage-side observation

The animals were monitored for behavioural parameters like Alertness, abnormal Gait (rolling and tiptoe), aggressiveness, akinesia, analgesia, catalepsy, convulsions, defecation, excitation, exopthalmos, head twitches, lacrimation, lethality, loss of corneal reflex, loss of righting reflex, loss of traction, piloerection, ptosis, reactivity to touch, respiration, salivation, scratching, sedation, stereotypies (chewing), stereotypies (head movements), stereotypies (sniffing), straub, tremor and writhes.

b. Gross necropsy:

At the end of the 14th day, all the animals were sacrificed by using the injection of Pentothal sodium. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, Lungs, heart, spleen, liver, kidneys, adrenals, uterus, of all animals. [25]

4.3.2. 90 DAYS REPEATED ORAL TOXICITY STUDIES OF VISHATHIRKU POORA MATHIRAI:

1. Experimental Animals:

Species: Wister albino Rats

Sex: Male and Female

Age / Weight at start of Test: 8- 12 weeks / 140 – 160 g b.wt.

Acclimatization Period: 7 Days prior to dosing.

Housing: Individually in polypropylene cages

Husbandry: 12 hour light/ 12 hour dark cycle.

Room temperature: 220 c (± 30)

Humidity: Relative 30 – 70 %

Feed and Water: Rodent pelleted feed, RO purified water *ad libitum.*

Identification: Animals will be kept in Individual cages and numbered. The Male & Female albino rats were obtained from the authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram Chennai and stocked in the animal house at National Institute of Siddha, Chennai – 47. Animals were housed in a cage at $22^{o}c \pm 3^{o}$ and relative humidity 30 – 70 % and have free access to standard rat pellet diet (Sai meera Feeds Pvt. Ltd, Bangalore).

2. EXPERIMENTAL DETAILS:

a) Animal selection and Identification:

The animals are randomly selected for each group. Each group contains Ten Male and Ten female animals. They were marked on head, body, tail, Head body, Body tail with picric acid solution prepared in water for identification in each group.

Test guideline: OECD guidelines (408)

Length of exposure to test substance: 90 days

No of animals: 10Male +10 Female / group

Control group: Warm water

Test groups: *VPM Low Dose, Mid Dose, High Dose*

Route of Administration: Oral

Groups No. of Rats:

Group I: Control Warm water -20(10M+ 10F)

Group II: Test drug (*VPM*) - Low dose 90 mg/kg b.wt 20(10M + 10F)

Group III: Test drug (*VPM*) - Mid dose 180 mg/kg b.wt 20(10M + 10F)

Group IV: Test drug (*VPM*) - High dose 360 mg/kg b.wt 20(10M + 10F)

3. EXPERIMENTAL PROCEDURE:

The 40 male and 40 female Wister albino rats are used for 90 days repeated oral toxicity study. The animals are divided into four groups. Each group contains 20 animals (10 Female and 10 Male). The first group treated as control and second, third, fourth groups were treated with Low dose 90 mg/kg/ b.wt, Mid dose 180 mg/kg/b.wt, High dose 360mg/ kg/ b.wt of Vishathirku Poora Mathirai mixed with RO water for 90 days. The low dose, mid dose and high dose of test drug will be calculated from human therapeutic dose based on by using the conversion table Paget and Barnes 1964. The control animals were administered with RO water. The administration was given by oral, once daily for 90 consecutive days. The animals were observed for the behavioural parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Feed & water intake were Calculated daily. All the animals were sacrificed at the end of the study 91st day by using the intra peritoneal injection of Pentothal Sodium as prescribed dose level. Blood was collected from the anesthetized animals from the abdominal aorta for the following investigations like Haemotology and Biochemical analysis. Gross pathological changes were monitored in all the organs and then the vital organs were preserved and subjected to Histopathological examination.

4. Observations:

Experimental animals were kept under observation throughout the course of study for the following:

-All rats were observed twice daily for 90 days

- Body weight were Calculated weekly once

- Feed & water intake were calculated daily

a) Cage side observation:

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

b) Laboratory Investigations:

On the 90^{th} day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

c) Hematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

d) Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of serum glutamate oxaloacetate transaminase/ Aspartate aminotransferase (SGOT/AST), serum glutamate pyruvate transaminase/ Alanine aminotransferase (SGPT/ALT) were estimated as per the colorimetric procedure.

e) Necropsy:

All the animals were sacrificed on the 91^{st} day. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and

abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, sex organs, of all animals were recorded.

f) Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs and stomach of the animals were preserved, and they were subjected to histopathological examination. Histopathological investigation of the vital organs was done. The organ pieces (35µm thick) of all the animals (low, mid, high) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technique 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^{o}C and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Hematoxylin-eosin. [26]

g) Statistical analysis:

Findings such as body weight changes, feed consumption, water intake, and hematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D Graph Pad Instat-3.

5. RESULTS

STANDARDIZATION OF THE TEST DRUG:

Standardization of the drug is more essential to derive the quality, purity and stability of the drug, which was analyzed by the various methods. Standardization of **Vishathirku Poora Mathirai** had been done and the results are tabulated. The results of Organoleptic characters (Table 1), Physicochemical (Table 2), Biochemical analysis (Table 3), Phyto chemical analysis(Table 4), Instrumental analysis such as Microbial contamination (Table 5), Pesticide residue (Table 6), Aflatoxin (Table 7) and heavy metal analysis (Table 8).

Organoleptic Character of Vishathirku poora Mathirai:

The following characters had been noted in **Vishathirku Poora Mathirai**

Table 1: Organoleptic characters of Vishathirku poora Mathirai

S.no	Test Performed	Result
1.	Colour in day light	Black
2.	Appearance	Round
3.	Nature	Hard
4.	Solubility	Sparingly soluble in water Completely soluble in acid

Physico-Chemical Properties of Vishathirku Poora Mathirai

Table 2: Physico-Chemical Properties of Vishathirku Poora Mathirai

S.no	Tests performed	Result
1.	Moisture Content	10 %
2.	Total Ash	1% Ash content
3.	Alcohol Soluble Extractive	0.4 %
4.	Water Soluble Extractive	0.7 %
5.	PH	6.45
6.	Disintegration time	12 min

BIOCHEMICAL ANALYSIS OF VISHATHIRKU POORA MATHIRAI

Table 3: Bio Chemical Analysis of Vishathirku Poora Mathirai

S.no	PROCEDURES	RESULTS
1.	Test for Magnesium	Absent
2.	Test for Aluminium	Absent
3.	Test for Calcium	**Present**
4.	Test for Ferrous Iron	**Present**
5.	Test for Zinc	Absent
6.	Test for Arsenic	Absent
7.	Test for Mercury	Absent
8.	Test for Lead	Absent
9.	Test for Sulphate	**Present**
10	Test for Chloride	Absent
11	Test for Phosphate	**Present**
12	Test for carbonate	Absent
13	Test for Fluoride & Oxalate	Absent
14	Test or Starch	Absent
15	Test for Reducing Sugar	Absent
16	Test for Alkaloids	**Present**
17	Test for Amino Acids	**Present**

PHYTOCHEMICAL ANALYSIS OF VISHATHIRKU POORA MATHIRAI

Table 4: Phytochemical analysis of Vishathirku Poora Mathirai

S.no	Phytochemicals	RESULTS
1.	Alkaloids	**Present**
2.	Flavonoids	Absent
3.	Glycosides	Absent
4.	Saponins	**Present**
5.	Steroids	Absent
6.	Triterpenoids	Absent
7.	Tannins	**Present**
8.	Phenol	**Present**
10	Coumarin	Absent
11	Protein	**Present**
12	Anthocyanin	Absent
13	Betacyanin	Absent

INSTRUMENTAL ANALYSIS

HEAVY METAL ANALYSIS OF VISHATHIRKU POORA MATHIRAI BY AAS METHOD:

Table 5: Test for Heavy Metals of Vishathirku Poora Mathirai

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Mercury	253.7 nm	0.09	1 ppm
Lead	217.0 nm	0.03	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm

BDL- Below Detection Limit

RESULT:

Results of the present investigation clearly showed that the VPM sample had no traces of heavy metals such as Arsenic and Cadmium. Further the results showed the presence of mercury and lead at 0.09 and 0.03 ppm in VPM those levels are below the prescribed limit.

AFLATOXIN OF VISHATHIRKU POORA MATHIRAI*:*

Table 6: Aflatoxin of Vishathirku Poora Mathirai*:*

Aflatoxin Sample	**VPM**	**AYUSH Specification Limit**
Aflatoxin B1	Not Detected - Absent	0.5 ppm
Aflatoxin B2	Not Detected – Absent	0.1 ppm
Aflatoxin G1	Not Detected - Absent	0.5 ppm
Aflatoxin G2	Not Detected - Absent	0.1 ppm

RESULT:

The results showed that there was no spots identified in the VPM test sample loaded on TLC plates when compare to the standard , which indicates that the VPM were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

PESTICIDE RESIDUE OF VISHATHIRKU POORA MATHIRAI

Table 7: Pesticide Residue of Vishathirku Poora Mathirai

PESTICIDE RESIDUE	**SAMPLE VPM**	**AYUSH LIMIT**
I.ORGANO CHLORINE PESTICIDES		
Alpha BHC	BQL	0.1
Beta BHC	BQL	0.1
Gamma BHC	BQL	0.1
Delta BHC	BQL	0.1
DDT	BQL	1
Endosulfan	BQL	3
II.ORGANO PHOSPHORUS PESTICIDES		
Malathions	BQL	1
Chlopyriphos	BQL	0.2
Dichlorovos	BQL	1
III. PYRETHROIDS		
Cypermethrin	BQL	1

BQL- Below Quantification Limit

RESULT:

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus and pyrethroids in the VPM.

TEST FOR SPECIFIC PATHOGEN

Table 8: Test for Specific Pathogen

Organism	Result	Specification
E-coli	Absent	Absent
Salmonella	Absent	Absent
Staphylococcus Aureus	Absent	Absent
Pseudomonas Aeruginosa	Absent	Absent

RESULT:

No growth / colonies were observed in any of the plates inoculated with the VPM test sample. A result shows that VPM is free from specific pathogens like *E-coli, Salmonella, Staphylococcus Aureus and Pseudomonas Aeruginosa.*

MICROBIAL CONTAMINATION OF VISHATHIRKU POORA MATHIRAI:

Table 9: Microbial Contamination of VPM:

Test	Result	Specification
Total Bacterial Count	Absent	NMT 10^5CFU/g
Total Fungal Count	Absent	NMT 10^3CFU/g

RESULT:

No growth / colonies were observed in any of the plates inoculates with the VPM test sample. A result shows that VPM is free from microbial contamination.

5.2. TOXICOLOGICAL STUDIES

The Safety profile of test drug was evaluated through Acute and Sub Chronic Toxicity study on Wister albino rats.

5.2.1. ACUTE TOXICITY STUDY:

The acute toxicity study was performed to evaluate the toxicity/ safety of the test drug. The test drug was administrated orally to Female Wister albino Rats with different single doses as per OECD guide line 423. Then the animal's behaviors were observed throughout the period. The behavioral sign was mentioned in Table.

Table 10: Behavioral Signs of Acute Toxicity Study of VPM

No	Dose Mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	300	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3.	2000	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased 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. Dyspnoea 20. Mortality.

Note: (-) - Absent of activity, & (+) - Present of activity.

All the data were summarized in the form of (Table-10) revealed that there was no abnormal signs and behavioral changes in all animals at the dose level of 300, 2000 mg/kg body weight administered orally, during the study period.

There was no mortality observed after dosing of Vishathirku Poora Mathirai (VPM) up to 2000mg/kg body weight during the study period of 14 days. This indicates that the LD50 of Vishathirku Poora Mathirai is more than 2000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group.

At the end of the 14 the day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

In group III, No animals were died within 14 day after dosing of Vishathirku Poora Mathirai (VPM) up to 2000mg/kg body weight. This indicates that the Toxic dose of Vishathirku Poora Mathirai is above 2000 mg/Kg b.wt.

5.2.2. 90 DAY REPEATED DOSE ORAL TOXICITY STUDY

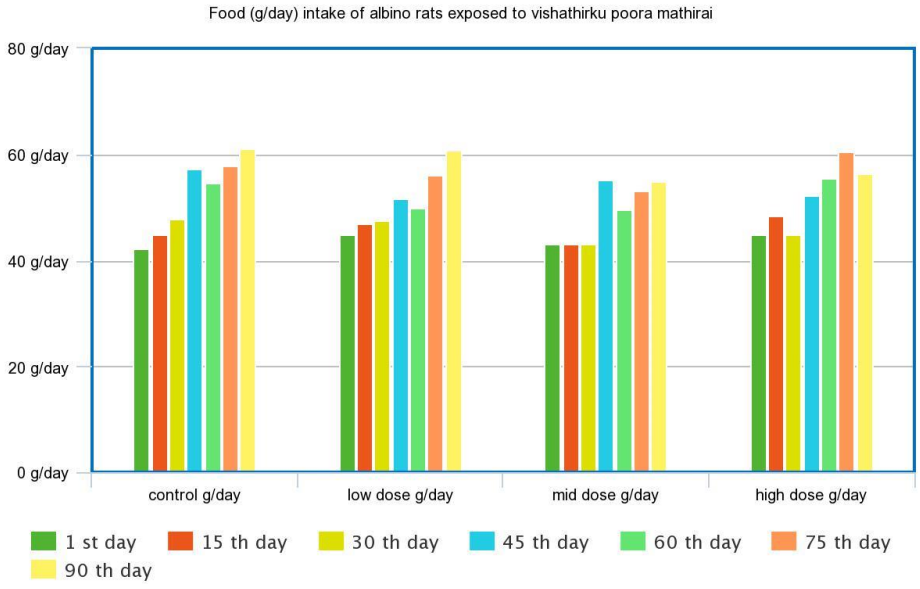
FEED CONSUMPTION

There was no significant difference in Feed intake the test group animals were observed when compared with control group during the study period. (Table 11),

Table: 11 Feed (g/day) intakes of albino rats exposed to Vishathirku poora Mathirai

	1st day	15th day	30th day	45th day	60th day	75th day	90th day
Control	42.15±1.8	45±1.4	47.75±1.4	57.25±1.08	54.75±1.47	58±1.5	61±1
Low dose	44.75±2.5	47±3.8	47.45±5.06	51.75±3.3	49.75±3.5	56±1.8	60.75±2.16
Mid dose	43±3.5	43±1.8	43.2±1.4	55.25±4.3	49.5±2.9	53±3.7	55±3.16
High dose	44.75±3.49	48.5±2.9	45±3.16	52.25±2.86	55.5±3.8	60.5±1.1	56.25±5.8

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01



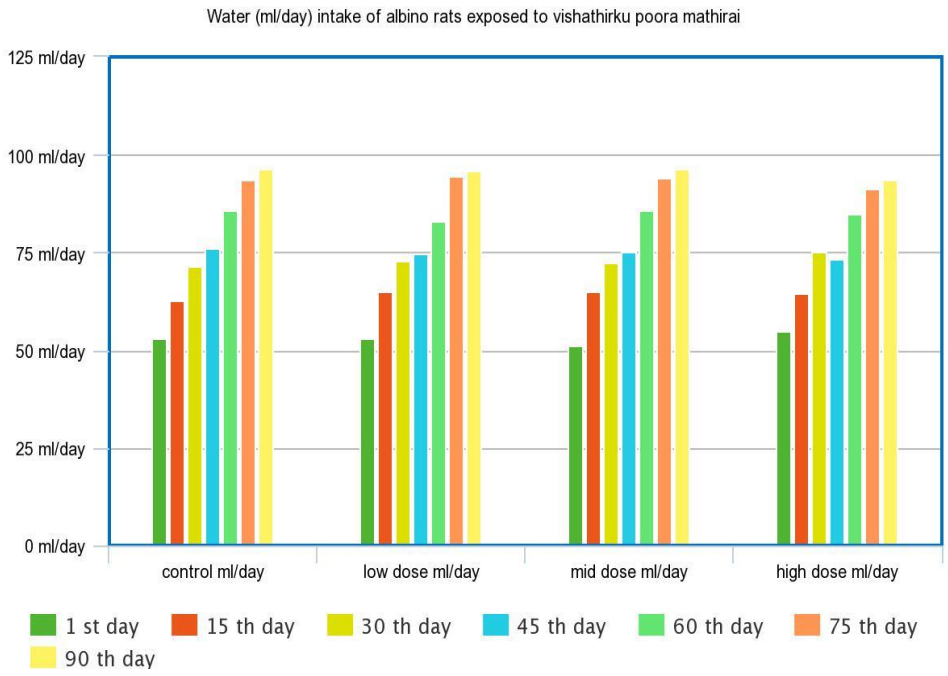
WATER INTAKE OF WISTER ALBINO RATS EXPOSED TO VPM FOR 90 DAYS

There was no significant difference in Water intake of test group of animals compared with control group of animals (Table 12).

Table: 12 Water (ml/day) intakes of albino rats exposed to Vishathirku poora Mathirai

	1st day	15th day	30th day	45th day	60th day	75th day	90th day
Control	53±1.87	62.75±1.92	71.5±3.9	76±2.45	85.75±1.48	93.5±1.11	96.5±1.12
Low dose	53.25±2.16	65.25±2.38	73±3.08	74.5±3.35	83±2.23	94.75±1.92	96±1.58
Mid dose	51.25±1.29	65±1.58	72.25±2.86	75±2.23	86±2.23	94.25±1.92	96.25±1.47
High dose	55±2.23	64.75±2.16	75±2.236	73.25±1.47	85±2.23	91.5±2.29	93.75±2.58

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01



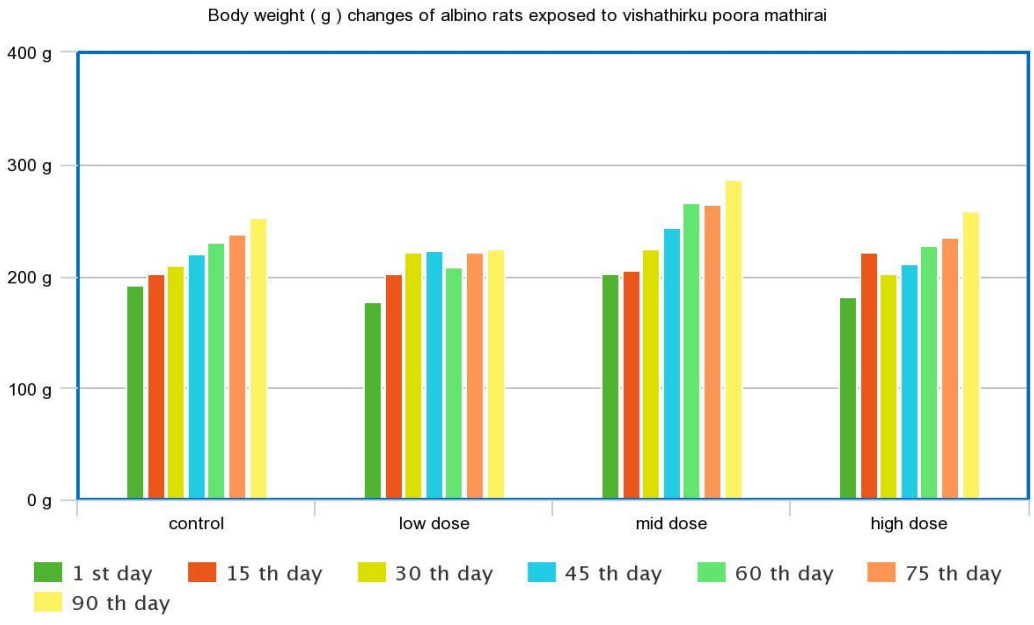
BODY WEGHT OF WISTER ALBINO RATS EXPOSED TO VPM FOR 90 DAYS

Body weight of both control and test dose group revealed normal body weight throughout the study .There is no significant change occur in body weight of test group compared with control group (Table 13).

Table: 13 Body weight (g) changes of albino rats exposed to Vishathirku poora Mathirai

	1st day	15th day	30th day	45th day	60th day	75th day	90th day
Control	191.35±27.05	202.15±25.40	209.85± 26.76	219.55± 29.71	230.6± 31.82	237.7± 34.78	251.8±32.5
Low dose	176.45±24.11	202.7±28.12	221.5± 28.16	222.25± 29.26	208.3± 22.24	221.7± 30.21	224.5±38.5
Mid dose	201.65±19.33	205.1±20.53	224.55± 26.07	243.7±2 6.98	265.35±33	264± 38.44	286.5±49.3
High dose	182.1±18.53	221.9±21.82	201.5± 17.94	211.06± 15.54	227.5±17.4	235.23± 10.23	258.73±12.

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01



90 DAY REPEATED DOSE ORAL TOXICITY STUDY – HEAMATOLOGICAL PARAMETER

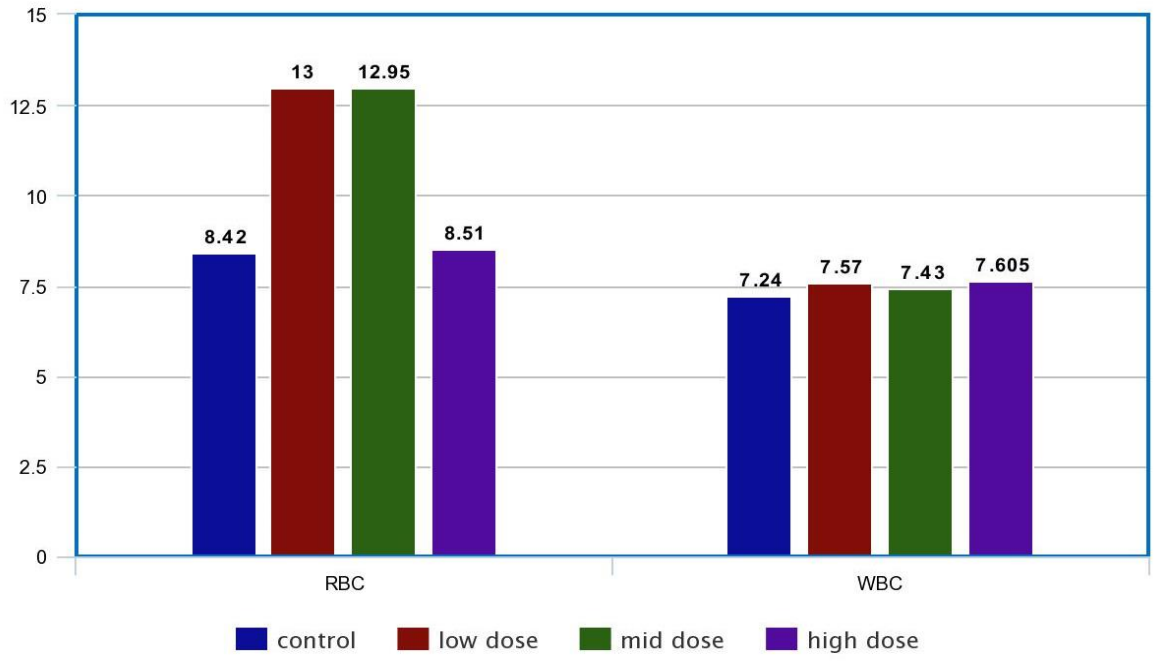
There were no significant changes in levels of hematological parameters in test group, when compared with control group.

Table 14: Effect of Vishathirku Poora Mathirai on Hematological Parameter

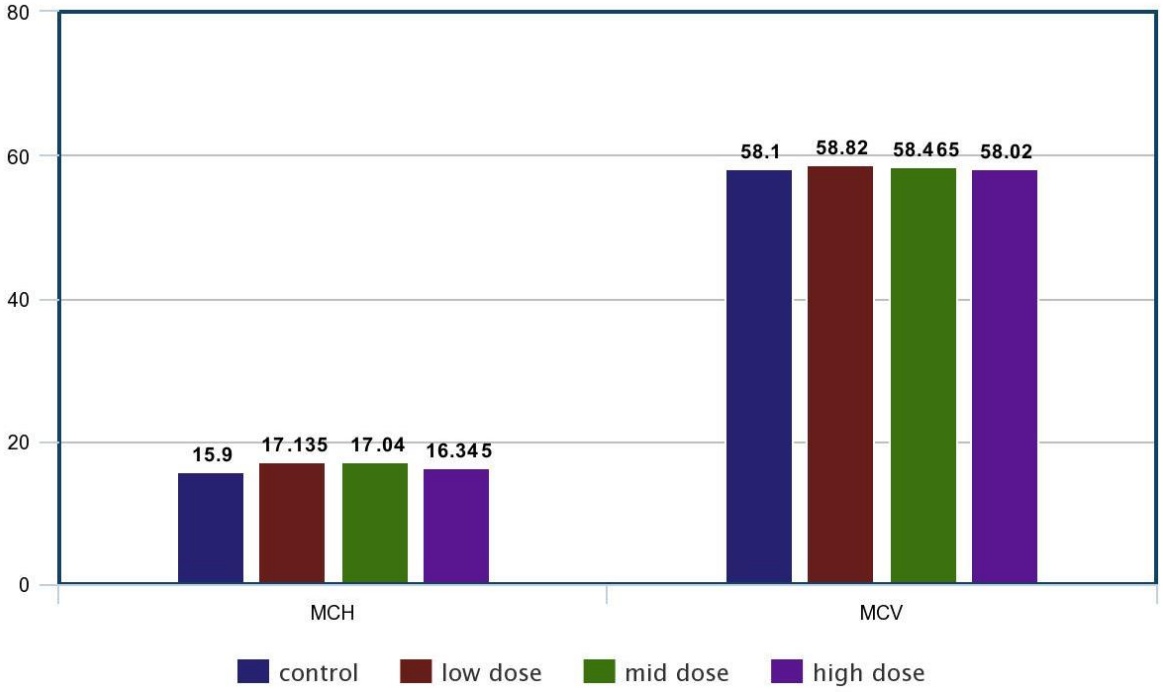
PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
RBC (X 10^6µl)	8.42±0.71	13±19.51	12.95±19.52	8.51±0.69
WBC (X 10^3 / µl)	7.24±0.95	7.57±0.89	7.43±0.92	7.605±0.95
PLT (X 10^3 / µl)	792±176	775.5±0.89	789.65±167.7	708.85±195.98
HB (g/dl)	13.6±1.38	14.05±1.57	13.845±1.55	13.5±1.07
MCH (pg)	15.9±3.87	17.135±2.38	17.04±2.26	16.345±1.92
MCV(fl)	58.1±5.17	58.82±4.92	58.465±5.05	58.06±4.47
Neutrophils (10^3/mm^3)	29.5±3.9	30.145±4.25	29.93±4.16	28.99±4.70
Eosinophils (%)	1.3±0.1	1.335±0.165	1.31±0.144	1.33±0.18
Basophils (%)	0.1±0.3	0.1±0.3	0.1±0.3	0.15±0.35
Lymphocytes (%)	67.3±3.27	68.1±2.8	67.9±2.78	68.92±3.08
Monocytes (%)	2.6±0.7	2.8±1	2.65±0.8	2.7±0.91

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

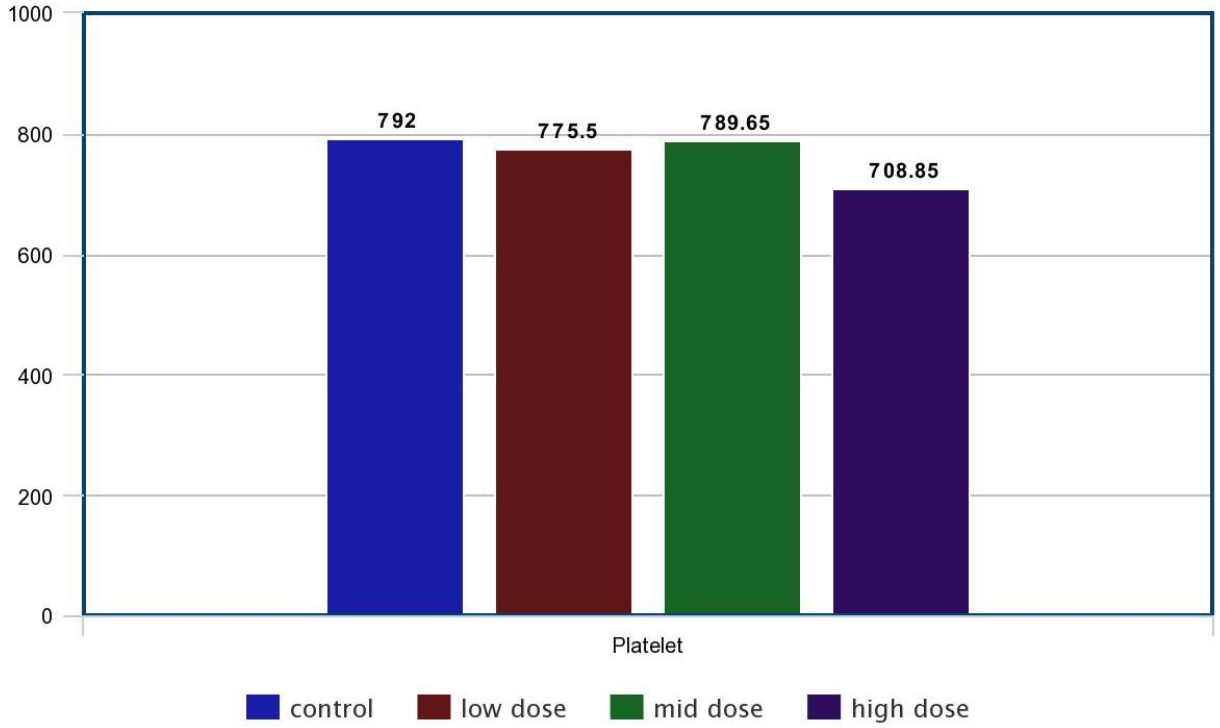
Effect of Vishathirku Poora Mathirai on RBC (10^6/µl) &WBC (10^3/ µl):



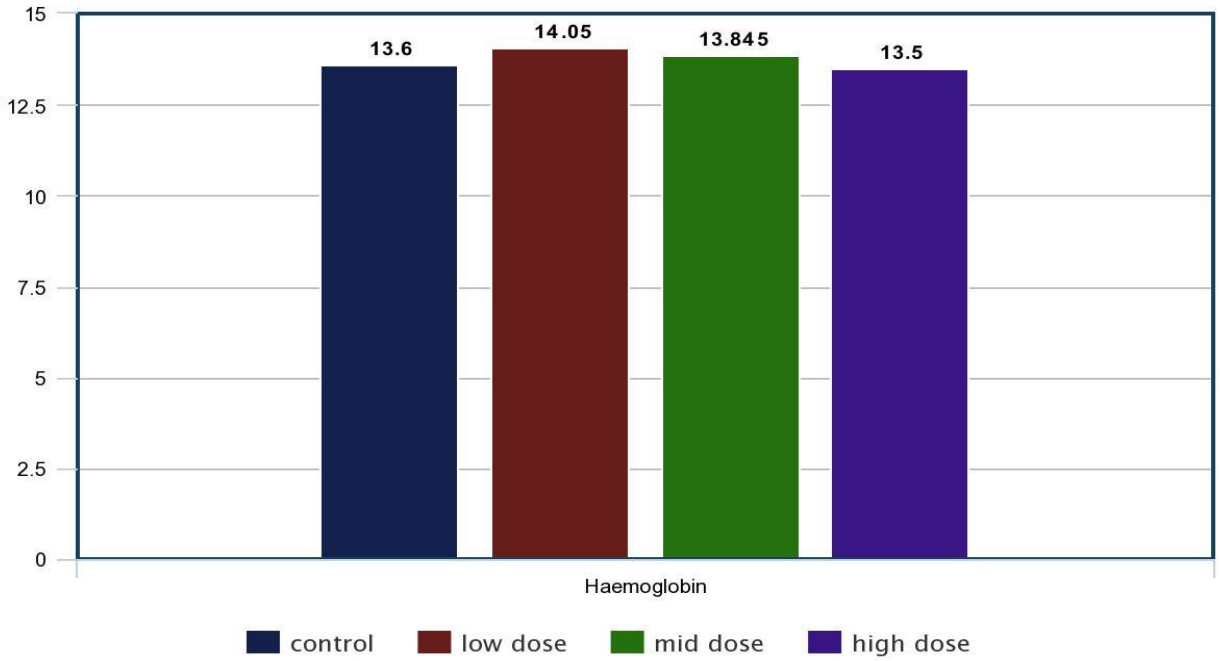
Effect of Vishathirku Poora Mathirai on MCH (pg) & MCV (fl):



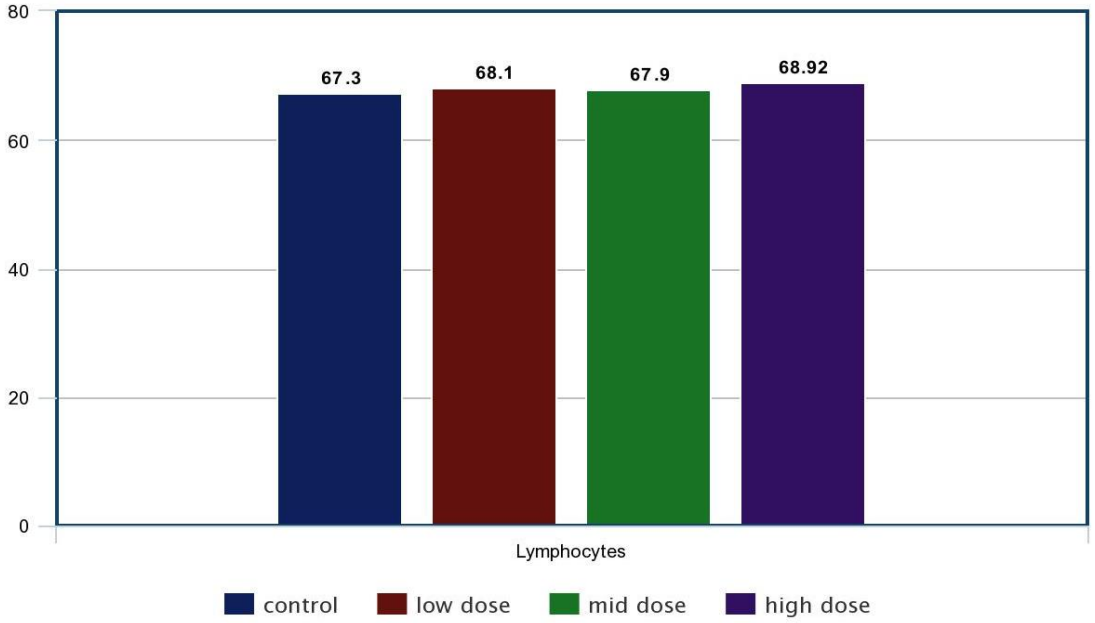
Effect of Vishathirku Poora Mathirai on Platelet (10^3/ µl):



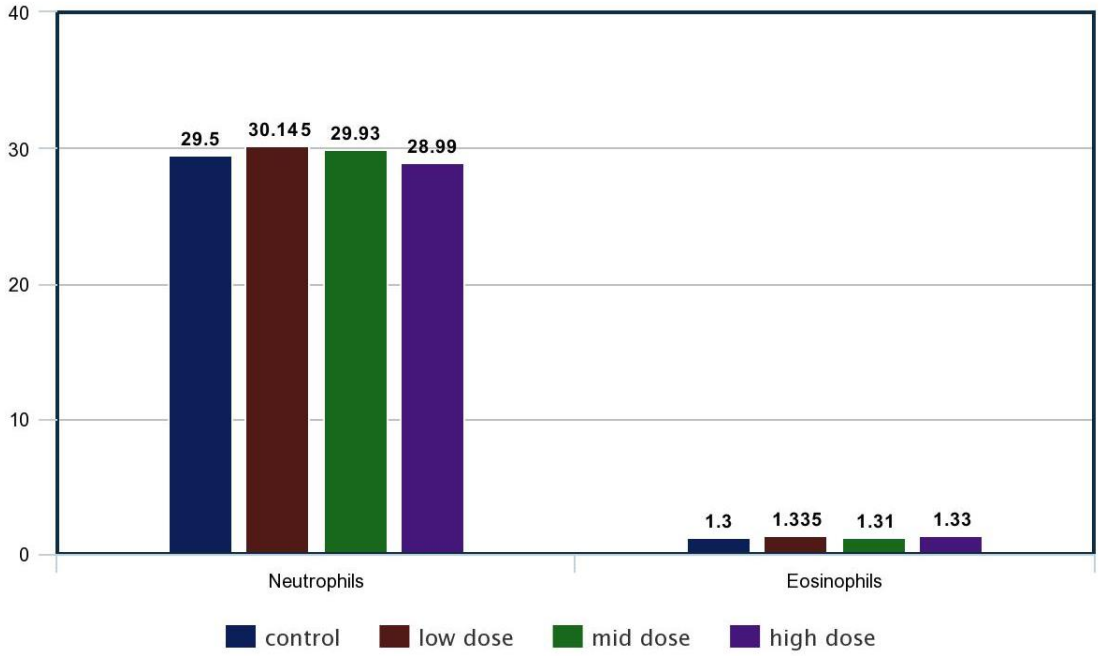
Effect of Vishathirku Poora Mathirai on Hemoglobin (g/dl):



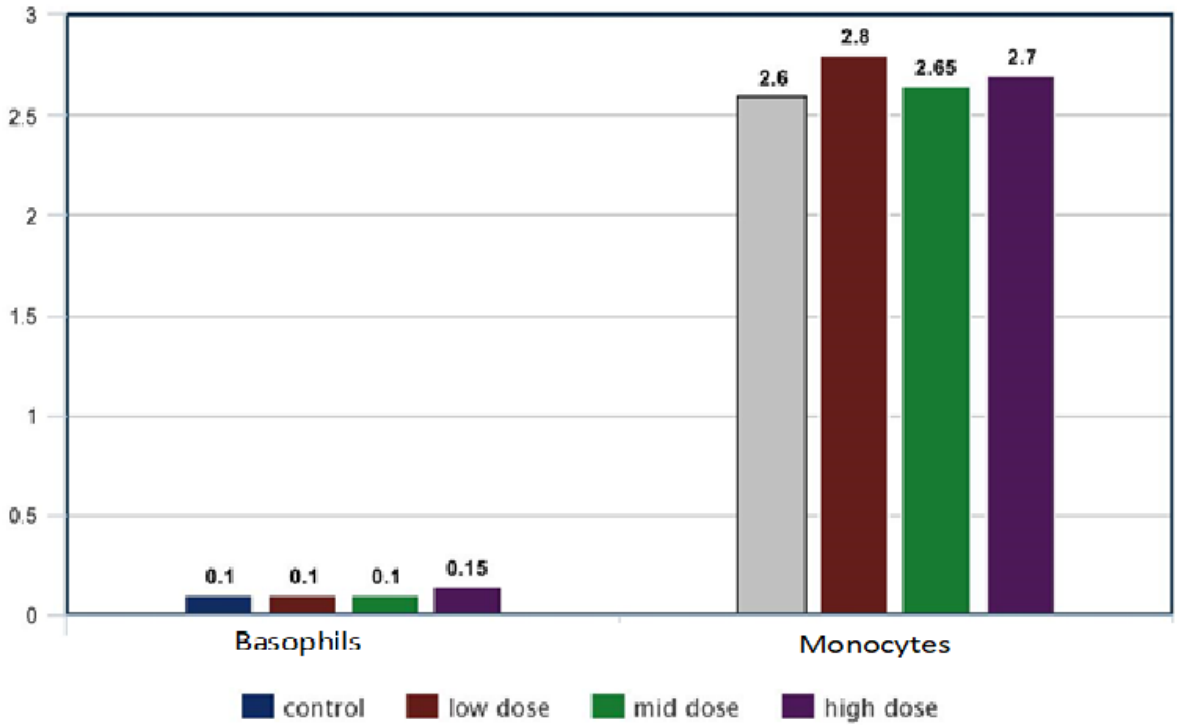
Effect of Vishathirku Poora Mathirai on Lymphocytes (%):



Effect of Vishathirku Poora Mathirai on Neutrophil (10^3/mm^3) and Eosinophils (%):



Effect of Vishathirku Poora Mathirai on Basophils (%) and Monocytes (%):



90 DAY REPEATED DOSE ORAL TOXICITY STUDY- BIOCHEMICAL PARAMETERS

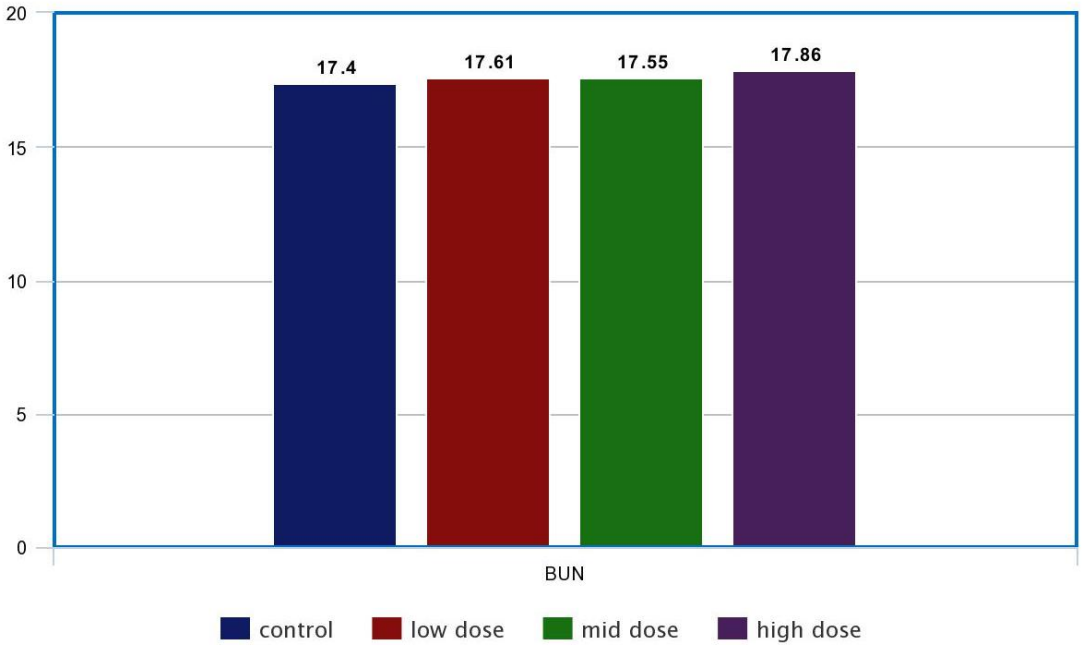
There were no significant changes in levels of biochemical parameters in test group, when compared with control group. (Table 15)

Table 15 Effect of Vishathirku Poora Mathirai on renal function test:

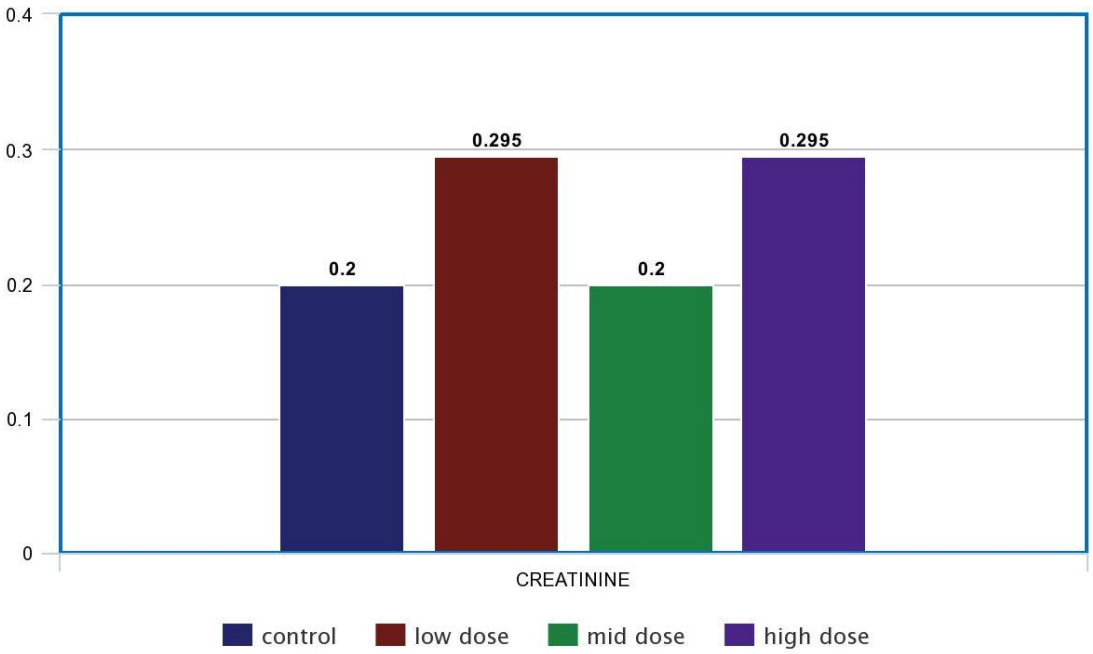
PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BUN (mg/dl)	17.4±2.2	17.61±2.42	17.55±2.3	17.86±2.38
CREATININE (mg/dl)	0.2±0.07	0.295±0.39	0.2±0.07	0.295±0.39

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Effect of Vishathirku Poora Mathirai on BUN (mg/dl):



Effect of Vishathirku Poora Mathirai on Creatinine (mg/dl):



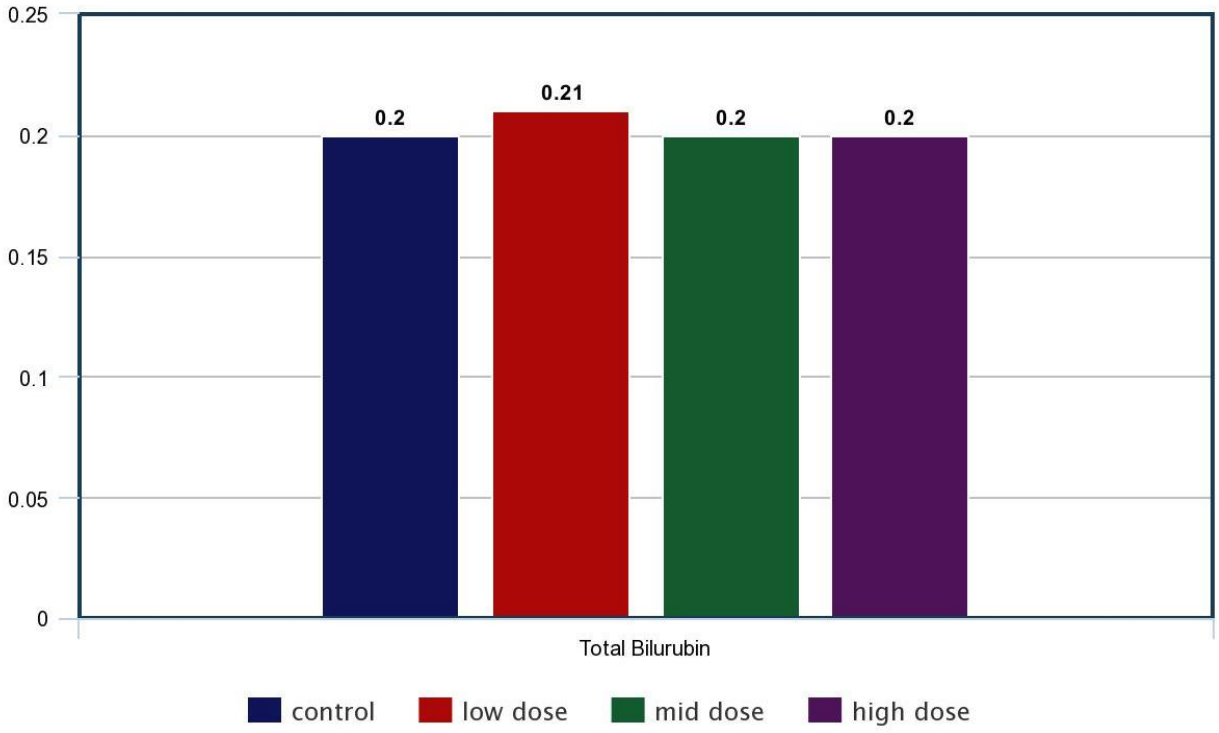
90 DAY REPEATED DOSE ORAL TOXICITY STUDY -LIVER FUNCTION TEST

Table 16 Effect of Vishathirku Poora Mathirai on liver function test:

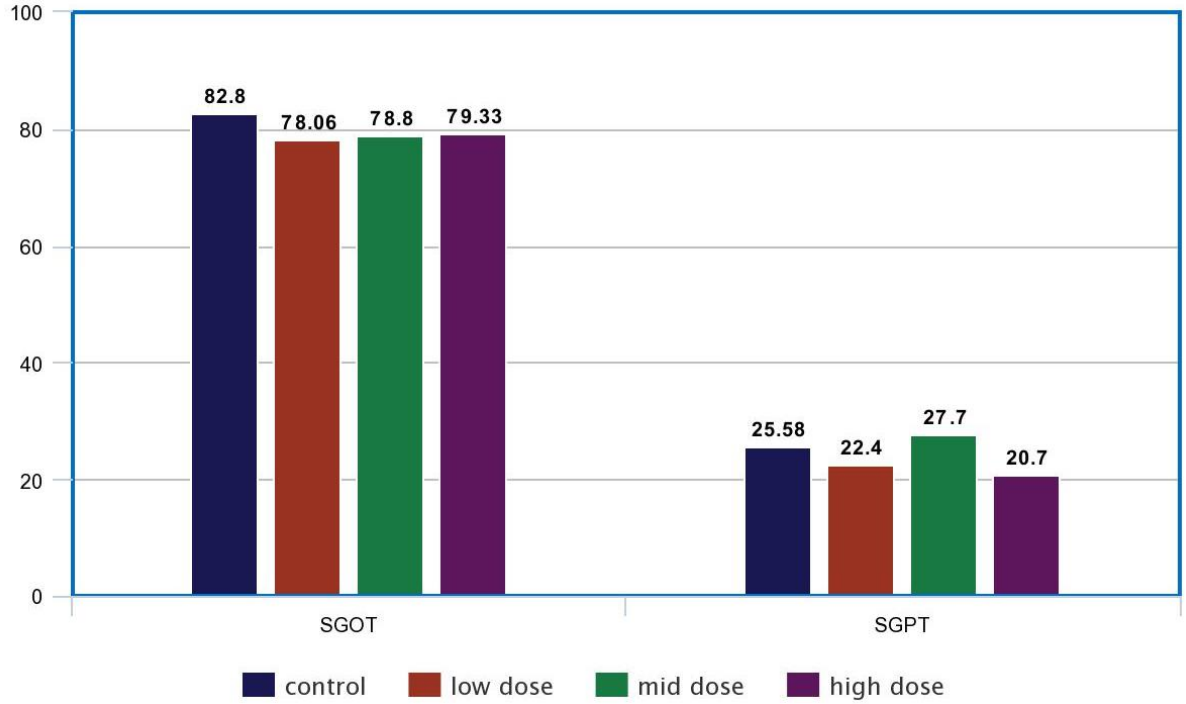
PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TOTAL BILURUBIN (mg/dl)	0.2±0.07	0.21±0.06	0.2±0.07	0.2±0.07
SGOT (U/dl)	82.8±6.4	78.06±4.45	78.8±3.7	79.33±4.73
SGPT (U/dl)	25.58±7.05	22.4±4.9	27.7±4.2	20.7±5.7

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

Effect of Vishathirku Poora Mathirai on Total Bilirubin (mg/dl):



Effect of Vishathirku Poora Mathirai on SGOT &SGPT (U/dl):



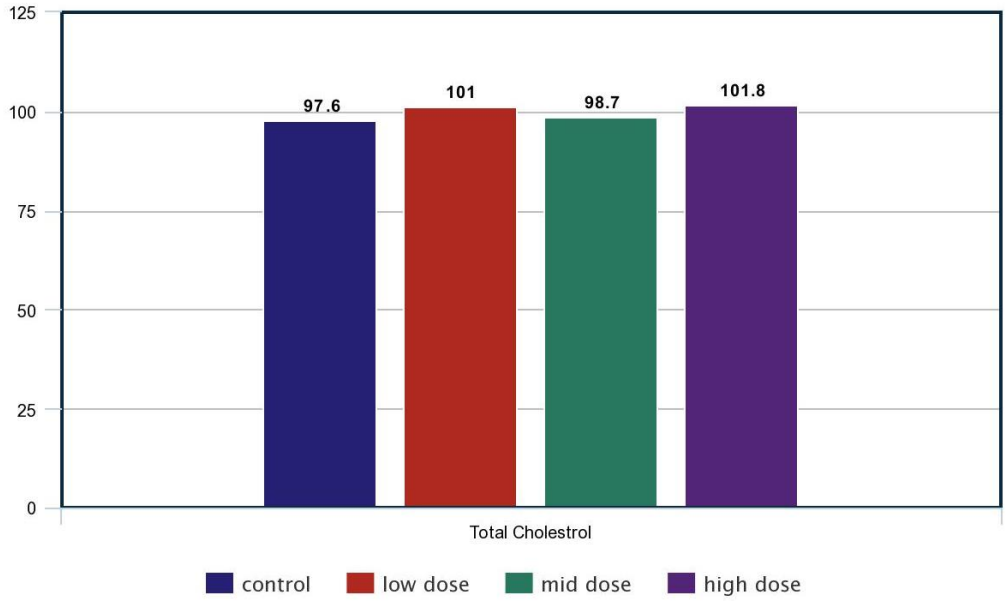
90 DAY REPEATED DOSE ORAL TOXICITY STUDY- LIPID PROFILE

Table 17 Effect of Vishathirku poora Mathirai on lipid profile:

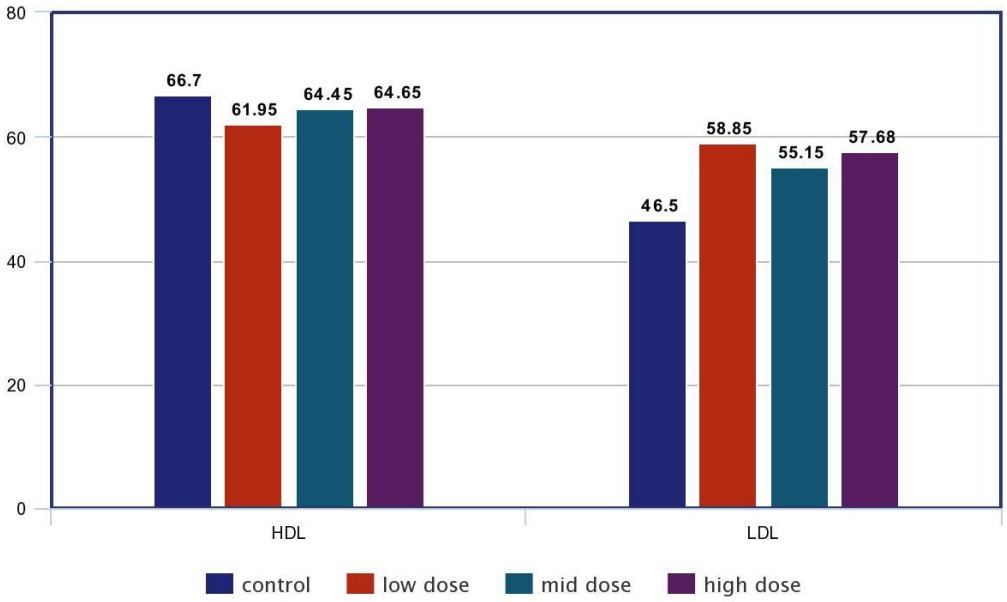
PARAMETER	**CONTROL**	**LOW DOSE**	**MID DOSE**	**HIGH DOSE**
TOTAL CHOLESTEROL (mg/dl)	97.6±18.9	101±15.8	98.7±17.04	101.8±16.6
HDL (mg/dl)	66.7±4.7	61.95±5.35	64.45±5.9	64.65±5.46
LDL (mg/dl)	46.5±7.59	58.85±7	55.15±11.2	57.65±9.57
VLDL (mg/dl)	17.06±1.48	16.4±1.02	17±1.4	16.52±1.14
TRIGLYCERIDES (mg/dl)	38.6±3.7	41.82±3.5	39.7±4.8	41±4.06

Values were expressed as mean± S.D. for N=20 rats in each group one-way ANOVA followed by Dunnet's test. Significant indicates that *P<0.05, **P<0.01

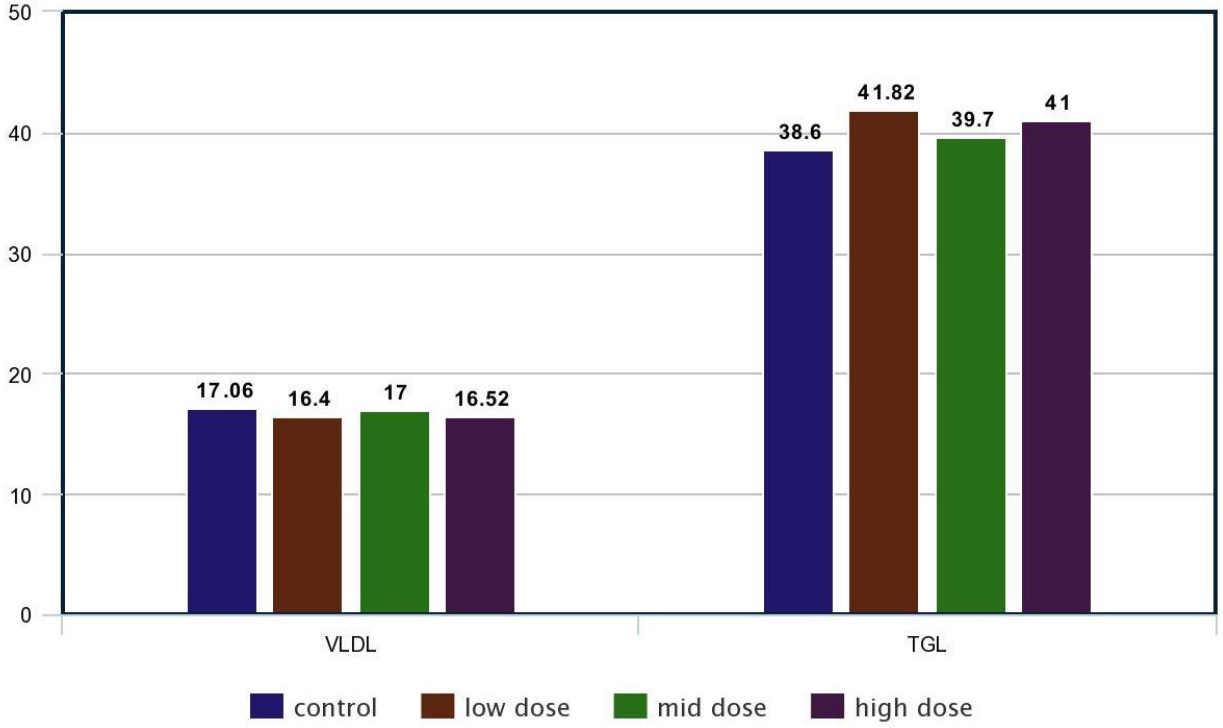
Effect of Vishathirku poora Mathirai on Total cholesterol (mg/dl):



Effect of Vishathirku poora Mathirai on HDL and LDL (mg/dl):



Effect of Vishathirku Poora Mathirai on VLDL & TGL (mg/dl):



EFFECT OF VPM ON HISTOPATHOLOGIC INVESTIGATON OF WISTER ALBINO RATS FOR 90 DAYS SUBCHRONIC TOXICITY STUDY

HISTOPATHOLOGY OF BRAIN

Plate A: Control Male

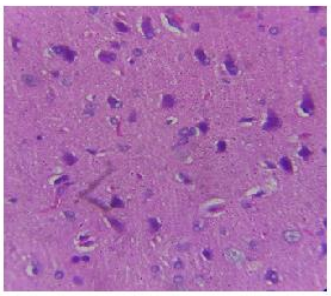


Plate B: Control Female

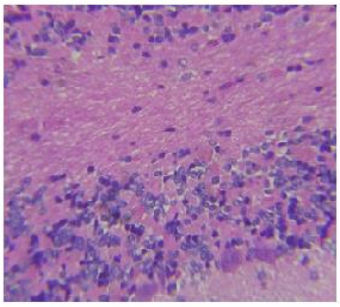


Plate C: High dose male

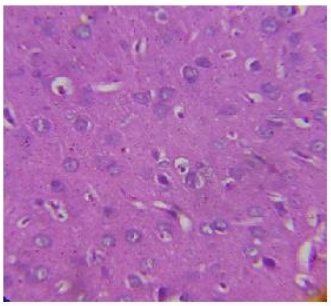


Plate D: High dose female

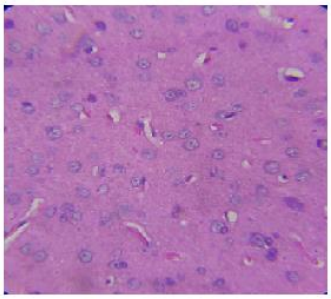


Plate A: Shows normal histology of striatum.

Plate B: Cerebellar section shows normal architecture of neurons.

Plate C: Normal arrangements of neurons with proper inter neuronal space were observed.

Plate D: The cerebral sections showed normal architecture in both cortex and medulla.

HISTOPATHOLOGY OF HEART

Plate A: Control male

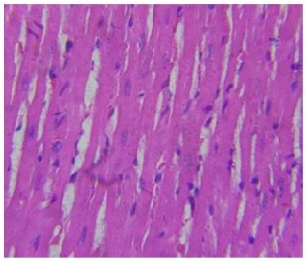


Plate B: Control female

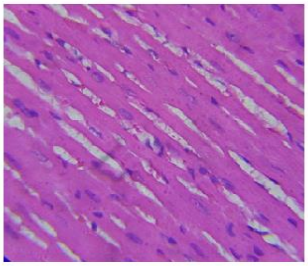


Plate C: High dose male

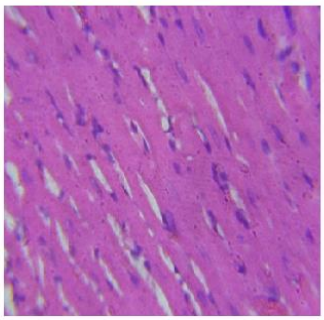


Plate D: High dose female

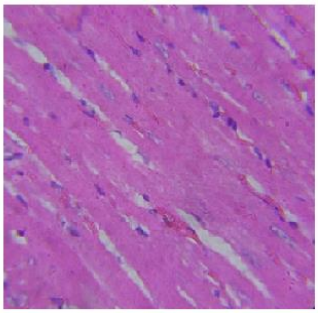


Plate A: Appearance of myofibrillar and cytoplasmic zone was normal

Plate B: Normal appearance of myocytes

Plate C: Fibres appears normal elongated and rod shaped

Plate D: Showing the normal histological structure of myocardium

HISTOPATHOLOGY OF LUNG

Plate A: Control Male

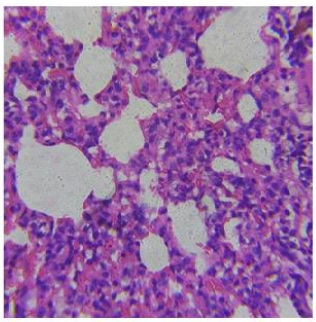


Plate B: Control Female

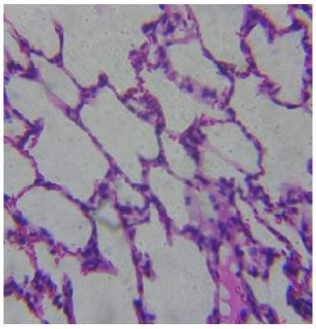


Plate C: High dose male

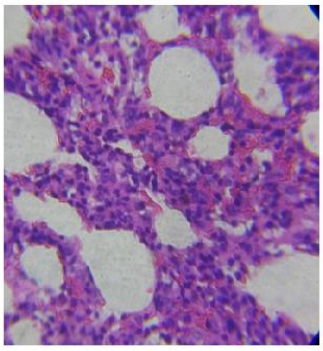


Plate D: High dose female

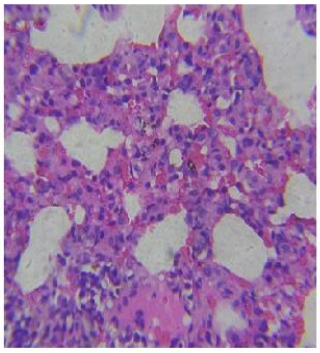


Plate A: Inter alveoli septum and bronchioles appears normal

Plate B: Alveolar epithelium and capillaries appears normal

Plate C: Alveolar septa and wall appeared widen and normal

Plate D: Regular arrangement of alveoli and alveolar sac

HISTOPATHOLOGY OF STOMACH

Plate A: Control Male

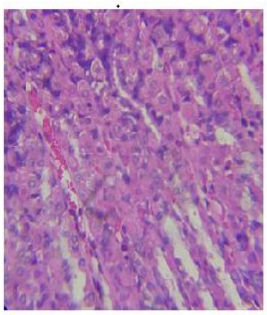


Plate B: Control Female

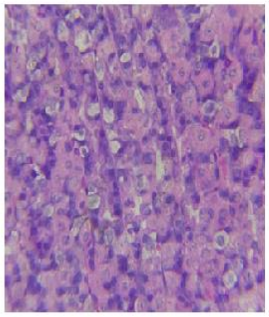


Plate C: High dose male

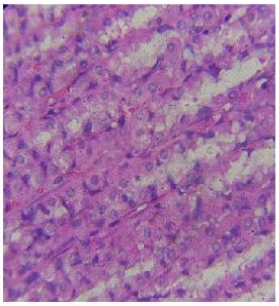


Plate D: High dose female

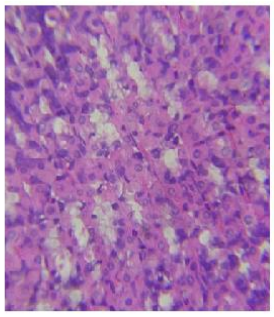


Plate A: Gastric epithelium and mucosa appears normal

Plate B: Mucosal wall appears normal with regular arrangement of connective tissue

Plate C: Normal gastric glands and gastric pits

Plate D: Normal surface epithelium, mucosa and sub-mucosa

HISTOPATHOLOGY OF LIVER

Plate A: Control Male

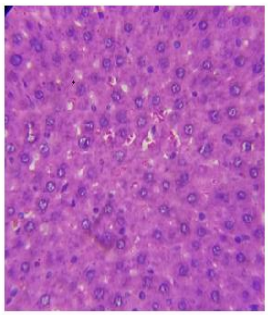


Plate B: Control Female

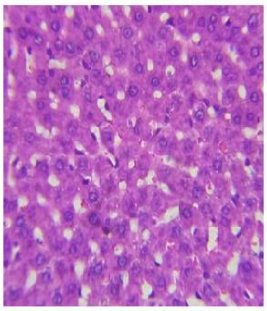


Plate C: High dose male

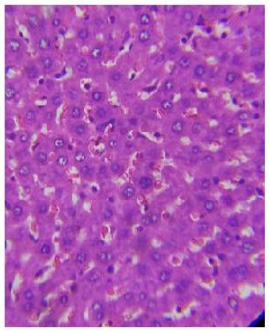


Plate D: High dose female

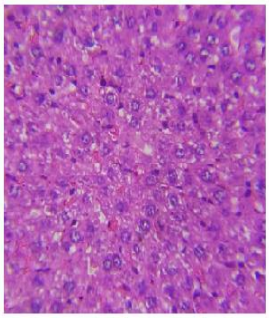


Plate A: Normal hepatocytes with occasional, appearance of variably pale necrotic changes were observed

Plate B: Showing normal hexagonal hepatic lobules with normal, regular radiated hepatic cords

Plate C: Cytoplasm appears normal with widen portal tract

Plate D: Hepatic cords appears normal with radiating morphology

HISTOPATHOLOGY OF KIDNEY

Plate A: Control male

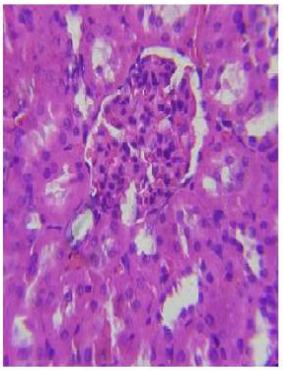


Plate B: Control female

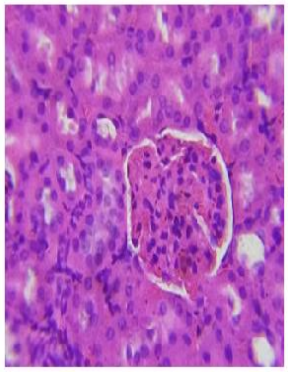


Plate C: High dose male

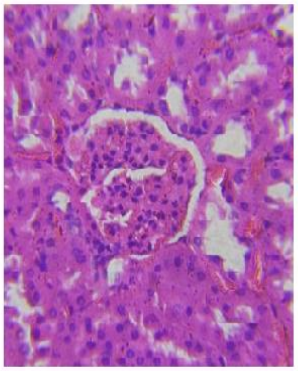


Plate D: High dose female

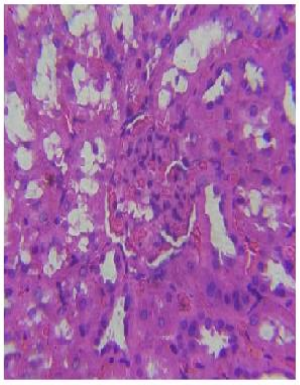


Plate A: Glomerular loop was normal with regular interstitium

Plate B: Showing normal, intact renal tubules as well as renal glomeruli.

Plate C: Lumen of distal convolutes tubule and collecting duct was normal.

Plate D: Appearance of proximal and distal convolutes tubules was normal

HISTOPATHOLOGY OF SPLEEN

Plate A: Control Male

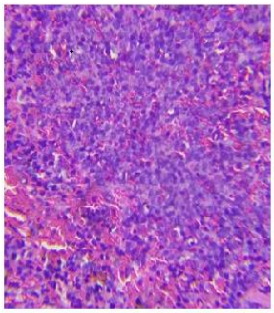


Plate B: Control Female

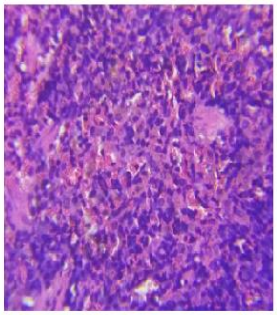


Plate C: High dose male

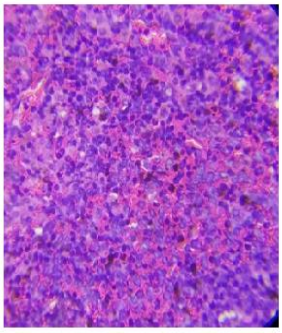


Plate D: High dose female

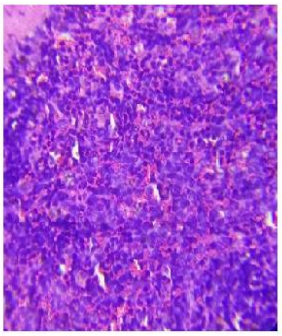


Plate A: Marginal sinus (MS) of the spleen and its sinus lining cells appears normal

Plate B: PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement

Plate C: Lymphoid follicles appears normal

Plate D: Regular appearance of red pulp

HISTOPATHOLOGY OF TESTES

Plate A: Control Male

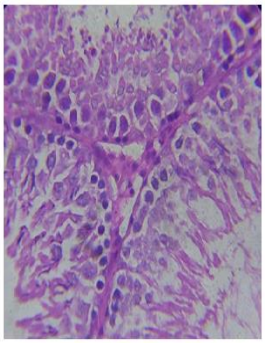


Plate B: High dose male

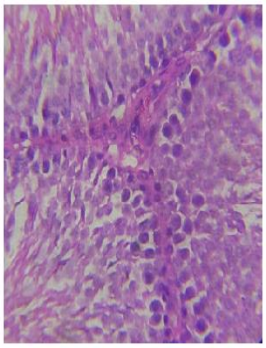


Plate A: Presence of mature somatic cells project the perfect histomorphology of testicular cells were observed

Plate B: Primary spermatocytes with large centered nucleus and dense chromatin were observed

HISTOPATHOLOGY OF UTERUS

Plate A: Control Female

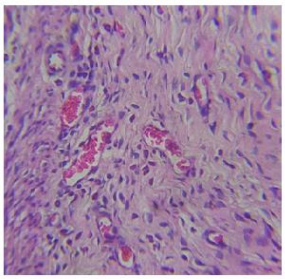


Plate B: High dose female

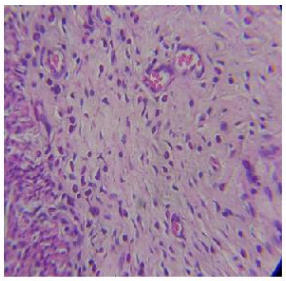


Plate A: Regular histology of uterine epithelium and endometrial glands

Plate B: Histopathological analysis of ovary showing normal corpus luteum

HISTOPATHOLOGY OF OVARY

Plate A: Control Female

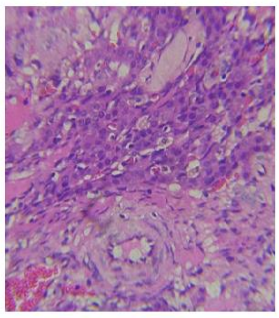


Plate B: High dose female

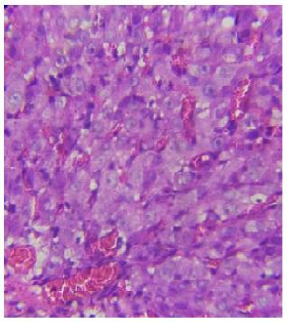


Plate A: Granulosa cells around oocyte was normal and regular

Plate B: Arrangement of stratum basale, functionale and surface epithelium seems normal

6. DISCUSSION

Metals and minerals are held in hand to hand in Siddha Pharmaceuticals with suitable as well as various process of purification. Therefore it has to be purified before using in the medicine preparation. In Siddha system of medicine one of the drug is Rasam, it has a long history in the treatment of many diseases among Siddha physicians. Pooram and Gandhagam is also used in many areas of Siddha medicine, it plays vital role in Siddha medicinal practice. Vishathirku Poora Mathirai is one of the traditional Siddha formulations which were indicated as an antidote to various to types of poisons of animals and other living creatures.

As an initial step, in this present study, a part of standardization of this drug and its safety has been confirmed through necessary analysis and Acute & 90 days Repeated Oral Toxicity studies as per OECD Test guidelines (423&408).[25, 26]

Standardization of the drugs means confirmation of its identity and determination of its quality and purity [27]**.** Standardization of the Mathirai was confirmed by the methodology of the Siddha text.

Physicochemical analysis of the Vishathirku Poora Mathirai from Table 1 showed that the organoleptic characters of Vishathirku Poora Mathirai which was black in colour and odorless. Tablet form of drug with pH of 6.45% revealed that, this is a slightly acidic. Hence in the oral administration, the drug is expected to be absorbed quickly in the stomach. It revealed that Vishathirku Poora Mathirai is expected to have better bio availability.

The loss on drying determines the amount of volatile matter (i.e. water drying off from the drug). This test is used to determine the stability and shelf-life of the drug. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus how moisture content could get maximum stability and better shelf-life. The loss on drying of Vishathirku Poora Mathirai was less than 1% w/w, which explains the moisture content of test drug is very low. [28]This result shows high stability and better shelf-life of VPM. It is easily soluble in water, alcohol and acetone and ether.

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form [29]**.** Total ash value was 1%, it implies that presence of inorganic constituents are low. This explains the purity of the test drug. (Table-2)

The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive. Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which correlates with the metabolism reactions. Water soluble and alcohol soluble extractive value plays an important role in evaluation of crude drugs. (Table-2) The extract value of alcohol in VPM is 0.4% and water is 0.7 %. The above result concludes that alcohol is little better solvent of extraction than water.

Qualitative analysis (Table-3) of Vishathirku Poora Mathirai for Acid radicals, Basic radicals, and other constituents demonstrated the presence of Calcium, Iron Sulphate, Phosphate, Alkaloids and Amino acids

Calcium is the major constituent of bones, teeth, muscle contraction and nerve transmission [30]. The total content of iron in an adult body is 3-5 g. About 70 % of this occurs in the erythrocytes of the blood as the constituents of hemoglobin. Peroxidase is the iron contained enzymes which is present in lysosomes. These enzymes are required for phagocytosis and killing of bacteria by neutrophil. Iron is associated with effective immune competence of the body so this iron is used to cure bacterial infection and boost the immunity. Sulphate is used to detoxify the toxic substance of the body by the conjugation process. So it eliminates the bacterial toxins in the body. Phosphate found in every cell of the body. Phosphate is present in bones, teeth, muscles and blood. It is important for the maintenance of ph in the blood as well as in the cell. Alkaloids are important secondary metabolite that is known to may possess curative properties. These are important therapeutic molecules that inhibit the growth and development of microorganisms including bacteria, fungi and protozoan. [30] Calcium, Iron Sulphate, Phosphate, Alkaloids and Amino acids were present in Vishathirku Poora Mathirai which suggests that this drug can be used to eliminate toxic symptoms from the body.

Phytochemical analysis showed the presence of Alkaloids, Saponins, Tannins, Phenol and Protein in Vishathirku Poora Mathirai (Table No.4)

The results of Heavy metal Analysis done by AAS method, results showed that Heavy metals such as Mercury, Lead were below the permissible limit Arsenic and Cadmium were found below the detectable limit so VPM is safe for oral consumption. (TableNo.5)

Test for Aflatoxin showed that the drug Vishathirku Poora Mathirai is free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2. (TableNo.6)

Pesticide residue analysis of the Vishathirku Poora Mathirai showed that it has below detectable limit for Organophosphorus, Organochlorine and Pyrethroid contents. (Table No.7)

Test for specific pathogens for the drug Vishathirku Poora Mathirai revealed that there is absence of E.coli, Salmonella, Staphylococcus Aureus and Pseudomonas Aeruginosa. (Table No.8)

Microbial contamination analysis of the Vishathirku Poora Mathirai showed that there was no bacterial and fungal count. (Table No.9)

In Acute toxicity study (Table -10); carried out as per OECD guideline 423, there was no treatment-related death or signs of toxicity developed in albino rats at dosage levels of 300mg and 2000mg/kg body weight throughout the study period. Further, no gross pathological changes had been seen in the internal organs of both control and treated groups. This study revealed the Safety of the drug.

To confirm the safety of Vishathirku Poora Mathirai **90 days Repeated Oral Toxicity** Study was also carried out as per OECD test guideline 408. This study was conducted for about 90 days as per OECD guidelines in 3 doses: Low dose (90 mg/kg.b.wt), mid dose (180 mg/kg.b.wt) and High dose (360 mg/kg.b.wt). Animals were observed throughout the period. After 90 days on the 91st day blood collection done for animals of all groups, all the animals were euthanized for gross pathological examinations of all major internal organs. The

blood samples were sent to a lab for hematological and biochemical analysis. The organs were weighed and preserved in 10% buffered formalin solution before sending for Histopathological study. All the reports were statistically evaluated.

There were no significant changes in feed intake (Table 11) and water intake (Table 12) of Wister albino rats compared to the control group. There were no significant changes in body weight of Wister albino rats compared to the control group. The Hematological results (Table 14) suggested that there were no significant changes compared to the control group. In biochemical parameter there were no significant changes in RFT (Table 15), LFT (Table 16) and Lipid profile (Table 17) compared to the control group. The histopathological study on the organs such as brain, heart, lungs, kidney, spleen, liver, stomach, uterus, ovary and testis was normal in high dose groups compared to the control groups. There was no pathological changes occur in all group of animals during the study period.

7. SUMMARY:

The test drug Vishathirku Poora Mathirai formulation was adopted from the text "Ramadevar Ennum Yakobhu eluthiya Vaithya Sindhamani 700'' for the evaluation of safety. The test drug was prepared with the ingredients of Purified Rasam (Mercury Hydragyrum), Purified Pooram (Calomel), and Purified Gandhagam (Sulphur), Kuppai Meni leaf juice (Acalypha indica). The toxicity of the test drug VPM was assessed by universal accepted scientific methods the ingredients were purchased from standard raw drug markets. The ingredients of VPM were identified and authenticated by the concerned departments. All the individual ingredients purified as per Siddha literature and formulation was prepared in NIS Gunapadam Lab. The preparation of trial drug was standardized primarily by physicochemical and biochemical analysis and then safety of the drug was evaluated by acute and sub chronic toxicity studies. The physicochemical analysis of the test drug showed increased bioavailability and purity of the test drug. The bio chemical analysis of VPM indicates the presence of Calcium, Ferrous Iron, Sulphate, Chloride, Phosphate, Oxalate and alkaloids. Microbial load, Aflatoxin and Pesticide residue were quantitatively measured in Vishathirku Poora Mathirai the result indicate the below detectable limit of them. Heavy metal analysis was carried out in Vishathirku Poora Mathirai by AAS to ensure the absence of Arsenic, Mercury, Cadmium and Lead. The acute toxicity study showed that Vishathirku Poora Mathirai did not produce any toxic effect at dose of 300mg/kg and 2000 mg/kg within 24 hours in Wister albino rats. No mortality and pathological changes had been noted in the internal organs of both control and treated groups on the 15^{th} day of the study. Body weight, feed intake and water intake is normal. In sub chronic toxicity study of test drug Vishathirku Poora Mathirai can be considered safe, as it did not cause either any lethality or adverse

changes with general behaviour of rats and also there was no observable detrimental effects **(90, 180 & 360 mg/kg body weight)** over a period of 90 days. Animals were observed throughout the period. There were no significant changes in feed intake, water intake, and no significant changes in body weight. On 91st day animals were sacrificed and blood samples were collected and investigated. The results suggest that there were no significant changes in biochemical parameter there were no significant changes in RFT, LFT, and Lipid profile. In organs of control group and drug treated groups no abnormality was detected. Histopathological examination revealed normal architecture in comparison with control and treated drug treated groups. These results had demonstrated that the Vishathirku Poora Mathirai is relatively safe when administered orally in rats.

8. CONCLUSION:

From the results of analytical evaluation of the test drug **Vishathirku Poora Mathirai,** it was inferred that quality and stability was good when prepared under the standard protocol mentioned in this study. Qualitative analysis of **VPM** revealed the purity and bioavailability of the drug. The heavy metals were found to be within the permissible limit so the drug is safe for oral consumption. In acute toxicity study revealed that the drug VPM did not show any mortality and signs of toxicity up to 2000 mg/kg bodyweight in acute oral administration. In 90 days repeated oral toxicity study there was no significant changes in hematological, biochemical parameter in **VPM (90mg, 180mg &360mg /Kg bodyweight)** treated group. The histopathology report of the internal organs also confirmed that there were no remarkable cellular changes at all the dose level. Based on the results, it can be concluded that, the dose level of Vishathirku Poora Mathirai is Panavedai alavu (488 mg) mentioned in "Ramadevar Ennum Yakobhu eluthiya Vaithya Sindhamani 700" is a safe dosage for human consumption.

In further study is to be carried out to evaluate the pharmacological activity of the test drug **Vishathirku Poora Mathirai**.

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10. ANNEXURE

The following certificates are enclosed,

1. Research Methodology and Biostatistics
2. Basic Research techniques and practices involved in Laboratory Animal care
3. IAEC Certificate for Acute and Sub Chronic toxicity study.
4. Authentication Certificate for herbal Plants.
5. Authentication Certificate for metal and mineral drug.

RESEARCH METHODOLOGY AND BIOSTATISTICS

CERTIFICATE

Ministry of AYUSH

NATIONAL INSTITUTE OF SIDDHA

Ministry of AYUSH, Government of India

Tambaram Sanatorium, Chennai - 600 047.

WORKSHOP ON

RESEARCH METHODOLOGY & BIOSTATISTICS

This is to certify that

Dr. R. PALRAJ

has participated in the above Workshop held from 16.04.2018 to 20.04.2018 conducted by the Dept. of Noi Naadal, at National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047.

Dr. G.J. Christian
Coordinator
HoD, Dept. of Noi Naadal,
National Institute of Siddha

Prof.Dr.V.Banumathi
Director,
National Institute of Siddha
Chennai – 600 047.

BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE

NATIONAL INSTITUTE OF SIDDHA

An Autonomous Body under Ministry of AYUSH
Govt. of India

Workshop on

Laboratory Animal Care and Basic Research Techniques

(11-15 February, 2019)

CERTIFICATE

This is to certify that

Dr.R.Palraj

has participated as Trainee in the workshop on

"Laboratory Animal Care and Basic Research Techniques"

held between 11.02.2019 & 15.02.2019 at National Institute of Siddha, Tambaram Sanatorium, Chennai.

Dr.V.Suba
Organising Secretary

Dr.B.R.Senthilkumar
Coordinator

Prof.Dr.V.Banumathi
Chairperson / Director, NIS

IAEC CERTIFICATE FOR ACUTE AND SUB CHRONIC TOXICITY STUDY.

(13)

CERTIFICATE

This is certify that the project title Preclinical Safety Evaluation of **"Vishathirku Poora Mathirai" has been approved by the IAEC.** Total No of animal approved: 86 (40♀+46♂)

Approval No: NIS/IAEC-VII/2808 2018/13

V. Banumathi

Prof. Dr. V. Banumathi,
Chairman IAEC

Prof .Dr. K. Nachimuthu
CPCSEA Nominee

28/8/18

Signature with Date

Chairman IAEC **CPCSEA Nominee**

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Name of the principle investigator: Dr. R. Palraj,
PG Scholar,
Department of Nanju Maruthuvam,
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Tambaram Sanatorium,
Chennai-47

Name of the Guide : Dr.R.Madhavan MD(S)
Lecturer Guide,
Department of Nanju Maruthuvam,
National Institute of Siddha,
Tambaram Sanatorium,
Chennai-47

AUTHENTICATION CERTIFICATE FOR HERBAL PLANTS

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **"Vishathirku Pooramathirai"** taken up for Post Graduation Dissertation studies by **Dr.R.Palraj** M.D.(S), II year, Department of Nanju Maruthuvam, 2019, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Acalypha indica Linn. (Euphorbiaceae), Whole plant

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

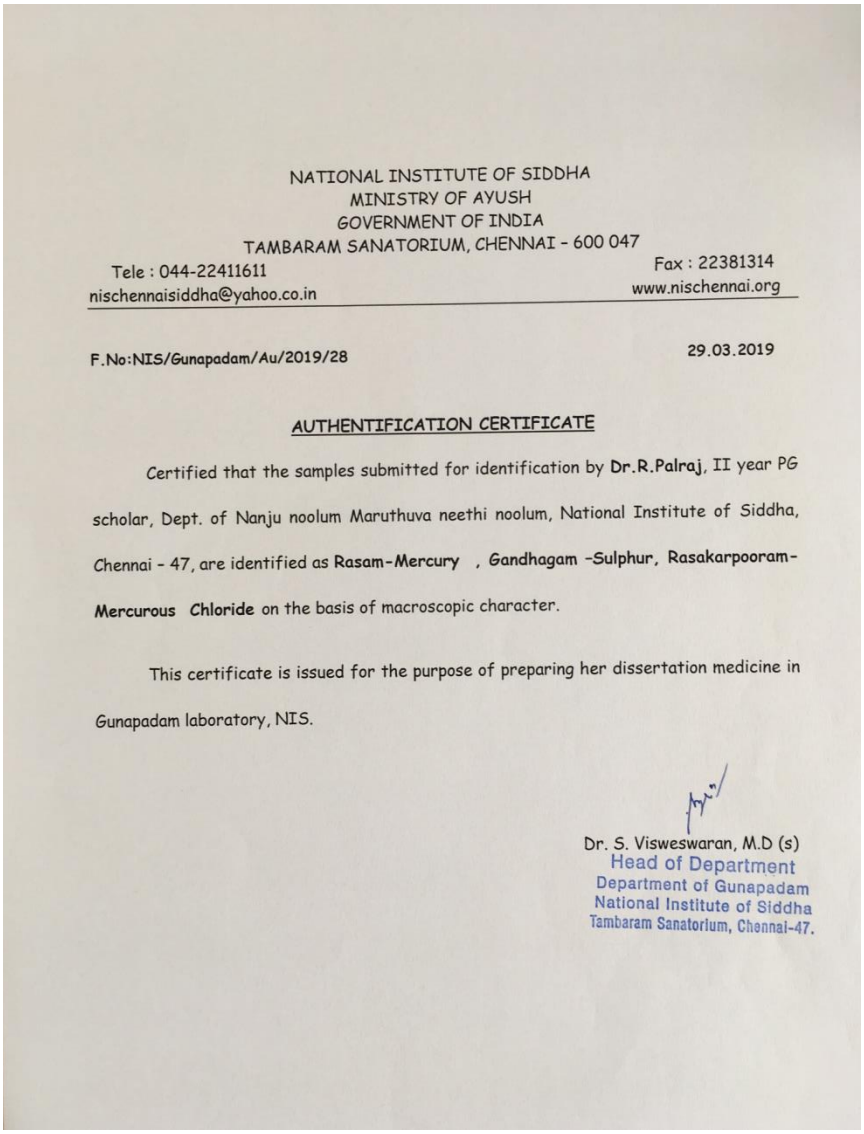
Certificate No: NISMB3812019

Date: 10-04-2019

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

AUTHENTICATION CERTIFICATE FOR METAL AND MINERAL DRUG



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F.No:NIS/Gunapadam/Au/2019/28 29.03.2019

AUTHENTIFICATION CERTIFICATE

Certified that the samples submitted for identification by **Dr.R.Palraj**, II year PG scholar, Dept. of Nanju noolum Maruthuva neethi noolum, National Institute of Siddha, Chennai - 47, are identified as **Rasam-Mercury , Gandhagam -Sulphur, Rasakarpooram-Mercurous Chloride** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

Dr. S. Visweswaran, M.D (s)
Head of Department
Department of Gunapadam
National Institute of Siddha
Tambaram Sanatorium, Chennai-47.