A CLINICAL STUDY ON

AAN MALADU

(MALE INFERTILITY)

WITH THE EVALUATION OF SIDDHA DRUG

VEERIYA VIRUTHI CHOORANAM

The dissertation submitted by

Dr.M.MEERAN GANI (Reg. No. 321411106)

Under the Guidance of

Prof. Dr. N. ANBU, M.D.(S)

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CERTIFICATE

This is to certify that the dissertation entitled "**A CLINICAL STUDY ON AAN MALADU "** is a bonafide work done by **Dr.M.MEERAN GANI.**, Government Siddha Medical College, Chennai – 600 106 in partial fulfillment of the University rules and regulations for award of **SIDDHA MARUTHUVA PERARIGNAR** under my guidance and supervision during the academic year 2014 – 2017.

Name & Signature of the Guide

Name & Signature of the HOD

Name & Signature of the Dean/ Principal

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INTRODUCTION

Siddha –The science of Holistic Health

The word "siddha" comes from the word "siddhi" which means "an object to be attained" or "perfection" or "heavenly bliss".[1] Siddhi generally refers to Ashtama siddhi i.e, the eight great super natural powers .Those who attained or achieved the above said powers are known as Siddhars.

Science of Siddha states that the 5 elements of Nature viz., earth, water, heat, air and space(panchapoodham) were the fundamentals of all the corporeal things in the world. It also states that there exists a close relationship between the external world and the internal system of human. To be precise, "Structure of the Human body is a miniature world by itself" [2].

According to panchapoodha pancheekaranam theory, each of these five elements said to posses two properties viz. Subtle and gross. Thus ,This theory proposes that 96 basic factors exist, which is the basic concept underlying this holistic medical science. The human body formed by these 96 basic factors . This 96 factor include physical, physiological, psychological, intellectual aspect of every human. The five primordial elements manifest themselves as a human through these 96 basic factor[3].

Man is said to be Microcosm(Pindam) and the world the Macrocosm(Andam) i.e., there is nothing in the Macrocosm of nature that is not contained in man. Disease, according to modern science is only a departure from a state of health and more frequently a kind of disturbance of the healthiness of the body to which any particular case of sickness is assigned.According to Siddhars Philosophy diseases in man do not originate in himself, but from the influences which act upon him.As already stated, man is compared to the world because the elements that exist in the world exist in man as well; and therefore any change in the elementary condition of the external world has its corresponding change in the human organism.There is the feeling of oneness between the external and the internal world of man; and it is upon this oneness that the doctrine of Humoral Pathology i.e., the theory of Tridosha is based.

மிகினும்குறையினும்நோய்செய்யும்நூலோர்
வளிமுதலாஎண்ணியமூன்று

-குறள்

The siddha medicine also recognises the role of three Humors (Vatham, Pitham and Kapham).These humors remain in a balanced state in normal healthy person and disturbance in their equilibrium leads to ill health.(4)

The characteristic of the three humors in the constitution of human beings is either hereditary or atavic. In scientific parlance, Vatham comprehends all the phenomena which come under the functions of the central and the sympathetic nervous system; Pitham, the functions of thermogenesis or heat-production, metabolisation within its limits, the process of digestion, coloration of blood, excretion and secretion etc., and Kapham, the regulation of the heat and the formation of the various preservative glands.When deranged, they bring about diseases peculiar to their influence ; when in equilibrium, freedom from disease; and when one or the other of the humours combine in such a way as to get derangedby aggravation, diminution etc., disease may result.In Siddha diagnosis is based on three humors .5,6,7)

Thirumoolar is one of the Siddhar. In his famous treatise called Thirumanthiram, he define medicine is one that give physiological effects, psychological effects, ensures prevention of diseases and ultimately grants Immortality. The treatment is mainly directed towards restoration of equilibrium of the three Humors .

The term **"infertility"** came from Latin word infertilis, which means "not fertile"(8)

Infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility, as well as being a medical condition, has a social dimension; it is a poorly-controlled, chronic stressor with severe long-lasting negative social and psychological consequences.(9)(10)(11)

In Siddha medicine, infertility in male is called as Aan maladu, Veeriyamantham, Anmai kuraipadu. Siddhar Yugimuni in his medical literature

"Yugimuni Sigicha Saaram" has described the male infertility due to problems in seminal fluid (Vindhu).

According to WHO in word wide 60 – 80 million couples are infertile and, in India 10 – 15 % of couples were infertile.[12] 1 in 6 couples are infertile and they have negative emotional responses such as stress, anxiety and depression.

The prevalence of infertility in the general population is 15%–20%. Of this, the male factor is responsible for 20%–40%. In Indian couples seeking treatment, the male factor is the cause in approximately 23%.[13]

In men, oligozoospermia, asthenozoospermia, teratozoospermia and azoospermia are the main causes of infertility, and these account for 20%-25% of cases.

Infertility is stressful life event and depressive symptoms are normal responses to the life crisis of the infertile couples. Now a days smoking, alcoholism, poor nutrition, pesticides level in food, overweight, stress are increasing, which are responsible for fertility problems and it ultimately leads to reduction in Birth Rate of our country. Reduction in Birth Rate along with already increasing life expectancy leads to increase in old age population which makes our country much older. As children are the future of our country, it is important to reduce infertility. There are many medicines available in Siddha system to treat male infertility. From those, the author has selected VEERIYA VIRUTHI CHOORANAM to study its safety and efficacy and hopes it will give fruitful results.

AIM & OBJECTIVE

AIM:

To study the safety & efficacy of Siddha medicine *VEERIYA VIRUTHI CHOORANAM* in Aan maladu (Male infertility)

OBJECTIVE:

- To evaluate the therapeutic efficacy of *VEERIYA VIRUTHI CHOORANAM* in Aan maladu(Male infertility) to increase the sperm concentration, viscosity of semen and to reduce premature ejaculation, nocturnal emission in Aan Maladu.
- To evaluate the safety profile of the trial drug *VEERIYA VIRUTHI CHOORANAM.*
- To collect the authorized measures and review the ideas of Aan maladu in Siddha and modern literatures.
- To have an idea about the relation of the disease with age, habits, occupation, economic states, family history and climatic conditions.
- To expose the efficacy of siddhars diagnostic principles such as mukkutram, envagai thervugal, eazhu udalthadhukkal, neerkuri and neikkuri.
- To evaluate,
    - ✓ Bio- chemical analysis
    - ✓ Physico-Chemical analysis
    - ✓ Toxicity study [acute & sub-acute]
    - ✓ Pharmacological action
    - ✓ Bio –statistical analysis
- To have detailed clinical investigations.
- To handle the modern parameters to confirm the diagnosis and prognosis of the study.
- To have a clinical trial on the disease "AAN MALADU**"** with the Siddha herbal formulation of "*VEERIYA VIRUTHI CHOORANAM***"**.

ஆண் மலடு

"பாரப்பா பெண்மலடாம் கர்ப்பக் கோணின்
பக்குவத்தை சொல்கிறேன் பண்பாய் கேளு
ஆரப்பா ஆண்மலடே யாருமல்லா மல்
அப்பனே பெண்மலடு யாருமில்லை".[14][15]

-அகத்தியர் வைத்திய சில்லறைக் கோவை

AAN MALADU GUNAM:

ஆண்மலடு குணம்:

"பார்க்கவேஆண்மகனின் விந்து தானும்
பதமான தித்திப்பு யில்லாததாலும்
ஏர்க்கவே சல மீதில் மிதந்தாலும்
எழிலாக உயிர்ப்பற்றுயி ருந்தாலும்
சேர்க்கவே மூத்திரத்தில் நுரைதான் போலும்
செயலான கருவதுவும் தரிக்கமாட்டா"[16]

-மகளிர் மருத்துவம்(யூகி முனி)

"கூறினார் புருஷருட விந்து தானும்
குணமக தித்திப்பு இல்லாத்தாலும்
மீறியதோர் சலமீது மிதந்தாலும்
மிகவாக உயிரற்று இருப்பதாலும்
சீறியதோர் மூத்திரத்தில் நுரைதான் போலும்
சிறப்பான கருவதுதான் தரித்திடாது
தேறியதோர் மங்கையர்கள் மலடே யாவாள்
தெளிவான ஆண்மலடின் தன்மைதானே"[17]

-அரிவையர் சிந்தாமணி,

According to " yugi" and the text "arivaiyar sinthamani"

- Lack of sweetness in semen
- Buoyancy on water
- Absence of virility
- Frothy maturation

The above told character of semen mainly contributes infertile man.

ACCORDING TO T.V.SAMBASIVAM PILLAI:

According to T.V.Sambasivam pillai dictionary, The semen in such cause will be devoid of sweetness and life and float on the surface of water. The urine also will be frothy. Such man is incapable to impregnate women[18]

FORMATION OF FOETUS COMBINES WITH PANICHABOOTHAM:

"உன்னிய கர்ப்பக் குழியாம் வெளியிலே
பன்னிய நாதம் பகர்ந்து பிருதிவி
வன்னியும் வாயுவும் மாயுறுஞ் சுக்கிலம்
மன்னிய சமனாய் வளர்க்குமுதகமே"[19]

- திருமந்திரம்

The ovum consists of the element earth, whereas the sperm consists of fire and air, the uterine wall nourishes it has water and uterine cavity is one of the element of space. Therefore in the formation of fetus all the five elements combine and create it.

THE ROLE OF VAAYUS IN FERTILIZATION:

"வேர்க்கவே வேலிபோல் வளைந்து காக்கும்
விந்துவுடன் பிராணவாயு விளக்கலாமே"[20][21]

- நோய் நாடல் நோய் முதல் நாடல் திரட்டு பகுதி-1

Abanan stays outside the zygote and protect it. The pranan goes along with spermatozoa and bisects the size of the zygote

According to siddha literature & siddhars view, vindhu or sukkilam(semen) is an important which is compared to shiva. Vindhu anotherwise known as "sukkilam" is the final and important constituent of 7 thathus, which giving birth to embryo.

Although all siddhars spoken about vindhu , some of them only briefly explained the formation, importance,embryo formation etc. For examble, the famous saint THIRUMOOLAR briefly explained the formation of vindhu(semen) , physiological importance,disadvantage of masturbation, timing for coitus, state of orgasm, embryo formation,determination of foetel sex by male partner,etc in Thirumandhiram. Some of the literature views from above told books and some other books are given below.

FORMATION OF VINDHU(SEMEN):

விந்து இயல் :

இரச முதலான ஏழ்தாது மூன்றின்
உரிய தினத்தின் ஒருபுற்பனி போல்
அரியதுளி விந்துவாகு மேழ் மூன்றின்
மருவிய விந்து வளரும் காயத்திலே

- திருமந்திரம் 1934

Although 7 thathus noursing our body after getting absorbed from GI Tract, 3 are the major things that is saram(chyme), raktham (blood)& vinthu (semen). The semen seems to be a waterglobules at the hit of grass. The sperm need 21 days for its full growth in our body.

FORMATION OF VINDHU(SEMEN):

விந்துற்பனம் (விந்துவின் தோற்றம்)

சகத்தின் தோற்றத்திற்கு மூலமான சுத்தம் முதலான மாயையினின்று விரியும் வகையும் விண்டத்தில் விந்து வினின்று புணர்ச்சியாகிய காரியத்தான் கரு உருக்கொள்ளுமாறு யாண்டு அண்டம் பிண்டம் இவற்றை இணைத்தே கூறுவராதலின் ஈண்டும் சில மந்திரங்களான அண்ட நிலைக்கு அடியான பர அபர விந்துவின் வெளிப் பாட்டையும், பிண்டத்திற்குக் காரணமான கரு விந்துவின் தோற்றத்தையும் உணர்த்துகிறார்

உதயத்தில் வந்துவில் ஓங்கு குண்டலியும்
உதய குடிலில் வயிந்தவம் ஒன்பான்
விதியில் பிரமாதி கள்மிகு சத்தி
கதியிற் கரணங் கலைவை கரியேஸூ(22)

-திருமந்திரம்(1898)

“அழிகின்ற விந்து அளவையறியார்
கழிகின்ற தன்னையுட் காக்கலுந் தேரார்
அழிகின்ற காயத் தழிந்தயர் வுற்றோர்
அழிகின்ற தன்மை யறிந்தொழியாரே”(23)

- திருமந்திரம்(1899)

According to siddhars 1 drop of venner made from 80 drops of senner (blood). 1drop of vindhu made from 80 drops of venner. So 6400 drops of senner (blood) needed to make one drop of vindhu. [23]

- உடல் தத்துவம்

விந்து புணர்ச்சியின் பயன் :

புறம் அகம் எங்கும் புகுந்துஒளிர் விந்து
நிறம் அது வெண்மை:நிகழ் நாதம் செம்மை
உற மகிழ் சத்தி சிவபாதம் ஆயுள்
திறனொடு வீடு அளிக்கும் செயல் கொண்டே.

- திருமந்திரம் 1929

The colour of semen s purely white & the colour of ovum is red . the joining of the both , semen &ovum leading to , fertilization of ovum by the sperm to form new energy, foetus.

விந்தினால் பிறப்பு:

துந்தை வித்ததி சாரத்திலரவமாயுதித்து
ஐந்து பூதம் தாக உப்பது உவர் படர்ந்து
விந்து மேலபி வருதல் தை மாசி பங்குனியும்
விந்து வாலுதித் திலகிய நாதமுமிதுவே[24]

-திருவள்ளுவ நாயனார் வைத்திய சிந்தாமனி 800

Birth by semen :

According to the saint Thiruvalluvar , The semen of male partner helps to embryo after fertilization . The embryo grows up by the influence of 5 elements (pancha bootham).The birth of foetus should takes place in the tamil month named “thy” and so the fertilization must be on the month of “maasi & panguni”

AETIOLOGY:

திருமூலர் கூறும் நோய் காரணம்:

"ஓரெட்டுச் சன்னி உழண்டது பெண்ணுக்கும்
வாரெட்டுஆணுக்கும் மகத்தாம் சுகசன்னி
நேரிட்டுப் பார்க்கில் நிகழ்ந்தது வெவ்வேறு
பாரெட்டு மெய்ச்ச பகுத்த முறைபாரே".(25)

-திருமூலர் கருக்கிடை வைத்தியம் 600,

பொருள் விளக்கம்:

பெண்களுக்கு 8 வகை சன்னியாலும்,ஆண்களுக்கு சுக சன்னிமுதற்கொண்ட பலவகை சன்னியாலும் கர்ப்பம் வாய்க்காமல் போகும்.(26)

MALE INFERTILITY DUE TO INFECTIONS:

கரும்பனிசையம்மை :

"அறிந்தபின் இவர்களுட நப்பா
அந்தந்த சரீ ரத்திற் கடுத்த வாராய்
தெரிந்ததொரு குணக்குறிகள் தோன்றுமப்பா
திறமான கருன்பனிசை விந்தைக் கொல்லும்
பரிந்த்தொரு கர்ப்பத்தை அழியப் பண்ணும்
பண்பாக யவர்களுக்கு பிள்ளை யில்லை"(27)(28)

- அகத்தியர் வைசூரி நூல்

The complications of the karumpanisai ammai are,

- Death of sperm cells in male
- Abortion in pregnant women
- Produce sterility in both men and women.(28)

MALE INFERTILITY DUE TO TRAUMATIC LESIONS:

1.கல்லிடைகாலம் (அண்ட வர்மம்,பீச காலம்)-

அடிபடுவதால் வர்ம குறிகுணம்:

விதை இரண்டும் காணாது. விதை ஏறிக்காணப்படும்.வர்மம் அதிகமானால் ஆண் குறி பலனற்று போகும்.நீர் பிடிக்கும்.விதை மேலேறிய பகுதியில் சதை வளர்ந்துமூடும்.(29)

2.உச்சி பதப்பு காலம்-

அடிபடுவதால் வர்ம குறிகுணம்:

கொண்டை குழைந்து போகும்.விந்து வெளிப்படும்.ஸ்திரி போகம் குழைந்து போகும்.சன்னி,சீதம் வந்து சேரும்.(30)

- வர்மம் 108

TOPICS RELATED TO MALE INFERTILITY IN SIDDHA LITERATURE:

SUKKILA VATHAM:

சுக்கில வாதம்:

"வாதமா முடலுருகி மிகவும் வற்றி
மலமூத்திரம் சிக்கியே கீழ்விழாமல்
நாதமாம் நாக்கொடுமூக்கு தன்னில்
நுணுக்கமாம் வுதிரம்தானருவி பாயும்
சேதமாம் சேட்டுமம் கோழையுண்டாஞ்
செயலொடு சுவாசமாம் யருசி யுண்டாஞ்
சூதமாய் சுக்கிலந்தான் ருன்னி யாகுஞ்
துரிய சுக்கில வாதம் சூட்சந்தானே."(31)

- யூகி முனி

- நோய் நாடல் நோய் முதல் நாடல் திரட்டு பகுதி-2,

Emaciation, constipation, oliguria, bleeding from the nose, phlegm accumulation due to increased kapham,breathlessness,loss of taste. All the symptoms are associated with affected sukkilam, according to yugi. (32)

சுக்கில வாதம்:

உடல் வற்றல், மலச்சிக்கல், மூக்கில் இரத்தம் வடிதல், ஐயம் பெருகல், மேல் மூச்சு வாங்கல், சுவையின்மை என்னும் குறிகுணங்களை காட்டும்.வெண்ணீர் (சுக்கிலம்) கட்டும்.

சுக்கில வாதம்:

"வாயு வாதம் காற்றினிடை வந்தால் அவயங்கள்
பாயும்கால் வலிக்கும் பண்ணுகுணம்- காயத்தின்
சுக்கிலக் காலந்திரத்திற் துன்று துரித மின்னம்
புக்கி நிறத்தாது கெட்டுப்போம்.(33)

-அகத்தியர் வைத்திய சிந்தாமணி வெண்பா-4000 பாகம்-1.

DIAGNOSTIC METHODS IN SIDDHA SYSTEM:

UDAL KATTUGAL: (SEVEN PHYSICAL CONSTITUENTS)

1. SAARAM – CHYLE (PLASMA):

It is responsible for the growth & development. It keeps the individual in good spirit and nourishes the blood.

In Aan Maladu, Saaram affected.

2. SENNEER – BLOOD:

Blood imparts color to the body and nourishes the muscle for the ability.

3. OON – MUSCLE:

Gives shape to the body.

4. KOZHUPPU – FAT:

It helps in lubricating the different organs and maintains oily matter of the body.

5. ENBU – BONE:

It supports the system and responsible for the posture movement of the body.

6. MOOLAI – MARROW:

It fills the bone cavity, nourishes semen and imparts strength, endurance and shiny appearance.

7.SUKKILAM (SPERM):

It is responsible for the reproduction.

In Aan Maladu, Sukkilam affected.[34]

சுக்கில குணம்:

‘உண்மையான சுக்கில முபாயமா யிருந்ததும்.
வெண்மையாகி நீரிலே விரைந்து நீரதானதும்
தண்மையான காயமே தரித்துருவ மானதும்
தென்மையான ஞானிகள் தெளிந்துரைக்க வேணுமே”[35].

- சிவவாக்கியர்

(Sivavakkiar padal –moolamum uraiyam)

At the time of copulation, the semen is ejaculated. The prostatic fluid gives the semen a milky appearance. In the early minutes after ejaculation, the sperm remains immotile, possibly because of the viscosity of the coagulum. As the coagulum dissolves the sperm become highly motile.[36]

சுக்கிலம் குறை குணம்

புணர்ச்சியில் வெண்ணீரும் சுரோணிதமும் பொறுத்துப் பொறுத்துப் துளித் துளியாய் விழுதல், அல்லது செந்நீர் வெளிப்படல், விதையில் குத்தலுடன் வலி, குறியில் அழற்சி மிகுதிப்படல்.

At the time of copulation insufficient quantity of semen is ejaculated with pain, pricking pain in the scrotum, irritation of the penis[36].

இந்திரிய பரிட்சை:

"ஐய மலை பால்மோர் தேனாச் சியங்கலா கம்மிவையை
யையமளை யாதறிந்து கொள்ளுவா யையமளை
யுத்தமத்தை முன்னேருன யிந்திரிய பரிட்சை
யுத்தமத்தை நூலா தரையோர்".(37)

-தேரன் யமக வெண்பா பக்க எண்.65

Examination of semen:

If the semen is,

1. White and akin to the butter, it is excellent.
2. White and akin to curd, it is very good.
3. White and akin to the milk, it is good.
4. White and akin to the butter milk, it is fair
5. Akin to the honey in color and consistency, it is average.
6. Akin to the ghee in color and weight, it is poor.
7. Akin to the toddy is color and weight, it is very poor.
8. Akin to the water, it is very bad.(38)

- நோய் நாடல் நோய் முதல் நாடல் திரட்டு பகுதி -1

சுக்கிலத்தை அடக்கினால் உண்டாகும் நோய்கள் :

"சுக்கில ந்தனை அடக்கின்
சுரமுடனீர்க் கட்டாகும்
பக்கமாய் கைகால் சந்து
பாரமாய் வழி இறங்கும்
மிக்க மார் நோயுண்டாகும்
மிகுந்திடும் பிரமேகந்தான்
தக்கதோர் போதுமாகின்
தரித்ததோர் வாயுவின் கூறே".(39)

- உடல் தத்துவம்

Uyir thathukkal / Mukkutram:

These are all the main three pillars which functioning the body with an equilibrium state. Any disturbance in that state leads to diseased condition in our body.

The three pillars are,

1. Vali
2. Azhal
3. Iyam

1. Vali or Vaayu:

Vali is not mere wind, but also that which causes motion, energy and sensation of every cell in the body. Vaayu relates to nerve force. It is responsible for all movements in the mind and the body. In human body it controls the Gnanendriyam (sensory actions) & Kanmendriyam (motor activities)

Vali generally lives in,

Abanan, Edakalai, Kamakodi, Undhiyin Keezh Moolam, Hip region, Bones, Muscles, Nerves, Joints, Skin, Hair follicles, Stools.

Varieties of Vali:

According to their location and functions they are classified into 10 types.

1. Uyirkkaal (Pranan)
2. Kezhnokkum Kaal (Abanan)
3. Paravukaal (Viyanan)
4. Melnokkum Kaal (Udhanan)
5. Nadukkaal (Samanan)
6. Naagan
7. Koorman
8. Kirugaran
9. Devadhathan
10. Thananjeyan

1. Uyirkkaal (Pranan)

It regulates the respiratory, cardiac and digestive system. By joining with pingalai it forms azhal naadi. It is responsible for bio confusion in the body.

2. Kezhnokkum Kaal (Abanan)

It regulates the defecation, micturition, menstruation, parturition and ejaculation. It corresponds to the pelvic plexus and the lower part of the gut.

3. Paravukaal (Viyanan)

It spreads all over the body and all nerve endings. It regulates constriction and relaxation of the voluntary and involuntary muscles. The neurological problems were due to this Vaayu. It spreads the nutrients to all over the body from the digested food.

4. Melnokkumkaal (Udhanan)

It is responsible for speech, vomiting, hiccough and sneeze.

5. Nadukkaal (Samanan):

It is responsible for digestion and it spreads the nutrients to all over the body. Joining with suzhumunai it forms the Kabha naadi. It neutralizes the other four Vaayus.

6. Naagam:

It is responsible for the intelligence and derangement of this Vaayu causes impaired memory. It helps to opening and closure of eyelids.

7. Koorman:

This is responsible for the vision, Yawning and Lacrimal secretions.

8. Kirugaran:

It is responsible for salivation, nasal secretion, hunger, sneeze, cough and concentration.

9. Devadhathan:

It is responsible for laziness and anger.

10. Thananjeyan:

It produces swelling all over the body and leaves from cranium only after the 3rd day after death. It is responsible for the decay of the body after death.

In Aan Maladu,Kezhnokkum Kaal & Paravukaal affected.[(40)]

II.Azhal:

This is nothing but the characteristics of fire such as burning, boiling and heating etc. It corresponds to the functions so thermo genesis production of heat necessary to maintain integrity of the human circulatory system. Azhal is classified into 5 types. In mainly governs enzymes & hormones.

Azhal lives in:

Between heart & the navel, Sweat, lymph, blood, stomach, urinary bladder, saliva eye and skin

In Aan Maladu, Sathaga Pitham affected.[(40)]

Name	Location	Function
1.Aakkanal (Analagam)	**Stomach, Small Intestine**	**Dissolvent& Digestive**
2.Vanna eri (Ranjagam)	**Liver, Spleen, Stomach**	**Colouring, Pleasing, Gratifying**
3.Aatralangi (Sathagam)	**Heart**	**Effective Efficient**
4.Nokkazhal (Alosagam)	**Eyes**	**Seeing, Consideration**
5.Ollolithee (Prasagam)	**Skin**	**Complexion Of The Skin**

Iyyam:

It imparts moisture. Iyam is located in samanan semen, head, tongue, flat, bone marrow, blood, nose, chest, nerves, brain, large intestine, eyes, stomach& pancreas.

Name	Location	Function
1.Alli Iyam (Avalambagam)	**Lung**	**Supports all the others**
2.Neerpi Iyam (Kilethagam)	**Stomach**	**Moistens and nourishes the food**
3.Suvaikanna Iyam (Pothagam)	**Tongue**	**Take care of perception**
4.Niraivu Iyam (Tharpagam)	**Head**	**Refrigerant effect of eyes**
5.Ondri Iyam (Sandhigam)	**Joints**	**Stability, lubrication, movement of joints**

In Aan Maladu, Tharpagam & Santhigam affected.[40]

ENN VAGAI THERVUGAL

நாடி பரிசம் நாநிறம் மொழிவிழி
மலம் மூததிரமிவை மருத்துவராயுதம்[41]

-தேரையர்

-நோய் நாடல் நோய் முதல் நாடல் திரட்டு பகுதி-1,

It is the unique and special method in siddha .Envagai thervugal is the specialty of siddha diagnosis. These are the instruments for the physician to diagnose disease.[42]

NAADI:

நோயின் நாடி:

"ஆகிய வாதமும் வாயுவும் கூடிடில்
தாகிய வெள்ளை தடையற்று மெத்தவாய்
போகிய மேனி பொருமி கருப்பேறும்
வாகிய தாது வழங்காது நஷ்டமே".[?(43)]

-பதினெண்சித்தர் நாடி சாஸ்திரம்,

The three uyir thaadukkal felt through the pulse is called naadi

Naadi		**Vaayu**		**Uyir thathu**		**Ratio**
Edakalai	+	Abanan	-	Vatham	–	1
Pinkalai	+	Piranan	-	Pitham	–	½
Suzhumunai	+	Samanan	-	Kapam	–	¼

PARISAM:

Observations by touch, temperature, sensory impairments, masses, nodes, swelling and texture of the skin, pain, hardness, edematous and dullness shall be noted.

NAA:

Signs and symptoms in the tongue are considered here. Size, appearance, thickness, color (pigmented, magenta) fissured (longitudinal, transverse) coated, geographical patches, oral hairy leukoplakia, candida, aphthous ulcer, sense of taste, saliva secretion.

NIRAM:

The color of skin is mainly considered here but also the change in other organs.

MOZHI:

The change in the normal sound of voice mainly uratha olli (Valithel), thazhntha olli (Melithal), physiological and mental status can also be noted during conversation.

VIZHI:

Color, warm, burning sensation, irritation, visual perception

MALAM:

Nature, quantity, color, odour, froth, consistency are noted.

MOOTHIRAM:

The urine examination is classified into two types.

NEERKKURI:

"வந்தநீர் கரி எடை மணம் நுரை எங்கலென்"[44]

-தேரன்

-நோய் நாடல் நோய் முதல் நாடல்,

Urine is to be observed for the following characters

- Niram (color)
- Edai (specific gravity)
- Manam (smell)
- Nurai (froth)
- Enjal (deposit)[45]

NEIKKURI:

It is an important test to assess the predominantly affected humour.

“அருந்து மாறி ரதமும் விரோதமதாய்
அஃகல் அலர்தல் அகால வூண்தவிர்தழல்
குற்றளவருந்தி வுறங்கி வைகறை
ஆடிக்கலசத் தாவியேகாது பெய்
தொருமுகூர்த்தக் கலைக்குட்படு நீரின்
நிறக்குறி நெய்க்குறி நிருமித்தல் கடனே”.[44][45]

- தேரன்

- நோய் நாடல் நோய் முதல் நாடல் திரட்டு பகுதி-1,

On the day before the urine test one should take food, consisting of all the six tastes at the regular time based on one’s digestive fire; after a sound overnight sleep,urine should be collected in a glass ware and the test should be done before 90 minutes from dawn.

A drop of oil is dropped at the center of urine (bowl) without any shake. It should be ensured that the Sunlight falls on it, but is not disturbed by the wind. A keen

observation of the oil drop suggests the condition of the patient. If the oil drop takes the shape of a snake, it indicates Vaadha disease.

If it spreads like a ring it indicates Pitha and if it stands like a pearl it indicates Kapha disease.If there is a combined shape like a ring in a snake, or snake in the ring, snake and a pearl or a pearl in the ring, it indicates combined derangement of humors. White layer starts with disturbed Azhal and eventually involves all the three uyir thathus -thus resulting in various patterns of oil spread in the urine surface.

NOI NEEKAM (TREATMENT):

In Siddha system the main aim of treatment is not only for the removal of Physical illness but also the mental illness. Treatment is considered with prevention and improvement of the general body condition (rejuvenation) also.

This is said as follows

Kappu - Prevention

Neekkam - Treatment – curative

Niraivu - Restoration – promotive

While treating the disease the following principles must be noted.

So it is essential to diagnosis properly to know about the etiology, the nature of the patient, the severity of illness, the seasons and the time of the occurrence of the disease.

LINE OF TREATMENT:

1. To bring the three Kutrams in equilibrium
2. Medicine (Internal)
3. Diet and advise

TO BRING THE THREE KUTRAMS IN EQUILIBRIUM:

Since Siddha system of medicine is based on Mukkutra theory, the purgation (Kalichal Maruthuvam) was given by for the vitiation of three humours.

Agasthiyar Kuzhambu 65mg was administrated at early morning as a purgative in the prior day of treatment.

மருந்துகள்:

உள் மருந்து : வீரீயவிருத்தி சூரணம்

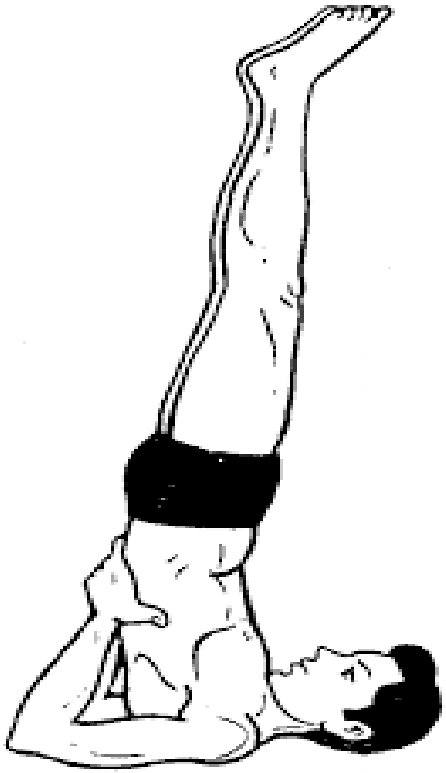
அளவு : 1 கிராம், இருவேளை

அனுபானம் : பால்

YOGA AND RELAXATION THERAPY:

Yoga has been practiced in India for a number of centuries. There are several methods of yogic practice originating from different school of thoughts. Yogic exercises improve the psychological functions of the individual[(46)]. I shall deal with only such asanaas are useful in curing ailments and maintaining good health for the male infertility.

1. SARVANGASANA:



It is stimulates the pituitary and thymus glands and keeps the prostate gland healthy. This asana keeps the sex glands healthy. Sexual weakness in the case of male can be overcome by the practice of asana.

2. SIRASASANAM:

Sirasasanam will prevent or give relief in neurasthenia, dyspepsia, seminal weakness, spermatorhoea, varicose veins. It will prevent the enlargement of prostate gland.

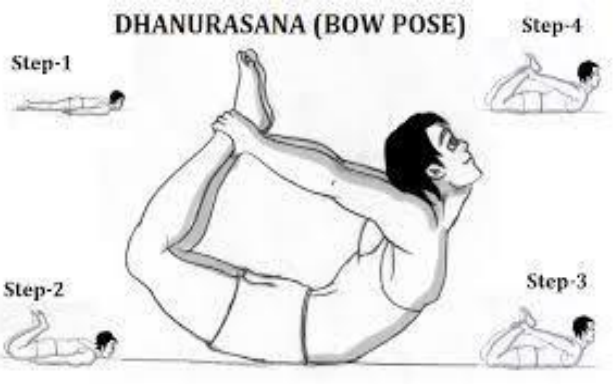
3. SAVASANAM:

Savasanam is a Powerful Tranquilizer. It pacifies the body and a mind by eliminating muscular, Nervous, Mental and Emotional tension almost immediately.

4. BHADRASANA:

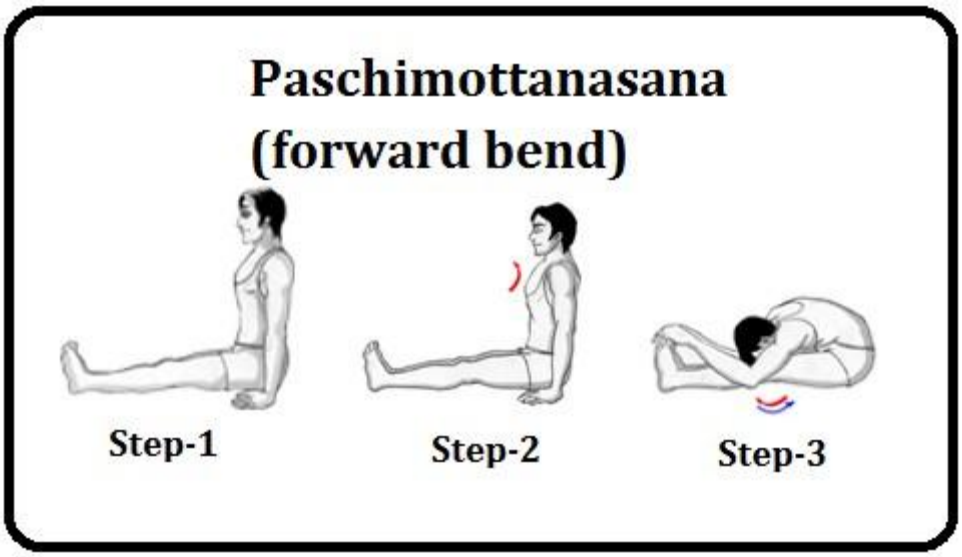
It promotes fresh Blood supply to Urogenital organs. It will effectively cure Nocturnal discharges and sexual debility.

5. DHANURASANA:



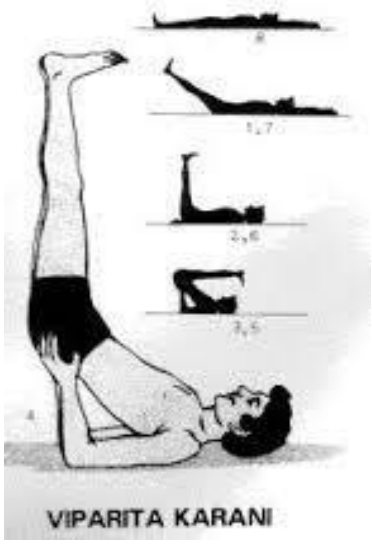
It improves functions of the reproductive system in male and female.

6. PASCHIMOTTASANA:



Relieves dyspepsia, Strengthens urogenital system.

7. VIPARETHA KARANI:



It is called as **"Sakala Roga Nivarini"** as it is curing all diseases.

8. PARSVAKONASANA:

Expands the chest. Knee pain, Back pain are eradicated.Nervous system is stimulated. Impotency is corrected.

9. ARDHA HALASANA:

Digestion is improved. Liver, spleen, kidneys and reproductive organs are activated.

MUDHRAS FOR MALE INFERTILITY:

1. YOGA MUDRA:

Yoga Mudra tones up the pelvic organs on account of the pressure of the heels on the groins. It is useful in seminal weakness.

2. ASWINI MUDRA:

Aswini Mudra gives tone to the reproductive organs and Nerves and removes seminal insufficiency and sterility.[47]

-Yoga for health, Pg no:23-94

PRANAYAMA:

- It gives a feeling of freshness, energy and lightness of body and mind.
- Strengthens the lungs. Increases its capacity and cures the disorders.
- Digestion is improved.
- Excretory system is stimulated. Toxins are removed from the body.
- Skin tone is well maintained.
- All the endocrine glands are stimulated.
- It makes the nervous system more energetic.
- It increases concentration and helps meditation.[48]

COOLING PRANAYAMA:

They are Cooling Pranayamas because of their cooling effect, and the help in calming down the mind by removing the mental anxiety & tension.[48]

MEDITATION:

It makes the mind calm and steady. It helps us to face the battle of life. It kills the pain and sorrow. It is a powerful nervine tonic. It increases memory power. It increases social harmony. It prevents and cures all psychosomatic diseases. It provides a healthy happy, long life. It gives positive attitude towards life. It increases creativity and alertness. It helps to fight the stress successfully and quickly. It increases will power and so one is able to overcome bad habits.[48]

DIET AND ADVICE:

DIETS TO BE ADDED:

"தாளி நன்முருங்கை தழைதூது ளம்பசலை
வாளி லறுகீரை நெய்வார்த்துண்ணி லாளியென
விஞ்சுவார் போகத்தில் வீம்புரைத்த பெண்களெல்லாம்
கெஞ்சுவர் பின்வாங்கிக் கேள்".[16]

-குணபாடம் மூலிகை வகுப்பு

KARPAM:

- உருளைக்கிழங்கு (அபிசித மூலம், மதனகாமியாதி மூலம்)
- மலட்டுக்குக் கற்பம்
- சுக்கிலத்தம்பனம்[49]

-சித்த மருத்துவம் சிறப்பு.

VEGETARIAN DIET:

- கீரை - தாளி,முருங்கை,பசலை,தூதுளம்,அறுகீரை,
- காய் - முருங்கைக்காய்,முருங்கைபிஞ்சு, வாழை
- பழம் - பேரிச்சு,மாதுளை,திராட்சை,நாவல்,மாம்பழம்
- வித்துக்கள்- முந்திரி, வாதுமை, முருங்கை
- பால் மற்றும் பால் பொருட்கள்[50]

NON - VEGETARIAN DIET:

- பறவை-கோழி,காடை, கௌதாரி,வானம்பாடி
- மீன் - வாளை, விலாங்கு, விறால்
- இறைச்சி- வெள்ளாடு [50]

DIETS TO BE RESTRICTED:

- கொள்ளு
- பாகல்
- அகத்தி
- புளிப்பான பதார்த்தங்கள்
- மாங்காய்
- காபி,டீ
- போதை பொருட்கள்[50]

PATIENTS ADVICED TO FOLLOW:

மங்கையரை கூட வேண்டிய காலம்:

சேய்யும் அளவில், திருநான் முகூர்த்தமே
எய்யும் கலை, காலம், இந்து பருதிகால்
நைய்யும் இடத்து ஓடி, நான் காமனூல் நெறி
செய்க, வலம் இடம் தீர்ந்து விடுகவே.

- திருமந்திரம் 1941

Time for coitus:

It is noted that 6 hours before sun rising is the apt time for sexual intercourse. During ejaculation it is better to maintain our breath either in idakalai/ pinkalai/ suzhumunai to make the purpose better.

விந்து ஒழியாதபடி புணர்தல்:

யோகம் அவ்வியந்து ஒழியாவகை புணர்ந்து
ஆகம் இரண்டும் கலந்தாலும ஆங்கு உறாப்
போகம் சிவபோகம்:போகி நற்போகமாம்
மோகம் கெட முயங் கார் மூடர்மாதர்க்கே

- திருமந்திரம் 1960

State of orgasm:

and it is a kind of art &stage of yoga , that is to reach the stage of orgasm in female without losing a drops of semen.

விந்து நீக்கம் கூடாது:

வித்துக்குற்று உண்பான் விளைவு அறியாதவன்
வித்துக்குற்று உண்ணாமல், வித்து சுட்டு உண்பவன்
வித்துக்குற்று உண்பானில், வேறு அலன் ஈற்றவன்
வித்துக்குற்று உண்ணாமல், வித்து வித்தான் அன்றே .

- திருமந்திரம் 1964[51]

it is important that seeding the seeds , instead of eating it by frying/raw ,to get the maximum benefits of this . similar to that everyone should utilize the semen as purposeful one, without losing it unnecessarily.

.

- பகல் உறக்கம் கூடாது
- பகல் புணர்ச்சி கூடாது
- வாரம் இருமுறை எண்ணெய் குளியல்
- மூத்தமகளிரை புணர்தல் கூடாது
- பெண்களிடத்தில் மாதம் ஒருமுறை மட்டும் புணர்தல் வேண்டும்
- பிராணாயாமம்,தியானம், யோகாசன பயிற்சிகளை மேற்கொள்ள வேண்டும்[52]

MALE INFERTILITY

DEFINITION:

Inability to produce offspring is called infertility. It is the inability to conceive a child by natural process or the inability to carry a pregnancy till the completion of term. Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female. In humans it accounts for 40-50% of infertility. Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity.

According to statistics collected from The World Health Organization (WHO) estimates that 60 to 80 million couples worldwide currently suffer from infertility[12]. The prevalence of infertility in the general population is 15%–20%. Of this, the male factor is responsible for 20%–40%[53]. In Indian couples seeking treatment, the male factor is the cause in approximately 23%[54]. In a World Health Organization multicenter study, 45% of infertile men were found to have either oligo-zoospermia or azoospermia[55]. A study from a tertiary care hospital in India reported 58% azoospermia and 24% oligozoospermia in infertile men[56].

INFERTILITY CAN BE OF THREE DIFFERENT TYPES:

PRIMARY INFERTILITY:

When a woman has never achieved conception in her life it is known as Primary Infertility.

SECONDARY INFERTILITY:

When a woman has given birth to a child in the past but is facing difficulty to conceive again it is called Secondary Infertility.

RECURRENT MISCARRIAGE:

Women who experience recurrent miscarriages may also receive a diagnosis of infertility if they experience two or more successive miscarriages. While miscarriage is not uncommon (occurring in up to 25% of recognized pregnancies),

less than 5% of women will experience two miscarriages in a row, and less than 1% three or more successive miscarriages.

ETIOLOGY:

Factors relating to male infertility include:-

PRE TESTICULAR CAUSES:

a) Hypothalamo pituitary diseases

b) Hyperprolactinemia

c) Isolated FSH deficiency

d) Congenital hypogonadotropic syndrome

e) Exogenous hormones (estrogen- androgen excess)

f) Glucocorticoid excess

g) Hyper – and hypothyroidism

h) Drugs like phennytoin, androgens and estrogens

i) Alcohol, smoking

j) Strenuous riding (bicycle riding, horseback riding)

TESTICULAR FACTORS:

a) Testicular atrophy

b) Cryptorchidism

c) Varicocele

d) Trauma

e) Hydrocele

f) Mumps

g) Malaria

h) Spermatogenesis arrest

POST TESTICULAR CAUSES:

a) Vas deferens obstruction

b) Lack of Vas deferens, often related to genetic markers for Cystic Fibrosis

c) Infection, e.g. prostatitis

d) Retrograde ejaculation

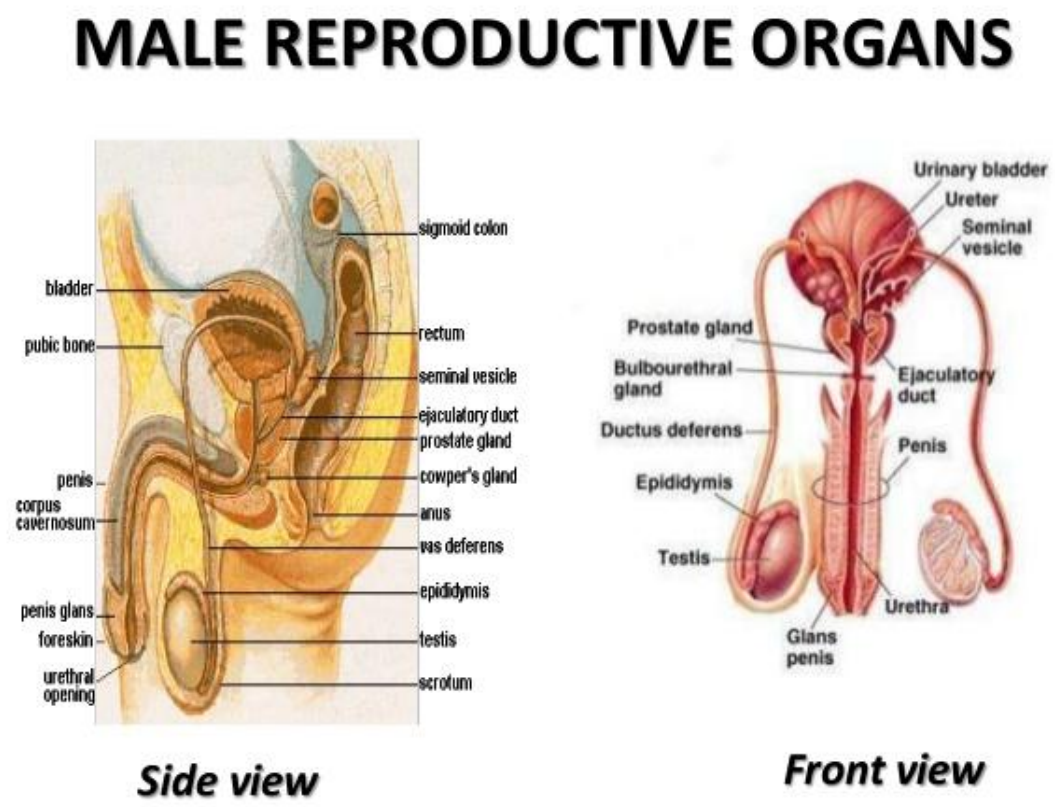
e) Hypospadias

f) Impotence

g) Acrosomal defect/egg penetration defect [57].

ANATOMY OF MALE REPRODUCTIVE ORGANS

The male reproductive system includes the testis, epididymis, ductus (vas)deferens, seminal vesicles, prostate and penis



TESTES:

The testes are the primary reproductive organs or gonads in the male. They are ovoid reproductive and endocrine organs responsible for sperm production. They are suspended in the scrotum by scrotal tissues including the dartos muscle and the spermatic cords. Average testicular dimensions are 4-5 cm in length, 2.5 cm in breadth and 3cm in antero posterior diameter; their weight varies from 10.5-14g. The left testis usually lies lower than the right testis. Each testis lies obliquely within the scrotum, its upper pole tilted antero laterally and the lower posteromedially

The testis is invested by three coats;

Tunica vaginalis

Tunica albuginea

Tunica vasculosa

TUNICA VAGINALIS

It is the lower end of the peritoneal process vaginalis, whose formation proceeds the decent of the fetal testis from the abdomen to the scrotum. The visceral layer covers all the aspect of the testis except most of the posterior aspect. The more extensive parietal layer reaches below the testis and ascends in front of and medial to the spermatic cord. The Inner surface of the tunica vaginalis has a smooth, moist mesothelium the potential space between its visceral and parietal layers is termed the cavity of the tunica vaginalis.

TUNICA ALBUGINEA

It is a dense, bluish white covering for the testis. It is composed mainly of interlacing bundles of collagen fibres. It is covered externally by the visceral layer of the tunica vaginalis, except at the epididymal head and tail and the posterior aspect of the testis, where vessels and nerves enter. It covers the tunica vasculosa and, at the posterior borders of the testis, project in to the testicular interior as a thick, incomplete fibrous septum, the mediastinum testis.

TUNICA VASCULOSA

It contains a plexus of blood vessels and delicate loose connective tissue, and extend over the internal aspect of the tunica albuginea, covering the septa and therefore all the testicular lobules.

EPIDIDYMIS

The epididymis lies posteriorly and slight lateral to the testis, with vas deferens along its medial side. It has an expanded head superiorly, a body and a tail. Its overall length is 6-7 cm and it consists of the single convoluted ductus epididymis formed by the union of the efferent ductus of the testis, which attach to the rete testis. From the tail the vas deferens ascends medially to the deep inguinal ring, within the spermatic cord[58].

TESTICULAR AND EPIDIDYAMAL APPENDICES

At the Upper extremities of the testis and epididymis are two small stalked bodies the appendix testes and appendix epididymis. They are developmental remnants of the para meso nephric ducts (mullerian) ducts and meso nephrons respectively.

TESTICULAR TORSION

The testis and epididymis are usually fixed to their surrounding tissues. In some patients this fixation may be insufficient, a condition which allows the structures to twist within the tunica vaginalis. This is termed testicular torsion and normally results in severe scrotal pain. Fertility may be affected by an episode of torsion.

SEMINAL VESICLES:

The two seminal vesicles are sacculated, contorted tubes located between the bladder and rectum. Each vesicle is 5 cm long, somewhat pyramidal, the base being directed up and posterolaterally. Essentially the seminal vesicle is a single coiled tube with irregular diverticula. The coils and the diverticula are connected by the fibrous tissue. The diameter of the tube is 3-4 mm and its uncoiled length is 10-15cm[59].

VAS DEFERENS:

It is a muscular tube, 45 cm long, which conveys sperm to the ejaculatory ducts, and its distal continuation of the epididymis, starting at the epididymal tail. At first it is very tortuous, but it becomes straighter, and ascends along the posterior aspect of the testis. From the superior pole of the testis it ascends in the posterior part of the spermatic cord, and traverses the inguinal canal. At the internal inguinal ring the vas deferens leaves the cord, curves round the lateral side of the inferior epigastric artery. It then turns back and inclines slightly down and obliquely across the external iliac vessels to enter the lesser pelvis. It crosses the ureter and bends acutely to pass anteromedially between the posterior surface of the bladder and upper pole of the seminal vesicle. It finally descends to the base of the prostate, where it joins to the duct of the seminal vesicle at an acute angle to form the ejaculatory duct[59].

EJACULATORY DUCTS

The ejaculatory ducts are formed on each side by the union of the duct of the seminal vesicle with ampulla of the vas. Each is almost 2 cm in length, starts from the base of the prostate, runs anteroinferiorly between its median right or left lobes.

SPERMATIC CORD:

At the testis traverse the abdominal wall into the scrotum during early life, it carries its vessels, nerves and vas deferens with it. These meet at the deep

inguinal ring to form the spermatic cord, which suspends the testis in the scrotum and extends from the deep inguinal ring to the posterior aspect of the testis. The left cord is a little longer than the right. Between the superficial ring and testis the cord is anterior to the rounded tendon of adductor longus. The spermatic cord contains the vas deferens, testicular artery and veins, cremastric artery and artery to the vas deferens, genital branches of the genitofemoral nerve, cremastric nerve and sympathetic components of the testicular plexus.

ABERRANT DUCTLESS:

A narrow, blind caudal aberrant ductile often occurs usually connected with the caudal part of the epididymal duct or with the start of the vas deference.

PARADIDYMIS:

The paradidymis is a small collection of convoluted tubules found anteriorly in the spermatic cord above the epididymal head.

SCROTUM

The scrotum is a cutaneous fibro muscular sac containing the testes and lower parts of the spermatic cords. It hangs below the pubic symphysis between the anteromedial aspects of the thighs. It is divided in to right and left halves by a cutaneous raphe, which continues ventrally to the inferior penile surface and dorsally along the midline of the perineum to the anus.

It consists of skin, dartos muscle and external spermatic, cremastric and internal spermatic fasciae. The scrotal skin is thin, pigmented and often rugose. It bears thinly scattered, crisp hairs. It has sebaceous glands, numerous sweat glands, pigment cells and nerve endings. The left side of the scrotum is usually lower because the left spermatic cord is longer.

PENIS

The penis is the male organ of copulation, consists of an attached root in the perineum and a free, normally pendulous body which is completely enveloped in skin. The penile skin is remarkably thin, dark and loosely connected to the tunica albuginea. At the corona of the penis it is folded to form the prepuce or foreskins, which variably overlap the glans. The prepuce and glans penis enclose a potential cleft, the preputial sac and the two shallow fossae flank the frenulum.

Root of the penis:

The root of the penis is situated in the superficial perineal pouch. It is consists of three masses of erectile tissue in the urogenital triangle, namely the two crura and one bulb, each crus is firmly attached to the margins of the pubic arch and is covered by the ischiocavernosus. The blub is attached to the perineal membrane in between the two crura. It is covered by the bulbospongiosus.

Body of the penis:

The body of the penis composed of three elongated masses of erectile tissue. During erection of the penis these masses become engorged with blood leading to considerable enlargement. When flaccid the penis is cylindrical, but when erect it is triangular with rounded angles.

Corpora cavernosa:

The corpora cavernosa of the penis form most of the body. On the urethral surface their combined mass has a wide median groove, adjoining the corpus spongiosum.

Corpus spongiosum:

The corpus spongiosum of the penis is traversed by the urethra. Near the end of the penis it expands in to a somewhat conical enlargement, called the glans penis[58].

REPRODUCTIVE AND HORMONAL FUNCTIONS OF THE MALE:

The reproductive functions of the male can be divided in to three major subdivisions:

(1) Spermatogenesis
(2) Male sexual cycle
(3) Regulation of male reproductive functions by various hormones

GAMETOGENIC FUNCTIONS OF TESTES – SPERMATOGENESIS:

The production of gamete cells is called the gametogenic function. Spermatogenesis is the process by which spermatozoa are developed from the primitive germ cells called the spermatogonia of testis.

STAGES OF SPERMATOGENESIS:

Spermatogenesis occurs in four stages:

1. Stage of proliferation
2. Stage of growth
3. Stage of maturation
4. Stage of transformation.

1. STAGE OF PROLIFERATION:

The spermatogonia near the basement membrane of seminiferous tubule are larger. Each spermatogonium contains diploid number of chromosomes (23 pairs in man) One member of each pair is from maternal origin and the other one from paternal origin. During the proliferative stage, the spermatogonia divide by mitosis without any change in chromosomal number. During this stage, the spermatogonia migrate along with sertoli cells towards the lumen of seminiferous tubule.

2. STAGE OF GROWTH:

In this stage, the primary spermatocyte grows into a large cell. Apart from growth, there is no other change in spermatocytes during this stage

3. STAGE MATURATION:

After reaching the full size, each primary sepermatocyte quickly undergoes meiotic or maturation division, which occurs in two stages.

I. First stage – two secondary spermatocytes are formed

II. Second stage – each secondary spermatocyte divides into two spermatids.

4. STAGE OF TRANSFORMATION:

The spermatids do not divide further but transform into matured spermatozoa (sperms) by a process called spermatogenesis. The changes which take place during maturation of sperm are,

a) Condensation of nuclear material

b) Formation of acrosome. Mitochondrial spiral filament tail structure

c) Removal of extraneous cytoplasm

The matured sperms are released from sertoli cells into the lumen of seminiferous tubules. The process by which the sperms are released into the lumen of seminiferous tubules. For the transport out of testis is called, spermination.

STAGE OF SPERMATOGENESIS- NECESSARY HORMONES

Stage of spermatogenesis	Hormones necessary
1. Stage of proliferation	FSH, Growth Hormone
2. Stage of growth	Testosterone, Growth Hormone
3. Stage of maturation	Testosterone, Growth Hormone
4. Stage of transformation	Testosterone, Estrogen

ROLE OF HORMONES IN SPERMATOGENESIS:

Spermatogenesis is influenced by many hormones which act either directly or indirectly. The hormones necessary for spermatogenesis are,

1. Follicle stimulating hormone (FSH)
2. Testosterone
3. Estrogen
4. Luteinizing hormone (LH)
5. Growth hormone (GH)

1. FSH:

FSH is responsible for the initiation of spermatogenesis. It binds with Sertoli cells and induces the proliferation of spermatogonia. It also stimulates formation of estrogen and androgen binding protein from sertoli cells.

2. TESTOSTERONE:

It stimulates the spermatogenesis. It is also necessary for the formation of secondary spermatocyte from primary spermatocyte.

3. ESTROGEN:

This is secreted by Sertoli Cells. This is also necessary for spermeogenesis

4. LH:

This hormone is essential for the secretion of testosterone from Leydig cells

5. GH:

GH is essential for back ground metabolism of testis. It is also necessary for proliferation of spermatogonia. In pituitary dwarfs, the spermatogenesis is severely affected

MATURATION OF SPERM IN THE EPIDIDYMIS:

After formation in the seminiferous tubules, the sperm require several days to pass through the 6-meter long tubule of the epididymis. Sperm removed from the seminiferous tubules and from the early portions of the *epididymis* are non-motile, they cannot fertilize an ovum. However after the sperm have been in epididymis for some 18 to 24 hours they develop the capability of motility even though several inhibitory proteins in the epididymal fluid still prevent final motility until after ejaculation.

STORAGE OF SPERM:

The two testes of the human adult form up to 120 million sperm each day. A small quantity of these can be stored in the epididymis but most are stored in the vas deferens. They can remain stored maintaining their fertility for at least a month. During this time they are kept in a deeply suppressed inactive state by multiple inhibitory substances in the secretions of the duct. Conversely with a high level of sexual activity and ejaculations storage may be no longer than a few days. After ejaculation the sperm become motile and they also become capable of fertilizing the ovum a process called maturation. The sertoli cells and the epithelium of the epididymis secrete a special nutrient fluid that is ejaculated along with sperm. This fluid contains hormones and enzymes and special nutrients that are essential for sperm maturation.

PHYSIOLOGY OF THE MATURE SPERM:

The activity of sperm is greatly enhanced in a neutral and slightly alkaline medium as exists in the ejaculated semen but it is greatly depressed in a mildly acidic medium. A strong acidic medium can cause rapid death of sperm. The activity of sperm increases markedly with increasing temperature. Although the sperm can live for many weeks in the suppressed state in the genital ducts of the testes. Life expectancy of ejaculated sperm in the female genital tract is only 1 to 2days (24 to 48 hours).

ROLE OF SERTOLI CELLS IN SPERMATOGENESIS:

Sertoli cells influence spermatogenesis by the following ways :

1. Sertoli cells provide nutrition to the developing sperms.
2. sertoli cells secrete estrogen, which is essential for spermatogenesis.
3. sertoli cells secrete hormone binding proteins. These proteins bind with testosterone and estrogen and carry the hormones into the fluid of somniferous tubules.
4. sertoli cells make these hormones available for the maturation of sperms.

FUNCTIONS OF SEMINAL VESICLE SECRETION: NUTRITION TO SPERMS:

The fructose and other nutritive substances from seminal vesicles are utilized by sperms after being ejaculated into female genital tract.

CLOTTING OF SEMEN:

The fibrinogen from secretions of seminal vesicle is converted in to the coagulum as soon as semen is ejaculated.

ON FERTILIZATION:

The prostaglandin of seminal vesicle fluid enhances the fertilization of ovum by the following processes:

1. Increasing the receptive capacity of cervical mucosa for Sperms.
2. Causing reverse peristaltic movement of uterus and fallopian tubes. This, in turn, increases the rate of transport of sperms in female genital tract during coitus.

FUNCTIONS OF PROSTATIC FLUID:

MAINTENANCE OF SPERM MOTILITY:

The prostatic fluid provides optimum pH for the motility of sperms. Due to the metabolic end products from sperm, the fluid in vas deferens acidic in nature. This inhibits the motility of sperms. The Vaginal secretions in females are highly acidic with a pH of 3.0 – 4.0. This also inhibits the motility of sperms. Generally, the sperms are non- motile at a pH less than 6.0.The prostatic secretion neutralizes the acidity of

vaginal secretions and maintains a pH of 6-6.5. At this pH the sperm become motile and chances of fertilization are enhanced.

CLOTTING OF SEMEN:

The clotting enzymes in prostatic secretion cause conversion of fibrinogen into coagulum. It is essential for holding the sperms in uterine cervix.

LYSIS OF COAGULUM:

The coagulum is dissolved by fibrinolysin of the prostate secretion so that the sperm become motile.

SEMEN:

Semen is a white or grey fluid that contain spermatozoa .It is the collection of fluid from testis, seminal vesical, prostate and bulbourethral gland. Semen is discharged during sexual act and the process of discharge is called ejaculation. At the time of ejaculation, human semen is liquid in nature. Immediately, it coagulates and some time it undergoes a secondary liquefaction.

PROPERTIES OF SEMEN:

1. Specific gravity: 1.028
2. Volume: 2 to 6 ml/ejaculation
3. Reaction:

Alkaline pH of 7.5.the alkalinity is due to the secretions from prostate.

COMPOSITION OF SEMEN:

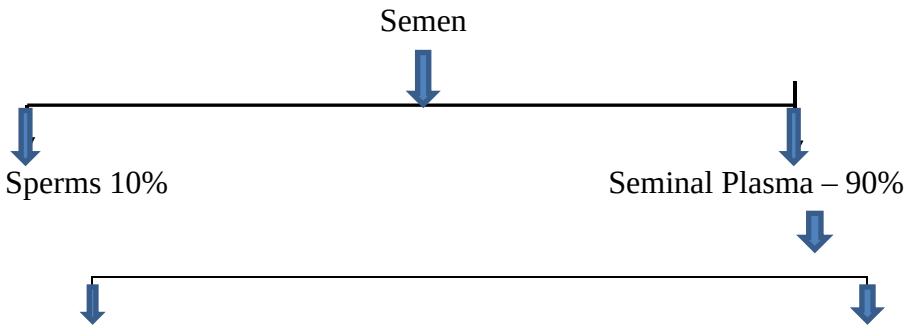
Semen contains

1. Sperms : 10%
2. Products from seminal vesicle : 60%
3. Products from prostate gland : 30%

SPERM:

The total count of sperm is about 100 to 150 million /ml of semen. Male sterility occurs, when the sperm count is less than 15 million/ml. After ejaculation of sperm, the survival time is only about 24 to 48 hours at a temperature equivalent to body temperature. The rate of motility of sperm in female genital tract is about3mm/minute. The sperm reach the fallopian tube in about 30 to 60 minute after

sexual intercourse. The Uterine contraction during sexual act facilitates the movement of sperms.

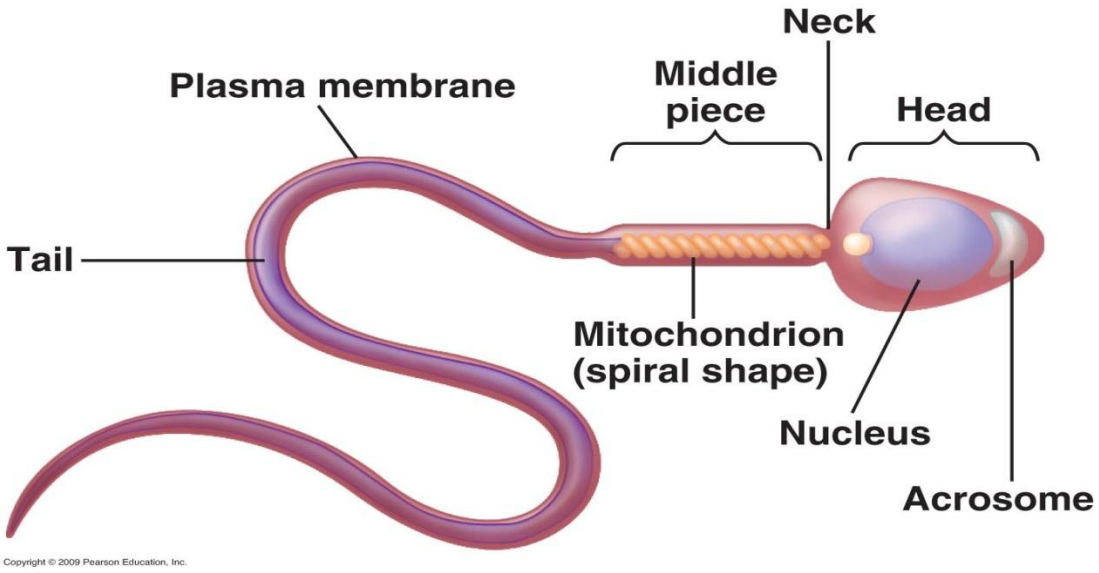


Products from seminal vesicles – 60%

1. Ascorbic acid
2. Fibrinogen
3. Flavin
4. Fructose
5. Inositol
6. Pepsinogen
7. Phosphorylcholine
8. Prostaglandian(PGE-2)
9. Citrate
10. Citric acid

Products from prostate gland – 30%

1. Acid phosphatase
2. Cholestrol
3. Clotting enzymes
4. Fibrinolysin
5. Glucose
6. Lactate dehydrogenase
7. Phospholipids
8. Plasminogen activator
9. Seminin
10. Spermine
11. Bicarbonate
12. Calcium
13. Citrate
14. Sodium
15. Zinc



Sperm cell

CAPITATION OF THE SPERMATION:

Mature sperm, even when they are coming out of the male genital tract are incapable of fertilizing the ovum unless the further changes or capitation takes place for a variable period 1 to 10 hours in the female genital tract. The membrane of the sperm thus become progressively permeable to calcium ion that enters in abundance to initiate the powerful whiplash forward movement of the flagellum or tail instead of its previous undulating motion. Calcium has a further role to bring about further chance in the acrosome intracellular membrane for helping to releasing its enzyme very rapidly in female genital tract.

THE ACROSOME REACTION:

The lytic enzyme involved in the sperm penetration is mostly located in the anterior sperm head, whereas other such as acrosin are primary contained within the acrosome. The anterior surface of the head needs to be removed allowing liberation of acrosin before the sperm can be penetrating zona pellucida. Removal of this anterior surface of the head is the process called acrosome reaction.

MECHANISM OF ERECTION:

The male erectile response is a vascular event initiated by neuronal action and maintained by a complex interplay between vascular and neurological, and perhaps humoral phenomenons resulting in cascade of events. Erection of penis in simple terms consists of trapping pressurized blood within the confines of a limited space provided by the spongy corpora cavernosa. This blood – filled spaces relax and open up, allowing free inflow of blood leading to expansion of the chambers pulling the tunica albuginea tight. The tensed tunica albuginea makes the corpora hard (resistant to indentation) and rigid (resistant to flexion). Secondarily, it pinches off the veins (that normally let blood leave the chambers) trapping blood inside and contributing to the state of engorgement .The valves (actually flaps), according to some experts) that control the flow of blood, however are opened and closed by nerves that run through the spinal cord to the brain.

Activation by the nervous system causes a rapid increase in the blood flow into the penis. During erection, as blood flows into the penis, the holes in the spongy tissue in the penis get filled in with it. At the same time, flaps in the veins leading out of the penis enlarge, cutting off the outflow. Thus, more blood flows in than out, and the penis are compressed from the increased pressure from the erection itself. In addition, the heart rate and blood pressure increase the pressure of blood into the penis increases to maintain its hardness.

MECHANISM OF EJACULATION:

The emission phase is the first phase. It involves deposition of seminal fluid from the ampullary vas deferens, seminal vesicles and prostate gland into posterior urethra. The second phase is the expulsion phase. It involves closure of bladder neck followed by the rhythmic contractions of the urethra by pelvicperineal and bulbospongiosus muscle, and intermittent relaxation of external urethra sphincters. It is believed that the neurotransmitter serotonin (5HT) plays a central role in modulating ejaculation. Several animal studies have demonstrated its inhibitory effect on ejaculation. Therefore, it is perceived that low level of serotonin in the synaptic cleft in these specific areas in the brain could cause premature ejaculation.

This theory is further supported by the proven effectiveness of selective serotonin reuptake inhibitors (SSRIs), which increase serotonin level in the synapse, in treating PE. Sympathatic motor neurons control. The emission phase of ejaculation reflex and expulsion phase is executed by somatic and autonomic motor neurons. These motor neurons are located in the thoraco lumbar and lumbo sacral spinal cord and are activated in a coordinated manner when sufficient sensory in a coordinated manner when sufficient sensory input to reach the ejaculatory threshold has entered the central nervous system. Several areas in the brain, and especially the nucleus paragigantocellularis, have been identified to be involved in ejaculatory control[59].

MALE SEXUAL HORMONE:

The testes secrete the male sex hormones are called the androgens. The testicular Androgens are:

1. Testosterone
2. Dihydrotestosterone
3. Androstenedione

SOURCE OF SECRETION OF ANDROGENS:

The androgens are secreted in large quantities by testes and in small quantities by adrenal cortex.

TESTES:

In testes, the androgens are secreted by the interstitial cells of leydig. This forms 20% of mass of adult testis, leydig cells are numerous in newborn male infant and in adult male after puberty.

ADRENAL CORTEX:

Zona reticularis of adrenal cortex also secretes androgens called testosterone, androstenedione and dehydro-epiandrosterone.

FUNCTION OF TESTOSTERONE:

In general, testosterone is responsible for the distinguishing characters of masculine body. In the fetal life, the tests are stimulated by human chorionic gonadotropins secreted by placenta.

SEX DIFFERENTIATION IN FETUS:

Testosterone is responsible for the sex differentiation.

Mullerian Duct

From this duct, female accessory sex organs like vagina, uterus and fallopian tube are developed.

Wolffian Duct

From this, male accessory sex organs like epididymis, vas deferens and seminal vesicles are developed.

.**DESCENT OF TESTES:**

Initially, testes are developed in the abdominal cavity and are later pushed into the scrotum through inguinal canal just before birth. This is called the descent of testes. Testosterone is necessary for this. If a male child is born with undecended testes, the condition is called „cryptorchidism".

FUNCTION OF TESTOSTERONE IN ADULT LIFE:

Testosterone has two important functions in adult,

i. Effect on sex organs

ii. Effect on secondary sexual characters

ON SEX ORGANS:

Testosterone increases the size of penis, scrotum and the testes after puberty.

ON SECONDARY SEXUAL CHARACTERS:

Testosterone causes development of secondary sexual characters at puberty, which distinguishes the male from female. The secondary sexual characters developed by testosterone are as follows:

MUSCULAR GROWTH:

One of the most important male sexual characters is the development of musculature after puberty. The mass of the muscle increases by about 50% is due to the anabolic activity of testosterone on proteins.

BONE GROWTH:

After puberty the bones grow in thickness with deposition of calcium. The increase in thickness is due to increase in total content of bone matrix which is because of protein anabolic activity of testosterone. Testosterone causes broadening of shoulders and it has a specific effect on pelvis which results in,

a) Narrowing of pelvic outlet

b) Lengthening of pelvis

c) The funnel like shape of pelvis

Pelvis in males is different from that of females, which is broad and ovoid in shape. Testosterone also causes early fusion of epiphyses of long bones with shaft.

CHANGES IN SKIN:

Testosterone increases the thickness of skin over the entire body surface and the ruggedness of subcutaneous tissue. These changes in skin are due to deposition of proteins in skin.

HAIR DISTRIBUTION:

The testosterone causes male type of distribution of hair on the body. i.e. hair growth over the pubis, along linea alba up to umbilicus, on face, on chest and other parts of the body like back, in males, the pubic hair has the base of the triangle downwards.

CHANGE IN VOICE:

At puberty, testosterone causes hypertrophy of laryngeal muscles, the enlargement of larynx and lengthening and thickening of vocal cords.

BASAL METABOLIC RATE:

At the time of adolescence and earlier part of adult life, the testosterone increases the BMR rises 5 – 10%. This is mostly due to anabolic effects of testosterone on protein metabolism.

ELECTROLYTE AND WATER BALANCE:

Testosterone increases the retention of sodium by reabsorption in renal tubules. The action is very mild.

BLOOD:

After puberty, testosterone causes slight increase in blood volume by increasing water content and by increasing the number of RBCs.

CONTROL OF MALE SEXUAL FUNCTIONS BY HORMONES

A major share of the control of sexual functions in both male and female begins with secretion of gonadotropin releasing hormone (GnRH) by the hypothalamus. This hormone in turn stimulates the anterior pituitary gland to secrete two other hormones called the gonadotropic hormones.

1. Luteinizing hormone (LH) – This hormone is essential for the secretion of testosterone from leydig cells.
2. Follicle stimulating hormone (FSH) – it accelerates the process of spermatogenesis, Combination with testosterone.

NEGATIVE FEEDBACK CONTROL OF TESTOSTERONE:

Testosterone regulates its own secretion by negative feedback mechanism. It acts on hypothalamus and inhibits the secretion of LHRH. When LHRH secretion is inhibited, LH is not released from anterior pituitary resulting in stoppage of testosterone secretion from testes. On the other hand, when testosterone production is low, lack of inhibition of hypothalamus leads to secretion of testosterone through LHRH and LH.

ABNORMALITIES OF SEXUAL FUNCTION:

ENLARGEMENT OF PROSTATE GLAND:

Enlargement of prostate gland occurs due to**:**

1. Hyperplasia of glandular structures and connectivity tissues benign (non-malignant) enlargement
2. Cancer – malignant enlargement

HYPOGONADISM IN MALES:

Hypogonadism is a condition characterized by reduction of functional activity of gonads.

SIGNS AND SYMPTOMS:

The clinical picture of male hypogonadism depends upon whether the testicular deficiency develops before or after puberty.

BEFORE PUBERTY:

The symptoms of hypogonadism are similar to those developed due to extirpation of testes before puberty.

AFTER PUBERTY:

The symptoms are similar to those developed due to removal of testes after puberty.

IN ADULT:

Hypogonadism caused by testicular disorder increases the gonadotropin Secretion and the condition called hypergonadotropic hypogonadism. Hypogonadism that occurs due to deficiency gonadotropin is called hypogonadotropic hypogonadism.

FROHLICHS SYNDROME:

It is the disorder characterized by obesity and hypogonadism in adolescent boy also called adiposo genital syndrome or hypothalamic eunuchism.

EFFECT OF TEMPERATURE ON SPERMATOGENESIS:

Increasing the temperature of the testes can prevent spermatogenesis by causing degeneration of most cells of the seminiferous tubules besides the spermatogonia. It has often be stated that the reason, the testes are located in the dangling scrotum is to maintain the temperature of these glands, below the internal temperature of the body, although usually only about 2º C below the internal temperature. On cold days scrotal reflexes cause the musculature of the scrotum to contract, pulling the testes close to the body to maintain this 2º C differential. Thus the scrotum theoretically acts as a cooling mechanism for the testes.

CRYPTORCHIDISM:

Cryptorchidism means failure of the testis to descent from the abdomen in to the scrotum at or near the time of birth of a fetus. During development of the male fetus, the testes are derived from the genital ridges in the abdomen. A testis that remains throughout the life in the abdominal cavity is incapable of forming sperm. The tubular epithelium undergoes degeneration, leaving only the interstitial structures of the testis.

SEMEN ANALYSIS:

COLLECTION:

The semen specimen should be collected in a small clean wide mouthed jar of 10 to 20 ml (using larger jar may cause drying of some portion, when it is transported to the laboratory). The container must be spotlessly clean. Masturbation (self- stimulation) is the most preferred method. Coitus interrupts (withdrawal of penis just prior to ejaculation during sexual intercourse) may be used, but there is always a possibility of loss of the sperm rich initial portion. The container must be ideally warmed to the body temperature, as sperms are especially susceptible to cold. The slide must be warmed, as otherwise motility studies may show erroneous result. Masturbation can be very stressful for some men especially when they know their counts are low, or if they have had problems with masturbation on demand for semen analysis in the past. The condition of the toilet room, where the patient has to go for procuring the specimen, is often related to their not providing the proper specimen. Men failing to provide a specimen could be advised either to have female partners beside or to see sexually arousing pictures for helping them to provide sample. They can also use a mechanical vibrator to get an erection.

In some cases, additional assistance by using liquid paraffin helps in masturbation. The infertility centres should have a special private room to allow the patients for the masturbation on demand.

The semen samples must be collected after a sexual abstinence 3 to 5 days, or at least 72 hours after the last ejaculation (no sex or masturbation). It is very important to keep with the chosen abstinence schedule, because variations in the time period between ejaculations interfere with the accuracy of test results. For up to one week, semen characteristics, such as volume and sperm concentration, may increase with each day of abstinence; but after that period, the sperm motility is usually impaired.

COMPONENTS OF SEMEN ANALYSIS:

- Sperm count
- Motility
- Morphology
- Volume
- Fructose level
- PH

SPERM COUNT:

Sperm count, or sperm concentration to avoid mix-up, measures the concentration of sperm in a man's ejaculate, distinguished from *total sperm count*, which is the sperm count multiplied with volume. Anything over 15 million sperm per milliliter is considered normal. Anything less is considered „oligospermia". The average sperm count today is around 60 million per milliliter in the Western world, having decreased by 1-2% per year from substantially higher number decades ago.

MOTILITY:

A more specified measure is *motility grade*, where the motility of sperm is divided into four different grades:

 Grade 4: Sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility **a**.

 Grade 3: (non-linear motility): These also move forward but tend to travel in a curved or crooked motion. Sometimes also denoted motility **b**.

 Grade 2: These have non-progressive motility because they do not move forward despite the fact that they move their tails.

 Grade 1: These are immotile and fail to move at all.

MORPHOLOGY:

i. Head - The head should be oval and smooth.

ii. Mid piece - the mid piece should be straight and slightly thicker than the tail.

iii. Tail - the tail should be single, unbroken, straight and without coils.

VOLUME:

The volume of the sample is measured between 1.0 mL and 6.5 mL is normal; WHO criteria specify that any volume greater than 1.5 mL is normal. Low volume may indicate partial or complete blockage of the seminal vesicles, or that the man was born without seminal vesicles. In clinical practice, a volume of less than 1.5 mL in the setting of infertility and absent sperm should prompt an evaluation for obstructive azoospermia.

FRUCTOSE LEVEL:

The normal level of fructose in the semen is at least 3 mg/ml. WHO specify a normal level of 13 μmol per sample. Absence of fructose may indicate a problem with the seminal vesicles.

PH:

The normal range of pH of the sample is 7.1-8.0; WHO criteria specify normal as 7.2-7.8. Acidic ejaculate (lower pH value) may indicate one or both of the seminal vesicles are blocked. A basic ejaculate (higher pH value) may indicate an infection. A pH value outside of the normal range is harmful to sperm.

LIQUEFACTION:

The liquefaction is the process when the gel formed by proteins from the seminal vesicles is broken up and the semen becomes more liquid. It normally takes less than 20 minutes for the sample to change from a thick gel into a liquid. An abnormally long liquefaction (more than 30 minutes) time may indicate an infection.

TOTAL MOTILE SPERMATOZOA:

Total motile spermatozoa (TMS) or *total motile sperm count* (TMSC) is a combination of sperm count, motility and volume, measuring how many million sperm cells in an entire ejaculate is motile. Use of approximately 20 million grade 3+4 sperm in ICI, and 5 million ones in IUI may be an approximate recommendation.

OTHERS:

The sample is tested for white blood cells. A high level of white blood cells (over 1 million per milliliter) may indicate an infection.

ABNORMALITIES:

i. Aspermia: absence of semen

ii. Azoospermia: absence of sperm

iii. Oligospermia: low number of sperm

iv. Asthenozoospermia: poor sperm motility

v. Teratozoospermia: sperm carry more morphological defects than usual[60].

ADVANCED SPERM FERTILITY TESTES: COMPUTER-ASSISTED SEMEN ANALYSIS (CASA)

The new technologies such as CASA incorporate the video systems to measure the types and the speed of sperm motility. Normal sperms swim faster and straighter than the abnormal ones. The average speed of a human sperm is roughly 48 to 96 mm per second. CASA permits the measurement of additional motility parameters such a curvilinear velocity (VCL), straight-line velocity (VSL), linearity, and flagellar beat frequency. CASA Measures the parameters such as VCL, VSL and amplitude of lateral head (ALH). The quality of sperm movement is based on a classification system of 0 to 4, wherein 0 represents no movement and 4 represents excellent forward progression; for example, a semen sample with 60% motility would be characterized as 3+ to 4.2.

QUALITY ASSESSMENT OF CASA:

Three levels of quality assessment are generally accepted: structure, process and results.

SPERM CLUMPING OR AGGLUTINATION:

The sperms may clump head-to-head, tail-to-tail, or head-to-tail. In particular tail-to-tail agglutination of motile sperm is noteworthy and usually is followed up with Sperm Function.

SEMEN CULTURE TEST:

In a semen culture test, the semen sample is tested for the presence of bacteria. Testing the bacterial sensitivity to antibiotics is mandatory if there is any presence of bacteria. Whether the bacteria present in the specimen is that are usually seen in normal semen or those of a bacterial disease, without the evidence of

inflammation or infection, there is no indication for routine culture or antibiotic treatment in infertile men. If urine analysis is abnormal or bacterial prostatitis is suspected from the history or the physical examination, semen culture is certainly indicated. The common sexually transmitted organisms such as Chlamydia trachomatis, Mycoplasma hominus and Ureaplasma urealyticulum have been implicated in reproductive failure in animals and humans.

BIOCHEMICAL TESTS:

Biochemical analysis of seminal plasma mostly provides insights into the function of the accessory sex glands. The fructose content of semen (normal value -250-400 mgm or 4-28 mmol/litre) should be routinely tested. Low fructose content (less than120 mgm) is often due to seminal vesiculitis, androgen deficiency or partial ejaculatory duct obstruction. Its absence indicates complete obstruction either due to a congenital block at the level of ejaculatory duct or proximal to it like agenesis of the vas and the seminal vesicle or following acquired post-infective cicatrisation. Almost invariably, these conditions are associated with azoospermia or severe oligospermia. The epididymis is represented by glyceryphosphorylcholine (GPC), the seminal vesicles by fructose, and the prostate gland by zinc.

IMMUNOLOGICAL TESTS:

In the enzyme-linked immune sorbent assay (ELISA) test, the antisperm antibodies measuring up to 20 units/m in 32 or 64 dilutions is considered normal.

SPERM FUNCTION TESTS:

The sperm function tests assess the sperm‟s ability to fertilize the ovum. There is a drawback that these tests are often not standardized adequately.

SPERM VIABILITY OR SPERM SURVIVAL TEST:

The sperm viability may be determined by two methods-Eosin Y stain exclusion and hypo-osmotic swelling or HOS assay.

BOVINE CERVICAL MUCUS TEST:

The bovine cervical mucus test is another form of testing for the ability of the sperms to penetrate and swim through cervical mucus. These tests to assess the fertilizing potential of sperms. This in vitro functional test measures the ability of penetration of the sperms. The end point of this assay is penetration of the ovum and decondensation of sperm heads. Men with sperm of low SPA score are less likely to achieve a spontaneous pregnancy than those with high SPA score.

SPERM CHROMATIN STRUCTURAL ASSAY (SCSA):

To measure the level of DNA fragmentation in the sperm is to help the diagnosis and treatment for male infertility. The sperm with high levels of DNA fragmentation have a lower probability of producing a successful pregnancy. Vitamin C protects the sperms from endogenous oxidative DNA damage that could affect sperm quality and increased risk of genetic defects, particularly in population with low ascorbic acid like smokers against free radical damages. 24 Studies show that a daily dose of 1000 mg showed statistically significant improvement of sperms.

THE POSTCOITAL TEST (PCT):

It is first performed by Sims, has traditionally been a common way to determine cervical mucus/sperm interaction. This test evaluates sperm concentration and motility in an aspirate of cervical mucus at midcycle shortly after the couple has intercourse. Results of a normal PCT would show the presence of 20 or more spermatozoa per high-power field. An abnormal PCT results most commonly is secondary to inappropriate timing of coitus. Other causes include ASA, an ovulation, an abnormal hormonal milieu, female or male genital tract infections, poor semen quality, and male sexual dysfunction.

Sperm Penetration Assay (Spa):

The SPA was developed to measure the functional properties of sperm and was initially developed following the observation that, upon the removal of the zona pellucida of hamster ova, the species specificity of fertilization and the block to polyspermy are lost. In particular, heterologous penetrations between hamster ova and sperm from a variety of species, including humans, has been observed. Ideally, human ova should be used for this assay, but they are not widely available, and there are ethical problems associated with their use. Therefore, hamster ova have provided a useful model for the measurement of human sperm function. For fertilization to occur

in vivo, the sperm must first be capacitated and have undergone the acrosome reaction. The physiology of sperm capacitation is not clearly defined. In particular, it is not known whether capacitated sperm that have gained the ability to penetrate human ova have undergone the acrosome reaction, or whether this occurs as a local event at the time of gamete fusion. The use of SPA as a measure of potential fertility is based on the theory that fertile sperm samples will either penetrate most hamster ova or result in a significant amount of polyspermy of the penetrated ova. Infertile sperm samples are expected to penetrate a lower percentage of ova or result in a lesser degree of polyspermy. Consideration should be given to obtaining the SPA in couples with unexplained infertility or in couples in whom the decision is being made to precede with intrauterine insemination (IUI) or IVF, since lower SPA results have been predictive of poor success with IVF and lower pregnancy rates in couples attempting conception through intercourse.

REACTIVE OXYGEN SPECIES (ROS) ASSAY:

For cells living under aerobic conditions, oxygen represents a paradox: While it is required for survival and normal function, its metabolites can be potentially toxic due to the generation of oxygen-free radicals. Some of these metabolites, called ROS, have been shown to be produced by spermatozoa and to generate toxic effects on sperm function. However, when produced at the right time and amount, these ROS can also initiate and promote normal physiologic reactions such as sperm hyperactivation and capacitation. In human semen, high ROS formation was detected in 40% of semen samples from an unselected population of men consulting an infertility clinic.

NUTRITION:

ZINC:

Zinc is the most important nutrient mineral influencing male fertility. Zinc level in the seminal plasma is directly related to sperm motility. Dietary zinc restriction reduces both sperm count and seminal plasma volume. Zinc levels in seminal plasma of normal, oligospermic, asthenospermic and azoospermic subjects show that a linear direct relationship seems to exist between zinc in seminal plasma and motility of spermatozoa. Dietary restriction of zinc can affect testicular

function adversely. The serum testosterone concentration and seminal volume are most sensitive to zinc depletion in men in the reproductive period.

VITAMIN B 12:

Vitamin B 12 deficiency also plays a role in fertility. "Intrinsic factor" is necessary for the proper absorption of B12 and its deficiency is one of the causes of secondary infertility in male.

VITAMIN C:

Studies have shown the concentration of ascorbic acid in seminal plasma directly reflects dietary intake, and lower levels of vitamin C may lead to infertility and increased damage to the sperm‟s genetic material.36 Fraga et al demonstrated this by reducing ascorbic acid intake in healthy men from 250 mg to 5 mg per day. Seminal plasma levels of vitamin C decreased by 50 percent, with a concomitant 91- percent increase in sperm with DNA damage.

L-ARGININE:

The biochemical and physiological relevance of L-arginine lies in its role as the precursor in the synthesis of polyamines and testosterone. The polyamines putrescence and spermidine are organic components important to sperm motility. An arginine metabolism is a factor in normal sperm production being involved as a source of nitric oxide within spermatozoa. Nitric oxide (at endogenous concentrations) appears to be necessary for adequate sperm motility. The endothelial (eNOS) and brain (b NOS) nitric oxide syntheses are abundant in normozoospermic samples but is low in asthenozoospermic patients. Consequently, an adequate dietary amount of Larginine is necessary for normal spermatogenesis, especially for the sperm motility and arginine aspartate (9 g daily) has been found to be effective in some cases of asthenospermia. Larginine, 4 gm. daily has been shown to improve sperm counts in men with oligospermia. Nuts, oilseeds, flesh foods, pulses and legumes are common sources of L-arginine.

VITAMIN E:

The membranes of the germ cells and spermatozoa are very sensitive to oxidation because of their high content of PUFA (Polyunsaturated fatty acids).Vitamin E is a major lipophilic chain-breaking anti-oxidant, which protects tissue PUFA against peroxidation, a property that is beneficial in the male reproductive physiology. Oral78 administration of vitamin E significantly improves

the in vitro function of human spermatozoa as assessed by the zona binding test. Vitamin E antioxidant therapy is however, dependent on the dosage or the in vitro concentration of the vitamin. Vitamin E in a dose of 200 IU twice daily acts an antioxidant and improves sperms‟ ability to impregnate.

SELENIUM:

Men with reduced sperm motility, supplementation with selenium (100 mcg per day for three months) significantly increased sperm motility, but it had no effect on sperm count. Selenium is one of the important ingredients that is very often lacking in order mean and can be found in horsetail, which has been used with success in ED following prostatic enlargement.

L- Carnitine:

Sperm motility also increased both in quantitative and qualitative manners. In a multicentric study, increase in the sperm motility was also observed in terms of both rapid linear progression and linearity index along with that the sperm output after oral administration of L -carnitine in patients with idiopathic asthenozoospermias. Two amino acids Iysine and methionine that is necessary for the biosynthesis of L-carnitine in the body.

ANTIOXIDANTS:

Polyunsaturated fatty acids and phospholipids are key constituents in the sperm cell membrane and are highly susceptible to oxidative damage. Sperm produce controlled concentrations of reactive oxygen species, such as the superoxide anion, hydrogen peroxide, and nitric oxide, which are needed for fertilization; however, high concentrations of these free radicals can directly damage sperm cells. Disruption of this delicate balance has been proposed as one of the possible etiologies of idiopathic male infertility. About some Anti-oxidants,

- Vitamin A alone improved sperm function and IVF rates in studies.
- Vitamin A, Vitamin E, and essential fatty acids (omega-3 fats) were shown to increase sperm count in another study.
- Folic acid and zinc may increase sperm concentration.
- The bottom line: having a healthy diet is important for male fertility. You should have a diet rich in a variety fruits and vegetables and take a good quality multivitamin daily. You may also consider taking an omega-3 supplement, if your intake of fish is low.

COENZYME Q-10:

In sperm cells, coenzyme Q10 (CoQ10) is concentrated in the mitochondrial midpiece, where it is involved in energy production. It also functions as an antioxidant, preventing lipid peroxidation of sperm membranes. When sperm samples from 22 asthenospermic men were incubated *in vitro* with 50 micro CoQ10, significant increases in motility were observed. CoQ10 (60 mg) was given to 17 infertile patients for a mean 103 days, and although there were no significant changes in standard sperm parameters, there was a significant improvement in fertilization rate ($p<.0.05$).52 In another study, 10 mg/day of coenzyme Q7 (an analog of CoQ10) was given to infertile men, with resulting increases in sperm count and motility.

NORMAL VALUES - WHO CRITERIA:

The WHO reference values for a normal semen analysis are defined as given below:

- Volume – 1.5ml or more
- Total sperm count - 39 million per ejaculate or more
- Sperm concentration - 15 million per ejaculate or more
- pH – 7.2 or higher
- Motility - 40 % or more motile
- 32% or more with progressive motility, within 60 minutes of ejaculation

Motility is graded from a to d according to WHO manual criteria,

- a – Fast progressive. Sperms are those which swim forward fast in a straight line, like guided missiles
- b – Slow progressive

  Sperms swim forward, but either in a curved or crooked line or slowly.
- c – Non progressive.

  Sperms move their tails, but do not move forward. (local motility only)
- d– Immotile. Sperms do not move at all.

  Sperms of grade c & d are considered poor.

MORPHOLOGY:

- Head - The head should be oval and smooth.
- Mid piece - the mid piece should be straight and slightly thicker than the tail.
- Tail - the tail should be single, unbroken,straight and without coil.

PREPARATION AND PROPERTIES OF TRIAL DRUG

DRUG NAME **:** *VEERIYA VIRUTHI CHOORANAM*

TEXT REFERENCE : REF. AATHMARATCHAMIRTHUM –PART 2 PAGE:243

INGREDIENTS:

Poonaikali vithai - 50grams

Nilapannai kizhangu - 50grams

Boomichakkarai kizhangu - 50grams[(61)]

STANDARD OPERATING PROCEDURE:

Source of raw drugs:

The required raw drugs are procured from a wall reputed indigenous drug shop. The raw drug will be authenticated by the botanist, Dept. of Medicinal Botany, Govt. Siddha Medical College, Arumbakkam, Chennai – 106.

Purification of raw drugs:

Raw drugs are purified as mentioned in Sikitcha Ratna Deepam Sarakku Suthi Muraigal.

Preparation:

The ingredients of trial drug should be powdered individually and strained using a fine cloth and the mixed in equal ratio then bottled up.

Drug Storage:

The trial drug is stored in clean dry air tight container and it is dispensed to the patients in packets.

Dose:

1 gm., twice a day with cow‟s milk after meals.

POONAIKAALI VITHAI

BOTANICAL NAME -*Mucuna pruriens*
SUVAI - THUVARPPU
THANMAI - THATPAM
PIRIVU - INIPPU
ACTION - ASTRINGENT, APHRODISIAC, NERVINE TONIC

GUNAM:

தழுதளைநாற் றத்தோடு சாரிரத்தப் போக்கும்
பழுதுபுரி கின்றகரப் பானும் – அழுதேகுந்
தூலமிசை விந்துவுமாஞ் சாற்றற் கரும்பூனைக்
காலி விதையைக் கழறு[62]

- குணபாடம் – மூலிகை வகுப்பு பக்கம் எண் : 707

NILAPPANAI KIZHANGU

BOTANICAL NAME -*Curculigo orchioides*
SUVAI - INIPPU
THANMAI - THATPAM
PIRIVU - INIPPU
ACTION - TONIC, CARMINATIVE, ASTRINGENT

GUNAM:

மேகவனல் தணியும் வெண்குட்டந் தான்விலகும்
போக மிகவுமுறும் பொற்கொடியே! – போகாத
சூலைமே கங்களோடு துன்னுகரும் புள்ளியும்போஞ்
சால நிலப்பனைக்குந் தான். [63]

-குணபாடம் – மூலிகை வகுப்பு பக்கம் எண் : 576

BOOMI CHAKKARAI KIZHANGU

BOTANICAL NAME - *Maerua oblongifolia(* Forssk) A.Rich
SUVAI - INIPPU
THANMAI - THATPAM
PIRIVU - INIPPU
ACTION - APHRODISIAC,LAXATIVE,TONIC.[64]

GUNAM :

மேகமுறு முள்ளுருக்கு வெட்டை யனற்றணியும்
போகுமே மூலம் புகலக்கேள் - பாகுமொழிப்
போன்னனையாய் பூமிச் சருக்கரைக் கிழங்குக்கு
வுன்னவுடல் பருக்கும் வாழ்த்து[65]

-பதார்த்த குணபாடம் -pg no :183


Nilapanai Kizhangu


poonaikaali vithai


Boomichakkarai Kizhangu


veeriya viruthi
chooranam

MATERIALS AND METHODS

STUDY DESIGN:

A Clinical study on AAN MALADU was conducted at the OPD section of Post Graduate, Pothu Maruthuvam Department attached to Arignar Anna Hospital Of Indian Medicine, Chennai-106, during the period of 2015 – 2017. The study was approved by Institutional Ethical Committee(IEC) and the approval number is GSMC-CH-ME-4/2015/007. it was registered in clinical trials registry-india(ctri) and the register number is CTRI/2017/05/008448

POPULATION AND SAMPLE:

The population consists of all patients who were attending the OPD section of Post Graduate, Pothu Maruthuvam Department attached to Arignar Anna Hospital Of Indian Medicine, Chennai.-106 sample consists of Aan maladu who satisfying the inclusion and exclusion criteria mentioned below.

SAMPLE SIZE:

The trial size will be 20 patients.

INCLUSION CRITERIA:

- Age 21-45 years
- Oligospermia (low number of sperm <15 millions)
- Oligo asthenospermia
- Willing to give semen for the investigation

EXCLUSION CRITERIA:

- Azoospermia
- Teratospermia
- Hydrocele
- Varicocele
- Diabetes mellitus
- Hypertension
- Cardiac disease

WITHDRAWAL CRITERIA:

- Intolerance to the development of adverse reactions during the drug trial (If ADR is reported the patient will be directed to RPC).
- Patients turned unwilling to continue in the course of clinical trial.
- Any other acute illness.

DURATION OF TREATMENT:

48 days.

EVALUATION OF CLINICAL PARAMETERS:

The history includes past, personal, family, occupation, dietary habits, seasonal history and associated history.

Clinical investigation:

Blood:

TC,DC,ESR,Hb,VDRL,Sugar,Urea,Serum,Creatinine,Cholesterol

Urine:

Albumin, Sugar, Deposit

SEMEN ANALYSIS

- The volume, colour, appearance of the SEMEN sample
- Approximate number of total SPERM CELLS
- SPERM MOTILITY/ FORWARD PROGRESSION
- Percentage of sperm with NORMAL MORPHOLOGY & MOTILITY
- VISCOSITY of semen
- pH of the Seminal fluid
- Liquification time
- Antisperm antibody

SIDDHA ASSESSMENT:

- Envagai Thervugal
- Neerkuri
- Neikkuri

A case sheet format was prepared on the basis of the Siddha methodology example envagai thervugal, mukkutram, nilam, kaalam, udal thathugal, including neerkuri and neikuri. Individual case sheet was maintained for each patient at outpatient department.

Data collection forms:

Required information will be collected from each patient by using following forms.

Form I : Screening and selection proforma

Form II : History taking proforma

Form III : Clinical assessment proforma

Form IV : Clinical assessment during and after trial

Form V : Laboratory Investigation proforma

Form VI : Informed consent

Form VII : Withdrawal form

Form VIII : Patient information sheet

Form IX : Diet sheet

Data Analysis:

After enrolling the patients in the study a separate file for each patient will be maintained and all forms will be kept in the file. Whenever the patient visits OPD during the study period necessary entries will be made in the assessment forms. The data entries and adverse events if any will be monitored by the Head of the Department.

Outcome of Treatment

The outcome treatment is mainly assessed to improve the sperm count at least 20% and by comparing the reduction in clinical symptoms and recurrence before and after treatment and assessed by comparing the safety parameters before and after treatment.

Adverse effect and Serious effect Management:

If the trial patient develops any adverse reactions the patient will be referred to the Pharmacovigilance department of SCRI and documented. For any adverse effect the investigator will give the proper management in the OPD.

Ethical issues

1. Informed consent will be obtained from the patient after explaining about the clinical trial in an understandable language.
2. After the consent of the patient (through consent) if they fit in the criteria they will be enrolled in the study.
3. Treatment will be provided free of cost.
4. Concomitant medicines will be used if there is any need.
5. The patients who are excluded (as per the exclusion criteria) will be referring to OPD.

Analysis of Trial medicine:

1. The acute and sub-acute toxicity study was carried out in centre for laboratory animal technology and research ,Sathyabama university,kelambakkam, Chennai-119 .
2. The Pharmacological analysis of trial drug for its Spermatogenic activity was carried out in centre for laboratory animal technology and research ,Sathyabama university,kelambakkam, Chennai-119 .
3. The physiochemical analysis was performed in Siddha Central Research Institute, Chennai.
4. Observation made from patients with sign and symptoms of the disease and their prognosis were recorded.

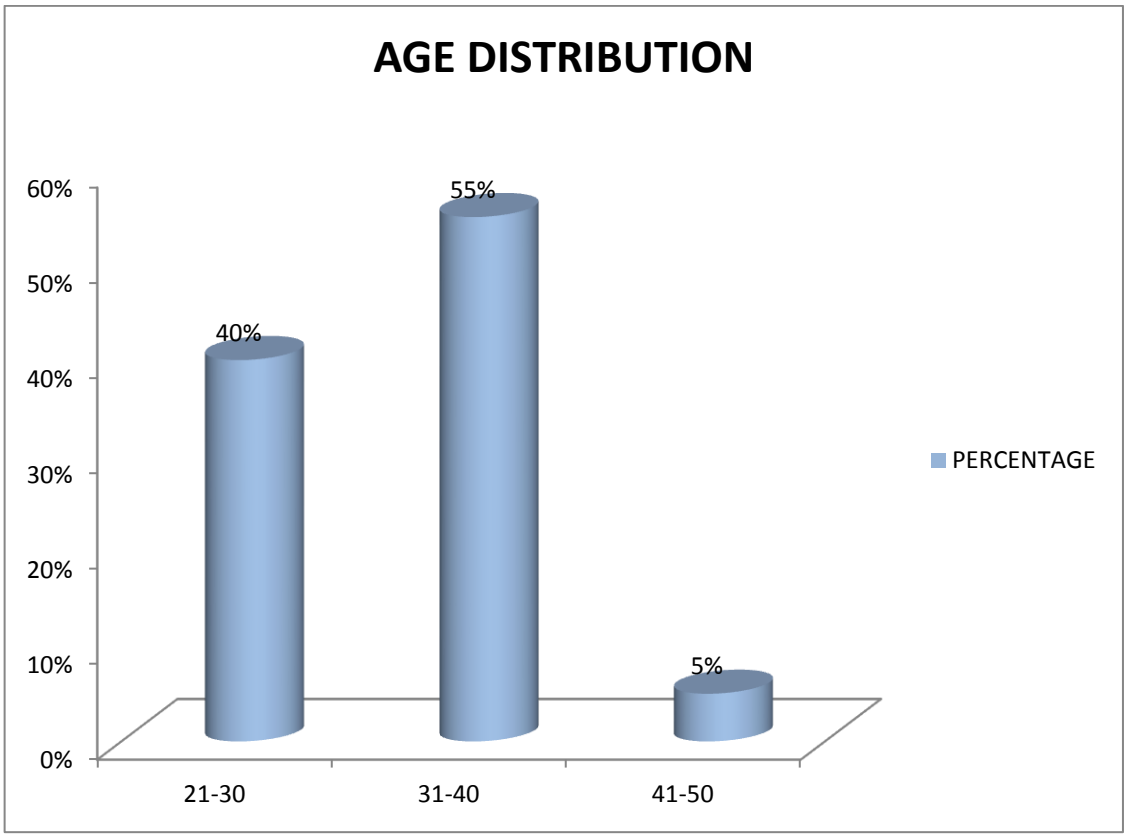
RESULTS AND OBSERVATION

The study on Aan maladu was carried out in 20 patients in the Department of Pothu Maruthuvam, Government Siddha Medical College, Chennai-106 attached to Arignar Anna Hospital during 2015-2017 were analyses. The observation were made and tabulated with following criteria.

- Age Distribution
- Kaalam
- Thinai
- Occupational status
- Socio economic status
- Food habits
- Personal habits
- Symptoms
- Mukkutram- Vaatham, Pitham, Iyyam
- Ezhu Udal Kattugal
- Envagai Thervugal
- Naadi
- Neikuri
- Clinical Progress
- Grading of Results

AGE DISTRIBUTION:

Sl.No	Age	No. of Patients / 20	Percentage
1.	21-30	8	40%
2.	31-40	11	55%
3.	41-50	1	5%

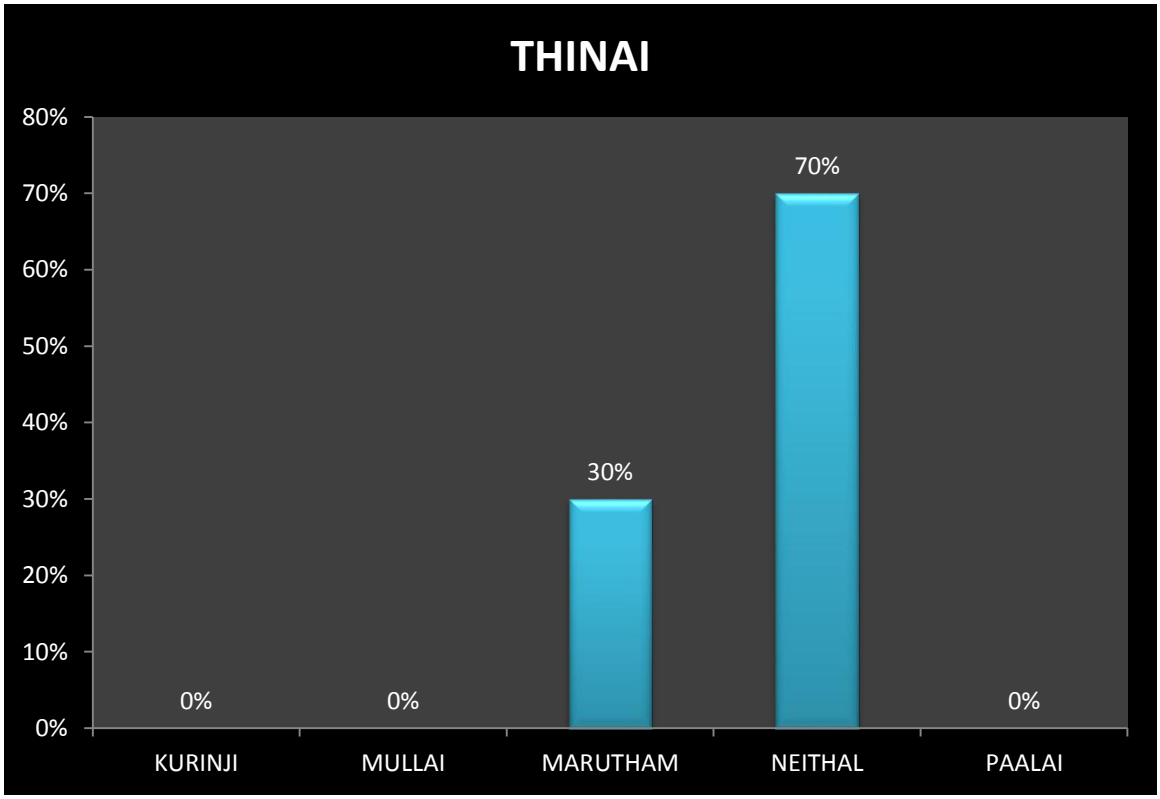


Inference:

According to the above mentioned data 40% of patients were in age group 21-30 years, 55% of patients were in age group 31-40 years and 5% of patients were in age group 41-50 years.

THINAI:

Sl. No	Thinai	No. of Patients/20	Percentage
1	Kurinji	0	0%
2	Mullai	0	0%
3	Marutham	6	30%
4	Neithal	14	70%
5	Paalai	0	0%

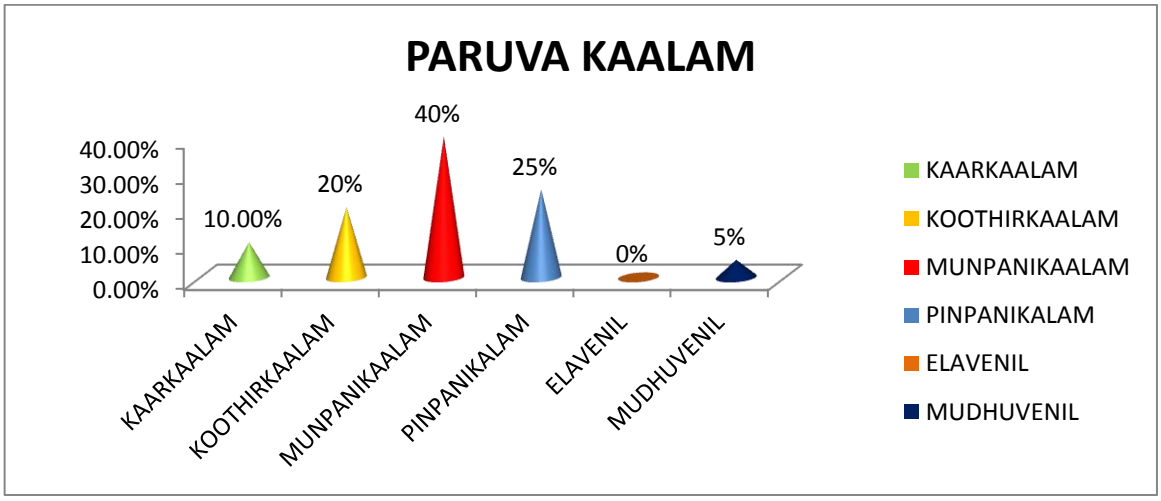


Inference:

According to the above mentioned data 70% of patients were from Neithal, and 30% of patients were from Marutham.

PARUVA KAALAM:

Sl. No	Paruva Kaalam	Months	No. Of Patients/20	Percentage
1.	Kaar kaalam	Avani, puratasi Mid Aug- Mid Oct	2	10%
2.	Koothir kaalam	Iyppasi, Kaarthigai Mid Oct- Mid Dec	4	20%
3.	Munpanikaalam	Margazhi, Thai Mid Dec- Mid Feb	8	40%
4.	Pinpani kaalam	Maasi, Panguni Mid Feb- Mid April	5	25%
5.	Elavenil kaalam	Chithirai, Vaigasi Mid April- Mid June	0	0%
6.	Mudhuvenil kaalam	Aani, Aadi Mid June- Mid Aug	1	5%

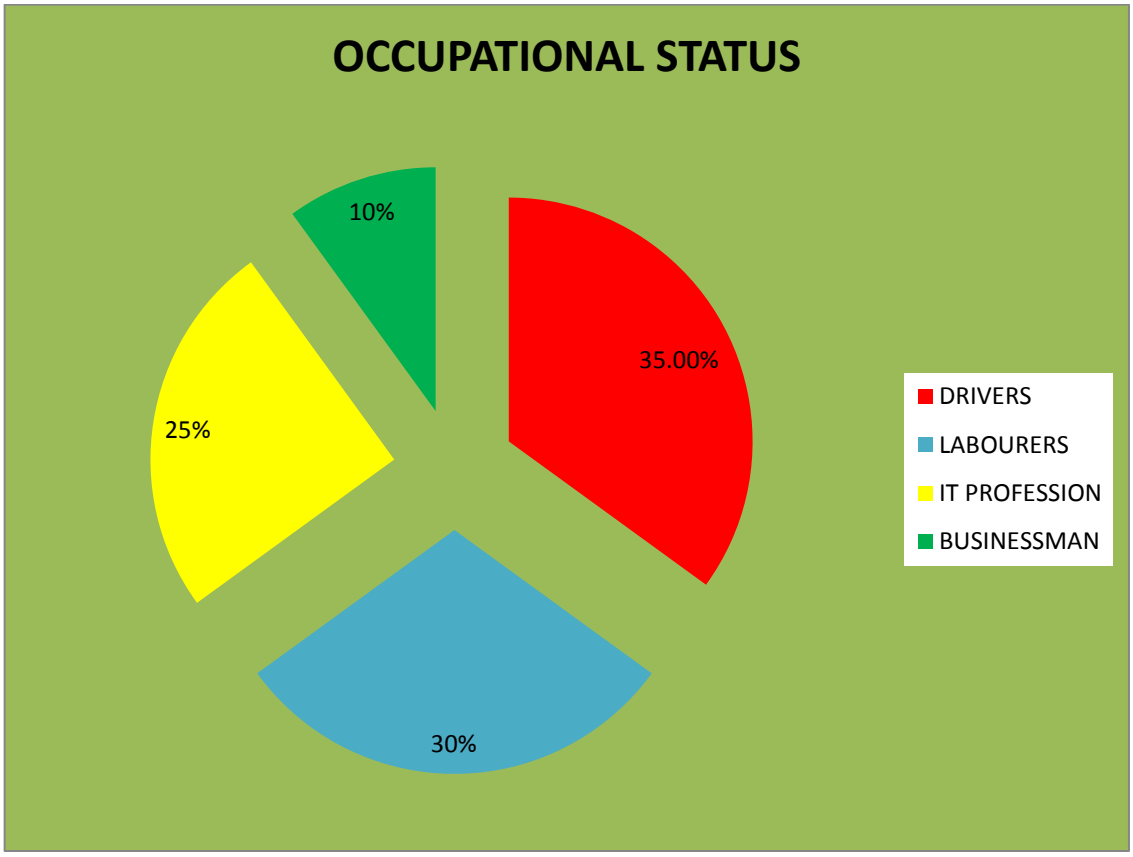


Inference:

40% of cases came in Munpani kaalam, 25% of cases in Pinpani kaalam, 20% of cases came in Koothir kaalam, 10% of cases came in Kaarkaalam and 5% cases in Mudhuvenil kaalam

OCCUPATIONAL STATUS:

Sl. No	Occupational Status	No. of Patients/20	Percentage
1.	Drivers	7	35%
2.	Labourers	6	30%
3.	IT Profession	5	25%
4.	Businessman	2	10%

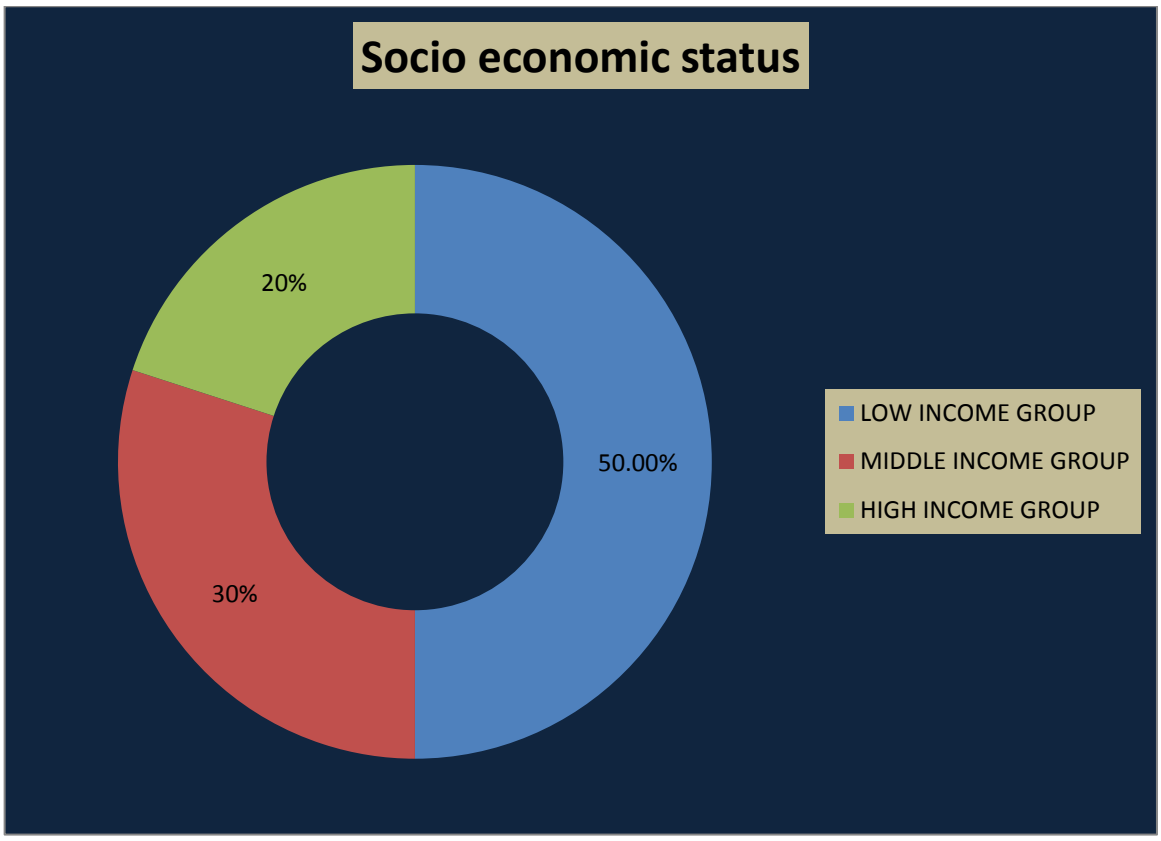


Inference:

35% of cases were Drivers, 30% of cases were Labourers, 25% of cases were IT Profession and 10% of cases were Businessman.

SOCIO ECONOMIC STATUS:

Sl. No	Socio Economic Status	No. of Patients/20	Percentage
1.	Low income group (upto 2,00,000/ annum)	10	50%
2.	Middle income group (2,00,000- 5,00,000/ annum)	6	30%
3.	High income group (above 5,00,000/ annum)	4	20%

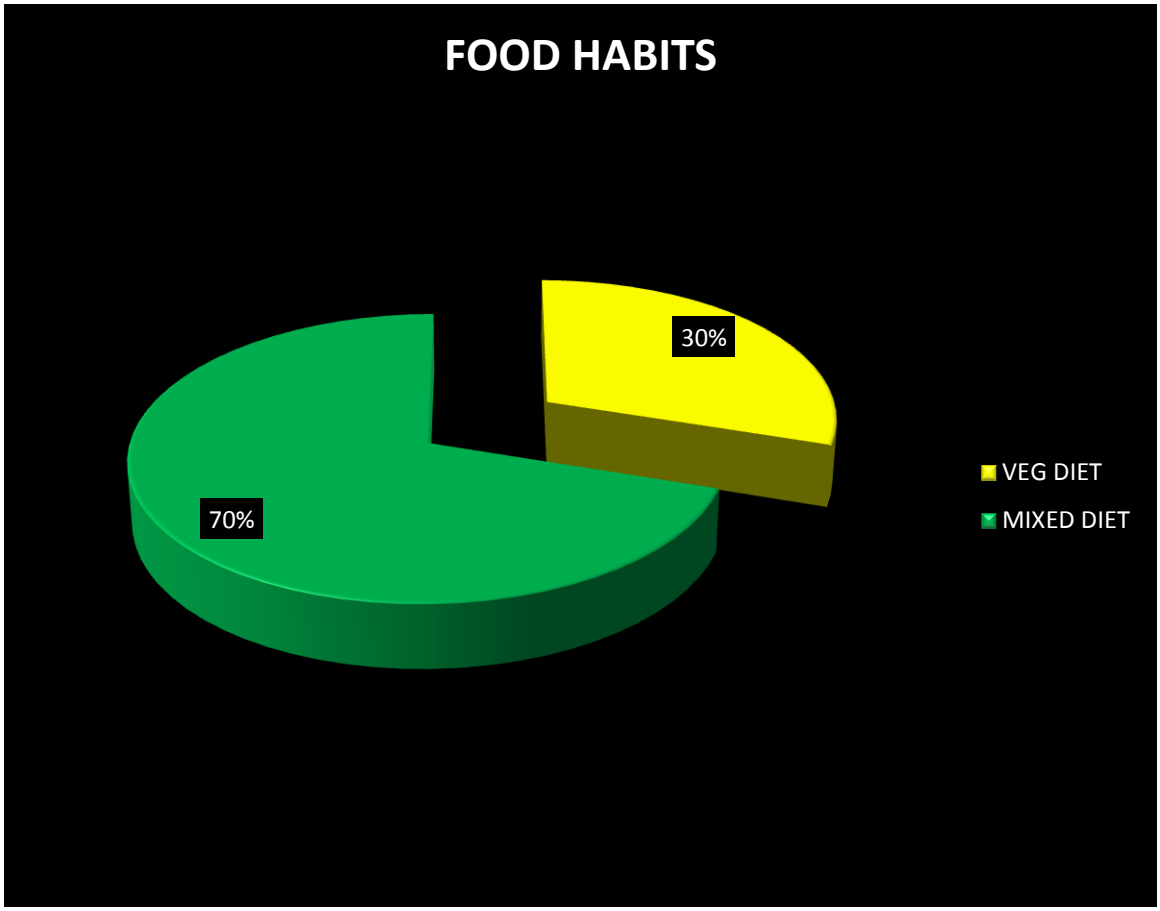


Inference:

50% of patients belong to low income group, 30% of patients belongs to middle income group and 20% of patients belongs to high income group.

FOOD HABITS:

Sl. No	Food Habits	No. of Patients/20	Percentage
1.	**Vegetarian Diet**	**6**	**30%**
2.	**Mixed Diet (including non-veg)**	**14**	**70%**



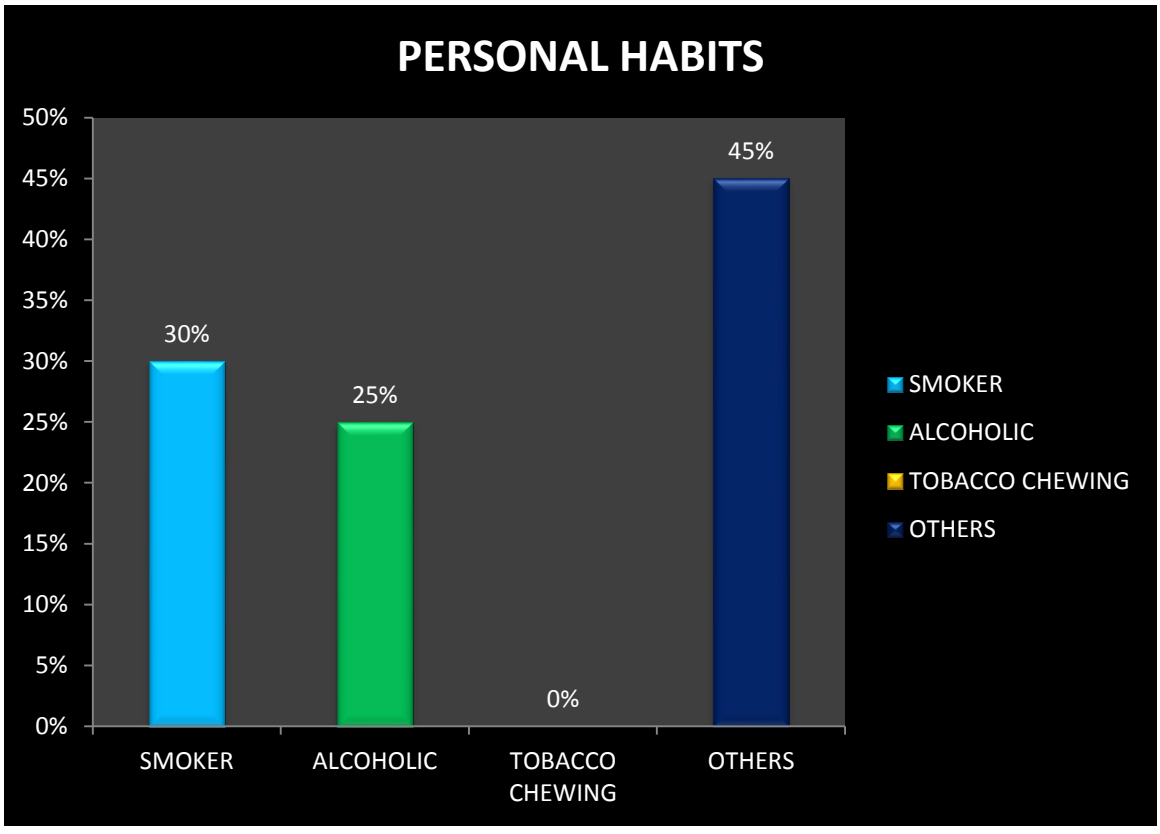
Inference:

70% of patients were mixed diet (including non-vegetarian)

30% of patients were vegetarian.

PERSONAL HABITS:

Sl. No	Personal Habits	No. of Patients/20	Percentage
1.	Smoker	6	**30%**
2.	Alcoholic	5	**25%**
3.	Tobacco chewing	0	**0%**
4.	No other habits	9	**45%**



Inference:

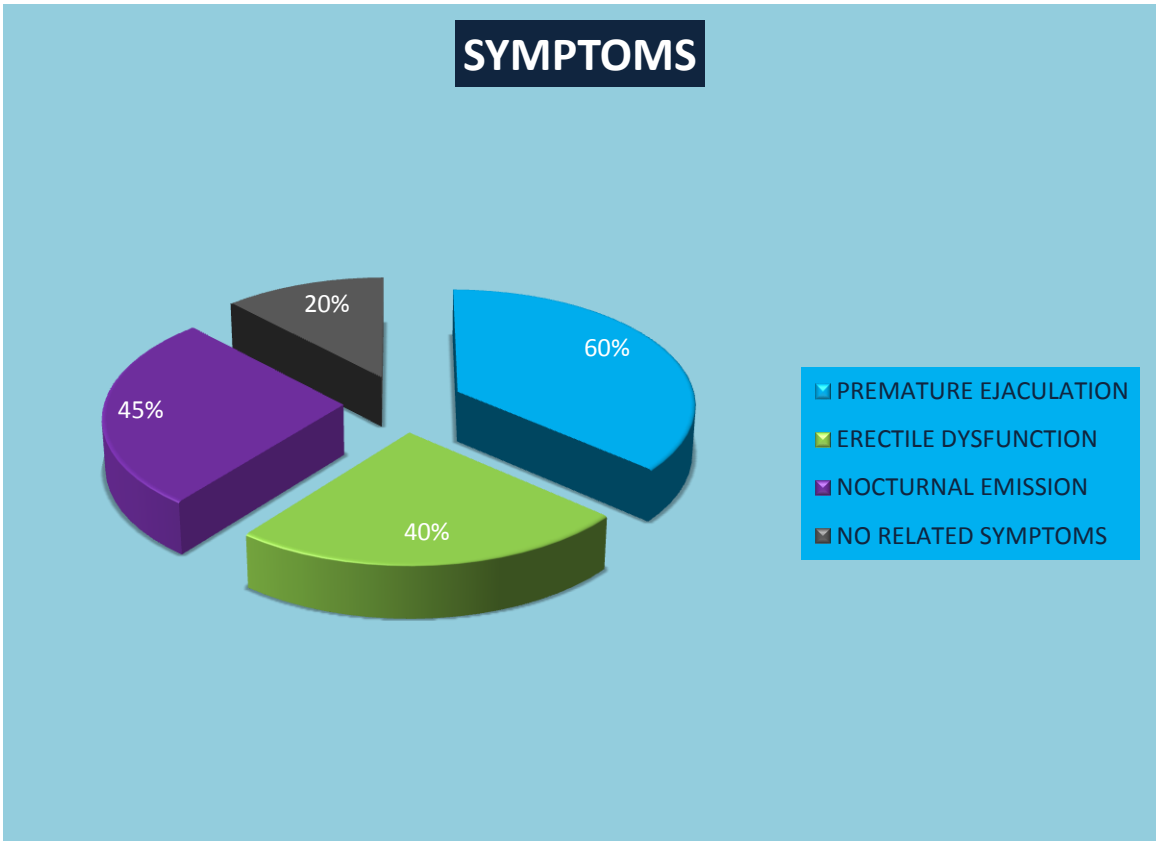
30% of cases were smokers

25% of cases were alcoholic

And 45% of cases were having No other above habits.

SYMPTOMS:

Sl. No	Symptoms	No. of Patients/20	Percentage
1.	Premature Ejaculation	12	60%
2.	Erectile Dysfunction	8	40%
3.	Nocturnal Emission	9	45%
4.	No related Symptoms	4	20%

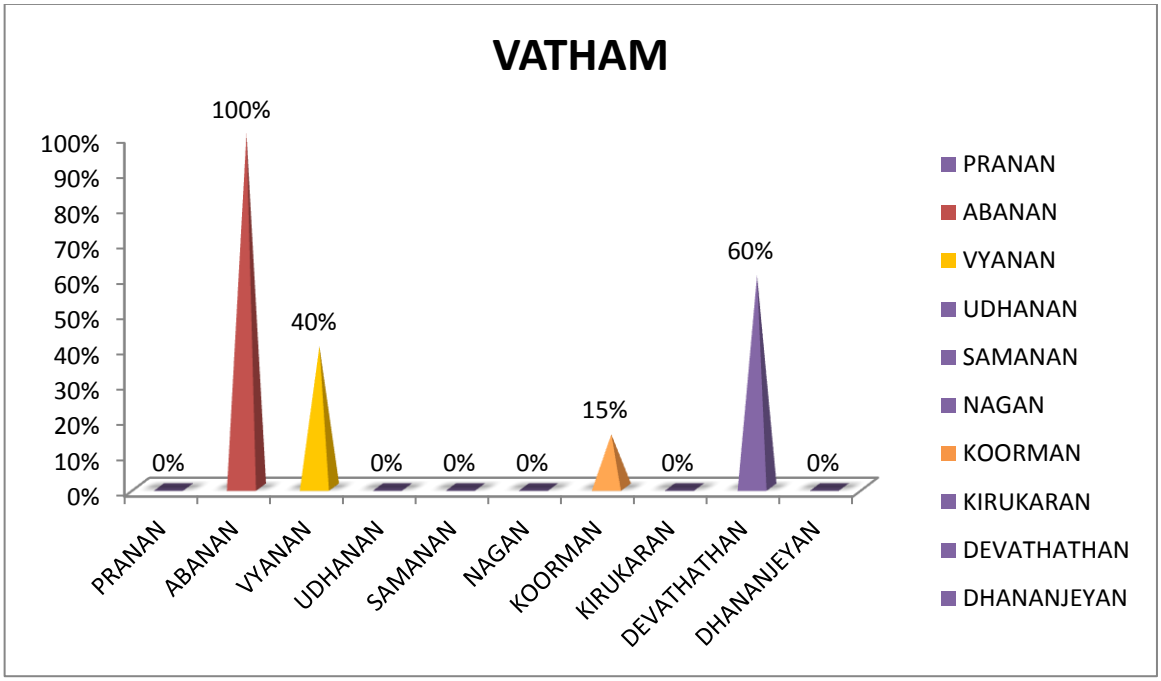


Inference:

60% of cases came with complaints of Premature Ejaculation, 40% of cases came with complaints of Erectile Dysfunction, and 45% of cases came with complaints of Nocturnal Emission 20% of cases had No related Symptoms

VAATHAM:

Sl. No	Vaatham	No. of Patients/20	Percentage
1.	Pranan	0	0%
2.	Abanan	20	100%
3.	Vyanan	8	40%
4.	Udhanan	0	0%
5.	Samanan	0	0%
6.	Nagan	0	0%
7.	Koorman	3	15%
8.	Kirukaran	0	0%
9.	Devathathan	12	60%
10.	Dhananjeyan	0	0%

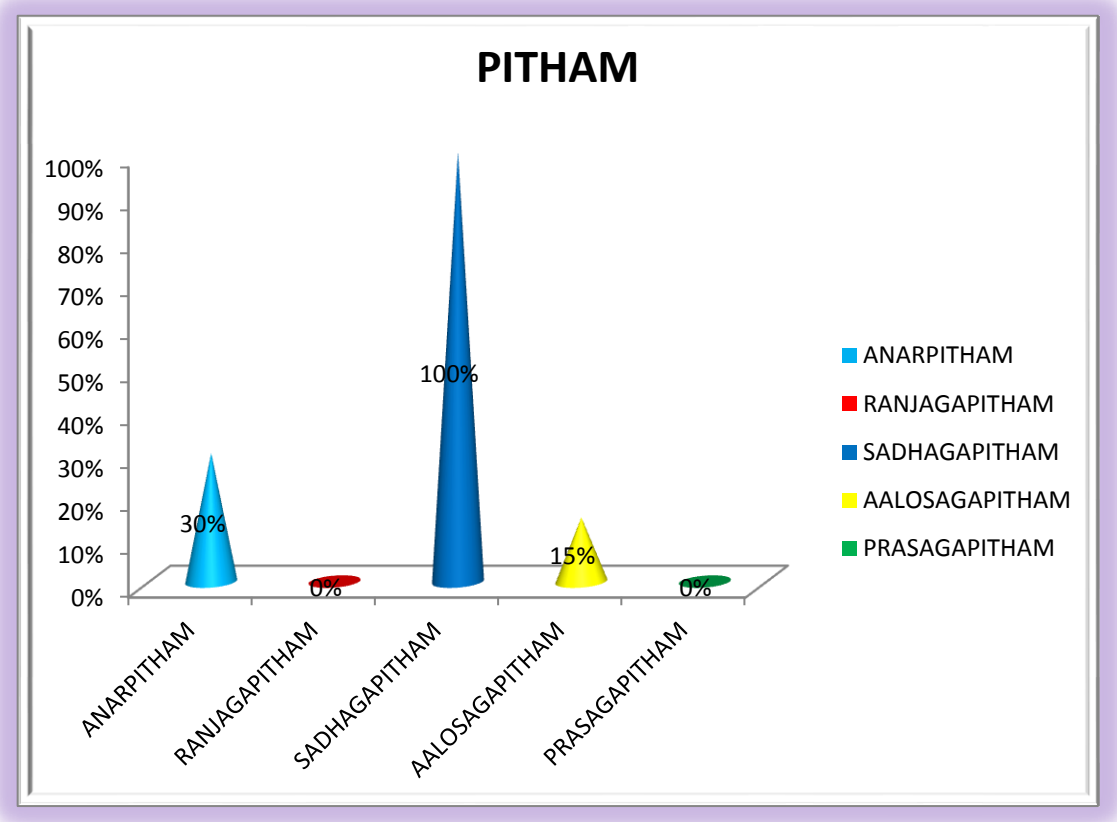


Inference:

Abanan was affected in 100% of patients, koorman was affected in 15% of patients , vyanan was affected in 40% of patients and devathathan was affected in 60% of patients.

PITHAM:

Sl. No	Pitham	No. of Patients/20	Percentage
1.	Anal Pitham	6	30%
2.	Ranjaga Pitham	0	0%
3.	Sadhaga Pitham	20	100%
4.	Aalosaga Pitham	3	15%
5.	Prasaga Pitham	0	0%

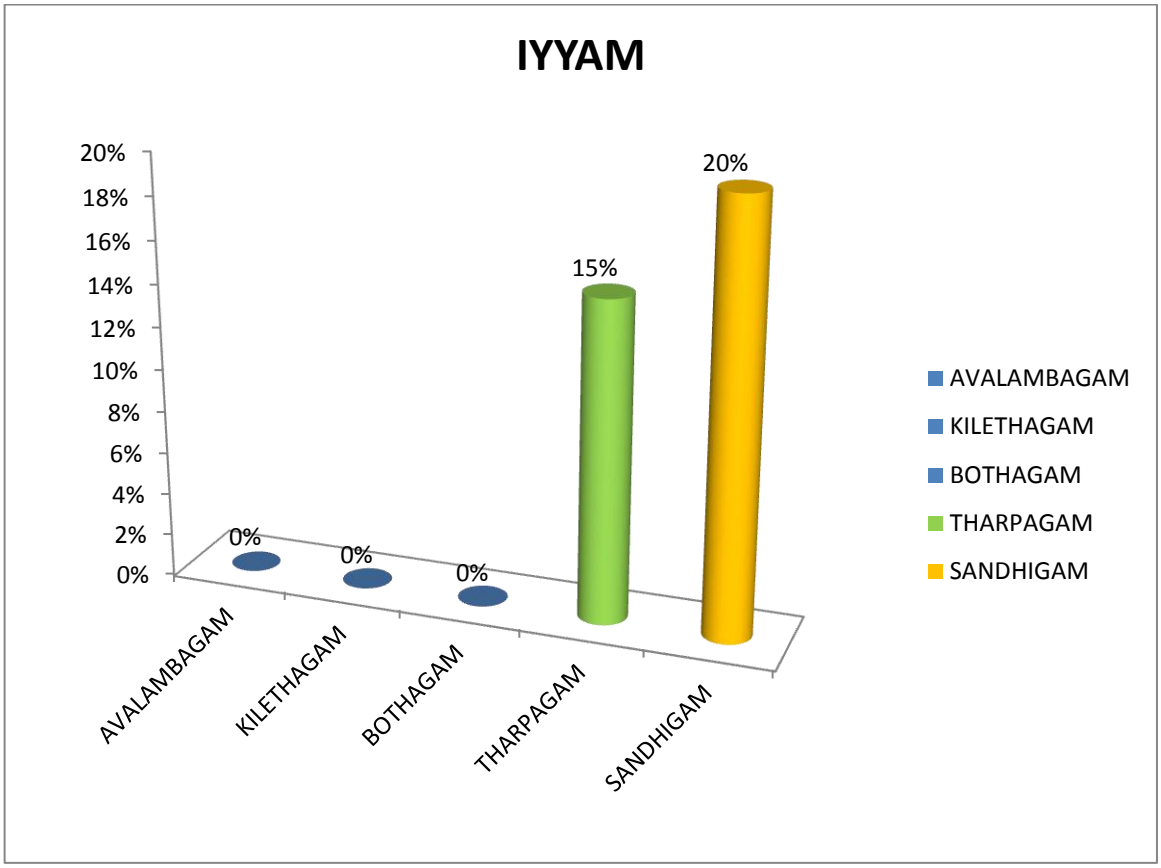


Inference:

Anar pitham was affected in 30% of patients, sadhaga pitham was affected in 100% of patients and Aalosaga Pitham was affected in 15% of patients.

IYYAM:

Sl. No	Iyyam	No. of Patients/20	Percentage
1.	Avalambagam	0	0%
2.	Kilethagam	0	0%
3.	Bothagam	0	0%
4.	Tharpagam	3	15%
5.	Sandhigam	4	20%

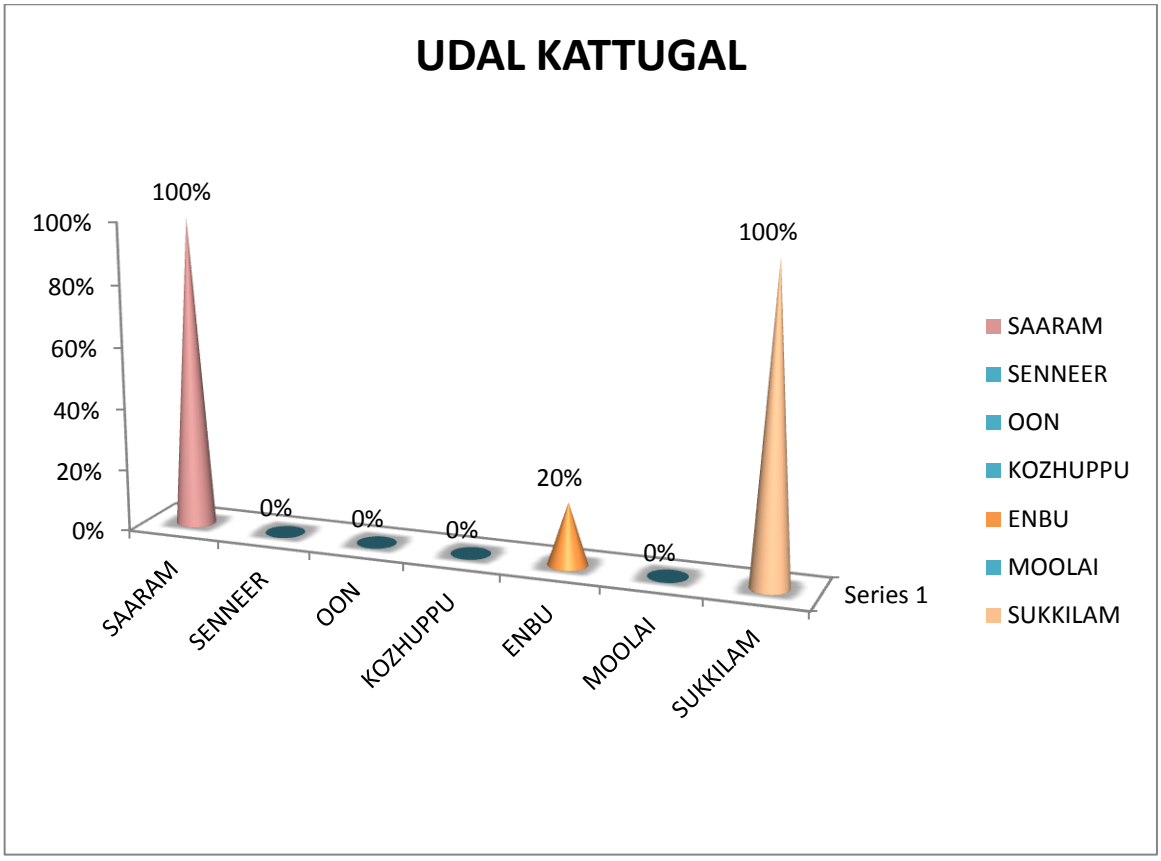


Inference:

Tharpagam was affected in 15% of patients and Sandhigam was affected in 20% of patients.

UDAL KATTUGAL:

Sl. No	Udal Kattugal	No. of Patients/20	Percentage
1.	Saaram	20	100%
2.	Senner	0	0%
3.	Oon	0	0%
4.	Kozhuppu	0	0%
5.	Enbu	4	20%
6.	Moolai	0	0%
7.	Sukkilam	20	100%

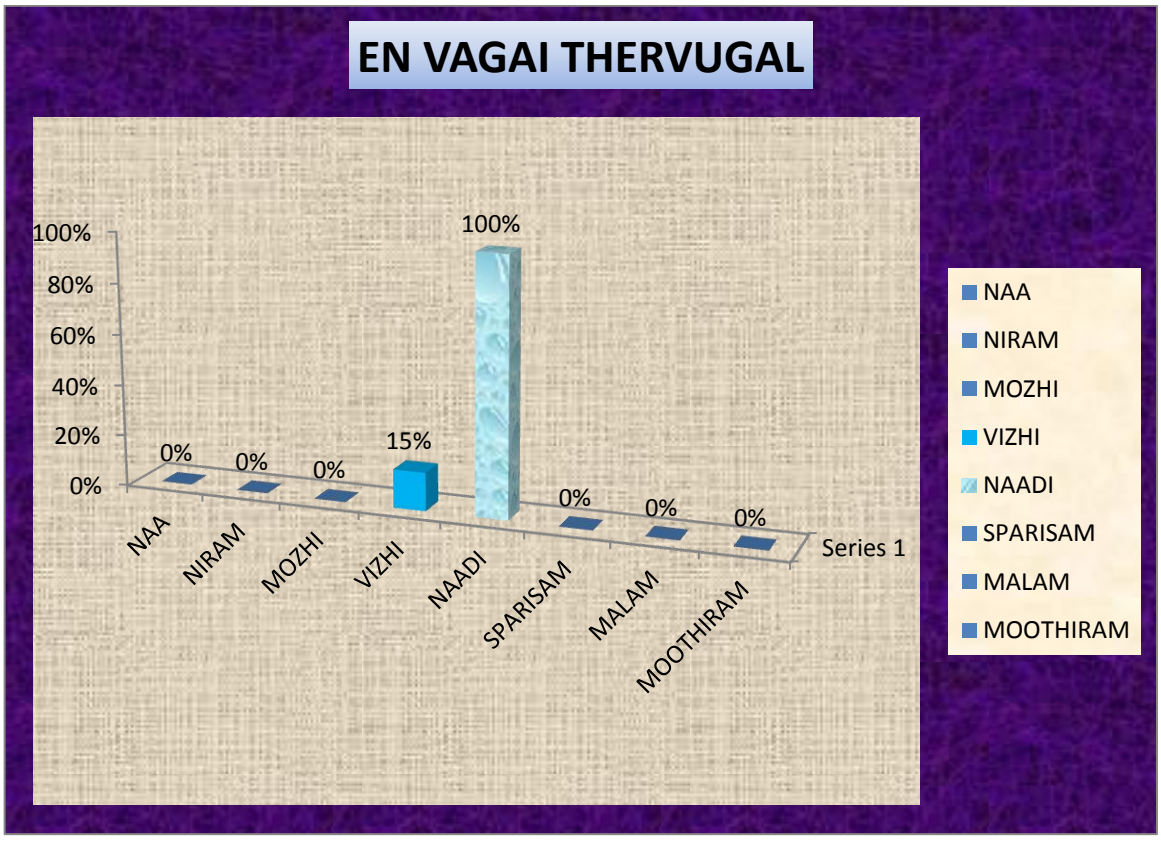


Inference:

Saaram and Sukkilam were affected in 100% of patients and Enbu was affected in 20% of patients.

ENN VAGAI THERVU:

Sl. No	Enn Vagai Thervu	No. of Patients/20	Percentage
1.	Naa	0	0%
2.	Niram	0	0%
3.	Mozhi	0	0%
4.	Vizhi	3	15%
5.	Naadi	20	100%
6.	Sparisam	0	0%
7.	Malam	0	0%
8.	Moothiram	0	0%

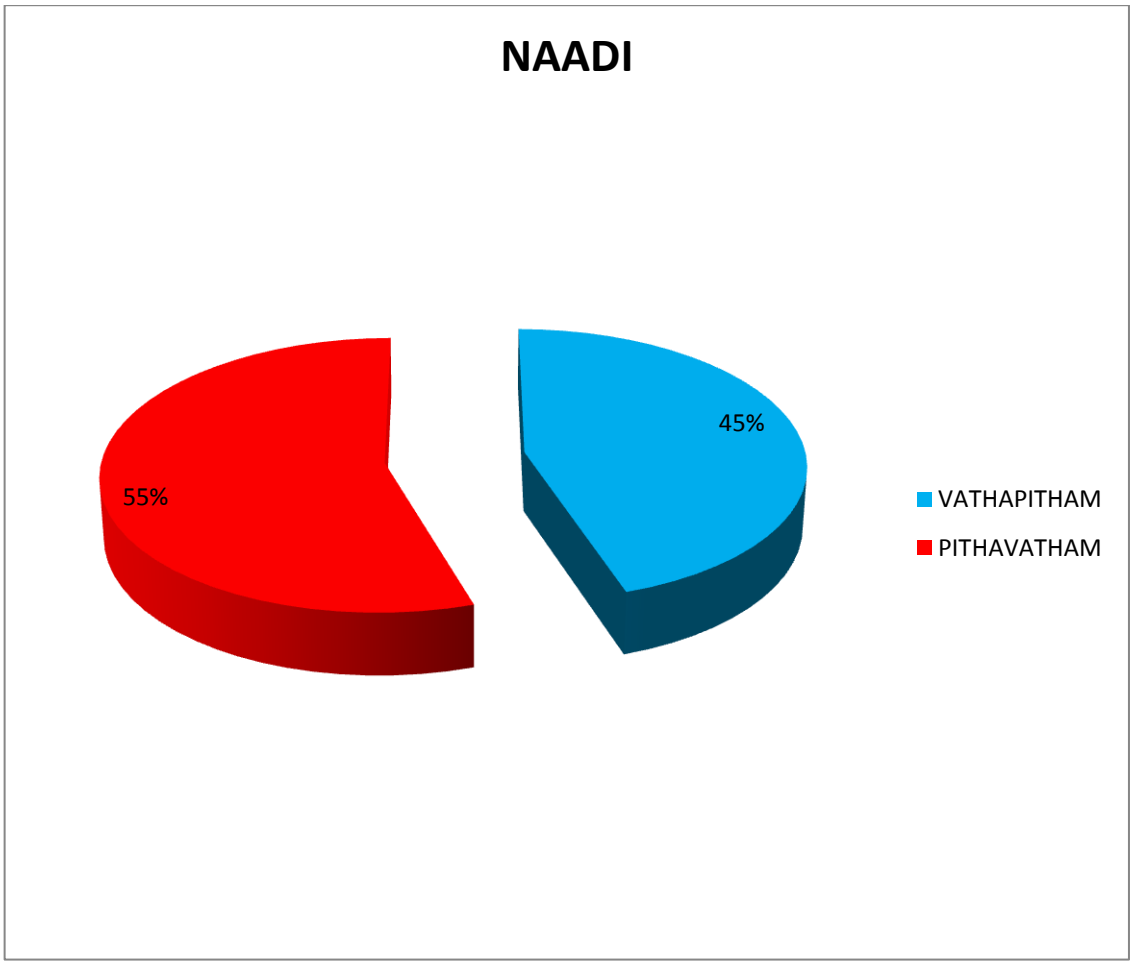


Inference:

Naadi was affected in 100% of patients and Vizhi was affected in 15% of patients.

NAADI:

Sl. No	Naadi	No. of Patients/20	Percentage
1.	Vaathapitham	9	45%
2.	Pithavaatham	11	55%

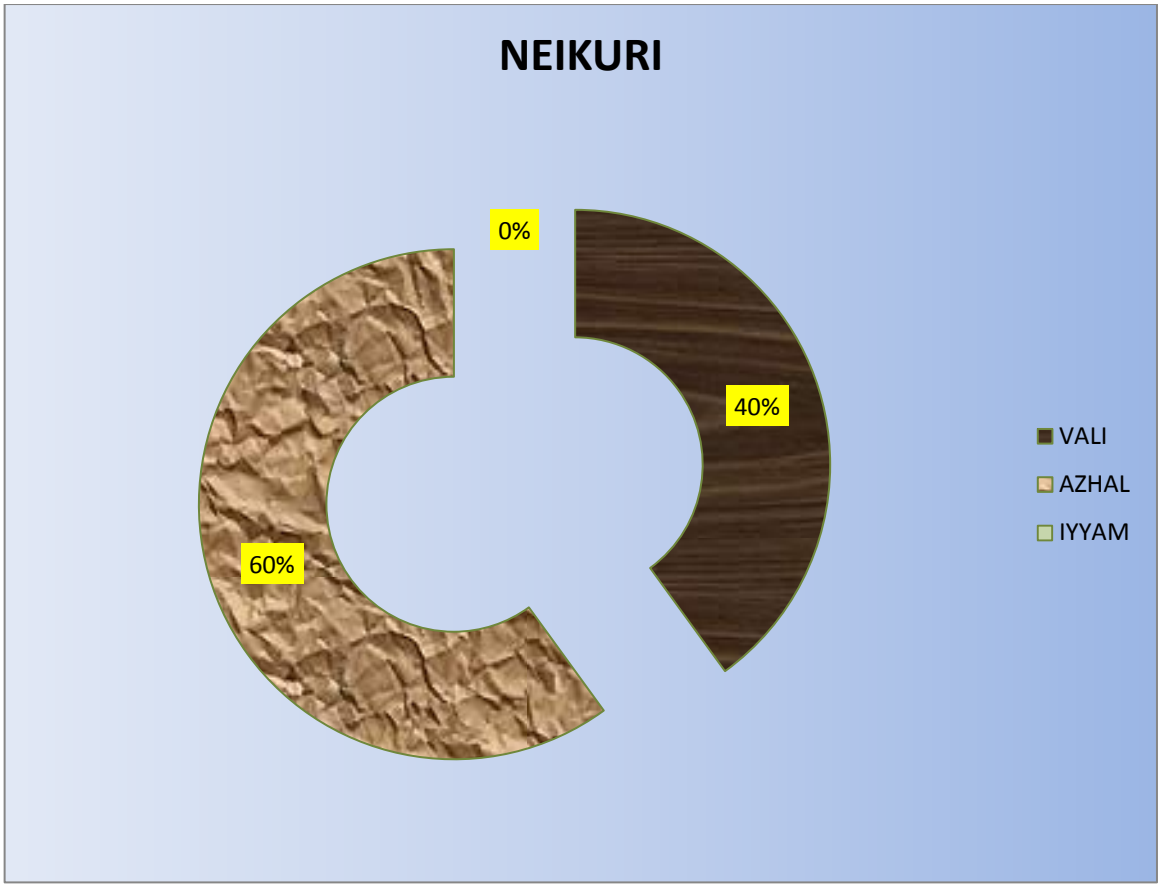


Inference:

45% of patient's Vaathapitham Naadi was felt and 55% of patient's Pithavaatham Naadi was felt.

NEIKKURI:

Sl. No	Neikkuri	No. of Patients /20	Percentage
1.	Vali (spreads like snake)	8	40%
2.	Azhal (spreads like ring)	12	60%
3.	Iyyam (spreads like pearl)	0	0%

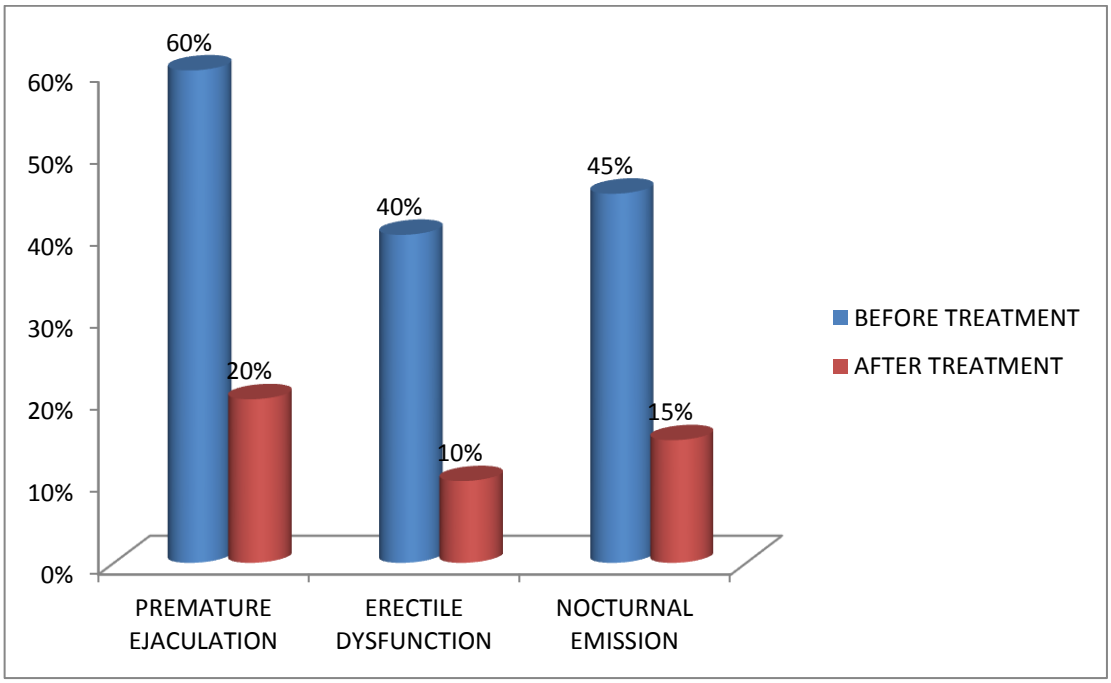


Inference:

40% of cases show Vali Neikkuri and 60% of cases show Azhal Neikkuri

CLINICAL PROGRESS:

Sl. No	Symptoms	No. of Patients/20		Percentage	
		BT	AT	BT	AT
1.	Premature Ejaculation	12	4	60%	20%
2.	Erectile Dysfunction	8	2	40%	10%
3.	Nocturnal Emission	9	3	45%	15%



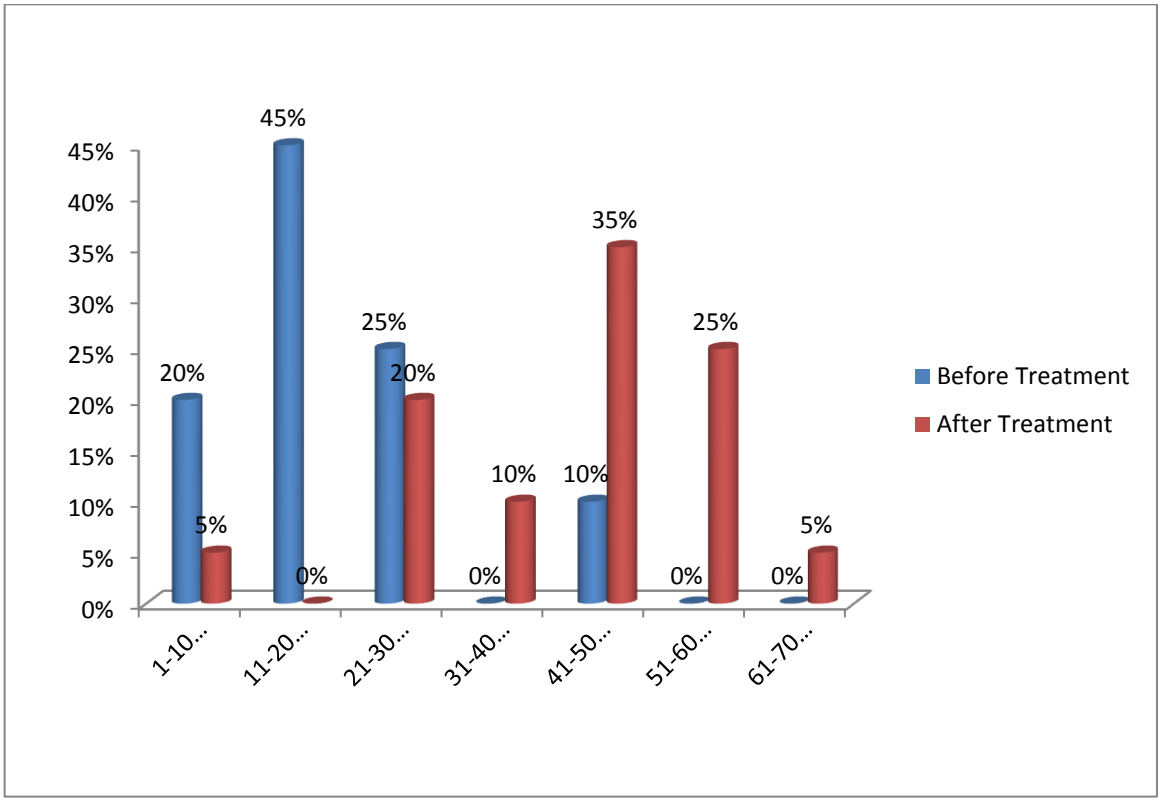
Inference:

Before treatment 60% of cases had Premature Ejaculation, 40% of cases had Erectile Dysfunction and 45% of cases had Nocturnal Emission.

After treatment Premature Ejaculation having 20% of cases, Erectile Dysfunction having 10% cases and Nocturnal Emission having 15% cases .

SEMEN ANALYSIS BEFORE AND AFTER TREATMENT

S.No	Sperm count Million/ cu mm	No. of patients / 20		Percentage	
		BT	AT	BT	AT
1.	1-10 million/cumm	4	1	20%	5%
2.	11-20 million/cumm	9	0	45%	0%
3.	21-30 million/cumm	5	4	25%	20%
4.	31-40 million/cumm	NIL	2	NIL	10%
5.	41-50 million/cumm	2	7	10%	35%
6.	51-60 million/cumm	NIL	5	NIL	25%
7.	61-70 million/cumm	NIL	1	NIL	5%



Inference:

Before treatment:

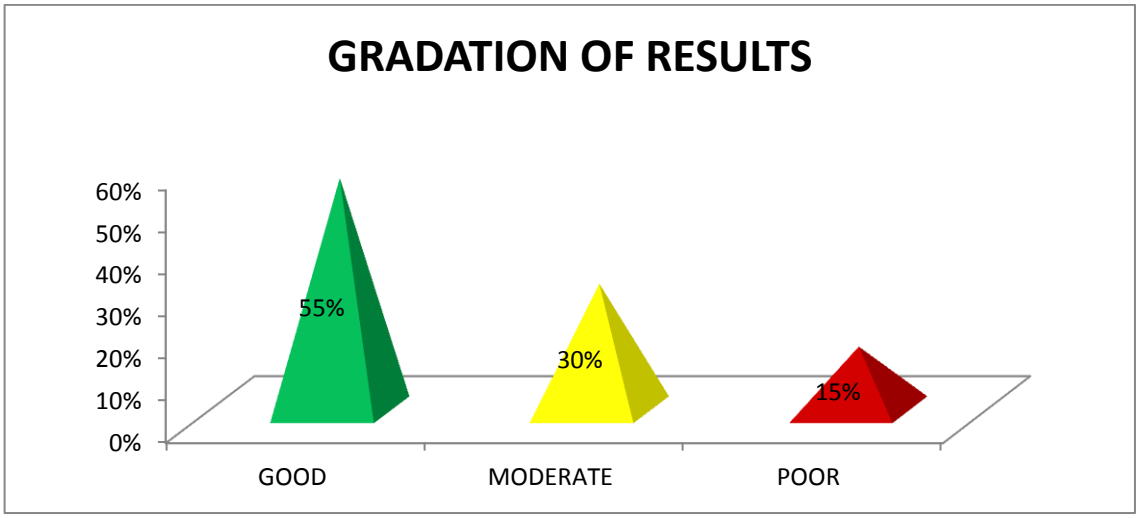
20% of cases sperm count had 1 -10 million/cumm, 45% of cases sperm count had 11 – 20 million/cumm, 25 % of cases sperm count had 21 – 30 million/cumm, 10% of cases sperm count had 41-50 million/cumm.

After treatment:

5% of cases sperm count had 1-10 million/cumm, 20% of cases sperm count had 21 - 30 million/cumm, 10% of cases sperm count had 31-40 million/cumm, 35% of cases sperm count had 41-50 million/cumm, 25% of cases sperm count had 51- 60 million/cumm, 5% of cases sperm count had 61-70 million/cumm.

GRADATION OF RESULTS

S.No.	Results	No.of.patients / 20	Percentage
1	Good	11	55%
2	Moderate	6	30%
3	Poor	3	15%



Inference :

55% of patients show good result, 30% of patients show moderate result and 15% of patients show poor result.

BEFORE TREATMENT

S.No.	OP.No.	Age	HEMATOLOGICAL REPORT							URINE ANALYSIS			STOOL EXAMINATION	
			Tc Cu/mm	Dc			ESR		Hb (Gm.)	Alb	Sug	Dep	Ova	Cyst
				P	L	E	1/2hr	1 hr.						
1.	8921	35	9400	58	39	3	5	12	12.4	Nil	Nil	OPC	Nil	Nil
2.	8894	38	9800	55	42	3	5	22	12.2	Nil	Nil	OPC	Nil	Nil
3.	1978	29	9600	58	36	6	7	13	12.4	Nil	Nil	OPC	Nil	Nil
4.	601	28	9000	60	32	8	6	12	12.6	Nil	Nil	OPC	Nil	Nil
5.	3458	34	9700	54	42	4	10	22	14	Nil	Nil	OPC	Nil	Nil
6.	8566	37	10700	66	30	4	2	3	14	Nil	Nil	OPC	Nil	Nil
7.	3805	35	9300	57	38	5	10	20	13.8	Nil	Nil	OPC	Nil	Nil
8.	4567	27	9400	56	39	5	12	24	13	Nil	Nil	FPC	Nil	Nil
9.	7467	30	9400	58	39	3	10	22	12.4	Nil	Nil	OEC	Nil	Nil
10.	9485	40	9400	58	36	6	2	5	13	Nil	Nil	OPC	Nil	Nil
11.	4564	38	9600	55	42	3	2	5	14	Nil	Nil	Nil	Nil	Nil
12.	8740	30	9600	59	37	4	7	12	13	Nil	Nil	OPC	Nil	Nil
13.	3806	30	9000	59	35	6	6	10	14.6	Nil	Nil	FPC	Nil	Nil
14.	7265	29	10100	59	37	4	15	25	13.2	Nil	Nil	OEC	Nil	Nil
15.	7932	25	9000	58	38	4	2	4	14.8	Nil	Nil	FPC	Nil	Nil
16.	4099	36	9200	56	38	6	5	10	12.4	Nil	Nil	OPC	Nil	Nil
17.	4100	39	10700	62	31	7	15	20	15	Nil	Nil	OPC	Nil	Nil
18.	3007	40	9700	58	37	5	10	18	12.5	Nil	Nil	OPC	Nil	Nil
19.	7386	42	10600	62	34	4	7	17	14	Nil	Nil	OPC	Nil	Nil
20.	6784	35	9400	57	38	5	3	5	12.8	Nil	Nil	Nil	Nil	Nil

AFTER TREATMENT

S.No.	OP.No.	Age	HEMATOLOGICAL REPORT							URINE ANALYSIS			STOOL EXAMINATION	
			Tc Cu/mm	Dc			ESR		Hb (Gm.)	Alb	Sug	Dep	Ova	Cyst
				P	L	E	1/2hr	1 hr.						
1.	8921	35	9450	56	42	2	4	8	12	Nil	Nil	Nil	Nil	Nil
2.	8894	38	9750	54	42	4	4	10	12.5	Nil	Nil	Nil	Nil	Nil
3.	1978	29	9600	60	35	5	13	6	13	Nil	Nil	Nil	Nil	Nil
4.	601	28	8800	62	32	6	5	10	12.7	Nil	Nil	Nil	Nil	Nil
5.	3458	34	9600	58	40	2	8	18	13.8	Nil	Nil	OPC	Nil	Nil
6.	8566	37	10800	65	30	5	4	6	14	Nil	Nil	Nil	Nil	Nil
7.	3805	35	9400	60	35	5	9	18	13.7	Nil	Nil	FPC	Nil	Nil
8.	4567	27	9600	58	36	6	24	10	13	Nil	Nil	Nil	Nil	Nil
9.	7467	30	9000	60	38	2	9	18	12.5	Nil	Nil	Nil	Nil	Nil
10.	9485	40	9200	60	34	6	4	10	12.8	Nil	Nil	OPC	Nil	Nil
11.	4564	38	9700	58	40	2	3	6	14.2	Nil	Nil	Nil	Nil	Nil
12.	8740	30	9550	60	35	5	10	14	12.8	Nil	Nil	Nil	Nil	Nil
13.	3806	30	9100	58	37	5	5	12	14.5	Nil	Nil	Nil	Nil	Nil
14.	7265	29	10300	60	36	4	10	13	13	Nil	Nil	Nil	Nil	Nil
15.	7932	25	9200	58	38	4	3	6	15	Nil	Nil	Nil	Nil	Nil
16.	4099	36	9350	55	42	3	8	16	12.7	Nil	Nil	Nil	Nil	Nil
17.	4100	39	10500	60	34	6	12	18	14.5	Nil	Nil	Nil	Nil	Nil
18.	3007	40	9700	58	38	4	15	20	12.4	Nil	Nil	FPC	Nil	Nil
19.	7386	42	10450	55	40	5	8	16	14.5	Nil	Nil	OPC	Nil	Nil
20.	6784	35	9600	60	36	4	3	8	13.1	Nil	Nil	Nil	Nil	Nil

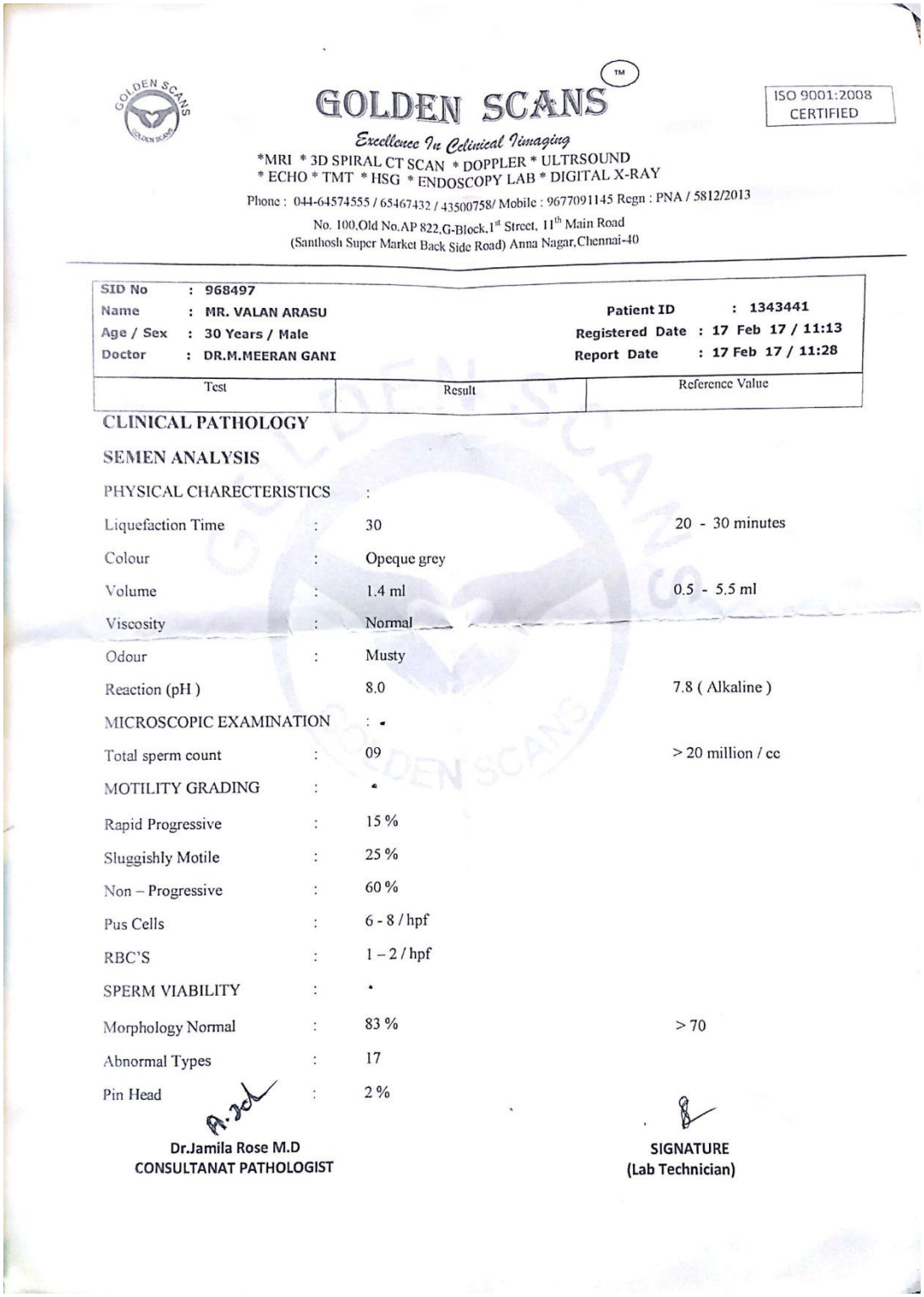
TC- Total Count, DC- Differential Count, P- Polymorph, L- Lymphocytes, E- Eosinophil, HB- Haemoglobin, ESR- Erythrocytes Sedimentation Rate, Alb- Albumin, Sug- Sugar, Dep- Deposits, OPC- Occasional Pus Cells, OEC- Occasional Epithelial Cells, FPC- Few Pus Cells

NO. OF OP PATIENTS BEFORE TREATMENT AND AFTER TREATMENT

SL. NO.	OP.NO.	AGE	SEMEN ANALYSIS BEFORE TREATMENT	SEMEN ANALYSIS AFTER TREATMENT	TOTAL NO. OF DAYS	RESULT
1.	8921	35	TSC – 22million/cu mm AM-18 %	TSC – 50million/cu mm AM- 58%	48 Days	Good Improvement
2.	8894	38	TSC –13 million/cu mm AM- 8%	TSC – 30million/cu mm AM- 32%	48 Days	Moderate improvement
3.	1978	29	TSC – 25million/cu mm AM-18 %	TSC – 58 million/cu mm AM-56 %	48 Days	Good Improvement
4.	601	28	TSC –12 million/cu mm AM-24 %	TSC –55 million/cu mm AM- 60%	48 Days	Good Improvement
5.	3458	34	TSC –22 million/cu mm AM-10 %	TSC – 24million/cu mm AM-22 %	48 Days	Poor Improvement
6.	8566	37	TSC –19 million/cu mm AM- 20%	TSC – 60million/cu mm AM- 55%	48 Days	Good Improvement
7.	3805	35	TSC –42 million/cu mm AM-10 %	TSC – 60million/cu mm AM- 35%	48 Days	Moderate Improvement
8.	4567	27	TSC –15 million/cu mm AM- 18%	TSC – 48million/cu mm AM- 65%	48 Days	Good Improvement
9.	7467	30	TSC – 21million/cu mm AM- 8%	TSC –40 million/cu mm AM- 40%	48 Days	Moderate Improvement
10.	9485	40	TSC –45 million/cu mm AM-15 %	TSC –48 million/cu mm AM-25 %	48 Days	Poor Improvement

SL. NO.	OP.NO.	AGE	SEMEN ANALYSIS BEFORE TREATMENT	SEMEN ANALYSIS AFTER TREATMENT	TOTAL NO. OF DAYS	RESULT
11.	4564	38	TSC – 18million/cu mm AM-10 %	TSC – 50million/cu mm AM- 55%	48 Days	Good Improvement
12.	8740	30	TSC –9 million/cu mm AM- 15%**	TSC – 52million/cu mm AM- 55%	48 Days	Good Improvement
13.	3806	30	TSC – 16million/cu mm AM-12 %	TSC – 48 million/cu mm AM-53 %	48 Days	Good Improvement
14.	7265	29	TSC –15 million/cu mm AM-15 %	TSC –45 million/cu mm AM- 58%	48 Days	Good Improvement
15.	7932	25	TSC –8 million/cu mm AM-22 %**	TSC – 50million/cu mm AM-62 %	48 Days	Good Improvement
16.	4099	36	TSC –14 million/cu mm AM- 15%	TSC – 67million/cu mm AM- 60%	48 Days	Good Improvement
17.	4100	39	TSC –10 million/cu mm AM-13 %	TSC – 25million/cu mm AM- 55%	48 Days	Moderate Improvement
18.	3007	40	TSC –23 million/cu mm AM- 15%	TSC – 38million/cu mm AM- 45%	48 Days	Moderate Improvement
19.	7386	42	TSC – 3million/cu mm AM- 7%	TSC –5 million/cu mm AM- 15%	48 Days	Poor Improvement
20.	6784	35	TSC –15 million/cu mm AM-17 %	TSC –30 million/cu mm AM-40 %	48 Days	Moderate Improvement

BEFORE TREATMENT



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No. 100,Old No.AP 822,G-Block,1st Street, 11th Main Road
(Santhosh Super Market Back Side Road) Anna Nagar,Chennai-40

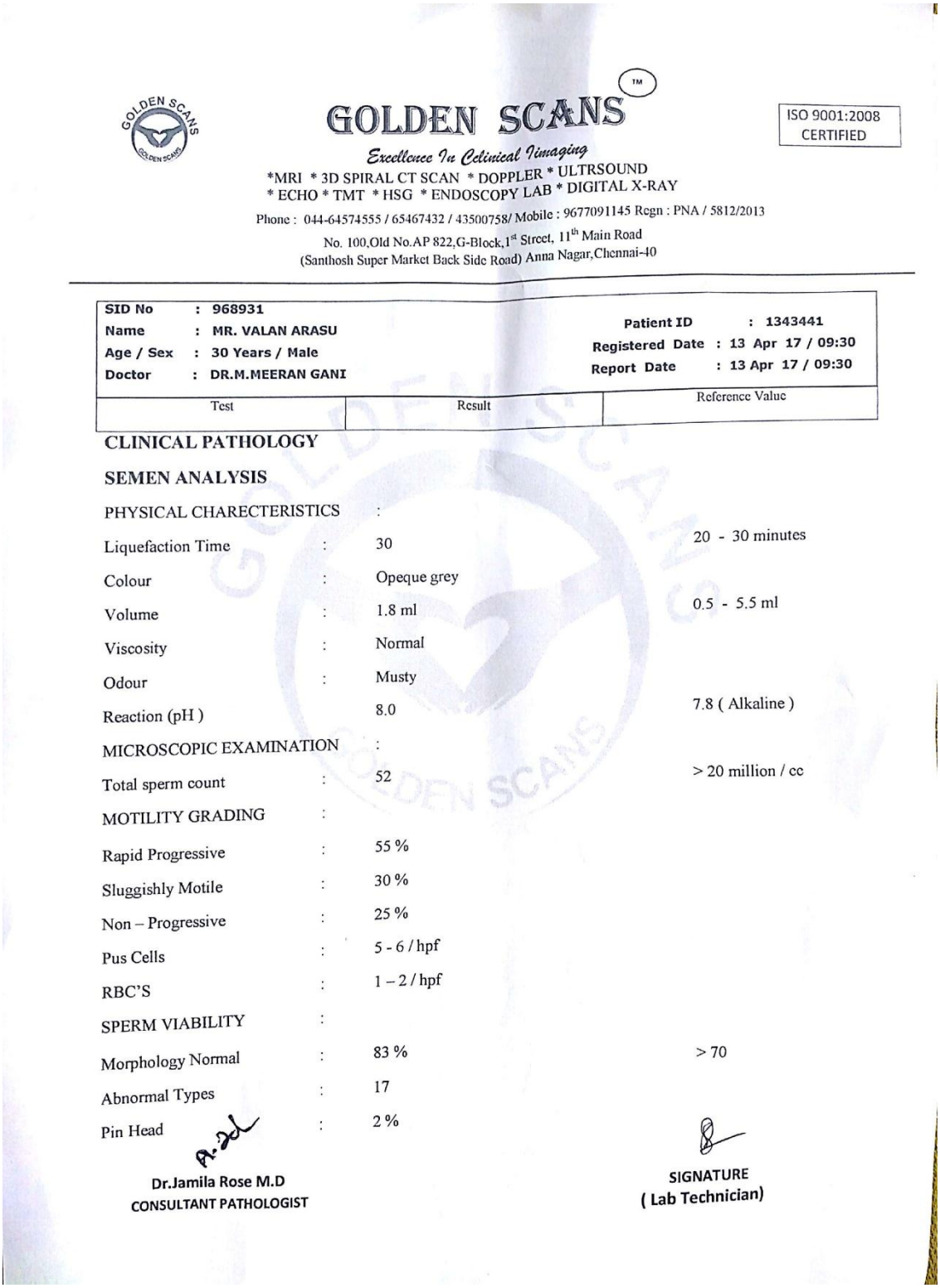
SID No	: 968497		
Name	: MR. VALAN ARASU	Patient ID	: 1343441
Age / Sex	: 30 Years / Male	Registered Date	: 17 Feb 17 / 11:13
Doctor	: DR.M.MEERAN GANI	Report Date	: 17 Feb 17 / 11:28

Test	Result	Reference Value
CLINICAL PATHOLOGY		
SEMEN ANALYSIS		
PHYSICAL CHARECTERISTICS :		
Liquefaction Time :	30	20 - 30 minutes
Colour :	Opeque grey	
Volume :	1.4 ml	0.5 - 5.5 ml
Viscosity :	Normal	
Odour :	Musty	
Reaction (pH)	8.0	7.8 (Alkaline)
MICROSCOPIC EXAMINATION :	-	
Total sperm count :	09	> 20 million / cc
MOTILITY GRADING :	-	
Rapid Progressive :	15 %	
Sluggishly Motile :	25 %	
Non – Progressive :	60 %	
Pus Cells :	6 - 8 / hpf	
RBC'S :	1 – 2 / hpf	
SPERM VIABILITY :	-	
Morphology Normal :	83 %	> 70
Abnormal Types :	17	
Pin Head :	2 %	

Dr.Jamila Rose M.D
CONSULTANAT PATHOLOGIST

SIGNATURE
(Lab Technician)

AFTER TREATMENT



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No. 100,Old No.AP 822,G-Block,1st Street, 11th Main Road
(Santhosh Super Market Back Side Road) Anna Nagar,Chennai-40

ISO 9001:2008 CERTIFIED

SID No	: **968931**	**Patient ID**	: **1343441**
Name	: **MR. VALAN ARASU**	**Registered Date**	: **13 Apr 17 / 09:30**
Age / Sex	: **30 Years / Male**	**Report Date**	: **13 Apr 17 / 09:30**
Doctor	: **DR.M.MEERAN GANI**		

Test	Result	Reference Value
CLINICAL PATHOLOGY		
SEMEN ANALYSIS		
PHYSICAL CHARECTERISTICS :		
Liquefaction Time :	30	20 - 30 minutes
Colour :	Opeque grey	
Volume :	1.8 ml	0.5 - 5.5 ml
Viscosity :	Normal	
Odour :	Musty	
Reaction (pH)	8.0	7.8 (Alkaline)
MICROSCOPIC EXAMINATION :		
Total sperm count :	52	> 20 million / cc
MOTILITY GRADING :		
Rapid Progressive :	55 %	
Sluggishly Motile :	30 %	
Non – Progressive :	25 %	
Pus Cells :	5 - 6 / hpf	
RBC'S :	1 – 2 / hpf	
SPERM VIABILITY :		
Morphology Normal :	83 %	> 70
Abnormal Types :	17	
Pin Head :	2 %	

Dr.Jamila Rose M.D
CONSULTANT PATHOLOGIST

SIGNATURE
(Lab Technician)

DISCUSSION

One of the Predominant disorders that endanger human species is **INFERTILITY** in Both men and women. The incidence of infertility is comparatively higher in males became of the drastic changes in human life style – irregular food habits, high calorie food items, fast food behavioral changes. Environmental toxins and changed compounds used for dispensing various ailments. It has been suggested that the average sperm count has been decreasing over the past 50 years.

Aan maladu as stated in Yugi Vaidya Chindhamani has close resemblance with male infertility in modern system.

In my study 20 patients were treated in outpatient department of Post Graduate Pothumaruthuvam Department, Govt. Aringar Anna Hospital attached with Govt. Siddha Medical College, Chennai – 106.

All patients were subjected to preliminary investigations which include hematological, urine examination, Semen Analysis before and after Treatment.

The Trial Medicine *VEERIYA VIRUTHI CHOORANAM* was administered 48 days.

Veeriya viruthi Chooranam was justified for Aan Maladu through various process Drug Authentication, Toxicity study, Pharmacological Activity, Biochemical Analysis, Physico chemical Analysis, , Clinical study and Biostatistical analysis.

DRUG AUTHETICATION:

Herbal drugs like *Mucuna pruriens, Curculigo orchioides, Maerua oblongifolia* were procured from a well reputed indigenous drug shop and authenticated by the Botanist, Dept.of Medicinal Botany, Govt. Siddha Medical College, Arumbakkam, Chennai – 106.

PRE CLINICAL SCREENINGS:

PHSICOCHEMICAL ANALYSIS:

Loss on Drying(at 105^0C) was 2.235%, The total ash value of *veeriya viruthi* chooranam was 6.02%, The water soluble ash value was 1.56% The acid soluble ash value was 1.01%, The water soluble extractive was 19.55%, alcohol soluble extractive was 14.40%, pH value was 5.61%.

TOXICITY STUDY:

IAEC:

I got IAEC approval for toxicological and pharmacological studies at Sathyabama University ,Chennai. IAEC NO: SU/CLATR/IEAC/VII/048/2016

ACUTE TOXICITY:

Acute and sub acute toxicity studies were conducted on experimental rats at Sathyabama University, Chennai, Tamilnadu.

Acute toxicity study of the drug *Veeriya Viruthi Chooranam* with cow's milk was carried out as the OECD guideline - 423 (Organisation for Economic Co-operation and Development).

The acute toxicity study of *Veeriya viruthi Chooranam* was studied and the drug was proved safer for long term administration, as it did not exhibit any significant toxicity at 2000 mg / kg body weight.

SUB ACUTE TOXICITY:

Sub acute toxicity study as per the OECD guideline of – 407. Under the dosage of trial drug 200mg / kg (Low dose), 400mg / kg (High dose) it did not exhibit any significant.

HISTO PATHOLOGY:

At the end of toxicity studies the animal were sacrificed and they were subjected to hematological parameters (TC, DC & Hb) chemical parameters (LFT, RFT) and histopathology of vital organs like Liver, Kidney, Spleen, Lungs were carried out. The studied did not exhibit the evidence of remarkable pathological lesions in the tissues.

PHARMACOLOGICAL ACTIVITY:

The spermatogenic activity of *Veeriya viruthi Chooranam* was carried out in wistar rats through Gentamicin-Induced testicular toxicity method. Then trial drug was administrated shows a potent spermatogenic activity during the studies. The pharmacology studies of trial medicine *Veeriya viruthi Chooranam* showed significant spermatogenic Activity in wistar rats.

The result of preclinical screening, the result of chemical analysis, Toxicological studies, Pharmacological studies were shown in annexures.

BIOCHEMICAL ANALYSIS:

Veeriya viruthi Chooranam contains iron, sulphide, calcium, Phosphate and reducing sugar.

IEC AND CTRI:

Study Design

The study was approved by Institutional Ethics Committee (IEC) and the approval number is GSMC-CH-ME-4/2015/007.It was registered in Clinical Trials Registry – India (CTRI) and the reference number is CTRI/2017/05/008448

Population and sample :

The population consists of all patients satisfying the inclusion and exclusion criteria mentioned below. Sample consists of 20 AAN MALADU patients who were attending the OPD of Arignar Anna Hospital, Arumbakkam, Chennai – 106.

CLINICAL STUDY:

All the necessary investigation were carried out to all patients and *Veeriya viruthi Chooranam* were given. Weekly once follow up were done. Total duration of treatment ranges from 48 days. All the patients were strictly advised to follow diet restriction and peaceful life style to normalize the immune mechanism.

Age Distribution:

According to this study age distribution was 40% of patients were in age group 21-30 years, 55% of patients were in age group 31-40 years and 5% of patients were in age group 41-50 years.

High incidences of cases were noted in age ranging of 31 – 40 years during the studies

Distribution of Thinai:

According to this study 70% of the Patients came from Neithal because Chennai and surrounding areas come under Neithal thinai, and 30% of patients were from Marutham.

Paruva kalam:

According to this study 40% of cases came in Munpani kaalam, 25% of cases in Pinpani kaalam, 20% of cases came in Koothir kaalam, 10% of cases came in Kaarkaalam and 5% cases in Mudhuvenil kaalam. Seasonal incidence is not affected their disease, male infertility.

Occupational Status:

35%of the patients were Drivers, 30% of patients were working as Labourers, 10% of patients are Businessman, and 25% of patients are IT Profession. Drivers and IT people mostly affected as they are having sleeplessness, stressful and night based work .

Socio Economic Status:

The majority of the Patients affected are from poor socio economic status. Poor hygienic conditions expose to polluted atmosphere and lower immune response made them prone to the disease.

Food Habits:

30% Patients were pure vegetarian, 70% were Mixed Diet (including non-vegetarian). Though a non-vegetarian diet account is not a reason for the occurrence of male infertility.

Personal Habits:

In my study 25% of the Patients were using alcohol, 30%were smoker, and 45% were having others. The observation coincides with the conception that male infertility the disease may be due to smoking, Alcohol consumption.

Classification of Results According To Vali, Azhal and Iyyam:-

Vali:

- Spermatogenesis, Premature Ejaculation, Nocturnal Emission is due to deranged Abana Vayu.
- Erectile dysfunction is due to deranged viyanan.
- In 100% patients abanan was affected, viyanan was affected 40% of patients.
- Koorman affected in 15% of cases.
- Devathathan affected in 60% of cases, Affected Devathathan produced insomnia, tiredness.

Azhal:

Sadhaga Pitham was affected in 100% of Patients, Aalosaga Pitham was affected in 15% of patients and Anal Pitham was affected in 30% of patients.

Affected analagam produced loss of appetite.

All the cases were unable to carryout regular works properly. Sathagam indicates this one. So 100% were affected in Sathaga pitham.

Affected Alosagam produced impairment of eye sight.

Iyyam:

15% of patients Tharpagam were affected. 20 % of Patients santhigam was affected.

Affected Tharpagam produced impairment of eye sight.

Santhigam iyam gives stability, lubrication and movements of joints.

Affected Santhigam produced joint pain.

Udal Kattugal:

Both bodily and mental weakness arises when saaram was affected. In 100% of patients both the saaram and sukkilam were affected. In 20% of cases enbu was affected.

Envagai Thervu

Naadi was affected in 100% of patients and 15% of patients vizhi was affected.

Naadi

In 45% of patients Vaathapitha Naadi was felt and 55% of patients Pithavaatha Naadi were felt.

According to Sathaganadi,

(பொருளான வாதத்தில் பித்தஞ் சேர்ந்து
.....தாதுநட்டம்,)

(சிறப்பான பித்தத்தில் வாத நாடி
சேரிலுறு தாதுநட்ட முதர பீடை……..)

Neikuri

60% of cases show azhal neikuri (spreads like ring) and 40% of cases Show vali neikuri (spreads like snake).

Clinical Progress:

Before treatment 60% of cases had Premature Ejaculation, 40% of cases had Erectile Dysfunction and 45% of cases had Nocturnal Emission. After treatment Premature Ejaculation having 20% of cases, Erectile Dysfunction having 10% cases and Nocturnal Emission having 15% cases .

Trial Medicine:

All the 20 patients treated with the Trial Medicine *VEERIYA VIRUTHI CHOORANAM* with milk, twice a day for 48 days. The disease and treatment are

based primarily on the derangement of Mukkutram, which again is based on the Pancha bootham theory. Incidence of Aan maladu and treatment are also based on these primary principles of Siddha medicine. The bootham raises azhal kuttram in the body and so as lead to general weakness and reduced sperm cell production. Increased azhal kuttram is brought to normal mainly by enippu suvai and thuvarpu suvai. These sweet and astringent tastes have the cool potency by nature.

A. Earth + Water = Sweet

B. Earth + Air = Astringent.

Thus they decrease the azhal kuttram. Sweet taste increases the spermatogenesis. So I conclude the trial drugs cures the Aan Maladu and it comes under the Ethirurai Maruthuvam.

IMPROVEMENT:

Among the total 20 patients all were improved both subjectively and objectively. Clinical symptoms before and after treatment were noted. To obtain prognosis of each clinical symptom, the following formulae was used

$$\left.\frac{\text{No. Of cases after treatment}}{\text{No. of cases before treatment}}\right\} \times 100$$

Semen Analysis:

Before treatment

20% of cases sperm count had 1-10 million/cumm, 45% of cases sperm count had 11-20 million/cumm, 25% cases sperm count had 21-30 million/cumm, 10% of cases sperm count had 41-50 million/cumm

After treatment

5% of cases sperm count had 1-10 million/cumm, 20% cases sperm count had 21-30 million/cumm, 10% of cases sperm count had 31-40 million/cumm, 35% of cases sperm count had 41-50 million/cumm, 25% of cases sperm count had 51-60 million/cumm, 5% of cases sperm count had 61-70 million/cumm,

Bio Statistical study:

The p value is highly significant ($p<0.001$). So the treatment was significantly improving the Semen count (millions/cu mm) and semen motility.

Since the p value is significant in all symptoms. So there is significant reducing of symptoms among the patients for the treatment of Aan Maladu(Male Infertility). Hence it is concluded that the treatment was effective and significant.

OVER ALL RESULT:

Out of 20 patients, 11 cases (55%) shows good result, 6 cases (30%) shows moderate result, 3 cases (15%) shows poor result.

SUMMARY

The aim of the study is to increase the sperm count and sperm motility in male infertility patient. The trial medicine *Veeriya viruthi Chooranam* was prepared as per literature. The duration of the trial period is 48 days. The trial dose is *Veeriya viruthi Chooranam* 1 gm. twice a day with cow‟s milk.

I had selected 20 patients for the trial based on Inclusion and Exclusion criteria. Before treatment routine blood, urine and semen analysis taken in all 20 patients. Siddha methods like udal thathukkal, Envagai thervu, Neerkuri and Neikuri were noted in case sheet proforma. Patients were instructed to come for next review once in 7 days.

Age:

Most of the patients were in the age group between 31-40 years.

Thinai:

Most of the patients were from Neithal Thinai 70%.

Kaalam:

Seasonal Variances do not have any impact for affecting the people.

Occupation:

The disease is more common in people working in hot atmosphere like Drivers and Labourers.

Diet & Personal habits:

People with habit of taking Vegetarian and Mixed Diet, smoking, Alcoholic has more incidence of the disease.

Mukkutrum:

In Vali, Abaanan, Koorman, Viyanan and Devathathan, In Pitham Anal, Sadhagam, Aalosagam, and in Iyyam Tharpagam and Sandhigam were affected in most of the cases.

Udal Thathugal:

Saaram, and sukkilam were affected in all the patients.

Naadi:

Pithavaatham naadi was most common naadi felt.

Results after treatment:

Veeriya Viruthi Chooranam showed good results with relieving symptoms in almost 70% patients.

55% of patients show good improvement, 30% of patients shows moderate improvement and in 15% of patients poor improvement was observed.

.

The pharmacological studies reveal that *Veeriya Viruthi Chooranam* had good spermatogenesis effect in rats. The toxicity study revealed that there were no signs of toxicity as could be judged by the absence of undesirable clinical manifestations and no alteration in bio chemical markers.

The bio-statistical report of the clinical trial shows significant result.

CONCLUSION

- **AAN MALADU** (Male infertility) is primarily due to the derangement of pitham.
- *Veeriya viruthi Chooranam* predominating with Inippu and Thuvarppu taste respectively neutralizes the pitham.
- From the pre-clinical pharmacological studies it is evident that the medicine was significant Spermatogenic activity.
- The *Veeriyaviruthi Chooranam* does not produce any toxicity in preclinical study. So it is non-toxic and safe drug for Aan maladu.
- From the preclinical study *Veeriyaviruthi Chooranam* increase the sperm motility.
- No adverse effect was reported during the course of the treatment.
- The trial medicine gave maximum relief from the symptoms of Aan maladu.
- The preparation of trial drug is too easy and the cost effect is economically.
- Therefore the author concluded that the trial medicine *VEERIYA VIRUTHI CHOORANAM* should be a very good remedy for Aan maladu (Male Infertility).

The Tamil Nadu Dr.M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./Mr./Ms. ....M. MEERAN GANI.....

for participating as ~~Resource Person~~ / Delegate in the First Workshop on

"Pre-clinical Studies in Research"

for Faculties & PG students of ASU Systems

Organised by the Department of Siddha,

The Tamil Nadu Dr. M.G.R. Medical University on 16.12.2014

Dr. N. KABILAN M.D. (Siddha)
Reader, Dept. of Siddha

Dr. JHANSI CHARLES, M.D.
Registrar

Prof. Dr. D. SHANTHARAM, M.D., D.Diab.,
Vice-Chancellor

Government Siddha Medical College
Department of Medicinal Botany

Dr.S.Sankaranarayanan M.Sc., M.Phil., Ph.D.,
Asst. Professor
Head of the Department

6, Anna Arch Rd,
NSK Nagar,
Arumbakkam, Chennai,
Tamil Nadu 600106.

AUTHENTICATION CERTIFICATE

Based upon the organoleptic/macroscopic/microscopic examination of fresh/market sample, it is certified that the specimen given by Dr. M. Meeran gani BSMS studying MD (S), Government Siddha Medical College, Arumbakkam, Chennai is identified below

Binomial name	Family	Regional names
Mucuna pruriens L. DC.	Fabaceae	Punai kalli
Maerua oblongifolia (Forssk)A. Rich	Capparidaceae	Pumi sakkarai
Cuculigo orchioides	Hypoxidaceae	Nelapanai kizhangu

13/6/2016

Dr. S. Sankaranarayanan M.Sc., M.Phil., Ph.D.,

Dr. S. SANKARANARAYANAN, M.Sc., M.Phil., Ph.D.,
Assistant Professor
Dept. of Maruthuva Thavaraiyal
(Medicinal Botany and Pharmacognosy)
Govt. Siddha Medical College,
Arumbakkam, Chennai-600 106.

GSMC/MB-10/2016

Date:13.06.2016

CERTIFICATE

This is to certify that the project entitled "**TOXICITY EVALUATION OF *VEERIYA VIRUTHI CHOORNAM* BY ACUTE TOXICITY -OECD 423 AND SUB-ACUTE REPEATED DOSE ORAL TOXICITY STUDY- OECD 407 IN RATS**" has been approved by the IAEC of Sathyabama University, Chennai.

IAEC Approval No.: **SU/CLATR/IAEC/IV/021/2016**

Animal Sanctioned: *Rattus norvegicus* / Wistar albino rats

Male: 6; Female: 12; Total: 18 (Eighteen)

Date: 5.3.2016

DR.B.SHEELA RANI
Chair Person

DR.R.ILAVARASAN
CPCSEA Main Nominee

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH (CLATR) ★ SATHYABAMA UNIVERSITY Chennai - 600 119

ACUTE TOXICITY STUDY

Acute toxicity study of the study drug *Veeriya Viruthi Chooranam* was carried out as per OECD guideline (Organization for Economic Co-operation and Development) Guideline-423.

Animal

Healthy adult Wistar albino rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^0 C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study.

The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India.

Acute toxicity Study

Acute toxicity study will be carried out in accordance with OECD guideline 423 [66]. The animals were fasted overnight with free access to water. The study was conducted with single oral dose administration of Veeriya Viruthi Chooranam.

IAEC SU/CLATR/IAEC/IV/021/2016

Animal Grouping

One group consist of 6 female rats were used for this study. The dose utilized for evaluation of acute toxicity study is about 2000 mg/kg higher than that of the therapeutic dose.

Animal Grouping

GROUP I : Animals received Test drug 2000 mg/kg (p.o)

The animals were fasted overnight (12- 16 hrs) with free access to water. The study was conducted with single oral administration of study drug *Veeriya Viruthi Chooranam* 2000mg/kg (p.o). The animals were observed continuously for first 72 h

and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.

Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S,C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention.

Body weight was recorded periodically. At the end of the experiment all animals were subjected for gross necropsy and observed for pathological changes.

SUB-ACUTE TOXICITY STUDY

Sub-acute toxicity study was carried out as per OECD guidelines Guideline-407 [67].

Animals

Healthy adult Wistar albino rat weighing between 170-200 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit (AHU). A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^{o} C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study.

The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India.

IAEC SU/CLATR/IAEC/IV/021/2016

Animal Grouping

Animals were divided into three groups of 06 animals each consist of 3 male and 3 female rats.

GROUP I **:** Animals received saline 5 ml/kg b.w (p.o)

GROUP II **:** Animals received low dose of test drug 200 mg/kg (p.o)

GROUP III **:** Animals received high dose of test drug 400 mg/kg (p.o)

The animals were randomly divided into control group and drug treated groups for two different doses viz. low dose (200 mg/kg b.w) and high dose (400 mg/kg b.w).

The animals were administrated with the study drug once daily for 28 days. The animals in group I (control group) received normal saline 5 ml/kg b.w. The animals in group II received low dose of *Veeriya Viruthi Chooranam* 200 mg/kg b.w (p.o) and group III received high dose of *Veeriya Viruthi Chooranam* 400 mg/kg b.w (p.o).

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28th day, the animals were fasted for overnight with free access to water. On 29th day the animals were sacrificed with excess anesthesia. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra actate) for Hematological analysis and for serum generation for biochemical analysis.

The vital organs including heart, brain, lungs, spleen, kidneys, liver, stomach, testes, and ovary were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation.

Hematological analysis

Blood samples were analyzed using established procedures and automated Bayer Hematology analyzer. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil’s, Basophils, Lymphocytes and Monocytes.

Biochemical analysis [68]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL) , Very low density Lipoprotein (VLDL) , Triglycerides (TGL), Total Cholesterol , Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline Phosphatase (ALP) using Mind ray auto analyzer model BS 120.

Histopathological evaluation [69]

Organs included of heart, brain, lungs, spleen, kidneys, liver, stomach, testes and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

Statistical analysis

The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error .A statistical comparison was carried out using the Dunnet's test for the control and treatment group.

Fecal Pellet Analysis

Methodology

Rats of control and treatment group were allowed to explore to open field on clean and sterile cage with blotting paper. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc

Acute Toxicity Study

Analysis	**Group I**
Consistency	Soft
Shape	Point ended
Colour	Dark Green
Mucous Shedding	Mild
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Group I**	**Group II**	**Group III**
Consistency	Soft	Soft	Soft
Shape	Oblong	Oblong	Oblong
Colour	Brownish green	Greenish Brown	Greenish Brown
Mucous Shedding	Absence	Absence	Absence
Blood Cells	Absent	Absent	Absent
Signs of Infection	None Observed	None Observed	None Observed

Muscle Grip Strength Analysis

Methodology

The grip strength test is a simple non-invasive method designed to evaluate rat muscle force in vivo. Rats of control and drug treated group was allowed to hold the pull bar with both the hind limbs firmly then the animal was gently pulled back with the tail until the animal lost the grip toward the bar. The procedure was repeated to get the average value. Muscle grip ness of the drug treated group was compared to that of the control rat to ensure the change in coordination.

Metabolic Cage for Urine Collection

Rat of control and treatment group was placed individually in metabolic cage with free access to feed and water. Urine dropping from the animal was collected using specialized wire mesh system fixed at the base of the cage having provision to trap the fecal pellet mixed with urine sample. The collected urine sample was subjected to analysis with respect to colour, pH, glucose, ketone bodies, pus and blood cells.

RESULTS

Assessment of clinical signs in rats treated with *Veeriya Viruthi Chooranam* **on Acute toxicity study**

Parameter	**Group I**
Clinical Signs Parameters for the duration of 14 days	Test Drug 2000mg/ Kg
Number of animals observed	6 Female
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Absence
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Color	Normal
Stereotype behavior	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behavior	Normal
Gait Balancing	Normal
Freezing Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	7
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

Quantitative data on the body weight of rats treated with *Veeriya Viruthi chooranam* in Acute toxicity study

Group I	Before Treatment Weight in Gms	After Treatment Weight in Gms
Mean	184.2	187
Std. Deviation	7.026	6.164
Std. Error	2.868	2.517

Values are mean ± S.D (n = 6 per group). Control and treatment group were compared statistically using one way ANOVA followed by Dunnett's test.

Assessment of clinical signs in rats treated with *Veeriya Viruthi Chooranam* on Sub-Acute toxicity study

Parameter	Group I	Group II	Group III
Clinical Signs Parameters for the duration of 28 days	Control	Test Drug 200mg/ Kg	Test Drug 400mg/ Kg
Number of animals observed	3 Male and 3 Female	3 Male and 3 Female	3 Male and 3Female
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Absence	Absence
Animal appearance	Normal	Normal	Normal
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Absence	Absence
Touch Response	Normal	Normal	Normal
Response to Sound	Normal Response	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response	Normal Response
Mobility	Normal	Normal	Normal
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Gait Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal

Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	6	6	6
Urine - Glucose	Absence	Absence	Absence
Urine - Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

Effect of *Veeriya Viruthi Chooranam* on Body weight of Rats in Sub-acute toxicity study

Group I	**Before Treatment Weight in Gms**	**After Treatment Weight in Gms**
Mean	189.5	193.8
Std. Deviation	5.788	5.492
Std. Error	2.363	2.242
Group II	**Before Treatment Weight in Gms**	**After Treatment Weight in Gms**
Mean	183	195.2
Std. Deviation	5.831	4.875
Std. Error	2.38	1.99
Group III	**Before Treatment Weight in Gms**	**After Treatment Weight in Gms**
Mean	176	183
Std. Deviation	6.356	7.563
Std. Error	2.595	3.088

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Quantitative data on the food and water intake of rats treated with *Veeriya Viruthi Chooranam* for 28 days in Sub-acute toxicity study

GROUP I	Food intake	Water intake
Mean	17.33	33.92
Std. Deviation	3.82	2.727
Std. Error	1.91	1.363
GROUP II	**Food intake**	**Water intake**
Mean	16.58	31.33
Std. Deviation	4.324	1.054
Std. Error	2.162	0.527
GROUP III	**Food intake**	**Water intake**
Mean	17.08	31.33
Std. Deviation	2.132	1.305
Std. Error	1.066	0.6526

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Veeriya Viruthi Chooranam* on Haematology profile of rats in sub-acute toxicity study

GROUP I	WBC count ($\times 10^3$ µl)	RBC ($\times 10^6$ µl)	PLT ($\times 10^3$ µl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	HGB (g/dl)
Mean	12.1	6.867	649.7	64.62	18.48	32.88	12.15
Std. Deviation	1.334	1.084	144.9	2.501	2.137	1.61	1.42
Std. Error	0.5447	0.4425	59.14	1.021	0.8723	0.6575	0.5795
GROUP II	**WBC count ($\times 10^3$ µl)**	**RBC ($\times 10^6$ µl)**	**PLT ($\times 10^3$ µl)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HGB (g/dl)**
Mean	12.07	6.233	612.5	63.68	17.97	32.39	11.5
Std. Deviation	0.9812	0.8618	137.6	3.539	2.037	1.903	1.064
Std. Error	0.4006	0.3518	56.16	1.445	0.8317	0.777	0.4344
GROUP III	**WBC count ($\times 10^3$ µl)**	**RBC ($\times 10^6$ µl)**	**PLT ($\times 10^3$ µl)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**HGB (g/dl)**
Mean	12.78	6.633	854.8	61.6	20.05	32.32	12.98
Std. Deviation	2.004	1.316	134.7	4.856	1.468	1.609	1.238
Std. Error	0.8183	0.5371	54.97	1.983	0.5993	0.657	0.5056

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Veeriya Viruthi Chooranam* on Haematology profile of rats in sub-acute toxicity study.

GROUP I	**Lymph (%)**	**Mon (%)**	**Neutrophils (X 10^3/mm^3)**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Mean	77.38	3.15	2.517	1.583	0.3333	5.567
Std. Deviation	7.903	0.8264	0.9196	0.2787	0.5164	1.181
Std. Error	3.226	0.3374	0.3754	0.1138	0.2108	0.4821
GROUP II	**Lymph (%)**	**Mon (%)**	**Neutrophils (X 10^3/mm^3)**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Mean	79.77	2.5	2.133	1.583	0.5	4.817
Std. Deviation	8.493	0.506	0.6861	0.2041	0.5477	1.761
Std. Error	3.467	0.2066	0.2801	0.08333	0.2236	0.719
GROUP III	**Lymph (%)**	**Mon (%)**	**Neutrophils (X 10^3/mm^3)**	**Eosinophils (%)**	**Basophils (%)**	**MPV (fl)**
Mean	71.48	5.1	1.967	1.35	0.1667	5.167
Std. Deviation	5.504	0.6723	0.5125	0.3146	0.4082	1.414
Std. Error	2.247	0.2745	0.2092	0.1285	0.1667	0.5772

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Veeriya Viruthi Chooranam* on Serum Bio-chemistry profile of rats in sub-acute toxicity study

GROUP I	**Blood sugar ® (mg/dl)**	**BUN (mg/dl)**	**Serum creatinine (mg/dl)**	**Serum total cholesterol (mg/dl)**	**Serum triglycerides level (mg/dl)**	**Serum HDL cholesterol (mg/dl)**	**Serum LDL cholesterol (mg/dl)**	**Serum VLDL cholesterol (mg/dl)**
Mean	79.83	18.17	0.8333	125.8	88.17	61.67	45	17.57
Std. Deviation	12.17	2.927	0.1862	9.827	13.17	10.89	5.02	3.546
Std. Error	4.969	1.195	0.07601	4.012	5.375	4.447	2.049	1.447
GROUP II	**Blood sugar ® (mg/dl)**	**BUN (mg/dl)**	**Serum creatinine (mg/dl)**	**Serum total cholesterol (mg/dl)**	**Serum triglycerides level (mg/dl)**	**Serum HDL cholesterol (mg/dl)**	**Serum LDL cholesterol (mg/dl)**	**Serum VLDL cholesterol (mg/dl)**
Mean	79.67	10.67	0.8333	104.2	79.17	52	40	19.97
Std. Deviation	7.891	2.658	0.2875	22.34	12.95	12.51	17.67	1.306
Std. Error	3.221	1.085	0.1174	9.119	5.288	5.106	7.216	0.5333

GROUP III	Blood sugar ® (mg/dl)	BUN (mg/dl)	Serum creatinine (mg/dl)	Serum total cholesterol (mg/dl)	Serum triglycerides level (mg/dl)	Serum HDL cholesterol (mg/dl)	Serum LDL cholesterol (mg/dl)	Serum VLDL cholesterol (mg/dl)
Mean	76.17	15.33	0.8167	97.67	82.33	58	45.67	13.97
Std. Deviation	11.37	3.266	0.2137	8.311	9.438	9.099	7.285	1.763
Std. Error	4.643	1.333	0.08724	3.393	3.853	3.715	2.974	0.7196

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Effect of *Veeriya Viruthi Chooranam* on Serum Bio-chemistry profile of rats in sub-acute toxicity study

GROUP I	Serum total protein (g/dl)	Serum albumin (g/dl)	(AST) (IU/ml)	(ALT) (IU/L)	(ALP)(IU/L)
Mean	3.833	4.183	104	33.67	186
Std. Deviation	0.432	0.5879	21.16	10.48	41.02
Std. Error	0.1764	0.24	8.637	4.279	16.75
GROUP II	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**(AST) (IU/ml)**	**(ALT) (IU/L)**	**(ALP)(IU/L)**
Mean	6.017	3.9	81.67	32.17	170.2
Std. Deviation	0.7985	0.8462	6.947	9.453	50.65
Std. Error	0.326	0.3454	2.836	3.859	20.68
GROUP III	**Serum total protein (g/dl)**	**Serum albumin (g/dl)**	**(AST) (IU/ml)**	**(ALT) (IU/L)**	**(ALP)(IU/L)**
Mean	3.833	2.85	104.3	18.67	194.2
Std. Deviation	0.7118	0.9607	27.66	3.67	35.95
Std. Error	0.2906	0.3922	11.29	1.498	14.68

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

Quantitative data on absolute organ weight of rats treated with *Veeriya Viruthi Chooranam* for 28 days in Sub-acute toxicity study

GROUP I	**HEART (gms)**	**LIVER (gms)**	**KIDNEYS (gms)**	**SPLEEN (gms)**	**BRAIN (gms)**	**LUNG (gms)**	**STOMACH (gms)**	**TESTES (gms)**	**UTERUS & OVARY (gms)**
Mean	0.6833	6.335	1.607	0.5167	1.567	1.683	1.25	3.067	1.167
Std. Deviation	0.1065	1.142	0.2337	0.1941	0.216	0.1722	0.345	1.172	0.05774
Std. Error	0.04349	0.4663	0.09542	0.07923	0.08819	0.07032	0.1408	0.6766	0.03333
GROUP II	**HEART (gms)**	**LIVER (gms)**	**KIDNEYS (gms)**	**SPLEEN (gms)**	**BRAIN (gms)**	**LUNG (gms)**	**STOMACH (gms)**	**TESTES (gms)**	**UTERUS & OVARY (gms)**
Mean	0.7067	5.428	1.438	0.5667	1.45	1.6	1.383	2.867	1.367
Std. Deviation	0.128	1.119	0.27	0.2066	0.2258	0.2098	0.3869	0.4726	0.05774
Std. Error	0.05226	0.4568	0.1102	0.08433	0.0922	0.08563	0.1579	0.2728	0.03333
GROUP III	**HEART (gms)**	**LIVER (gms)**	**KIDNEYS (gms)**	**SPLEEN (gms)**	**BRAIN (gms)**	**LUNG (gms)**	**STOMACH (gms)**	**TESTES (gms)**	**UTERUS & OVARY (gms)**
Mean	0.6733	6.702	1.405	0.7	1.633	1.633	1.283	3.067	1.2
Std. Deviation	0.1082	0.8958	0.1963	0.1897	0.1506	0.2338	0.4355	0.8505	0.1
Std. Error	0.04417	0.3657	0.08016	0.07746	0.06146	0.09545	0.1778	0.491	0.05774

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females) for Heart, Liver, Kidney, Brain, Spleen, Lung, Stomach. Values are mean ± S.D (n = 3 per group per sex) for testes , ovary and uterus for Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

HISTOPATHOLOGY REPORT

BRAIN

Histology of brain revealed the presence of normal cortex showing neurons, glial cells and capillaries. Section of cerebellum shows distinct molecular and granular layer. Neuronal architecture appears normal with sufficient numbers. Arrangement of the neurons appears intact with no sings of degeneration or apoptotic changes were observed in sample belongs to group I,II and III.

LUNG

Light microscopic examination of lung revealed normal alveoli and alveolar sac with no signs of infiltration in both control and treated rats.

HEART

Atrial and ventricular wall of both the heart sample appears normal. Sarcoplasmic region of myocardium appears normal. Appearance of cardiomyocyte was normal with dark nuclear region in samples belongs to group I, II and III.

STOMACH

Light microscopic observation stomach reveals normal histology of gastric wall composed of normal mucosa, muscularismucosa, submucosa, muscularispropiria and adventitia. No signs of ulceration were observed in sample belongs to group I, II and III.

SPLEEN

Appearance of central artery and marginal sinus are normal . Lymphoid follicles appears normal . Marginal sinus (MS) of the rat and its sinus lining cells appears normal. Erythropoietic cells (EP) are scattered throughout the red pulp of both control and treated rats.

LIVER

Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal Central vein appears prominent with no signs of cellular infiltration were observed in sample belongs to group I, II and III.

KIDNEY

Lumen of vessels and bowman’s space appears normal. Appearance of Podocytes and parietal epithelium in the glomeruli appears normal in sample belongs to group I,II and III.

TESTES

Presence of mature somatic cells project the perfect histomorphology of testicular cells in this group. Primary spermatocytes with large centered nucleus and dense chromatin were observed in sample belongs to group I,II and III.

UTERUS

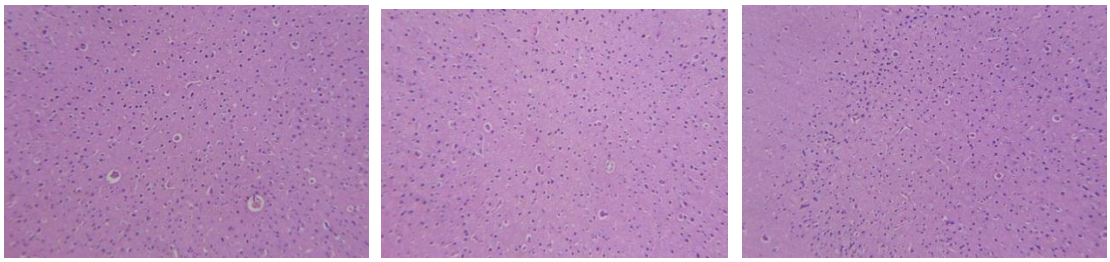
Appearance of endometrium, myometrium and uterine glands was normal. Arrangement of stratum basale, functionale and surface epithelium seems normal in samples belongs to group I,II and III.

OVARY

Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality. Appearance of antral follicle, primary oocyte and secondary follicles are normal in sample belong to group I,II and III.

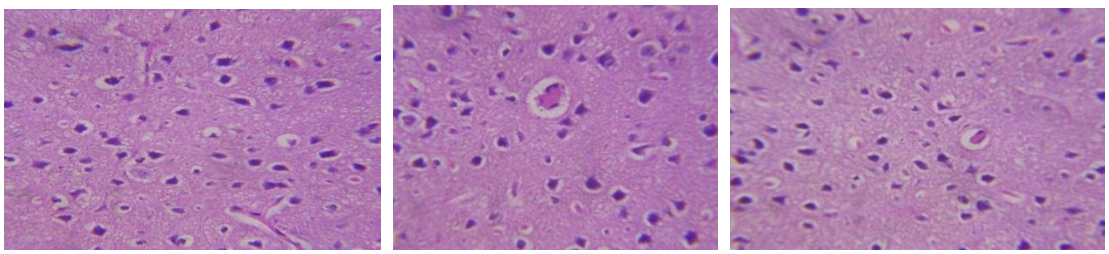
Histopathology of Brain (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

Histopathology of Heart (Male Rat) in Sub-acute toxicity Study

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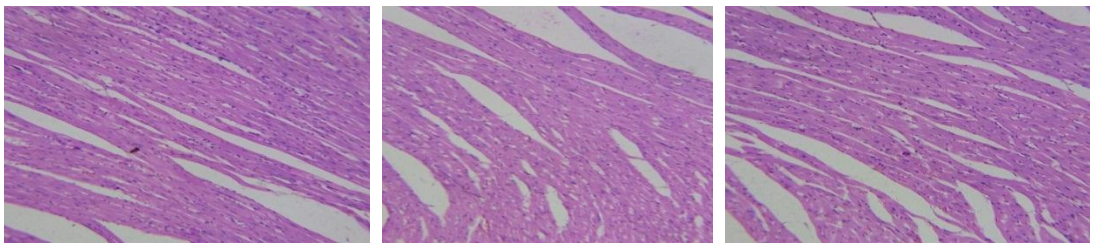

GROUP I **GROUP II** **GROUP III**

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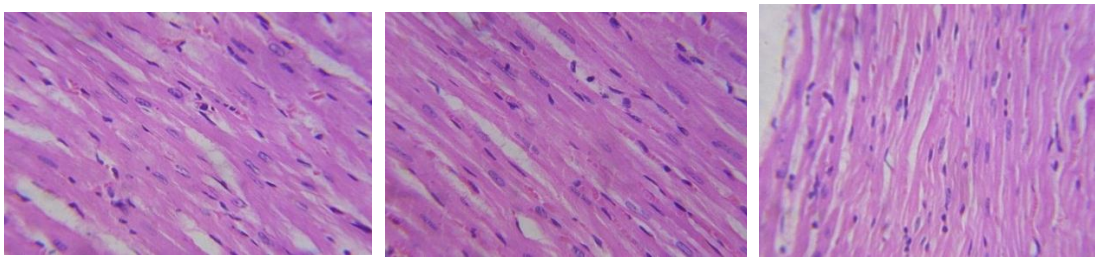

GROUP I **GROUP II** **GROUP III**

Histopathology of Kidney (Male Rat) in Sub-acute toxicity Study

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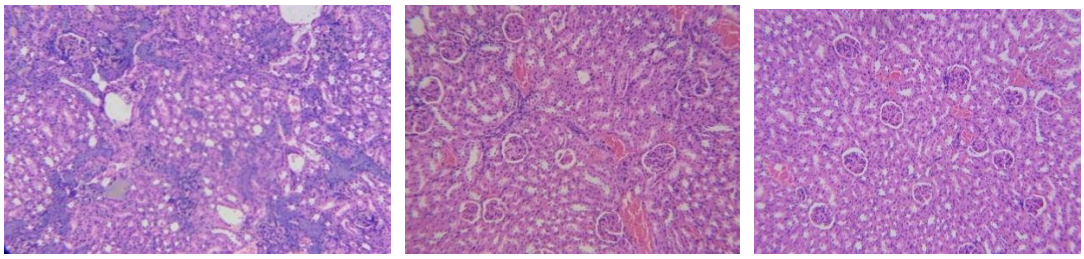

GROUP I **GROUP II** **GROUP III**

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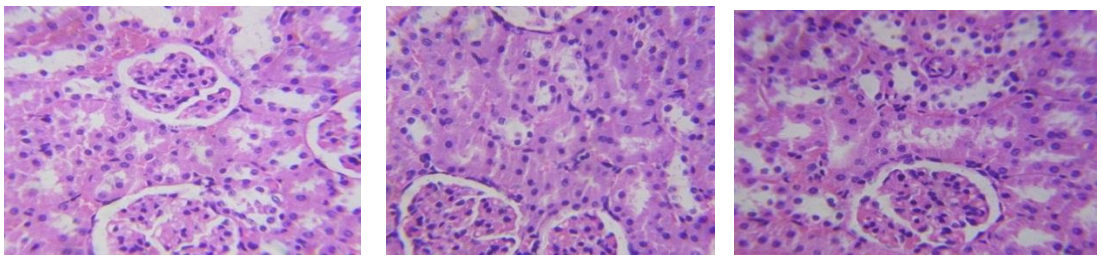

GROUP I **GROUP II** **GROUP III**

Histopathology of Liver (Male Rat) in Sub-acute toxicity Study

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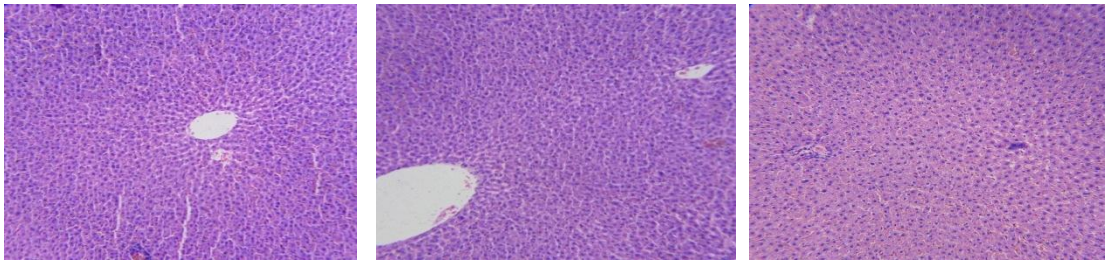
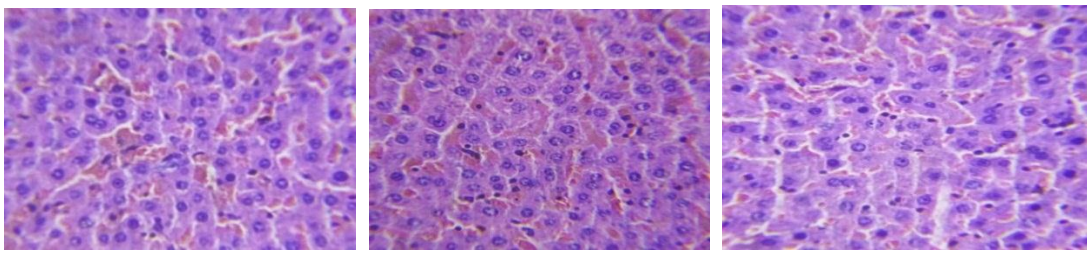


GROUP I **GROUP II** **GROUP III**

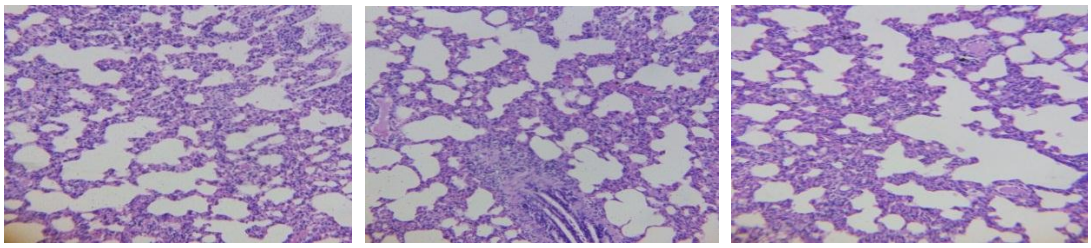
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

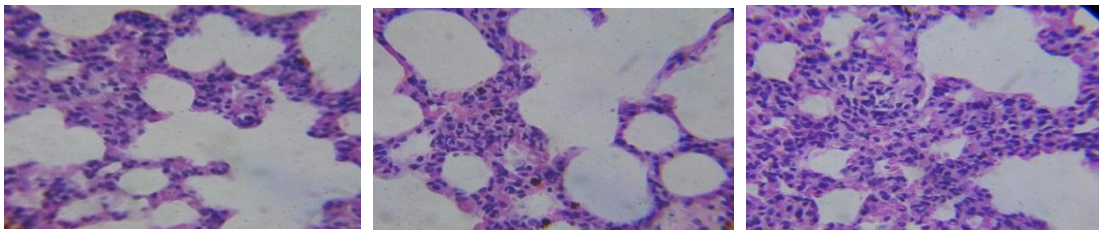
Histopathology of Lung (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

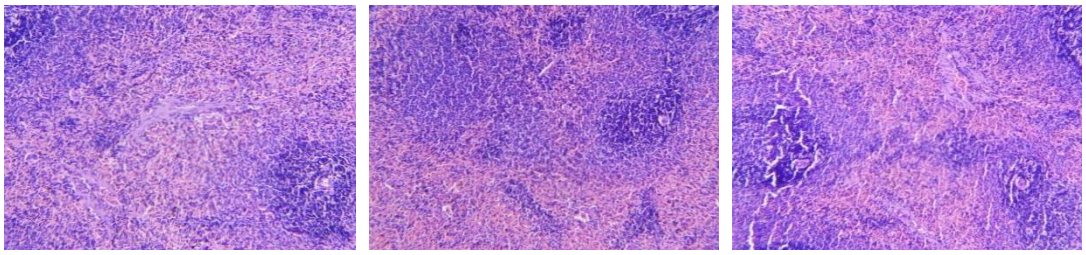
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

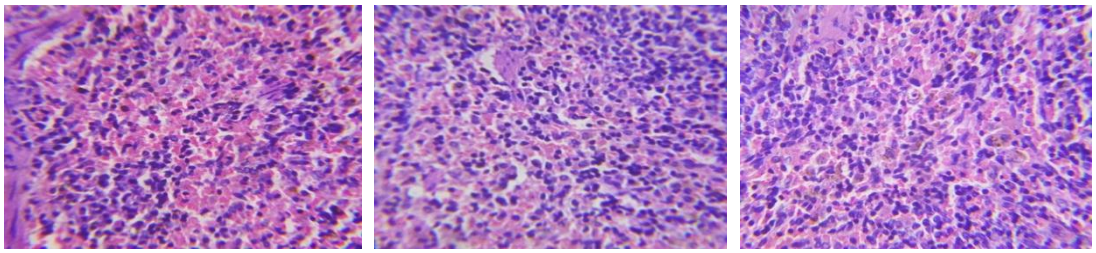
Histopathology of Spleen (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

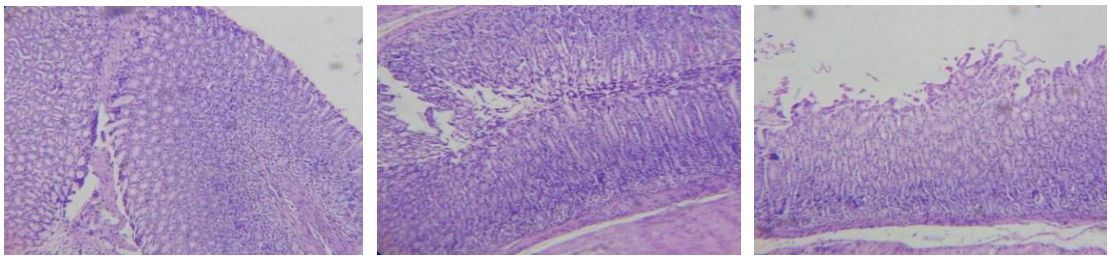
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

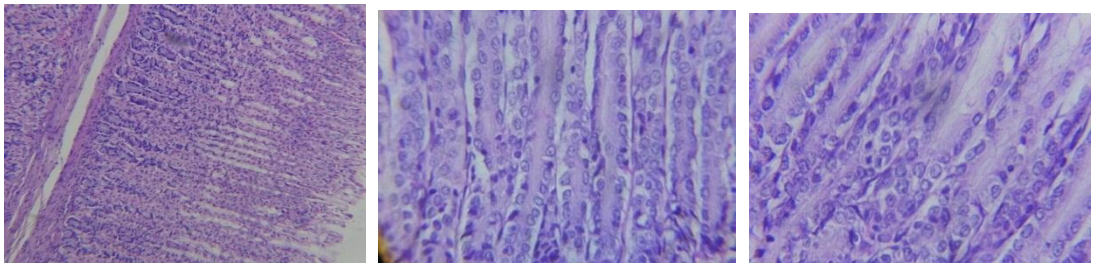
Histopathology of Stomach (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

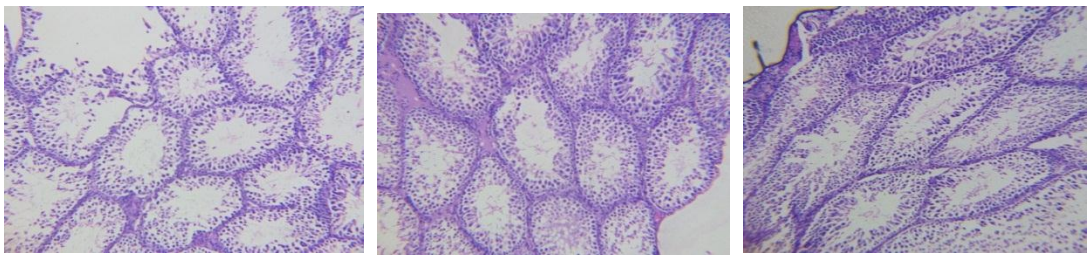
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

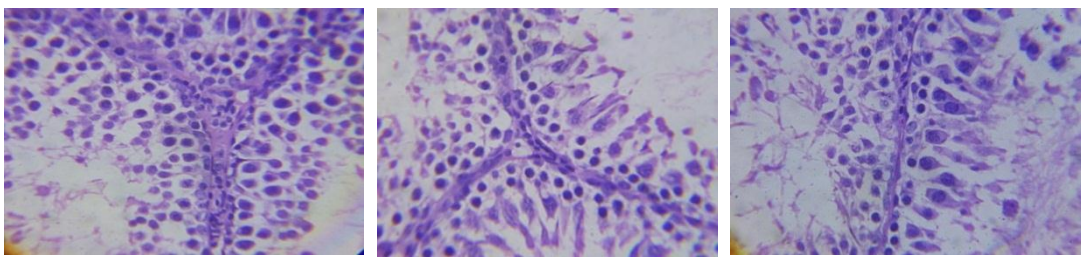
Histopathology of Testes (Male Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

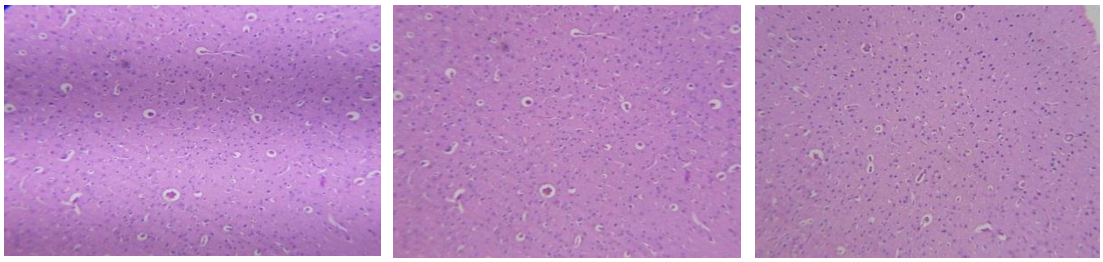
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

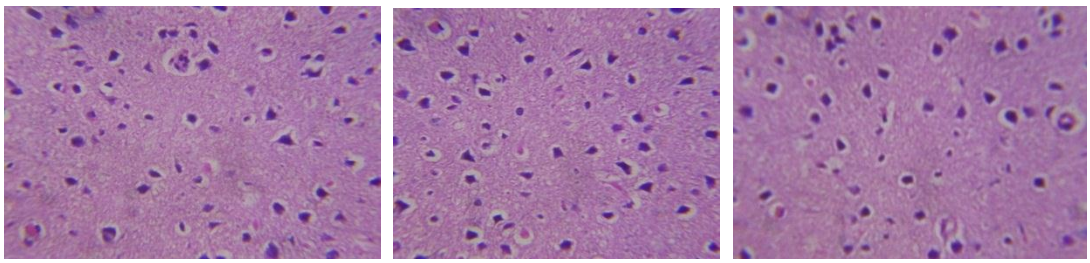
Histopathology of Brain (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

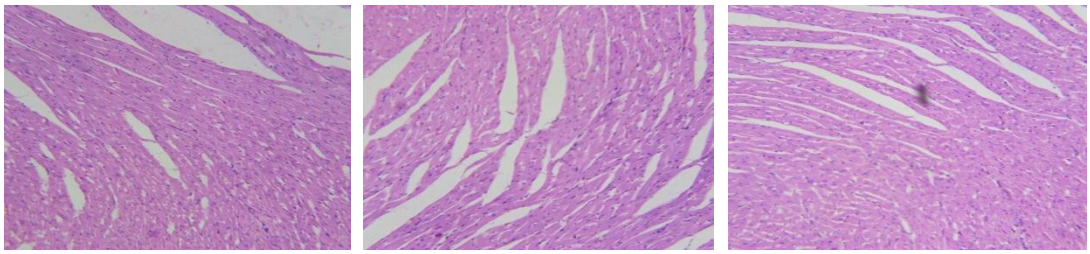
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

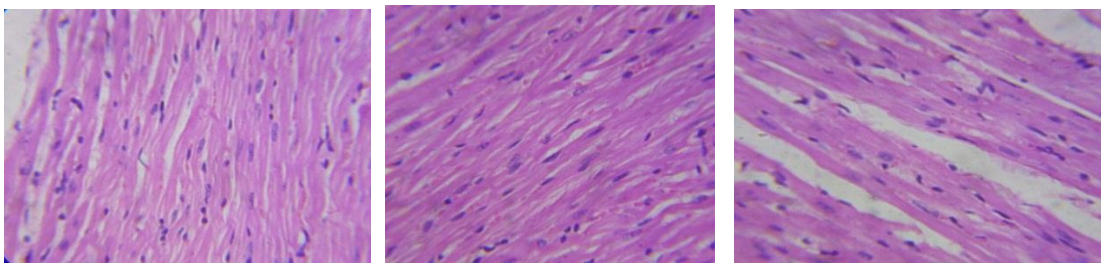
Histopathology of Heart (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

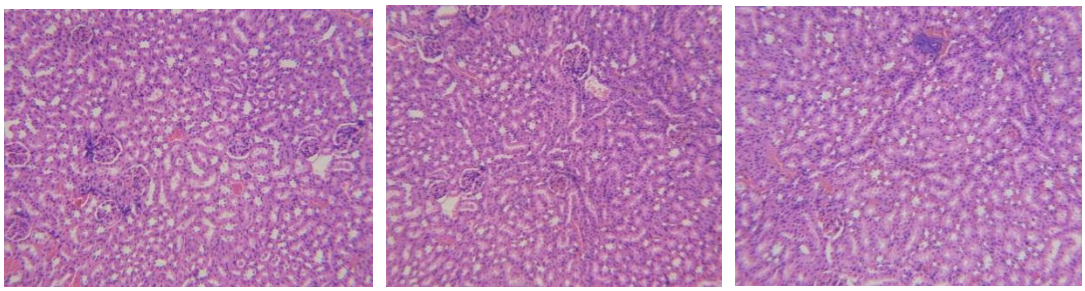
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

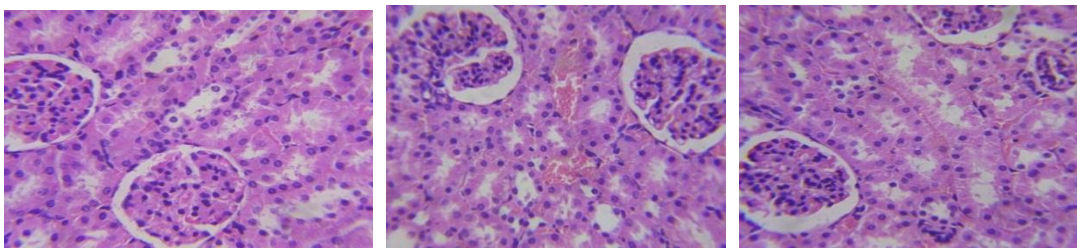
Histopathology of Kidney (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

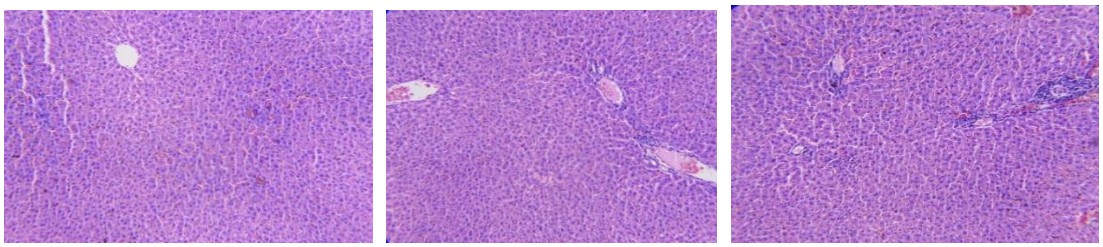
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

Histopathology of Liver (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

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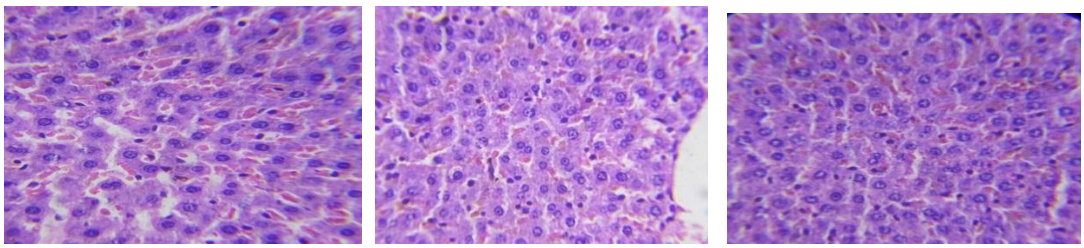
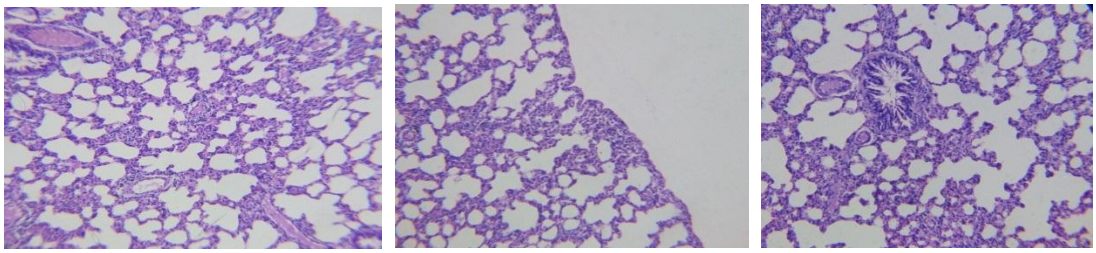


GROUP I **GROUP II** **GROUP III**

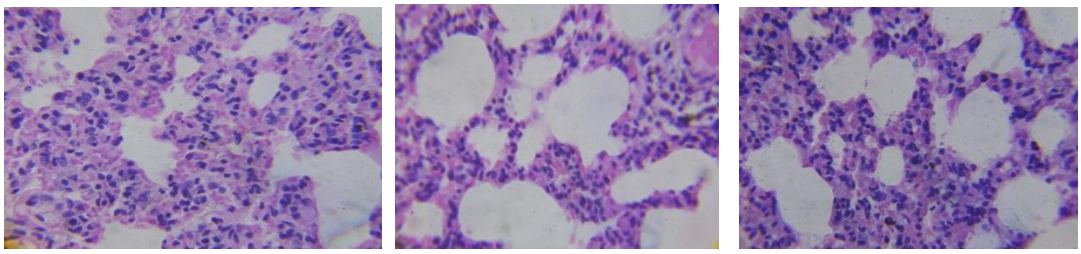
Histopathology of Lung (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

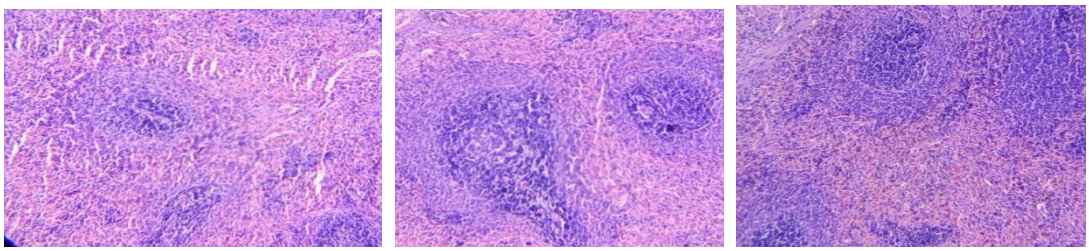
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

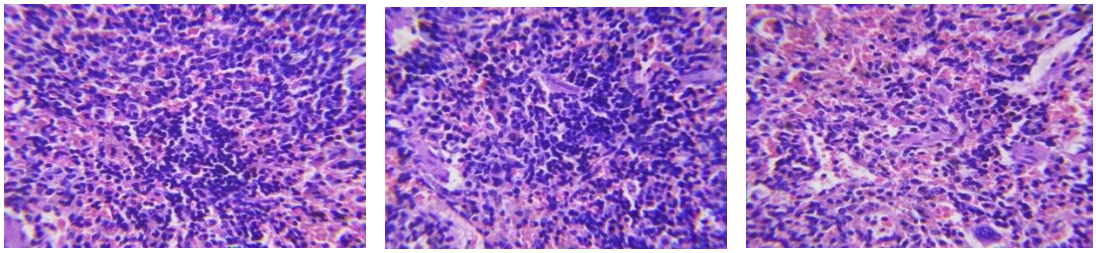
Histopathology of Spleen (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

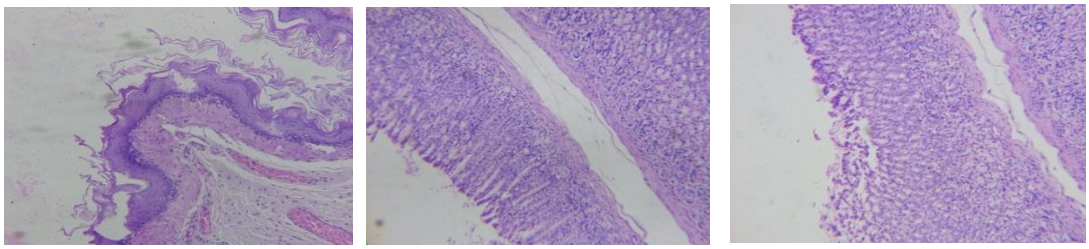
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

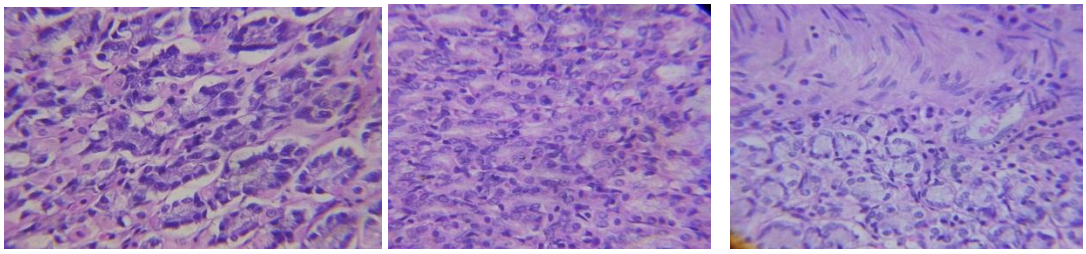
Histopathology of Stomach (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

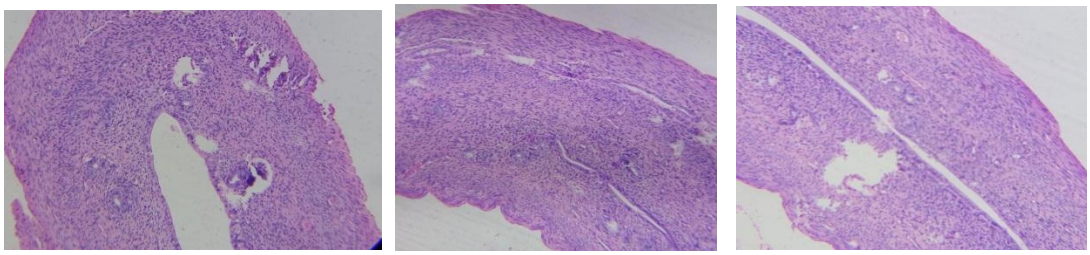
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

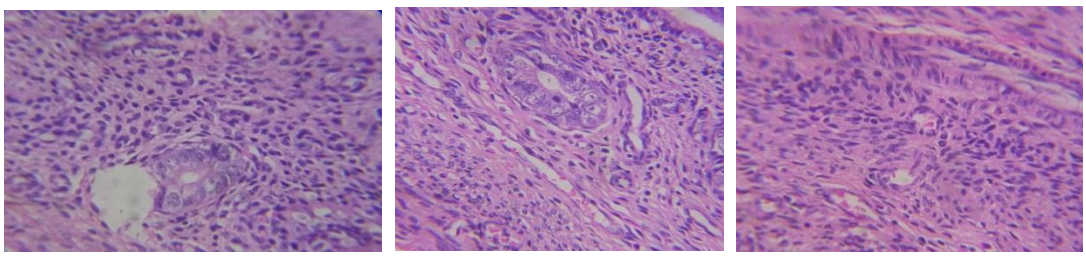
Histopathology of Uterus (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

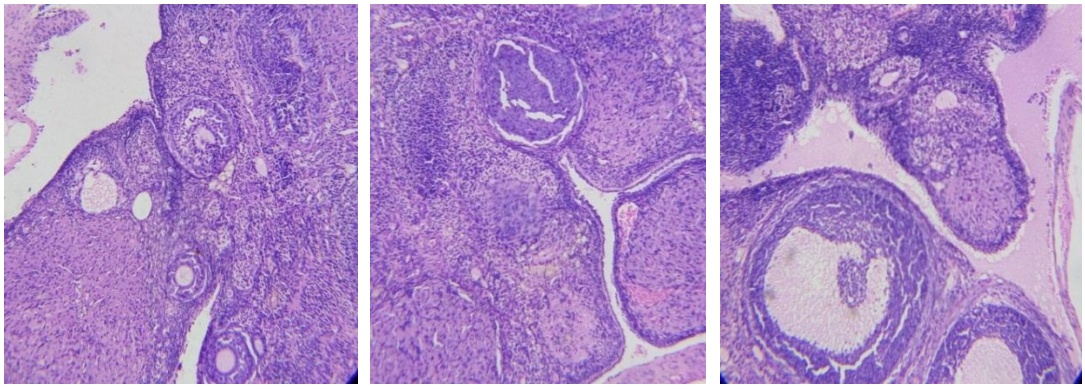
High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

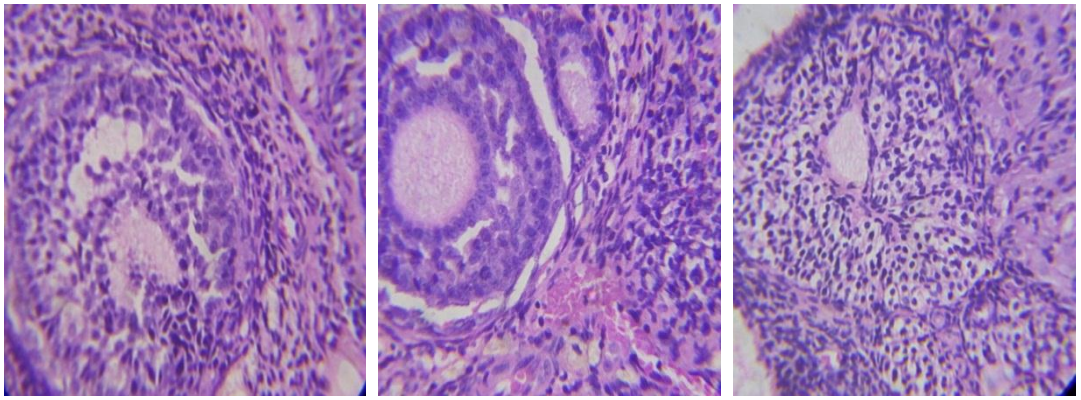
Histopathology of Ovary (Female Rat) in Sub-acute toxicity Study

Low Power Magnification 10X



GROUP I **GROUP II** **GROUP III**

High Power Magnification 40X



GROUP I **GROUP II** **GROUP III**

CERTIFICATE

This is to certify that the project entitled "**PHARMACOLOGICAL EVALUATION OF VEERIYA VIRTHI CHOORNAM ON GENTAMYCIN INDUCED TESTICULAR TOXICITY IN WISTER RATS**." has been approved by the Institutional Animal Ethics Committee of Sathyabama University, Chennai.

IAEC Approval No.: **SU/CLATR/IAEC/VII/048/2016**
Principal Investigator: Dr. M. Meeran Gani
Animal Sanctioned: *Rattus norvegicus* / Wistar Albino rats
Male: 24; Total: 24 (Twenty Four)
Date: 05.10.2016

DR. B. SHEELA RANI
Chairperson

DR. R. ILAVARASAN
CPCSEA Nominee

Pharmacological Evaluation of Veeriya *Viruthi Chooranam* on Gentamicin-Induced testicular toxicity in wistar rats

IAEC : SU/CLATR/IEAC/VII/048/2016

Animals

Healthy adult Wistar albino male rats weighing between 230-250 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit . A 12 light / dark cycle were maintained .Room temperature was maintained between 22 $\pm$ 2^{o} C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study.The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama University, Chennai, Tamil Nadu, India.

IAEC: SU/CLATR/IEAC/VII/048/2016

Experimental Methodology

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Disease control group rats received Gentamycin 50mg/kg, i.p from day 1 to 30. Group III (low dose treatment group) animal received 200 mg/kg of *Veeriya Viruthi Chooranam* 1 hr prior to gentamycin injection from day 1 to 30. Group III (High dose treatment group) animal received 400 mg/kg of *Veeriya Viruthi Chooranam* 1 hr prior to gentamycin injection from day 1 to 30.

Sample Collection

Determination of sperm count and motility

24 hrs after last dose of drug administration the animals was sacrificed for the assessment of sperm count. The testis was be decapsulated by isolating cauda epididymis the sperm will be released by cutting into phosphate buffer saline and 0.5% bovine serum albumin of pH 7. The homogenate was under room temp for 5 min and an aliquot was taken in leukocyte hemocytometer and discharged in neuabaurs counting chamber and count will be done in 4 chambers. Remaining part of 0.3 ml of plain slide and the sperm motility was analyzed with microscope.For morphological analysis PBS sperm suspension was admixed with 4 drops of eosin and thin smear of the same was made on to the glass slide and allowed to dry for further morphological analysis.

Determination of Sperm Morphology

Homogenate of about 0.5 ml was stained with eosin at room temperature for 30 mins and the slide was observed under microscope for sperm morphology.

Histopathology of testes

Testes was dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation, a tissue was embedded in paraffin. Fixed tissues were cut at 10 µm and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs will be taken.

Effect of *Veeriya Viruthi Chooranam* on Sperm Count, Motility and Viability parameters on Gentamicin-Induced rats

Group I	**Sperm Count X 10 6**	**Sperm Motility (%)**	**Viability (%)**
Mean	**57.67**	**87.5**	**73**
Std. Deviation	**3.933**	**8.503**	**2.53**
Std. Error	**1.606**	**3.471**	**1.033**
Group II	**Sperm Count X 10 6**	**Sperm Motility (%)**	**Viability (%)**
Mean	**16.67**	**17.67**	**14.17**
Std. Deviation	**4.082**	**5.854**	**3.189**
Std. Error	**1.667**	**2.39**	**1.302**
Group III	**Sperm Count X 10 6**	**Sperm Motility (%)**	**Viability (%)**
Mean	**29.5**	**41**	**35.33**
Std. Deviation	**3.619**	**8.099**	**5.203**
Std. Error	**1.478**	**3.307**	**2.124**
Group IV	**Sperm Count X 10 6**	**Sperm Motility (%)**	**Viability (%)**
Mean	**45**	**54**	**45.5**
Std. Deviation	**4.195**	**7.071**	**5.32**
Std. Error	**1.713**	**2.887**	**2.172**

Values are mean ± S.D / S.E (n = 6 per group)

Microscopic View of Neubauer's Chamber on Sperm Count

Control Group

Gentamycin Induced group

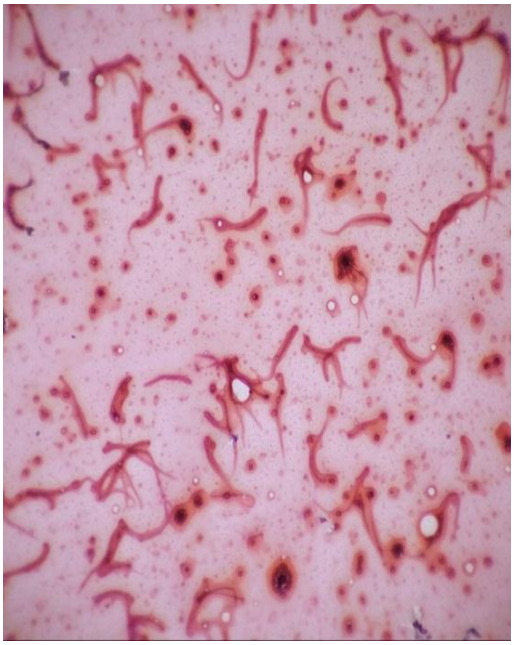
Gentamycin+ 200 mg/kg of VVC

Gentamycin+ 400 mg/kg of VVC

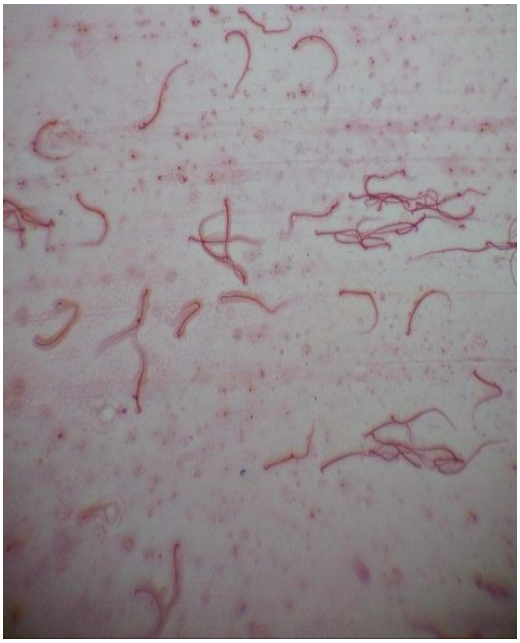
Microscopic View of Sperm morphology Stained with Eosin dye

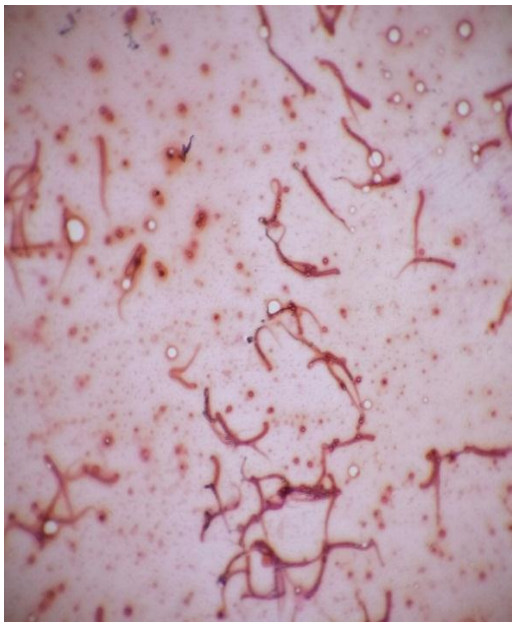
(Low Magnification)

Control Group

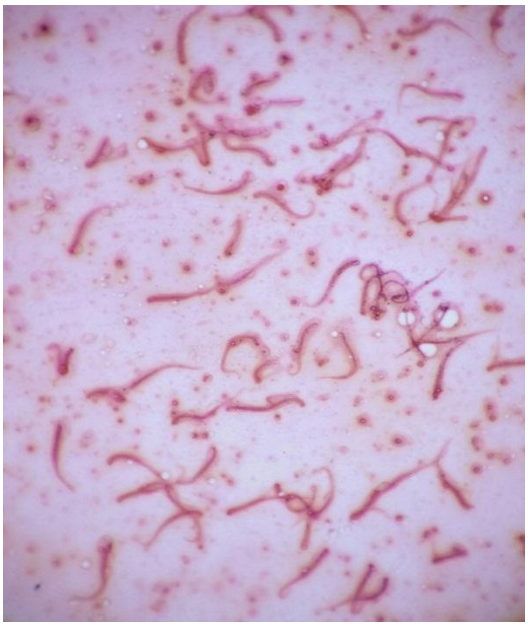


Gentamycin Induced group





Gentamycin+ 200 mg/kg of VVC

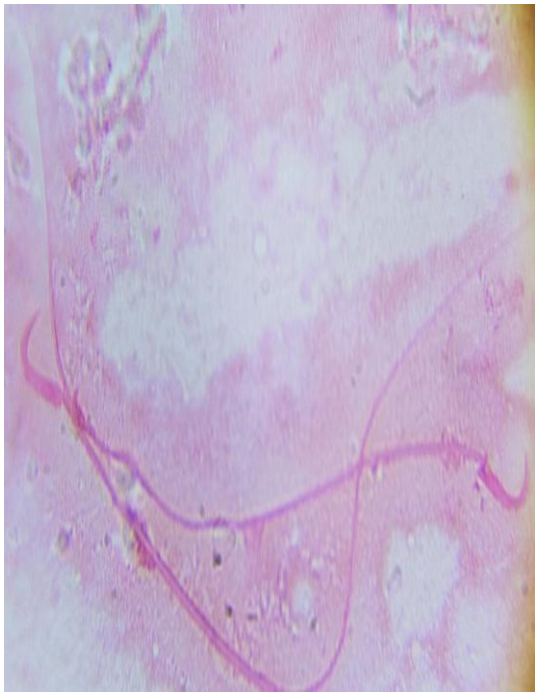


Gentamycin+ 400 mg/kg of VVC

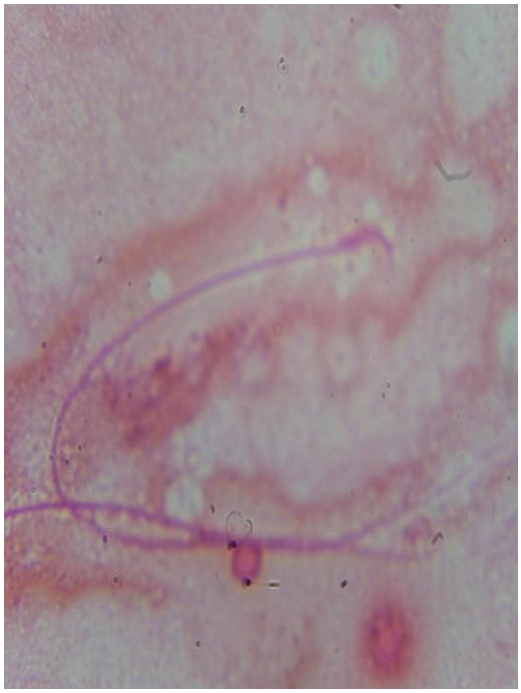
Microscopic View of Sperm morphology Stained with Eosin dye

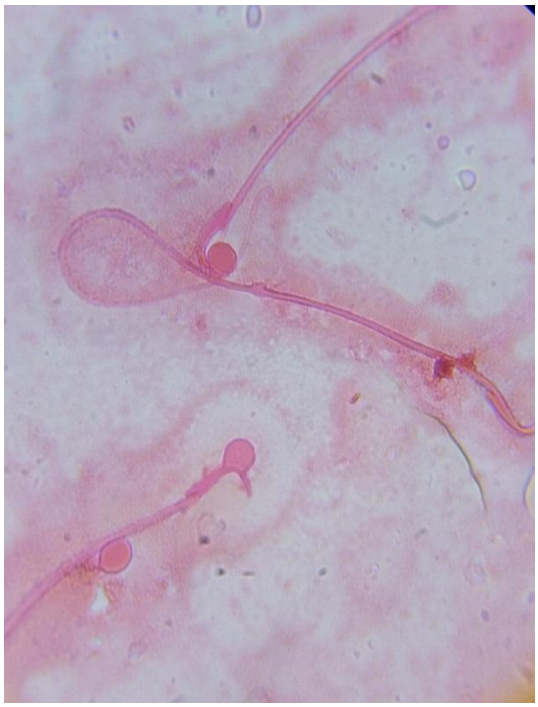
(High Magnification)

Control Group

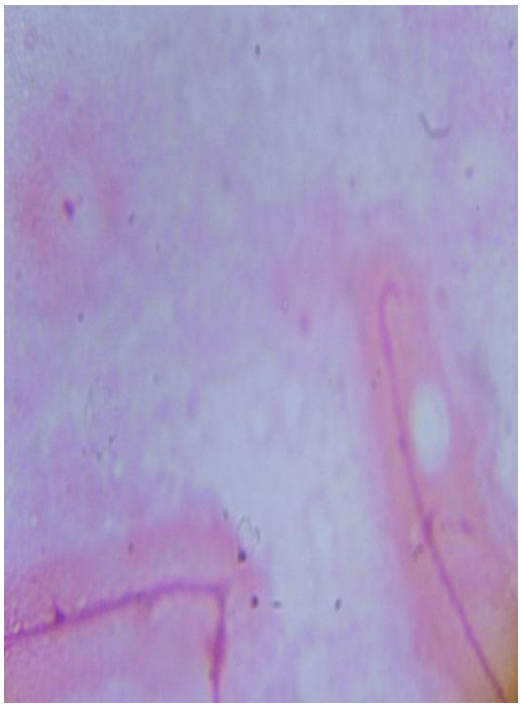


Gentamycin Induced group





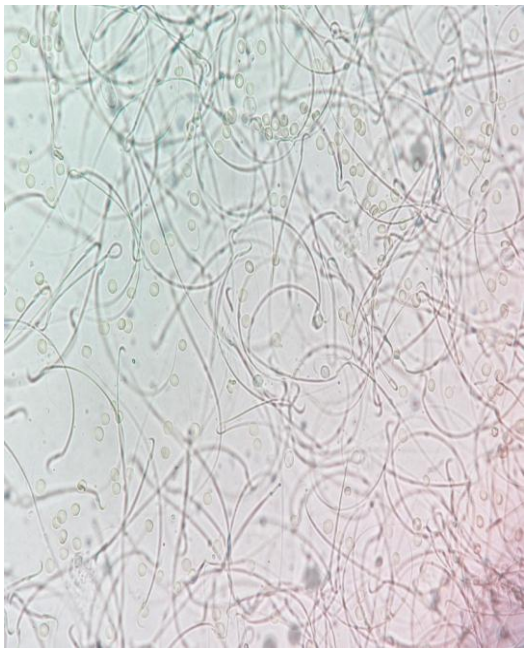
Gentamycin+ 200 mg/kg of VVC



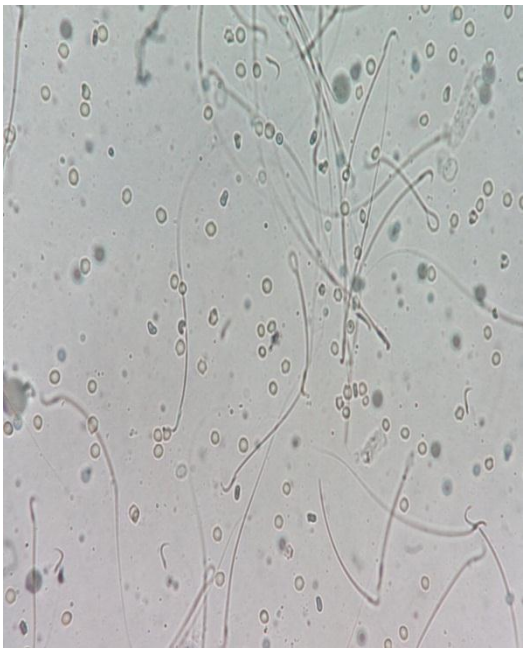
Gentamycin+ 400 mg/kg of VVC

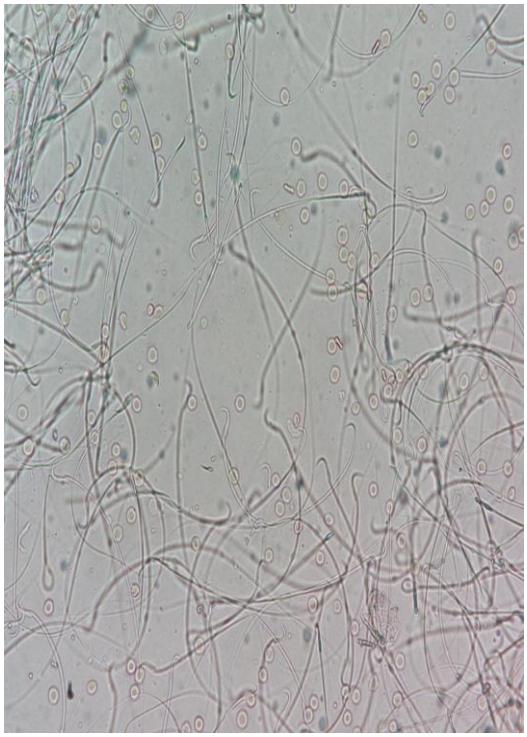
Microscopic View of Sperm Motility of Control and drug treated rats

Control Group

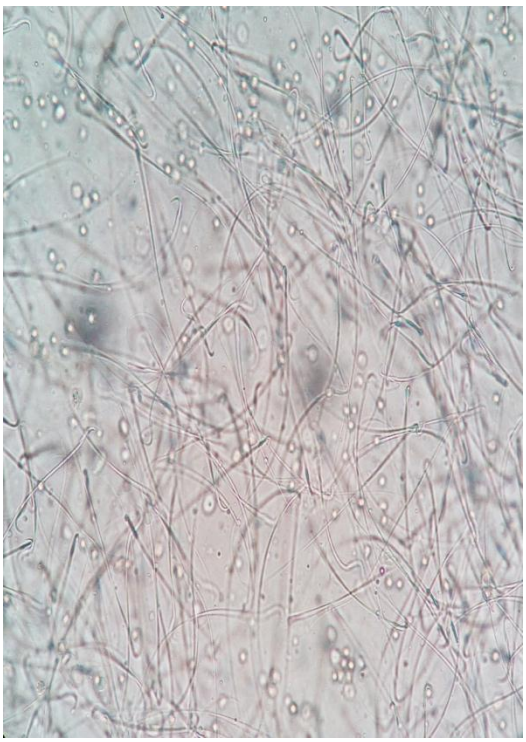


Gentamycin Induced group





Gentamycin+ 200 mg/kg of VVC

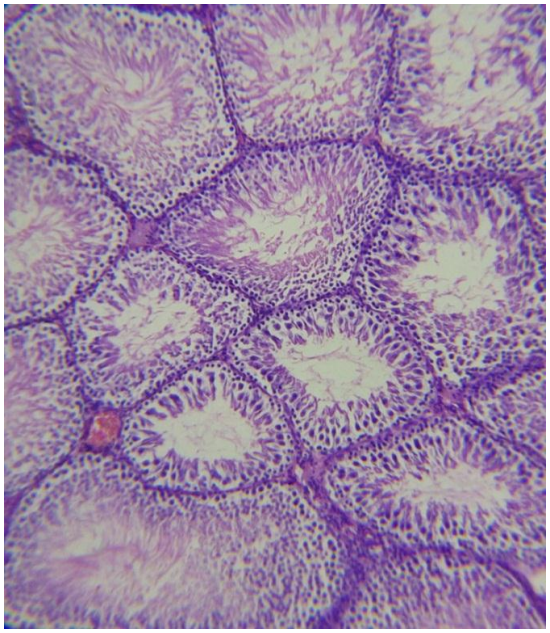


Gentamycin+ 400 mg/kg of VVC

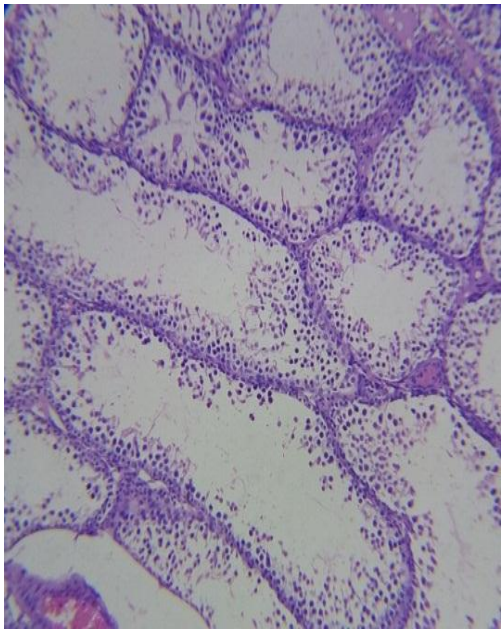
Histopathology of Rat Testes (H&E) Staining

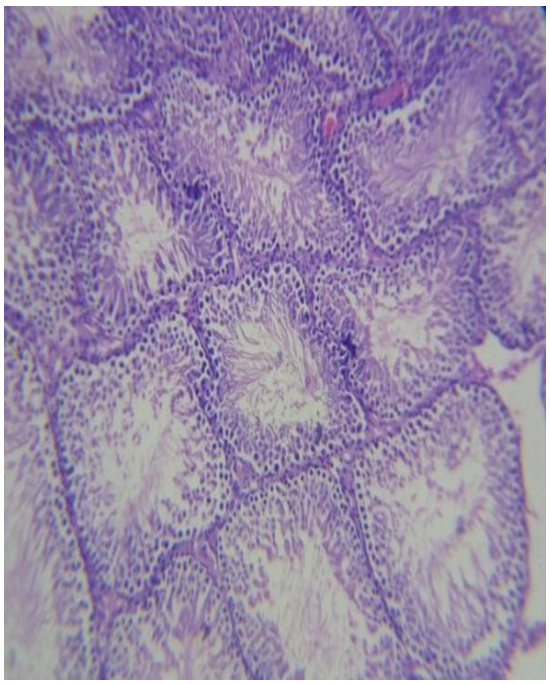
Low Power Magnification 10 X

Control Group

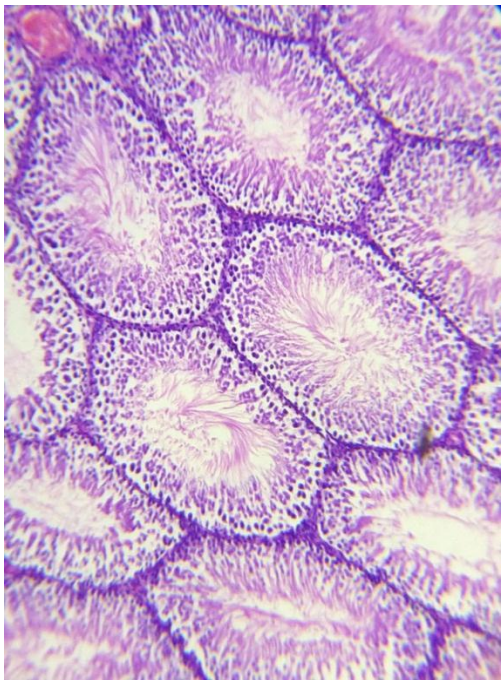


Gentamycin Induced group





Gentamycin+ 200 mg/kg of VVC

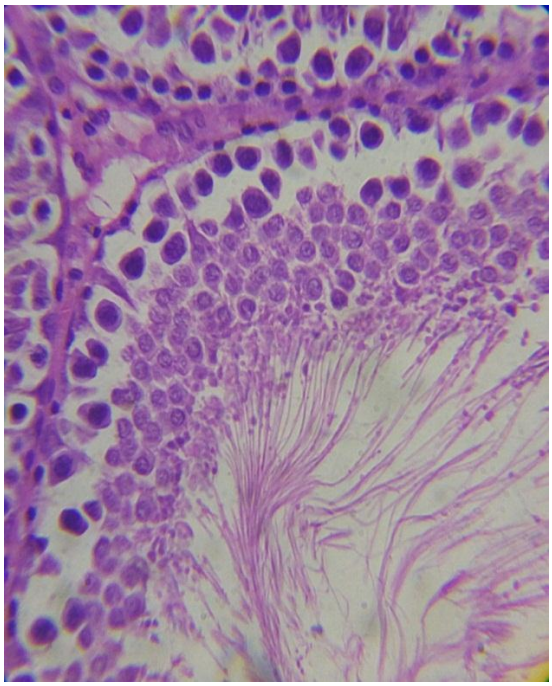


Gentamycin+ 400 mg/kg of VVC

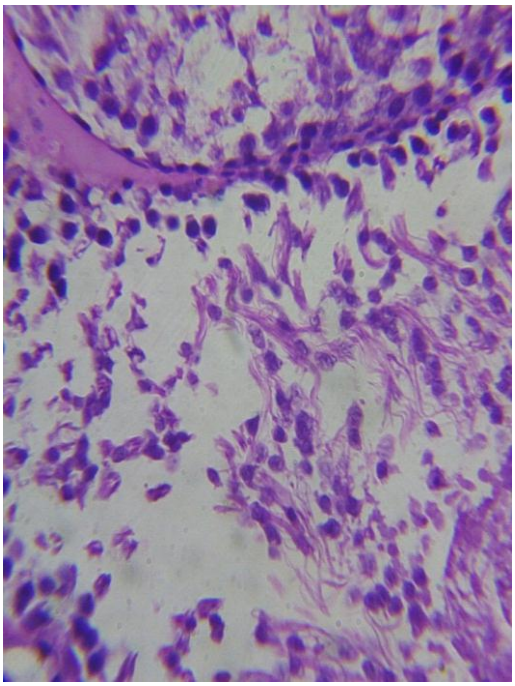
Histopathology of Rat Testes (H&E) Staining

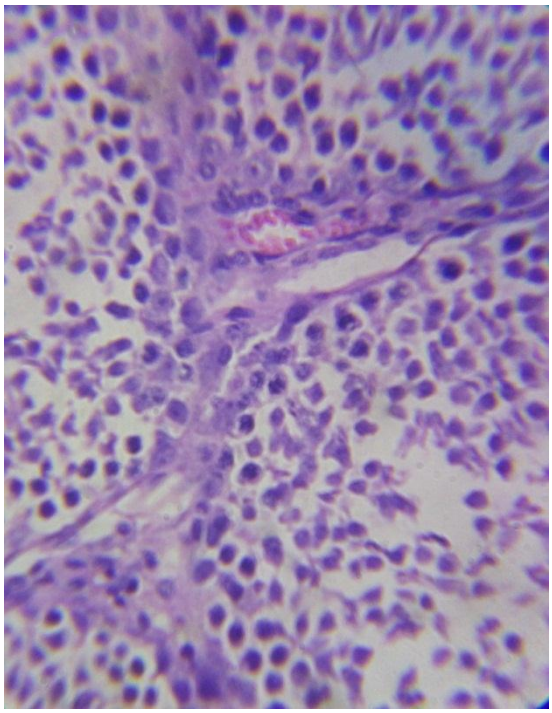
High Power Magnification 40 X

Control Group

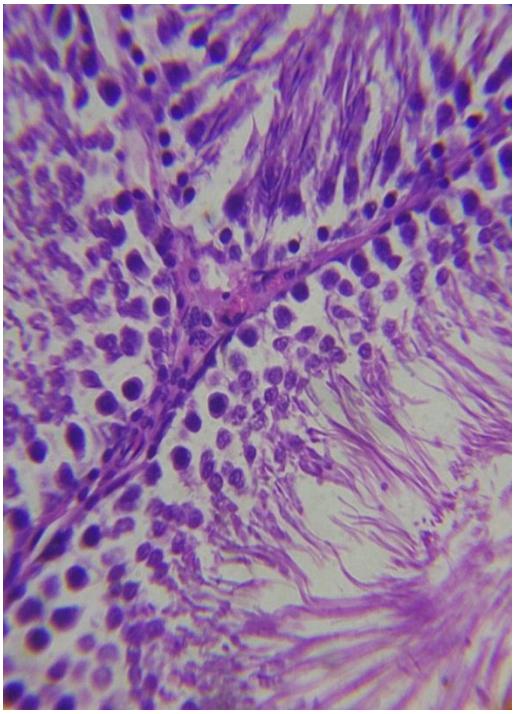


Gentamycin Induced group





Gentamycin+ 200 mg/kg of VVC

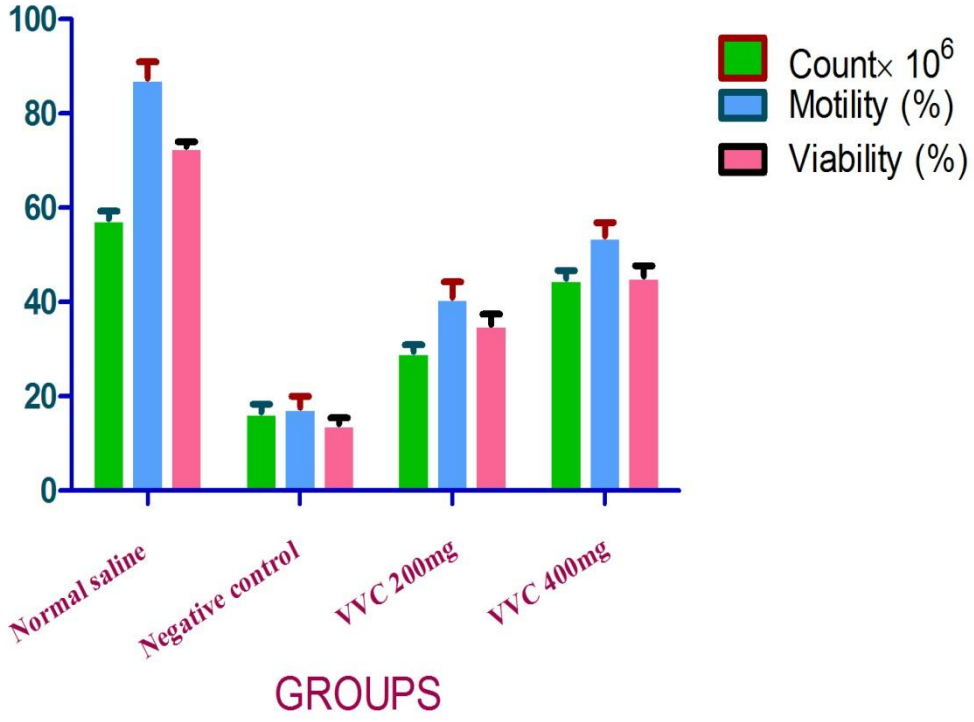


Gentamycin+ 400 mg/kg of VVC

Pathology Report

- Light microscopic observation of group I rats revealed well differentiated germ cells with respect of spermatogonia includes spermatid and sperm. Normal sertoli cell properly aligned on the basement membrane with oval dome shaped nucleus shows the normal morphology of the seminiferous tubule.
- Marginal interstitial fibrosis was observed in testicular tissue sample of rats belongs to group II. Significantly reduced number of primary spermatocytes was observed. Hypertrophic swollen seminiferous tubule with spacious lumen of sertoli cells was observed in sample belongs to group II.
- Seminiferous tubule and interstitial leydig cells appears atrophic with occasional evidence of collagen deposition. Increased number of early spermatids as an evidence of sperm rejuvenation was observed in the sample belongs to group III.
- Significantly increased level of pooled sperm oriented towards the center of sertoli cells with cluster of tail projected outside was observed in sample belongs to group IV. Primary spermatocytes with large centered nucleus and dense chromatin were observed in this group.

Effect of VVC in Sperm count, Motility and viability



சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், சென்னை - 600 106
सिद्ध केंद्रीय अनुसन्धान संस्थान,
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई – 600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106
Phone: 044-2621 4925, Fax: 044-2621 4809

21.6.2017

CERTIFICATE

Name of the student: Dr. M. Meeran Gani, III year PG student, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Name of the sample: Veeriya Viruthi Chooranam

Name of the Parameter	I	II	Mean
Loss on drying(at 105°C)	2.23%	2.24%	2.235%
Total ash	6.07%	5.97%	6.02%
Water soluble ash	1.59%	1.54%	1.56%
Acid insoluble ash	1.09%	0.94%	1.01%
Water soluble extractive	19.60%	19.50%	19.55%
Alcohol soluble extractive	12.10%	12.70%	14.40%
pH value (10%)	5.61		
TLC/HPTLC	Report Enclosed		

(R. Shakila)
Research Officer (Chemistry) & Head,
Department of Chemistry

21/6/17

(Dr. P. Sathiyarajeswaran)
Assistant Director (Siddha) I/c

1. Determination of Loss on Drying

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2. Determination of Total Ash

Incinerate 2 g accurately weighed, of the drug in a tared silica dish at 450ºC until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450ºC. Calculate the percentage of ash with reference to the air-dried drug.

3. Determination of Water Soluble Ash

Boil the ash obtained in the above test for 5 minutes with 25 ml of distilled water repeatedly; collect the insoluble matter on an ashless filter paper, and ignite to constant weight. Calculate the percentage of water soluble ash with reference to the air dried drug.

4. Determination of Acid Insoluble Ash

Boil the ash obtained in the above test for 5 minutes with 25 ml of dilute hydrochloric acid repeatedly; collect the insoluble matter on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

5. Determination of Alcohol Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

6. Determination of Water Soluble Extractive

Proceed as directed for the determination of Alcohol-soluble extractive, using distilled water instead of ethanol.

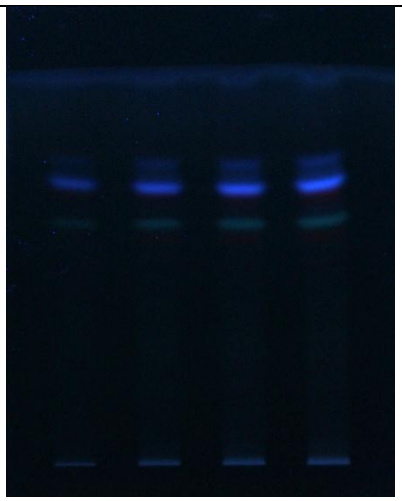
7. Determination of pH

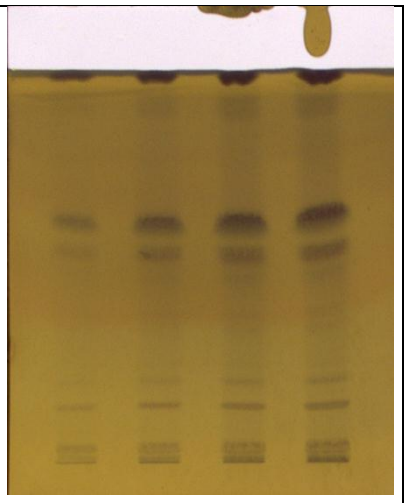
Take 10g of sample, add 100 ml of distilled water, stir well and filter. Use the filtrate for the experiment. Switch on the instrument. Give 30 minutes time for warming pH meter. Introduce the pH 4 solution first and adjust the pH meter by using the knob to 4.00 for room temperature 20°C, 4.01 for room temperature 25°C, 4.02 for room temperature 30°C. Introduce the pH 7 solution and adjust the pH meter to 7 by using the knob. Introduce the pH 9.2 solution and check the pH reading without adjusting the knob. Then introduce the sample solution and note the reading. Repeat the test four times and take the average reading as result.

Sample Name: *Veeriya Viruthi Chooranam*

Stationary Phase - Silica Gel 60 F_{254}

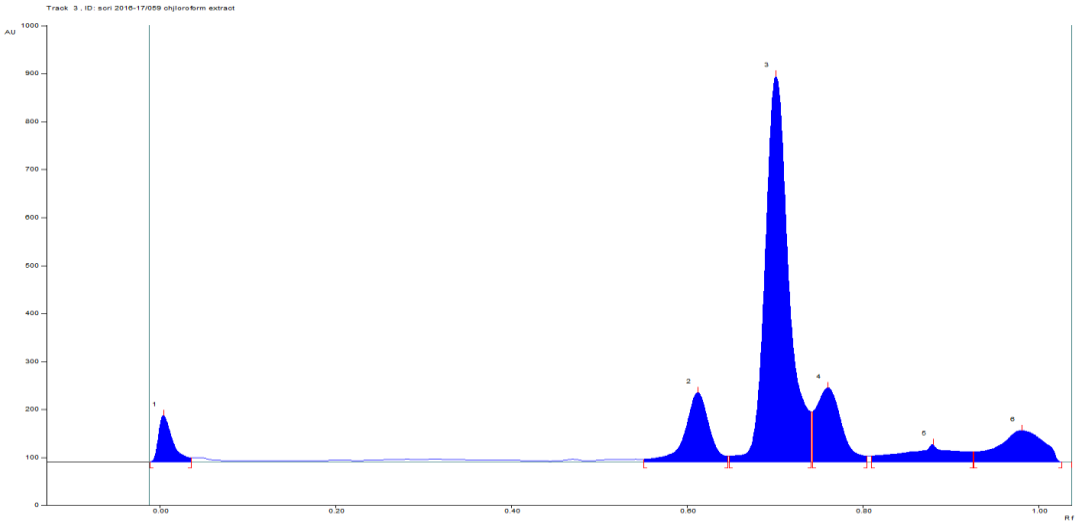
Mobile Phase - Toulene : Ethyl Acetate : Formic Acid (5:1;0.2 v/v/v)





λ = 366 nm		**λ = 575 nm (Derivatized)**	
Color	**R_f value(s)**	**Color**	**R_f value(s)**
Maroon	0.59	Ash	0.04
Light Green	0.62	Ash	0.15
Pink	0.67	Ash	0.21
Sky Blue	0.71	Light Blue	0.53
Sky Blue	0.75	Light Blue	0.61

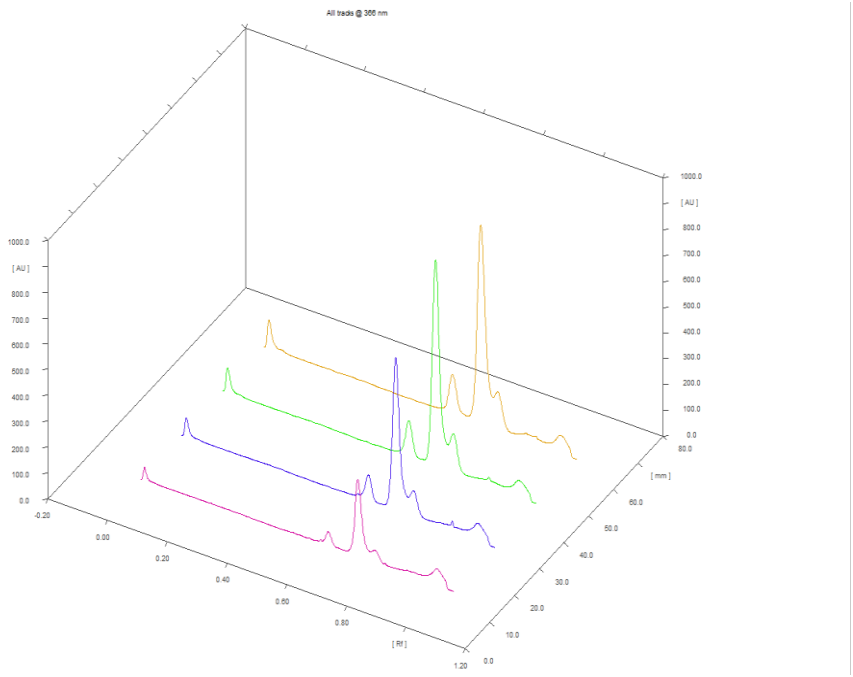
HPTLC Chromatogram @ 366 nm:



Peak Table @ 366 nm:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.5 AU	0.00 Rf	96.3 AU	7.41 %	0.04 Rf	8.7 AU	1360.2 AU	4.12 %
2	0.55 Rf	5.6 AU	0.61 Rf	144.3 AU	11.10 %	0.65 Rf	12.2 AU	3559.2 AU	10.79 %
3	0.65 Rf	12.3 AU	0.70 Rf	803.6 AU	61.80 %	0.74 Rf	04.2 AU	19144.4 AU	58.03 %
4	0.74 Rf	104.3 AU	0.76 Rf	154.6 AU	11.89 %	0.81 Rf	12.7 AU	4009.3 AU	12.15 %
5	0.81 Rf	12.8 AU	0.88 Rf	36.1 AU	2.77 %	0.93 Rf	20.8 AU	1888.7 AU	5.72 %
6	0.93 Rf	20.9 AU	0.98 Rf	65.5 AU	5.03 %	1.03 Rf	0.0 AU	3029.7 AU	9.18 %

3D Chromatogram @ 366 nm:



BIO-CHEMICAL ANALYSIS

BIO-CHEMICAL ANALYSIS OF TRIAL MEDICINES

Preparation of Sodium Carbonate extract:

2 gm of the sample drug is mixed 5 gm of Sodium carbonate and taken in a 100 ml beaker and 20 ml of distilled water is added. The solution is boiled for 10 minutes, cooled and then filtered. The filtrate is called sodium carbonate extract.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
I	**TEST FOR ACID RADICALS**		
1a	**Test for Sulphate** 2 ml of the above prepared extract is taken in a test tube. To this add 2ml of 4% Ammonium oxalate solution.	Absence of White Precipitate	Absent
b	2ml of extract is added with 2ml of dilute hydrochloric acid until the effervescence ceases off. Then 2ml barium chloride solution is added.	Absence of White Precipitate	Absent
2	**Test for Chloride:** 2ml of extract is added with dilute nitric acid till the effervescence ceases. Then 2ml of silver nitrate solution is added.	Absence of white precipitate Obtained	Absent
3	**Test for Phosphate** 2ml of the extract is treated with 2 ml of Ammonium molybdate solution and 2ml of concentrated nitric acid.	Yellow precipitate Obtained	Present

4	**Test for Carbonate:** 2ml of the extract is treated with 2ml of magnesium sulphate solution.	Absence of white Precipitate	Absent
5	**Test for Sulphide:** 1 gm of the substance is treated with 2ml of concentrated Hydrochloric acid	Rotten egg smelling	Present
6	**Test for Nitrate:** 1gm of the substance is heated with copper turnings and concentrated sulphuric acid and viewed the test tube vertically down.	Absence of reddish brown gas.	Absent
7a	**Test for Fluoride and oxalate** 2ml of the extract is added with 2ml of dilute acetic acid and 2ml of calcium chloride solution and heated.	White precipitate	Absent
b	5 drops of clear solution is added with 2ml of dilute sulphuric acid and slightly warmed to this, 1 ml of dilute potassium permanganate solution is added.	KMNO4 solution Discolourisation obtained	Absent
8	**Test for Nitrite** 3 drops of the extract is placed on a filter paper. On that, 2 drops a Acetic Acid and 2 drops of Benzidine solution is placed.	Absence of yellowish red colour	Absent
9	**Test for Borate** 2 pinches of the substance is	Absence of Green tinged flame	Absent

	made into paste by using Sulphuric acid and Alcohol (95%) and introduced into the blue flame.		
II	**TEST FOR BASIC RADICALS**		
10	**Test for lead** 2 ml of the extract is added with 2 ml of Potassium iodide solution.	Absence of Yellow precipitate	Absent
11a	**Test for Copper** One pinch of substance is made into paste with concentrated Hydrochloric acid in a watch glass and introduced into the non luminous part of the flame.	Absence of Bluish green coloured flame.	Absent
b	2ml of the extract is added with excess of Ammonia solution	Absence of deep blue	Absent
12	**Test for Aluminium** To the 2 ml of extract. Sodium Hydroxide solution is added in drops to excess	Absence of White Precipitate.	Absent
13a	**Test for Iron** To the 2 ml of extract, 2 ml of Ammonium Thiocyanate Solution is added.	Blood red colour	Present
b	To the 2 ml of extract, 2 ml of Ammonium Thiocyanate solution and 2 ml of concentrated Nitric Acid is added.	Blood red colour obtained	Present
14	**Test for Zinc** To the 2 ml of extract Sodium	Absence of White precipitate.	Absent

	Hydroxide solution is added in drops to excess.		
15	**Test for Calcium** 2 ml of the extract is added with 2 ml of 4% Ammonium Oxalate solution.	Absence of White precipitate.	Present
16	**Test for Magnesium** 2ml of extract, Sodium Hydroxide solution is added in drops to excess.	Absence of White precipitate.	Absent
17	**Test for Ammonium** 2 ml of extract few ml of Nessler‟s Reagent and excess of Sodium Hydroxide solution are added.	Absence of Reddish brown precipitate	Absent
18	**Test for Potassium** A pinch of substance is treated with 2 ml of Sodium Nitrite solution and then treated with 2 ml of Cobal Nitrate in 30% glacial Acetic acid.	Absence of Yellow precipitate	Absent
19	**Test for Sodium** 2 pinches of the substance is made into paste by using Hydrochloric acid and introduced into the blue flame	Absence of Yellow colour flame	Absent
20	**Test for Mercury** 2 ml of the extract is treated with 2 ml of Sodium Hydroxide solution.	Absence of yellow Precipitate	Absent
21	**Test for Arsenic** 2 ml of extract is treated with 2	Absence of Yellow precipitate	Absent

	ml of silver Nitrate solution		
22	**Test for Starch** 2ml of extract is treated with weak iodine solution	Absence of Blue colour	Absent
23	**Test of reducing Sugar** 5ml of Benedicts qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 10 drops of the extract and again boiled for 2 minutes. The colour changes are noted.	Green colour	Present
24	**Test of the alkaliods** 2ml of the extract is treated with 2ml of potassium lodide solution.	Absence of Red colour	Absent
25	**Test of the proteins** 2ml of the extract is treated with 2ml of 5% NaOH ,mix well and add 2 drops of copper sulphate solution.	Absence of Violet colour	Absent

RESULTS:

The given sample (*VEERIYA VIRUTHI CHOORANAM*) contains

Phosphate, Sulphide, , Iron,Calcium, Reducing sugar.

GOVERNMENT SIDDHA MEDICAL COLLEGE
Arumbakkam, Chennai-106

Communication Of The Decision Of Institutional Ethics Committee (IEC)

IEC No: GSMC-CH-ME-4/2015/007

Protocol title: A CLINICALSTUDYON AAN MALADU (MALE INFERTILITY) WITH THE EVALUATION OF SIDDHA DRUG VEERIYA VIRUTHI CHOORANAM
Principal Investigator: DR.M. MEERAN GANI
Name & Address of Institution : Government siddha medical college, Arumbakkam, Chennai-106
☑ New Review ☐ Revised Review ☐ Expedited Review
Date of review (DD/MM/YY): 26-03-2015
Date Of Previous Review, If Revised Application :
Decision of the IEC
☑ Recommended ☐ Recommended with suggestions
☐ Revision ☐ Rejected
Suggestions / Reasons / Remarks : 1. In Inclusion Criteria, add patients with sperm count less than 20 million, not 30 million.
Recommended for a period of 1 year from date of completion of preclinical studies:

Please Note:

- Inform IEC immediately in case of any adverse events/serious drug reaction.
- Seek IECapproval in case of any change in the study procedure, site and investigator
- This approval is valid only for period mentioned above
- IEC member have the right to review the trial with prior intimation.

26/3/15.

Dr.P.Jeyaprakashnarayanan
Chairman

Dr.V. Banumathi
Member Secretary

INSTITUTIONAL ETHICS COMMITTEE

Date:

Sub: IEC review of research proposals.

Ref:Your letter dated

MEMBERS	PARTICIPATION	SIGNATURE
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DR.V.BANUMATHI M.D(S)., Member Secretary		
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DR.P.SATHIYA RAJESWARAN M.D(S)., Clinician- Siddha	✓	26/3/15
DR.G.AADINAAATH REDDY,M.Pharm, Ph.D., Pharmacologist	✓	Aadinaathy 26/03/2015
DR.S.THILAGAVATHY Msc.,Ph.D., Social Scientist	✓	26/3/15
DR.T.MAHALAKSHMI M.A.,Ph.D., Linguistic Expert	✓	26/3/15
DR.P.VIDYA M.B.B.S., DMRD., Modern Medicine Expert	✓	26/3/15
MR.P.SARAVANAN., Puplic Person	✓	

26/3/15.

Dr.P.Jeyaprakashnarayanan
Chairman

Dr.V.Banumathi
Member Secretary

BIO STATISTICAL ANALYSIS

CLINICAL PROGNOSIS

Treatment for Aan Maladu:

The most popular non parametric statistical tool, namely, McNemar Test analysis has been employed to analyses the effectiveness with the help of a hypothesis.

S. No	Symptoms	Before Treatment	After Treatment
		n%	n%
1.	Premature Ejaculation	12(60)	4(20)**
2.	Erectile Dysfunction	8(40)	2(10)**
3.	Nocturnal Emission	9(45)	3(15)**

McNemat test, C.I: 95%, *P<0.05; **P<0.01

Software: spss17 version

Number of cases: 20

Inference:

Since the p value is significant in all symptoms. So there is significant reducing of symptoms among the patients for the treatment of Aan Maladu(Male Infertility). Hence it is concluded that the treatment was effective and **significant.**

BIOSTATISTICAL ANALYSIS

Effect of Medicine… on Semen Count in human subjects

S.No	Semen Count(million/cumm)	
	Before Treatment	After Treatment
1	22	50
2	13	30
3	25	58
4	12	55
5	22	24
6	19	60

7	42	60
8	15	48
9	21	40
10	45	48
11	18	50
12	9	52
13	16	48
14	15	45
15	8	50
16	14	67
17	10	25
18	23	38
19	3	5
20	15	30

Software: spss17 version

Variables: Semen Count (millions/cu mm) – before treatment, after treatment

Number of cases: 20

Test: Paired t test

Confidence Interval: 95%

Correlation coefficient (r): 0.436

Before and after treatment mean difference: 25.35±14..04

P Value (2 tailed): $p<0.001$.

Inference:

The p value is highly significant ($p<0.001$). So the treatment was significantly improving the Semen count (millions/cu mm).

BIOSTATISTICAL ANALYSIS

Effect of Medicine… on Semen Motility(%) in human subjects

S.No	Semen Motility (AM%)	
	Before Treatment	After Treatment
1	18	58
2	8	32
3	18	56
4	24	60
5	10	22
6	20	55
7	10	35
8	18	65
9	8	40
10	15	25
11	10	55
12	15	55
13	12	53
14	15	58
15	22	62
16	15	60
17	13	55
18	15	45
19	7	15
20	17	40

Software: spss17 version

Variables: Semen Motility (%) – before treatment, after treatment

Number of cases: 20

Test: Paired t test

Confidence Interval: 95%

Correlation coefficient (r): 0.578

Before and after treatment mean difference: 33.30±2.80

P Value (2 tailed): $p<0.001$.

Inference:

The p value is highly significant ($p<0.001$). So the treatment was significantly improving the Semen Motility (%).

GOVERNMENT SIDDHA MEDICAL COLLEGE

ARIGNAR ANNA GOVERNMENT HOSPITAL OF INDIAN MEDICINE

CHENNAI – 600 106

A CLINICAL STUDY ON **"*VEERIYA VIRUTHI CHOORANAM*"** IN THE TREATMENT OF

"AAN MALADU" (MALE INFERTILITY)

INFORMED CONSENT FORM

"I have read the foregoing information. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction.

I consent voluntarily to participate in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care".

"I have received a copy of the information sheet/consent form".

Date:

Station:

Signature of participant:

Signature of the Guide: Signature of the Investigator:

அரசு சித்த மருத்துவக் கல்லூரி, சென்னை-106

அறிஞர் அண்ணா மருத்துவமனை, சென்னை

ஆண் மலடு நோய்க்கான சித்த மருந்தின் (வீரீயவிருத்தி சூரணம்)

பரிகரிப்புத் திறனைக் கண்டறியும் மருத்துவ ஆய்விற்கான தகவல் படிவம்.

ஒப்புதல் படிவம்

ஆய்வாளரால் சான்றளிக்கப்பட்டது

நான் இந்த ஆய்வு குறித்த அனைத்து விபரங்களையும் நோயாளிக்கு புரியும் வகையில் எடுத்துரைத்தேன் என உறுதியளிக்கிறேன்.

தேதி: **கையொப்பம்:**

இடம்: **பெயர் :**

<u>நோயாளியின் ஒப்புதல்</u>

என்னிடம் இந்த மருத்துவ ஆய்வின் காரணத்தையும், மருந்தின் தன்மை மற்றும் மருத்துவ வழிமுறை பற்றியும், தொடர்ந்து எனது உடல் இயக்கத்தை கண்காணிக்கவும், அதனை பாதுகாக்கவும் பயன்படும் மருத்துவ ஆய்வுக்கூட பரிசோதனைகள் பற்றி திருப்தி அளிக்கும் வகையில் ஆய்வு மருத்துவரால் விளக்கிக் கூறப்பட்டது.

நான் இந்த மருத்துவ ஆய்வின் போது, காரணம் எதுவும் கூறாமல், எப்பொழுது வேண்டுமானாலும் இந்த ஆய்விலிருந்து என்னை விடுவித்து கொள்ளும் உரிமையை தெரிந்திருக்கின்றேன். நான் என்னுடைய சுதந்திரமாக தேர்வு செய்யும் உரிமையைக் கொண்டு ஆண் மலடு நோய்க்கான வீரீயவிருத்தி சூரணம் மருந்தின் பரிகரிப்புத் திறனைக் கண்டறியும் மருத்துவ ஆய்விற்கு என்னை உட்படுத்த ஒப்புதல் அளிக்கிறேன்.

தேதி : **கையொப்பம் :**

இடம் : **பெயர் :**

தேதி : **சாட்சிக்காரர் கையொப்பம் :**

இடம் : **பெயர் :**

உறவுமுறை :

துறைத்தலைவர் கையொப்பம் : ஆராய்ச்சியாளர்
கையொப்பம்:

ANNEXURE

CASE SHEET PROFORMA FOR "AAN MALADU"

GOVT.SIDDHA MEDICAL COLLEGE&HOSPITAL, CHENNAI-106

POST GRADUATE DEPARTMENT BRANCH –I MARUTHUVAM

Duration: 2015 - 2017

Op No/ Ip No	:	Occupation	:
Ward No	:	Income	:
Bed No	:	Nationality	:
Name	:	Religion	:
Age	:	D.O.A	:
Sex	:	D.O.D	:
Address	:	Diagnosis	:

1. Complaints and duration:

2. History of present illness :

3. History of past illness	**:**	**Yes**	**No**
Mumps / Orchitis	:		
Prostatitis	:		
STD	:		

Diabetes mellitus :

Hypertension :

Cardiac diseases :

4. Surgical history :

Hydrocele :

Varicocele :

Blockage of vas :

Hernia :

Obstruction of ejaculatory duct :

Trauma :

5. Personal history

A.Diet : Veg Non veg

B.Marital status : Single Married

C.Durationofmarriage(yr):

D.Consanguineous : Yes No

6. Sexual history

Erectile function	:	Normal	Affet
Ejaculatory effect	:	Normal	Affec
Frequency of intercourse	:	(per/month)	
Frequency of masturbation	:	(per/month)	
Lubricant	:	Yes	No
Nocturnal Emission	:	Yes	No

7. Respiratory rate :

8. Blood pressure :

9. Pallor :

10. Cyanosis :

11. Jaundice :

12. Clubbing :

13. Lymphadenopathy :

14. Acanthosis nigricans :

15. Hisutiom :

EXAMINATION OF VITAL ORGANS

1. CVS : Normal Abnormal

If abnormal details……..

2. CNS : Normal Abnormal

If abnormal details……..

3. Respiratory system : Normal Abnormal

If abnormal details……..

4. Digestive system : Normal Abnormal

If abnormal details……..

5. Urogenital system : Normal Abnormal

If abnormal details……..

LOCAL EXAMINATION

INSPECTION: Yes No

Hydrocele **:** Yes No

Varicocele : Yes No

Inguinal Hernia : Yes No

Filarial Scrotum : Yes No

Both testicles present in scrotum : Yes No

Painful coitus : Yes No

Burning Micturition : Yes No

Spermaturia : Yes No

Emotional stress : Yes No

Addiction : Yes No

If yes specify:_____

Bowel habit : Regular

Constipation

Sleep : Good Disturbed

Insomnia

Presence of anxiety Yes No

FAMILY HISTORY:

- No. of abortions his wife had Yes No
- Cardiovascular disease Yes No
- Tuberculosis Yes No
- Others Yes No

If yes specify :

HISTORY OF CONGENITAL ANOMALIES

Cryptorchidism	:	Yes	No
Hypospadias	:	Yes	No

DRUG HISTORY

Steroids	Yes	No
Anti depressant	Yes	No

GENERAL EXAMINATION

Physical build

Body weight

Temperature

Pulse rate

Heart rate

PALPATION

Size and Consistency of testicles

SIDDHA ASPECT NILAM

Kurinchi(Hills Areas) :

Mullai(Forest Areas) :

Marutham(Fertile Areas) :

Neithal(Sea Areas) :

Palai(Desert Areas) :

PARUVA KALAM

Kaar (Aavani-Puratasi) Aug-Sep :

Koothir (Iypasi-Karthigai)Oct-Nov :

Munpani (Maargazhi-Thai)Dec-Jan :

Pinpani (Maasi-Panguni)Feb-Apr :

Elavenil (Chithirai-Vaikasi)Apr-May:

Mudhuvenil (Aani-Adai)Jun-Jul :

YAAKKAI (UDAL NILAI)

Vaatham :

Pittham :

Kabam :

Kalappu :

GUNAM

Satthuvam :

Rajotham :

Thamasam :

PORI/PULANGAL (SENSORY ORGANS)

Mei –Sensation Vaai – Taste Kan – Vision

Mooku - Smell Sevi – Hearing

KANMENTHRIYAM/KANNMAVIDAYAM [MOTOR ORGANS]

Kai- Dhaanam Kaal-Kamanam Vaai-Vasanam

Eruvaai- Visarkkam Karuvaai-Aanantham

UTHKAAYA ATHAKAAYAM

Puyam[forearm] Sayam[arm] Kaal[leg]

Paaatham[feet]

UYIR THATHUKKAL

A.VATHAM

Piranan ……. Abanan …….

Viyanan ……. Udanan ……..

Samanan…….. Nagan…….

Koorman ……. Kirukaran…….

Devathathan…… Thananjeyan ……

B.PITHAM

Anar pitham :

Ranjaga pitham :

Saathaga pitham :

Pirrasaga pitham :

Alosaga pitham

C.KAPAM

Avalambagam :

Kilethagam :

Pothagam :

Tharpagam :

Santhigam :

UDALTHAATHUKKAL

Saaram :

Senner :

Oon :

Kozhuppu :

Enbu :

Moolai :

Sukkilam :

ENVAGAI THERVUGAL

1.Naa :

2.Niram :

3.Mozhi :

4.Vizhi :

5.Sparisam :

6.Malam :

7.Moothiram :

8.Naadi

a)Neer Kuri :

b)Nei Kuri : :

MALAM

Niram :

Edai :

Erugal :

Elagal :

MOOTHIRAM

1. Neerkuri

Niram :

Manam :

Edai :

Nurai :

Enjal :

2. Neikuri

SIGNS AND SYMPTOMS:

	Clinical Features	Before Treatment	During Treatment of every 7days							
	Premature ejaculation									
	Erectile dysfunction									
	Painful coitus									
	Nocturnal emission									
	Burning micturition									

LABORATORY INVESTIGATION

1. BLOOD : AT BT

TC

DC

ESR

Bleeding time

Clottingtime

Blood sugar

Blood urea

Serum cholesterol

VDRL

2. URINE

Albumin Sugar Deposits

3.SEMEN ANALYSIS

Semen analysis	Before Treatment	After Treatment
Count		
Viscosity		
Volume(ml)		
Liquification time		
Sperm concentration(millions/cumm)		
Motility(%)		
Active motile(%)		
Sluggish motile(%)		

Morphology

Other Investigation

TRIAL DRUG : *VEERIYA VIRUTHI CHOORANAM*

Dose : 1Gm (bd)

Anubanam : Milk

Duration of Treatment : 48 Days

Pathiam (Do's and Don'ts)

Prognosis at the end of the Treatment.

MEDICAL OFFICR **HEAD OF THE DEPARTMENT**

SIGNATURE **SIGNATURE**

DATE	DAILY REPORT	MEDICINE

ADVICE:

MEDICAL OFFICER:

H.O.D/Guide

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A CLINICAL STUDY ON

AAN MALADU

(MALE INFERTILITY)

WITH THE EVALUATION OF SIDDHA DRUG

VEERIYA VIRUTHI CHOORANAM

The dissertation submitted by

Dr.M.MEERAN GANI (Reg. No. 321411106)

Under the Guidance of

Prof. Dr. N. ANBU, M.D.(S)

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GOVERNMENT SIDDHA MEDICAL COLLEGE

CHENNAI – 106

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