

**PART I**

**HAEMATINIC ACTIVITY OF  
*ECHURAMOOI ILAI CHOORANAM***

*( Aristolochia indica Linn)*

**&**

**PART II**

**SPERMATOGENIC ACTIVITY OF**

**“*ANDA ODU PARPAM*”**

The dissertation Submitted by

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*Under the Guidance of*

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**GOVT. SIDDHA MEDICAL COLLEGE,  
CHENNAI-106**

**CERTIFICATE BY THE GUIDE**

This is to certify that the dissertation entitled “**Haematinic activity of *Echuramooli ilai chooranam (Aristolochia indica linn.)***” and “**Spermatogenic activity of *Anda odu parpam***” is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.I.S.Gnanavel** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

**Date:**

**Seal and Signature of the Guide**

**Place:** Chennai

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**ENDORSEMENT BY THE HOD**  
**PRINCIPAL/HEAD OF THE INSTITUTION**

This is to certify that the dissertation entitled “**Haematinic activity of *Echuramooli ilai chooranam (Aristolochia indica. Linn)*” and “Spermatogenic activity of *Anda odu parpam*” is a bonafide work carried out by **Dr.I.S.Gnanavel** under the guidance of **Dr.V.Velpandian, M.D(S), Ph.D.**, Lecturer, Post graduate department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.**

**Seal and Signature of the HOD**

**Seal and Signature of the Principal**

**GOVT. SIDDHA MEDICAL COLLEGE,**

**CHENNAI-106**

**DECLARATION BY THE CANDIDATE**

I hereby declare that this dissertation entitled “**Haematinic activity of *Echuramooli ilai chooranam (Aristolochia indica. Linn)*” and “Spermatogenic activity of *Anda odu parpam*”** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian, M.D(s), Ph.D.,** Lecturer, Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

**Date:**

**Signature of the Candidate**

**Place:** Chennai

**Dr. I.S.GNANAVEL**

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# 1. INTRODUCTION

Before entering into the study, let us know the basic concepts like origin of earth, origin of life in it, origin of medicine in mankind. Knowing these basics concepts reveals us some truths which glorify our great *Siddhars* that they are the pioneers to all modern scientists invariable to the department they belong, and also invariable to the country they belong. For many scientific evolutions even for today's nano concepts our *Siddhar* thoughts are the basics tools for the modern scientists which help them in their researches.

The age of earth still is a made of great debate. Some people calculated the age of the earth based on Bible that the earth was created in 4000BC. Later on in the middle of the nineteenth century, the great scientist, Charles Darwin believed that the earth must be extremely old because he recognized that natural, selection and evolution required vast amounts of time.

The history of earth describes the most important events and fundamental stages in the development of the planet. Nearly all the branches of natural science contribute in understanding the Earth's history. Biological and geological changes have been constantly occurring on our planet since the time of its formation. Organisms continuously evolve, taking on new forms or going extinct in response to an ever changing planet. This is quoted by our *Siddhars* as “மாற்றத்தால் ஆயது உலகம்”. Life on earth starts as small and microscopic and then into complex multicellular life's and experienced a rapid diversification into the most major phyla and then into chimpanzees to modern humans it was quoted as “பல்லாகி பூடாகி”. As soon as the humans originated in the earth many dramatic changes occurred in the aspects of knowledge of the living things. Sympathy, caring and love all these contribute more to show the difference between the mankind and their primitives. These feelings particularly sympathy on others pay the way for the origin of medicines. Thus sympathy is the basic step to treatment.

Crystallization of knowledge of early humans about plants and minerals helps to invent medicines. As soon as the man moves against the nature the word “disease” invades the mankind. According to World Health Organization, the definition of health is a state of complete physical, mental and social well being. So any system of medicine which fulfills this definition of health is said to be the best system of medicine. In this

way our Siddha systems is found to the best system of medicine as it heals the man in all aspects by its many wings like internal medicine, external medicine, *varma*, *yoga* and *noi illa neri* (social and preventive measures by holistic way).

In Siddha system of medicine all the diseases of human being can be categorized into 147 and classified into 4448 diseases. (Uthamarayan, 1953). The Siddha system is capable of treating all these types of diseases very effectively. *Paandu noi* is one among the important disease classified in the Siddha system of medicine based on the symptoms which literally means the pallor, which can be correlated with modern classification of Anaemia. A detailed description of signs, symptoms, aetiological factors and treatment have been described in detail by great Saint *Yoogi Muni* in his '*Yoogimuni Vaidhya Chinthamani*'.

Greek word anaemia means without blood. It is defined as a qualitative or quantitative deficiency of hemoglobin, a molecule found inside the red blood cells. Common causes are usually malnutrition, low intake of iron content food and worm infestations. So it is a very common disease in the developing countries like India where poverty and poor socio-economic conditions plays a vital role in causes of many diseases.

Iron deficiency is the most common and widespread nutritional disorder in the world. As well as affecting a large number of children and women in developing countries, it is the only nutrient deficiency which is also significantly prevalent in industrialized countries. The numbers are staggering: 2 billion people – over 30% of the world's population – are anaemic, many due to iron deficiency, and in resource-poor areas, this is frequently exacerbated by infectious diseases. Malaria, HIV/AIDS, hookworm infestation, schistosomiasis, and other infections such as tuberculosis are particularly important factors contributing to the high prevalence of anaemia in some areas. Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions (WHO Global Database on Anaemia, 2013). It also remains a problem even in developed countries where other forms of malnutrition have already been virtually eliminated.

Anaemia is characterized by a decrease of the concentration of hemoglobin and then the lack of erythrocytes. The anaemia prevalence remains high in India, with an overall incidence of 64.6% in children, 55.8% among pregnant women and 44.4% among young girls (WHO, 2011). The Ministry of Public Health of India investigated in

September 2011 an anaemia frequency of 56.9% among children, 27.3% among women of reproductive age and 19.2% among men. Factors contributing to anaemia may be related not only to malnutrition and poverty, but also from the free radicals due to the excessive consumption of drugs as well as the viral and parasitic infections.

I have witnessed many patients in the outpatient department during my graduation, who are the victims of anaemia and most of them are below the poverty line. This spirit made me to carry out a work in anaemia and come out with a most effective medicine at low cost effect. “Only healthy people can make a healthy India”. This is the main motivation for the work done.

I selected *Paandu noi*, which can be correlated with the clinical condition called iron deficiency anaemia for this dissertation work. Any failure to treat *Paandu* can lead to dangerous end often fatal consequences. So I selected *Echuramooli ilai chooranam* to study for its therapeutic efficacy on *Paandu noi*.

## 2. AIM AND OBJECTIVES

### Aim

The main aim of this dissertation is to do a scientific review, to validate the safety and efficacy of the *Echuramooli ilai chooranam* for *Paandu noi* (Anaemia) by pre clinical as well as clinical studies.

### Objectives

- Besides the scientific study, basic concepts of Siddha science and treatment aspects also our aim. Hence, the following methodology was adopted to evaluate the safety and efficacy of the test drug.
  - ❖ Identification of the herbal drug *Echuramooli*.
  - ❖ Collection of various Siddha and modern scientific literature.
  - ❖ Preparation of drug according to the text.
  - ❖ Physio-chemical analysis of *Echuramooli Ilai*.
  - ❖ Phyto chemicals analysis test drug.
  - ❖ Evaluation of the toxicity of test drug.
  - ❖ Evaluation of Haematinic activity of test drug
  - ❖ Clinical assessment of *Echuramooli Ilai* on Anaemia.

### 3. REVIEW OF LITERATURE

#### 3.1 Gunapadam aspect

ஈசுரமூலி

Other names:

பெருமருந்து

பெருங்கிழங்கு

தலைச்சுருளி

தராசுக் கொடி - குணபாடம் (மூலிகை வகுப்பு) முருகேச முதலியார்

Orgonoleptic Characters:

சுவை	-	கைப்பு
தன்மை	-	வெப்பம்
பிரிவு	-	கார்ப்பு
செய்கை	-	வெப்பமுண்டாக்கி உரமாக்கி ருது உண்டாக்கி

குணம்

“பாண்டகற்று மெய்யிற் படர்குட்ட நோய் விலக்கம்  
நீண்ட விருதயநோய் நீக்குங்காண் - தாண்டதப்பை  
முன்னே யொழித்துவிடு மூவாத் தலைச்சுருளி  
யென்னே யுலகி லிசை”

(அ.கு)

இதன் இலைக்கு கைப்புச்சுவையிருப்பதால் இலையை உலர்த்திப் பொடி செய்து 2 கிராம் வீதம் காலை மாலை கொடுக்க சொறி, சிரங்கு, படர்குட்டம், நஞ்சு, இருதயநோய், பாண்டு, சோபை, சுரம் நீங்கும்.

- சித்த வைத்திய பதார்த்த குண விளக்கம் மூல வர்க்கம்

ஈசுரமூலி சேரும் மருந்துகள் :

1. ஐந்தெண்ணையத் தைலம் - சொறி சிரங்குக்கு

தானென்ற ஐந்தெண்ணையத் தைலம் கேளு  
சாரியாம் நிம்பெண்ணைய இலுப்பை எண்ணெய்  
தேனென்ற நல்லெண்ணைய தேங்காய் எண்ணெய்  
சிற்நேரண் டத்தெண்ணைய ஐந்துங் கூட்டு  
மூலமொடு குங்கிலியம் நவச்சா ரந்தான்  
முனையான ஆனைதிப் பிலியும் சேரத்  
தாலமிகு சிற்றரத்தை பேர ரத்தை  
தனியான பெருமருந்து முசுறு முட்டை

கோலமிகு மயிலிறகு அண்டத் தோடு  
குதிரைக்காற் குளம்புசீவல் ஆகா சத்தூள்  
ஞாலமிதிற் பூத்திடுகா ளானும்மூட  
நற்கடலின் நுரையோடு சமுத்ராப் பழமே.  
பறந்துபோம் உள்குத்து புறவீச் சோடு  
**பாண்டு**இசிவு பிரமேகம் சிரசு ரோகம்  
இறந்துபோம் பெருவயிறு சோகைகா மாலை  
ஏகாத நீர்க்கோவை குமர கண்டம்  
பறந்துபோம் குதிரைவலி முயல்வ லிப்பு  
பழுதான காக்கைவலி நடுக்கு வாதம்  
துறந்தோடும் சூனியமும் பில்லி சிரங்கு  
சொறிகரப்பான் குஷ்டம்வண்டு எலியும் போமே.  
- போகர்வைத்தியம் 700 (Page 149)

## 2. இரத்த குன்மாதிக்கு மருந்து

வெள்ளைச் சாரணை வேர்  
கொடிவேலி வேர்  
கள்ளிக் கொழுந்து  
சாதிக்காய்  
**ஈசுவரமூலி**  
கருவேல் இலை  
எருக்கின் வேர் பட்டை  
சீரகம்  
கருஞ்சீரகம்  
அரிசித்திப்பிலி  
சுக்கு  
இந்துப்பு  
வசம்பு  
மிளகு  
பொரித்த பெருங்காயம்

இவைகள் வகைக்கு 1 பலம்

கடுகு - 1 உழக்கு  
வெள்ளைப்பூண்டு - 1 பலம்  
நல்ல பிண்ணாக்கு - ஒரு கையளவு

இவைகளை ஒன்றிரண்டாகப் பொடித்துக் கல்வத்திலிட்டுக் குப்பைமேனிச் சாற்றினால் நன்கு அரைத்து சுண்டைக்காய்ப் பிரமாணம் உருட்டவும். இதை நல்லெண்ணையிலுர வைத்துத் தினந்தோறும் அதிகாலையில் சாப்பிட இரத்த குன்மம் தீரும்.

- சரபேந்திரர் வைத்திய முறைகள்(Page :120)

3. சிலந்திக்கு எண்ணெய்

நன்னாரி, சிறுகுறிஞ்சான் வேர், நிலவேம்புவேர். அந்தரத்தாமரை வேர், நெல்லிமுள்ளி, ஈசரமுலி, கொழிஞ்சிவேர், பருத்திவேர், பேய்ப்பீர்க்கு வேர், மணத்தக்காளி வேர் இவற்றை ஓரளவாகக் கூட்டியரைத்து அத்துடன் வெள்ளாட்டு நீர் வார்த்துப் பிசைந்து வடிகட்டவும். அதில் வேப்பெண்ணெய், நல்லெண்ணெய், தேங்காயெண்ணை, புங்கெண்ணெய் இவை நான்கும் ஓரளவாய் எடுத்துச் சேர்த்து முறைப்படி வடித்திறக்கவும். அளவு : தேவையான அளவு உள்ளூக்குக் கொடுத்து மேலேயும் பூசி வெந்நீர் விட்டு குளிக்கத் தீரும்.

- அகத்தியர் வைத்திய காவியம் 1500(Page: 624)

4. கரப்பானுக்கு மருந்து :

நன்னாரிவேர் நெடுஞ்சில் வேர்  
சங்கம் வேர் நத்தைச்சூரி வேர்  
**பெருமருந்து வேர்** வெள்ளைப் பூண்டு  
ஆவாரைவேர் வசம்பு

இவைகளை வகைக்கு 1 வராகனடை வீதமெடுத்து தூள் செய்து, ஆமணக்கெண்ணெய் 1 சேரில் குழப்பி அடுப்பின் மேலேற்றி எரித்துப் பதத்திலிறக்கவும். இதில் ஒரு துட்டெடையும் கொள்ளக் கரப்பான் தீரும். இத்தைலத்தை கரப்பான் புண்களிலும் இடலாம்.

- சரபேந்திரர் வைத்திய முறைகள்  
(விரணம், கரப்பான் ரோக சிகிச்சை)

5. செங்கரப்பானுக்கு மருந்து

செங்கத்தாரிவேர் எருக்கம் வேர்  
சிறுகுறிஞ்சான் குறட்டை வேர்  
**பெருமருந்துவேர்** பூண்டு  
வசம்பு

இவைகளைச் சமனெடுத்து (1 பலம்) பசுவின் பால் விட்டு நன்றாக அரைத்து நல்லெண்ணெய் 1 1/2 சேருடன் கூட்டி அடுப்பின் மேலேற்றி எரித்துப் பதத்திலிறக்கவும். இந்த எண்ணெயை ஒரு ஸ்பூனிலெடுத்து உள்ளூக்குச் சாப்பிடவும். இதையே செங்கரப்பான் புண்களின் மீது பூச தீரும்.

- சரபேந்திரர் வைத்திய முறைகள் (Page: 256)

6. ஓடுகரப்பானுக்கு மருந்து

நிலவேம்பு **பெருமருந்து**  
சிவனார்வேம்பு அந்தரத்தாமரை  
சிறுகுறிஞ்சான் உத்தாமணி  
நீர் முள்ளி துளசி



மணத்தக்காளி நன்னாரி  
பேய்ப் பீர்க்கங்காய்

- இவைகளின் வேர் வகைக்கு 1 வராகனெடை

வசம்பு, பூண்டு இவ்விரண்டும் வகைக்கு 1 வராகனெடையும் எடுத்து இடித்துத் தண்ணீர் விட்டு நன்றாக அரைத்து,

தேங்காயெண்ணெய்	-	1 உழக்கு
இலுப்பெண்ணெய்	-	1 உழக்கு
புங்கெண்ணெய்	-	1 உழக்கு
நல்லெண்ணெய்	-	1 உழக்கு

முதலானவைகளுடன் ஒன்ற கலந்து அடுப்பின் மேல் ஏற்றி எரித்துப் பதத்திலிறக்கவும். இந்த எண்ணெயில் ஒரு வராகனெடையெடுத்து உட்கொண்டு, புண்களின் மேலேயும் போட ஓடுகரப்பான் தீரும்.

- சரபேந்திரர் வைத்திய முறைகள் .

7. விப்புருதிக்குத் திருகுக்கள்ளியெண்ணெய்

திருகுக்கள்ளிவேர்	அமுக்கிராகிழங்கு
கொடிவேலிவேர்	பெருமருந்து வேர்
ஆதண்டை வேர்	உத்தாமணி வேர்
சங்கங்குப்பிவேர்	பிரண்டைவேர்
பெருங்காயம்	மிளகு
கருஞ்சீரகம்	வெங்காயம்
கடுக்காய்த்தோல்	சுத்திசெய்த காந்தம்
பூதகரப்பான்பட்டை	இந்துப்பு
ஓமம்	சுக்கு

முதலான சரக்குகளை வகைக்கு ஒரு பாக்களவெடுத்து இடித்து பின்பு அம்மியின் மேல் வைத்துத் தண்ணீர் விட்டு அரைத்து வேப்பெண்ணெய் ஆமணக்கெண்ணெய் இவ்விரண்டும் தனித்தனியே 1¼ சேர் வீதமெடுத்து ஒன்று கூட்டி அதனுடன் குழப்பி அடுப்பின் மேல் வைத்து பதமாகக் காய்ச்சி இறக்கவும்.

இந்த எண்ணெயில் ஒரு ஸ்பூன் அளவெடுத்துத் தினந்தோறும் அதிகாலையில் உட்கொள்ளவும்.

**பத்தியம்**

உப்பு, புளி, முதலியவைகளை நீக்கவும். விப்புருதிகள் தீரும்.

- சரபேந்திரர் வைத்திய முறைகள் (Page: 324)

8. கரப்பானுக்கு மருந்து

கருஞ்சீரகம்	பூதகரப்பான் பட்டை
பற்படாகம்	வெங்காயம்
பெருங்காயம்	வசம்பு
கௌரி	காட்டு மொச்சை

சடைச்சி	மிளகரணை வேர்
சிறுகாஞ்சொறி	சத்திச்சாரணை
பொன்முசுட்டை	பெருங்குரும்பை
புனலி தண்டு	<b>பெருமருந்து</b>
நன்னாரி வேர்	கொடிவேலி வேர்
சிறு குறட்டை	குப்பைமேனி வேர்
சிற்றவரை கிழங்கு	

இவை அனைத்தும் வகைக்கு ஒரு பலம் வீதம் எடுத்து கற்கமாக்கி பசுநெய் ஒரு படியுடன் கலந்து அடுப்பிலேற்றி வடிக்கவும்.

அளவு : 1 காசெடை 2 வேளை

அனுபானம் : பால்

- சரபேந்திரர் வைத்திய முறைகள் கர்ப்பிணி

பாலரோக சிகிச்சை (பாகம் 4) (Page 235)

## 3.2 Botanical Aspect

### Classification

1. According to Bentham & Hooker's (1876) classification "*Aristolochia indica*" is classified as follows:

Kingdom	:	Plant Kingdom
Division	:	Angiosperms
Group	:	Dicotyledons
Class	:	Monochlamydeae
Series	:	Multiovulate terrestris
Family	:	Aristolochiaceae
Genus	:	<i>Aristolochia linn</i>
Species	:	<i>indica linn</i>

### Vernacular Names

Tamil	:	<i>Isvaramooli, Karuta kodi, Garudakkodi, Perumkizhangu</i>
English:		Indian Birth wort

As the name *Iswari* indicates the plant is believed to have the power to neutralize or resist snake poison.

### Habitat

- The plant is distributed in all the provinces of India and in Srilanka, Nepal and Bangladesh.
- It is usually found scrambling over hedges and bushes.
- It can be propagated by seeds, which generates in and about two weeks.

### Habit and General Features

*Aristolochia indica linn* is a glabrous perennial twiner with a long slightly tuberous or stout root that penetrates deep into the soil. The younger branches and tender shoots are slender striated and smooth while stems and older branches are woody strong and flexure and covered with corky bark. The former bear simple alternate short-petioled entire, membranous very variable three nerved leaves, the shape varying from linear to

obovate or sub-panduri form and from 10 x 1.2 or 1.8 cm to 18 x 8 cm in size. The flowers are irregular, greenish-white to light Purplish and 2.5 cm or more long. These have a tubular perianth with a swollen base a long narrow neck and an obliquely trumpet-shaped mouth or lip. The fruits are septicidally dehiscent pendent capsules tightly packed with numerous flat triangular broadly winged or very thin seeds. All parts of the plant have a bitter taste and emit when crushed a characteristic sharp nauseous odour.

## **External Morphology**

### **Leaves**

Simple, alternate, short-petioled, the petiole- from 6 to 8 mm long, very slender with its base dilated and often with a stipule like prophyll or reduced leaf of the undeveloped auxiliary bud; blade somewhat wedge-shaped or obovate, very variable in shape and size. The shape varies from linear to obovate, oblong or sub-panduri form and the size in the narrowest forms are about 10 cm by 1.2 or 1.8cms and in the larger and broader forms from 10 to 18 cm by 7.5 cm at the broadest part which may be at the base, middle or above the middle, entire membranous or thin, smooth, the base cuneate rounded or shallowly or slightly cordate and mostly three or occasionally five-nerved and the tip obtuse or abruptly or gradually obtusely acuminate or even apiculate tender leaves are light purplish.

### **Ethno Medical Information:**

Extracts or Isolates of *Aristolochia indica* containing aristolochic acid.

The chief active principle of the drug is aristolochic acid, though aristolic acid and p-coumaric acids also appear to contribute to the activities of the drug.

Aristolochic acid is 8-methoxy-3; 4-methylene – dioxy – 10 – nitrophenanthrene – 1 – carboxylic acid.

It is intensely bitter and is optically inactive. It is the same as isoaristolochic acid, aristolochia yellow, aristic and aristolochic acids, but is different from aristolochine now identified as 1-curine.

Isolation of the acid by column chromatography and crystallization from the mixture of dimethyl formamide and ethanol resulted in yellow-orange crystals with mp 275-78<sup>0</sup>

Aristolochic acid can also be isolated by extracting the plant material with alcohol (95%) or ammonium hydroxide (5%) and chromatographing the extract.

Aristolochic acid possess anti cancer activity.

It is the active against adeno carcinoma and ascetic hepatoma in rats.

It is also active against Ehrlich ascities carcinoma in mice but is inactive against a wide spectrum of experimental neoplasms.

It stimulates phagocytic activity in guinea pigs administered chloromphenicol, cyclophosphamide and to a limited extent prednisone.

Aristolochic acid increased the defense mechanism of the eye against virus infections. The application of Aristolochic acid leads to a more rapid healing of the lesions. The animals treated with aristolochic acid show increased number of micro and macrophages in the cornea and in the vitreous body respectively.

Aristolochic acid is used for stimulating phagocytosis in infectious diseases in formulation with antibiotics.

Aristolochic acid stimulates phagocytic function of reticulo – endothelial system in rats.

### Phytochemical studies:

Constituents	Mol. Formula	mpoC
Aristolochic acid 1a, 2, 3a, 4a	$C_{17}H_{11}NO_7$	275-78
Aristolochic acid – D 3a – 4	$C_{17}H_{11}NO_8$	269-72
Aristolochic acid – D Me either lactam 4	$C_{18}H_{11}NO_5$	350-55
Aristololactam3	$C_{17}H_{11}NO_5$	315-17
Aristolochic b-D-glucoside 3a,4	$C_{23}H_{21}NO_9$	330-33
Aristolic acid	$C_{17}H_{12}O_5$	292
Aristoloamide 3a	$C_{18}H_{14}O_5$	172
Aristoloamide3a	$C_{17}H_{13}NO_4$	293-94
Methyl aristolochate	$C_{18}H_{13}NO_7$	285-86
6-Methoxy Me-aristolate	$C_{19}H_{16}O_6$	206-07

## Alkaloids:

Constituents	Mol. Formula	mpoC
1 – Curine (aristolochine) 5a, 1c, *	$C_{36}H_{38}N_2O_6$	215 (MeOH)
Unidentified Sesquiterpenoids	(Mol wts, 608; 622, 636)	Bp oC
Ishwarane6a	$C_{15}H_{24}$	80-82/1 mm
Ishwarane1b	$C_{15}H_{24}$	104-05/1 mm
Aristolochane 6a	$C_{15}H_{24}$	85/0.5 mm
Selina-4(14), 11-diene 5b+	$C_{15}H_{24}$	80-85/0.3 mm
Sesquiterpene A7	$C_{15}H_{24}$	112-12.5/6 mm
Sesquiterpene B7	$C_{15}H_{24}$	113/6 mm
Ishwarol 1c 5c	$C_{15}H_{24}O$	110/1 mm
Ledol 7,5d, 3a	$C_{15}H_{26}O$	mp 103-04
Isomer of ledol 3a	$C_{15}H_{26}O$	mp 150
Ishwarone 1c, 6b, 8	$C_{15}H_{26}O$	mp 57

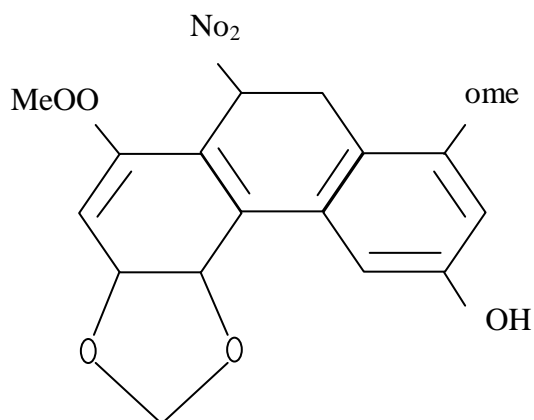
## Steroids

Constituents	Mol. Formula	mpoC
b-Sitosterol-1a	$C_{29}H_{50}O$	134-35
Strerol glycoside 1a	$C_{29}H_{50}O$	285-90
Stigmast-4-en-3-ohne3a,b	$C_{29}H_{47}O$	80-81

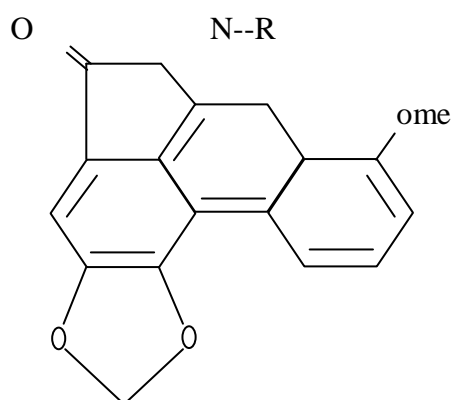
## Others

Constituents	Mol. Formula	mpoC
Ceryl alcohol-1a	$C_{26}H_{54}O$	79
Allantoin-1a	$C_4H_6N_4O_3$	232 (decomp)
p-Coumaric acid 3a	$C_9H_8O_3$	210-13 (206)
d-Camphor 1b, 7	$C_9H_8O_3$	177

**Structure elucidation of Phytochemicals in *Aristolochia indica***



**Aristolochic acid**



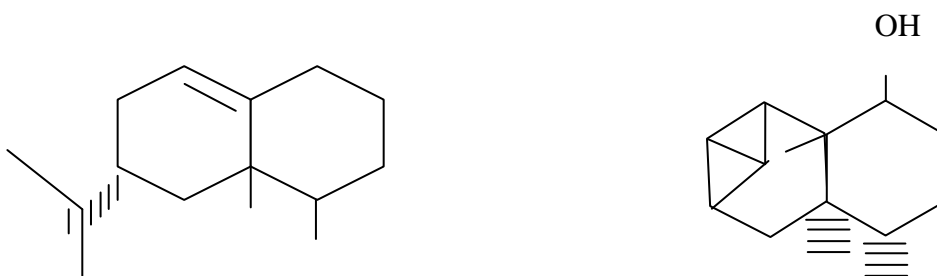
R = H

R<sup>1</sup> = ome Aristolactam

- β - D - glucoside

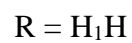
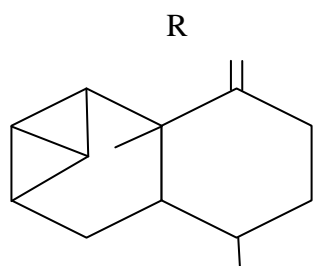
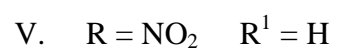
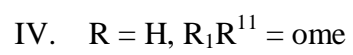
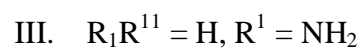
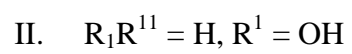
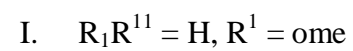
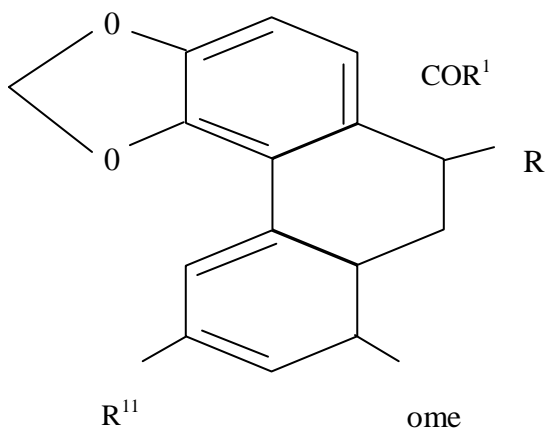
R - Glu R<sup>1</sup> = H

**Aristolochic acid Ome ether lactam**



**Aristolochine (C<sub>17</sub>H<sub>19</sub>O<sub>3</sub>N)**

**Ishwarol**





### 3.3. Siddha aspect of the disease

#### பாண்டு

#### வேறு பெயர்

வெண்மை நோய்  
வெளுப்பு நோய்

#### இயல்

“கழிவாகுந்த தேகமப்பா காணத்தச வத்தாய்

வற்றிவிடு மன்னவாசல் கேட்கில்

பழிகாரர் முகத்தினால் முரியார் போல பாண்டெல்லாம்

வெளுத்து குடித்தது ரத்தம்”

- அகத்தியர் வைத்திய காவியம்

“தேகத்திலே இரத்தம் வற்றி

தீங்கான விந்த நோய் காணுமப்பா”

- அகத்தியர் குணவாகடம்

இயற்கை நிறம் மாறி, உடல் வெளுத்து கண், நகக்கண் இவைகளை நீக்கிப் பார்த்தால் குருதியின்றி வெளுத்திருக்கும் நோயாகும்.

#### நோய் வரும் வழி

அறிந்துமே உற்பத்தி சொல்லக் கேளாய்

அதிசாரமலமிளகி யெந்நேரந்தான்

பிறிந்துமே புளியுப்பு பெருத்தலாலும்

பெத்தமாமக்கினி யிருத்தலாலும்

மிறிந்து தாம் பூலமிக அருந்தலாலும்

மீறியெமதுக் களைத்தான் புசித்தலாலும்

பறிந்துமே பகல் நித்திரை செய்தலானும்

பாண்டுவந்து பாரிலுள்ளோர் படும் பாடாமே

பாடான பஞ்சு தனைத்தாருடினோர்க்கும்

பாங்கான சிவதுகிலைப் பற்றினோர்க்கும்

மாடான பசுவதைப் பட்டினியதாக

வைக்கின்றோர் மறைவழியே நடத்திடாதார்

காடான வாரணியந்தனிற்வழி பறித்துக்

கடுவதைகள் செய்கின்றோர்கண் காணாத

கோடான பழி சொல்லிக் குடிகெடுக்குங்

கொடும்பாவி பாண்டுவினாற் குதிகொள்வாரே

- யுகி வைத்திய சிந்தாமணி

### குறிகுணங்கள்

உணவு முதலிய வேறுபாடுகளால், தீக்குற்றம் மிகுந்து குருதியின் நிறத்தையும் எடையையும் கெடுத்து, உடற்க்கு வேண்டிய ஊட்டத்தையும் கொடாமல், உடலை வெளுக்கச் செய்யும்.

சிறிது தொலைவு நடக்கினும் கால் ஓய்ந்து போதல், பெருமூச்சு வாங்கல் உணவில் விருப்பமின்மை, வாய்க்குமட்டல், தலைசுற்றல், கண் இருளல், அடிக்கடி மயக்கமாதல், மார்பு துடித்தல், உடல் இளைத்தல் ஆகிய குறிகுணங்களையும் காட்டும்.

### நோய் எண்.

குற்றத்தால் வருவன நான்கும், நஞ்சால் வருவன ஒன்றும் கூடி ஐந்தாகும். 1.வளி(வாதப் பாண்டு) 2.(பித்தப்) பாண்டு 3.ஐய(கபப்) பாண்டு 4.முக்குற்றப் (திரிதோஷ) பாண்டு 5.நஞ்சு(விட) பாண்டு என ஐந்தென வகுத்துள்ளார். இன்னும் மண்ணுண் வெளுப்பு (பாண்டு நோய்) ஒன்றுள்ளது. குருதிக் குறைவால் உடற்கட்டுகள் மெலிந்து உடல் வீங்கி, நிறம்மாறி மஞ்சள் அல்லது நீலநிறம் பெற்றும், மிகுந்த நீர்வேட்கை, அடிக்கடி மயக்கம் வருதல், மனச்சோர்வடைதல், அறிவு தடுமாறல், ஆண்மை குறைதல் என்னும் குறிகளையும் காட்டும்.

### பொதுக்குறி குணங்கள்

உடல் வன்மை நாளுக்கு நாள் குறைந்து நடக்க இயலாமை, தலைநோதல், மார்பு துடித்தல், கண் அடிக்கடி இருளல், தலைச்சுற்றல், மயக்கமுண்டாதல், மூச்சுத் தடுமாறல், பசித்தீக் கெடல், உணவு வேண்டாமை, உண்ட சிறு உணவும் வாந்தியாதல் ஆகிய குறிகள் தோன்றும்.

இன்னும், மிகவும் வெளுத்துத் தோல் சுருங்கல், உடல் மெலிந்து பளபளத்து வெளுப்பாதல், நகக் கண்கள் தடித்து வெளுத்தல், நாவெடித்துப் புண்ணாதல், தொண்டை கட்டல் ஆகியவை உண்டாகும்.

இந்நோய் பெண்களுக்குண்டாயின், சூதகத்தில் வெளியாகும் குருதி, தன்னிறம், எடை, அளவு முதலியன குறைந்து வெளியாகும். சிலருக்கு அளவு கடந்து வெளியாகும்.

குழந்தைகளுக்கும் பெரியவர்களுக்கும் உண்டாகும் வயிற்றுப்புழு நோய், குருதியழல் நோய் ஆகியவற்றிற்கும் துணைநோயாக அமையும்.

அழல் உடலோர்க்கு இந்நோய் உண்டாகுமாயின், முதலில் பசித்தீயைக் கெடுத்து உண்ட உணவு செரியாமை, உடல் எரிச்சல், சுரம் உள்ளது போன்று வெப்புத் தோன்றல், நாவெளுத்துச் சிவத்தல், அல்லது பட்டுத் துணிபோல் வழுவுமுத்தல், உணவை மெல்லவும் விழுங்கவும் முடியாமை, சிறிது பித்த நீர்கலந்து அடிக்கடி வாந்தியாதல், வாய் கைப்பு, வயிற்று நோய், வயிறு கடுத்து நுரை நுரையாகக் கழிதல் என்னுங்குறிகள் உண்டாகும்.

#### நாடி நடை

“சேத்தும நாடி யிளகினால் பாண்டாகும்

கண்டாயோ சிலேற் பனத்தில் வாதநாடி கலந்திடுகில்  
..... பாண்டு

தானமுள்ள சேத்துமந்தானிளகில் பாண்டுரோகம்

இடமான சேத்துமத்திற் வாதநாடி  
எழுந்தணுகில் பாண்டாகும்

உண்டாயோ சேத்துமத்தில் வாதநாடி  
கலந்திடுமேல் பாண்டுபிறக்குந்தானே

வாதத்தில் சீதஞ்சேர்த்தல் பாண்டுண்டாமே

#### உணவு

நோய் தொடக்கத்தில் பசியைத் தூண்டக் கூடியதும் உடலின் குருதியைப் பெருக்கக்கூடியதுமான உணவு பொருள்களைப் பெரிதும் கொடுத்தல் வேண்டும். வயிற்றுப் புழுக்கள் வெளியாயின், அகத்தி கீரையை அடிக்கடி சேர்த்து வருவது நன்று. உணவு எளிதில் செரிக்க கூடியதாகவே இருத்தல் வேண்டும்.

கீரைவகைகளில், கரிசாலை, பொன்னாங்காணி, அறுகீரை, சிறுகீரை, மணத்தக்காளி, தாளிக்கீரை, இவைகளை கொடுக்க வேண்டும்.

காய்கறிகளில், கத்தரிப்பிஞ்சு, முருங்கைப்பிஞ்சு, வாழைக்கச்சல், அவரைப்பிஞ்சு முதலியன கொடுக்க வேண்டும்.

நோய்வன்மை மிகுந்திருப்பின் பசித்தீ குறைந்து, உணவில் வெறுப்பு, வாந்தி இவைகள் உண்டாகாமையால், எளிதில் செரிக்கக்கூடிய உணவுகளாகிய கஞ்சி வகைகளையும், ஊனின் இரசத்தையும் கொடுக்க வேண்டும்.

ஆட்டு ஈரலை சிறு சிறு துண்டுகளாகத் துண்டித்து, அதன் அளவிற்கேற்ப நீர்கூட்டி ஒரு கொதிவந்தபின் எடுத்து நன்றாகப் பிசைந்து அந்நீரை வடித்து வேண்டிய அளவு பழரசங்களுள் கொடிமுந்திரி, சாத்துக்குடி, ஆப்பிள் இவற்றில் ஏதேனும் ஒன்றை சேர்த்து கொடுக்க வேண்டும்.

### மோர் அனுபானமாக செயல்படும் விதம்

வீக்க மகோதரமுள் வீறு குன்மம் பாண்டு பித்தந்

தாக்குமருந் திட்டத்தி சாரமொடு - கூக்குரலே

மாறாத்திரிதோஷ மந்தமனற்தகம் போம்

வீளுவின் மோர்க்கு மெய்”

பசுவின் மோருக்கு வீக்கம், மகோதரம், வயிற்றுவலி, பாண்டு, ரோகம், பித்த கோபம், இடுமருந்தால் வரும் நோய்கள், பேதி, திரிதோஷம், அக்கினி மந்தம், வெப்பம் தாகம் போகும்.

சிறிது புளித்த மோரை உண்பதினால் இரத்த தாரைகளின் மாசுக்களை நீர்மூலமாய் வெளியாக்கி இரத்த ஓட்டத்தை சீர்படுத்தும், மலசலகட்டுகள் உண்டாகாமல் தடுக்கும். இது மகோதரம், வயிற்றுவலி, பாண்டு, அக்கினி மந்தம் முதலியவற்றிற்கு சிறந்த பத்தியமாகும்.

### **3.4 Modern aspect of the disease**

#### **Anaemia**

##### **Definition:**

It is a condition of reduction in the haemoglobin concentration of the peripheral blood below the normal level in relation to age and sex. However, it should be remembered that anaemia is not a disease by itself but an expression (or) sign of an underlying disease.

##### **Classification:**

Based on,

- I. Etiology
- II. Red Cell Morphology

##### **I. Etiological Classification:**

- 1) Due to blood loss
  - a) Acute
  - b) Chronic
- 2) Haemolytic anaemia due to destruction of RBC
- 3) Impaired RBC production.
  - a) Defective proliferation and differentiation of stem cells.
    - i) Aplastic anaemia
    - ii) Chronic renal failure
    - iii) Endocrinopathy
  - b) Defective proliferation and maturation of differentiated erythroblasts.
    - i) Defective DNA synthesis vitamin B12, Folic acid deficiency
    - ii) Defective haemoglobin synthesis.
    - iii) Iron deficiency anaemia and pyridoxine deficiency.
    - iv) Sideroblastic anaemia
    - v) Anaemia due to chronic infection and inflammation (or) neoplasia.
  - c) Myelophthistic anaemia due to infiltration of bone marrow by various agents.
- 4) Anaemia of uncertain etiology  
E.g.: Rheumatoid arthritis, Pre-maturity, burns etc.

## **Iron Deficiency Anaemia**

It is a commonest type of anaemia

### **Cause:**

- Low intake of iron content food.
- Defective Absorption
- Gastrectomy
- Gastro jejunostomy.
- Sprue Syndrome
- Growing worm
- Bleeding piles
- Menorrhagia
- Hamotemesis,
- Malaena
- Oesophageal virus
- Gastro Intestinal malignancy etc,

### **Based on RBC morphology**

#### **1) Normocytic anaemia**

- a. Acute blood loss
- b. Liver disease
- c. Endocrinopathy
- d. Infection
- e. Deficient diet
- f. Less absorption
- g. Increase demand e.g.: Pregnancy, Lactation.

#### **2) Macro anaemia**

- a) Megaloplastic
- b) Non megaloplastic

### 3) Microcytic anaemia

- a) Iron deficiency
- b) Thalasaemia
- c) Sideroplastic anaemia
- d) Pyridoxine deficiency
- e) Anaemia of chronic disease

### 4) Sideroplastic Anaemia

Haemoglobin Synthesis is low

#### Cause:

- 1. Hereditary
- 2. Acquired sideroplastic anaemia
  - i) Drug – Isoniazid Chloro Phenicol
  - ii) Lead poisoning
  - iii) Haemolytic anaemia
  - iv) Chronic alcoholism

### 5) Megaloplastic Anaemia

It is characterized by megaloplastic reaction of the bone marrow due to slow DNA Synthesis. Megaloplastic macrocytic anaemia is deficiency of vitamin B12 and folic acid.

### 6) Haemolytic anaemia

It happens due to increased breakdown of RBC. Therefore there will be anaemia, hyperbilirubinemia, haemolytic jaundice excessive stercobilinogen in the stool. Excessive urobilinogen in urine and high reticulocyte count in blood.

### 7) Sickle – cell Anaemia

The basic fault is abnormal haemoglobin called HBS in the RBC which makes the letter to assume a sickle shape at a lower physiological range of oxygen pressure.

### 8) A Plastic Anaemia

There is a type of anaemia associated with aplasia (or) hypoplasia of the bone marrow resulting in reduction of all the formed elements of blood usually.

## **Clinical Features:**

### **General**

Weakness fatigue, Lassitude, Oedema of the body, pallor, Dry skin, Lusterless hair, Spoon shaped nails (Koilonychias).

### **Cardio vascular**

Palpitation, breathlessness, Angina pain, Sinus tachycardia etc.

### **GI tract**

Anorexia, acidity, heart burn, palpable spleen, tachycardia etc.

### **Neurological**

Dizziness, grittiness, numbness, insomnia, diminished vision, Lack of concentration.

### **Reproductive**

Amenorrhea, menorrhagia, abortion, infertility.

### **Special Investigations**

#### **Blood:**

Hb % and RBC are low

MCV low, 50-80fl

MCH low, 15-20pg

MCHC low, 24-30 gm/dl

Peripheral Smear study – Hypochromic

Reticulocyte count – low

Platelet count – Normal

Sr. Iron level is below 60mg/dl



## 4. MATERIALS AND METHODS

### 4.1 Preparation of the drug

#### Drug selection:

In this dissertation the leaves of *Echuramooli* (*Aristolochia indica*) and its preparation were taken as a single drug for *Paandu noi* (Anaemia) from the Siddh literature '*Gunapaddam, First Part- Mooligai Vaguppu (Porutpanbu Nool)*' written by **K.S.Murugesu Mudaliyar**, Page no 129& 130.

#### Collection of the Drug

The leaves of *Echuramooli* were collected from Idappadi, Salem district and shade dried.

#### Identification and Authentication of the drug:

The plant *Echuramooli* was identified and authenticated by Director, Plant anatomy research centre (PARC), Tambaram, Chennai-600045 and Gunapadam experts, P.G *Gunapadam* branch, GSMC, Arumbakkam, Chennai-106. The specimen sample of the herb has been preserved in PG *Gunapadam* department for future reference.

#### Preparation of the Drug

The dried leaves were made into a fine powder form (*Echuramooli ilai chooranam*) and sieved through cloth.

#### Purification of the Drug: (*Pittaviyal murai*)

The *Echuramooli ilai chooranam* was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. The same drug was later dried and powdered then sieved again.

#### Storage of the drug:

The test drug was stored in a clean, dry glass container.

The contents were inspected frequently to avoid moisture and insects.

**Administration of the Drug:**

Form of the medicine : *Chooranam*

Route of Administration : Enteral

Dose : 500 mg

*Anubanam* (Vehicle) : Butter milk

Time of Administration : twice in a day; before food

Duration : 1-3 months



**Fig no: 1** *Echuramooli* Plant (*Arishtalochia indica*)



**Fig no: 2** *Echuramooli Ilai* (Leaf of *Arishtalochia indica*)



**Fig no: 3** *Echuramooli ilai chooranam* (Powder form of *Arishtalochia indica*)

## **4.2. Standardization of the drug**

Standardization is the first step for the establishment of a consistent biological activity, a consistent chemical profile, standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects.

Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of marker or bioactive compounds and other major constituents, without consistent quality of a phytochemical mixture, a consistent pharmacological effect is not expected (M.Mosihuzzaman et.al 2008).

#### **4.2.1. Pharmacognosy study of *Aristolochia indica* - Leaf**

##### **T.S of Leaf:**

The leaf consists of prominent midrib (Fig: 4) and thick lamina. The midrib has wide and thick adaxial hump and broad semicircular abaxial part. The midrib is 900 $\mu$ m thick and 1mm wide. The adaxial epidermis of the midrib consists of squarish or rectangular thick walled cells with thick cuticle (Fig: 7). The abaxial epidermis has smaller, squarish thick walled cells. The inner layer of the abaxial epidermis includes squarish thick walled cells (Fig: 7). The adaxial hump comprises wide, angular, compact collenchymas cells. The remaining part of the midrib has large, thin walled, compact polygonal cells. The palisade tissue extends upto the lateral portions of the collenchymatous adaxial hump. The vascular bundle is single, broadly arc shaped and collateral. The bundle consists of several short, parallel lines of circular thick walled xylem elements and thick abaxial zone of phloem elements. The vascular strand of the midrib is 200 $\mu$ m and 400 $\mu$ m wide.

##### **Lamina:**

The surfaces of the lamina are smooth and even. It is 270 $\mu$ m thick. The adaxial epidermis is quite thick comprising large, thin walled, elliptical cells with thick cuticle (Fig: 5). The cells are 40 $\mu$ m thick. The abaxial epidermis is thin and includes narrow, thin rectangular cells. The abaxial epidermis is stomatiferous. The mesophyll tissue consists of two horizontal bands of cylindrical, less compact palisade cells. The lower part of the lamina has about ten layers of small, lobed, spongy parenchyma cells forming air chambers.

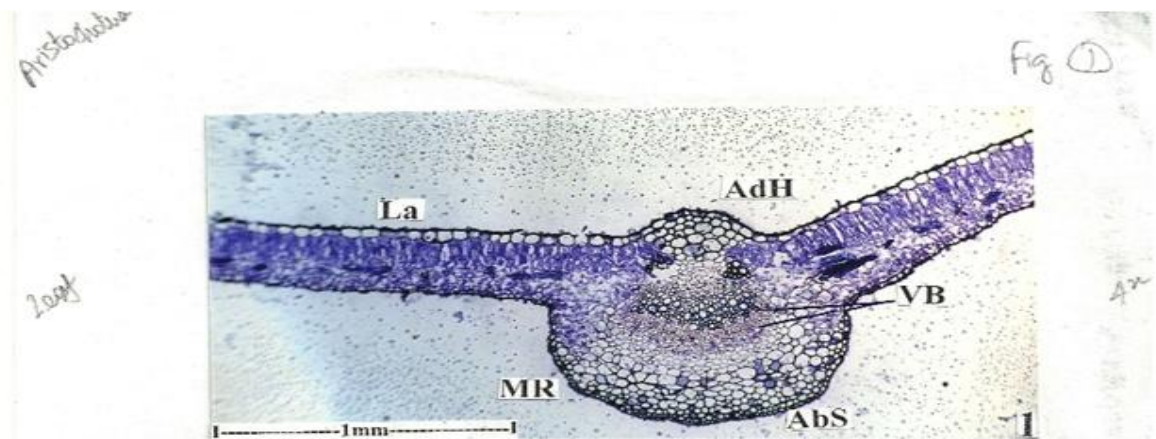
##### **Leaf Margin:**

The Marginal part of lamina is slightly bent down and become conical. It is 210 $\mu$ m thick. The epidermal layer of the leaf margin consists of squarish, thick walled cells with thick cuticle. The mesophyll tissue at the marginal part remains unchanged. (Fig: 6).

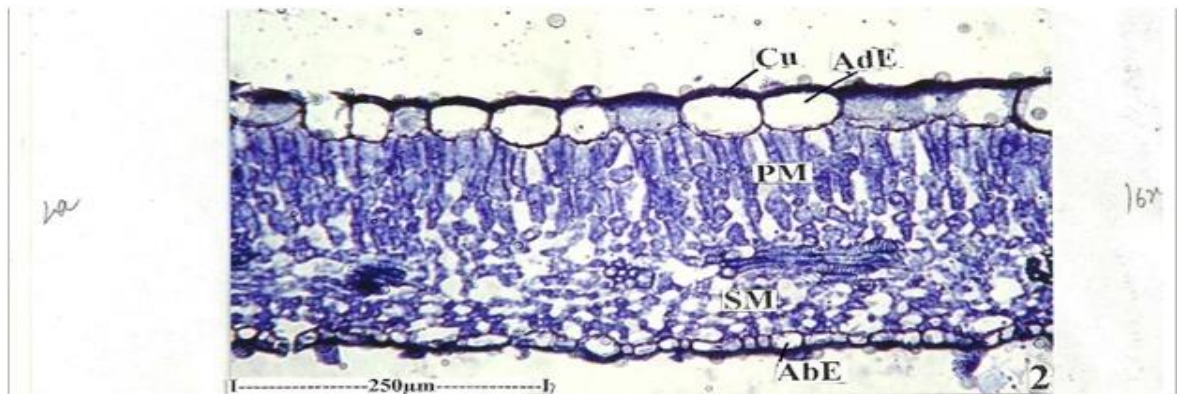
Calcium oxalate crystals are fairly common in mesophyll tissue of the leaf, the crystals are druses. Each druse has four crystals aggregated to form a four petal of the flower. (Fig: 8). The crystal is 10 $\mu$ m in diameter.



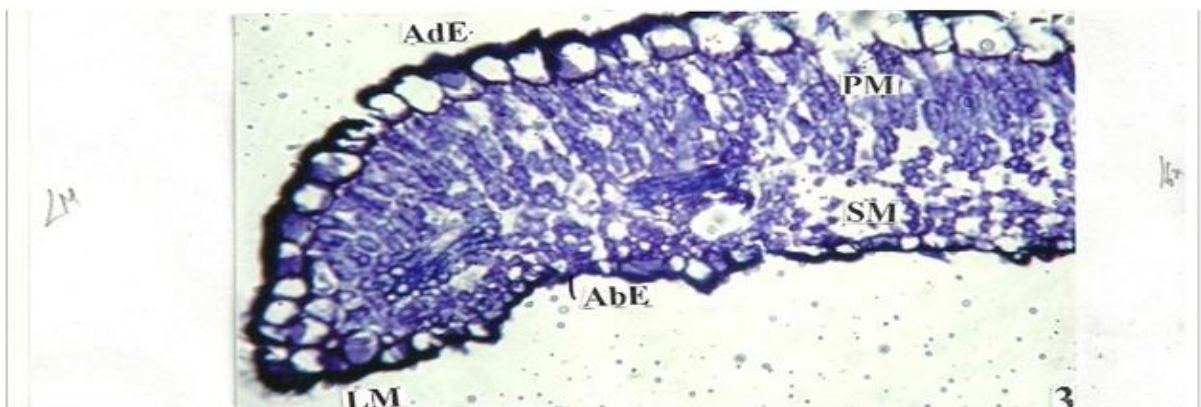
**Legend for the figures**



**Fig: 4. T.S. of leaf through midrib**

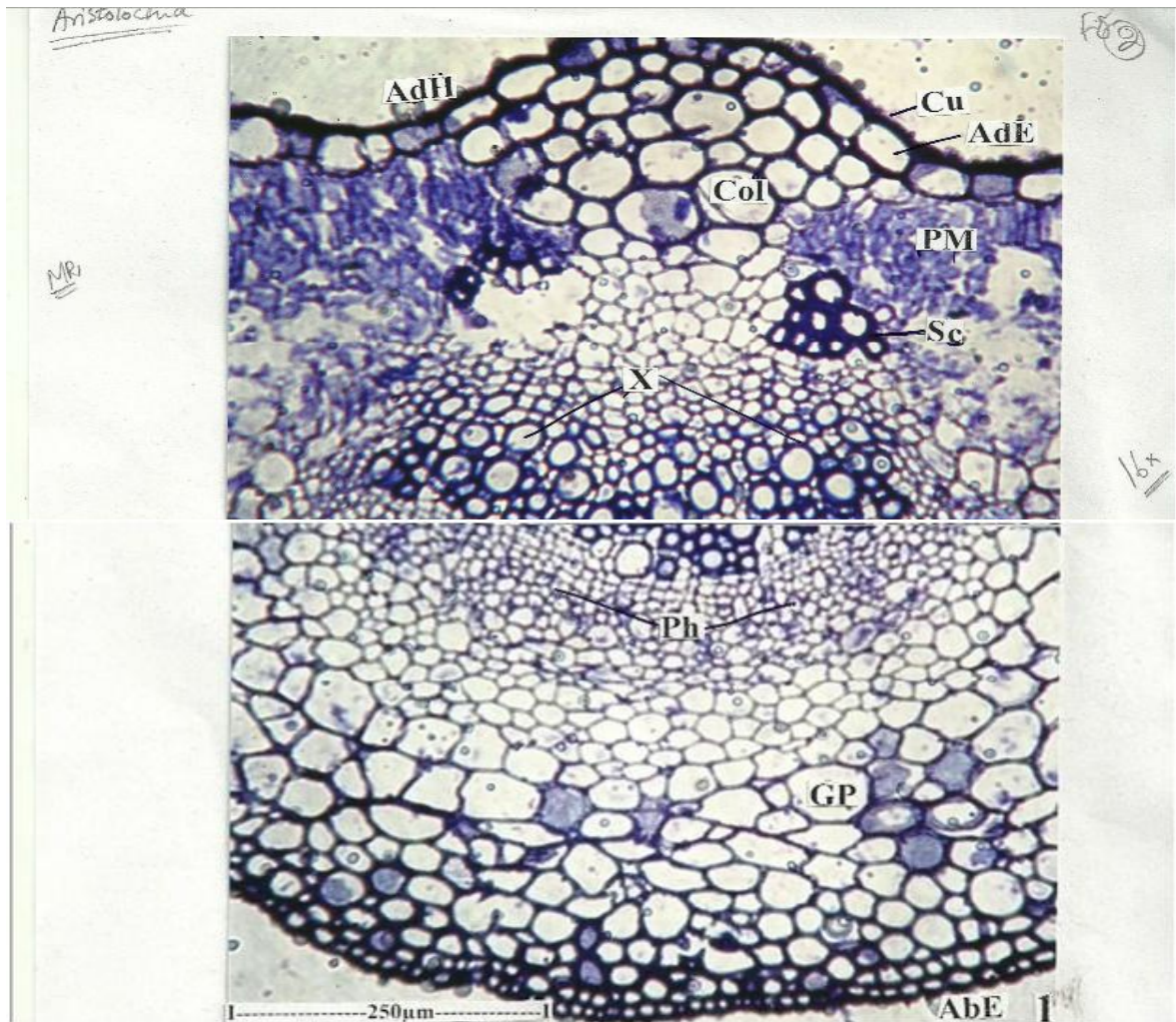


**Fig no: 5 T.S. of lamina**

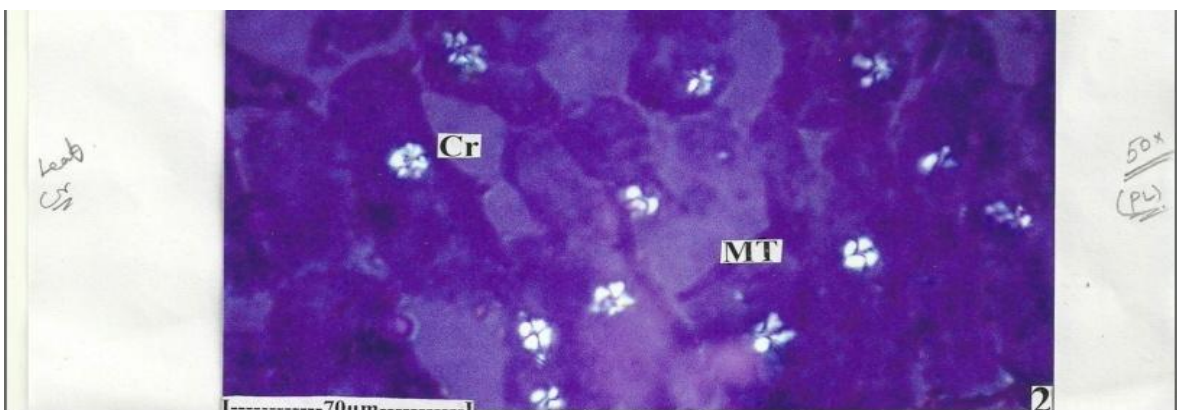


**Fig no: 6 T.S. of lamina through marginal part.**

(AbE – Abaxial Epidermis, AdE- Adaxial Epidermis, AdH- Adaxial hump, cu – cuticle, La- Lamina, LM – Leaf Margin, MR – Midrib, PM- Palisade Mesophyll, SM- Spongy Mesophyll, VB – Vascular bundle).



**Fig no: 7 T.S of Midrib – Enlarged.**



**Fig no: 8 Crystal distribution in the leaf mesophyll as viewed under polarized light.**

(AbE – Abaxial Epidermis, AdE- Adaxial Epidermis, AdH- Adaxial hump, col- collenchymas, cu – cuticle, GP- Ground Parendryma, Cr-Crystals, MT- Mesophyll Tissue, Ph – Phloem, PM- Pallisade Mesophyll, Sc- Sclerenchyma, X- Xylem)



#### **4.2.2. Phyto chemical analysis**

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are phytochemicals. These are non-nutritive chemicals that have protective or disease preventive property. The most important of these phytochemicals are alkaloids, flavonoids, tannins and phenolic compounds (Hill, 1952).

Chemical tests were carried out using the aqueous extracts from Plants and or the powdered specimens, using standard procedures to identify the constituents as describe by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

##### **Test for Flavonoids (Shinoda test)**

Substance is dissolved in alcohol, added with magnesium bits and concentrated hydrochloric acid. On heating over a water bath, the appearance of magenta colour shows the presence of flavonoids.

##### **Triterpenoids (Noller's Test)**

To few mg of extract, add tin and thionyl chloride and heat in water bath. Purple colour indicates the presence of tritepenoids.

##### **Test for Proteins (Biuret test)**

To the sample solution in a test tube, add sodium hydroxide solution and then add a few drops of very dilute (1 %) copper II sulphate solution and mix gently. Appearance of purple colour indicates the presence of protein.

##### **Test for Reducing sugar (Fehling's Test)**

To the sample solution, Fehling's reagent is added. The appearance of brick red precipitate or coloration indicates the presence of reducing sugar.



### **Test for Anthraquinones**

Few milligram of crude powder is shaken with 10 ml of benzene and filtered. To this filtrate, 0.5 ml of 10 % ammonia solution is added and the mixture is shaken well and the presence of the violet colour in the layer phase indicates the presence of the anthraquinone.

### **Test for Alkaloids (Dragendorff's Test)**

Few mg of extract in separate test tube was warmed with 2% Sulphuric acid for 2 minutes. And it was filtered in separate test tube and few drops of Dragendorff's reagent were added. The presence of orange red precipitates indicates the presence of alkaloids.

### **Test for Saponins**

To few mg of extract distilled water is added and shaken well. The formation of foam indicates the presence of saponin.

Results are shown in page no: 47

### 4.2.3. Physio-chemical analysis

#### Ash and acid insoluble ash:

To the ash add 1:5 HCl: Distilled water 15 ml boil, cooled and then filtered using whatman filter paper (No.41) repeat 3 to 4 times till the yellow colour disappear or colourless, then remove the filter paper and add to the filter to the original dish and keep it in the muffle furnace at 600° C and take constant weight and calculate the acid insoluble ash value.

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of acid insoluble residue} \times 100}{\text{Weight of the sample}}$$

Acid insoluble residue = Acid insoluble ash value – Empty weight of the dish

#### Loss on drying:

3gm of the drug is heated in a hot oven at 105° c to constant weight. The % of weight was calculated.

Loss on drying value at 105° c – 9.485 %w/w

#### Potential of hydrogen (pH):

The pH scale is logarithmic and runs from 0.0 to 14.0 with 7.0 being neutral. Readings less than 7.0 indicate acidic solutions, while higher readings indicate alkaline or base solutions. pH of *Echura mooli ilai chooranam* – 6.5

#### Thin layer chromatography (TLC)

##### Solvent system:

Toluene: Ethyl acetate (6:1.5).

##### TLC plate:

Aluminium plate precoated with silica gel 60F<sub>254</sub> of 0.2 mm thickness (Merck).

**Developing chamber:**

Camag's twin trough chamber.

**Visualizing reagent:**

Vanillin-sulphuric acid reagent.

**Extract Preparation:**

4 g of the *chooranam* was soaked overnight in chloroform. After that it boiled on water bath for 10 min, and then it filtered and concentrated to 10 ml.

**Procedure:**

The extract was applied on the TLC using capillary and developed in the solvent system. The developed TLC plate was air dried and photograph was taken in white light. Then it dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C until the development of coloured spots and photograph taken.

Results are shown in page no: 48 & 49

#### 4.2.4. Chemical Analysis

##### Proximate Chemical Analysis of the drug

##### Preparation of Extract

5gm of sample is weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 20 minutes. Then it is cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

S.No	Experiment	Observation	Inference
1.	<b>Test for reducing Sugar :</b> To 5ml of Benedicts qualitative reagent, add 10 drops of extract, then boil for two minutes	Green / Yellow / Red precipitation	Presence of Reducing Sugar
2.	<b>Test for Starch :</b> To 5 ml of extract add 2ml of acetic acid and then add few drops of N/50 Iodine Solution.	Blue Colour	Presence of Starch
3.	<b>Test for Proteins :</b> To 2 ml of extract, add 2ml of 5% Sodium Hydroxide mix and add 2 drops of Copper Sulphate Solution.	Violet or Purple Colour	Presence of Proteins
4.	<b>Test for amino Acid :</b> Place 2 drops of extract on a filter paper and allow to dry well. Then spray 1% ninhydrin over the same and allow drying.	Violet Colour	Presence of Amino Acid
5.	<b>Test for Albumin :</b> To 2 ml of extract, add 2ml of Esboch's reagent.	Yellow precipitation	Presence of Albumin

S.No	Experiment	Observation	Inference
6.	<b>Test for Phosphate :</b> To 2ml of extract, add 2ml of ammonium Molybdate solution and 2ml of concentrated Nitric Acid.	Yellow precipitation	Presence of Phosphate
7.	<b>Test for Sulphate :</b> To 2 ml of extract add 2ml of 4% ammonium oxalate solution.	White precipitation	Presence of Sulphate
8.	<b>Test for Chloride :</b> Add 2ml of extract to dilute nitric acid till the effervescence ceases. Then add 2 ml of Silver Nitrate Solution.	Cloudy White precipitation	Presence of Chloride
9.	<b>Test for Iron :</b> To 2ml of extract, add 2ml of ammonium thio cynate solution and add 2ml of concentrated Nitric Acid.	Red Colour	Presence of Iron
10.	<b>Test for Calcium :</b> To 2 ml of extract, add 2 ml of 4% ammonium Oxalate Solution.	White precipitation	Presence of Calcium
11.	<b>Test for Sodium :</b> Make a paste with 2 pinches of the sample with Hcl and Introduce it into the blue flame.	Yellow Flame	Presence of Sodium
12.	<b>Test for Potassium :</b> Add a pinch of the sample to 2 ml of Sodium Nitrate Solution. Then add 2ml of Cobalt Nitrate in 20% acetic acid.	Yellow precipitation	Presence of Potassium

S.No	Experiment	Observation	Inference
13.	<b>Test for Zinc :</b> To 2ml of extract, add few drops of Sodium Hydroxide.	White precipitation	Presence of Zinc
14.	<b>Test for Magnesium :</b> To 2ml of extract, add few drops of Sodium Hydroxide Solution.	White precipitation	Presence of Magnesium
15.	<b>Test for Alkaloids :</b> a. To 2ml of extract, add 2ml of Potassium Iodide Solution b. To 2ml of extract add 2ml of Picric Acid. c. To 2 ml of extract add 2ml of Phospho tungstic Acid.	Red Colour  Yellow Colour  White precipitation	Presence of Alkaloids  Presence of Alkaloids  Presence of Alkaloids
16.	<b>Test for Tannic Acid :</b> To 2ml of extract add 2 ml of Ferric Chloride Solution	Black precipitation	Presence of Tannic Acid

Results are shown in page no: 50

#### **4.2.5. Toxicological study**

##### **Introduction**

Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug–drug and drug–food interactions if not properly assessed. Assessment of the safety of herbal products, therefore, is the first priority in herbal research. There are various approaches to the evaluation of safety of herbal medicines.

Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phytochemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely be due to misidentification and overdosing of certain constituents

According to the literature, many Siddha preparations are effective in curing the anaemia with better palatability, as well as cost effectiveness. In our study, the *Aristolochia Indica* leaf *Chooranam* was evaluated in phenyl hydrazine induced anaemic rats to verify the acclaimed haematinic property.

##### **Materials and methods**

###### **Drug and Stock solution**

The *Aristolochia Indica* leaf *Chooranam* was prepared as per the procedure in traditional Siddha text recommendation and made into suspension form using CMC as a suspending agent and used in this study. The resulting suspension was then grounded and filtered. The filtrate was stored in a refrigerator until use. The suspension was further diluted with 2% CMC so as to achieve 200mg/ml stock concentration.

###### **Animals**

Male albino rats (150-180g) and Mice of either sex weighing 25-30g were used for the study. The rats were housed in wire-mesh cages with a 12 h light/dark cycle. They had continuous access to food and water during the entire period of experimentation. (Approval number: XIII/VELS/PCOL/66/2000/CPCSEA/IAEC/08.08.2012).

**Acute toxicity study:**

Acute oral toxicity test for the *Aristolochia Indica* leaf *Chooranam* was carried out as per OECD Guidelines 425. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead.

All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death was recorded.



#### **4.2.6. Pharmacological study**

##### **Haematinic activity of *Aristolochia indica* leaf chooranam in Phenylhydrazine induced anaemic rats**

#### **Introduction**

According to the literature, many Siddha preparations are effective in curing the anaemia with better palatability, as well as cost effectiveness. In our study, the *Aristolochia Indica* leaf *Chooranam* was evaluated in phenyl hydrazine induced anemic rats to verify the acclaimed haematinic property.

#### **Materials and methods**

##### **Drug and Stock solution**

The *Aristolochia Indica* leaf *Chooranam* was prepared as per the procedure in traditional Siddha text recommendation and made into suspension form using CMC as a suspending agent and used in this study. The resulting suspension was then grounded and filtered. The filtrate was stored in a refrigerator until use. The suspension was further diluted with 2% CMC so as to achieve 200mg/ml stock concentration.

##### **Animals**

Male albino rats (150-180g) and Mice of either sex weighing 25-30g were used for the study. The rats were housed in wire-mesh cages with a 12 h light/dark cycle. They had continuous access to food and water during the entire period of experimentation. (Approval number: XIII/VELS/PCOL/35/2000/CPCSEA/IAEC/08.08.2012).

##### **Acute toxicity study**

Acute oral toxicity test for the *Aristolochia Indica* leaf *Chooranam* was carried out as per OECD Guidelines 425. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead.

All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention

was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death was recorded.

### **Evaluation of Haematinic Activity**

Six rats were kept as normal control group (Group 1), while 24 rats were made anaemic by oral intubations of phenyl hydrazine (10 mg/kg body weight) daily for seven days. Rats that developed anaemia with haemoglobin concentration <14 g/dl were recruited for the study. Anaemic rats were randomly divided into 5 groups (2 to 6) and treated as follows: Group 1: received distilled water (1 ml) daily (normal control), Group 2: received 2% CMC (1 ml) daily (anaemic control), Group 3: received oral single dose of the *Aristolochia Indica* leaf *Chooranam* 100 mg/kg body weight/day Group 4: received oral single dose of the *Aristolochia Indica* leaf *Chooranam* 200 mg/kg, Group 5: received oral single dose of the *Aristolochia Indica* leaf *Chooranam* 400 mg/kg Group 6: received oral single dose of the haematinic syrup 2ml/kg body weight/day. The treatment was continued for 2 weeks.

### **Haematological investigation**

Before and after treatment with drug *Aristolochia Indica* leaf *Chooranam* blood was collected from the retro orbital vein of experimental animals after an overnight fast (T=0) and after 1 and 2 weeks of treatment with *Aristolochia Indica* leaf *Chooranam*, was used for the determination of red blood cell count (RBC), haemoglobin (Hb) concentration and packed cell volume (PCV). The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated.

### **Statistical analysis**

Experimental data was analysed using analysis of variance (ANOVA) and Dunnet's test to determine significant differences between means. The statistical analysis system (INSTAT-V3) package was used for this analysis.

### **4.3. Clinical assessment**

#### **Aim**

A systematic study of pharmaceutical products on human subjects (whether patients or non-patient volunteers) in order to discover or verify the clinical, pharmacological (including pharmacodynamics / pharmacokinetics), and or adverse effects, with the object of determining their safety and or efficacy.

This study is intended to provide adequate information on the Clinical trial of *Echuramooli Ilai chooranam* with Haemoglobin enhancing potentials.

#### **Objectives:**

- ◆ To evaluate the Haematinic effect of *Echuramooli Ilai chooranam*
- ◆ To explore the efficacy of *Echuramooli Ilai chooranam* in patients with anaemia.

#### **Design of the Study:**

The Open clinical trial phase-2B

Study period was 2-3 months

#### **Study Centre:**

Govt. Siddha medical college hospital, Arumbakkam, Chennai – 106.

#### **Study Participants:**

Male and female patients in all races and ethnic groups were eligible for this trial. Treatment was administered both on an inpatient and outpatient basis. The patients will be selected from the Outa-patient department of Government Siddha Medical College Hospital, Chennai – 106.

#### **Number of Subjects:**

Number of participants were 40-50.

#### **Registration Process:**

To register a patient, the following documents were completed by the investigator.

- ◆ Copy of required laboratory tests

- ◆ Signed patient consent form
- ◆ Other appropriate forms (e.g., Trial profoma).

This Clinical trial is an ethical and scientific quality standard for designing, conducting and recording trials that involve the participation of human subjects. Compliance with this standard provides assurance to public that the rights, safety and well being of trial subjects are protected, consistent with the principles enshrined in the Declaration of Helsinki and ensures that clinical trial data are credible.

### **Consent form**

Patients were included in this clinical study only after getting the concern form accordance of ‘Helsinki’. Voluntary written assent of a subject’s willing to participate in this study and in its documentation. The confirmation is sought only after information about the trial including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, alternative treatment that may be available and of the subject’s rights and responsibilities has been provided to the potential subject.

### **Selection of Patients:**

50 Patients were selected in both male and female, the selection of patients were carefully examined before treatment for correct diagnosis and ruled out any other co-existing systemic illness. Selection of patients was based on the following inclusion and exclusion criteria.

### **Inclusion Criteria:**

1. Pallor of skin and conjunctiva
2. Loss of appetite
3. Tiredness
4. Patients having haemoglobin level 7-10 g/dl

### **Exclusion Criteria:**

- i) Iron deficiency Anaemia due to oesophageal varices, hiatus hernia, peptic ulcer, Ca of stomach, colon, caecum, ulcerative colitis, haemorrhoids, haematuria, haemoptysis, repeated epistaxis

- ii) Megaloplastic anaemia
- iii) Pernicious anaemia
- iv) Haemolytic anaemia
- v) Sickle cell anaemia
- vi) Bone marrow disorder
- vii) Leukaemia
- viii) Pregnant women
- ix) Lactating women

**Withdrawal Criteria:**

- Irregular treatment
- Patients who followed dual treatment

**Line of treatment:**

The patient was orally administrated with *Echuramooli Ilai chooranam* 500mg with buttermilk, twice a day before food.

**Criteria for Assessment of Response to Therapy:**

- 1) Good relief : 91-100% complete relief in signs and symptoms.
- 2) Moderate relief : 76-90% relief in signs and symptoms.
- 3) Mild relief : 51-75% relief in signs and symptoms.
- 4) Poor relief : <50% relief in the signs and symptoms.

**Investigation Parameters:**

Before treatment a detailed clinical history was taken by considering the history of present and past illness, personal history, family history, menstrual history and associated history such as occupational, socio-economical status etc.

The following lab investigations were carried out before and after treatment:

- Urine routine
- Blood - TC, DC, ESR, Hb%, PCV, Sugar, Urea, Cholesterol
- Motion - Ova, Cyst

### **Medical Advice and Diet:**

- Patients were advised to take cereals, milk, tomatoes, beans, walnuts, vegetable leaves, cauliflower, radish and pomegranate regularly.
- Patients were advised to take mutton, liver, kidney, brain, egg yolk and fishes.
- Patients were advised to take rich source of Vitamin C like citrus fruits which promotes iron absorption.
- In severe cases with anorexia only *Kanji* (rice gruel) and soup are advised.
- Daily consumption of dates supports the therapy.
- Easily digestible food is preferred.
- Iron rich greens like *Karisalai* (*Eclipta prostrate*), *Ponnanganni* (*Alternanthera sessilis*), and *Manathakaali* (*Solanum nigrum*) also support the therapy.

### **Ethical Review:**

The protocol and any amendments have been submitted to the Govt. Siddha Medical College, Chennai-106. Institutional Ethical Committee (IEC) approval obtained to conduct the study. All subjects for this study were provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form was also submitted with the protocol for review and it was approved by the IEC. The formal consent of a subject, using the IEC-approved consent form, has been obtained before that subject was admitted to any study procedure. This consent form was signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

### **Statistical analysis:**

The data were subjected to paired student 't' test to determine the significance of changes followed by comparisons to analyze the significance of difference within the before and after treatment. P values of <0.05 were taken as significant. The results are shown in page no. 60.

## 5. RESULTS & DISCUSSION:

Various studies have been carried out in this trial drug *Echuramooli ilai chooranam*. The study includes literary collections, Pharmacognostic study, physico and Phyto chemical analysis, toxicological study, pharmacological study and clinical study. *Echuramooli ilai chooranam* was taken for the treatment of Anaemia. The drug has been selected for the treatment of Anaemia in reference with *Gunapadam-Mooligai vaguppu (Porut panbu Nool )* - First part written by K.S.Murugesu Mudaliyar.

Literary collections about the drug from various text books were done. It indicates the efficacy of the drug in the treatment of Anaemia. Botanical aspect deals with the identification, description, cultivation and ethno medicinal importance of the plant. *Gunapadam* aspect expressed that the drug possess good Haematinic property.

Since the trial drug, *Echuramooli ilai chooranam* is very easy to prepare, the drug was prepared according to the classical methods. Then it was purified by *pittaviyal* method. This method helps to vitalize the drug.

The trial drug was studied for its clinical importance in the management of *Paandu noi* (Anaemia).

### Siddha aspect:

From the literatures, the trial drug has bitter taste, the potency of the drug is hot and Bio- transformation of the drug is *karppu*. As per the Siddha concept, this disease occurs due to the derangement of the *pitha* humor.

“பித்த மதிகரிப்பின் பேசும் பரிகாரம்

சுத்தத் துவரோடு சொல்லிணிப்புச்- சத்தாகும்

கைப்புச் சுவையே கருதவதன் வீறு ” - கண்ணுசாமியம்

The properties of bitter taste are decreases the *Iyam* and *Pitha* humour, increases the *Vatha* humour. Bitter taste has the property to kills the worms which seems to be the main causes for anaemia and also purifies the blood. By giving this drug it normalise the deranged humours, reduces the signs and symptoms.

Therefore the drug *Echuramooli ilai Chooranam* with butter milk acts on the basis of *Ethirurai way* and is very effective in treating *Paandu noi*.

**Modern aspect of *Aristolochia indica ilai chooranam*:**

**Table No. 1 : Phyto Chemical Constituents of *Aristolochia indica ilai chooranam***

<b>Phyto Chemical Constituents</b>	<b>Results</b>
1.Alkaloids	<b>Present</b>
2.Triterpines	<b>Present</b>
3.Flavanoids	Absent
4.Saponin	<b>Present</b>
5.Steroids	<b>Present</b>
6.Protein	<b>Present</b>
7.Anthraquinones	Absent
8.Coumarin	<b>Present</b>

From the above results alkaloids, triterpines, saponin, steroids, protein and coumarin are present in the *Echura mooli ilai chooranam*.

By the available phytochemicals, the trial drug has the therapeutic potency of increasing haemoglobin level in the blood.



## Physio chemical analysis



சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106

सिद्ध केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नई- 600106

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(Central Council for Research in Siddha, Department of AYUSH,  
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Web: www.crisiddha.tn.nic.in

**Table: 2 Physio chemical results of *Aristolochia indica ilai chooranam***

S.No	Parameter	Mean
1.	Loss on Drying at 105°C	9.485%
2.	Total Ash	17.725%
3.	Acid insoluble Ash	9.172%
4.	Water Soluble Extractive	21.45%
5.	Alcohol Soluble Extractive	16.75%
6.	pH	6.5
7.	TLC	Enclosed



**Fig. No: 9 TLC of *Aristolochia indica ilai chooranam***

After spray with visualizing agent

**Table No: 3 TLC result of *Aristolochia indica ilai chooranam***

Sl.No	After Dipping in Vanillin-Sulphuric acid	
	Rf value	Colour of the spot
1	0.14	Grey
2	0.28	Blue
3	0.34	Grayish green
4	0.46	Purple
5	0.51	Purple
6	0.67	Purple
7	0.70	Greyish green
8	0.80	Violet

By the above results, the trial drug has very low foreign matter and acid insoluble ash, indicates that trial drug will digest completely in human GI tract. The analytical parameters like total Ash value, Acid insoluble ash value, Loss on drying values are helping us to interpret the digestion and solubility capacity of the crude extract. As per the result the tested sample contains good percentage of solubility as well as digestive capacity.

**Table No.4 Chemical analysis result of *Aristolochia indica leaf chooranam***

S.No	Experiment	Observation	Inference
1.	Test for reducing Sugar :	Green precipitation	Presence of Reducing Sugar
2.	Test for Starch :	Absence of Blue Colour	Absence of Starch
3.	Test for Proteins :	Absence of Violet or Purple Colour	Absence of Proteins
4.	Test for amino Acid :	Absence of Violet Colour	Absence of Amino Acid
5.	Test for Albumin :	Absence of Yellow precipitation	Absence of Albumin
6.	Test for Phosphate :	Absence of Yellow precipitation	Absence of Phosphate
7.	Test for Sulphate :	White precipitation	Presence of Sulphate
8.	Test for Chloride :	Cloudy White precipitation	Presence of Chloride
9.	Test for Iron :	Red Colour	Presence of Iron
10.	Test for Calcium :	White precipitation	Presence of Calcium
11.	Test for Sodium :	Absence of Yellow Flame	Absence of Sodium
12.	Test for Potassium :	Absence of Yellow precipitation	Absence of Potassium
13.	Test for Zinc :	Absence of White precipitation	Absence of Zinc
14.	Test for Magnesium :	Absence of White precipitation	Absence of Magnesium
15.	Test for Alkaloids :	Absence of Red Colour Absence of Yellow Colour Absence of White precipitation	Absence of Alkaloids Absence of Alkaloids Absence of Alkaloids
16.	Test for Tannic Acid :	Black precipitation	Presence of Tannic Acid

## Results

From the preliminary chemical analysis of *Echura mooli ilai chooranam* have an idea that the trial drug has reducing sugar, Sulphate, Chloride, Iron, Calcium and Tannic acid.

The above result, the major ions are performing an important role in the blood and promotes haemoglobin level. They decrease the symptoms and signs.

## Scanning Electron Microscope images

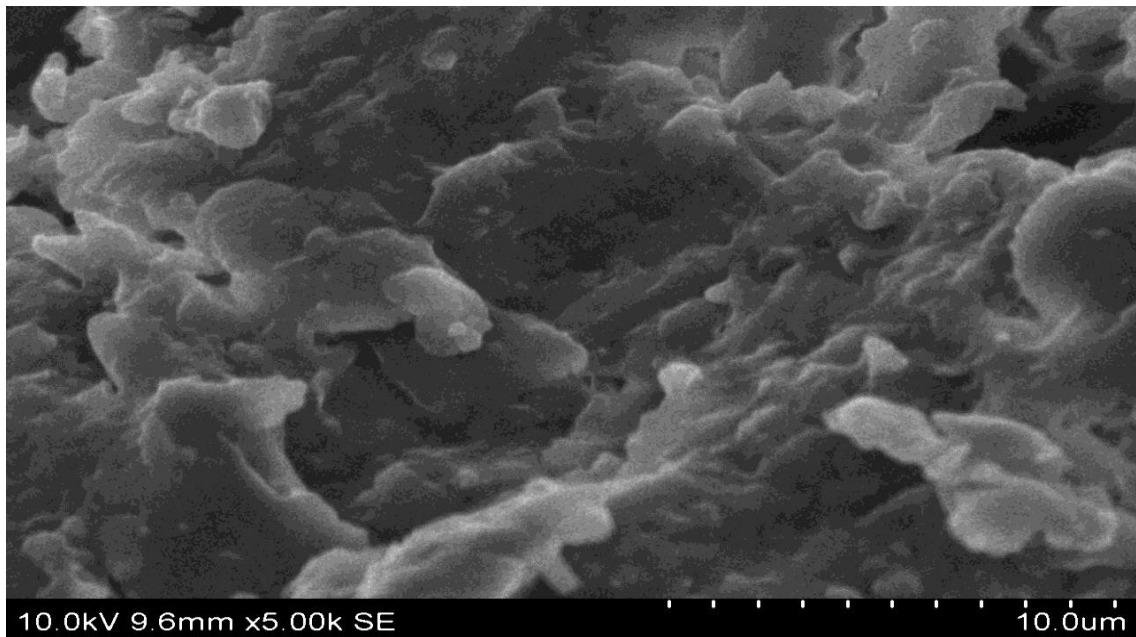


Fig. No: 10 SEM image of *Aristolochia indica ilai chooranam* at 10.0um

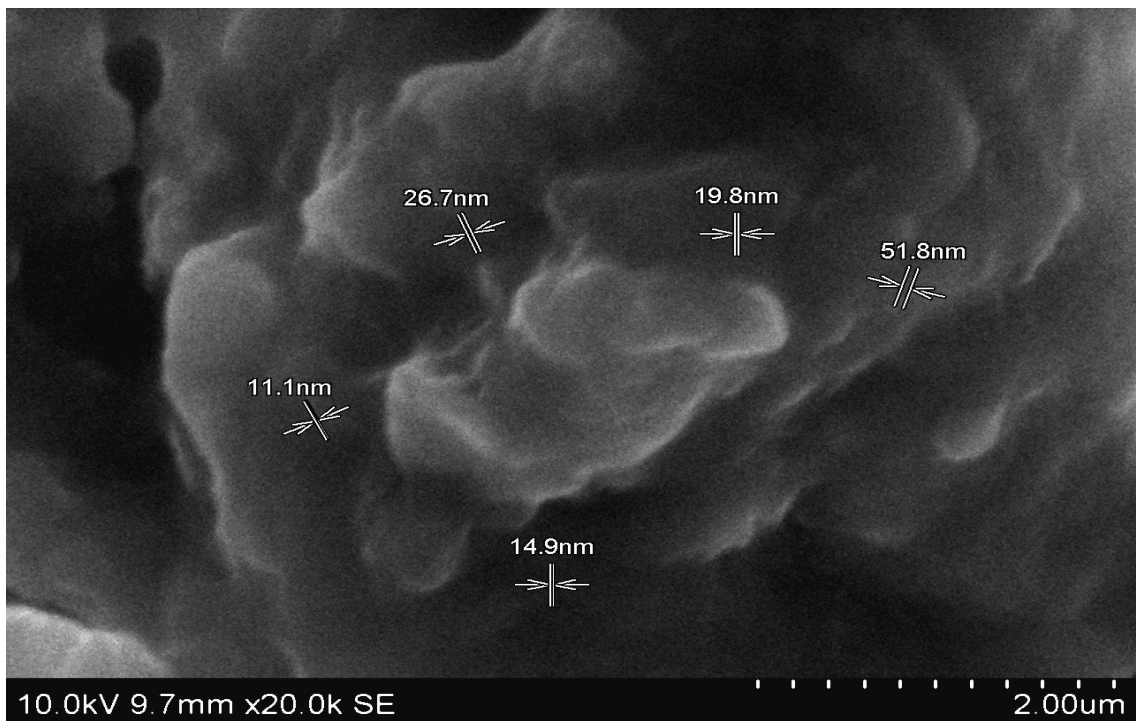


Fig. No: 11 SEM image of *Aristolochia indica ilai chooranam* at 2.0um

### Result:

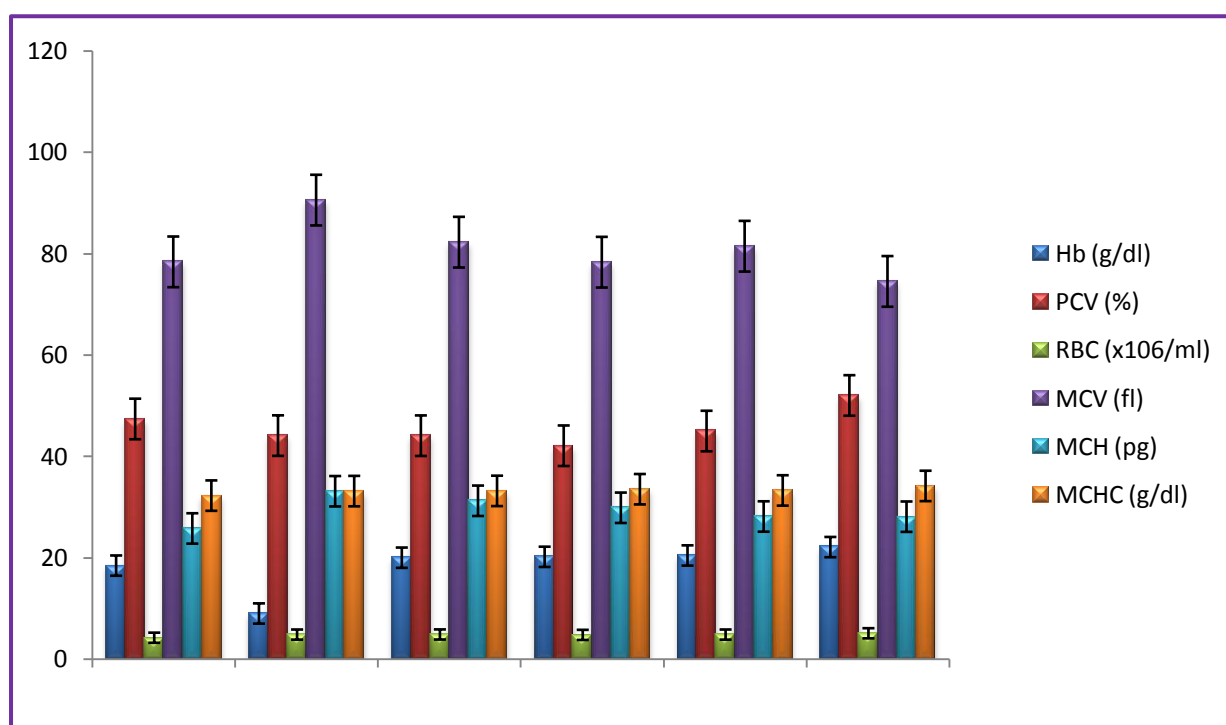
The nano particle size of the *Aristolochia indica ilai chooranam* is 11.1nm, 14.9nm, 19.8nm, 26.7nm and 51.8nm. Because of these smaller particle sizes the *Aristolochia indica ilai chooranam*, is easily absorbed in the digestive system.

**Table 5: Effect of Phenylhydrazine (10mg/kg, p.o. daily for 7 days) alone on Haematological parameters.**

Parameters	Group 1 (Normal)	Group 2 (Anaemic)	Group 3 (Anaemic)	Group 4 (Anaemic)	Group 5 (Anaemic)	Group 6 (Anaemic)
Hb (g/dl)	18.64 ± 0.46	13.85±0.32**	13.76±0.30**	13.64±0.34**	11.75±0.32**	12.56 ± 0.30**
PCV (%)	52.32 ±1.44	40.41 ± 2.21**	41.52 ± 2.11**	41.20 ± 2.79**	40.31 ± 2.02**	40.34 ± 2.55**
RBC (x10 <sup>6</sup> /ml)	6.88 ± 0.14	4.42 ± 0.30**	4.28 ± 0.20**	4.46 ± 0.22**	4.57 ± 0.28**	4.39 ± 0.31**
MCV (fl)	74.62 ± 2.74	85.23±4.18	86.99±4.36	85.20±3.12	85.48±4.19	88.14± 3.12
MCH (pg)	25.10 ± 1.56	27.18±1.31	27.40±1.51	29.30±1.32	30.40±1.20	30.18 ± 2.34
MCHC (g/dl)	32.55 ± 0.40	34.12±0.6	34.22±0.92	34.11±1.18	34.45±3.24	30.42 ± 1.74

Values are mean ± S.E.M. (Dunnet't test). \*\*P<0.01 Vs Control N=6.

**Graph No.1 Effect of Phenylhydrazine alone on Haematological parameters**

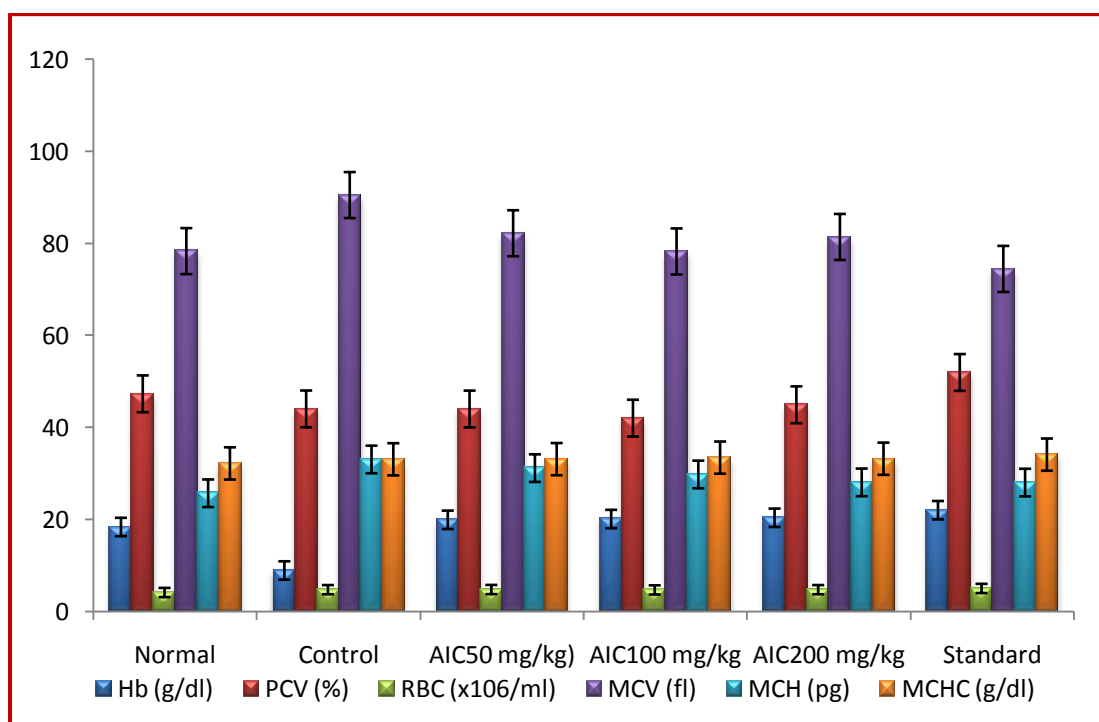


**Table No. 6 Hematological parameter of rats after Seven days treatment with *Aristolochia indica* Chooranam.**

Parameters	Group 1 (Normal)	Group 2 (Anemic Control)	Group 3 (50 mg/kg)	Group 4 (100 mg/kg)	Group 5 (200 mg/kg)	Group 6 (Heamatinic syrup)
Hb (g/dl)	18.98 ±1.45	14.20±1.32	17.26±0.43	16.78±0.48	17.04 ± 0.77	22.15 ±2.32**
PCV (%)	54.20 ±1.98*	45.27±2.11	46.24±2.20	48.64±2.26	46.45±3.00	55.48 ±2.24*
RBC (x10 <sup>6</sup> /ml)	7.18±0.24**	5.12±0.22	6.47±0.28**	7.26±0.25**	7.88±0.23**	8.00±0.22**
MCV (fl)	78.56±2.13	75.21±2.12	80.10±2.11	77.45±2.02	75.74±3.12	72.52±2.32
MCH (pg)	25.15±1.44	26.78±1.28	25.80±1.67	28.02±1.22	30.00±2.00	24.36±1.28
MCHC (g/dl)	32.02±1.30	29.33±1.20	26.32±1.05	26.17±1.28	27.2±1.47	32.41±2.00

Values are mean ± S.E.M. (Dunnet't test). \*P<0.05; \*\*P<0.01 Vs Control N=6.

**Graph No. 2 Changes in Hematological parameters after 7 days treatment with *Aristolochia indica* leaf Chooranam**

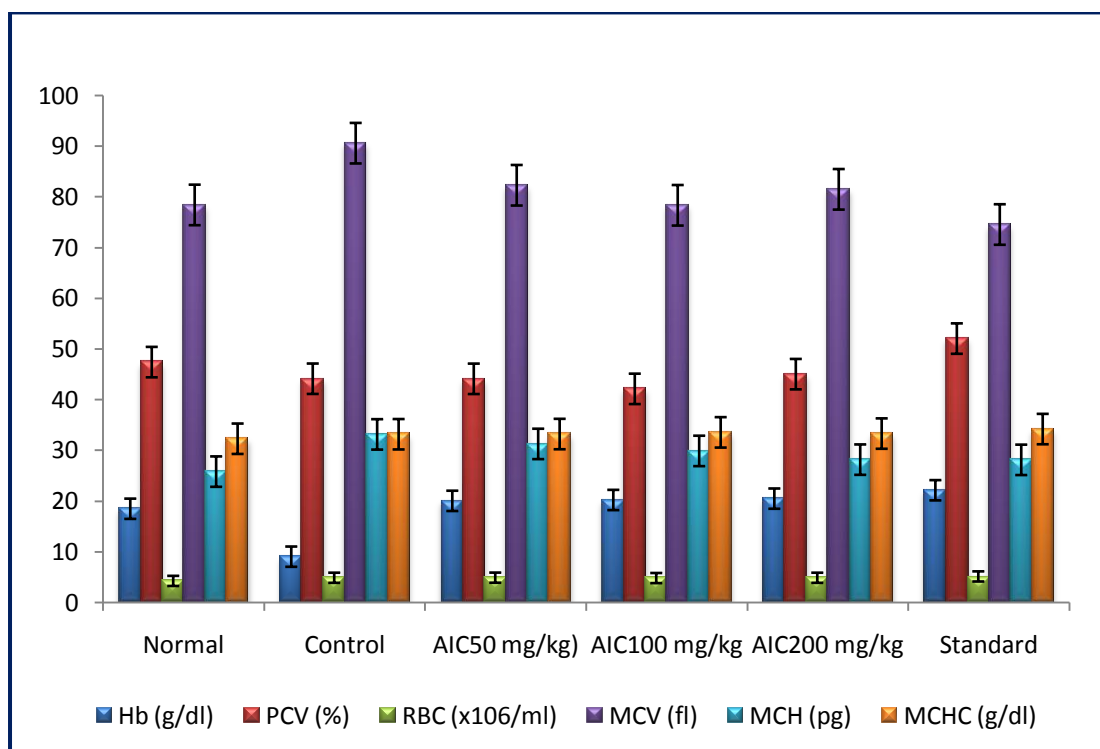


**Table 7: Hematological parameters of rats after 14 days treatment with *Aristolochia indica* leaf Chooranam.**

Parameters	Group 1 (Normal)	Group 2 (Anemic Control)	Group 3 (50 mg/kg)	Group 4 (100 mg/kg)	Group 5 (200 mg/kg)	Group 6 (Heamatinic syrup)
Hb (g/dl)	18.45 ± 1.32**	9.00 ± 1.12	20.01 ± 0.74**	20.18 ± 0.80**	20.45 ± 1.95**	22.10 ± 1.88**
PCV (%)	47.38 ± 1.10	44.11 ± 1.30	44.08 ± 2.4	42.10 ± 2.2	45.00 ± 1.2	52.02 ± 1.46*
RBC (x10 <sup>6</sup> /ml)	4.22 ± 0.24	4.84 ± 0.30	4.86 ± 0.33	4.78 ± 0.28	4.84 ± 0.29	5.10 ± 0.28
MCV (fl)	78.38 ± 2.12**	90.56 ± 2.42	82.26 ± 2.21*	78.31 ± 1.72**	81.46 ± 2.23*	74.52 ± 2.21**
MCH (pg)	25.78 ± 2.64	33.12 ± 2.17	31.24 ± 1.96	29.86 ± 1.82	28.14 ± 1.70	28.10 ± 2.53
MCHC (g/dl)	32.26 ± 1.11	33.15 ± 1.20	33.19 ± 1.23	33.52 ± 0.65	33.28 ± 2.30	34.17 ± 2.20

Values are mean ± S.E.M. (Dunnet't test). \*P<0.05; \*\*P<0.01 Vs Control N=6.

**Graph No. 3 Hematological parameters after 14 days treatment with *Aristolochia indica* leaf Chooranam**



Toxic compounds like phenylhydrazine could also destroy red blood cell and generate anaemia. The mechanism of oxidative hemolysis Produced by Phenylhydrazine is known. Phenyl diazene is a phenyl free radical produced via the 2-electron oxidation of phenylhydrazine by oxyhemoglobin. This free radical was found to hemolyze red cells rapidly and convert oxyhemoglobin into methemoglobin. Then it is the active hemolytic agent in red blood cells which could lead to anaemia. The RBC, Hb, and PCV of rats administered Phenylhydrazine decreased significantly ( $P < 0.01$ ) while the MCV and MCH increased giving rise to macrocytic anaemia ( $P < 0.05$ ). *Aristolochia Indica ilai Chooranam* at the dose of 100-400 mg/kg showed good percentage of improving in haemoglobin level, which was almost equivalent to standard treated group indicating correction of anaemia induced by Phenyl hydrazine after 14 days treatment.

Treatment with *Aristolochia Indica* leaf *Chooranam* at the dose levels 200 and 400mg/kg for 14 day showed significant increase in Hb ( $p < 0.01$ ) compared to positive control and it was comparable to standard drug used in this study. Phenylhydrazine altered the haematological parameters by haemolysis characterized by decrease in haemoglobin concentration, total RBC counts and PCV on day 7. However, the haematological parameters were restored to normal range after treatment with *Aristolochia Indica* leaf *Chooranam* for 14 days. Effective changes was observed after one week of treatment of anaemic rats with *Aristolochia Indica* leaf *Chooranam* reversed the influence of Phenylhydrazine resulting to a significant ( $P < 0.05$ ) increase in RBC, Hb, and PCV. The Hb, RBC and PCV reached near normal at the second week of the treatment.

Rats treated with Phenylhydrazine (10mg/kg/day for 7 days) resulted in a marked haemolytic anaemia characterised by decreased RBC, Hb and PCV. The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study Phenylhydrazine altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. However, this effect was restored after one week of *Aristolochia Indica ilai Chooranam* treatment. Also the recovery was progressive such that after 1week of continuous treatment, the Hb concentration and PCV were higher in the treated groups than in the normal control group.

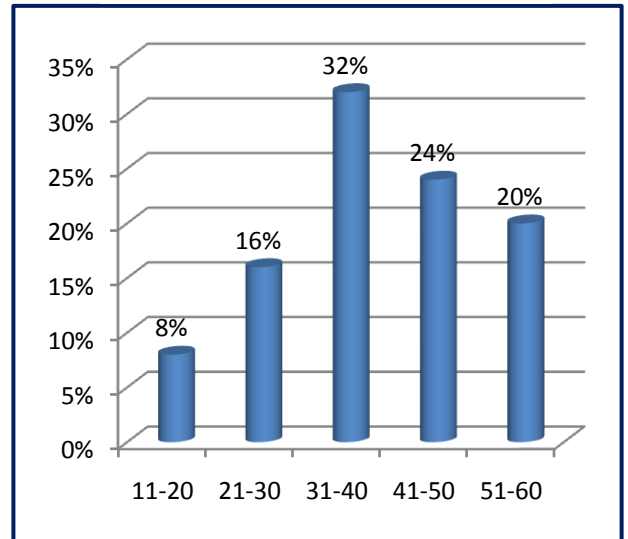


## Clinical results of *Aristolochia indica ilai chooranam* on anaemic patients

**Table No: 8 Age distribution**

Sl. No	Age in Years	No. of Patients/50	Percentage (%)
1.	11-20	4	8
2.	21-30	8	16
3.	31-40	16	32
4.	41-50	12	24
5.	51-60	10	20

**Graph No. 4 Age distribution**



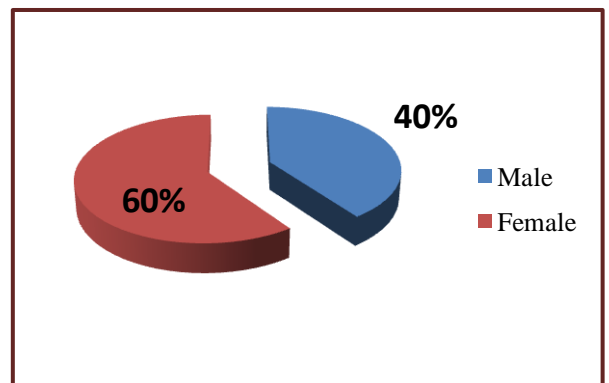
### Inference

Among 50 patients, 4 patients(8%) belongs to the age group 11-20 and 8 patients (16%) belongs to the age group 21-30 and 16 patients (32%) belongs to the age group of 31-40 and 12 patients (24%) belongs to the age group of 41-50 and 10 patients (20%) belongs to the age group of 51-60.

**Table No: 9 Sex distribution**

Sl. No	Sex	No. of Patients/50	Percentage (%)
1.	Male	20	40
2.	Female	30	60

**Graph No. 5 Sex distribution**



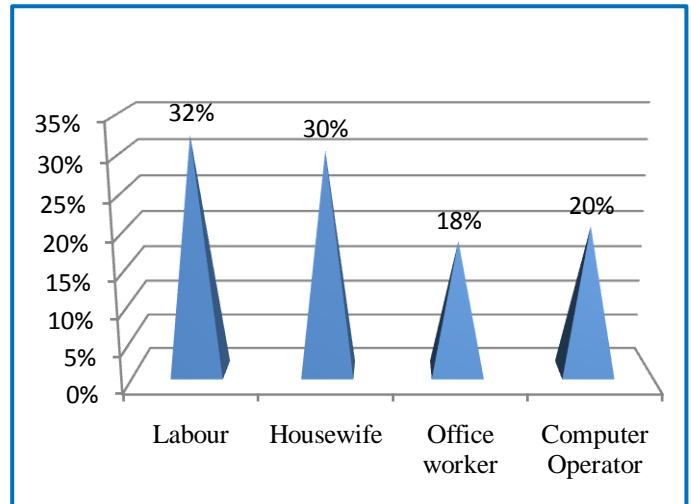
### Inference

Among 50 patients, 20 patients (40%) were male and 30 patients (60%) were female.

**Table No: 10 Occupational distribution**

Sl. No	Occupation	No. of Patients/50	Percentage (%)
1.	Labour	16	32
2.	Housewife	15	30
3.	Office worker	9	18
4.	Computer Operator	10	20

**Graph No. 6 Occupational distribution**



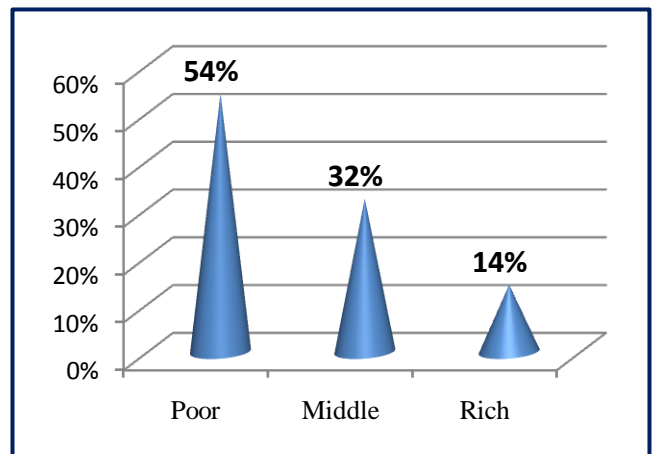
**Inference**

Among 50 patients, 16 patients (32%) were labour and 15 patients (30%) were housewife, 9 patients (18%) were office workers and 10 patients (20%) were computer operator.

**Table No: 11 Socio economic status**

Sl. No	Socioeconomic Status	No. of Patients/50	Percentage (%)
1.	Poor	27	54
2.	Middle	16	32
3.	Rich	7	14

**Graph No. 7 Socio economic status**



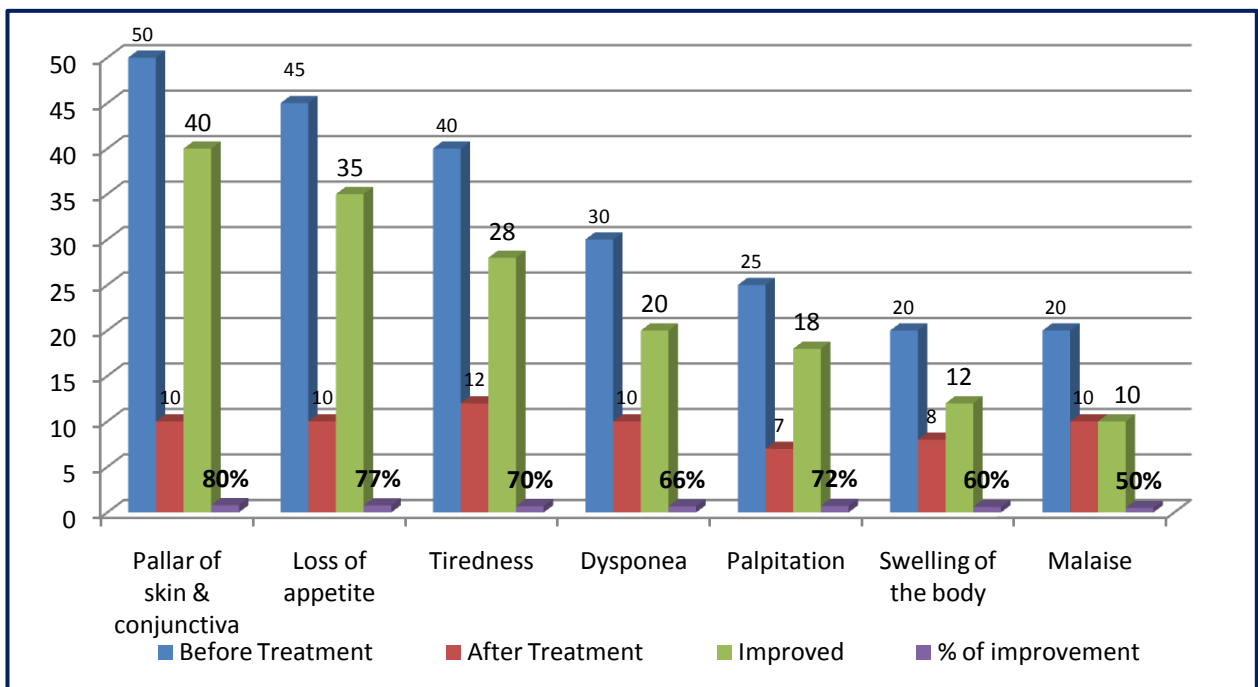
**Inference**

Among 50 patients, 27 patients (54%) were poor and 16 patients (32%) were middle and 7 patients (14%) were rich.

**Table No. 12 Improvement of Signs & Symptoms**

Sl.No	Symptoms	No. of Patients/50			% of improvement
		Before Treatment	After Treatment	Improved	
1.	Pallor of skin & conjunctiva	50	10	40	80
2.	Loss of appetite	45	10	35	77
3.	Tiredness	40	12	28	70
4.	Dyspnoea	30	10	20	66
5.	Palpitation	25	7	18	72
6.	Swelling of the body	20	8	12	60
7.	Malaise	20	10	10	50

**Graph No. 8 Improvement of signs & symptoms**



**Inference** – The improvement % are

Pallor skin & conjunctiva - 80%

Loss of Appetite - 77%

Tiredness - 70%

Dyspnoea - 66%

Palpitation - 72%

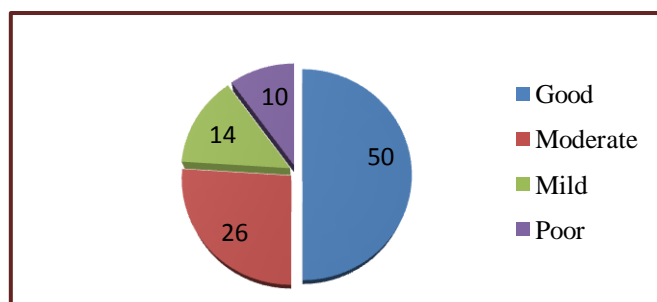
Malaise - 50%

Swelling of the body - 60%

**Table No: 13 Extent of relief in %**

Sl. No	Total No. of Patients	Extent of Relief in Percentage			
		Good	Moderate	Mild	Poor
1.	50	50	26	14	10

**Graph No. 9 Extent of relief in %**



### **Inference**

Among 50 patients, 50% have good relief, 26% have moderate relief, 14% have mild relief and 10% poor relief.

The *Aristolochia indica ilai chooranam* was studied for its clinical importance in the management of *Paandu noi* (Anaemia).

It was administered in 50 patients at a dose of 500-1gm, twice a day with butter milk. After 3 weeks of administration most of the patients showed good result apart from their immune system.

Among the 50 patients, 25 showed good results (50%), 13 showed moderate reliefs (26%), only 7 patients took partial clinical response (14%) and other 5 patients did not respond even after 4 weeks of administration (10%).

## Biostatistical Analysis

Effect of *Aristolochia indica ilai chooranam* on Hb level (gm %) in human subjects

Sl.No	Hb level (gm %)	
	Before Treatment	After Treatment
1	10.00	14.20
2	11.20	13.70
3	12.00	9.20
4	13.10	16.20
5	9.50	11.50
6	10.00	11.60
7	11.50	12.50
8	14.00	15.20
9	13.30	14.80
10	7.50	10.00
11	13.00	14.50
12	7.50	10.80
13	11.00	12.70
14	8.00	11.10
15	10.00	11.90
16	14.40	15.20
17	14.50	15.90
18	12.00	15.40
19	9.00	11.40
20	12.60	14.30
21	10.20	12.10
22	11.30	12.50
23	11.60	13.70
24	13.00	15.10
25	10.50	11.90

Sl.No	Hb level (gm %)	
	Before Treatment	After Treatment
26	10.20	12.70
27	10.90	13.20
28	10.80	12.20
29	12.00	14.20
30	10.40	11.70
31	12.00	14.10
32	12.30	14.50
33	11.00	12.60
34	12.00	12.80
35	11.80	14.00
36	12.10	14.70
37	12.40	15.10
38	13.00	14.70
39	10.00	12.40
40	13.70	16.10
41	11.00	13.20
42	10.20	12.70
43	8.60	9.20
44	13.10	16.20
45	9.00	11.50
46	9.00	10.60
47	11.30	14.80
48	13.00	14.20
49	13.00	16.50
50	7.40	8.00

**Software:** spss17 version

**Variables:** Hb level (gm %) – before treatment, after treatment

**Number of cases:** 50

**Test:** Paired t test

**Confidence Interval:** 95%

**Correlation coefficient (r):** 0.847

**Before and after treatment mean difference:**  $1.97 \pm 1.06$  (gm%).

**P Value (2 tailed):**  $p < 0.01$ .

**Inference:**

The p value is significant ( $p < 0.01$ ). So the treatment was significantly improving the Hb level (gm %).

By knowing the above clinical studies, administrating the *Aristolochia indica ilai chooranam* increases the haemoglobin level in blood and showed promising result in anaemia.

## 6. CONCLUSION

The trial drug *Echuramooli ilai chooranam* (*Arishtolochia indica*) is selected from the classical Siddha text *Gunapadam- Mooligai vaguppu* (*porut panbu nool*)-First part written by K.S.Murugesu mudhaliyar for the evaluation of safety and efficacy in the management of Haematinic action.

The trial drug was duly identified and authenticated by the botanist and *Gunapadam* experts.

Since the trial drug, *Echuramooli ilai chooranam* is very easy to prepare and the drug was prepared according to the classical methods. Then it was purified by *pittaviyal* method. This method helps to vitalize the drug.

Though the drug has bitter taste, the hot potency and bio-transformation into pungent clearly indicates its activity on red blood cells.

The presence of ferrous ions indicated that they help in maintaining the hemoglobin level. The other components Calcium, Chloride, Sulphate, Reducing sugar, Zinc and Magnesium are also responsible for its haematinic property.

In order to provide effective, safe and cheap drug and to prove the traditional claim for the treatment of anemic conditions the *Aristolochia Indica Chooranam* at a dose of 100, 200 and 400 mg/kg (p.o.), was evaluated and found significantly increased the haemoglobin, haematocrit value and RBC count in anaemic rats indicating the haematinic effect. The rapid and progressive recovery of anaemic rats responding to treatment of *Aristolochia indica Chooranam* may be due to its erythropoietic activity. However, the mechanism of action by which *Aristolochia indica Chooranam* produced its effect on increasing RBC, Hb and PCV in experimental animals need to be evaluated in a scientific manner using specific experimental animal models and also multi centre clinical trials are required to understand the exact molecular mechanisms of action. Based on the results it can be concluded that the *Aristolochia Indica Chooranam* is a good drug of choice for the anaemia at the dose level of 200mg/kg.

The open clinical trial results revealed that 76% of patients were having improvement in the clinical features and biochemical reports. The study validates the effectiveness of herb in improving hemoglobin level and also helps for curing certain causes like worm infestation etc.

The drug is easily available and preparation is very simple. The trial medicine is cost effective. No adverse effects were produced during the entire course of treatment. Conclusively, that the drug "*Echuramooli ilai chooranam*" (*Aristolochia indica*) gives a new hope in the field of Anaemia treatment.



## 7. SUMMARY

“*Echuramooli ilai chooranam*” were collected from Idappadi, Salem district and powdered then purified and stored. This drug was subjected for various studies by the author.

*Echuramooli ilai chooranam* was selected by the author for this study to establish the Haematinic activity.

To collect the information about the drug, various text books, literature were referred. From them, the author came to an idea about the drug and its efficacy on Anaemia.

A brief description about botanical aspect of the *Echuramooli ilai chooranam* and its identifying characters and Phyto chemical data were given.

The wide use of *Echuramooli* according to *Gunapadam* aspect as well as in various Siddha literatures were discussed with much importance to that of preparation related to *Paandu noi*.

The Phyto chemical analysis of the drug showed that it contains Iron, Calcium, Chloride, Sulphate, Reducing sugar, Zinc and Magnesium. It is related in treatment of Anaemia.

The preclinical study showed that the drug has got safety and significant haematinic activity.

The patients were responding well from the beginning of the treatment and no adverse effects were reported.

The clinical result revealed that 76% of patients were having improvement in the clinical futures and biochemical reports.

This present study confirmed that *Echuramooli ilai chooranam* has the remarkable Haematinic activity and high therapeutically value against the clinical symptom of Anaemia.

## 1. INTRODUCTION

Siddha system of medicine, being the oldest traditional system in the world has a strong significance in detoxification, anti-oxidation, immune modulation and metabolic balance. It is a carefully guarded medical system given away incredible and rapid outcomes in various unremitting ailments. During the period of *Siddhars* there was not even a microscope, but were able to identify, as many as 3500 herbs, their properties, purification process, also administered plant based and mineral-based medicine with suitable adjuvant and they documented all these things. The therapeutic effect has been proved for thousands of year that were never changed for centuries. Like other medical systems based on the foundations of logic and scientific methods, Siddha medicine had theoretical foundations as well as deserves to be called a medical science.

Since, Siddha medicine, based an individualistic medicine, on patients constitution and syndrome differentiation that we cannot make use of modern medical system as a standard to explain in all levels. Our Siddha medicine not only pay attention to the preventive and curative methods, but also focused on the physical, mental, spiritual and psychological well-being thus giving a total perfection in life.

Ancient India is the creator of sexual education. The sex is considered as a part of life. It was wide-open in all kind of arts, like literatures, sculptures etc. An American professor Wendy Downier states that, “For Hindus, the phallus in the back ground, the archetype of which their own penises are manifestations, is the phallus (called the *lingam*) of the god *Siva*”.

The term infertility has traditionally been used to describe the inability to make off-spring. For life to continue, an organism must reproduce itself before it dies. A male cannot make his off-spring is known as male infertility.

A fall in the sperm count in the reproductive years is now a serious global problem and public health warning and unfortunately the crisis is equally acute in India. According to specialists, the sperm count of a normal adult in India has plunged to around 20 million per ml, one-third of what it was three decades ago. This trend could be linked to diet, lifestyle modification (The Times of India, Health & Fitness, Dec 26, 2012).

The World Health Organization's guidelines specify the normal, healthy sperm count to be 20 million sperm per milliliter in a total semen volume of two ml. This indicates that how overtime, sperm counts have gone down across the world. "A decade or two ago, 40-50 million was the normal sperm count. However, WHO has changed the definition of what's 'normal' and has brought it down to 20 million. Urban-influenced lifestyles along with food, water and milk contamination have adversely affected sperm health over the years (WHO, 2011).

Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. The alterations in motility, viability and morphology of spermatozoa in treated rats are likely the result of adverse effect of the treatment on epididymal functions. Inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova. Some diseases such as coronary heart diseases; diabetes mellitus and chronic liver diseases have been reported to cause deleterious effects on spermatogenesis.

So, there is an urgent need to establish the causes of male infertility so measures can be taken to prevent further damage.

In this present scenario, male infertility can be managed by the use of Spermatogenesis activity drug. This can also be viewed as any food, drug, scent or device that can increase Sperm quality.

Research during past two decades has an unfolded focus on male infertility, impotence (erectile dysfunction), and pre mature ejaculation. There are a number of prescription drugs which may act as increasing sperm count, the use of medicines have not shown significant improvement in treating infertility, at the same time there are large number of side effects. These include arrhythmias, suicide tendency, mental confusions and tremors etc...

A recent study estimated that 152 million men worldwide experience some degree of infertility. In addition, based on population projections, it is likely that the prevalence of the condition will more than double over the next 25 years. Today, the development of numerous treatments has allowed the social stigma to subside and both the patient and the physician some choice in how to manage the condition.

Successful treatment of infertility may improve not only sexual relationships, but also the overall quality of life. This is very important because of the side effect associated

with other treatment options and the readily available drugs. The increasing incidence of male infertility is necessitating more and rapid search into drugs with spermatogenesis potentials with negligible side effects.

There are many spermatogenic medicines in Siddha which is given for many years. Except few, most of the drugs were not yet validated scientifically. Among them *Anda odu* is one of the known Spermatogenesis, mentioned in classical Siddha literature.

By the literature evidence and the scientific background all parts of the eggs are medicinally useful, in that the *Anda odu* are particularly mentioned for male infertility in *Anuboga Vaidhiya Navaneedham*- Part 3 written by Hakeem Mohammed Abdhulla Shahibu. So I have chosen the *Anda odu Parpam* (Egg shell of *Gallus domesticus*) in my present study for *Vindhu kuraivu* (Oligospermia). This study is intended to provide adequate information on the screening of *Anda odu Parpam* with sperm enhancing potentials since attention is now being focused on the use of Siddha drugs as Indian traditional medical practice in the management of this high rising incidence of infertility.

## 2. AIM AND OBJECTIVES

### Aim

Infertility, Sexual dysfunction and other related problems are eluding scientific community and medical practitioners since time memorial. There has been constant exploration for newer medicines or herbs to overcome these age-old problems of infertility. A variety of plants, herbo-minerals and animal origins have been used as fertile drugs in Siddha medicines. There is no scientific evaluation on *Anda odu Parpam* substantiating its usage as sexual stimulant.

The main aim of this dissertation is to do a scientific review, to validate the safety and efficacy of the *Anda odu Parpam* for *Vindhu kuraiivu* (Oligospermia).

### Objectives

- Besides the scientific study of *Siddha* science and medicine also our aim, the following methodology was adopted to evaluate the safety and efficacy of the test drug.
  - ❖ Identification of the *Anda odu* (Egg shell of *Gallus domesticus*)
  - ❖ Collection of various Siddha and modern scientific literature
  - ❖ Preparation of drug according to the text
  - ❖ Physio chemical analysis of *Anda odu Parpam*
  - ❖ Phyto chemicals analysis test drug
  - ❖ Evaluation of the toxicity of test drug
  - ❖ Evaluation of Spermatogenesis activity of test drug
  - ❖ Evaluation of Spermatogenic activity of test drug in experimental animal model
  - ❖ Clinical assessment of *Anda odu Parpam* on Oligospermia
  - ❖ Statistical analysis

### 3. REVIEW OF LITERATURE

#### 3.1. Gunapadam Aspect:

##### A) முட்டை ஓடு

##### (Hen's Egg Shell)

120 உபரச சரக்குகளில் ஒன்றான முட்டை ஓடு, சித்தமருத்துவத்தில் சுண்ணம், பற்பம் போன்ற மருந்துகளாக செய்யப்பட்டு, இரசவாதத் தொழிலுக்கும், மருத்துவ தொழிலுக்கும் மிகுந்த பயனை தருகின்றது. சித்த மருத்துவத்தில் முட்டை ஓடு பல நோய்களை நீக்க பயன்பட்டாலும், சிறப்பாக விந்துவை அதிகரிப்பதற்கு நல்ல மருந்தாக அகத்தியர் தனது அகத்தியர் பன்னிரு காண்டம் 200 நூலில் குறிப்பிடுகின்றார்.

#### வேறு பெயர்கள் :

“முட்டையுட பேர்தனையே மொழியக் கேளு

மும்மூர்த்தி, மஞ்சள் வெள்ளைகருவுமாகும்

நிட்டையாங் கடுங்கார நீறு மானோன்

நீற்றுண்டை யுமையா முருளையாகும்

குட்டை யாஞ்சுண்ணத்துக்கு உயிருமானோன்

கொடு வண்டம் மெழுகுக்கு கூர்மையானோன்

கட்டையாம் லோகங்கள் தகர்ந்து சாடுங்

காரத்தின் சத்துருவாம் முட்டைதானே

“ஊமை யுறுவ ஞயர்ந்த நீற்றுண்டைதான்<sup>47</sup>

துமய வண்ட முறுவான மும்மூர்த்தி

தாமய மஞ்சற் றனிவெள்ளை யாங்கரு

காமய வித்தைக் குகைகண்ட வண்ணமே”

மும்மூர்த்தி	சுண்ணத்திற்கு உயிருமானோன்
மஞ்சள் வெள்ளைகரு	அண்டம்
கடுங்கார நீறுமானோன்	மெழுகுக்கு கூர்மையானோன்
நீற்றுண்டை	லோகங்கள்
ஊமை	சாரத்தின் சத்துரு
உருளை	குகை
காமயவித்தை	ஞாணியண்டம்
முக்கருவு	மையண்டம்

**முட்டை ஓடு :**

அண்டவோடு

அண்டகுகை

அண்டதோல்

சித்தண்டு

நந்திரி

பஞ்ச சுண்டு

முட்டை - முட்டைதோடு, வெண்கரு, மஞ்சள்கரு

பொதுவாக முட்டை அல்லது சிற்றண்டம் என்பது மேற்கூறிய மூன்றையும் குறிப்பிடப்படுகின்றது.

**முட்டை பொதுக்குணம் :**

பெற்றக்கருவுக்கு கருவாய்ப் பேதமிலா நற்பொருளாய்

வற்ற மருந்துக் குறவாகிப் - பற்று

மினமா யவிழ்த விருப்பா யிருக்குஞ்

சினை முட்டை யண்ட மெனுஞ் சீர்.

சினை, அண்டம் எனப்படுகின்ற முட்டை கருவுக்கு கருவாயும், மருந்துக்கு பேதமிலா உறவாயும், பொருந்திய இனமாயும், அவிழ்தத்தின் இருப்பிடமாயும் சீர் பெற அமைந்திருக்கும்.

**பஞ்சபூத உபரச சரக்கு :**

அப்பு பூதம்

**முட்டை ஓடு சுத்திமுறைகள் :**

அழுக்ககற்றும் பூகற்சுண்ணம் நேராயிட்டு

அதையளந்து சட்டியிட்டு பாணிரெட்டி

விழுக்கருத்த ஞானிசினை பத்துமே தான்

விட்டு நீ கொதிக்கவைக்க யெண்ணெய் கக்கும்

(அழுக்ககற்றும் பூ - பூநீறு, கற்சுண்ணம், ஞானி சினை - கோழிமுட்டை)

கற்சுண்ணாம்பு, பூநீறு கலந்த கலவையில் கொதிக்க வைத்து சவ்வு நீக்கிக் கொள்ளுதல்

அமுரிநீர் அல்லது அப்பளகாரத்தில் கொதிக்க வைத்து கழுவி எடுத்துக் கொள்ளுதல்.

**பகைச்சரக்குகள் :**

‘இரும்புக்கு சத்துரு ஏற்றினன் என்னந்தி

அரும்பியே அண்டத்திண் வொடு அப்பிரகம்

- இரும்பு முட்டை ஓட்டிற்கு சத்துருவாம்.

அண்டதோல் சத்துருக்கள் -

காரம் வெங்காரம்

சாரம் சீனம்

தங்கம் கோழிமுட்டைவெண்கரு

காரீயம் நாகம்

காந்தம் வெண்கலம்

பித்தளை தரா

துருசு வெடியுப்பு

முட்டையில் உள்ள

காரசரக்கு - அண்ட ஓடு, நாகம்

சாரசரக்கு - மஞ்சள் கரு, கெந்தி



**கோழிமுட்டையில் நாகம், ரசம், கெந்தி உள்ளது**

**ஓட்டித்த ஓடதுவு நாகமாச்சு**  
யுத்தமனே வெண்கருதான்குதமாச்சு  
சுட்டித்த மஞ்சள் கரு கெந்தியாச்சு  
அப்பனே அண்டத்தின் பிறவி தானே

மேற்கண்ட பாடலில் முட்டை ஓட்டில் நாகம் உள்ளது எனக் கூறப்படுகிறது.

**இந்நாகம் இயற்கை நாகத்தை விட உயர்வுடையதாகும் என்பதை**

**ஆமென யின்னம் சொல்லி அறைகிறே னிந்த மட்டும்**  
தாமென அண்டத் துள்ளே தனித்த வெண்கருவே உப்பு

வாமென மஞ்சள் மார்க்கம் வழங்கிய புளியே யாச்சு  
**ஓமென மேலினோடு வசந்திடும் நாகமாமே**

என்ற பாடலின் மூலம் அறியலாம்.

**முட்டை ஓட்டில் 2 சவ்வுகள் உள்ளது**

1. கோரை
2. ஜவ்வு

சுத்தி செய்யும்போது 2 சவ்வினையும் நீக்குதல் வேண்டும்

**பஞ்ச சுண்ண குகையில் முட்டை ஓடு சேருகிறது.**

**குயினுமாம் கோழிமுட்டை சரக்குக்கெல்லாம் சுண்ணமாம்.**

கோழி முட்டை சரக்குகளை சுண்ணமாக்கும் தன்மை உடையது.

**முட்டை ஓடு சேர்ந்த மருந்துகளில் சில**

**சுண்ண மருந்துகள் :**

- ❖ அண்டசுண்ணம் - பற்பல விதமான வாயுக்கள்  
(எலுமிச்சைசாறு+இஞ்சிசாறு) மேகங்கள் தீரும்
- ❖ அண்டசுண்ணம் - பலநோய்கள் தீர்க்கும்
- ❖ சிற்றண்ட சுண்ணம் - தீராத நோய் எல்லாம் தீரும்.
- ❖ சிற்றண்ட சுண்ணம் - வாய்வுகள் தீரும்.
- ❖ அண்ட சுண்ணம் - பற்பல கொடியரோகங்கள்  
(நஞ்சறுப்பான்) அண்ட நோய் தீரும்.

- ❖ சிற்றண்ட சுய சுண்ணம் - வாய்வுகள் தீரும்.  
(பாவைசாறு)
- ❖ அண்டசுண்ணம்(எருக்கம்பால்) - இரசவாத தொழில் செய்யவும்  
நோய் தீர்க்கவும் உதவும்
- ❖ தனி அண்ட சுண்ணம் - இரசவாத தொழில் செய்யவும்  
நோய் தீர்க்கவும் உதவும்
- ❖ கற்பூர அண்டசுண்ணம் - இரசவாத தொழில் செய்யவும்  
நோய் தீர்க்கவும் உதவும்
- ❖ அண்டகுரு சுண்ணம் - இரசவாத தொழில் செய்யவும்  
நோய் தீர்க்கவும் உதவும்
- ❖ அண்டசுண்ணம் - பித்தவாயுவை குணப்படுத்தும்  
அதிவியர்வையை நிறுத்தும்
- ❖ வங்க சுண்ணம் - மேக சூலை தீரும்
- ❖ வெள்ளி சுண்ணம் - பல நோய்களை தீர்க்கும்.

#### பற்ப வகைகள்

- ❖ முட்டை ஓட்டு பற்பம் - **விந்து குறைவு**, அத்திசுரம், மூர்ச்சைபோகும்
- ❖ வெள்ளிபற்பம் - மேகம் போகும்
- ❖ தாமிரபற்பம் - மேகம், குன்மம் போகும்
- ❖ தங்கபற்பம் - **தாதுசோர்வு நீக்கும்**
- ❖ அண்டபற்பம் - கல்லடைப்பு, நீரடைப்பு, நீர்கட்டு தீரும்
- ❖ வங்க தாம்பர பற்பம் - மேகஊறல், இடுப்பு வாதம்,  
சர்வாங்க நடுக்கம் தீரும்
- ❖ வெள்ளிபற்பம் - நரம்பு தளர்ச்சி நீங்கி **தாது வலுக்கும்**
- ❖ வெள்வங்கபற்பம் - மேகரணம், கைகால் பிடிப்பு,  
குடைச்சல், வெள்ளை தீரும்

- ❖ சிற்றண்ட கல்நார்பற்பம் - நீர்கட்டு, நீர் எரிச்சல், வெள்ளை, வெட்டை தீரும்
- ❖ ஆறாதார்பற்பம் - நீரடைப்பு, கல்லடைப்பு, நீர்கட்டு, நீர் எரிச்சல் போகும்
- ❖ மகா தாம்பர பற்பம் - மேகம் 20, பிரமேகம் 21, வாதம் 80, பித்தம் 40, சிலேத்துமம் 20 போகும்.
- ❖ இராஜ தாம்பிர பற்பம் - மேகம், சகல உதர ரோகங்கள் போக்கும்
- ❖ கருவங்க பற்பம் - நீரிழிவுக்கு சிறந்தது
- ❖ தாமிர பற்பம் - மேகம் 20, பிரமேகம் 21, மகா பயங்கரமான ரோகங்கள் தீரும்.

## B)எலுமிச்சை

**வேறுபெயர்கள் :**

சம்பீரம்  
தேசிப்பழம்

**பயன்படும் உறுப்பு :**

இலை, காய், பழம், பழரசம், எண்ணெய்

**சுவை :**

இலை : புளிப்பு  
தன்மை : வெப்பம்  
பிரிவு : கார்ப்பு

**செய்கை:**

குளிர்ச்சியுண்டாக்கி

**காய், பழம் :**

சுவை : புளிப்பு  
தன்மை : வெப்பம்

பிரிவு : கார்ப்பு  
செய்கை : குளிர்ச்சியுண்டாக்கி

பழம் :

பொதுகுணம் :

“தாகம் குதநோய் தாழாச் சிலிபதநோய்  
வேகங்கொள் உன்மாதம் வீறுபித்தம் - மா கண்ணாய்  
கண்ணோய் வாந்தியும்போங் கட்டுவா தித்தொழிலில்  
மன்னெலுமிச் சங்கனியை வாழ்த்து”

தாகம், குதநோய், சிலிபதம், உன்மாதம், பித்தம், கண்ணோய், வாந்தி,  
வயிற்றுப்போக்கு.

சகல மேகரோகங்களுக்கும் நெய் :

அளவு : 2 தோலா, 2 வேளை

தீரும் நோய் : பிரமியம், தந்திபிரமியம், கை கால் எரிவு

கருவங்க பற்பம்:

எலுமிச்சை சாறு, கருவங்கம்

அளவு : 1 பணவெடை, 2 வேளை

அனுபானம் : தேன்

10 நாள் வயிற்று வலி தீரும்.

அதிமதுரம், முள்ளங்கிசாறு, பற்பம் - 21 வித மேகம் தீரும்.

பொதுமுறை :

வெள்ளாட்டுப்பால், எலுமிச்சைசாறு, மாம்பட்டை சாறு

1. நிறை 3 நாள்
2. எலுமிச்சம் சாறு, ஈருள்ளிச்சாறு, பனங்கருப்புக்கட்டி, 1 நிறை  
இரவில் பனியில் வைத்துக் காலையில் 3 நாள் சாப்பிடத் தீரும்

பிரமேகம், சுரப்பிரமேகம் தீரும்.

1. எலுமிச்சை எண்ணெய் :

2. நல்லெண்ணெய்

3. எலுமிச்சைச்சாறு சம அளவு

அளவு : 1-2 தேக்கரண்டி

அனுபானம் : நீராகாரம்

தீரும் நோய் : வெள்ளை

சூடு, கடுப்பு தீர ஏறண்ட எண்ணெய்

ஆமணக்கெண்ணெய்

தேங்காய் பால்

எலுமிச்சம் பழச்சாறு

வெங்காயச் சாறு

இரண்டு வேளை

நெல்லி கந்தகம்

பசும்பால்

எலுமிச்சைபழச்சாறு

கற்சண்ணதெளிநீர்

கற்றாழைச்சாறு சம அளவு பிரமேகம் தீரும்

எலுமிச்சை லேகியம் - பித்தம் தீரும்

### 3.2 Modern Aspect

#### A) முட்டை ஓடு

#### Zoological Aspect

#### Egg Shell (Or) Ovi Testa of *Gallus Domesticus*

#### Economy of *Gallus Domesticus*

*Gallus domesticus* is a domestic cock and hen. Taxonomy followed Howard & Moure (1994) species name updated according to Peters and Scissors (1931-1987)

Kingdom	:	Animal kingdom
Class	:	Aves
Order	:	Galliformis
Family	:	Phasianidae
Genus	:	Gallus
Species	:	Domesticus
Synonyms	:	Gallus & Gallus Gallus Gallus domesticus

#### Vernacular Names:

English	:	Egg
Sanskrit	:	Anda
Hindi	:	Anda
Bengali	:	Anta
Tamil	:	Muttai
Canada	:	Mottey

Maharashtra	:	Ande
Telugu	:	Gadda
Malayalam	:	Mutta

### **Structure:**

Egg consists of a shell, membrane, white and yolk. Hen and duck egg of average size are made up of,

Albumin	:	57%
Yolk	:	32%
Shell	:	11%

The shell of an egg has a rigid yet porous structure and is composed mainly of organic salts (Chiefly calcium carbonate). It has great resistance to the entry of microorganism. The surface of egg shell is covered by a thin cuticle.

Inside the shells are two tough and fibrous membranes are present. One attached to the shell and the other to the thick white at the small end of the egg.

### **Egg Shell Layers**

There are several layers in egg shell each with a different function. Different egg shells are made from slightly different materials. A chicken egg is much more rigid than other eggs.

**5 layers** morphologically differentiated from the inner to outer layer are, in consecutive order.

Inner egg membrane (innermost)



Outer egg membrane



The mammillae



Matrix



Finally cuticle (Outer most)

- The porous white finally surrounds the calcined egg shell on the outside the  $\text{CO}_3$  ion as well as calcium is equal significance in the calcification process of egg shell.
- Surrounding the egg white was two shell membranes each composed of a network of fibres. Outer membrane consists of 3 layers. **Outer must keratin fibres & 2 mucin fibres & inner membrane consist of keratin & mucinfibres.**
- The cuticle to be mainly mucin the presence of protein containing many disulphide links and free sulphydryl groups and some evidence for the presence of phospholipids was shown by Simkiss (1958).

### Chemical Constituents:

The shell of egg consists of predominately of calcium carbonate-96%. It has following composition.

Ca	–	38%
Mg	–	0.6%
$\text{CO}_3$	–	55%
Protein	–	1.5% & Reminder with water, trace minerals.



- ❖ Carbonate of lime
- ❖ Phosphate of lime
- ❖ Trace amount of Sulphur
- ❖ Iron
- ❖ Some Organic matters
- ❖ Salts like Chlorides, Iodides, Sulphates and Phosphate of K, Mg, Ca
- ❖  $\text{CaCO}_3$  – 88- 97%
- ❖ Ca and Mg, Phosphate (0.5 to 5%)
- ❖  $\text{MgCO}_3$  – (0.2%)
- ❖ Organic substance – 2 – 5%

#### **Action & Uses:**

- ❖ Useful in **Oligospermia**
- ❖ The carbonate of calcium renders the shell absorbent and antacid
- ❖ Shell sometimes used as antacid
- ❖ Bhasma of the shell is antacid and styptic, useful in diabetes, stops excessive urination and strengthen the kidney.

#### **Egg Shell Proteins**

Protein contain egg shell membrane and it contains several bactericidal enzymes and other membrane component, which may alter the thermal resistance of gram +ve, gram –ve bacterial pathogens in the thermal resistance of microorganisms might lead to the use of more moderate process.

#### **Egg Shell Collagen**

Egg Shell membrane comprises about 10% collagen, it is used in the bio-medical field, research to produce skin and tissue replacement Egg shell collagen economical acceptable than bovine collagen and protected from allergy of bovine collagen.

## **Egg Shell Minerals**

- It is considered as a good source of highly bio available calcium.
- Also contains significant quantities of strontium, which increases bone density in humans.
- A lower level of lead, aluminium, cadmium and mercury that normally occurs in egg shell may be advantage of egg shell powder over other natural calcium sources.
- Calcium – used as an oral phosphate binder. Patient suffering from renal failure often require oral phosphate binder to supplement other treatments to prevent hyper phosphatemia
- Egg shell calcium was shown to be more efficient as an oral phosphate binder than calcium carbonate.

## **Egg Shell Carbohydrates**

Glycosaminoglycons, have a wide range of applications in the pharmaceutical, cosmetics and food industries, including moisturizers in cosmetics treatment of osteoarthritis and as an emulsifying agent in mayonnaise and dressings.

**B) எலுமிச்சை**

**Lemon**

*(Citrus Limon)*

**Scientific classification:**

Kingdom : Plantae  
Division : Magnoliophyta  
Class : Magnoliopsida  
Subclass : Rosidae  
Order : Sapindales  
Family : Rutaceae  
Genus : *Citrus limon*

Binomial name : *Citrus limon*

**Other names:**

English : Lime  
Malayalam : Cheru-Naranga  
Hindi : Ninbu Limu  
Telugu : Nimma  
Kannada : Nimbe  
Sanskrit : Jambira

**Plant description:**

1. Grow up to 10mt but usually smaller.
2. Leaves are green shining and elliptical acuminate

3. Flowers are white violet streaked interior with strong fragrance.
4. On a Lemon tree, flowers and ripe fruits can be found at the same time.

**Fruits raw without peel:**

**Nutritional value per 100gm**

Energy	:	30 Kilo Calories (120KJ)
Carbohydrates	:	9 g
Sugars	:	2.5 g
Dietary fibers	:	2.8 g
Fat	:	0.3 g
Protein	:	1.1 g
Water	:	89 g
Vitamin C	:	53 mg (88%)
Citric acid	:	5 g
pH	:	2-3

**Medicinal uses of lemon:**

Lemon has been used for centuries for its protective and therapeutic qualities. Fresh lemon juice squeezed into water is often promoted to aid fertility health – plus it's refreshing.

A very common and easy to find fruit. Lemons are packed full of vitamin C which is an important antioxidant. They also contain the B vitamins, potassium, calcium, iron, magnesium, bioflavonoids, and phytochemicals, among other beneficial nutrients for spermatogenesis.

Antioxidants in lemon help protect our cells from free radical damage. This is important for the health of sperm cells and the female ovum (egg cell) including the DNA within.

The high vitamin C content in lemon juice helps ward off and relieve the symptoms of infections such as colds, sore throats, and influenza. Sickness if bad enough can upset the body's natural rhythms, including ovulation or sperm production. In fact a bout of flu/fever can lower a man's sperm count for up to three - four months after the ailment has passed!

Getting adequate amounts of vitamin C through lemon juice and other foods in diet may improve male fertility. A study published in the July 2011 issue of "Reproductive Biology and Endocrinology" investigated the effect of vitamin C on hyperglycemic rats and found it decreased the amount of abnormal sperm and boosted testosterone levels, both of which may contribute to male infertility.

Consume a serving of lemon juice have 3 percent of the thiamin for each day. This thiamin may contribute to a protective effect when it comes to testicular injury. Research featured in the February 2009 "Journal of Huazhong University of Science and Technology" indicates that thiamin consumption -- in conjunction with vitamin C -- benefits sperm count and motility in male rats with impaired fertility triggered by lead exposure.

## **C) Ghee**

Ghee is a class of clarified butter. It is composed almost entirely saturated fat.

Ghee is rich with antioxidants and acts an aid in the absorption of vitamins and minerals from other foods, serving to strengthen the immune system.

Ghee is an oil that offers important nutritive benefits, even for lacto-vegetarian diets, helping cell membranes maintain a low state of oxidation by replacing oxidized fats.

### **Calories and Fat**

Ghee contains 112 calories per tbsp. and 33 mg of cholesterol. The total fat content is 12.7 g, of which almost 8 g are saturated fat, 3.7 g monounsaturated fat and .5 g polyunsaturated fat. Ghee provides many essential fatty acids, such as omega-3 and omega-6 that provide anti-oxidant properties, regulate DNA production and assist with cellular communication.

### **Nutrition**

The protein content of ghee is .04 g per tbsp., which includes 17 amino acids, essential for good health. Ghee contains 3 percent linoleic acid, an antioxidant. Ghee provides 393 IU of vitamin A per tbsp., including 105 mcg of retinal and 25 mcg of beta-carotene. Other vitamins include .36 mg vitamin E per tbsp., 1.1 mcg of vitamin K, and small amounts of riboflavin and pantothenic acid. Minerals in ghee include 1 mg of calcium and potassium per tbsp.

### **Health Benefits**

Ghee is a good choice for the lactose intolerant because all the milk proteins are removed during the clarifying process, making it lactose free. Although ghee contains a high amount of fat -- about 65 percent saturated, 25 percent monounsaturated and about 5 percent polyunsaturated -- fats are essential to life and are needed to assimilate vitamins A, D, E and K, and help nourish the skin, hair, cell membranes and help to increase the sperm production. Fats promote a healthy body temperature, help nourish and protect internal organs and the brain and store energy.

### 3.3 Siddha Aspects of the disease (*Vindhu kuraivu*)

#### Evolution Theory

பராபர மாகிய பரமேஸ்வரன்

தராதாம் படைக்கத் தானினைத் தருளி

-Jeevorpathi Chintamani(A compendium of *Siddha* Doctreine) Pg.42.

According to the work *Jeevorpathi Chintamani* the *paraparam* (the God Supreme) the *Sivam* emerges from *Para*, the *Sakthi* from *Siva*, then *Nandha* from *Sakthi*, the *Vindhu* from *Nandha*, the *Sadasivam* from *Vindhu* the *Maheswara* from *Sadasiva*, the *Rudra* from *Maheswara*, the *Vishnu* from *Rudra*, the *Brahma* from *Vishnu*, then the *Akash* (Space) comes out from *Brahma*, *Vayu* (Air) from *Akash*, *Agni* (Fire) from *Vayu*, *Appu* (Water) from *Agni*, then *Prithivi* (Earth) from *Appu*, then the Food (*Annam*) emerges from *Prithivi* then the Food gives rise to *Sangama* objects like Human, Animal, Birds And Vegetation.

#### *Athuva tathuvam:*

From the Food, *Saaram* (Chyle) is produced, from *Saaram Senneer* (Blood) is formed, from that *Oon* (Muscle), *Kozhuppu* (Fat), *Enbu* (Bone), *Moolai* (Bone marrow), *Sukkilam* (or) *Suronitham* [Semen (or) Ovum] are formed in that order. The fusion of these *sukkila* and *Suronitha* constitutes the human body.

#### விந்து (Semen)

இரத முதலான ஏழ் தாது மூன்றின்  
உரிய தினத்தின் ஒரு புற் பனி போல்  
அரிய துளி விந்து வாகு மேழ் மூன்றின்  
மருவிய விந்து வளருங்கா யத்திலே

-திருமந்திரம்-1897

இரதமாகிய சாரம் முதலாக சொல்லப்படும் ஏழு முதற் பொருள்களால் ஆக்கப்பட்டதில் வுடல் இவ்வேழு உடற்கட்டுகளுள் சாரம், செந்நீர், வெண்ணீர் ஆகிய மூன்றும் ஒர் நாள் ஒரு புற்பனிபோல் திரளும்.

இத்திரட்சியே விந்து எனப்படும். இவ் விந்து ஏழ் மூன்றாகிய இருபத்தி ஒன்று நாட்கள் வரை வளரும் என திருமூலர் கூறுகின்றார்.

“அழிகின்ற விந்து அளவையறியார்  
சுழிகின்ற தன்னையுட் காக்கலுந் தேரார்  
அழிகின்ற காயத் தமிழந்தயர் வற்றோர்  
அழிகின்ற தன்மை யறிந் தொழியாரே”

-திருமந்திரம்-1899

As per the *Thirumoolar Thirumandiram* it has been described that 6400 drops of Blood Cells make one drop of Vindhu (Example: 80 drops of red cell make one drop of white corpuscle and 80 drops of white corpuscle make one drop of *vindhu*)

Thus  $80 \times 80 = 6400$  drops of blood cells makes one drop of *vindhu*.

If extensive loss of *vindhu* occurs in one human body naturally it will reflect on blood cells.

*Siddhar Therayar*: also mentioned about the importance of *vindhu*

‘வெல்லும் புவியில் விளங்கிய தாபரம்  
புல்லிடுஞ் சங்கமம் பொறியிடும் விந்தே  
விந்தினாலல்லோ மேதினி யாச்சுது  
செந்துக்களெஞ்லார்ஞ் சிவமயமாச்சுது (மேதினி- உலகம்)  
வந்திடும் நாதம் மௌனம் கலந்திது  
சிந்தையிலுள்ளத் தெளிவாகச் செப்புமேசு

-நோய் நாடல் நோய் முதனாடல் திரட்டு 1 -பக்கம் 94,

விந்துவினால்தான் உலகம் (மேதினி) உண்டாச்சு என்று சித்தர் தேரையர் கூறுகிறார்.

*Agathiyar Vaidya Valladhi* -600:

‘ஆண்மையென்று மங்கையர்கள் பூக்குங்காலம்  
அன்று முதல் பதினாலு நாளும் அந்தத்  
தான்மையன்றிப் பதினாறு இதமாய் நின்ற  
தாமரைபோல் மலர்ந்திருக்குஞ் சாற்றக் கேளு  
காண்மையன்றி தினமொன்று இதழ் தானென்று  
கருவான கருக்குழிதான் இந் நாட்டுக்குள்ளே  
பான்மையென்ற விந்தங்கே யூறும்போது  
பாயும்பா வன்னியோடு வாயுதானே !

-சித்த மருத்துவாங்க சுருக்கம் - பக்கம் 36.

According to *Agathiyar Vaidya Valladi* – 600 – The *vindhu* (Semen) is chiefly constituted by the Fire (*Vanni*) and Air (*Vayu*) elements.



### **Siddhar Kaya Karpam-300:**

“யோகியும் ஞானியும் உத்தம சித்தனும்  
யோகியும் ஞான புரந்தர னாவோனும்  
மோக முறினு முரையமிர் துண்போனும்  
ஆகிய விந்து அழியாத அண்ணலே  
ஒழியாத விந்துவுடன் நிற்கும்  
அழியாப் பிராணன் ஆதி பலஞ் சத்தி  
ஒழியாத புத்தி தபஞ்செப மோனம்  
அழியா சித்தியுண்டாம் விந்து வற்றிலே”

-சித்தர் காய கற்பம் 300-பக்கம் 8-9

In this poem *Siddhars* emphasized the need to control, the *vindhu* through *siva bhogam*, for a healthy and long life. *Thirumoolar*, *Therayar* and *Agathiyar*, *Bhogar* recommended the *Kayakarpam* therapy to rejuvenate *vindhu*.

### **Saint Yoogi said:**

முனையறுகு நுனிபனிபோல் சரோணிதத்தில் சேர்ந்து  
தாக்கவே சுரோணிதந் திரண்டு தன்ரூபமாகித்  
தமர்வாசல் தனைமூடும் வாயு தானும்

The Semen and Ovum are like dew on the sharp tip of a grass. (முனையறுகு நுனிபனிபோல்) which means the Semen and Ovum merges and that the Sperms in the Semen moves in almost swiftness and the Spermatozoa enters by penetrating the wall of the Ovum and merges with in it.

“வேர்க்கவே வேலிபோல் வளைந்து காக்கும்  
விந்துவுடன் பிராணவாயு விளக்க லாமே”

-சித்த மருத்துவாங்க சுருக்கம் - பக்கம்-3.9

According to Saint Yoogi, just as the Fence guards the garden the air surrounds the zygote and guards and prevents other sperms entering it.

### **According to Thiruvalluvanayanar Gnanavettiyan – 1500**

*Thiruvalluva Nayanar* clearly explained that the hormonal influence and Brain stem is charactgerized with the following features.

“விந்து குடியிருந்த திருநாட்டை விட்டேன்  
மாறுகின்ற கத்தரிக்கோல் பட்டந்தன்னில்  
விந்து நின்று விளங்கு நதி மையத்துள்ளே  
விளங்கு சுவாதிஷ்டான வெளியிலேதான்”

In this poem we may understand that;

Spermatogenesis is stimulated by commands from the cerebral cortex. Decussating of the fibers in the brain stem explains this. Spermatogenesis is controlled by pituitary gland and Hypothalamus.

### ***Thiruvalluva Nayanar – Navarathina Chinthamani – 800***

திருவள்ளுவ நாயனார் தந்தை விந்தினால் பிறப்பு உண்டாகிறது என்பதை கீழ் வரும் பாடல் மூலம் சொல்கிறார்.

தந்தை விந்ததி சாரத்திலரவமா யுதித்து  
ஐந்து பூதம் தாக வுப்பது வுவர் படர்ந்து  
விந்து மேலபி வருதல்தை மாசி பங்குனியும்  
விந்து வாலுதித் திலகிய நாத முமிதுவே

-திருவள்ளுவ நாயனார் நவரத்தின வைத்திய சிந்தாமணி- 800 பக்கம் -3

தந்தையின் விந்துவின் சாரத்தினால் உயிர்கள் உருவாகி உயிர் 5 பூதங்களினால் வளர்ந்து வரும் எனவும், குழந்தை தை மாதத்தில் பிறக்க விந்துவானது மாசி, பங்குனியில் நாதமுடன் சேர வேண்டும் என திருவள்ளுவர் நாயனார் நவரத்தின சிந்தாமணி - 800 யில் கூறுகிறார்.

“பின்னுமாம் சுக்கிலத்திற் பிராணவாயு தான் சென்று

பின்னுமாம் இரத்தம் சூழ்ந்து உதானவாயு வளர்க்கும்”

-தன் வந்திரி நாடி- சித்த மருத்துவாங்க சுருக்கம்- பக்கம்-39

### **இந்திரியப் பரிட்சை**

“ஐயமளை பால் மோர்தே னாச்சியங்கள் கம்மி வையை  
யையமளை யாதறிந்து கொள்ளுவா- யையமளை  
யுத்த மத்தை முன்னே குணரிந்திரி யப்பரிட்சை  
யுத்தமத்தைநூலதரை யோர்

தண்ணீரைப் போலிருப்பின் சாரம் முதலிய  
உடல் தாதுக்களிலும் சற்றும் சாரமற்றவனென்றும்  
உயிருடன் இருந்தாலும், பிணத்துக்கு ஒப்பானவன்  
என்றும் உணரக்கடவாய்

-தேரையர் யமக வெண்பா- பக்கம் -65 பாகம்-1

இந்திரியப் பரிட்சை அதன் பக்குவமான நிலையை, ஆயுள்வேத மென்கிற வாகட  
சாத்திரப்படியே கூற வேண்டும் என்று கூறுகிறார்.

### சுக்கிலத்தன்மை

#### பிணியாளனின் சுக்கிலம்

வெண்மையும் வெண்ணைக்கு நிகராயுமிருப்பின்  
உத்தமோத்தமமென்றும்

வெண்மையும் தயிருக்கு நிகராயுமிருப்பின்  
உத்தமத்தில் இரண்டாம் தர உத்தமமென்றும்

வெண்மையும் பாலுக்கு ஒப்பாகவுமிருப்பின்  
மத்தி மோத்தம மென்றும்

தேனையும் அதன் நிறத்தையும் கனத்தையும்  
ஒத்திருந்தால் அதம மத்திமமென்றும்

நெய்யையும் அதன் நிறத்தையும் கனத்தையும்  
ஒத்திருந்தால் அதம மத்திமமென்றும்

கள்ளைப் போன்ற நிறமும் தடிப்புமிருப்பின்  
அதமாத மோத்தமம் என்றும்

- நோய் நாடல் நோய் முதனாடல் திரட்டு -1-பக்கம் -197

தண்ணீரைப் போலிருப்பின் சாரம் முதலிய உடற்தாதுக்களிலும் சற்றும் சாரமற்றவை  
என்றும், உயிருடனிருந்தாலும் பிணத்துக்கு ஒப்பானவனென்றும் என தேரையர் கூறுகிறார்.

1. It is good if semen is white and resembles like butter or curd.
2. If it is thin like honey and ghee in its colour and densities, it is not considered as good quality semen.
3. It is suppose to be worst if the semen resembles like toddy or water in its colour.

The disease “*Aan Maladu*” (Male Infertility) is well described in *Yoogi Vaidya Chindamani* as

“பார்க்கவே ஆண்மகனின் விந்து தானும்  
பதமான தித்திப்புயில்லாத தாலும்  
எற்கவே சலமிதில் மிதந்தாலும்  
எழிலாக உயிர்ப்பற்று இருப்பதாலும்  
சேர்க்கவே மூத்திரத்தில் நுரைதான் போலும்  
செயலான கருவதுவும் தரிக்க மாட்டா  
தீர்க்கவே யூகி முனி சிகிச்சாசாரம்  
தெளிவாக பாடி வைத்தார் திறமிதானே”

- மகளிர் மருத்துவம்- பக்கம் -45

The characteristics of “*Aan maladu*” (Male infertility) as for as the Semen is concerned are

- 1) Lack of Sweetness
- 2) lacking of motility of the semen
- 3) lack of virility and frothy micturition
- 4) buoyancy on water

ஆண்களின் விந்துவை நீரிலிடமிதந்தாலும், இனிப்பு சுவையில்லாமல் இருந்தாலும், உயிர்பில்லாமல் இருந்தாலும், புணர்ச்சியுரினும் கருத்தரிக்காமல் இருக்கின்ற குறிகுணங்களை கொண்ட செய்கைக்கு ஆண்மலடு என்று யூகிமுனிவர் தெளிவாக கூறியுள்ளார்.

அகத்தியர் வைத்திய சிந்தாமணி வெண்பா - 4000

**சுக்கில வாதம்:**

“வாயு வாதம் காற்றினிடை வந்தால் அவயங்கள்  
பாயுங்கால் வலிக்கும் பண்ணுகுணம்- காயத்தின்  
சுக்கிலக் காலந்திரத்திற் துன்னு துரிதமின்னம்  
புக்கி நிறத் தாது கெட்டுப்போம்”

- அகத்தியர் வைத்திய சிந்தாமணி வெண்பா- 4000 - பக்கம் -60-பாகம்-1

பின் இடுப்பில் கல்லை கட்டியது போன்ற பாரம் உண்டாகி இடுப்பு வலிக்கும். உடல் வலி ,சோர்வு உண்டாகும்.

சுக்கிலம் விரைவில் வெளியாதல், தாது கெடல் போன்ற குறிகுணங்கள் சுக்கில வாதத்தில் வரும். ஆண்மலடு தாது கெடல் - ஆல் வரும் என அகத்தியர் வைத்திய சிந்தாமணி வெண்பா நூல் கூறுகிறது.

**சுக்கில சிலேத்துமம்:**

“மேன் மூச்சு நோவிருமல் மெய் சூம்பல் கணயோதல்

ஈனத் தொனியும் சீரணமாம் - மேன் கழுத்து

சுட்கித்து மார்பு நேரம் சீரெரிச்சல் தாகமுமாம்

சுக்கில சிலேத்தும மெனச் சொல்”

-அகத்தியர் வைத்திய சிந்தாமணி வெண்பா- 4000 பக்கம் - 76- பாகம்-1

**பதினாக்கு வேகங்களில் ஒன்றான சுக்கிலத்தை அடக்கினால் உண்டாகும் நோய்கள்:**

“சுக்கிலந் தனையடக்கின்

சுரமுடனீக் கட்டாகும்

பக்கமாங் கை கால் சந்து

பாரநோய் வழியிறங்கும்

மிக்க மார் நோயுண்டாகும்

மிகுந்திடும் பிரமேகந்தான்

தக்கதோர் போதுமாகின்

தரித்திடும் வாயுக் கூறே”

-உடல் தத்துவம்- பக்கம் -337

If semen is controlled by man against nature, it may lead to fever, oliguria, joint pain, urinary infection, spermatorrhoea, leucorrhoea and chest pain.

**திருமூலர் திருமந்திரம்:**

“பாய்ந்த பின் அஞ்சோடில் ஆயுளும் நூறாகும்

பாய்ந்த பின் நாரோடில் பாரினில் எண்பதாம்

பாய்ந்திடும் வாயும் பகுத்தறிந் திவ்வகை

பாய்ந்திடும் யோகிக்கும் பாய்ச்சலுமாமே”

-திருமந்திரம்- 463 பக்கம் - 290, பாகம் -2.

ஆணிமருந்து வெளிப்படும் விந்து ஐந்து விரற்கிடை பாய்ந்தோடில் பிறக்கும் குழந்தைக்கு ஆயுள் நூறு. நாலுவிரற்கிடை பாய்ந்தோடனால் ஆயுள் எண்பது. இவ்வாறு சுக்கிலம் விழும் வேகத்திற்கு தகுந்தவாறு சிசுவின் ஆயுள் நிர்ணயம் செய்யப்படுகிறது என விந்துவின் முக்கியத்துவத்தை திருமூலர் கூறுகிறார்.

**தாழ்வு மனப்பான்மையால் மலடனாவது பற்றி திருமூலர்,**

ஆண்மிகில் ஆணாகும் பெண்மிகில் பெண்ணாகும்

பூனிரண் டொத்துப் பொருந்தில் அலியாகுந்

தாண்மிகு மாகில் தரணி முழுதாளும்

பாணவ மிக்கிடில் பாய்ந்ததும் இல்லையே

- திருமந்திரம் - 462, பக்கம் 290, பாகம் -2.

ஆணின் சக்தி மிகுந்திருந்தால் ஆண் குழந்தை பிறக்கும். பெண்ணின் சக்தி மிகுந்திருந்தால் பெண் குழந்தை பிறக்கும். இரண்டும் சமமாக ஒன்றோடு ஒன்று போட்டி போட்டு அழிக்க முற்படும் நிலையில் பிறக்கும் குழந்தை அலியாகும்.

கலவியின்போது ஆணின் சக்தி நீடித்து நின்றால் பிறக்கும் குழந்தை உலகையே ஆளக்கூடிய ஆற்றல் உள்ளவனாக இருப்பான்.

ஆண் தாழ்வு மனப்பான்மை ஏற்பட்டு துவண்டு போவானானால் சக்சிலம் வெளிப்படுவதும் நின்றுவிடும் என திருமூலர் கூறுகிறார்.

### அகத்தியர் வைசுரி நூலில் - அகத்தியர்

அறிந்த பின் இவர்களுட குணந்தானப்பா  
அந்தந்த சரீரத்திற் கடுக்க வாராய்  
தெரிந்த தொரு குணக்குறிகள் தோன்றுமப்பா  
திறமான கரும்பினிசை விந்தைக் கொல்லும்  
பிரிந்ததொரு கெர்ப்பத்தை யழியப் பண்ணும்  
பண்பாக யவர்களுக்குப் பிள்ளையில்லை  
முரிந்த தொருயிவர்களுட குணமுஞ் சொன்னேன்  
முறைமையுடன் மருந்து வகை சொல்லக் கேளேசு

-நோய் நாடல் நோய் முதனாடல் திரட்டு II பக்கம்-66.

அகத்தியர் தனது அகத்தியர் வைசுரி என்ற நூலில் கரும்பணிசை அம்மை நோய் பாதித்தவர்கள் ஆண்களாக இருந்தால் விந்து அழியும் எனவும், பெண்களுக்கு கர்ப்பமும் அழியும் எனவும் இந்நோயில் வருந்தியவர்கள் பெரும்பாலும் மலடாகவே இருப்பார்கள் என கூறுகிறார்.

அம்மை நோயில் இன்னொரு வகையான புட்டாலம்மை (Mumps) நோய் பாதித்தவர்களும் பெரும்பாலும் மலடாக இருப்பார்கள் என சித்த மருத்துவம் கூறுகிறது.

### T.V. சாம்பசிவம்பிள்ளை அவர்களின் கூற்று

#### ஆண் மலடு:

ஆண் பிள்ளைக்கு இயற்கையாகவே அமைந்த மலட்டுத்தன்மை, இதனால் அவர்களின் விந்து தித்திப்பு இல்லாமல் தண்ணீரில் விட்டால் கரைந்து மிதப்பதும், உயிர்ப்பற்றதும், மூத்திரத்தில் நுரை கட்டுவதுமாக இருக்கும். இவ்விந்துவினால் பெண்களுக்கு ஒரு காலும் கருப்பந்தரிக்க மாட்டாது.

Want of fertility of fecundation in a man's Semen. The semen in such cases will be devoid of sweetness, immotile and will float on the surface of water and the urine also will be frothy. Such man will be incapable to impregnate women.

### **The variations of the *Sukkilam* – physical constituents**

#### ***Sukkilam* excess:**

Excess *sukkilam* causes love and lust towards women and also urinary calculi.

#### ***Sukkilam* decreased:**

Decreased *sukkilam* causes failure in reproduction, pain in the genitalia etc.

### **Diagnosis (பிணியறிமுறைமை):**

Diagnostic methods in Siddha medicine are very unique and are mainly based on the clinical aspect.

பலிப்பதற்கு வியாதியுள்ளோர் தேகக் கூறும்  
பருத்தவுடல் மெலிந்தவுடல் நோயின் கூறும்  
சலிப்பில்லா மலக்கூறும் சலத்தின் கூறும்  
சாத்தியம் அசாத்தியமும் தத்துவக் கூறும்  
வலிப்பில்லாத் தாது நித்திரையின் கூறும்  
மங்கையர்கள் ஆசையின் மேல் மயக்க கூறும்  
பலிப்பில்லாக் கன்மதன்மை நாட்டக் கூறும்  
பார்த்தறிந்த பிறகு பார் மருந்தின் கூறே!

சித்த மருத்துவாங்க சுருக்கம்

.பக்.309.

According to this the constitution of body types of disease, examination of stools and urine, whether it is curable or not, sleep nature of the patient, Sexual indulgence and karma should be analyzed and then only treatment should be given to the patient.

### **3.4 Modern aspects of the disease**

#### **Infertility**

The WHO defines infertility as a disease if the reproductive system that impairs the body's ability to perform the basic function of reproduction. Although conceiving a child may seem to be simple and natural, the physiological process is quite complicated and depends on the proper function of many factors, including the following,

- Production of healthy sperm by the man
- Production of healthy ovum by the woman
- Unblocked fallopian tubes that allow the sperm to reach the ovum
- The sperm's ability to fertilize the ovum
- The ability of the fertilized ovum to become implanted in the uterus
- Adequate embryo quality

#### **Male Infertility**

##### **Definition**

Male factor infertility is said to be present when a couple fails to achieve pregnancy after one year of unprotected coitus and a problem is identified in the male partner.

Causes of infertility in men can be explained by deficiencies in ejaculate volume causing low sperm production (Oligospermia), poor sperm motility (asthenospermia), abnormal morphology (teratospermia), and abnormal sperm function or by preventing sperm transport to vagina.

##### **Classification**

###### **Primary Infertility**

This is when the man has never impregnated women.

###### **Secondary Infertility**

This is when the man has impregnated women, irrespective of whether she is the present partner and irrespective of the outcome of the pregnancy. Men with secondary infertility in general have a better chance of future fertility.



## **Structures of Male Genital Organ**

### **Male reproductive system**

The male reproductive system consists of the primary reproductive organs, the testis and the secondary reproductive organs, which include the,

- ✓ Scrotum
- ✓ Epididymis
- ✓ Ductus deferens
- ✓ Seminal vesicles
- ✓ Prostate gland
- ✓ Urethra
- ✓ Bulbo-urethral glands and
- ✓ Penis

The tests in which the sperm cells develop are located outside the body cavity in the scrotum where the temperature is lower.

Sperm cells are transported from the testes to the epididymis, which lies on the external surface of each testis and then through the ductus deferens into the prostate.

Just before the ductus deferens enters the prostate gland, the ductus deferens increases in diameter to become the ampulla of the ductus deferens. A short duct of the seminal vesicle joins the ampulla of the ductus deferens to form the ejaculatory duct at the prostate, which then projects through the prostate gland and empties into the urethra, within the prostate gland. The urethra exists from the pelvis and passes through the penis to the outside of the body.

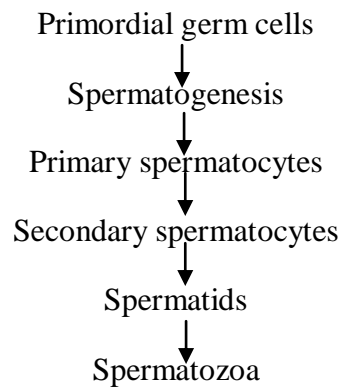
### **Physiology**

- ❖ Spermatogenesis
- ❖ Performance of the male sexual act
- ❖ Regulation of male reproductive functions by the various hormones.

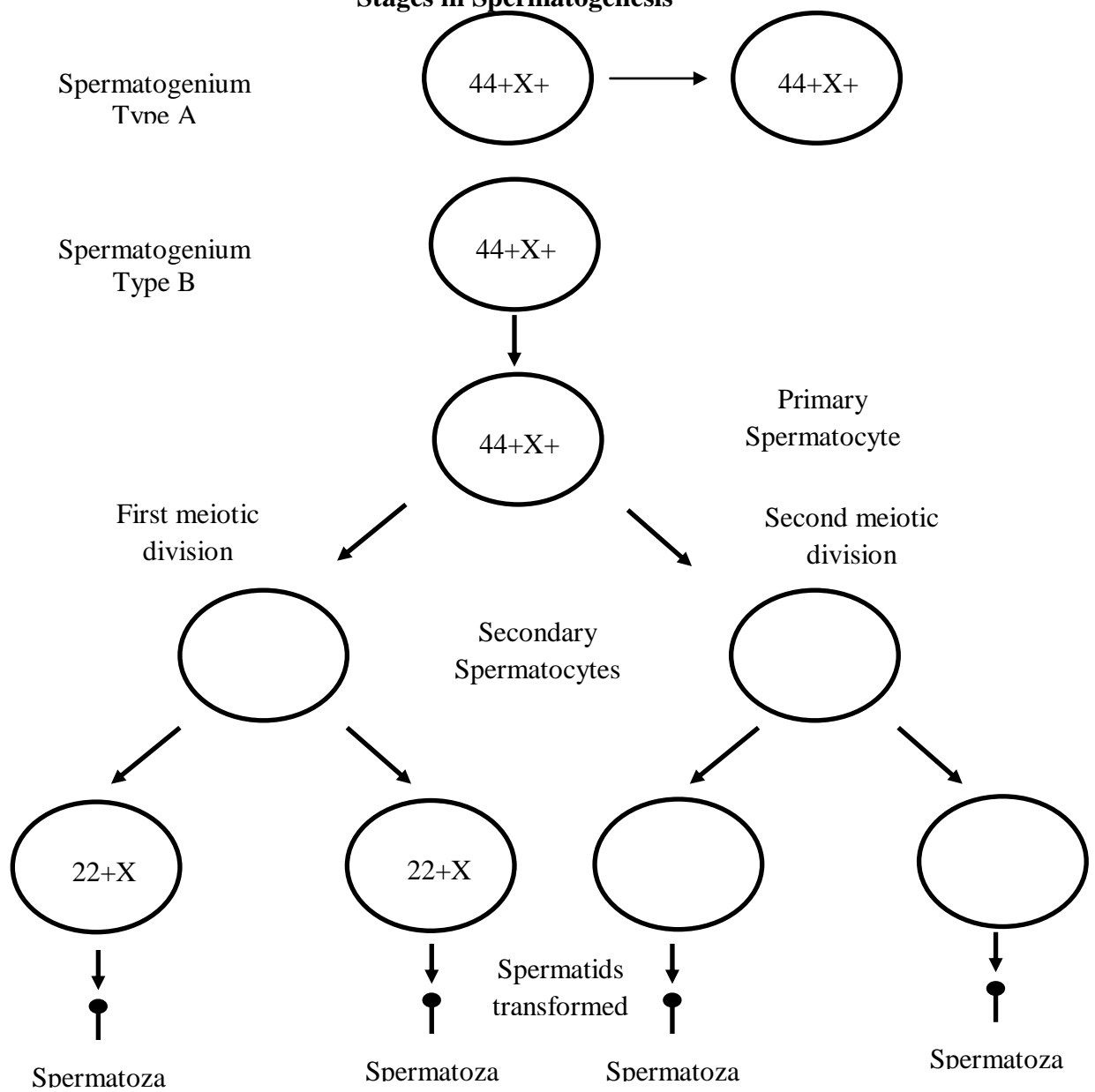
### **Spermatogenesis**

Spermatogenesis occurs in all the seminiferous tubules during active sexual life as the result of stimulation by anterior pituitary gonadotropic hormones, beginning at an average age of 11-13 years and continuing throughout the remainder of life.

## Stages of Spermatogenesis



### Stages in Spermatogenesis



The process of spermatogenesis takes approximately 60-70 days from the beginning of the differentiation of the spermatocyte to a completion of the motile sperm. When the sperm leave the testis they are relatively immature and have a poor capacity to fertilize. The transport of the sperm through the epididymis to the ejaculatory duct requires an additional 12 to 21 days.

### Secretary products of the cells in the testis

Sl.No	Cell Products	Proposaed Function
A.	<b>Leydig Cells</b> <ol style="list-style-type: none"> <li>1. Androgens</li> <li>2. Proopiomelanocortin</li> <li>3. Inhibin</li> <li>4. IGF – I</li> <li>5. IL - 1<math>\beta</math></li> </ol>	<ol style="list-style-type: none"> <li>1. Endocrine, paracrine, autocrine control</li> <li>2. Opioids, <math>\alpha</math>-MSH, ACTH</li> <li>3. Endocrine, paracrine activity</li> <li>4. Growth, differentiation</li> <li>5. Kinin activity</li> </ol>
B.	<b>Sertoli Cells</b> <ol style="list-style-type: none"> <li>1. ABP</li> <li>2. Transferrin</li> <li>3. Ceruloplasmin</li> <li>4. Estrogen/Aromatase</li> <li>5. Laminin, Collagen</li> <li>6. Proteoglycans</li> <li>7. TFG-<math>\alpha</math>, TFG-<math>\beta</math>, IGF-I, IL-I</li> </ol>	<ol style="list-style-type: none"> <li>1. Binding of androgens</li> <li>2. Iron Transport</li> <li>3. Copper Transport</li> <li>4. Endocrine, paracrine regulation</li> <li>5. Extracellular matrix</li> <li>6. Growth factors which inhibit or stimulate cell physiology and proliferation</li> </ol>
C.	<b>SC-EGF</b> <ol style="list-style-type: none"> <li>1. Inhibin, Activin</li> <li>2. Mullerian, inhibitory factor</li> <li>3. LHRH-Like substance</li> <li>4. Lactate / Pyruvate</li> </ol>	<ol style="list-style-type: none"> <li>1. Endocrine, paracrine</li> <li>2. Fetal Sertoli cell development</li> <li>3. Binds of leydig cells in rats</li> <li>4. Metabolites, nutrients for gem cells.</li> </ol>
D.	<b>Peritubular Cells</b> <ol style="list-style-type: none"> <li>1. P-Mod-S</li> <li>2. Fibronectin</li> <li>3. Proteoglycans</li> <li>4. TFG-<math>\alpha</math>, TFG-<math>\beta</math>, IGF-I</li> </ol>	<ol style="list-style-type: none"> <li>1. Paracine regulation od\f Sertoli cells</li> <li>2. Extra cellular matrix</li> <li>3. Growth factors</li> </ol>

During passage through the epididymis the spermatozoa has the following features,

- Maturation takes place
- Sustained motility
- Modification of nuclear chromatin and tail organelles
- Loss of spermatid cytoplasm

### **Hormonal factors that stimulate Spermatogenesis**

#### **1. Testosterone**

It is secreted by the Leydig cells. Located in the interstitium of the testis, it is essential for growth and division of the germinal cells in forming sperm.

#### **2. Luteinizing Hormone**

Secreted by the anterior pituitary gland, it stimulates the Leydig cells to secrete testosterone.

#### **3. Follicle – Stimulating Hormone**

FSH is also secreted by the anterior pituitary gland, it stimulates the Sertoli cells. Without this stimulation the conversion of the spermatids to sperm will not occur.

#### **4. Estrogens**

It is formed from testosterone by the Sertoli cells, when FSH stimulates them. It is probably also essential for spermatogenesis.

The Sertoli cells also secrete an androgen-binding protein that binds both testosterone and estrogens and carries these into the fluid in the seminiferous tubular lumen thus making both these hormones available for maturing the sperm.

#### **5. Growth Hormone**

It is necessary for controlling the background metabolic functions of the testes. Growth hormone especially promotes early division of the spermatogonia themselves. In its absence as in pituitary dwarfs, spermatogenesis is severely deficient or absent.

## **The Hypothalamic – Pituitary – Testicular Axis**

The hypothalamic – pituitary – testicular axis is physiologically a closely integrated system.

Testicular function is regulated by a series of closed-loop feedback systems involving the higher centre in the central nervous systems (CNS), the hypothalamus, the pituitary and the testicular, endocrine and germinal compartments.

The hypothalamus is the site of production of gonado-tropin-releasing hormone (GnRH). GnRH binds to GnRH receptors in the pituitary gland and stimulates the synthesis and release of the Gonadotropic Hormones, Luteinizing Hormone (LH) and Follicle stimulating Hormone (FSH). LH and FSH are secreted by the pituitary gland into the general circulation and carried to the testes. In the testis they stimulate gonadal secretion of steroid hormones (testosterone and estradiol) that are important in the maturation and maintenance of spermatogenesis.

Testosterone is the major steroid hormone produced by the testis. 98% of testosterone circulates bound either to sex hormone – binding globulin (SHBG) or to albumin. The testis secretes only 25% of circulating estradiol. Dihydro testosterone (DHT) another potent androgen is derived from the peripheral conversion of testosterone. This DHT is necessary for external virilization during embryogenesis and androgen action during puberty and adulthood.

The testis also produces non-steroid substance inhibin, secreted by the sertoli cells. Inhibin may also exert local regulatory effects on spermatogenesis.

Prolactin a polypeptide hormone is synthesized and secreted from the pituitary gland. Prolactin stimulates lactation in women. Elevated levels of prolactin suppress testosterone synthesis in man. Control and coordination of testicular function occur via feedback signals both positive and negative exerted by the hormones secreted at each level of the hypothalamic-pituitary-testicular axis.

## **Infertility History**

### **1. History of Infertility**

- Duration
- Prior Pregnancies
- Previous treatments
- Evaluation and treatment of wife
- Present partner
- Another partner

### **2. Sexual History of the Man**

- Frequency of masturbation
- Frequency of intercourse,
- Timing of intercourse
- Potency
- Lubricants

### **3. Childhood and Development**

- Undescended testicles
- Herniorraphy
- Testicular trauma
- Testicular torsion
- Y-U plasty of bladder
- Onset of puberty-early, normal or delayed

### **4. Family History**

- History of infertility in his family members
- Cystic fibrosis
- Androgen receptor deficiency

### **5. Infections**

- Viral infections
- Febrile
- Sexually transmitted disease

- Tuberculosis
- Chicken pox
- small pox
- Mumps
- Orchitis

## **6. Surgical History**

- Pelvic injury
- Orchiectomy
- Herniorrhaphy
- Pelvic injury or scrotal swelling
- Y-V plasty, Transulatal
- Retro peritoneal surgery

## **7. Gonadotoxins**

- Thermal exposure
- radiation
- Smoking
- Chemicals – Pesticides
- Drugs, Chemotherapeutic, Marijuana, Sulfasalazine, nitro furantoin, androgenic steroids.

## **Causes of Infertility**

### **Varicocele**

A varicocele is defined as a dilation of the veins of the pampiniform plexus of the scrotum. Varicocele is present in 15% of the male population. Dilated spermatic vein leads to the reflux of toxins (impure blood-increased CO<sup>2</sup> concentration produces excessive heat) down through the spermatic vein to the testis. That increases scrotal temperature caused by varicocele.

### **High Fever:**

A high fever exceeding 38<sup>0</sup>C may suppress spermatogenesis over a period of 6 months. Example: Influenza, malaria.

## **Testicular trauma**

Testicular trauma is the second most common acquired cause of infertility the testes are at risk for both thermal and physical trauma because of their exposed position.

## **Orchitis**

The most common cause of acquired testicular failure in adults is viral orchitis usually caused by the mumps virus, echovirus or group B arbovirus.

## **Down syndrome**

These patients have mild testicular dysfunction with varying degrees of reduction in germ cell number. LH and FSH are usually elevated.

## **Sertoli – cell only syndrome (Germinal cell aplasia)**

Patients with germinal cell aplasia have LH and testosterone levels within the reference range but have an increased FSH level. The etiology is unknown but is probably multi-factorial patients present with small to normal sized testes and azoospermia.

Secondary sex characteristics are normal. Histology reveals seminiferous tubules lined by sertoli cells and a normal interstitium although no germ cells are present.

## **Commoner Abnormalities of Genital Organs**

- Local infection
- Idiopathic
- Testicular trauma of Torsion
- Varicocele
- Obstruction of epididymis,
- Obstruction of vas deferens
- Cryptorchidism

## **Chronic Diseases**

- Mumps
- Tuberculosis
- Leprosy
- Epididymitis



- Prostatitis
- Diabetes mellitus
- Hypertension
- Sexual transmitted diseases

## **Impaired Sperm Production and Function**

### **1. Hypothalamic pituitary disorders**

Congenital hypo-gonadotropic hypogonadism Kallmann's syndrome due to deficiency of gonadotrophin releasing hormone (GnRH). In the X-linked form of this disease, deletion of a gene KAL1 has been found. This gene encodes for neurons involved in production of GnRH. Acquired hypogonadotropic hypogonadism pituitary adenoma (including prolactinomas) craniopharyngiomas other brain tumours, intracranial radiation therapy.

### **2. Genetic Factors**

#### **Sex chromosome abnormalities**

- a) 47-XXY karyotype (Klinefelter syndrome) is almost always associated with azospermia (destruction of seminiferous tubules at puberty leading to shrinkage of testes).
- b) Extra Y chromosome results in various degrees of impairment of spermatogenesis. Characteristic decreased sexual function, gynecomastia, decreased length of penis and testis and decreased testosterone level.

#### **Other chromosomal abnormalities**

- a) Translocations cause more severe impairment in male than female.
- b) Impaired chromosome pairing in meiosis leads to azospermia.
- c) Deletions corresponding to AZF (Human azospermia factor) region on long arm of chromosome. This genetic region controls spermatogenesis in human beings.

### **3. Undescended Testis ( Cryptorchidism)**

Extent of impairment of spermatogenesis is variable from a complete Sertoli-cell only pattern to only a slight reduction in the number of germ cells. Spermatogenesis is also impaired in contralateral testis in patients with unilateral mal-descent. Early treatment before age of two years is advocated.

#### **4. Testicular Cancer**

This is associated with increased risk of impaired spermatogenesis oligospermia is observed in more than 40% of patients at the time of Diagnosis of testicular cancer.

#### **5. Germ cell Aplasia**

Seminiferous tubules contain only sertoli cells. Absence of germ cells may be due to factor present during fetal life. Leydig cell insufficiency may also be associated.

This cytological appearance can also result from cryptorchidism, cytotoxic drugs or irradiation.

#### **6. Drugs**

Sulphasalazine, used to treat inflammatory bowel disease can markedly reduce semen quality. The effect is reversible if smaller doses are used for limited time else it may be permanent, B-Blockers may cause importance Anabolic steroids may cause oligo (or) azospermia and cytotoxic drugs especially the alkylating agents (Cyclophosphamide, cisplatin and procarbazine) also cause gonadal failure.

#### **7. Environmental Factors**

Exogenous heat can impair spermatogenesis, Pesticides (Chlorinated nematocide dibromochloropropane – DBCP, Chlordane, carboxyl and ethylenedibromide) glycol ethers (used in paintings, painting and adhesives) and metals (lead, calcium and mercury) have adverse effect on sperm production. Male metal welders may be at increased risk of sub-fecundity. Several other environmental toxins can also have an adverse effect on male reproductive organs. Various hormonal metabolic and neural signals like stress, under nutrition, emotional upset and drugs can affect hypothalamic-GnRH pulse generator and thus, spermatogenesis.

### **Impaired sperm transport**

#### **1. Autoimmune Infertility**

Spermatozoal antigens are shielded inside the testes and are not recognized by the immune system. Autoimmune reaction against sperms is manifested as circulating sperm antibodies. These antibodies may be associated with vasectomy, unilateral or bilateral obstruction of genital tract, Epididymis and varicocele.

## **2. Obstructive Azoospermia**

Sexually transmitted diseases may cause epididymitis and block the ductal system. Agenesis of epididymis and other parts of ductal system, congenital bilateral agenesis of vas deferens is found in many patients with cystic fibrosis.

## **3. An Ejaculation / Retrograde Ejaculation**

- ❖ Diabetic patients
- ❖ Retroperitoneal lymph-node dissection causing neural damage.
- ❖ Spinal cord injury
- ❖ Bladder neck surgery
- ❖ Other sexual dysfunction-including impotence
- ❖ Kartagener's syndrome (immotile cilia syndrome) sperms are immotile due to missing dyne in arms.

## **4. Disturbance in sperm Oocyte Function**

Complementary adhesion molecules are present on surface of oocytes and spermatozoa. These molecules interact and cause fusion of gametes. Abnormalities in these molecules may potentially contribute to infertility.

## **5. Unexplained Infertility**

Semen quality is normal and no effect can be found in the female partner.

## **Evaluation of the infertile man**

### **I. This includes,**

- ❖ A comprehensive history,
- ❖ Physical examination
- ❖ Multiple semen analysis and
- ❖ Endocrine evaluation

### **II. In special circumstances, further specific investigations may also be indicated.**

- ❖ Bacterial examination
- ❖ Genetic assessment

- ❖ Testicular biopsy
- ❖ Sperm function tests
- ❖ Ultrasound
- ❖ Other parameters

## a) History

### **Sexual history**

About 5 percent of all couples are barren due to sexual dysfunction.

### **Smoking**

Reduces sperm density, reduces the proportion of motile sperms and increase level of abnormal morphology.

### **Alcohol**

Excess intake impairs liver function leading to increased estrogen levels, decreased sexual performance and depressed spermatogenesis.

### **Drug abuse**

Marijuana can lead to impotence and infertility marijuana inhibits the secretion of GnRH and can suppress reproductive functions in both men and women and cocaine use is known to reduce – spermatogenesis.

### **Medical treatment**

Psychiatric drugs like phenothiazines, anti-hypertensives like B-blockers, epileptic drugs like diphenyl hydantoin, anti-bacterials like Sulphasalazine and nitrofurantion, H<sub>2</sub> antagonist's cimetidine and ranitidine, erythromycin tetracycline's anabolic steroids and chemotherapeutic agents can depress sperm quantity and quality.

### **Past history of surgery**

For hernia, hydrocele may lead to vassal damage. Orchidopexy for undescended testis (If done late in life) and surgery far varicocele, may all affect the semen quality. Past history of mumps, orchitis, epididymitis, prostatitis, sexual transmitted disease and testicular injury.

### **Exposure to excessive heat**

A small rise in scrotal temperature can adversely affect spermatogenesis and a febrile illness can produce striking changes in sperm count and motility. The effect of the illness can be seen in the sperm count and motility even 2-3 months later. Environmental sources of heat such as the use of khaki shorts instead of boxer shorts, excessively hot baths, hot tubs, or occupation that require long hours of sitting, e.g. drivers, may all decrease fertility potential. Severe allergic reactions and exposure to radiation or to industrial or environmental toxins-

A study from Scandinavia did show lower sperm counts in males from an urban area compared to males in rural areas, suggesting an effect of urban pollutants. In any case the clinician should determine if a male with an abnormal semen specimen had exposure to industrial or environmental.

Exposure to diethyl stilbestrol in utero has been suggested (but not proven) as a cause of male infertility. Indeed in the largest follow-up of men born to the women treated with diethylstilbestrol, no impairment of fertility or sexual function was detected.

### **Coital frequency**

Counts at the lower levels of the normal range may be depressed to below normal levels by ejaculations occurring daily or more frequently. Most couples every 36 hours around the time of ovulation will give the optimal chance for pregnancy. However studies in men with oligospermia fail to detect a decline in sperm numbers with sequential ejaculations suggesting that limitations on coital frequency are not necessary.

## **B. Examination**

### **Bodily habitus**

- ❖ Size of testes estimated by comparing with Orchidometer normal volume is – 150-200ml.
- ❖ Scrotal palpation for vas deferens and varicocele.

- ❖ Presence of penis abnormalities like hypospadias, scar and induration.
- ❖ Shrunken testis (5ml) with infantile genitalia sparse body hair, gynaecomazia and low testicular volume are seen in congenital gonadotrophin deficiency.
- ❖ Eunuchoid habits with infantile genitalia sparse body hair, gynaecomazia and low testicular volume are seen in congenital gonadotrophin deficiency.
- ❖ Androgenized man with normal sized testis and distended epididymis may indicate obstructive azoospermia.
- ❖ Androgenized man small sized testes may have seminiferous tubular failure.
- ❖ Absence of cord like feel of vas of the neck of the scrotum indicates vassal aplasia.

### **Endocrine Evaluation**

Serum follicle-stimulating hormone (FSH) helps to distinguish patients with azoospermia who have obstruction (normal FSH) from those with seminiferous tubule destruction (Raised FSH). Low levels of FSH, LH and testosterone suggest acquired hypo gonadotrophin hypogonadism.

### **C. Semen Analysis:**

The Semen analysis is the corner stone of the male infertility work up should be performed according to the WHO recommended procedure. Computer assisted semen analysis (CASA) is considered mainly a research tool and is not used routinely. If abnormal results are obtained semen analysis is repeated after 6-12 weeks.

<b>Semen volume</b>	: Normal ejaculate volume is 1.5-5ml.
<b>Colour</b>	: Grey, yellow or opalescent
<b>Ph</b>	: 7.2 – 8
<b>Liquefaction</b>	: Coagulation occurs soon after ejaculation but semen liquefies within 5-20 minutes failure to liquefy after 30 min is abnormal.
<b>Sperm concentration</b>	: Sperm per ml of semen
	Normal - $20 \times 10^6$ sperms/ml
	Oligozoospermia - $20 \times 10^6$ /ml
	Polyzoospermia - $350 \times 10^6$ /min

- Total sperm count** : Sperm concentration x volume of semen (Normal)  $40 \times 10^6$  sperms ejaculate.
- Sperm Motility** : At least 100 sperms are evaluated. Normal is 50% with forward progression within 60min of ejaculation.
- Morphology** : Assessed by light microscopy (hematoxylin, eosin, geimsa or papanicoloau stain) or electron microscopy. At least 100 sperms are examined. Normal is 30% with normal forms.
- White blood cells** : have to be differentiated from immature germ cells. Normal ( $1 \times 10^6$  / ml peroxidase staining technique). If excessive, semen culture should be performed.
- Sperm antibodies** : Detected by immunobead or mixed antiglobulin reaction which localizes IgG or IgA specific regions of spermatozoa. Normal for immunobead test for antiglobulin reaction test – 10% spermatozoa with adherent particle.
- Accessory gland functions:** Assessed by measuring seminal fructose for seminal vesicles (Normal  $13\mu\text{mol/ejaculate}$ ) and acid phosphatase, zinc citrate for prostate. Post ejaculatory urine should be examined for presence of sperms if retrograde ejaculation is suspected.

**Collection of semen:**

- ✓ The sample should be obtained atleast in three occasions.
- ✓ With an interval of atleast two months of each specimen.
- ✓ Atleast 4 days abstinence from sexual activity.
- ✓ A sample to be collected by masturbation into a clean, dry, sterile container.
- ✓ The specimen (semen) should be examined should not be missed since it contains the highest concentration of spermatozoa.

**Other Investigations**

**Bacteriological examination**

Bacterial examination of semen in patients with leukocytospermia.

## **Chromosomal and genetic assessment**

Chromosomal analysis is essential in men with azoospermia with raised FSH levels and small testicular volume, in order to diagnose klinefelter syndrome. Screening for cystic fibrosis-by-cystic fibrosis transmembrane conductance regulating (CFTR) gene analysis is done in men with congenital absence of vas deferens.

## **Testicular biopsy**

Can confirm the diagnosis of obstructive azoospermia (Normal testis size with azoospermia) before reconstructive surgery.

## **h) Sperm function**

- Strict morphology evaluation
- Acrosomal assessment
- Sperm – zone binding tests
- Production of reactive oxygen species by sperms.

## **Ultrasound**

Transrectal and scrotal ultrasound can be used to assess prostate and seminal vesicle and diagnose ejaculatory duct obstruction. Testicular ultrasound can locate impalpable testes or those with hydrocele.

## **Other Parameters**

Although all of the major elements of the semen analysis have some bearing on fertility, especially when markedly deficient the lack of precise correlations have led to a search for tests of the functional capacity of sperm. Despite enthusiasms generated by a variety of assays over the past four decades, no test has emerged as a reliable standard for the fertilizing ability of sperm.

## **Measurement of sperm velocity**

The CASA systems are best at supplying information on sperm velocity and specific movements such as lateral head displacement however; it is unlikely that these measurements provide information that cannot be obtained with less expensive methods.



### **Hypo-osmotic swelling test**

When sperm are placed in a hypo-osmotic solution of sodium citrate and fructose, a sperm tail with normal membrane function will swell and coil as fluid is transported across the membrane. Conversely, if there is a functional disturbance of the tail membrane the tail will appear unaffected.

### **Measurement of acrosin**

Acrosin is a proteolytic enzyme associated with the acrosome that may be important for aiding sperm to traverse the zona. Low acrosin concentrations could be associated with infertility. Although theoretically appealing, the test has little application in clinical practice.

### **Measurement of the acrosome reaction**

The acrosome reaction that allows the release of enzymes from the acrosome occurs on or near the zona pellucida. However a low percentage of sperm will also become reactive while in media or following treatment with calcium, ionophore that includes capacitation. Although the artificial initiation of the acrosome reaction has been correlated with IVF results the relatively small difference in an acrosome reactive sperm in the different groups indicates that this approach is not clinically important.

### **Sperm penetration assay (SPA)**

The zona pellucida of most mammalian species presents not only a block to polyspermia but also a barrier to fertilization of an ovum by sperm of a different species. However, foreign sperm can fuse with and penetrate an ovum if the zona is removed by gentle enzyme digestion. In the sperm penetration assay, ovum are collected from super ovulated golden hamsters the zona are removed by enzyme digestion, and the denuded ovum are cultured for 2-3 hours with human sperm that have been washed and incubated over night in culture media. Presence of a swollen sperm head in the ovum cytoplasm is evidence of successful penetration. Most laboratories report the percentage of ovum penetrated and compare this figure to the percent penetrated by a known fertile sperm specimen.

### **Human zona binding assay**

Whereas the SPA tests the ability of sperm to penetrate or to be engulfed by the ovum it does not test the critical ability to pass through the zona pellucida.

The zona is of course, removed in preparation for the SPA because it is, with rare exceptions, impervious to foreign sperm. Thus, to test zona penetrating or zona binding ability of human sperm requires the use of human zona.

One approach is to use zona obtained from surgically removed ovarian tissue and slit them in half so that both patient sperm and donor sperm can be tested in parallel on different portions of the same zona.

The ratio of the number of sperm bound for the test subject to the number of sperm bound for fertile control sperm bound for fertile control sperm has been labeled the hemizona assay index (HZI) a break point at an HZI value of 36 has provided a good correlation with results in human IVF.

The limited availability of the zona and the technical requirements of the assay will always restrict its application to a small number of committed laboratories.

In the future, development of materials that mimic the properties of the zona could lead to simple tests.

However the widespread use of ICSI, which by passes the zona, renders such, tests superfluous, unless they can determine with certainty which couples require ICSI.

### **Measurement of the adenosine triphosphate (ATP)**

ATP is an important component of sperm metabolism related to tail movement. In one reports levels of ATP in semen were a strong discriminator between populations of fertile and infertile males. A multicentre study sponsored by the world health organization concluded. However, those levels of semen ATP could not predict the occurrence of pregnancy when the female was normal and the male partner had a sperm concentration greater than 20million/ml.

### **Immunological factors: Anti sperm antibodies**

Immunological factors have been impacted in the causation of human infertility. In men, this may present as anti-sperm antibodies in the semen, serum or on the surface of

the sperm. Anti-sperm antibodies have also been demonstrated in the cervical mucus and the serum of the female partner.

Though there are several tests to detect anti-sperm antibodies the exact significance of these tests is not known. Besides there is no satisfactory way to treat these couples, though immunosuppressive therapy with corticosteroids, testosterone therapy, intrauterine artificial insemination with husbands sperms (IUAID) and have all been suggested as appropriate therapy. The pregnancy rate has been variable.

The role of anti-sperm antibody is significant since the sperm is not recognized, as self-antigen in our body immunologically speaking the spermatogenesis does not occur, when the ontogeny of the 'T' lymphocytes happens. At this time of ontogeny of the 'T' lymphocytes our body proteins are started to recognize as self-antigens. If any antibody arises against our own protein it will be destroyed by clonal energy.

The sequestered sperm protein also causes the immune response, especially vasectomy like surgeries.

### **Non-surgical factors related to male infertility**

Gonado-toxine	Ejaculatory dysfunction
Genital tract infection	Hormonal imbalances
Coital timing	Testicular hyperthermia

### **Retrograde ejaculation**

Disruption of the innervations of the vasa deferentia and bladder neck can result in retrograde ejaculation. Diabetes mellitus complicated by peripheral neuropathy, multiple sclerosis, medical therapies interfering with sympathetic tone, Tran urethral resection of the prostate bladder neck surgery retroperitoneal lymph node dissections and extensive pelvic surgery also can lead to retrograde ejaculation.

The diagnosis is confirmed by identification of large numbers of sperm in a post ejaculate urine specimen.

### **Microbiology**

The presence of white blood cells (WBC's) in the patient's semen may indicate an infection. Accordingly the semen should be evaluated for bacterial growth, mycoplasma and chlamydia.

## **Distribution of final diagnostic categories**

### **Found in male fertility clinic**

#### **Diagnosis**

Varicocele	Endocrine
Testicular failure	Cryptorchidism
Ejaculatory Failure Agglutination	Low Volume
Sexual Dysfunction Viscosity	Idiopathic

#### **Necropermia**

Relative frequency of causes and associated conditions in men who present with infertility.

#### **Conditions**

Varicocele	Viral Orchitis	immotile sperm
Coital disorders	Abdrogen resistance	
	Radiation/chemotherapy	
Obstruction of epididymis or of vas deferens		Klinefelters syndrome

#### **Surgical operations in the male possibility associated with male infertility**

1. Hydrocele
2. Varicocele
3. Inguinal hernia
4. Vasectomy
5. Prostatectomy
6. Sympathectomy
7. Testicular torsion
8. Hypospadias
9. Urethral strictures or diverticula
10. Bladder neck operation

#### **WHO manual – semen analysis – nomenclature's**

1. **Normozoospermia** - Normal semen
2. **Aspermia** - Absence of ejaculation
3. **Azoospermia** - Absence of sperms in the semen
4. **Oligo zoospermia** - Less than 20 millions count / ml
5. **Astheno zoospermia** - Less than 5.0% spermatozoa with forward Progression

- 6. **Terato zoospermia** - Less than 30% spermatozoa with normal head morphology
- 7. **Oligoastheno teratozoosperima** - Signifies the disturbance of all the above variables

**Sub fertility options – Abbreviations**

For infertility disorders there are so many latest and advanced techniques are available and some of them are listed below,

- IVF** : In Vitro Fertilization
- GIFT** : Gamete Intra Fallopian Transfer
- TET** : Tubal Embryo Transfer
- TUFT** : Trans-Uterine Fallopian Transfer
- ICSI** : Intra-Cytoplasmic Sperm Injection
- SUZI** : Sub Zonal Sperm Injection (Directly into Ovum)
- ZIFT** : Zygote Intra-Fallopian Transfer
- POST** : Peritoneal Oocyte Sperm Transfer
- PESA** : Percutaneous Epididymal Sperm Aspiration
- MESA** : Micro Epididymal Sperm Aspiration –from testis

**Sperm Function Tests**

- Sperm migration test : More than 150 million in 250 micl-fertile
- Hypo osmotic test : More than 60% with HOS positive - Fertile

**Acrosomal intactness**

- Test nuclear chromatin : More than 60% with halo 30micm – Fertile
- Decondensation test : More than 70% NCD – Fertile

**Sperm mitochondrial**

- Activity Index : More than 50 SMAI – Fertile

**In vitro cervical mucus**

- Penetration test : Score: 0-Negative, 3-Poor, 6-Good, 9-Excellent
- Post coital test : More than 10 rapid forward progressing  
sperms/HPF
- Post ejaculatory urine : No spermatozoa in urine

## 4. MATERIALS AND METHODS

### Drug selection:

In this dissertation the stamens of *Anda odu Parpam* (Egg shells of *Gallus domesticus*) was taken as a compound drug for *Vindhu kuraivu* (Oligospermia) from the literature of the preparation was collected from *Anuboga vaidhya navaneedham*-Third part written by **Hakeem Mohamed Abdhulla Shahib**, Page no.106 Ingredients of the test drug are Egg shells of *Gallus domesticus* and Lemon juice.

### Source of collection:

The hen egg shells (Ovitesta of *Gallus Domesticus*) were collected from the hatchery at Namakkal and the lemon were collected from a form house at Idappadi, Salem District.

### 4.1 Preparation of the drug

#### Purification of egg shell:

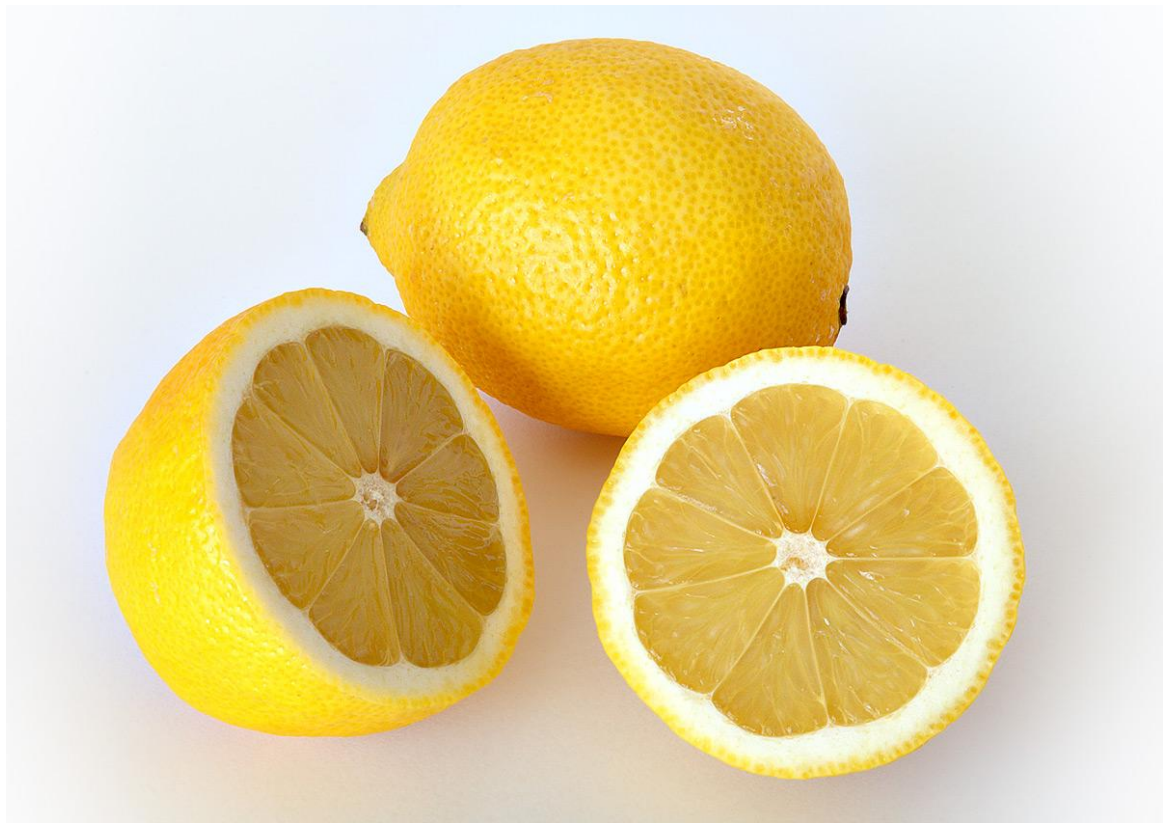
The egg shells were boiled with *Appalakaram* water (Sodium Carbonate) for 20minutes. Then the membranous layer is peeled out and then washed with water for two times.

#### Preparation of *parpam* and storage:

The purified dried egg shells were powdered and sieved in clean white cloth (*Vastharakayam*). This fine powder about 320gms is kept in a vessel and 500ml of lemon juice is poured into the powder and kept in sunlight. It is repeated for seven times. After then the product was made into *Villai* and dried in sunlight and kept in an *Agal* (mud pan) with suitable cover. Make 10 mud smeared cloths around the *Agal* and kept for *pudam* (calcinations process) in 20 *varatties* (cow dung cakes). After calcination it was powdered and kept in dry clean air tight container (*Anuboga Vaidhaya Navaneetham Part III*).



**Fig No.1 *Anda odu* (Egg Shell of *Gallus domesticus*)**



**Fig No.2 *Elumichai Pazham* (Lemon - *Citrus Limon*)**





**Fig No.3 *Anda odu parpam***

**Administration of the Drug:**

Form of the medicine	: <i>Parpam</i> (Powder)
Route of Administration	: Enteral
Dose	: 130 mg
<i>Anubanam</i> (Vehicle)	: Ghee
Time of Administration	: Twice in a day; before food
Duration	: 48 days

## 4.2 Standardization of the drug

Standardization is the first step for the establishment of a consistent biological activity, a consistent chemical profile standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects.

### 4.2.1. Physio-chemical analysis of test drug

#### Quantitative analysis

##### Ash and acid insoluble ash:

To the ash add 1:5 Hcl: Distilled water 15 ml boil, cooled and then filtered using whatman filter paper (No.41) repeat 3 to 4 times till the yellow colour disappear or colourless, then remove the filter paper and add to the filter to the original dish and keep it in the muffle furnace at 600° C and take constant weight and calculate the acid insoluble ash value.

$$\text{Acid insoluble ash (\%)} = \frac{\text{Weight of acid insoluble residue} \times 100}{\text{Weight of the sample}}$$

Acid insoluble residue = Acid insoluble ash value – Empty weight of the dish

##### Loss on drying:

3gm of the drug is heated in a hot oven at 105° c to constant weight. The % of weight was calculated.

Loss on drying value at 105° c – 9.485 %w/w

##### Potential of hydrogen (pH):

The pH scale is logarithmic and runs from 0.0 to 14.0 with 7.0 being neutral. Readings less than 7.0 indicate acidic solutions, while higher readings indicate alkaline or base solutions. pH of *Anda odu Parpam* – 13.

## 4.2.2 Chemical analysis

### Methodology for chemical analysis

#### Preparation of extract

5gm of sample is weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 20 minutes. Then it is cooled and filtered in a 1000ml volumetric flask and made up to 100ml distilled water.

S.No	Experiment	Observation	Inference
1.	<b>Test for reducing Sugar :</b> To 5ml of Benedicts qualitative reagent, add 10 drops of extract& boil for 2 min.	Green / Yellow / Red Precipitation	Presence of Reducing Sugar
2.	<b>Test for Starch :</b> To 5 ml of extract add 2ml of acetic acid and then add few drops of N/50 Iodine Solution.	Blue Colour	Presence of Starch
3.	<b>Test for Proteins :</b> To 2 ml of extract, add 2ml of 5% Sodium Hydroxide mix and add 2 drops of Copper Sulphate Solution.	Violet or Purple Colour	Presence of Proteins
4.	<b>Test for amino Acid :</b> Place 2 drops of extract on a filter paper and allow to dry well. Then spray 1% ninhydrin and allow drying.	Violet Colour	Presence of Amino Acid
5.	<b>Test for Albumin :</b> To 2 ml of extract, add 2ml of Esboch's reagent.	Yellow Precipitation	Presence of Albumin
6.	<b>Test for Phosphate :</b> To 2ml of extract, add 2ml of ammonium Molybdate solution and 2ml of concentrated Nitric Acid.	Yellow Precipitation	Presence of Phosphate
7.	<b>Test for Sulphate :</b> To 2 ml of extract add 2ml of 4% ammonium oxalate solution.	White Precipitation	Presence of Sulphate
8.	<b>Test for Chloride :</b> Add 2ml of extract to dilute nitric acid till the effervescence ceases. Then add 2 ml of Silver Nitrate Solution.	Cloudy White Precipitation	Presence of Chloride

S.No	Experiment	Observation	Inference
9.	<b>Test for Iron :</b> To 2ml of extract, add 2ml of ammonium thio cyanate solution and add 2ml of concentrated Nitric Acid.	Red Colour	Presence of Iron
10.	<b>Test for Calcium :</b> To 2 ml of extract, add 2 ml of 4% ammonium Oxalate Solution.	White Precipitation	Presence of Calcium
11.	<b>Test for Sodium :</b> Make a paste with 2 pinches of the sample with Hcl and Introduce it into the blue flame.	Yellow Flame	Presence of Sodium
12.	<b>Test for Potassium :</b> Add a pinch of the sample to 2 ml of Sodium Nitrate Solution. Then add 2ml of Cobalt Nitrate in 20% acetic acid.	Yellow Precipitation	Presence of Potassium
13.	<b>Test for Zinc :</b> To 2ml of extract, add few drops of Sodium Hydroxide.	White Precipitation	Presence of Zinc
14.	<b>Test for Magnesium :</b> To 2ml of extract, add few drops of Sodium Hydroxide Solution.	White Precipitation	Presence of Magnesium
15.	<b>Test for Alkaloids :</b> a. To 2ml of extract, add 2ml of Potassium Iodide Solution b. To 2ml of extract add 2ml of Picric Acid. c. To 2 ml of extract add 2ml of Phospho tungstic Acid.	Red Colour  Yellow Colour  White Precipitation	Presence of Alkaloids  Presence of Alkaloids Presence of Alkaloids
16.	<b>Test for Tannic Acid :</b> To 2ml of extract add 2 ml of Ferric Chloride Solution	Black Precipitation	Presence of Tannic Acid

Results are discussed in table 1.1

### 4.2.3 Instrumental analysis



**Fig No. 4 Fourier Transform Infrared Spectroscopy (FTIR)**

Instrument details:

<b>Model</b>	<b>: Spectrum one: FT-IR Spectrometer</b>
<b>Scan Range</b>	<b>: MIR 450-4000 cm<sup>-1</sup></b>
<b>Resolution</b>	<b>: 1.0 cm<sup>-1</sup></b>
<b>Sample required</b>	<b>: 50 mg, solid or liquid.</b>

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique used to identify mainly organic materials. FTIR analysis results in absorption spectra which provide information about the chemical bonds and molecular structure of a material. The FTIR spectrum is equivalent to the "fingerprint" of the material and can be compared with cataloged FTIR spectra to identify the material.

#### **Applications:**

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH<sub>2</sub>, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

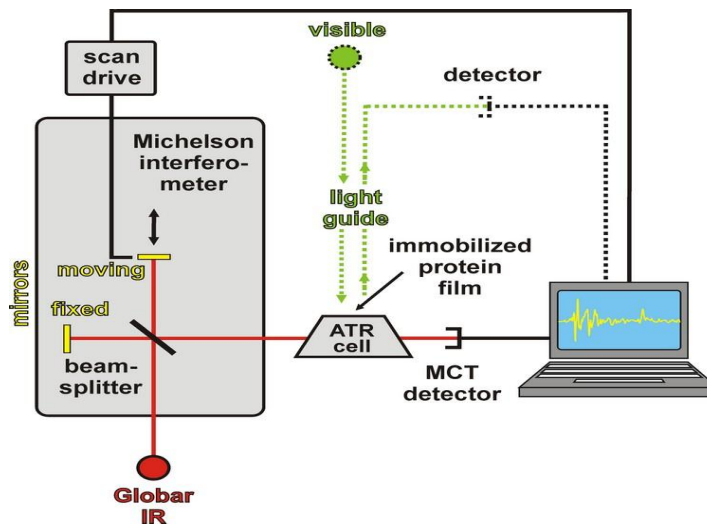


Fig No. 5 Mechanism of FTIR analyzer

**Fourier Transforms Infrared Spectroscopy analytical capabilities:**

- Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond
- Especially capable of identifying the chemical bonds of organic materials
- Detects and Identifies organic contaminants
- Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
- Detection limits vary greatly, but are sometimes  $<10^{13}$  bonds/cm<sup>3</sup> or sometimes sub monolayer
- Useful with solids, liquids, or gases

Results are shown in Graph no: 1

#### **4.2.4. Toxicological study**

##### **Acute and sub acute toxicity study on *Anda odu parpam***

##### **Animals**

Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vels University. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28<sup>0</sup>C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group.

##### **Acute toxicity study**

Acute oral toxicity test for the *Anda Odu Parpam* was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavages using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behaviour and other physiological activities. Single animals are dosed in sequence usually at 48 hr intervals. However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal.

##### **Observation of toxicity signs:**

General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.

### **Sub-acute toxicity**

In a 28-days sub acute toxicity study, twenty four either sex rats were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the *Anda Odu Parpam* (p.o.) for 28 days at a dose of 50, 100 and 200mg/kg respectively. The animals were then observed daily for gross behavioural changes and any other signs of sub acute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at 4°C for 10 min to separate the serum and used for the biochemical assays.

### **Hematological and blood biochemical analyses:**

At the end of the study, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semiautomated hematology analyzer. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis (glucose, creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP)) were automatically determined using autoanalyzer.

### **Necropsy:**

All rats were sacrificed after the blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The Spleen, Testes, Pancrea, Lung, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, and Kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for histopathological assessment. The tissues were



embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

### **Statistical analysis**

Values were represented as mean  $\pm$  SEM. Data were analyzed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparison Test using Graph Pad InStat-V3 software. P values  $< 0.05$  were considered significant.

#### **4.2.5. Pharmacological study**

##### **Spermatogenic activity of *Anda odu parpam* against 2, 3, 7, 8 -Tetrachlorodibenzo-p-dioxin induced Oligospermic Rats**

###### **Introduction**

Increasing concern expressed about the declining sperm counts of humans in the last few decades. This is hypothesized to be as a result of the rising incidence of both testicular cancers and subfertility caused by exposure of the developing male embryo to certain potential environmental estrogenic agents that disrupt normal hormonal balance in the body. Male reproductive function seems to have deteriorated considerably in the past 4 to 5 decades. It was observed that the significant decline in mean sperm concentrations from  $113 \times 10^6/\text{ml}$  in 1940 to  $66 \times 10^6/\text{ml}$  in 1990; a fall of  $0.94 \times 10^6/\text{ml}/\text{year}$ .

Infertility is one of the major health problems in life and approximately 30% of this problem is due to male factors. Several factors can interfere with the process of spermatogenesis and reduce sperm quality and quantity. The alterations in motility, viability and morphology of spermatozoa in treated rats are likely the result of adverse effect of the treatment on epididymal functions. Inadequate concentration, sluggishly motile or immotile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova. Some diseases such as coronary heart diseases; diabetes mellitus and chronic liver diseases have been reported to cause deleterious effects on spermatogenesis. The present study was conducted to evaluate the possibility of using *Anda Odu Parpam* as a therapeutic agent to treat spermatogenic disorders in the animal models.

## **Materials and methods**

### **Chemicals**

The 2, 3, 7, 8-Tetrachloro dibenzo-p-dioxin (tcdd) and necessary chemicals and reagents were obtained from Sigma chemicals. All other solvents and Analytical Kits were of analytical grade and obtained from Qualigen fine chemicals and Artek laboratories.

### **Animals**

Adults male rats weighing between 140-160g and albino mice weighing between 28-32g (For acute toxicity study) were maintained in a well ventilated animal house under standard condition of humidity, temperature and a constant 12 hour light:12 hour dark lighting schedule. The animals were housed in clear polypropylene cages. The animals were maintained with standard pellet feed (Sai Durga Feeds and Foods, Bangalore, India) and water ad libitum. The health, normal behaviour and reproductive status of the animals were assessed and only healthy animals were selected for the experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethical Committee (IAEC). IAEC approval no: (XIII/VELS/PCOL/65/2000/CPCSEA/IAEC/08.08.2012).

### **Drug Stock solution**

The powdered form of *Anda Odu Parpam* was mixed uniformly in 2% CMC and made into uniform suspension to achieve 200mg/ml as main stock solution and used in this study.

### **Animal grouping and Treatment**

Twenty four adult male rats were randomly divided into four groups of six animals each. Group 1 (control) was administered with the vehicle (2% CMC suspension) while groups 2 and 3 were given suspension of *Anda Odu Parpam* at 50mg/kg and 100mg/kg. One rat was sacrificed to ensure the oligospermic induction at the beginning of the experiment i.e. after one week of TCDD injection. Two rats from each treatment group were randomly sacrificed after 14 days of *Anda Odu Parpam* administration while

the remaining rats treatment were continued up to 28 days. Treatment was done daily using oral dosing needle and twenty four hours after the last dose, blood was collected and the animals were sacrificed. All procedures regarding handling of the test animals were in accordance with the existing CPCSEA and IAEC guidelines.

### **Induction of Oligospermia by TCDD in rats**

Initially, rats were injected with 40µg TCDD/kg i.p. At one week after TCDD exposure, a rat from each group was selected and tested for induction of Oligospermia and after ensuring the Oligospermic conditions the study was proceeded further.

### **Blood sample and organ collection**

After the last dosing of *Anda Odu Parpam*, all the animals were sacrificed by employing euthenesia procedure and the testes, epididymis, vas deferens, seminal vesicles and ventral prostates were identified, dissected out, blotted free of blood and cleared of connective tissue or fat. The organs were weighed immediately using an electronic digital balance. Blood samples were collected by retro-orbital puncture into anticoagulant pre-coated and also in plain sterile eppendorff tubes and allowed to clot at room temperature. Serum samples were separated by centrifugation at 3000 rpm for 10 min and stored at -20°C until testosterone assay. Anticoagulant added blood samples were used for the studying haematological parameters

### **Sperm collection and Measurement of sperm parameters**

The rats were anaesthetized with anesthetic ether and sacrificed after the last day of administration and weighed for the essential reproductive organs, such as testis, caudal epididymis, seminal vesicle and prostate glands. A scrotal incision was made to exteriorize the testis and epididymides. The epididymides were carefully dissected out of the testes and blotted free of blood. To prepare sperm suspension, epididymal sperm were obtained by mincing cauda epididymis of each rat in pre-warmed beaker containing 2 ml of physiological saline (maintained at 37°C). Several incisions were made on it to allow sperm swim out.

Sperm characteristics were determined according to the standard protocols derived by the previous investigators in this research area. Sperm motility was also assessed immediately by counting both motile and immotile spermatozoa per unit area at the 40x magnification. Sperm count was done using the improved Neubauer's haemocytometer under the light microscope at 100x magnification. The count was expressed as million/ml of suspension. Sperm viability was assessed using eosin-nigrosin test. The percentages of unstained (alive) and stained (dead) spermatozoa were calculated by counting 100 spermatozoa randomly per sample. Morphological appearance of normal and abnormal spermatozoa was determined by examining stained smears under the oil immersion (100x) and their percentages were calculated.

### **Testosterone Assay**

Blood samples were spun at 2500rpm for 10 minutes in a table top centrifuge. The serum samples obtained were analyzed to determine the concentration of testosterone. The analysis was carried via the tube-based enzyme immunoassay method as described in the kit.

### **Collection of tissues and histological analysis**

The testes were collected and immediately fixed in Bouins fluid for 6 h and transferred to 70% alcohol for histological processing. And following fixation of the testes from both control and test animals, tissue sections were processed by dehydration in 95% and absolute alcohol, cleared in xylene and embedded in pure clean molten paraffin wax from which blocks of tissues were made for sectioning. Ribbon slices of about 5.0 $\mu$ m in thickness were made with the aid of a microtome and the sections picked with slides which were dried in oven. The slices were then stained with Haematoxylin and Eosin, and then mounted using DPX onto a light microscope (magnification 40x) for histopathological and morphological changes. The changes observed were recorded and photomicrographs of the most prominent pathological alterations.

## **Statistical Analysis**

The results were analyzed by one-way analysis of variance using INSTAT version 3 for Windows. Significant differences within group variables were determined by Tukey's multiple comparison tests. Results were considered significant at 5% level of probability ( $P < 0.05$ ). The data were presented as mean  $\pm$  SEM.

### **4.3 Clinical assessment**

#### **Aim**

Male factor infertility is said to be present when a couple fails to achieve pregnancy after one year of unprotected coitus and a problem is identified in the male partner.

Causes of infertility in men can be explained by deficiencies in ejaculate volume causing low sperm production (oligospermia), poor sperm motility (asthenospermia), abnormal morphology (teratospermia), and abnormal sperm function or by preventing sperm transport to vagina.

The increasing incidence of male infertility is necessitating more and rapid search into drugs with spermatogenic potentials with negligible side effects. This study is intended to provide adequate information on the Clinical trial of *Anda odu Parpam* with sex enhancing potentials.

#### **Objectives:**

- ◆ To evaluate the spermatogenic effect of *Anda odu Parpam*
- ◆ To explore the efficacy of *Anda odu Parpam* in patients with infertility.

#### **Design of the Study:**

The Open clinical trial phase-2B

Study period was 1-3 months (according to prognosis)

#### **Study Centre:**

Govt.Siddha Medical College Hospital, Arumbakkam, Chennai – 106.

#### **Study Participants:**

Male patients in all races and ethnic groups were eligible for this trial. The patients will be selected from the Out-patient department of Govt Siddha Medical College Hospital, Chennai.

**Number of Subjects:**

Number of participants were 50.

**Registration Process:**

To register a patient, the following documents should be completed by the investigator.

- ◆ Copy of required laboratory tests
- ◆ Signed patient consent form
- ◆ Other appropriate forms (e.g., Trial profoma).

This Clinical trial is an ethical and scientific quality standard for designing, conducting and recording trials that involve the participation of human subjects. Compliance with this standard provides assurance to public that the rights, safety and well being of trial subjects are protected, consistent with the principles enshrined in the Declaration of Helsinki and ensures that clinical trial data are credible.

**Selection of patients**

The patients were selected for clinical trial, those who had the following clinical features,

- ◆ Decreased fertility
- ◆ Presence of nocturnal emission
- ◆ Premature ejaculation
- ◆ Decreased sexual desireness

**Consent form**

Patients were included in this clinical study only after getting the consent form (both English and Tamil). The confirmation is sought only after information about the trial including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, alternative treatment that may be available and of the subject's rights and responsibilities has been provided to the potential subject.

The patients were selected for clinical trials as per the following criteria, which are listed below.

**Inclusion criteria**

- ❖ Age-25-50
- ❖ Low sperm count
- ❖ Willing to give semen for the investigation

**Exclusion criteria**

- ❖ Age below 25yrs and above 50yrs
- ❖ Azoospermia
- ❖ Cardiac diseases
- ❖ Diabetic mellitus
- ❖ Hypertension
- ❖ Mumps
- ❖ Orchitis
- ❖ Varicocele
- ❖ Hydrocele

**Withdrawal criteria**

- ❖ Irregular visit
- ❖ Uncooperative patient
- ❖ Drug abuse
- ❖ Deterioration of vital signs
- ❖ Any adverse effects during the treatment period

**Investigation criteria:**

- ❖ Complete blood count
- ❖ Lipid profile
- ❖ Blood glucose level
- ❖ Semen analysis

**Diagnosing of infertility Patient history**

- ❖ Is the patient has oligospermic or from loss of libido or disorder of ejaculation
- ❖ No child birth
- ❖ Prior history of smoking, heart attacks, strokes
- ❖ Is the patient taking medications that can contribute to infertility



**Laboratory tests:**

- ❖ Complete blood counts
- ❖ Blood glucose level
- ❖ Semen analysis

**Semen analysis:**

- ❖ Volume
- ❖ Colour
- ❖ Appearance
- ❖ Viscosity
- ❖ Liquefaction time
- ❖ Motility
- ❖ Sperm count

**Management:** The aim of the *Noinekkam* (Treatment) is based on

- ❖ To bring the three *Thathus* in equilibrium
- ❖ Treatment of the disease
- ❖ *Paththiyam* (diet restriction)

**Drug and Dosage:**

- ❖ The test drug *Anda odu Parpam* was given to the patients at the dose level of 130 mg once in a day with ghee before food.
- ❖ The duration of the treatment varies according to the severity and response of the treatment.
- ❖ It had been given minimum of 1-3 months.

**Dietary advice**

The patients are advised to take nuts (Walnuts & Pine nuts), fruits (Bananas, Dates, Figs, Grapes, Pomegranate, Strawberry, Peach and Mango) and Vegetables (Carrots, Fennel, Onions, Garlic, Cardamom, Asafoetida & pepper).

**Criteria for assessment of response to therapy:**

- 1) Marked response : 76%-90% relief in the presenting symptoms and marked normality in semen analysis.
- 2) Mild response : 50%-75% relief in the presenting symptoms and mild normality in semen analysis.
- 3) Fair response : Below 50% relief in the presenting symptoms and fair normality in semen analysis.

**Observation:**

- ❖ The duration of the treatment ranged between 30-90 days.
- ❖ At the time of treatment, no adverse effects were observed.
- ❖ The drug was well accepted by all the patients.

**Statistical analysis:**

The data were subjected to paired student 't' test to determine the significance of changes followed by comparisons to analyze the significance of difference between pre and post treatment. P value of <0.05 was considered as significant. The results are shown in page no. 168 and 169.

## 5. RESULTS AND DISCUSSIONS

Various studies have been carried out in this trial drug *Anda odu parpam*. The study includes literary collections, physio and bio chemical analysis, toxicological study, pharmacological study and clinical study. *Anda odu parpam* was taken for the treatment of Oligospermia. The drug has been selected for the treatment of Oligospermia in reference with *Anuboga vaidhya navaneedham*-Third part written by Hakeem Mohamed Abdhulla Shahib.

**Literary collections** about the drug from various text books were done. It indicates the efficacy of the drug in the treatment of Oligospermia. Zoological aspect deals with the identification, description, cultivation and ethno medicinal importance of egg shell. Gunapadam aspect expressed that the drug possess good Spermatogenic property.

Since the trial drug, *Anda odu parpam* is very easy to prepare, the drug was prepared according to the classical methods. This method helps to vitalize the drug.

The trial drug was studied for its clinical importance in the management of *Vindhu kuraivu* (Oligospermia).

## Physio chemical report of *Anda odu parpam*



சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600 106

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(Central Council for Research in Siddha, Department of AYUSH,

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**Table No.1 Results of Physio chemical report of *Anda odu parpam***

S.No	Parameter	Mean Value
1.	Loss on Drying at 105°C	0.38 %
2.	Total Ash	91.47 %
3.	Acid insoluble Ash	2.3 %
4.	Particle size	Completely passes through sieve no.44
5.	pH	13.0

By the above results, the trial drug has very low foreign matter and acid insoluble ash, indicates that trial drug will digest completely in human GI tract. The analytical parameters like total Ash value, Acid insoluble ash value, Loss on drying values are helping us to interpret the digestion and solubility capacity of the crude extract. As per the result the tested sample contains good percentage of solubility as well as digestive capacity.

## Proximate Chemical Analysis of a Drug

Department of Bio-Chemistry

Govt. Siddha Medical College, Chennai – 600 106.

### Preparation of Extract:

Add 5 gm of the *Anda odu parpam* to 50ml of distilled water. Boil the solution for 20 minutes, cool and then filter. Use the Extract for the following tests.

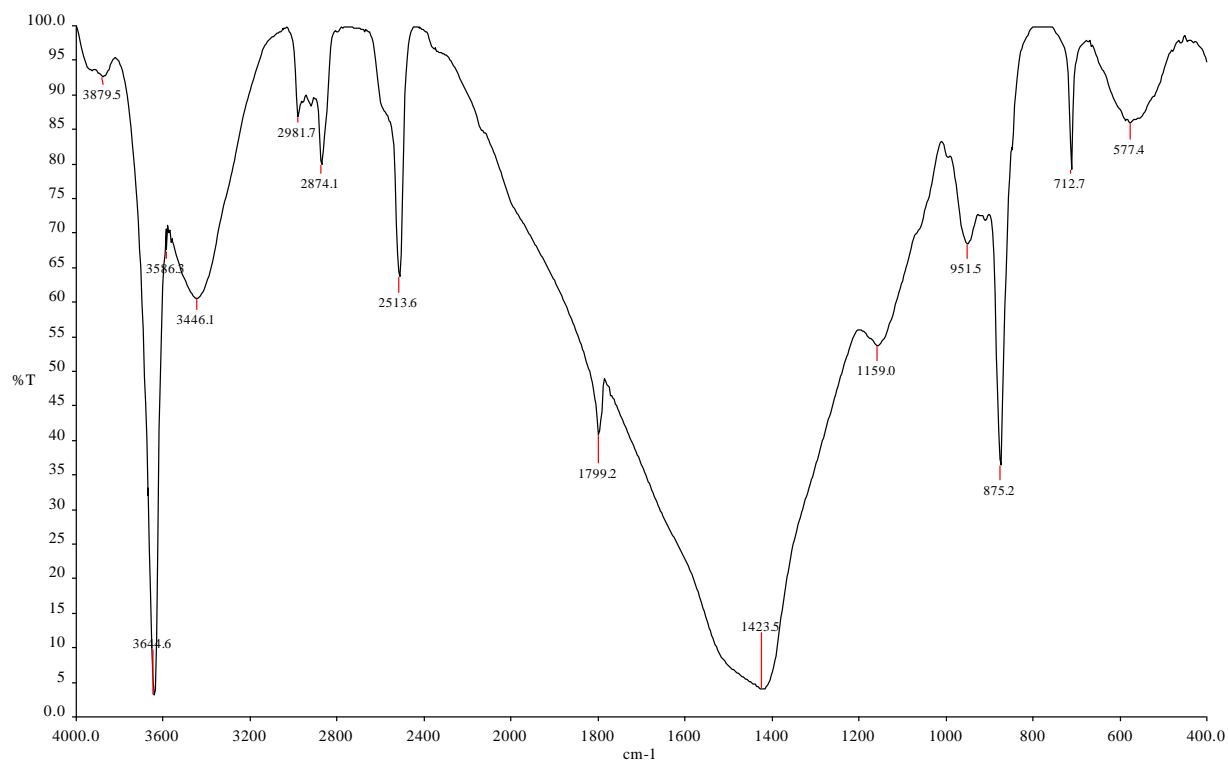
**Table No: 1.1 Chemical analysis of *Anda odu parpam***

S.No	Experiment	Observation	Inference
1.	Test for reducing Sugar	Absence of Green / Yellow / Red precipitation	Absence of Reducing Sugar
2.	Test for Starch	Absence of Blue Colour	Absence of Starch
3.	Test for Proteins	Violet Colour	Presence of Proteins
4.	Test for amino Acid	Violet Colour	Presence of Amino Acid
5.	Test for Albumin	Absence of Yellow precipitation	Absence of Albumin
6.	Test for Phosphate	Yellow precipitation	Presence of Phosphate
7.	Test for Sulphate	White precipitation	Presence of Sulphate
8.	Test for Chloride	Cloudy White precipitation	Presence of Chloride
9.	Test for Iron	Red Colour	Presence of Iron
10.	Test for Calcium	White precipitation	Presence of Calcium
11.	Test for Sodium	Absence of Yellow Flame	Absence of Sodium
12.	Test for Potassium	Yellow precipitation	Presence of Potassium
13.	Test for Zinc	White precipitation	Presence of Zinc
14.	Test for Magnesium	White precipitation	Presence of Magnesium
15.	Test for Alkaloids	Absence of Red Colour Absence of Yellow Colour Absence of White precipitation	Absence of Alkaloids Absence of Alkaloids Absence of Alkaloids
16.	Test for Tannic Acid	Absence of Black precipitation	Absence of Tannic Acid

**Results:** From the preliminary chemical analysis of *Anda odu parpam* contained Zn, Mg, K, S, Cl, Ca, Iron, Protein and Amino acid. The above result, the major ions are performing an important role in the spermatogenesis and promotes sperm count.

## Physio chemical result – FTIR

Graph No. 1 Physio chemical result (FTIR)



AOP 26.09.12.pk

SP 3601 4000.0 400.0 3.1 100.0 4.0 %T 4 2.0

REF 4000 100.0 2000 74.2 600

3879.5 92.6 3644.6 3.1 3586.3 67.5 3446.1 60.4 2981.7 86.7

2874.1 79.9 2513.6 63.8 1799.2 40.8 1423.5 4.0 1159.0 53.6

951.5 68.4 875.2 36.4 712.7 79.2 577.4 85.9

END 14 PEAK(S) FOUND

### Interpretation

The above result shows the *Anda odu parpam* contained amines, phenols, alcohols, alkanes, carboxylic acid, Esters (C-O stretch), aromatic and bromoalkanes compounds. All these compounds help the spermatogenesis activity.

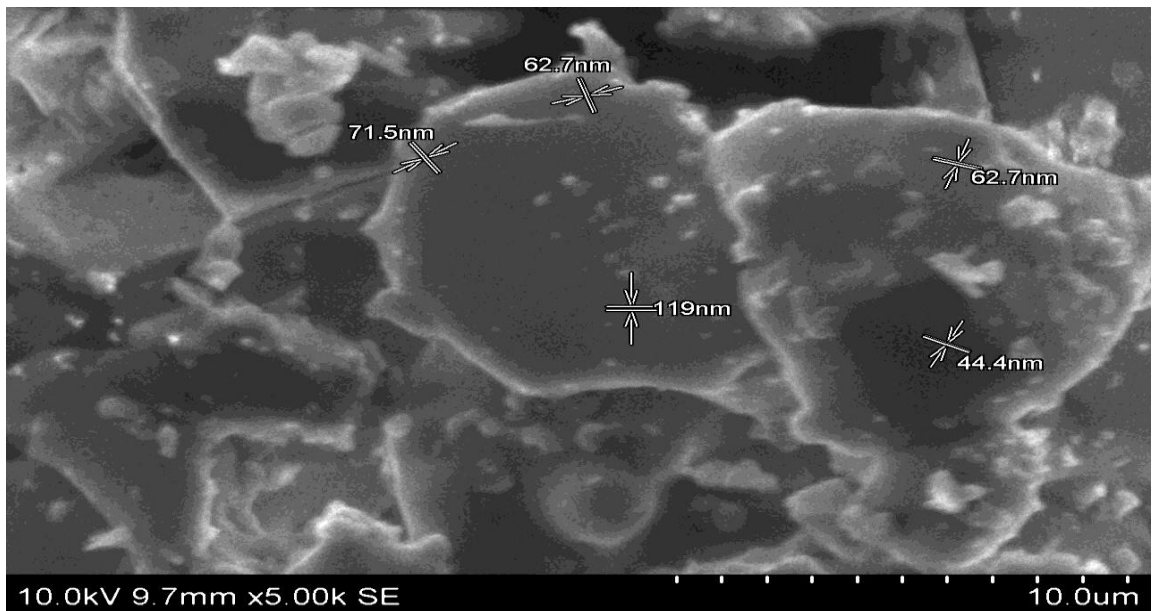


Fig. No: 6 Image of scanning electron microscope (SEM) for AOP at 10um

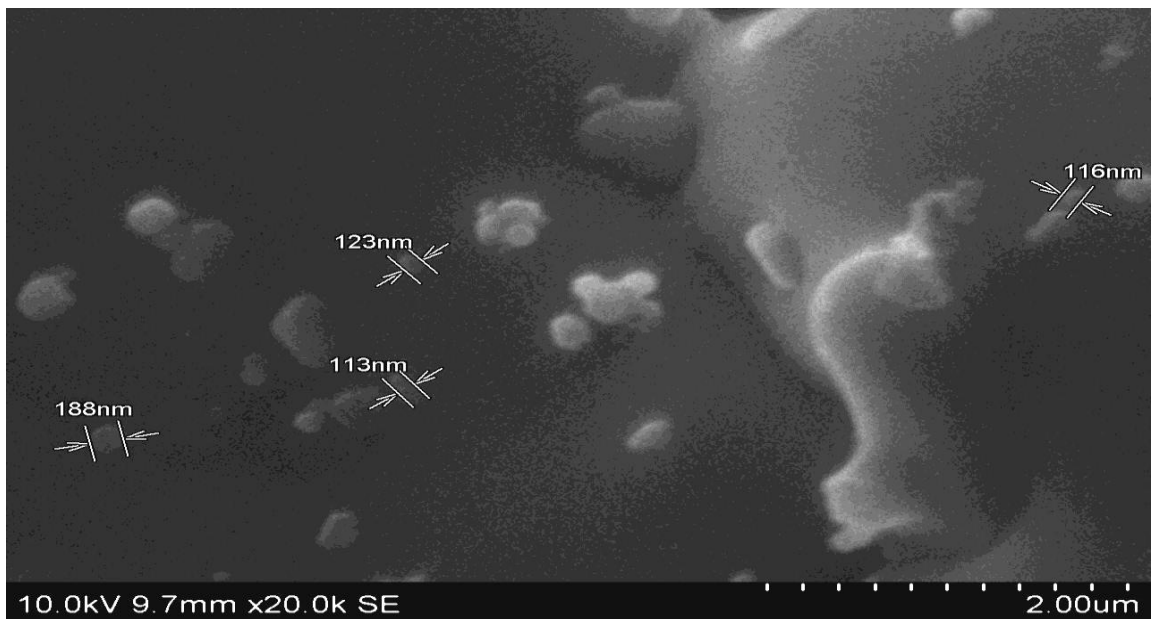


Fig. No: 7 Image of scanning electron microscope (SEM) for AOP at 2um

### Results:

The particle size of *Anda odu parpam* 113nm, 116nm, 123nm & 188nm. Because of these smaller size of the particles, it absorbs easily in the digestive system

## Toxicological Study

### Results

**Table 2: Dose finding experiment and its behavioral Signs of Toxicity**

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	500	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
2	1000	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+
3	2000	+	-	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+

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



**Table 3. Effect of *Anda Odu Parpam* on Hematological and Biochemical blood parameters of rats after 28 days oral administration.**

Parameter	Treatment and Dose			
	Control	<i>Anda Odu Parpam</i> (50mg/kg)	<i>Anda Odu Parpam</i> (100mg/kg)	<i>Anda Odu Parpam</i> (200g/kg)
WBC( $\times 10^3/\mu\text{L}$ )	11.1 $\pm$ 3.0	12.0 $\pm$ 1.4	11.5 $\pm$ 0.8	11.0 $\pm$ 0.6
RBC( $\times 10^{12}/\text{l}$ )	7.0 $\pm$ 0.15	6.66 $\pm$ 0.14	6.25 $\pm$ 0.19*	6.72 $\pm$ 0.22
Hemoglobin(g/dl)	14.0 $\pm$ 0.42	12.25 $\pm$ 0.30	12.59 $\pm$ 0.28	12.11 $\pm$ 0.82*
Hematocrit (%)	0.42 $\pm$ 0.02	0.35 $\pm$ 0.02	0.35 $\pm$ 0.02	0.37 $\pm$ 0.04
MCV (fl)	54.2 $\pm$ 0.3	52.4 $\pm$ 0.4*	52.1 $\pm$ 0.5**	52.2 $\pm$ 0.5*
MCHC (g/dl)	35 $\pm$ 0.1	36.2 $\pm$ 0.5	35.4 $\pm$ 1.2	34.1 $\pm$ 1.2
MCH (pg)	20 $\pm$ 0.2	20 $\pm$ 0.1	20 $\pm$ 0.2	20 $\pm$ 0.3
Platelet count ( $\times 10^9/\text{l}$ )	868.0 $\pm$ 102	862.1 $\pm$ 120	848.8 $\pm$ 136	838.4 $\pm$ 166
Bilirubin	1.60 $\pm$ 0.5	0.48 $\pm$ 0.04	1.00 $\pm$ 1.2	1.23 $\pm$ 1.4
ALT ( $\mu\text{l}$ )	70.2 $\pm$ 2.4	78.0 $\pm$ 5.2	75 $\pm$ 4.0	68.5 $\pm$ 3.5
AST ( $\mu\text{l}$ )	90.2 $\pm$ 3.4	98.17 $\pm$ 2.5	127.4 $\pm$ 2.8**	126.10 $\pm$ 3.0**
Creatinine ( $\mu\text{l}$ )	28.11 $\pm$ 2.0	28.14 $\pm$ 2.4	29.00 $\pm$ 4.0	28.19 $\pm$ 4.3
Cholesterol (mmol/l)	40.22 $\pm$ 1.2	47.15 $\pm$ 1.4*	45.92 $\pm$ 2.6	42.12 $\pm$ 2.0
Alkaline Phosphate( $\mu\text{l}$ )	72.24 $\pm$ 2.2	82.8 $\pm$ 3.9	90.44 $\pm$ 2.1**	91.35 $\pm$ 3.2**
Triglyceride ( $\mu\text{l}$ )	25.5 $\pm$ 4.0	26.11 $\pm$ 4.2	23.88 $\pm$ 4.1	24.10 $\pm$ 3.4

Values are mean of 6 animals  $\pm$  S.E.M. (Dunnett's test). \*P<0.05; \*\*P<0.01. Vs. control

**Table 4: Effect of *Anda Odu Parpam* on vital organ weight in 28 day sub-acute toxicity study**

<b>Organs</b>	<b>Control</b>	<b><i>Anda Odu Parpam</i> (50mg/kg)</b>	<b><i>Anda Odu Parpam</i> (100mg/kg)</b>	<b><i>Anda Odu Parpam</i> (200mg/kg)</b>
<b>Lung</b>	1.24 ± 0.05	1.21 ± 0.05	1.20 ± 0.03	1.12 ± 0.04
<b>Heart</b>	0.80 ± 0.04	0.81 ± 0.02	0.85 ± 0.05	0.84 ± 0.02
<b>Liver</b>	6.25 ± 0.20	6.45 ± 0.17	6.82 ± 0.31	6.22 ± 0.23
<b>Pancreas</b>	0.84 ± 0.05	0.86 ± 0.05	0.84 ± 0.04	0.81 ± 0.03
<b>Spleen</b>	0.61 ± 0.02	0.59 ± 0.02	0.60 ± 0.04	0.58 ± 0.04
<b>Testis</b>	0.50 ± 0.03	0.49 ± 0.03	0.51 ± 0.04	0.48 ± 0.04
<b>Kidney</b>	0.78 ± 0.02	0.76 ± 0.02	0.77 ± 0.02	0.76 ± 0.02
<b>Ovary</b>	0.08 ± 0.00	0.07 ± 0.01	0.08 ± 0.04	0.08 ± 0.02
<b>Brain</b>	0.47 ± 0.05	0.44 ± 0.03	0.45 ± 0.05	0.44 ± 0.04

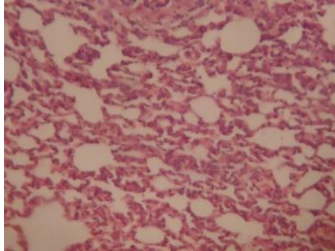
Values are mean of 6 animals ± S.E.M. (Dunnett's test). \*P<0.05; \*\*P<0.01. Vs. control

**Table 5- Urine Analysis**

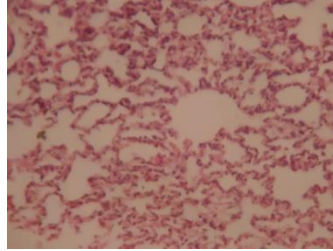
<i>Parameters</i>	<b>Control</b>	<i>Anda Odu Parpam (50mg/kg)</i>	<i>Anda Odu Parpam (100mg/kg)</i>	<i>Anda Odu Parpam (200g/kg)</i>
<b>Colour</b>	Yellow	Yellow	Yellow	Yellow
<b>Transparency</b>	Clear	Slightly turbid	cloudy	Slightly turbid
<b>Specific gravity</b>	1.010	1.010	1.010	1.010
<b>PH</b>	>7.2	>8.0	>8.0	>9.0
<b>Protein</b>	Nil	3+	3+	3+
<b>Glucose</b>	Nil	Nil	Nil	Nil
<b>Bilirubin</b>	-ve	-ve	-ve	-ve
<b>Ketones</b>	-ve	+ve	+ve	+ve
<b>Blood</b>	Absent	Absent	Absent	Absent
<b>Urobilinogen</b>	Normal	Abnormal	Abnormal	Abnormal
<b>Pus cells</b>	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
<b>RBCs</b>	Nil	Nil	0-1cells/HPF	Nil
<b>Epithelial cells</b>	Nil	1-cell/HPF	Nil	1-cell/HPF
<b>Crystals</b>	Nil	Nil	Nil	Nil
<b>Casts</b>	Nil	Nil	Nil	Nil

**Fig No: 8 Histopathological images of animals treated with *Anda odu parpam***

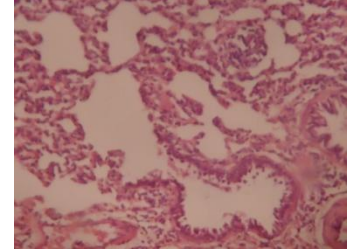
**LUNGS**



**50 mg**

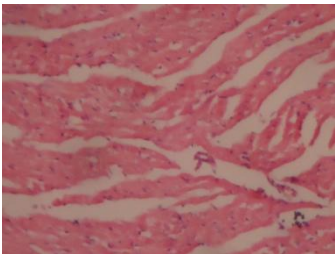


**100 mg**

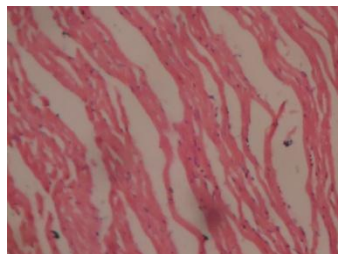


**200 mg**

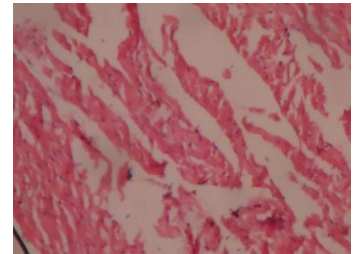
**HEART**



**50 mg**

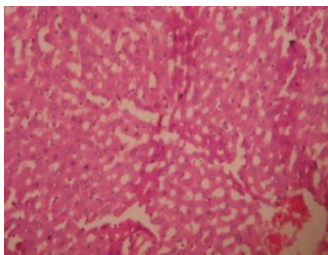


**100 mg**

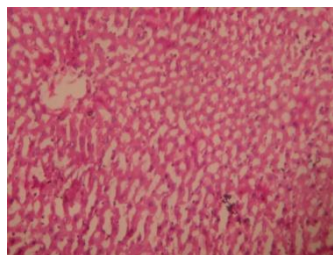


**200 mg**

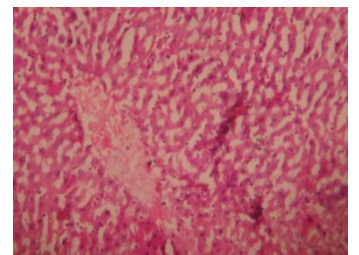
**LIVER**



**50 mg**

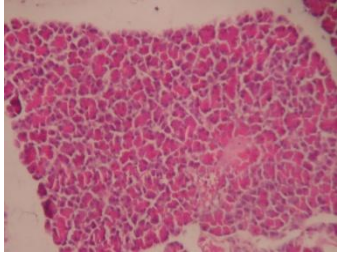


**100 mg**

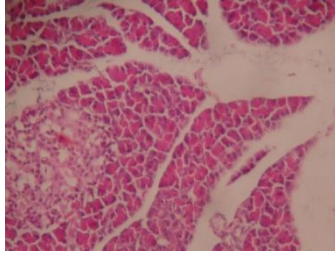


**200 mg**

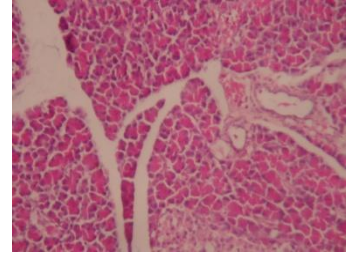
**PANCREAS**



**50 mg**

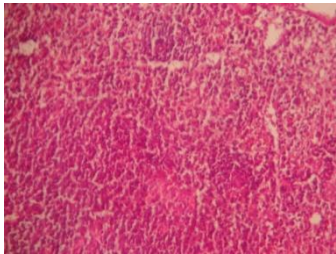


**100 mg**

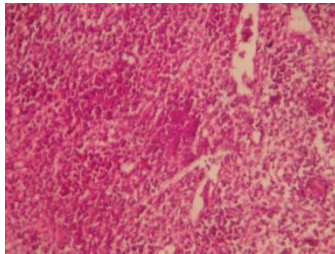


**200 mg**

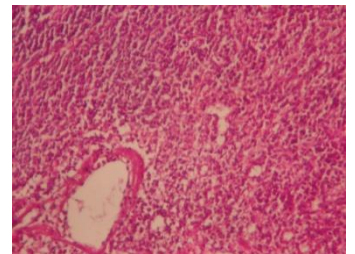
**SPLEEN**



**50 mg**

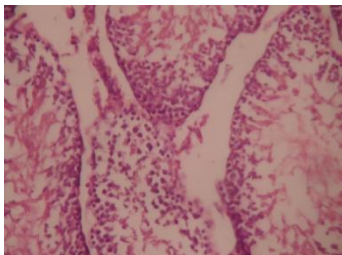


**100 mg**

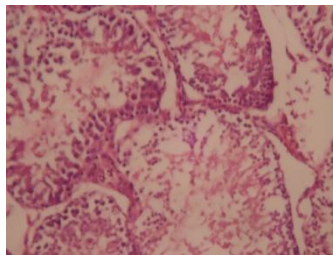


**200 mg**

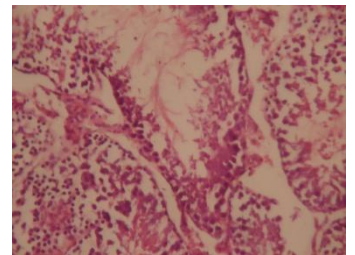
**TESTIS**



**50 mg**



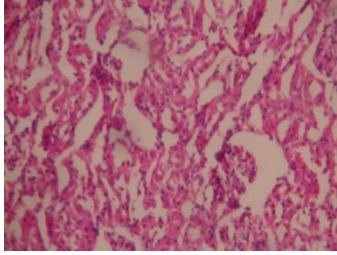
**100 mg**



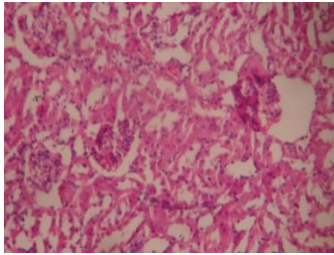
**200 mg**



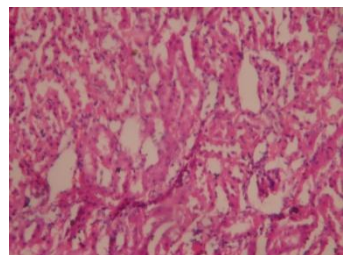
**KIDNEY**



**50 mg**

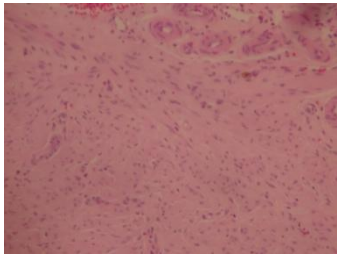


**100 mg**

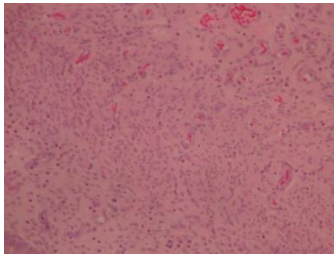


**200 mg**

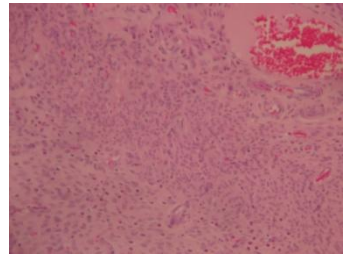
**OVARY**



**50 mg**

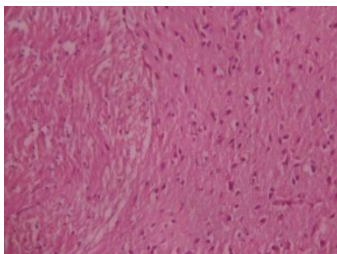


**100 mg**

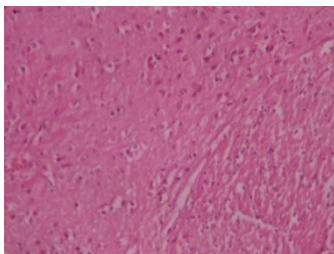


**200 mg**

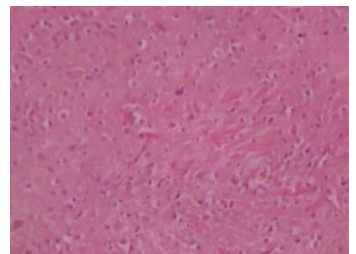
**BRAIN**



**50 mg**



**100 mg**



**200 mg**

Animals were shown significant toxic clinical signs during the dosing period of 28 days. All animals from treated dose groups not survived throughout the dosing period of 28 days and it was found two animal dead after 12days of treatment in mid and high dose. Results of body weight determination of animals of control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days. During dosing period, the quantity of food consumed by animals from different dose groups was found to be comparable and normal with that of control animals. Ophthalmoscope examination of animals in control and *Anda Odu Parpam* treated group revealed minor abnormality.

The results of haematological investigations conducted on day 28, revealed following significant changes in the values of different parameters investigated when compared with those of respective controls and, the increase or decrease in the values obtained was within normal biological and laboratory limits. A slight fall in total Heamatocrit, Hb and RBC count values were obtained for animals in the dose group of 100 and 200 mg/kg ( $P<0.05$ ). Results of Biochemical investigations conducted on days 28 revealed the significant changes in the values of different parameters studied when compared with those of respective controls; however, the values obtained were within normal biological and laboratory limits. Cholesterol level is elevated in animals of 50 and 100 mg/kg dose group ( $P<0.05$ ). AST and ALP levels slightly increased in animals of 100 and 200mg/kg group ( $P<0.01$ ). Urine analysis data of control group and treated group of animals determined in week 4 did not reveal any abnormalities. Comparison of organ weights of treated animals with respective control animals on day 28 was found to be comparable but the testis and kidney weight was increased. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

## Pharmacological Study - Results and discussion

**Table 6. Effect of *Anda Odu Parpam* on body weight of male albino rats.**

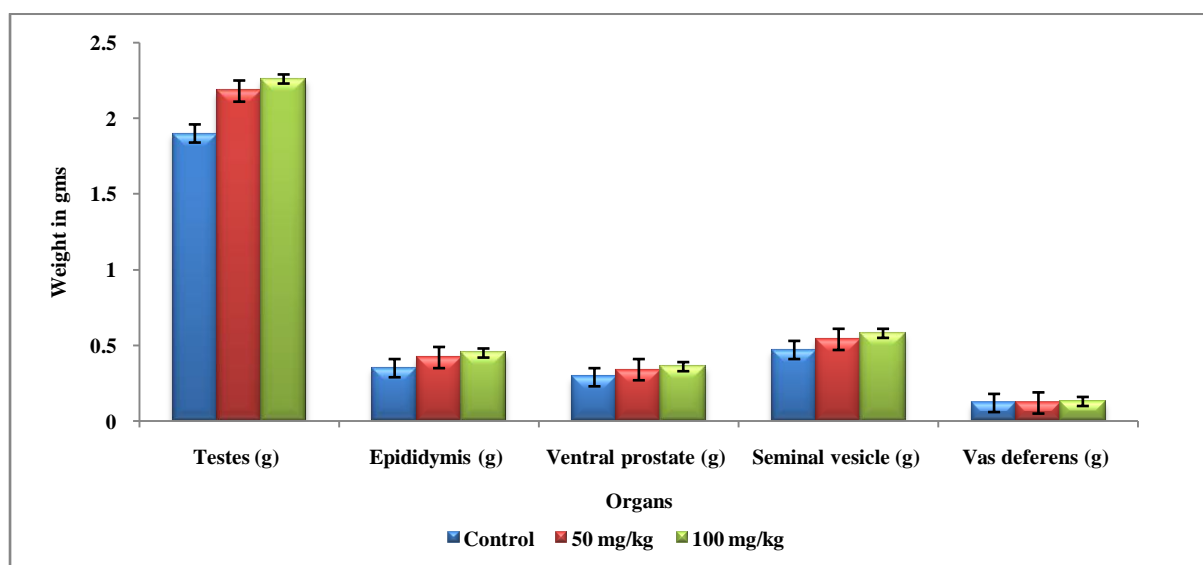
Parameter	Control	50 mg/kg	100 mg/kg
Initial Body Weight (g)	164.12 ± 1.30	160.10 ± 2.02	158.88 ± 1.29
Final Body Weight (g)	191.41 ± 1.22	180.35 ± 1.74**	174.20 ± 2.25**

\* $P < 0.05$ , values expressed as Mean ± SEM, n=6

**Table 7. Effect of *Anda Odu Parpam* on reproductive organ weights of male albino rats**

Organs	Control	50 mg/kg	100 mg/kg
Testes (g)	1.90 ± 0.02	2.18 ± 0.02**	2.26 ± 0.02**
Epididymis (g)	0.35 ± 0.01	0.42 ± 0.01**	0.45 ± 0.01**
Ventral prostate (g)	0.29 ± 0.02	0.34 ± 0.03	0.36 ± 0.02
Seminal vesicle (g)	0.47 ± 0.01	0.54 ± 0.01**	0.58 ± 0.01**
Vas deferens (g)	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01

**Graph No. 2 Effect of *Anda Odu Parpam* on reproductive organ weights of male**



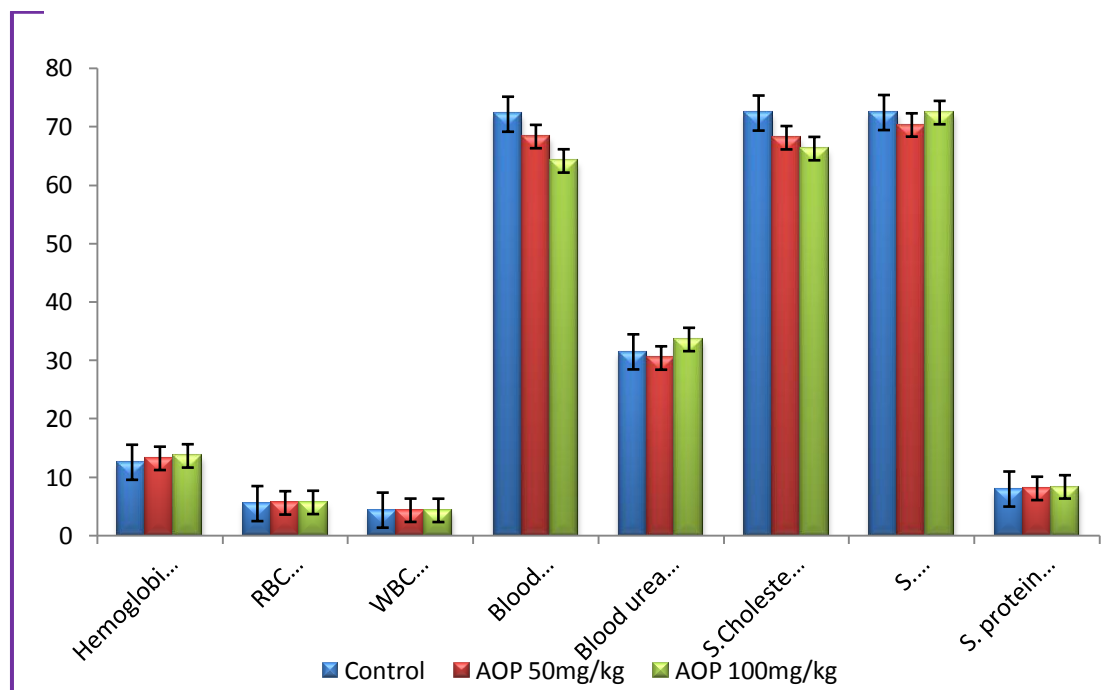


**Table 8. Effect of *Anda Odu Parpam* on hematological and biochemical parameters in male rats after 28days of treatment.**

Parameters	Control	AOP 50mg/kg	AOP 100mg/kg
Hemoglobin (gm %)	12.57± 0.22	13.24±0.18	13.66±0.20**
RBC (million/cu.mm)	5.50± 0.03	5.62± 0.03	5.70± 0.03
WBC (X10 <sup>3</sup> /cu.mm)	4.38± 0.50	4.36±0.42	4.34± 0.65
Blood sugar (mg/dL)	72.12± 1.25	68.30± 2.65	64.13± 3.12
Blood urea (mg/dL)	31.46± 2.42	30.41±3.14	33.58± 3.11
S.Cholesterol (mg/dL)	72.32± 0.45	68.10± 1.00**	66.24± 1.12**
S.phospholipids (mg/L)	72.41± 0.42	70.28± 0.40**	72.40± 0.45
S. protein (mg/dL)	7.99± 0.62	8.10±0.64	8.36± 0.48

\*P<0.05, values expressed as Mean±SEM, n=6

**Graph No. 3 Effect of *Anda Odu Parpam* on hematological and biochemical**

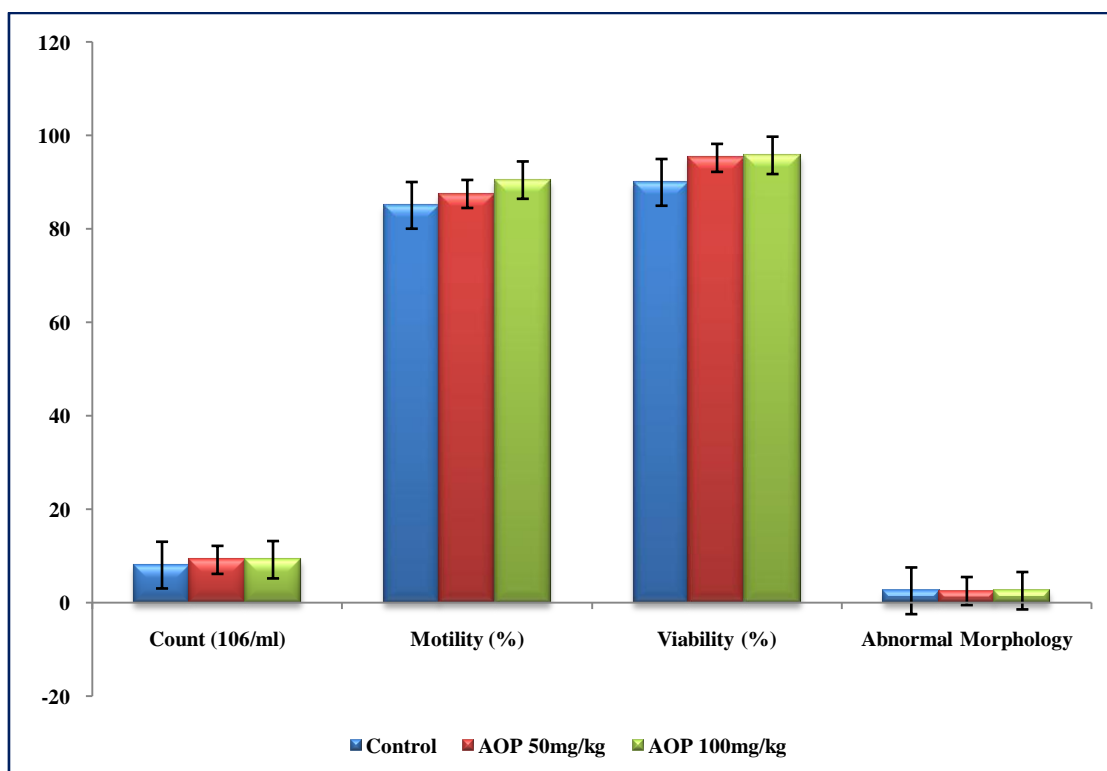


**Table 9: Effects of *Anda Odu Parpam* on sperm count, motility, viability and abnormal morphology after 14 days treatment.**

Groups	Count (10 <sup>6</sup> /ml)	Motility (%)	Viability (%)	Abnormal Morphology
Control	8.72 ±0.44	87.48±1.20	94.07 ±1.28	2.12 ±0.30
AOP 50mg/kg	8.90±0.70	89.52±2.18	95.24 ±1.52	2.27 ±0.28
AOP 100mg/kg	9.02±0.64	90.55±1.56	95.66 ±1.30	2.14 ±0.42

\**P*<0.05, values expressed as Mean±SEM, n=6

**Graph No. 4 Effects of *Anda Odu Parpam* on Semen quality after 14 days**

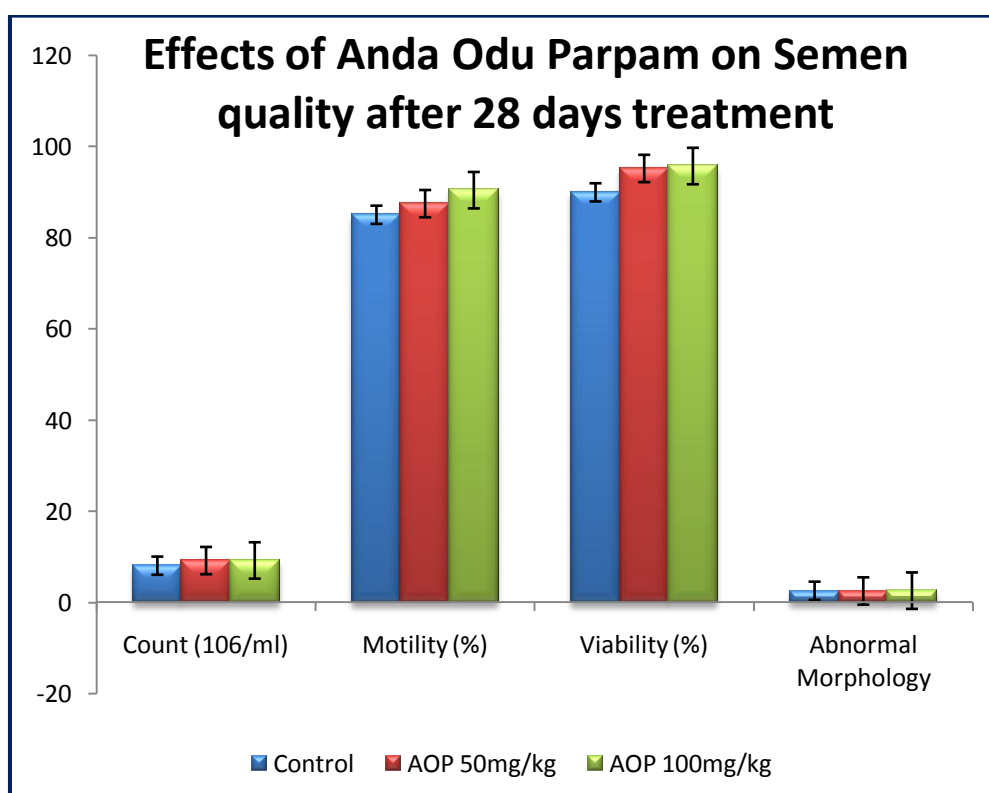


**Table 10: Effects of *Anda Odu Parpam* on sperm count, motility, viability and abnormal morphology after 28 days treatment**

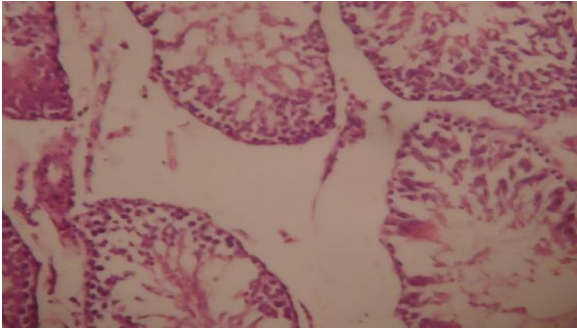
Groups	Count (10 <sup>6</sup> /ml)	Motility (%)	Viability (%)	Abnormal Morphology
Control	8.10±0.24	85.10±1.77	90.02 ±1.79	2.60 ±0.30
AOP 50mg/kg	9.22±0.25*	87.54±2.28	95.26 ±2.17	2.55 ±0.35
AOP 100mg/kg	9.25±0.34*	90.50±1.62	95.80 ±1.74	2.62 ±0.34

\**P*<0.05, values expressed as Mean±SEM, n=6

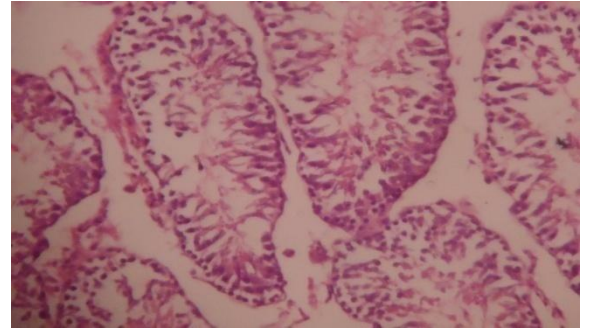
**Graph No. 5 Effects of *Anda Odu Parpam* on Semen quality after 28 days**



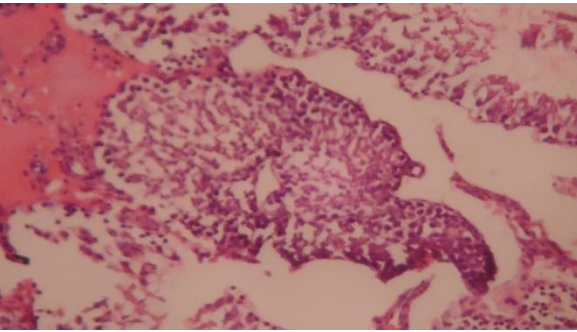
**Fig No: 9 Histo pathology study on AOP treated in animals at various dosages**



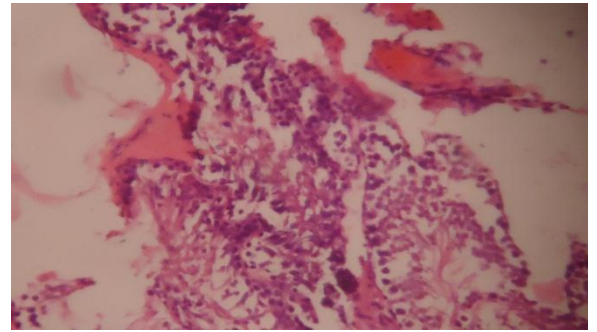
**Normal**



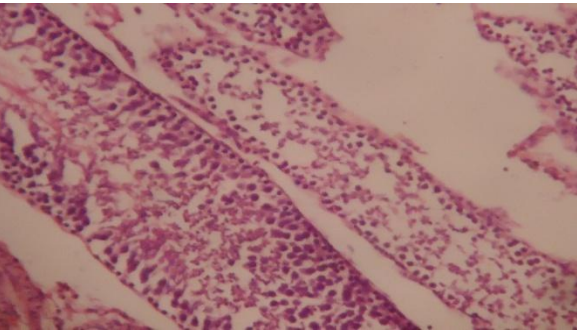
**Normal 1**



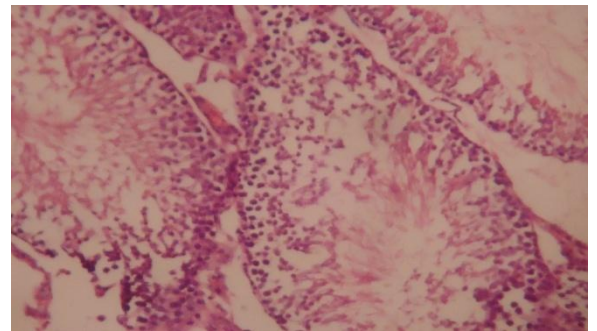
**TCDD 40µg treated**



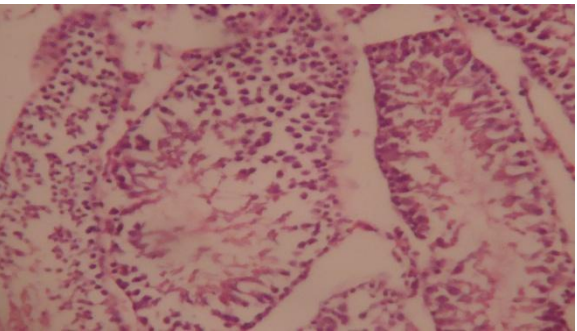
**TCDD 40µg treated control**



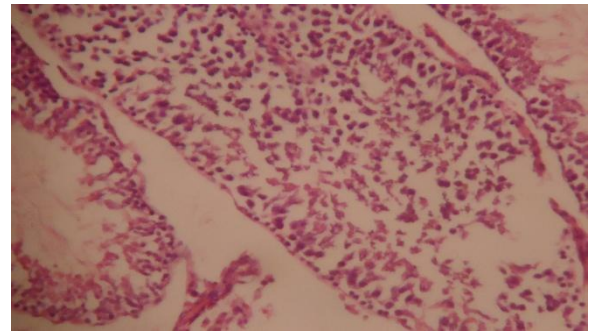
**AOP 50mg**



**AOP 50mg 1**



**AOP 100mg**



**AOP 100mg 1**

In the acute and sub acute toxicity study, Mortality and sign of toxicity, such as weight loss, abnormal grooming, reflex response and other behavioral manifestations was seen in the limit test dose at 2000 and 5000mg/kg. The mild behavioural change like lethargy was observed in the 500mg/kg treated animals. From the maximum tolerable dose 500mg/kg of *Anda Odu Parpam* one-fifth and one tenth of the dose was taken as the therapeutic dose levels for the further pharmacological study. The rats did not reveal observable signs of central nervous system and mortality during spermatogenic assessment. And also a significant increase in the average daily body weights indicating that the test animals were in healthy condition at the throughout the study. This indicates that the dosages administered were below toxic level.

TCDD and its related congeners have been shown to act as developmental and reproductive toxicants, which reduce testicular and accessory sex organ weights, alter testicular morphology, and decrease sperm production. Significant increase ( $P < 0.05$ ) in weights of testes, epididymides, ventral prostate, seminal vesicles and vas deferens were observed in the treated groups compared with the control. It is well established that androgens are the major regulators of the growth, structure and functions of accessory sex organs. In accessory sex organs it is not testosterone, but rather the  $5\alpha$ -reduced metabolites, dihydrotestosterone and  $3\alpha, 17\beta$ -androstenediol are the primary regulatory hormones controlling their structure and functions. A decrease in such androgen metabolites might eventually result in decreased accessory sex organs weight.

High levels of intratesticular testosterone are necessary for the proliferation and differentiation of spermatogenic cells and spermatogenesis. While high circulating testosterone concentration is required for functional integrity of androgen dependent accessory sex organs. Testosterone is synthesized and released by the Leydig cells in response to LH. The Leydig cells are able to respond to changes in LH secretion within half an hour and influence the seminiferous tubules by maintaining a high concentration of testosterone in the peritubular compartments of the testis. Spermatogenesis involves a complex interplay between the structural elements of testis and the endocrine system.

Hypothalamic gonadotrophic releasing hormone induces pituitary gonadotrophin. Abundance of spermatozoa in seminiferous tubule clearly indicates spermatogenesis which is regulated by hormone. Hypertrophy of Leydig cells is also suggestive of steroids synthesis. A significant decrease in the seminiferous tubular diameter, Leydig cell nuclear diameter and alterations in the Leydig cells differential counts probably

correspond to decrease in testosterone production and or inhibin of pituitary gonadotropin secretion, hence, disruption of spermatogenesis occurred. In the hematological investigations, no significant differences were found in the total leukocyte count, level of hemoglobin and in hematocrit value in *Anda Odu Parpam* treated rats compared to control. But, significant changes were noted in the levels of blood sugar, serum cholesterol and increase in the serum testosterone level in the rats treated with *Anda Odu Parpam* at the both dose levels used in this study.

Histological effect of *Anda Odu Parpam* on gonadal tissues at dose 0.1g/kg body weight showed spermatogenic series of cells seen at the various stages of transformation and differentiation with central tubule showing marked spermatogenic transformation of spermatids into spermatozoa. The supporting Sertoli cells are intact and also the basal lamina placed spermatogonia are intact and spermatids at various stages of differentiation filled up the central lumen of the tubules.

The outcome of all point to an increased activity of spermatogenesis. Similarly *Anda Odu Parpam* 50mg/kg treated male albino rats showed spermatogenic cells with central tubule showing marked spermiogenic transformation. Photomicrographs of the untreated control showed normal histoarchitecture of these structures. There were no treatment related adverse effects on seminal vesicles, prostate gland and vas deferens. Photomicrograph of normal testis of rat showing well layered seminiferous tubules with different stages of spermatogenic cells

The current investigation reveals that the therapeutic dose of *Anda Odu Parpam* has no toxic effects on rats administered orally. This was particularly based on the observation of the daily body weight changes which are not significantly different with the control. Although there was a slight decline in the reproductive organ weight to body weight ratio, reproductive organs in general were unaffected by oral administration of the *Anda Odu Parpam*. Further tests, however, should be conducted to confirm the precised effects on spermatogenesis. It is suggested that histological analysis be conducted to determine the effects of the *Anda Odu Parpam*, spermatogonia, epithelialization of spermatogonial cells, and lumen diameter. Our results indicated that administration of *Anda Odu Parpam* creates marked improvement in the sperm counts and motilities compared with control.

A significant enhancement in the number and motility of sperm ( $p < 0.05$ ) was observed in experimental animals, which could be due to the influence of the *Anda Odu Parpam* on the cell cycle, cell division and expression of genes necessary for the

spermatogenesis and also that these changes might be resultant effect of changes in the microenvironment of epididymis and creation of a pleasant environment influencing for the improvement of sperm count and motility. Reduced numbers of spermatozoa, abnormal spermatozoa or their reduced or insufficient motility are the leading causes of disturbed fertility or infertility in patient.

### **Clinical results and discussions of *Anda odu parpam***

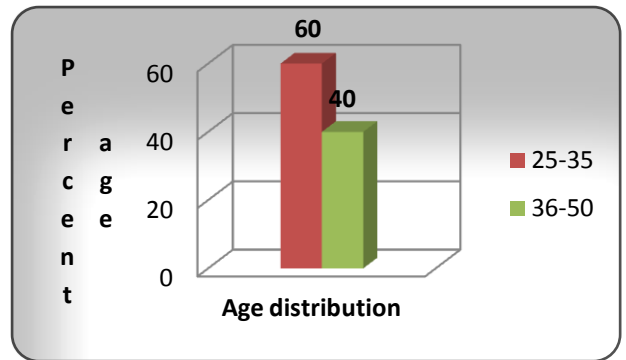
The clinical factors considered for the purpose of the study comprised as follows:

- ❖ Age Distribution
- ❖ Occupational status
- ❖ Socio economic Status
- ❖ Food habits
- ❖ Personal habits
- ❖ Symptoms
- ❖ *Udal kattugal*
- ❖ *Enn vagai thervu*
- ❖ *Naadi*
- ❖ Classification on the basis of *Neikkuri*
- ❖ Clinical progress
- ❖ Results after treatment.

**Table No. 11 Age Distribution**

Sl.No	Age	No. of patients/50	Percentage
1.	25-35	30	60%
2.	36-50	20	40%

**Graph No.6 Age Distribution**



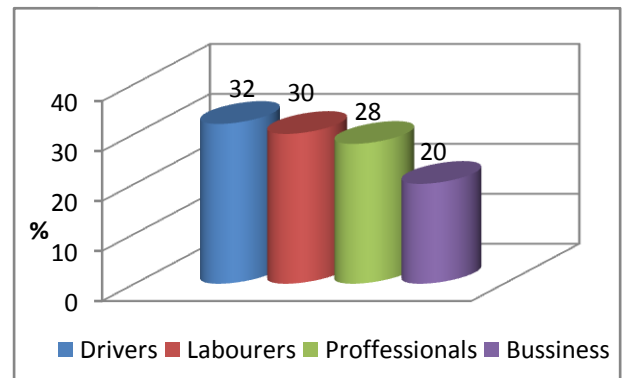
**Inference:**

According to the above mentioned data 60% of patients were in age groups 25-35 years, 40% of patients were in age group 36-50 years.

**Table No. 12 Occupational status**

Sl.No	Occupational status	No. of patients/50	Percentage
1	Drivers	16	32%
2	Labours	10	20%
3	Professionals	14	28%
4	Businessman	10	20%

**Graph No.7 Occupational status**



**Inference**

32% of cases were drivers.

20% of cases were labours.

28% of cases were Business Man.

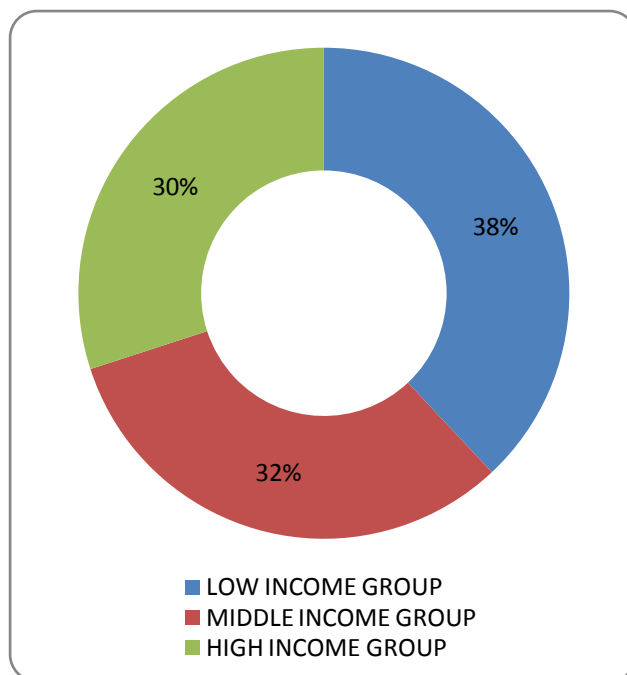
20% of cases were professionals.



**Table No. 13 Socio economic status**

Sl.No	Socio Economic Status	No. of patients/50	In %
1	Low income group (below 10000/month)	19	38%
2	Middle income group (below 15000/month)	16	32%
3	High income group (below 20000/month)	15	30%

**Graph No.8 Socio economic status**



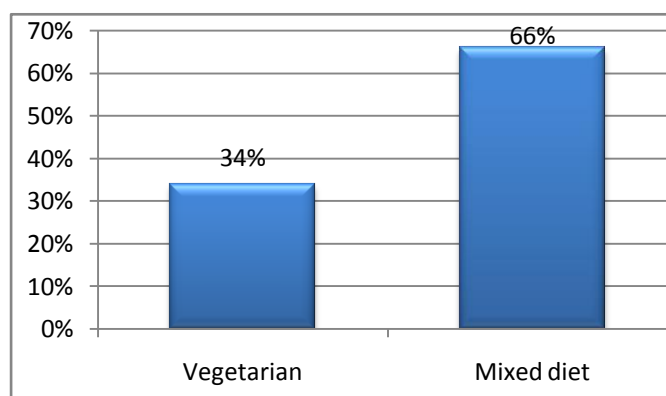
**Inference**

38% of cases belong to Low income group and 32% of patients belong to lower income group. 30% of cases belong to high income group.

**Table No. 14 Food habits**

Sl.No	Food Habit	No. of patients/50	Percentage
1.	Vegetarian Diet	17	34%
2.	Mixed Diet	33	66%

**Graph No. 9 Food habits**



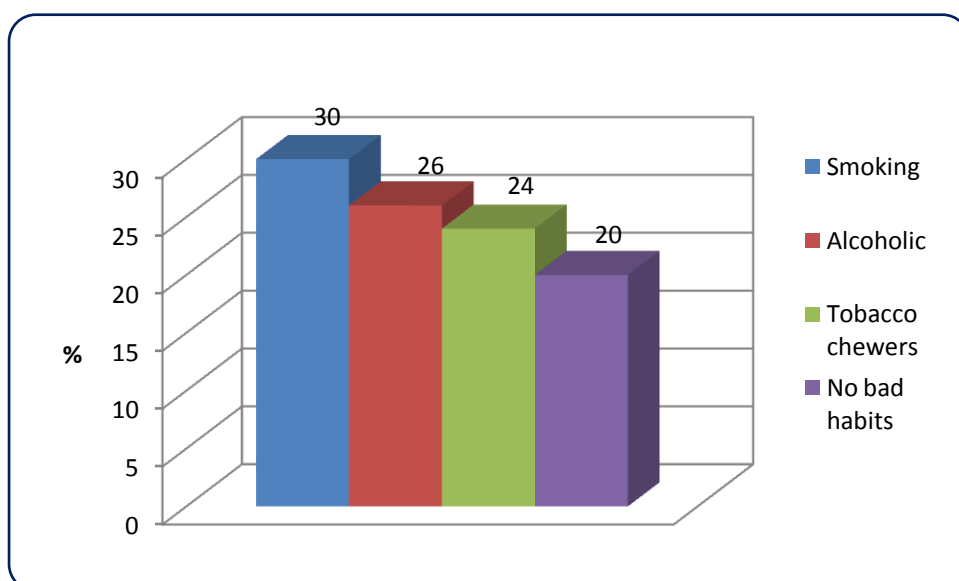
**Inference**

66% of cases were mixed diet and 34% of cases were Vegetarian.

**Table No. 15 Personal habits**

Sl.No	Personal Habits	No. of patients/50	Percentage
1	Smoker	15	30%
2	Alcoholic	13	26%
3	Tobacco chewing	12	24%
4	No bad Habits	10	20%

**Graph No.10 Personal habits**



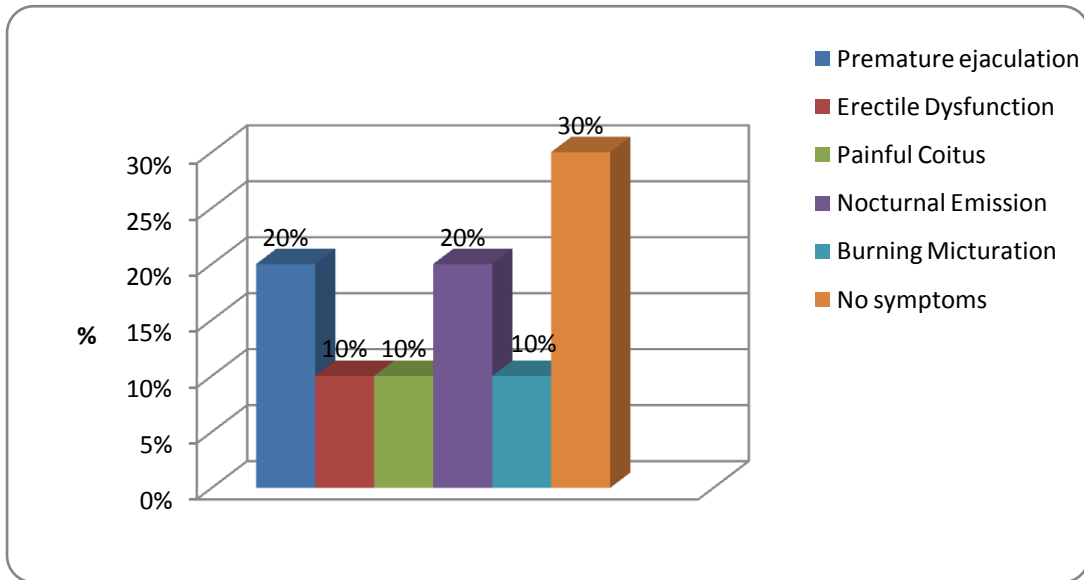
**Inference**

20% of patients had no bad habits, 30% of cases were smoker, 26% of cases were alcoholic and 24% were tobacco chewers.

**Table No. 16 Symptoms**

Sl.No	Symptoms	No. of patients/50	Percentage
1	Premature ejaculation	10	20%
2	Erectile Dysfunction	5	10%
3	Painful Coitus	5	10%
4	Nocturnal Emission	10	20%
5	Burning Micturation	5	10%
6	No symptoms	15	30%

**Graph No.11 Symptoms**



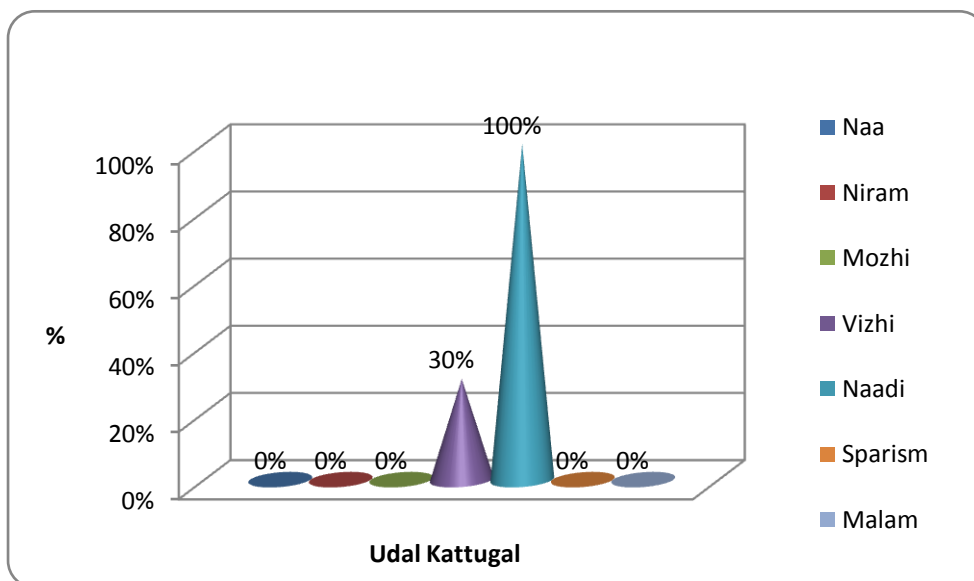
**Inference**

20% of cases came with complaints of premature ejaculation and 10% of cases with erectile dysfunction, 10% cases had painful coitus, 10% cases with burning micturation, 20% cases with nocturnal emission and 30% of cases had no symptoms.

**Table No: 17 Udal Kattugal**

Sl.No	Udal Kattugal	No. of patients/50	Percentage
1	Saaram	50	100%
2	Senner	0	0%
3	Oon	0	0%
4	Kozhuppu	0	0%
5	Enbu	6	12%
6	Moolai	0	0%
7	Sukkilam	50	100%

**Graph No: 12 Udal Kattugal**



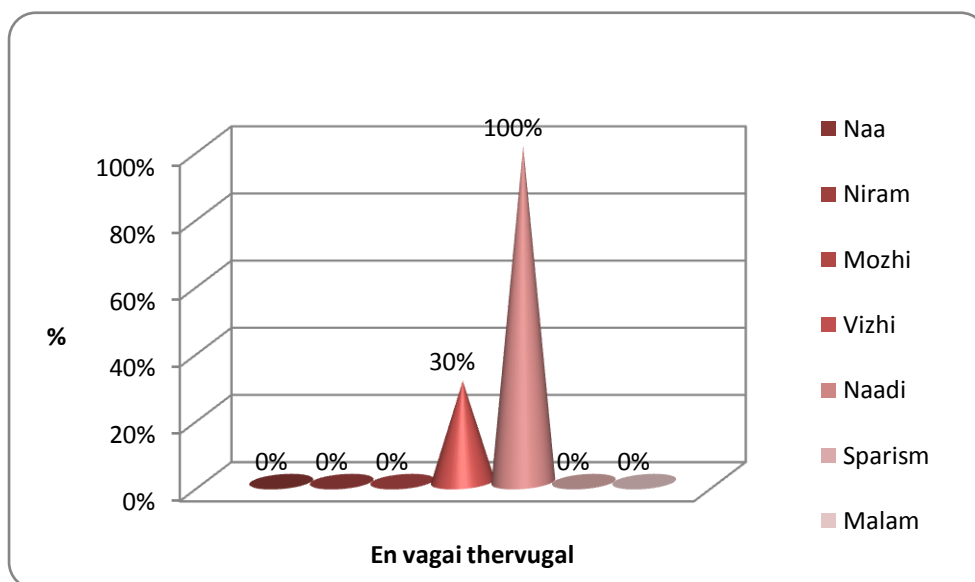
**Inference:**

Both *Saaram* and *Sukkilam* were affected in 100% of patients and *Enbu* was affected in 12% of patients

**Table No: 18 Enn Vagai Thervu**

Sl.No	Enn Vagai Thervu	No. of patients/50	Percentage
1	<i>Naa</i>	0	0%
2	<i>Niram</i>	0	0%
3	<i>Mozhi</i>	0	0%
4	<i>Vizhi</i>	15	30%
5	<i>Naadi</i>	50	100%
6	<i>Sparism</i>	0	0%
7	<i>Malam</i>	0	0%
8	<i>Moothiram</i>	0	0%

**Graph No: 13 Envagai Thervugal**



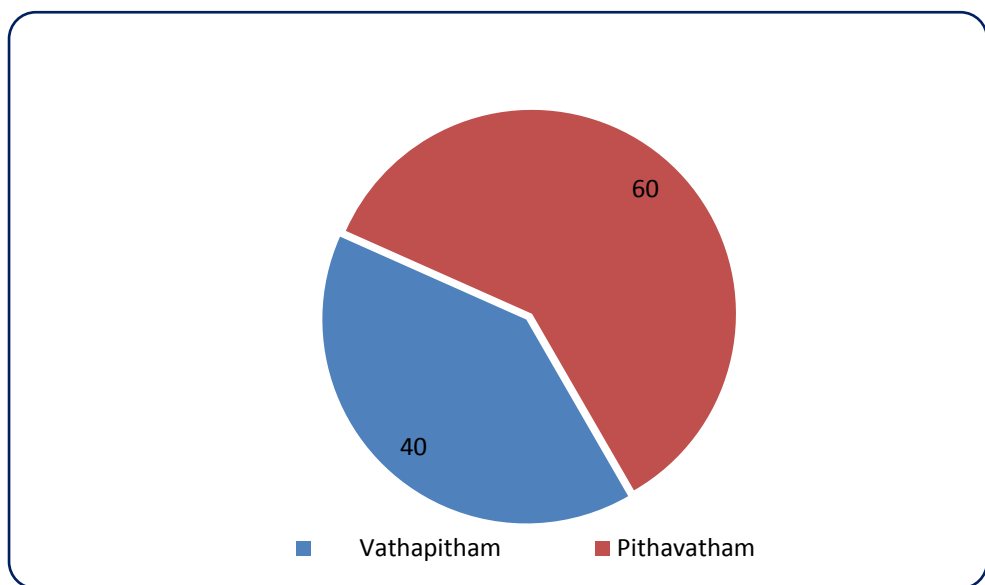
**Inference**

*Naadi* was affected in 100% of patients and 30% of patients *vizhi* was affected.

**Table No: 19 Naadi:**

<b>Sl.No</b>	<b>Naadi</b>	<b>No. of patients/50</b>	<b>Percentage</b>
1	<i>Vathapitham</i>	20	40%
2	<i>Pithavatham</i>	30	60%

**Graph No: 14 Naadi**

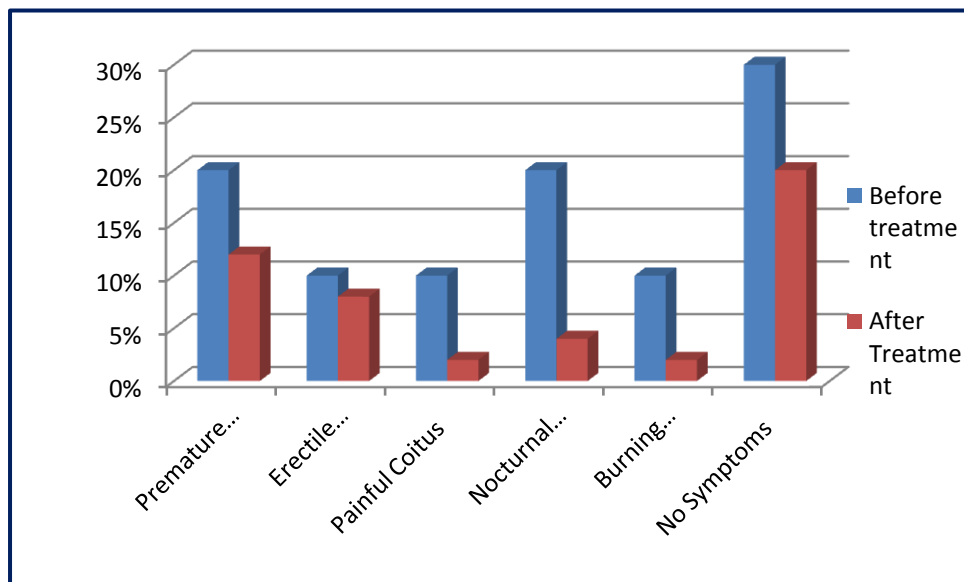


**Inference**

40% of patient's *vatha pitha naadi* was felt and 60% of cases *Pitha vatha naadi* was felt.

**Table No: 20 Clinical progress**

Sl.No	Symptoms	No.of patients/50		Percentage	
		BT	AT	BT	AT
1	Premature ejaculation	10	6	20%	12%
2	Erectile Dysfunction	5	4	10%	8%
3	Painful Coitus	5	1	10%	2%
4	Nocturnal Emission	10	2	20%	4%
5	Burning Micturation	5	1	10%	2%



**Graph No: 15 Clinical**

**Inference**

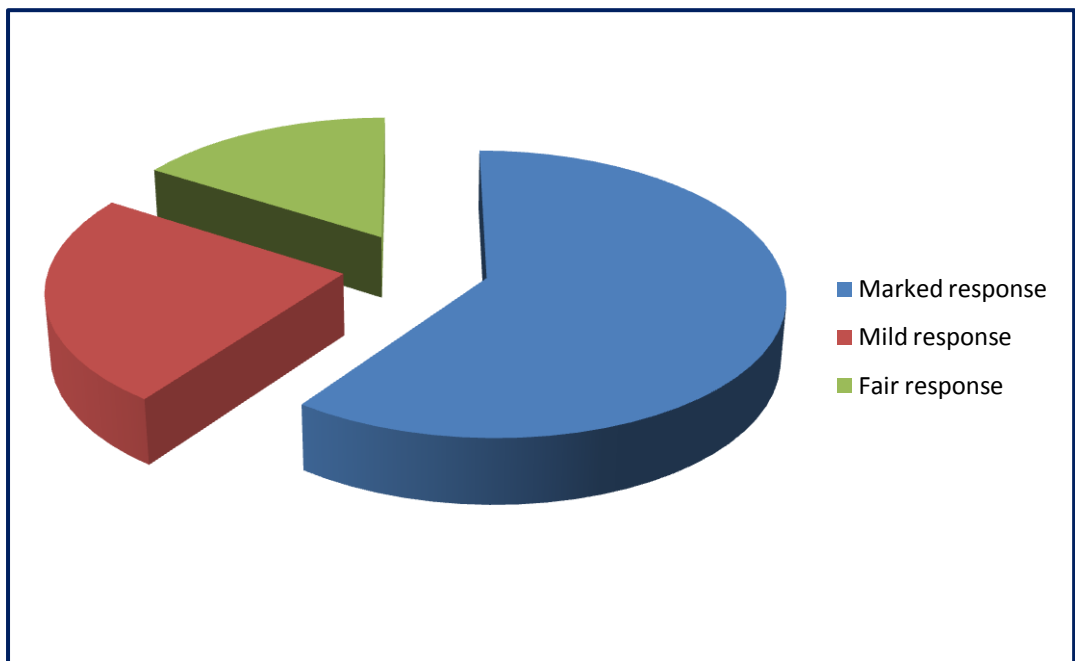
Before treatment 50% of cases had premature ejaculation, 50% of cases had nocturnal emission & 25% having erectile dysfunction.

After treatment premature ejaculation, nocturnal emissions were 15% and 10% of cases respectively & 10% having erectile dysfunction.

**Table No: 21 Gradation of results**

Sl.No	Gradation of results	No. of patients/50	Percentage
1	Marked response	30	60%
2	Mild response	12	24%
3	Fair response	8	16%

**Graph No: 16 Gradation of results**



**Inference**

60% of Patients show good improvement, 24% of shows moderate improvement and 16% of cases shows poor improvement.



## Biostatistical Analysis

### Effect of *Anda odu parpam* on Sperm Count in human subjects

Sl.No	Sperm Count (Million/Cumm)	
	BT	AT
1	6.2	12.6
2	8	42
3	17	26
4	18	60
5	10	50
6	62	70
7	16.2	49
8	34	52
9	53	86
10	10	14
11	6	40
12	49	67
13	46	71
14	14	44
15	50	76
16	25	50
17	19	56
18	25	30
19	17	20
20	65	75
21	44	72
22	37	48
23	20	25
24	29	59
25	15	45

Sl.No	Sperm Count (Million/Cumm)	
	BT	AT
26	60	70
27	20	45
28	40	50
29	65	85
30	12	16
31	6	45
32	55	68
33	48	70
34	35	45
35	59	80
36	26	52
37	10	56
38	25	30
39	16	20
40	67	75
41	44	70
42	35	48
43	18	25
44	20	59
45	26	45
46	60	70
47	18	45
48	40	55
49	45	80
50	12	16

**Software:** spss17 version

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

**Number of cases:** 50

**Test:** Paired t test

**Confidence Interval:** 95%

**Correlation coefficient (r):** 0.797

**Before and after treatment mean difference:** 20.04±12.44 (millions/cu mm).

**P Value (2 tailed):** p<0.01.

**Inference:** The 'p' value is significant (p<0.01). So the treatment was significantly improving the Semen count (millions/cu mm).

**Effect of *Anda odu parpam* on Sperm Motility in human subjects**

Sl.No	Sperm Motility (%)	
	BT	AT
1	10	40
2	50	58
3	8	10
4	22	45
5	4	10
6	20	30
7	6	27
8	35	55
9	25	45
10	2	4
11	9	40
12	20	30
13	30	54
14	10	20
15	15	40
16	10	50
17	10	55
18	28	25
19	20	15
20	12	34
21	30	48
22	50	58
23	7	10
24	22	50
25	3	30

Sl.No	Sperm Motility (%)	
	BT	AT
26	15	30
27	6	30
28	30	55
29	15	45
30	3	4
31	4	45
32	18	30
33	28	53
34	10	20
35	12	40
36	15	50
37	7	55
38	28	25
39	20	15
40	13	34
41	30	48
42	55	58
43	6	10
44	22	50
45	3	11
46	15	30
47	4	38
48	35	60
49	20	45
50	6	8

**Software:** spss17 version

**Variables:** Sperm motility (%) – before treatment, after treatment

**Number of cases:** 50

**Test:** Paired t test

**Confidence Interval:** 95%

**Correlation coefficient (r):** 0.617

**Before and after treatment mean difference:** 17.88±13.40 (%).

**P Value (2 tailed):** p<0.01.

**Inference:**

The p value is significant (p<0.01). So the treatment was significantly improving the sperm motility (%).

## 6. CONCLUSION

The trial drug *Anda odu parpam* (Egg shell of *gallus domesticus*) is selected from the classical Siddha text *Anuboga Vaidhaya Navaneetham*-Part III for the evaluation of safety and efficacy in the management of Male infertility.

The trial drug was duly identified and authenticated by the *Gunapadam* experts.

The presence of Zn ions indicates that they help improving the sperm count. The other components Mg, K, Ca, Protein, Amino acid, Phosphate, Sulphate, Chloride and Iron ions are also responsible for its spermatogenic property.

Toxic effect was observed at 100mg/kg of *Anda Odu Parpam* treated via oral route over a period of 28 days. So, it can be concluded that the *Anda Odu Parpam* can be prescribed for therapeutic use in human with the 30-40% dose reduction from median dose used in this study.

Oral administration of *Anda Odu Parpam* at 50 and 100mg.kg dose level increase the weight of testes and seminal vesicles, improve semen quality, quantity and increase testosterone levels. Therefore, this study recommends that intake of *Anda Odu Parpam* may be useful for patients who suffer from male infertility. Thus, the drug *Anda Odu Parpam* may provide an alternative for management of infertility due to reduced spermatogenesis or oligospermic condition at the prescribed dose level.

Single dose (100 mg/kg body weight, oral) treatment with the Siddha drug *Anda odu parpam* produced remarkable spermatogenic activity. Finally it can be concluded that *Anda odu parpam* was found to possess remarkable ( $P < 0.05$ ) in male rats.

The open clinical trial results reveal that 84% of patients were having improvement in the clinical futures and biochemical reports. The study validates the effectiveness of *Anda odu parpam* in improving the sperm count.

The drug is easily available and preparation is very simple. The trial medicine is cost effective. No adverse effects were produced during the entire course of treatment. Conclusively, that the drug “*Anda odu parpam*” (*Parpam of egg shell of Gallus domesticus*) gives a new hope in the field of Infertility treatment.

## 7. SUMMARY

The hen egg shells (*Ovitesta of Gallus Domesticus*) were collected from the hatchery at Namakkal and the lemon were collected from a farm house at Idappadi, Salem District and *Anda odu parpam* is prepared as quoted in the classical Siddha literature.

*Anda odu parpam* was selected by the author for this study to establish the Spermatogenic activity.

To collect the information about the drug, various text books, literature were referred. From them, the author came to an idea about the drug and its efficacy on Infertility.

A brief description about zoological aspect of the *Anda odu parpam* and its identifying characters and physio chemical and bio chemical data were given.

The wide use of according to *Gunapadam* aspect as well as in various Siddha literatures were discussed with much importance to that of preparation related to *Vindhu kuraivu*.

The bio chemical analysis of the drug shows that it contains Zn ions indicates that they help in maintaining the sperm count. The other components Mg, K, Ca, Protein, Amino acid, Phosphate, Sulphate, Chloride and Iron ions are also responsible for its spermatogenic property. It is related in treatment of male infertility.

The preclinical study showed that the drug has got safety and significant Spermatogenic activity.

The patients were responding well from the beginning of the treatment and no adverse effects were reported.

The clinical result reveals that 84% of patients were improvement in the clinical futures and biochemical reports.

This present study confirms that *Anda odu parpam* has the remarkable Spermatogenic activity and high therapeutical value against the clinical symptom of Oligospermia.

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## CONSENT FORM

I certify that I have disclosed all the details about the study in the terms readily understood by the patient.

DATE :  
PLACE:

SIGNATURE OF THE INVESTIGATOR  
NAME:

## CONSENT BY THE PATIENT

I have been informed to my satisfaction by the attending physician for the purpose of the clinical trial and the nature of the drug treatment and follow up including the lab investigation to be performed to monitor and safeguard my body functions.

I am aware of my right to opt out of the trial at any time during the course of the trial without having to give reasons for doing so.

I ,exercising my free power of choice, here by give my consent to be included as a subject in the clinical trial of *Echuramooli ilai Chooranam* for the treatment of *Paandu*.

DATE:

SIGNATURE

PLACE:

NAME

**GOVERNMENT SIDDHA MEDICAL COLLEGE AND HOSPITAL**

**CHENNAI – 600106.**

**M.D.(siddha) – BRANCH – II. GUNAPADAM**

Name of the Disease : *Paandu (Anaemia)*

Name of the Medicine: *Echuramooli ilai Chooranam (Aristolochia indica)*

Dose : 1g bd before food with honey

O.p.No		Address	
Date			
Name			
Age & Sex			
Occupation			
Income			
Marital status		Religion	
Body weight:	B.P:	P.R:	R.R:

<b>Sign/Symptoms/Day</b>	3	6	9	12	15	18	21	24	27	30	33	36	39	42
Pallor of conjunctivae														
Pallor of Nail beds														
Angular stomatitis														
Glossitis														
Anorexia														
Palpitation														
Swelling of the Body														
<b>Sign of MO</b>														

<b>Lab investigation</b>	<b>Particulars</b>	<b>Before Treatment</b>	<b>After treatment</b>
Blood	TC		
	DC		
	ESR		
	Hb		
	Sugar		
	Urea		
	MCV		
	PCV		
	Serum Ferritin		
	Urine	Albumin	
Sugar			
Deposits			
Motion	Ova		
	Cyst		
	Occult Blood		

<b>Siddha Aspect</b>	<b>Before treatment</b>	<b>After treatment</b>
<i>Na</i>		
<i>Niram</i>		
<i>Vizhi</i>		
<i>Parisam</i>		
<i>Malam</i>		
<i>Muthiram</i>		
<i>Nadi</i>		

**Signature of MO:**

**Signature of HOD**

## CONSENT FORM

I certify that I have disclosed all the details about the study in the terms readily understood by the patient.

DATE:

SIGNATURE OF THE INVESTIGATOR

NAME:

## CONSENT BY THE PATIENT

I have been informed to my satisfaction by the attending physician for the purpose of the clinical trial and the nature of the drug treatment and follow up including the lab investigation to be performed to monitor and safeguard my body functions.

I am aware of my right to opt out of the trial at any time during the course of the trial without having to give reasons for doing so.

I, exercising my free power of choice, here by give my consent to be included as a subject in the clinical trial of *ANDA ODU PAMPAM* for the treatment of **OLIGOSPERMIA.**

DATE:

SIGNATURE

NAME

**GOVERNMENT SIDDHA MEDICAL COLLEGE AND HOSPITAL**

**CHENNAI-600 106.**

**M.D (siddha) - BRANCH- II. GUNAPADAM**

Name of the Disease : *Aan maladu (Spermatogenesis activity)*

Name of the Medicine : *Anda odu parpam*

Dose : 130mg bd with ghee before food

O.p.No		ADDRESS	
Date			
Name			
Age & sex			
Occupation			
Income			
Marital status		Religion	
Body weight:	BP:	PR:	RR:

Clinical features	Before Treatment	During Treatment every 7 days						
		1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>
Premature ejaculation								
Erectile dysfunction								
Painful coitus								
Nocturnal emission								
Burning micturation								
<b>Sign. of M.O :</b>								

**Investigations**

Semen Analysis	Before Treatment	After Treatment
Colour		
Volume (ml)		
Viscosity		
Liquification time (min)		
Sperm concentration(million/cc)		
Motility (%)		
Active motile(%)		
Sluggish motile (%)		
Nonmotile (%)		



<b>Lab investigation</b>	<b>Particulars</b>	<b>Before Treatment</b>	<b>After treatment</b>
Blood	TC		
	DC		
	ESR		
	Hb		
	Sugar		
	Urea		

Urine	Albumin		
	Sugar		
	Deposits		
Motion	Ova		
	Cyst		
Ultra sonography of Scrotum			

<b>SIDDHA ASPECT</b>	<b>Before treatment</b>	<b>After treatment</b>
<i>Naadi</i>		
<i>Papisam</i>		
<i>Naa</i>		
<i>Niram</i>		
<i>Vizhi</i>		
<i>Malam</i>		
<i>Muthiram</i>		

**Signature of MO**

**Signature of HOD**

### Haemoglobin level of OP & IP patients-before treatment and after treatment

Sl.No	O.P. No.	Name	Age	Sex	Hb level before treatment	Hb level after treatemnt	Total no. of days	Result
1.	636	Lakshmi	45	F	12.0	14.2	70 Days	Good Improvement
2.	304	Avudayappan	50	M	11.2	13.7	65 Days	Moderate Improvement
3.	6280	Raj	45	M	6.6	9.2	76 Days	Poor Improvement
4.	1110	Seetha	55	F	13.1	16.2	50 Days	Good Improvement
5.	7400	Maharajan	23	M	9.5	11.5	70 Days	Good Improvement
6.	7732	Nallasivam	30	M	10.0	11.6	50 Days	Moderate Improvement
7.	5341	Lakshmi	40	F	11.5	13.5	56 Days	Good Improvement
8.	1012	Balakrishnan	40	M	14.0	15.2	62 Days	Moderate Improvement
9.	6922	Natrajan	60	M	12.3	14.8	49 Days	Good Improvement
10.	219	Meeran	34	M	9.0	10.0	65 Days	Mild Improvement
11.	4207	Periyasamy	46	M	12.0	14.5	63 Days	Good Improvement
12.	386	Amutha	30	F	7.5	10.8	87 Days	Moderate Improvement
13.	1070	Chitra	28	F	11.0	13.7	76 Days	Good Improvement
14.	5865	Rajesh	29	M	8.0	11.1	92 Days	Moderate Improvement
15.	1983	Pappurani	23	F	10.0	12.9	75 Days	Good Improvement
16.	4216	Shanthy	28	F	12.4	15.2	58 Days	Good Improvement
17.	5320	Abaragam	30	M	12.5	15.9	95 Days	Good Improvement
18.	581	Beerbathu	54	F	12.0	13.0	49 Days	Mild Improvement
19.	7013	Muneyansamy	59	M	9.0	11.4	56 Days	Poor Improvement
20.	2809	Muthammal	63	F	12.6	14.3	45 Days	Moderate Improvement
21.	726	Nalini	39	F	10.2	12.9	105 Days	Good Improvement
22.	420	Gomathy	45	F	11.3	12.5	65 Days	Moderate Improvement
23.	8716	Rekha	42	F	11.6	13.7	78 Days	Poor Improvement
24.	2227	Maharajan	65	M	13.0	15.1	115 Days	Good Improvement
25.	7171	Sayedbeeve	50	F	9.5	11.9	75 Days	Good Improvement

<b>S.No</b>	<b>O.P. No.</b>	<b>Name</b>	<b>Age</b>	<b>Sex</b>	<b>Hb level before treatment</b>	<b>Hb level after treatemnt</b>	<b>Total no. of days</b>	<b>Result</b>
26.	7722	Thenmozhi	51	F	10.2	12.7	90 Days	Moderate Improvement
27.	5199	Ponnammal	76	F	10.9	13.2	95 Days	Good Improvement
28.	1301	Parveen	36	F	10.8	12.2	110 Days	Moderate Improvement
29.	6464	Sudalai	75	M	12.0	14.2	85 Days	Good Improvement
30.	108	Cheenisamy	65	M	10.4	11.7	65 Days	Poor Improvement
31.	4827	Ilavarasi	59	F	12.0	14.1	62 Days	Good Improvement
32.	196	Pushpa	60	F	12.3	14.5	87 Days	Moderate Improvement
33.	1053	Sudha	60	F	11.0	12.8	76 Days	Good Improvement
34.	5865	Murugaiya	58	M	12.0	12.8	92 Days	Moderate Improvement
35.	1984	Mageswari	38	F	11.8	14.0	75 Days	Good Improvement
36.	4270	Devi	49	F	12.1	14.7	58 Days	Good Improvement
37.	5203	Juliet	23	F	12.4	15.1	95 Days	Good Improvement
38.	501	Gopalsamy	63	M	14.0	14.9	49 Days	Mild Improvement
39.	7310	Chellavalli	55	F	12.0	12.4	56 Days	Poor Improvement
40.	2408	Radhika	45	F	13.7	15.1	45 Days	Moderate Improvement
41.	711	Radha	36	F	11.0	13.2	105 Days	Good Improvement
42.	166	Gayathri	32	F	10.2	12.7	65 Days	Moderate Improvement
43.	6789	Latha	41	F	8.6	9.2	78 Days	Mild Improvement
44.	1100	Tamilarasi	43	F	13.1	16.2	115 Days	Good Improvement
45.	2406	Elizabeth	33	F	9.0	11.5	75 Days	Good Improvement
46.	5317	Thangaraj	44	M	9.0	10.6	90 Days	Mild Improvement
47.	5474	Gobi	34	M	11.3	14.8	95 Days	Good Improvement
48.	1201	Sathya	34	F	13.0	14.2	110 Days	Mild Improvement
49.	5282	Sivakumar	42	M	13.0	16.5	85 Days	Good Improvement
50.	801	Maniraja	31	M	7.4	8.0	65 Days	Mild Improvement







**NO .OF OP & IP PATIENTS-BEFORE TREATMENT AND AFTER TREATMENT**

S.No	O.P.NO.	NAME	AGE	SEMEN ANALYSIS BT	SEMEN ANALYSIS AT	NO. OF DAYS	RESULT
1.	636	Arul	29	TSC – 6.2 million/cu. mm SM - 10 %	TSC – 126 million/cu. Mm SM – 40 %	105 Days	Marked response
2.	304	Dhamodaran	26	TSC -08 million/cu. mm SM - 50 %	TSC – 42 million/cu. Mm SM - 58 %	65 Days	Mild response
3.	6280	Thangavel	33	TSC –17 million/cu. mm SM - 08 %	TSC – 26 million/cu. Mm SM - 10 %	76 Days	Fair response
4.	1110	Venkatesan	35	TSC – 18 million/cu. mm SM - 22 %	TSC – 60 million/cu. Mm SM - 45 %	50 Days	Marked response
5.	7400	Sudharsanam	40	TSC – 10 million/cu. mm SM - 04 %	TSC –50 million/cu. Mm SM - 10 %	70 Days	Marked response
6.	7732	Vijayakumar	27	TSC – 62 million/cu. mm SM - 20 %	TSC – 70 million/cu. Mm SM - 30 %	50 Days	Mild response
7.	5341	Rajmohan	38	TSC – 16.2 million/cu mm SM - 06 %	TSC – 49 million/cu. Mm SM - 27 %	56 Days	Marked response
8.	1012	Mohanasundaram	31	TSC – 34 million/cu. mm SM - 35 %	TSC – 52 million/cu. Mm SM - 55 %	62 Days	Mild response
9.	6922	Saravananakumar	30	TSC – 53 million/cu. mm SM - 25 %	TSC – 86 million/cu. Mm SM - 45 %	49 Days	Marked response
10.	219	Stalin	41	TSC – 10 million/cu. mm SM - 02 %	TSC – 14 million/cu. Mm SM - 04 %	65 Days	Fair response
11.	4207	Shanmugam	27	TSC – 06 million/cu. mm SM - 09 %	TSC –40 million/cu. mm SM - 40 %	63 Days	Marked response
12.	386	Aravindhhan	27	TSC – 49 million/cu. mm SM - 20 %	TSC –67 million/cu. mm SM - 30 %	87 Days	Mild response
13.	1070	Dhilipan	26	TSC –46 million/cu. mm SM - 30 %	TSC – 71 million/cu. mm SM - 54 %	76 Days	Marked response
14.	5865	Dharanidaran	40	TSC – 14 million/cu. mm SM – 10 %	TSC – 44 million/cu. mm SM – 20%	90 Days	Mild response
15.	1983	Sriganesan	30	TSC –50 million/cu. mm SM - 15 %	TSC –76 million/cu. mm SM - 40 %	75 Days	Marked response
16.	4216	Sundara moorthy	34	TSC – 25 million/cu. mm SM - 10 %	TSC – 50 million/cu. mm SM - 50 %	58 Days	Marked response

S.No	O.P.NO.	NAME	AGE	SEMEN ANALYSIS BT	SEMEN ANALYSIS AT	NO. OF DAYS	RESULT
17.	5320	Balasubramanian	30	TSC – 19 million/cu. mm SM - 10 %	TSC – 56 million/cu. mm SM - 55 %	95 Days	Marked response
18.	581	Mohammed maideen	42	TSC – 25 million/cu. mm SM - 28 %	TSC – 30 million/cu. mm SM - 25 %	49 Days	Fair response
19.	7013	Vinayaga sundaram	44	TSC – 17 million/cu. mm SM - 20 %	TSC – 20 million/cu. mm SM - 15 %	56 Days	Fair response
20.	2809	Balaganesan	47	TSC – 65 million/cu. mm SM - 12 %	TSC – 75 million/cu. mm SM - 34 %	45 Days	Mild response
21.	726	Iyyanar	33	TSC – 44 million/cu. mm SM - 30 %	TSC – 72 million/cu. Mm SM – 48 %	105 Days	Marked response
22.	420	John pandian	40	TSC -37 million/cu. mm SM - 50 %	TSC – 48 million/cu. Mm SM - 58 %	65 Days	Mild response
23.	8716	muthuraman	25	TSC –20 million/cu. mm SM - 07 %	TSC – 25 million/cu. Mm SM - 10 %	78 Days	Fair response
24.	2227	kamarajar	30	TSC – 29 million/cu. mm SM - 22 %	TSC – 59 million/cu. Mm SM - 50 %	110 Days	Marked response
25.	7171	Karthikaiyan	41	TSC – 15 million/cu. mm SM - 03 %	TSC –45 million/cu. Mm SM - 30 %	75 Days	Marked response
26.	7722	Sandresekar	28	TSC – 60 million/cu. mm SM - 15 %	TSC – 70 million/cu. Mm SM - 30 %	90 Days	Mild response
27.	5199	Rajkumar	31	TSC – 20 million/cu mm SM - 06 %	TSC – 45 million/cu. Mm SM - 30 %	95 Days	Marked response
28.	1301	Jayaraj	34	TSC – 40 million/cu. mm SM - 30 %	TSC – 50 million/cu. Mm SM - 55 %	70 Days	Mild response
29.	6464	Abdhul raafi	37	TSC – 65 million/cu. mm SM - 15 %	TSC – 85 million/cu. Mm SM - 45 %	85 Days	Marked response
30.	108	Dhurai	35	TSC – 12 million/cu. mm SM - 03 %	TSC – 16 million/cu. Mm SM - 04 %	65 Days	Fair response
31.	4827	Vadivelan	45	TSC – 06 million/cu. mm SM - 4 %	TSC –45 million/cu. mm SM - 45 %	60 Days	Marked response
32.	196	Anbarasan	31	TSC – 55 million/cu. mm SM - 18 %	TSC –68 million/cu. mm SM - 30 %	87 Days	Mild response
33.	1053	Kumar	33	TSC –48 million/cu. mm SM - 28 %	TSC – 70 million/cu. mm SM - 53 %	76 Days	Marked response



S.No	O.P.NO.	NAME	AGE	SEMEN ANALYSIS BT	SEMEN ANALYSIS AT	NO. OF DAYS	RESULT
34.	5865	Micheal	40	TSC – 35 million/cu. mm SM – 10 %	TSC – 45 million/cu. mm SM – 20%	92 Days	Mild response
35.	1984	Maniraja	34	TSC – 59 million/cu. mm SM - 12 %	TSC – 80 million/cu. mm SM - 40 %	70 Days	Marked response
36.	4270	Soundhar	25	TSC – 26 million/cu. mm SM - 15 %	TSC – 52 million/cu. mm SM - 50 %	58 Days	Marked response
37.	5203	Gopal	36	TSC – 10 million/cu. mm SM - 7 %	TSC – 56 million/cu. mm SM - 55 %	88 Days	Marked response
38.	501	Kannan	32	TSC – 25 million/cu. mm SM - 28 %	TSC – 30 million/cu. mm SM - 25 %	49 Days	Fair response
39.	7310	Mahendren	29	TSC – 16 million/cu. mm SM - 20 %	TSC – 20 million/cu. mm SM - 15 %	56 Days	Fair response
40.	2408	Venkatesan	37	TSC – 67 million/cu. mm SM - 13 %	TSC – 75 million/cu. mm SM - 34 %	45 Days	Mild response
41.	711	Radhakrishnan	36	TSC – 44 million/cu. mm SM - 30 %	TSC – 70 million/cu. Mm SM – 48 %	105 Days	Marked response
42.	166	Chandran	32	TSC - 35 million/cu. mm SM - 55 %	TSC – 48 million/cu. Mm SM - 58 %	65 Days	Mild response
43.	6789	Senthilkumar	41	TSC – 18 million/cu. mm SM - 06 %	TSC – 25 million/cu. Mm SM - 10 %	78 Days	Fair response
44.	1100	Tamilarasan	43	TSC – 20 million/cu. mm SM - 22 %	TSC – 59 million/cu. Mm SM - 50 %	96 Days	Marked response
45.	2406	Anbuvendhan	33	TSC – 26 million/cu. mm SM - 03 %	TSC – 45 million/cu. Mm SM - 11 %	75 Days	Marked response
46.	5317	Thangaraj	44	TSC – 60 million/cu. mm SM - 15 %	TSC – 70 million/cu. Mm SM - 30 %	90 Days	Mild response
47.	5474	Gobikrishnan	34	TSC – 18 million/cu mm SM - 04 %	TSC – 45 million/cu. Mm SM - 38 %	95 Days	Marked response
48.	1201	Sathyaprakash	34	TSC – 40 million/cu. mm SM - 35 %	TSC – 55 million/cu. Mm SM - 60 %	70 Days	Mild response
49.	5282	Sivakumar	42	TSC – 45 million/cu. mm SM - 20 %	TSC – 80 million/cu. Mm SM - 45 %	85 Days	Marked response
50.	801	Maniraja	31	TSC – 12 million/cu. mm SM - 06 %	TSC – 16 million/cu. Mm SM - 08 %	60 Days	Fair response











# VEL'S COLLEGE OF PHARMACY

Approved by the Government of Tamil Nadu  
Affiliated to The Tamil Nadu Dr. MGR Medical University

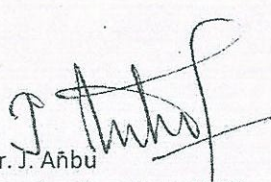
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S.No	Title of The Project	Name of The Investigator	Approval status/Remarks	Project Reference
62.	Role Of Nootropic Medicinal Plants In Scopalamine Induced Amnesia In Rodent Model	Mrs. M.Sumithra	Totally 48rats were proposed and sanctioned.	XIII/VELS/PCOL/62/2000/CPCSEA/AEC/08.08.12
63.	Role Of Phytoconstituents Of Medicinal Plants Against Cognitive Dysfunction Induced By Acute Ethanol treatment.	Mrs. M.Sumithra	Totally 45rats and 30mice were sanctioned.	XIII/VELS/PCOL/63/2000/CPCSEA/AEC/08.08.12
64.	Hepatoprotective Activity Of Indian Medicinal Plants	Dr. V.Ravichandran	Total number of animals proposed was 42 rats. But it is advised to share the common group data with similar pattern of projects if possible.	XIII/VELS/PCOL/64/2000/CPCSEA/AEC/08.08.12
65.	Spermatogenic activity of Anda Odu parpam in rats	Dr. Gnanavel	Totally 48rats and 30mice were proposed and sanctioned.	XIII/VELS/PCOL/65/2000/CPCSEA/AEC/08.08.12
66.	Heamatinic activity of Aristalochia Indica in rats	Dr. Gnanavel	Total number of animals proposed was 36 mice and 18 rats but 24 mice and 12rats were sanctioned.	XIII/VELS/PCOL/66/2000/CPCSEA/AEC/08.08.12

Encl: 1.Copy of the filled in application with protocol (Form-B)

  
Dr. J. Anbu

Member Secretary-IAEC

Dr. J. ANBU, M.Pharm., Ph.D., D.M.L.T.,MBA.


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Nominee-CPCSEA



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Herbal PARC

Director, PARC,  
Retd. Professor, Presidency College



## AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /microscopic examination of fresh /market

sample, it is certified that the specimen given by Dr. I. S. GINANAVEL,  
P.G. GUNAPADAM, Govt. Siddha Medical College, Chennai, is identified as below:

Binomial: Aristolochia indica L.

Family: Aristolochiaceae.

Synonym(s): Aristolochia lanceolata Wight,

Regional names: Tam: Adagam, Tsuraver, Karudakkodi.

Reg.No of the certificate: PARC/2012/1472

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: \_\_\_\_\_ .1983.  
Henry, A.N. et al. Ibid. II: p: 201 .1987.  
Ibid. III: \_\_\_\_\_ .1989.

Date: 04/07/12.

  
(Prof. P. JAYARAMAN)

Prof. P. Jayaraman, Ph.D.  
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सिद्ध केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नई- 600106

**Siddha Central Research Institute**

Arignar Anna Govt. Hospital Campus, Arumbakkam, Chennai-600 106  
(Central Council for Research in Siddha, Department of AYUSH,  
Ministry of Health & Family Welfare, Govt. of India)

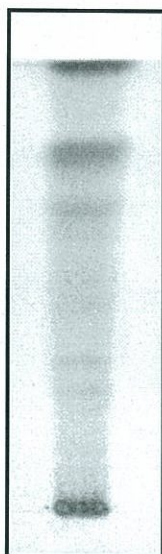
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31.12.2012

Name of the student: Dr. I. S. Gnanavel , Govt Siddha Medical College, Chennai-106

**REPORT OF ECHURAMOOLI ILAI CHOORANAM**

S.No	Parameter	Mean Value
1.	Loss on Drying at 105°C	9.485 %
2.	Total Ash	17.725 %
3.	Acid insoluble Ash	9.172 %
4.	Water Soluble Extractive	21.45 %
5.	Alcohol Soluble Extractive	16.75 %
6.	Particle size	Completely passes through sieve no.44
7.	pH	6.5
Qualitative Phytochemical Tests		
1.	Alkaloids	+ ve
2.	Triterpenes	+ ve
3.	Flavonoids	- ve
4.	Saponin	+ ve
5.	Steroids	+ ve
6.	Protein	+ ve
7.	Anthraquinones	- ve
8.	Coumarin	+ ve
TLC		
		As Below



After spray with visualizing agent

Sl.No	After Dipping in Vanillin-Sulphuric acid	
	Rf value	Colour of the spot
1	0.14	Grey
2	0.28	Blue
3	0.34	Greyish green
4	0.46	Purple
5	0.51	Purple
6	0.67	Purple
7	0.70	Greyish green
8	0.80	Violet

**Solvent system:**

Toluene : Ethyl acetate (6:1.5).

**TLC plate:**

Aluminium plate precoated with silica gel 60F<sub>254</sub> of 0.2 mm thickness (Merck).

**Developing chamber:**

Camag's twin trough chamber.

**Visualizing reagent:**

Vanillin-sulphuric acid reagent.



**Extract Preparation:**

4 g of the chooranam was soaked overnight in chloroform. Boiled on a water bath for 10 mins, filtered and concentrated to 10 ml.

**Procedure:**

The extract was applied on the TLC using capillary and developed in the solvent system. The developed TLC plate was air dried and photograph was taken in white light. Then dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C until the development of coloured spots and photograph taken.

**REPORT OF AANDA PARPAM**

S.No	Parameter	Mean Value
1.	Loss on Drying at 105°C	0.38 %
2.	Total Ash	91.47 %
3.	Acid insoluble Ash	2.3 %
4.	Particle size	Completely passes through sieve no.44
5.	pH	13.0



(R. Shakila )  
Research Officer (Chemistry)



(S. Jega Jothi Pandian)  
Research Officer (Scientist 2) I/c





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

69, Anna Salai, Guindy, Chennai-600 032

This Certificate is awarded to Dr. **I. S. GNANAVEL**.....

for participating as a **Resource Person** / Delegate in the V Workshop on

## **"Research Methodology & Biostatistics"**

for AYUSH Post-Graduates & Researchers

organized by the Department of Siddha,

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

from 8th August 2011 to 12th August 2011.

**Dr. MAYILVAHANAN NATARAJAN**

M.S.Orth. M.Ch.Orth. (L'pool) Ph.D. D.Sc. F.R.C.S. D.Sc. (Hon)<sup>3</sup>

**VICE CHANCELLOR**

**Dr. SUDHA SESHAYYAN, M.S.**

REGISTRAR (FAC)

**Dr. N. KABILAN, M.D. (Siddha)**

HOD, DEPT. OF SIDDHA





# THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

69, Anna Salai, Guindy, Chennai - 600 032.

## DEPARTMENT OF SIDDHA

### CERTIFICATE OF PARTICIPATION

This is to certify that Dr/Mr/Ms I. S. Gnanavel has participated in the CME on Good Clinical Practice conducted by Department of Siddha on 25-01-2011.

This educational activity has been awarded 2 Credit points by The Centre for Accreditation, The Tamil Nadu Dr. MGR Medical University.

Total Credits Claimed :

Participant's Signature

Date

  
Dr. N. KABILAN

Prof & Head  
Department of Siddha

  
Dr. SUDHA SESHAYYAN

Registrar i/c

  
Dr. MAYIL VAHANAN NATARAJAN

Vice Chancellor



# THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

69, Anna Salai, Guindy, Chennai - 600 032.

## DEPARTMENT OF SIDDHA

### CERTIFICATE OF PARTICIPATION

This is to certify that Dr/Mr/Ms I. S. Gnanavel has participated in the CME on Pharmacological and Toxicological Studies conducted by Department of Siddha on 29-11-2010.

This educational activity has been awarded 2 Credit points by The Centre for Accreditation, The Tamil Nadu Dr. MGR Medical University.

Total Credits Claimed :

Participant's Signature

Date

  
Dr. N. KABILAN

Prof & Head  
Department of Siddha

  
Dr. SUDHA SESHAYYAN

Registrar i/c

  
Dr. MAYIL VAHANAN NATARAJAN

Vice Chancellor