SCIENTIFIC VALIDATION AND ANALYTICAL EVALUATION OF SIDDHA HERBAL FORMULATION "*NILAPANAI KILANGU CHOORANAM*" FOR SPERMATOGENIC, APHRODISIAC AND ANTI-OXIDANT ACTIVITIES IN-VIVO AND IN-VITRO MODEL

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Scientific Validation And Analytical Evaluation Of Siddha Herbal Formulation "*Nilapanai Kilangu Chooranam*" for Spermatogenesis, Aphrodisiac And Anti-Oxidant Activities in Animal Models"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian M.D(S), Ph.D,** 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

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled "**Scientific Validation and Analytical Evaluation of Siddha Herbal Formulation "*Nilapanai Kilangu Chooranam*" for Spermatogenesis, Aphrodisiac and Anti-Oxidant Activities In Animal Models"** 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. S. Sudharsan** 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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ARUMBAKKAM, CHENNAI-106.

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation and Analytical Evaluation of Siddha Herbal Formulation "*Nilapanai Kilangu Chooranam*" For Spermatogenesis, Aphrodisiac and Anti-Oxidant Activities In Animal Models"** is a bonafide work carried out by **Dr.S.Sudharsan** under the guidance of **Dr.V.Velpandian M.D(s).,** Post graduate department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

Seal and Signature of the HOD **Seal and Signature of the Principal**

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ABBREVATION

ALT	**Alanine Amino Transaminase**
AST	**Aspartate Amino Transferase**
ANOVA	**Analysis of variance**
ATP	**Adenosine Triphosphate**
AYUSH	**Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy**
AZF	**Azoospermia Factor**
BHT	**Butylated Hydroxyl Toluene**
BSA	**Bovine Serum Albumin**
BUN	**Blood Urea Nitrogen**
CFTR	**Cystic Fibrosis Transmembrane Conductance Regulator**
CASA	**Computer Assisted Sperm Analysis**
CMC	**Carboxy Methyl Cellulose**
CPCSEA	**Committee for the Purpose of Control and Supervision of Experiments on Animals**
CNS	**Central nervous system**
DBCP	**Dibromochloropropane**
DHT	**Dihydrotestosterone**
DMT	**Dimethyl tryptamine**
DOPA	**Dihydroxyphenylalanine**

DPPH	**1,1-Diphenyl-2-picrylhydrazyl**
EDTA	**Ethylenediaminetetraacetic acid**
FSH	**Follicle-Stimulating Hormone**
FTIR	**Fourier Transform Infrared (spectroscopy)**
GIFT	**Gamete Intra Fallopian Transfer**
GOT	**Glutamate Oxaloacetate Transaminase**
GPT	**Glutamate Pyruvate Transaminase**
GIT	**Gastro Intestinal Tract**
GnRH	**Gonadotropin Releasing Hormone**
GLFT	**Gamete intrafallopian transfer**
HPTLC	**High performance thin layer chromatography**
HZI	**Hemizona Index**
IAEC	**Institutional animal ethical committee**
IUI	**Intrauterine Insemination**
IV	**In Vitro Fertilization**
ICSI	**Intra-Cytoplasmic Sperm Injection**
ICPOES	**Inductively Coupled Plasma Optical Emission Spectroscopy**
IIT	**Indian Institute Of Technology**
ISM	**Indian System Of Medicine**
LH	**Leutinizing hormone**
MESA	**Micro Epididymal Sperm Aspiration**
NOAEL	**No Observed Adverse Effect Level**

NPKC	***Nilapanai Kizhangu Chooranam***
OECD	**Organization for Economic Cooperation and Development**
PCV	**Packed cell volume**
POST	**Peritoneal Oozyte Sperm Transfer**
PESA	**Percutaneous Epididymal Sperm Aspiration**
RBC	**Red blood corpuscles**
SAIF	**Sophisticated Analytical Instrument Facility**
SPA	**Sperm Penetration Assay**
SEM	**Scanning Electron Microscope**
SUZI	**Sub Zonal Sperm Injection (Directly into Ovum)**
TET	**Tubal Embryo Transfer**
TLC	**Thin Layer Chromatography**
TUFT	**Trans-Uterine Fallopian Transfer**
WBC	**White blood corpuscles**
WHO	**World health organization**
XRD	**X-Ray Diffraction**
ZIFT	**Zygote Intra-Fallopian Transfer**

1. INTRODUCTION

Siddha system is one of the most conservative medical system in the world. Herbals plays a vital role in Siddha medicinal preparations. In Siddha system of medicine, the diagnostic methodology is based on the three humours namely "*Vatham*", "*Pitham*" and "*Kapham*"

Siddhars were incorporated some magical techniques in their formulation of medicines. The formulated Siddha medicines depends upon the factors of "*Panchaboothams*" and "*Arusuvai* .

According to Siddhar's concept the human body is made up of "*Saptha Udal Thaathukkal"* like *'Saaram', 'Saenneer', 'Oon', 'Kolupu','Ennbu', 'Moolai', 'Sukilam'*.

The role of '*sukkilam'* in reproduction was well explained in Siddha literatures in exclusive manner. The sperm count and its reduction are the major reason for causing infertility in males.

The drug which enhances the quality of the "*Sukkilam"* can act as Aphrodisiac. In Siddha there are lot of herbals, herbo mineral formulations labelled as Aprodisiacs. Nowdays the prevalence of male infertility is so high. The Siddha medical term "*Thathu viruthi"* is highly correlated with the modern term Spermatogenesis .

The great saint Yugi muni explained "*Aan maladu"* that the semen exhibits the following characters such as absence of sweetness, buoyancy on water etc.
According to World Health Organisation Infertility is defined as 'The inability of a sexually active non-contracepting couple to achieve spontaneous conception in one year'. Nearly 30 million men worldwide are infertile[1]. In 40% to 50% of infertile males, the etiology is unknown[2].

WHO categorised 6 factors affecting male infertility which are the following:

1. Congenital or acquired urogenital abnormalities
2. Urogenital tract infections
3. Increased scrotal temperature
4. Endocrine disturbances
5. Genetic abnormalities
6. Immunological factors

In male infertility, the pathophysiology having a number of cellular abnormalities manifesting at both the molecular and biochemical levels which result in decreased quality and quantity of sperm in the semen and thus producing an imbalance in the reproductive hormones. Reduced male infertility's major prevalence was observed to be oligospermia[3].

Pre-mature ejaculation, erectile dysfunction, varicocele are the major causes seen in male infertility. To be fertile, men must have enough sperm in their semen, and these sperm must be healthy. Sperm production is driven by hormones. In the brain, the pituitary gland makes 2 important hormones: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Both of these hormones affect the testicle, an important organ for fertility.

Sperm is produced in the testicle and travels through very small ducts (the epididymis and vas deferens) before it is released in semen. Sometimes men can be treated with medications to improve their hormone levels and increase sperm production.

Chemicals used in our routine life like the higher levels of Bisphenol-A (in plastic containers) in men's urine, lowers their sperm count. Other than this alcohol, nicotine, caffeine, trans fatty acids, obesity, oxidative stress and some medicines like spironolactone, Anti-androgens, high doses of nitrofurantoin, cimetidine are all affects spermatogenesis profoundly[4]. Treatment is available for male fertility problems. Surgery, with or without the use of an operating microscope (microsurgery), can be performed to correct a varicocele.

Microsurgery is also used to successfully repair certain types of blockage. Surgery, medication, hormone treatments, and donor sperm may be used alone or along with other treatments that help the egg and sperm unite. Two of the most common treatments include intrauterine insemination (IUI) and in vitro fertilization (IVF) $ 3-5 billion a year industry per a year for Gamete Intra Fallopian Tube Transfer (GIFT), Zygote Intra Fallopian Transfer (ZIFT) etc as infertility treatments found in some research[5].

Even though there are a lot of medications available in allopathy system of medicine, men are always facing some complications with those type of modern treatments.

The basic and fundamental purpose of sex and sexuality is the "continuation of progeny" and the survival of human race[6]. There are so many medicines indicated in our Siddha system for the treatment of male infertility there is a need for much more effective drug with easy affordablity and availablity. "*Nilapanai kilangu chooram"* was indicated as a best drug for male infertility in "*C.Kannusamy Parambarai Vaithiyam"*[7].

The ingredients of "*Nilapanai kilangu chooranam*" will be an effective drug for spermatogenesis without causing any adverse effects.

Hence, the author was interested in the evaluation of "*Nilapanai kilangu chooranam"* for Spermatogenesis , aphrodisiac and its anti-oxidant properties.

2. AIM AND OBJECTIVES

AIM

The aim of this thesis is to evaluate the Spermatogenic, Aphrodisiac and Anti - oxidant Activity of *Nilapanai Kilangu Chooranam* and to create the fingerprints to standardize this medicine with reference to the authentic drugs.

OBJECTIVES:

The key objectives of the study are:

- Having a collective review of the literature.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug into physico-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment through sophisticated analytical modern techniques like FTIR, ICPOES, SEM, XRD
- Studying the toxicity profile of *Nilapanai kilzhangu chooranam* according to OECD guidelines.
- Evaluation the pharmacological study of the test drug *Nilapanai Kilangu Chooranam* through the following activities
- Spermatogenic, Aphrodisiac Activities in Wistar albino rats
- Anti Oxidant Of - Through DPPH assay
- Evaluation of anti-microbial load for this formulation.
- Analyzing all the above study results to evaluate the benefits of *Nilapanai Kilangu Chooranam*

3. REVIEW OF LITERATURE

3.1 Drug Review

Gunapadam aspect[8]

3.1.1. *Nilapanai kizhangu (Curculigo orchioides)*

Alternative names

Vaaraaki, musali, thiralaaram, thirakathaaru, thiranaraasan, sakiyam, thalamooli, thalaiththaathu, nilavizhumi, naeyam, kurathi, sasiyam, siththi

Vernacular name

Eng name	:	Black musale,
Tel. name	:	Naelatadi
Mal.name	:	Nieppana
Sans.name	:	Musale
Hindi name	:	Musalikano
Kan name	:	Neladali
Part used	:	Tuber, root

Properties

Suvai	:	*Inippu,*
Thanmai	:	*Thatpam,*
Pirivu	:	*Inippu*

General character

மேக வனல்தணியிம் வெண்குட்டந்”தான்விலகும்

போக மிகவுமுறும் பொற்கொடியேபோகாத !

சூலைமே கங்களோடு துன்னுகரும் புள்ளியும்போஞ்

சால நிலப்பனைக்குத் தான்“

-அகத்தியர் குணவாகடம்

Actions

- Tonic
- Diuretic
- Astringent
- Carminative
- Emollient

Uses

It is used in the treatment of leucorrhea, diabetes, eye diseases and male infertility.

3.1.2. *Nerunjil (Tribulus terrestris)*

Alternative names

Thirikandam, Thirikandakam, Thirithandam, Naerinjipudhum, asuvasattiram, suvathattam, koekandam, kaamarasi, suvaathukandam, kittiram, koendam, sutham

Vernacular names

Eng	:	small caltrops, land caltrops, Puncl-ure-vine
Tel	:	Palleru
Sanskrit	:	gokshura
Hindi	:	Gakhru
Malayalam	:	Nerunji

Part used: Whole plant

Suvai	*:*	*Thuvarppu, inippu*
Thanmai	*:*	*Seetham*
Pirivu	*:*	*inipu*

General properties

நல்ல நெருஞ்சிலது நாளுங்கி ரிச்சரத்தை”

வல்ல சுரமனலை மாற்றுங்காண் - மெல்லியலே!

மாநிலத்தில் கல்லடைப்பும் வாங்காத நீர்க்கட்டும்

கூனுறுமெய் வாதமும்போக் கும்“

- அகத்தியர் குணவாகடம்

Actions

- Refrigerant
- Demulcent
- Diuretic
- Tonic
- Aphrodisiac
- Astringent

Uses

It is indicated as a best remedy for urinary tract disorders and male infertility.

3.1.3. *Nelli vatral (Phyllanthus emblica)*

Alternative names

Aamalakam, Aalakam, Aambal, Aamarikam, Thaaththaari, Thaathiri, Koerangam, Miruthupala, Meethuindhthu

Vernacular names

Eng	:	Indian gooseberry
Tel	:	usirika
Mal	:	nellikay
Kan	:	nellikai
Sans	:	amalaki
Hind	:	amlika
Arab	:	amlaj
Pers	:	amila

Part used: leaves, flower, barks, root, seeds

Suvai : *pulippu, thuvarppu, inippu*

Thanmai : *thatpam*

Pirivu : *inippu*

General properties

பித்தமன லையம் பீனசம்வாய் நீர் வாந்தி”

மத்தமலக் காடும் மயக்கமுமில் ஒத்தவுரு -

வில்லிக்கா யம்மருங்கா மென்னாட்கா லந்தோர்ந்தே

நெல்லிகா யம்மருந் துணீ.“

-தேரன் குணவாகடம்

Actions

- Astringent
- Refrigerant
- Laxative
- Diuretic

Uses

It is used in the treatment of delirium, sinusitis, constipation, hypertension, menorrhagia, eye diseases.

3.1.4. *Poonaikkaali vithai (Mucuna pruriens)*

Alternative names: *Kandoothi, Markadi*

Vernacular names:

Eng: common cowitch

Tel: Pilliadagu

Mal: Choraivalli

Sans: Atmagupta

Hind: Kavach

Kan: Nasugenne

Part used : seeds, root, sunai

Suvai : *Thuvarppu*

Thanmai **:** *Thatpam*

Pirivu : *Inippu*

General properties

"தழுதளைநாற் றத்தோடு சாரிரத்தப் போக்கும்
பழுதுபுரி கின்றகரப் பானும் - அழுதேகுந்
தாலமிசை விந்துவுமாஞ் சாற்ற கரும்பூனைக்
காலி விதையைக் கழறு"

- *அகத்தியர் குணவாகடம்*

Actions

- Astringent
- Nervine tonic
- Aphrodisiac
- Diuretic
- Vermifuge
- Irritant

Uses: It is used in the treatment of dropsy, leucorrhea, male infertility, diarrhoea

3.1.5. *Seenthil Sarkkarai* (*Tinospora cordifolia)*

Alternative names

Amirthavalli, Somavalli, Amirthai, Amirthakodi, Kundali

Vernacular names

Eng	:	Heart leaved moon seed, tinospora, gulancha tinospora
Tel	:	Tippa-tiga
Mal	:	Amruta
Sans	:	Guduchi
Hind	:	Gul-bc
Kan	:	Amruta-valli

Part used: leaves, climber, root (tuber)

General properties

”குட்டம் பதினெட்டும் குஞ்சரத்தின் றோற்சொறியுங்
கட்டம் பெரிதாங் கயநோயும் – பட்டவுடன்
செந்தீமுன் பஞ்செனவே சீந்தலுப் போட்ளைந்த
தந்தா வளநீர்க்குச் சாம்“

-தேரன் வெண்பா

Actions

- Alterative
- Antiperiodic
- Aphrodisiac
- Demulcent
- Stimulant (hepatic)
- Stomachic
- Tonic
- Mild diuretic

Uses

It is used in the management of fever, indigestion, diabetes, GIT disorders, Skin disorders.

3.1.6. *Mullilavam pisin* (*Bombax malabaricum)*

Alternative names: *Mul ilavam, Sanmali, Poorani, Pongar, Moesam*

Vernacular names:

Eng	:	The red silk-cotton tree
Tel	:	Mundla-buraga chettu
Mal	:	Pula-Maram, Mul-elava
Kan	:	Mullu-buraga Mara
Hind	:	Ragai-senbal, ragai semal
Duk	:	Kantno-ka-khatyan, Kanton-ka-sema
Sans	:	Kantaka-shalmali

Part used: Leaves, Flower, Seeds, Barks, Pisin, Root, *panju*

Suvai	*:*	*inippu, thuvarppu,*
Thanmai	*:*	*Thatpam*
Pirivu	*:*	*inippu*

General properties

”தந்துமே கஞ்சிறுநீர்த் தாரைவெப் பம்வாயு

வுந்தவரு பேதியிவை யோட்டுங்காண் – முந்திக்

கிளர்வள்ளை பாயும்வரிக் கெண்டை விழியாய்!

வளர்முள் ளிலவு மரம்“

-அகத்தியர் குணவாகடம்

Actions

- Refrigerant
- Demulcent
- Laxative
- Diuretic
- Styptic
- Aphrodisiac
- Astringent (mild)

Uses

It is used in the treatment of diarrhoea, leucorrhea

3.1.7. *Karkandu* (*Saccharum officinarum)*

Alternative names

Punarpoosam, ikku, vaei

Vernacular names

Eng	:	sugar cane, noble cane
Tel	:	cheruku, kanupula-cheruku
Mal	:	karinpa

Kan	:	khabbu
Sans	:	Ikshu, Rasalah
Arab	:	Qasabus-sakar, Qasabe-sakar
Pers	:	Nai-shakar
Hind	:	Ukh-ganna
Duk	:	Ganda

Part used

Juice, Sugar, Root

Suvai	*:*	*Inippu*
Thanmai	*:*	*Seetham*
Pirivu	*:*	*Inippu*

General properties

”சீனிச் சர்க்கரைக்குத் தீராத வன்சுரமுங்

கூனிக்கும் வாதத்தின் கூட்டுறவும் – ஏனிற்கும்

வாந்தி யொடுகிரும் மாறாத விக்கலுமே

போந்திசையை விட்டுப் புரண்டு“

-அகத்தியர் குணவாகடம்

Actions

- Demulcent
- Antiseptic
- Laxative
- Diuretic

Uses

It is used in the treatment of diabetes, leucorrhea, male infertility.

3.2 Botanical aspect [9] :

3.2.1 Curculigo orchioides (*Nilapanai kilangu*)

Curculigo orchioides Gaertn. (Fam. Amaryllidaceae), a small herb, upto 30 cm high with tuberous root stock, occurring wild in sub-tropical Himalayas from Kumaon eastwards, ascending upto 1830 m in Khasi hills, Manipur and the Eastern Ghats, also

from Konkan southwards; drug is collected from two year old plants, washed well and cleared of rootlets, sliced and dried in shade.

Scientific classification

Kingdom	:	Plantae
Clade	:	Angiosperms
Clade	:	Monocots
Order	:	Asparagales
Family	:	Hypoxidaceae
Genus	:	*Curculigo*
Species	:	*C.orchoides*

Synonyms

Sanskrit	:	Bhmitila
Assamese	:	Talmuli, Tailmuli
Bengali	:	Talmalu, Tallur
English	:	Black musale
Gujrati	:	Kalirnusali
Hindi	:	Syahmusali, Kalimusli
Kannada	:	Neltal, Neltathigodde, Nelatale, Nelatelegadde
Malayalam	:	Nilappenea
Marathi	:	Kali musali, Bhuimaddi
Oriya	:	Talamuli
Punjabi	:	Syah musali, Musali safed,
Tamil	:	Nilappanai
Telugu	:	Nel tadigadda
Urdu	:	Musali Siyah, Kali Musali

DESCRIPTION

a) Macroscopic

Drug occurs in transversely cut pieces of 2.5 to 5 cm long, cylindrical, straight to slightly curved, cut surface 1.0 to 4.5 cm in dia.; external surface blackish-brown, cut surface cream coloured; surface with numerous shallow wrinkles and transverse

cracks; with a few rootlets and root scars; nodes and internodes prominent; taste, mucilaginous and slightly bitter.

b) Microscopic

Shows a narrow strip of cork, consisting of 5 to 7 rows of light brown cubical to rectangular cells; secondary cortex consists of thin-walled, parenchymatous cells, densely filled with starch grains and acicular crystals of calcium oxalate, either isolated or in bundles, in a few cells; a few small, round to tangentially elongated, lysigenous cavities also found scattered in this region; a few vascular bundles found embedded in cortical region with phloem towards outer side, and consisting of a few xylem elements of calcium oxalate; numerous fibro-vascular bundles found scattered throughout the region, mostly towards peripheral region having phloem, almost encircled by xylem vessels having annular and spiral thickenings; starch grains simple, rounded to oval and also compound of 2 to 4 components, measuring 4 to 21 μ in dia., present in cortical and central region, a number of deep red, resin canals found throughout the region, mucilage in the form of colourless mass found in a few cortical parenchymatous cells.

Powder - Greyish; vessels with annular and spiral thickenings; simple, round to oval, starch grains measuring 4 to 21 μ in dia., and compound starch grains having 2 to 4 components and a few acicular crystals of calcium oxalate; mucilage in the form of colourless mass found in a few cortical parenchymatous cells

T.L.C:

T.L.C. of alcoholic extract of the drug on Silica gel 'G' plate using n-Butanol : Acetic Acid: Water (4:1:5) shows under U.V. (366 nm) four fluorescent zones at Rf. 0.39, 0.77, 0.90 and 0.97 (all yellow). On exposure to Iodine vapour twelve spots appear at Rf. 0.06, 0.13, 0.17, 0.25, 0.39, 0.50, 0.62, 0.70, 0.77, 0.88, 0.90 and 0.97 (all yellow). On spraying with Dragendorff reagent followed by sodium nitrite three spots appear at Rf. 0.39, 0.70 and 0.88 (all light purple).

Constituents - Tannin, Resin, Sapogenin and Alkaloid

Medicinal Uses

According to Siddha, root is heating, aphrodisiac, alternative, appetizer, fattening and useful in treatment of piles, biliousness, fatigue, blood related disorders etc.

3.2.2. Tribulus terrestris

Scientific classification

Kingdom	-	Plantae
Division	-	Phanerogams
Subdivision	-	Angiospermae
Class	-	Dicotyledonae
Subclass	-	Polypetalae
Series	-	Disciflorae
Order	-	Giraniales
Family	-	Zygophyllaceae
Genus	-	Tribulus
Species	-	terrestris Linn

SYNONYMS

Assamese	:	Gokshura, Gukhurkata
Bengali	:	Gokshura, Gokhri
English	:	Caltrops root
Gujrati	:	Be tha gokharu, Nana gokharu, Mithogokharu
Hindi	:	Gokhru
Kannada	:	Sannanaggilu, Neggilamullu, Neggilu
Kashmiri	:	Michirkand, Pakhda
Malayalam	:	Nerinjil
Marathi	:	Sarate, Gokharu
Oriya	:	Gukhura, Gokhyura
Punjabi	:	Bhakhra, Gokhru
Tamil	:	Nerinjil, Nerunjil
Telugu	:	Palleruveru
Urdu	:	Khar-e-Khasak Khurd

DESCRIPTION[10]

a) Macroscopic

Drug consists of root, 7-18 cm long and 0.3-0.7 cm in diameter, slender, cylindrical, fibrous, frequently branched bearing a number of small rootlets, tough, woody and yellow to light brown in colour, surface becomes rough due to presence of small nodules, fracture fibrous, odour aromatic, taste, sweetish and astringent.

b) Microscopic

Transverse section of primary roots show a layer of epidermis followed by 4-5 layers of thin-walled parenchymatous cortex, endodermis distinct, pericycle enclosing diarch stele, in mature root, cork 4-6 layered, cork cambium single layered follwed by 6-14 layers of thin-walled parenchymatous cells with varying number of fibres, distributed throughout, some secondary cortex cells show secondary wall formation an reticulate thickening, fibres found in groups resembling those of phloem, secondary phloem divided into two zones, outer zone characterised by presence of numerous phloem fibres with a few sieve tubes slightly collapsed, inner zone frequently phloem rays distinct, few cells get converted into fibres in outer region, cambium 3-5 layered, wood composed of vessels, tracheids , parenchyma and fibres and traversed by medullary rays, vessels scattered, arranged in singles or doubles towards inner side, in groups of three to four on outer side having bordered pits, tracheids long, narrow with simple pits, xylem parenchyma rectangular or slightly elongated with simple pits and reticulate thickening, xylem fibres few, trachieds elongated with simple pits, medullary rays heterogenous, 1-4 cells wide, starch grains and rosette crystals of calcium oxalate present in secondary cortex, phloem and medullary rays cells, few prismatic crystals also present in xylem ray cells.

Constituents - Alkaloids and saponins.

Dose - 20-30 g of the drug for decoction.

Major chemical constituents

The major constituents of the fruit are steroidal saponins including gitonin, protodioscin (0.245%, tribulosaponins A and B, tribulosin and terrestrosins A–K, among others. Other constituents include alkaloids, tribulusamides A and B, and trace amounts of harman and norharman; and flavonols such as kaempferol, quercetin and rutin.

Medicinal uses

Uses supported by clinical data None. Uses described in pharmacopoeias and well established documents Orally for the treatment of cough, headache and mastitis . Although clinical trials have assessed the use of the crude drug for the symptomatic treatment of angina pectoris and male infertility,randomized controlled clinical trials are needed before the use of the crude drug can be recommended for the treatment of these conditions. Uses described in traditional medicine Orally for the treatment of abdominal distension, diarrhoea, kidney stones, nosebleeds and vitiligo. Also used as an aphrodisiac, diuretic, galactagogue, general tonic and uterine tonic.

Dietary supplement

Some body builders use T. terrestris as post cycle therapy or "PCT". After they have completed an anabolic-steroid cycle, they use it under the assumption that it will restore the body's natural testosterone levels.The extract is claimed to increase the body's natural testosterone levels and thereby improve male sexual performance and help build muscle. Its purported muscle-building potential was popularized by American IFBB bodybuilding champion Jeffrey Petermann in the early 1970s. However, T. terrestris has failed to increase testosterone levels in controlled studies.It has also failed to demonstrate strength-enhancing properties,a finding indicating that the purported anabolic steroid effects of Tribulus terrestris are untrue.

3.2.3. ***Phyllanthus emblica***

Scientific classification

Kingdom : Plantae

Division : Angiosperms

Class : Eudicots

Subclass : Rosids

Order : Malpighiales

Family : Phyllanthaceae

Tribe : Phyllantheae

Subtribe : Flueggeinae

Genus : *Phyllanthus*

Species : *P. emblica*

Pericarp of dried mature fruits of Emblica officinalis Gaertn. Syn. Phyllanthus emblica Linn. (Fam. Euphorbiaceae); mostly collected in winter season after ripening and in Kashmir in summer, a small or medium sized tree, found both in natural state in mixed deciduous forests of the country ascending to 1300 m on hills; cultivated in gardens, homeyards or grown as a road side tree.

SYNONYMS

Sanskrit	:	Amataphala, Emalaka.
Assamese	:	Amlakhi, Amlakhu, Amlaku
Bengali	:	Amla, Dhatri
English	:	Emblic Myrobalan
Gujrati	:	Ambala, Amala
Hindi	:	Amla, Aonla
Kannada	:	Nellikayi, Bela nelli, pottadenollikayi
Kashmiri	:	Amli, Embali
Malayalam	:	Nellikka
Marathi	:	Anvala, Avalkathi
Oriya	:	Ainla, Anala
Punjabi	:	Aula, amla
Tamil	:	Nellikkai, nelli
Telugu	:	Usirika

Urdu : Amla, Amlaj

DESCRIPTION[11]

a) Macroscopic

Drug consists of curled pieces of pericarp of dried fruit occuring either as separated single segment; 1-2 cm long or united as 3 or 4 segments; bulk colour grey to black, pieces showing, a broad, highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface, external surface show s a few whitish specks, occasionally some pieces show a portion of stony testa (which should be removed before processing); texture rough, cartilaginous, tough; taste, sour and astringent.

b) Microscopic

Transverse section of fruit shows epicarp consisting of a single layered epidermis cell appearing tabular and polygonal in surface view; cuticle present; mesocarp cells tangentially elongated parenchymatous and crushed differentiated roughly into peripheral 8 or 9 layers of tangentially elongated smaller cells, rest consisting of mostly isodiametric larger cells with walls showing irregular thickenings; ramified vascular elements occasionally present; stone cells present either isolated or in small groups towards endocarp ; pitted vascular fibres, walls appearing serrated due to the pit canals, leading into lumen.

Powder: Fine powder shows epidermis with uniformly thickened straight walled isodiametric parenchyma cells with irregular thickened walls, occasionally short fibres

and tracheids.

Constituents - Ascorbic acid and gallotannins.

Dose - 3-6 g of the drug in powder form.

Traditional medicine

In traditional Indian medicine, dried and fresh fruits of the plant are used. All parts of the plant are used in various siddha and Ayurvedic medicine herbal preparations, including the fruit, seed, leaves, root, bark and flowers.

Chemical constituents

Fruit is a rich natural source of vitamin C. It also contains tannins and colloidal substances, phyllembic acid, lipids, gallic acid, ellagic acid, trigalloylglucose, terchebin, corilagin and emblicol. Phyllembin and mucic acid have been isolated from the fruit pulp. Seeds contain fixed oil, phosphatides, tannins and essential oil. Bark, fruits and leaves are rich in tannin. They also contain lupeol, β-sitosterol and ellagic acid. Bark also contains leucodelphinidin. Seed oil also contains linoleic acid (64.8%), closely resembled linseed oil.

3.2.4. Mucuna pruriens

Scientific classification

Kingdom	:	Plantae
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae
Tribe	:	Phaseoleae
Genus	:	Mucuna
Species	:	M.pruriens

Distribution

Native: Southern China and eastern India. Now widely distributed in the tropics.

DESCRIPTION[12]

a) Macroscopic

Root long, 7 mm or more in thickness, hard, having lateral roots, dark brown to black; fracture, fibrous; odour and taste not distinct.

b) Microscopic

Root shows a narrow cork consisting of 4 or 5 rows of tangentially elongated cells; secondary cortex narrow consisting of 2 to 5 rows of thin-walled, parenchymatous cells, a few containing brownish contents; secondary phloem wide,

forming bulk of the bark in the form of long, radial strips that are conical due to the medullary rays funneling out in the phloem region; phloem fibres are arranged in groups or occasionally single; phloem rays uni to biseriate; cambium distinct 1 or 2 layered; secondary xylem very wide composed of usual elements, vessels large as well as small, surrounded by xylem parenchyma and fibres; medullary rays in the xylem also mostly uniseriate, somewhat wavy, consisting of radially elongated thin-walled cells.

Powder - Grey to dark brown; shows fragments of cork, fibres singly or groups and xylem vessels.

T.L.C.

T.L.C. of the alcoholic extract on Silica gel 'G' plate using n-Butanol : Acetic acid: Water (4:1:5) shows under UV (366 nm) four fluorescent zones at Rf. 0.33, 0.51, 0.66 and 0.86 (all blue). On exposure to Iodine vapour seven spots appear at Rf. 0.10, 0.20, 0.38, 0.48, 0.59, 0.77 and 0.86 (all yellow). On spraying with Ninhydrin and on heating the plate at 110o C for ten minutes four conspicuous spots appear at Rf. 0.38, 0.48, 0.59 and 0.86 (all light pink).

Constituents: Choline

Dose: 3-6 g of the drug in the powder form for decoction

Chemical compounds

In addition to levodopa, it contains minor amounts of serotonin (5-HT), 5-HTP, nicotine, N,N-DMT (DMT), bufotenine and 5-MeO-DMT. As such, it could potentially have psychedelic effects, and it has purportedly been used in ayahuasca preparations.

The mature seeds of the plant contain about 3.1–6.1% L-DOPA, with trace amounts of 5-hydroxy tryptamine (serotonin), nicotine, DMT-n-oxide, bufotenine, 5-

MeO-DMT-n-oxide, and beta-carboline. One study using 36 samples of the seeds found no tryptamines present in them.

The leaves contain about 0.5% L-DOPA, 0.006% dimethyl tryptamine (DMT), 0.0025% 5-MeO-DMT and 0.003% DMT n-oxide. The ethanolic extract of leaves of Mucuna pruriens possesses anticataleptic and antiepileptic effect in albino rats. Dopamine and serotonin may have a role in such activity.

Functional components of *Mucuna pruriens*

In addition to the low levels of sulfur-containing amino acids in *M. pruriens* seeds, the presence of anti-physiological and toxic factors may contribute to a decrease in their overall nutritional quality. These factors include polyphenols, trypsin inhibitors, phytate, cyanogenic glycosides, oligosaccharides, saponins, lectins and alkaloids. Polyphenols (or tannins) are able to bind to proteins, thus lowering their digestibility. Phenolic compounds inhibit the activity of digestive as well as hydrolytic enzymes such as amylase, trypsin, chymotrypsin and lipase. Recently, phenolics have been suggested to exhibit health related functional properties such as anti-carcinogenic, anti-viral, anti-microbial, anti-inflammatory, hypotensive and anti-oxidant activities.

Trypsin inhibitors belong to the group of proteinase inhibitors that include polypeptides or proteins that inhibit trypsin activity. Tannins exhibit weak interactions with trypsin, and thus also inhibit trypsin activity. Phytic acid [myoinositol-1,2,3,4,5,6-hexa (dihydrogen phosphate)] is a major component of all plant seeds, which can reduce the bioavailability of certain minerals such as zinc, calcium, magnesium, iron, and phosphorus, as well as trace minerals, via the formation of insoluble complexes at intestinal pH.

Cyanogenic glycosides are plant toxins that upon hydrolysis, liberate hydrogen cyanide. The toxic effects of the free cyanide are well documented and affect a wide spectrum of organisms since their mode of action is inhibition of the cytochromes of the electron transport system. Hydrogen cyanide (HCN) is known to cause both acute and chronic toxicity, but the HCN content of *M. pruriens* seeds is far below the lethal level. Janardhan et al. (2003) have investigated the concentration of oligosaccharides

in *M. pruriens* seeds, and verbascose is reportedly the principal oligosaccharide therein.

Fatty acid profiles reveal that lipids are a good source of the nutritionally essential linoleic and oleic acids. Linoleic acid is evidently the predominant fatty acid, followed by palmitic, oleic, and linolenic acids . The nutritional value of linoleic acid is due to its metabolism at tissue levels that produce the hormone-like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and constriction of smooth muscle. Phytohemagglutinins (lectins) are substances possessing the ability to agglutinate human erythrocytes.

The major phenolic constituent of M. pruriens beans was found to be L-dopa (5%), along with minor amounts of methylated and non-methylated tetrahydro isoquinolines (0.25%). However, in addition to L-dopa, 5-indole compounds, two of which were identified as tryptamine and 5-hydroxytryptamine, were also reported in *M. pruriens* seed extracts Mucunine, mucunadine, prurienine, and prurieninine are four alkaloids that have been isolated from such extracts.

Uses

In many parts of the world, Mucuna pruriens is used as an important forage, fallow and green manure crop. Since the plant is a legume, it fixes nitrogen and fertilizes soil.

Cooked fresh shoots or beans can also be eaten. This requires that they be soaked from at least 30 minutes to 48 hours in advance of cooking, or the water changed up to several times during cooking, since otherwise the plant can be toxic to humans.

3.2.5. *Tinospora cordifolia*

Scientific classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta

Class	:	Magnoliopsida
Subclass	:	Ranunculidae
Order	:	Ranunculales
Family	:	Menispermaceae
Genus	:	Tinospora

Tinospora cordifolia (Willd.) (Family: Menispermaceae) commonly known, as "Amrita" or "Guduchi" is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis. The starch obtained from the stem known as "Guduchi-satva" is highly nutritive and digestive and used in many diseases. During last two decades, the drug has been subjected to extensive phytochemical, pharmacological and clinical investigations and many interesting findings in the areas of immunomodulation, anticancer activity, liver disorders and hypoglycaemic are reported.

SYNONYMS

Sanskrit	:	Amitavalle,Madhupar,, Gudichik, Chinnobhavi
Assamese	:	Siddhilata, Amarlata
Bengali	:	Gulanch
Gujrati	:	Galac, Garo
Hindi	:	Giloe, Gurcha
Kannada	:	Amrutaballi
Kashmiri	:	Amrita, Gilo
Malayalam	:	Chittamrutu
Marathi	:	Gulvel
Oriya	:	Guluchi

Punjabi	:	Gilo
Tamil	:	*Seendil, Seendil kodi*
Telugu	:	Thippateega
Urdu	:	Gilo

DESCRIPTION[13]

a) **Macroscopic**

Drug occurs in pieces of varying thickness ranging from 0.6-5 cm in diameter, young stems green with smooth surfaces and swelling at nodes, older ones show a light brown surface marked with warty protuberances due to circular lenticels, transversely smoothened surface shows a radial structure with conspicuous medullary rays traversing porous tissues, taste bitter.

b) **Microscopic**

Transverse section of stem shows outer-most layer of cork, differentiating into outer zone of thick-walled brownish and compressed cells, inner zone of thin walled colourless, tangentially arranged 3-4 rows of cells, cork broken at some places due to opening of lenticels, followed by 5 or more rows of secondary cortex of which the cells of outer rows smaller than the inner one, just within the opening of lenticels, groups of sclereids consisting of 2-10 cells found in secondary cortex region, outer zone of cortex consists of 3--5 rows of irregularly arranged, tangentially elongated chlorenchymatous cells, cortical cells situated towards inner side, polygonal in shape and filled with plenty of starch grains, simple, ovoid, or irregularly ovoid-elliptical, occasionally compound of 2-4 components, several secretory cells, found scattered in the cortex, pericyclic fibres lignified with wide lumen and pointed ends, associated with a large number of crystal fibres containing a single prism in each chamber, vascular zone composed of 10-12 or more wedge-shaped strips of xylem, externally surrounded by semi-circular strips of phloem, alternating, with wide medullary rays, phloem consists of sieve tube, companion cells and phloem parenchyma of polygonal or tangentially elongated cells, some of them contain cryste1s of calcium oxalate, cambium composed of one to two layers of tangentially elongated cells in each vascular bundle, xylem consists of vessels, tracheids, parenchyma and fibres, in primary xylem, vessels comparatively narrow devoid of tyloses, secondary xylem elements thick-walled, lignified, vessels cylindrical in shape bearing bordered pits on

their walls some large vessels possess several tyloses and often contain transverse septa, meduallry rays 15-20 or more cells wide containing rounded,
hemispherical, oblong, ovoid, with faintly marked concentric striations and central hilum appearing like a point, starch grains of 5.5-11.20 μ in diameter and 6-11.28 μ in length, pith composed of large, thin-walled cells mostly containing starch grains.

Constituents: Terpenoids and alkaloids.
Dose: 3-6 g of the drug in powder form.
20-30 g of the drug for decoction.

3.2.6. *Bombax malabaricum (mul ilavu)*

Scientific classification

Kingdom	:	Plantae
Classification	:	Angiosperms
Order	:	Malvales
Family	:	Malvaceae
Genus	:	Bombax
Species	:	*malabaricum*

Common name

Silk cotton tree, Salmali, Bombax ceiba, Simul tree

Chemical constituents

Bombax malabaricum is rich in many phytochemicals, among the active constituents found in this herb. They are the following:

- Steroids
- Tannins
- Flavonoids
- Triterpenoids
- Saponins
- Cholesterol

- Stigmasterol
- Campesterol
- A-Amyrin
- Hydrocarbons

Medicinal uses

It has the medicinal property of Anthelmintic, Anti-Helicobacter, Anti-Oxidant, Vermicide, Vermifuge

3.2.7. *Saccharum officinarum*[14]

Scientific classification

Kingdom	:	Plantae
Order	:	Poales
Family	:	Poaceae
Subfamily	:	Panicoideae
Tribe	:	Andropogoneae
Genus	:	Saccharum
Species	:	*S. officinarum*

SYNONYMS

Sanskrit	:	Asipatra, Bhurirasa, Derghacchada, Gudamula, Tarasa
Assamese	:	Kuhiyare
Bengali	:	Akh, Ganna
English	:	Sugar-cane
Gujrati	:	Sheradi
Hindi	:	Ganna, Ikh
Kannada	:	Ikshu, Kabbu
Malayalam	:	Karimpu
Punjabi	:	Ganna
Tamil	:	Karumbu Ver
Telugu	:	Cheraku, Cheruku
Urdu	:	Ganna, Naishkar

DESCRIPTION

a) Macroscopic

Drug occurs in form of root stock with attached yellowish-brown stem portion, having 10 to 15 cm long, numerous grey to blackish-brown fibrous roots; solid, jointed, more or less cylindrical, 2 to 2.5 cm thick and varying in length, rough; fracture,splintery; odour and taste, not distinct.

b) Microscopic

Root Stock - Shows single layered epidermis followed by 3 to 4 layers of oval to elliptical, lignified, thick-walled more or less radially elongated, sclerenchymatous cells; cortex consists of upper 12 to 15 layers oval to polygonal, thin-walled and lower 5 layers, elliptical, parenchymatous cells; endodermis single layered; pericycle 3 or 4 layers, sclerenchymatous; fibro-vascular bundle, covered with sclerenchymatous sheath, scattered throughout the ground mass of parenchymatous cells.

Root - Shows single layered epidermis of thin-walled, rectangular cells, followed by a layer of hypodermis of thin-walled, rectangular cells, outer cortex composed of 2 or 3 layers of thick-walled, polygonal to circular, sclerenchymatous cells filled with dark brown or blackish pigment, inner cortex composed of large aerenchymatous cells; endodermis composed of barrel-shaped, thin-walled cells, enclosing a layer of pericycle consisting of rectangular cells having inner wall thickened, and vascular tissue; xylem and phloem form an equal number of separate bundles. arranged in a ring; centre occupied by a large pith, composed of circular to oval, parenchymatous, thin-walled cells.

Powder: Blackish in colour; shows sclerenchymatous cells of cortex, xylem vessels and fibres, groups of spindle-shaped, elongated, epidermal cells in surface view.

T.L.C.

T.L.C. of alcoholic extract on Silica Gel 'G' using n-Butanol : Acetic acid : Water (4:1:5) shows under visible light two spots at Rf. 0.80 and 0.96 (both grey). Under U.V. (366 nm) four fluorescent zones are visible at Rf. 0.67 (light blue). 0.80 (dark blue). 0.86 (light blue) and 0.96 (dark blue). On exposure to Iodine vapour several spots appear out of which three spots are conspicuous at Rf. 0.30. 0.80 and

0.96 (all yellow). On spraying with 5% Methanolic- Sulphuric acid reagent and heating the plate for ten minutes at 110°C several spots appear out of which three are conspicuous at Rf. 0.10. 0.86 and 0.96 (all grey).

DOSE: 15-30 gm in decoction form.

The phytochemistry of sugarcane wax (obtained from the leaves and stalks of sugarcane), leaves, juice, and its products has revealed the presence of various fatty acid, alcohol, phytosterols, higher terpenoids, flavonoids, -O- and -C-glycosides, and phenolic acids.

Mollases have also been studied for their polyphenolic content. One novel O-glycoside, dehydroconiferylalcohol-9'-O-β-D-glucopyranoside along with the already reported isoorientin-7, 3'-O-dimethyl ether.

3.3 DISEASE REVIEW

3.3.1.SIDDHA ASPECT OF THE DISEASE "*AAN MALADU*"- MALE FERTILITY

Maladu

According to T.V.Sambasivam pillai dictionary, A disease by which men or women are rendered incapable of producing child by reason of defective semen is termed as *Maladu* in Siddha[15]. *Maladu rogam* can be classified into two types:

- *"Aan maladu"*
- *"Pen maladu"*

The term " *maladan"* means a male with no issues . *Aan Maladu*: *Agathiar* suggest that *maladu* is only for male and not for female.

Yugimuni explains about *aan maladu* in his treatise *yugimuni sikitcha saaram.*

ஆண் மலடின் குணம்

“பார்க்கவே ஆண்மகனின் விந்து தானும்

பதமான தித்திப்புயில் லாத்தாலும்

ஏற்கவே சலமீதில் மிதந்த தாலும்

எழிலாக வுயிர்ப்பற்று யிருப்பதாலும்

சேர்க்கவே மூத்திரத்தில் நுரைதான் போலும்

செயலான கருவதுவும் தரிக்க மாட்டா

தீர்க்கவே யூகிமுனி சிகிச்சா சாரம்

தெளிவாக பாடி வைத்தார் திறமி தானே[16]”.

மகளிர் மருத்துவம்

According to *yugimuni*, a person with semen of following qualities

- Lack of sweetness
- Buoyancy on water
- Absence of virility/viability
- And frothy micturition

SEMEN FORMATION

According to *Thirumoolar thirumanthiram*, at the time of *sirushti*, *kundali* appears in semen. Semen functions as God’s *kiriyashakthi* during formation of embryo.

விந்துவின் தோற்றம்

“உதயத்தில் விந்துவில் ஒங்கு குண்டலியும்

உதய குடிலில் வயிந்தவம் ஒன்பான்

விதியில் பிரமாதி கல்மிகு சத்தி

கதியிள் கரமங் கலைவை கரியே”

-திருமூலர்

” அழிகின்ற விந்து அளவை அறியார்

கழிகின்ற தன்னையுட் காக்கலுந் தேரார்
அழிகின்ற காயட் தழிந்தயர் வுற்றோர்
அழிகின்ற தன்மை யறிந்தொழி யாரே“

-திருமூலர்

In the above verses states the significance of sperm. In modern correlation spermatogenesis the process by which the male gamete called spermatozoa are formed by various stages like proliferation, growth, maturation, transformation. 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 x 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.

According to *Agathiyar Vaidya Valladi* – 600[17] – The *vindhu* (Semen) is chiefly constituted by the Fire (*Vanni)* and Air (*Vayu*) elements.

Development of Sperm

”உதயத்தில் விந்துவில் ஒங்கு குண்டலியும்
உதய குடிலில் வயிந்தவம் ஒன்பான்
விதியில் பிரமாதி கள்மிகு சத்தி
கதியிற் கரணங் கலைவை கரியே“

-திருமூலர்

In the above verses *Thirumoolar* states that the driving force the kundali arises in the sperm which in turn initiates the *Anthakaranam* to bring changes in the ova during fertilization.

CONFIGURATION OF SEMEN

Based on Siddha principles the configuration of semen is eighty drops of blood is equal to one drop of semen. therefore wasting a drop of semen is equal to wasting six thousand four hundred drops of blood. Different siddhars revealed their approach to a infertile patient in different aspects. Dhanwanthri explains a disease associated more with defective semen: sukkilapitham

சுக்கில பித்தம்

’தக்கதாங் கர்ப்பந் தன்னைத் தவிர்த்திடுங் கனவு தன்னில்

சக்கிடுஞ் சுக்கிலத்தை சுக்கில நாளங் காந்தும்

மிக்கசுகிலம் போல்னீரில் வெள்ளையுங் காணுங்கண்டாற்

சுக்கிலபித்தமென்றே சொல்லினர் சுருதிவல்லோர்“[18]

-தன்வந்திரி வைத்தியம்

Sukkilapitham is characterised by

- The semen is incapable to impregnate women
- Nocturnal emission
- Burning sensation in ejaculatory ducts

When a drop of semen is poured on water it will float with white colour. The views of *yugimuni* and *Dhanwanthri* synchronise in one thing that a semen which floats on a water surface will be incapable for fertility.

According to *Theraiyar yamagam*[19]

Theraiyar explains the different qualities of semen. He compared the physical nature of semen with known things proposed the ratings for the quality of semen.

Table 1 shows the nature and inference of the sperm

Nature of semen	Inference
White and akin to the butter	Excellent
White akin to curd	Very good
White and akin to milk	Good
White and akin to buttermilk	Fair
Akin to honey in colour and consistency	Average
Akin to ghee in colour and weight	Poor
Akin to toddy in colour and thickness	Very poor
Akin to water	Very bad

Siddhars thought about the basic units of reproduction- semen and ovum, crossed the limits of procreation and waved towards attaining their spiritual target. In Siddha, *sukkilam* is considered as *sivam* and *Naadham* is considered as *sakthi*. By which *karu* is considered as *sathasivam*.

According to *Agathiyar*, significance of sperm

” விந்துநிலை யறிந்து பாயுங்காலம்
வேதாந்த நாதமதுக்கு இருண்டாகி
சொந்தமுடநே இரண்டுமே நியுமாகி
சோதிமனி யானதுவிம்பி றையுமாகி
வட்னமதிப் பிறையதுவும் வட்டமாகி
வட்டமதி ரண்டுருவாய் மண்னுமாகி
அந்தமுள்ள மண்ணுதறி உப்பாய் நின்றே
யாதியென்ற பொருளான பிண்டமாச்சே“

-அகத்தியர்

After the penetration of the sperm into the ovum , the sperm head fuse with the oocyte to form a single cell. Then it undergoes several stages of cell division and finally form the embryo.

Sage *sivavaakiyar* and sage *thirumoolar* explains the role of semen after entering uterus

சுக்கில குணம்

”உண்மையான சுக்கில முபாயமா மிருந்ததும்
வெண்மையாகி நீரிலே விரைந்துனீர தானதும்
தண்மையான காயமே தரித்துருவமானதும்
தெண்மையான ஞானிக தெளிந்துரைக்க வேணுமே“

-சிவவாக்கியர்

At the time of copulation, the semen is ejaculated. The prostatic fluid gives the semen a milky appearance. In the early minutes after ejaculation, the sperm

remains immotile, possibly because of the coagulum. As the coagulum dissolves the sperm becomes highly motile.

”உன்னிய கர்ப்பக் குழியாம் வெளியிலே

பன்னிய நாதம் பகர்ந்த பிருத்திவி

வன்னியும் வாயுவும் மாயுருஞ் சுக்கிலம்

மன்னைய சமனாய் வளடர்க்கு முதகமே“

விழுந்தது இலிங்கம் விரிந்தது யோனி

ஒழிந்த முதல் ஐந்தும் ஈரைந்தோடு ஏறிப்

பொழிந்த புனல்பூதம் போற்றும் கரணம்

ஒழிந்த நுதல் உச்சி உள்ளே ஒளித்ததே“

-திருமூலர்

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

“வேர்க்கவே வேலிபோல் வளைந்து காக்கும்

விந்துவுடன் பிராணவாயு விளக்களாமே”.

-யூகிமுனி

Abanan stays outside and the pranan goes along with spermatozoa and bisects the size of the zygote. *Udhanan* helps in the growth of an embryo.

According to *Theraiyar*

- Food nourishes *saaram* or essence on the first day
- It then nourishes blood on the second day
- It then nourishes muscles on the third day
- It then nourishes adipose tissue on the fourth day
- It then nourishes bone on the fifth day
- It then nourishes bone marrow on the sixth day
- It then nourishes semen on the seventh day
- Concepts about formation of embryo by semen

The variations of the *Sukkilam* – physical constituents excess and decreased:
Excess *sukkilam* causes love and lust towards women and also urinary calculi.
Decreased *sukkilam* causes failure in reproduction, pain in the genitalia etc.

Sex variation in foetus

At the time of copulation if the male dominates then the foetus will be male and if the female dominates then it would be female foetus. If the both are in equl domination, the child would be eunuch.disease associated more with defective semen.
Sukkilavaatham

Symptoms of Sukkilavaatham: emaciation, constipation, oliguria, bleeding from the nose, phlegm accumulation due to increased kabam, breathlessness, loss of taste, abnormal semen.

Sukkilapittham

Sukkilapitham is characterised by the semen is incapable to impregnate women nocturnal emission burning sensation in ejaculatory ducts, when a drop of semen is poured on water it will float with white colour.

Vali Azhal Suram Characterised by fever, rigor, sneezing, restlessness, *thathunattam*, nausea etc.

Karumpanasai ammai
According to " *Agathiyar vaisoori* nool" *karumpanasai ammai"* will affect the semen and can make the patient maladu.
Iya maladu:

Indhiriya nashtam is one of the symptoms of iya paandu along with vomiting, sneezing, expectoration, hip pain etc.

Perumanjal kaamalai

This disease is characterized by *thaathunattam*, yellowish discolouration of face, eye, tongue, skin, loss of appetite, dyspnoea etc,These diseases mentioned above, if not properly treated may lead on to *Aan maladu.*

"வெல்லும் புவியில் விளங்கிய தாபரம்
புல்லிடுஞ் சங்கமம் பொறியிடும் விந்தே
"விந்தினாலல்லோ மேதினி யாச்சுது
செந்துக்களெஞ்லார்ஞ் சிவமயமாச்சுது
வந்திடும் நாதம் மௌனம் கலந்திது
சிந்தையிலுள்ளத் தெளிவாகச் செப்புமே"

- நோய் நாடல் நோய் முதனாடல் திரட்டு[20]

Siddhar *Therayar* says that the world originate from semen.

"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
பாயுமப்பா வன்னியோடு வாயு தானே!"

- சித்த மருத்துவாங்க சுருக்கம்[21]

According To *Agathiyar Vaidya Valladi* 600- The *Vindhu* (Semen) Is Chiefly Constituted By The Fire (*Vanni*) And Air (*Vayu*) Elements.

According to *Thiruvalluva Nayanar Gnanavettiyan* – 1500[22]

Thiruvalluva Nayanar Clearly Explained That The Hormonal Influence And Brain Stem Is Characterized 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 fibres in the brain stem explain this. Spermatogenesis is controlled by pituitary gland and hypothalamus.

”சுக்கிலந் தனையடக்கின்

சுரமுடனீர்க் கட்டாகும்

பக்கமாங் கை கால் சந்து

பாரநோய் வழியிறங்கும்

மிக்க மார் நோயுண்டாகும்

மிகுந்திடும் பிரமேகந்தான்

தக்கதோர் போதுமாகின்

தரித்திடும் வாயுக் கூறே[23]“

உடல் தத்துவம்

3.3.2. Modern aspect 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. The husband is responsible for the infertility of approximately one half of all childless marriages. There is usually defective development of the germinal epithelium in the somniferous tubules, with oligospermia or azoospermia[24].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 becme 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.

The formation of the male gamete from germ cell to mature spermatozoon takes approximately 70 days and proceeds within the confines of the seminiferous tubules[25].

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, with in 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. 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.

Table 2 Secretary products of the cells in the testis

Sl.No	Cell Products	Proposed Function
A.	Leydig Cells Androgens Proopiomelanocortin Inhibin IGF – I IL - 1β	Endocrine, paracrine, autocrine control Opioids, α-MSH, ACTH Endocrine, paracrine activity Growth, differentiation Kinin activity
B.	Sertoli Cells ABP Transferrin Ceruloplasmin Estrogen/Aromatase Laminin, Collagen Proteoglycans TFG-α, TFG-β, IGF-I, IL-I	Binding of androgens Iron Transport Copper Transport Endocrine, paracrine regulation Extracellular matrix Growth factors which inhibit or stimulate cell physiology and proliferation
C.	SC-EGF Inhibin, Activin Mullerian, inhibitory factor LHRH-Like substance Lactate / Pyruvate	Endocrine, paracine Fetal Sertoli cell development Binds of leydig cells in rats Metabolites, nutrients for gem cells.
D.	Peritubular Cells P-Mod-S Fibronectin Proteoglycans TFG-α, TFG-β, IGF-I	Paracine regulation od\f Sertoli cells Extra cellular matrix Growth factors

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

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

Hormonal factors that stimulate Spermatogenesis

Testosterone

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

Luteinizing Hormone

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

Follicle – Stimulating Hormone

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

Estrogens

It 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.

Growth Hormone

It is necessary for controlling the back ground metabolic functions of the testes. Growth hormone especially promotes early division of the spermatogonia themselves. In its absence as in pituitary dwarfs, spermatogenesis is severally 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.

Physiology of male sexual function:

Normal male sexual function requires coordinated regulation of the following physiologic events: libido or sexual desire, sustained penile tumescence or erection, ejaculation, orgasm, and detumescence[26].

The requirements for pregnancy to occur are several especially from male are the following:

- The male must produce adequate numbers of normal, motile, spermatozoa,

- The male must be capable of ejaculating the sperm through a patent ductal system,
- The sperm must be able to transverse an unobstructed female reproductive tract,
- The sperm must be able to fertilize the ovum.
- Infertility History
- History of Infertility
- Duration
- Prior Pregnancies
- Previous treatments
- Evaluation and treatment of wife
- Present partner
- Another partner
- Sexual History of the Man
- Frequency of masturbation
- Frequency of intercourse,
- Timing of intercourse
- Potency
- Lubricants
- Childhood and Development
- Undescended testicles
- Herniorraphy
- Testicular trauma
- Testicular torsion
- Y-U plasty of bladder
- Onset of puberty-early, normal of delayed

Family History

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

Infections

- Viral infections

- Febrile
- Sexually transmitted disease
- Tuberculosis
- Chicken pox
- small pox
- Mumps
- Orchitis

6. Surgical History

- Pelvic injury
- Orchitectomy
- Herniorraphy
- Pelvic injury or scrotal swelling
- Y-V plasty, Transulatal
- Retro peritoneal surgery

7. Gonadotoxins

- Thermal exposure
- radiation
- Smoking
- Chemicals – Pesticides
- Drugs, Chemotheraputic, Marijuana, Sulfasalazine, nitro furantoin, androgentic 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^2 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 38^0C 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 far 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 interstitum 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

Testicular Changes In Infertility

The cause of infertility are classified as pretesticular, testicular and posttesticular. pretesticular causes include various disturbances of the hypothalamic-pituitary-gonodal and adrenal hormonal axis. these diseases are usually considered under the heading of hypogonodotropic hypogonadism. testicular causes include idiopathic conditions affecting spermatogenesis and secondary testicular injury caused by inflammation, cryptorchidism, radiation therapy, drugs or varicocele. post testicular causes include various disorders that obstruct the outflow of sperm.

Post Testicular Causes Of Infertility

Azoospermia or severe oligospermia in a patient whose testicular biopsy findings are normal is indicative of obstructive, post testicular infertility. the site of obstruction is less determined during surgical exploration or by a vasogram.

On the basis of testicular morphology, primary disturbances of spermatogenesis can be subdivided into three groups, germ cell aplasia, maturation arrest of spermatogenesis and hypo spermatogenesis.

Germ cell aplasia, or sertoli syndrome, is the secere of these disturbances and is invariably accompanied azoospermia.

Maturation Arrest Of permatogenesis is characterized by incomplete spermatogenesis. the maturation arrest can be at any stage of spermatogenesis but most often occurs at the level of the primary spermatocytes. the term incomplete maturation arrest is used to refer those cases in which the tubules show arrest at different stages of spermatogenesis. in such cases, some tubules show spermatogenic cells arrested at one stage (for example, spermatids0 and adjacent tubules arrested at another stage (for example, primary spermatocytes).

Hypospermatogenesis is a quantitative reduction in the number of spermatozoa produced by spermatogenesis.

- Impaired Sperm Production and Function

- Hypothalamic pituitary disorders

Congenital hypo-gonadotropic hypogonadism kalimann's syndrome due to deficiency of gonadotrophin releasing hormone (GnRH). In the X-linked form of this disease, deletion of a gene kalig-2 has been found. This gene encodes for neurons involved in production of GnRH. Acquired hypogonadotrophic hypogonadism pituitary adenoma (including prolactinomas) cranio-pharingiomas other brain tumours, intracranial radiation therapy.

Genetic Factors

Sex chromosome abnormalities

47-xxy karyotype (Klinefelter syndrome) is almost always associated with azospermia (destruction of seminiferous tubules at puberty leading to shrinkage of testes).

Extra Y chromosome results in various degrees of impairment of spermatogenesis. Characteristic decreased sexual function, gynaecomazia, decreased length of penis and testis and decreased testosterone level.

Other chromosomal abnormalities

Translocations cause more severe impairment in male than female.

- Impaired chromosome pairing in melosis leads to azoospermia.
- Deletions corresponding to AZF (Human azoospermia factor) region on long arm of chromosome. This genetic region controls spermatogenesis in human beings.
- Undescended Tetsis (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 contra lateral testis in patients with unilateral mal-descent. Early treatment before age of two years is advocated.

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.

Germ cell Aplasia

Seminiferous tubules contain only sertoli cells. Absence of germ cells may be due to factor present during fetal life. Leydig cell insuffiency may also be associated. This cytological appearance can also result from cryptorchidism, cytotoxic drugs or irradiation.

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) azoospermia and cytotoxic drugs especially the alkylating agents (Cyclophosphamide, cisplatin and procarbazine) also cause gonadal failure.

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
- 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.

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.

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

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.

- Unexplained Infertility
- Semen quality is normal and no effect can be found in the female partner.
- Evaluation of the infertile man

This includes,

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

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

- Bacterial examination
- Genetic assessment
- Testicular biopsy
- Sperm function tests
- Ultrasound
- 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_2 antagonist's cimetidine and ranitidine, erythromycin tetracycilne'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, and 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 court 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 boxes shorts excessively hot baths hot tubes 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 courts in males from an urban area compared to males in rural areas, suggesting an affect of urban pollutants. In any case the clinician should determine it a male with an abnormal semen specimen had exposure to industrial or environmental.

Exposure to diethyl stillbestrol 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 of 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 couple's every 36 hours around the time of ovulation will give the optimal chance far pregnancy. However studies in men with Oligospermia fail to detect a decline in sperm-numbers with sequential ejaculations suggesting that in 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.

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

Total sperm count:

Sperm concentration x volume of semen Normal) 40 x 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 x 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 13µmol/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 day's 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.
- 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 far 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, innophore 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 (HZ1) a break point at on 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. It 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 chalmydia.

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
Necrospermia	

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

Hydrocele	Sympathectomy
Varicocele	Testicular torsion
Inguinal hernia	Hypospadias
Vasectomy	Urethral strictures or diverticula
Prostatectomy	Bladder neck operation

WHO manual – semen analysis – nomenclature's

Normozoospermia	-	Normal semen
Aspermia	-	Absence of ejaculation
Azoospermia	-	Absence of sperms in the semen
Oligo zoospermia	-	Less than 20 millions count / ml

Astheno zoospermia	-	Less than 5.0% spermatozoa with Forward Progression
Terato zoospermia	-	Less than 30% spermatozoa with Normal head morphology
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 Oozyte 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

3.3 PHARMACEUTICAL REVIEW

Chooranam

Definition

Chooranam is a fine powders of drugs. The "*Chooranam*" may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity. (Formulary Of Siddha Medicine,1993)

Method of preparation

- Equipment required
- The drug enumerated in the recipe in clean and well dried state.
- A mortar and pestle.
- A fine sieve or fine cloth of close mesh.

Process of preparation

The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storages or changed in colour, taste and scent, and those that are insects infested or attacked by fungi should be positively rejected.

However drugs like Embelia fruits, Senna, Long Pepper, Jaggery and cows ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed racidity.

In general the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.

The *chooranam* should be so fine to be called amorphous and should never be damp. The fineness of the sieve should be 100 mesh or still finer.

Purification of the prepared *chooranam*

”தானென்ற சூரணத்தின் சுத்திக்கேளு

தப்பாதே சரக்கெல்லாஞ் சூரணித்து

நானென்ற வாவின் பாலாற் பிசைந்து

நலமான சட்டியிலே பாலைவிட்டு

வானென்ற சுத்தசலம் பாதிவிட்டு

வளமாக மேற்சீலை கோடு கட்டிப்

பானென்ற சூரணத்தைப் பிட்டுபோல் வைது

பதறாதே வெந்தெடுக்கச் சித்தியமே“

-அகஸ்தியர் வைத்திய இரத்தினச் சுருக்கம்

The prepared *chooranam* is mixed with the milk .in a pot half a quantity milk and half a quantity water is taken. The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed *chooranam* is placed. The pot is placed over the stove and heated.

”ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து

அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து

நாமப்பா கொண்டு வர தோஷம் போச்சு

நன்றாக்ச் சுத்தி செய்யாச் சூரணந்தான்

தாமப்பா ரோகத்தை வெல்லா தப்பா

தளமான வியதி யெல்லாம் பாரிக்கும் பார்

வேமப்பா சுத்தி செய்து கொண்டாயனால்

வெகுசுறுக்காய் தீருமா வியாதி கேளு“

-அகஸ்தியர் வைத்திய இரத்தினச் சுருக்கம்

Then the *Chooranam* is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally. All type of diseases get cured. If the drug is taken without purification the disease does not cure. If taken after purification the disease cures easily.

Storage

The prepared *chooranam* should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cardboard boxes.

The *chooranam* to facilitate easy handling and to assure exact dosage administration, could be pressed into tablets, could be packed in bottles or tubes made either of glass or plastic or packed in strip of metal foil or plastic sheets.

In industry the tablets are made, counted & packed by electronic devices. Then *chooranam* is said to retain its potency for 2 months and then gradually deteriorate. However if properly packed & stored they keep good for a year. (Formulary of Siddha Medicines, 1993)

According to AYUSH guidelines shelf life of chooranam is one year.

Table : 3 Analytical Specifications Of Curna/Choornam[27]

Sl.No	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 105^0 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)
9.	Test for heavy/Toxic metals Lead Cadmium

	Mercury
	Arsenic
10.	Microbial contamination
	Total bacterial count
	Total fungal count
11.	Test for specific Pathogen
	E. coli
	Salmonella spp.
	S.aureus
	Pseudomonas aeruginosa
12.	Pesticide residue
	Organochlorine pesticides
	Organophosphorus pesticides
	Pyrethroids
13	Test for Aflatoxins (B1,B2,G1,G2)

4. MATERIALS AND METHODS

4.1 PREPARATION OF THE DRUG

Drug selection:

In this research work, the "*Nilapanai Kizhangu Choornam"*, a poly herbal formulation, has been selected to evaluate *Dhadhu Balaveenam* (Oligospermia), mentioned in "*Kannusamy Parambarai Vaithiyam"*.

Ingredients:

Nilapanai kizhangu (*Curculigo orchioides*)	-	1 *palam* (35 gram)
Nerunjil (*Tribulus terrestris*)	-	1 *palam* (35 gram)
Nelli vatral (*Phyllanthus emblica*)	-	1 *palam* (35 gram)
Poonaikaali vidhai (*Mucuna pruriens*)	-	1 *palam* (35 gram)
Seendhil sarkarai (*Tinospora cordifolia*)	-	1 *palam* (35 gram)
Mul Ilavam pisin (*Bombax malabaricum*)	-	1 *palam* (35 gram)
Karkandu (*Saccharum officinarum*)	-	1 *palam* (35 gram)

Source of Collection:

The drug was purchased from authorized country Raw Drug Store in Parrys corner Chennai.

Identification and Authentication of the drug

The collected raw materials and plants were identified and authenticated by Botanist and faculties of *Gunapadam* department, Government Siddha Medical College Chennai, Tamilnadu.

Purification of the ingredients

Nilapanai kizhangu

Tuber of *Nilapanai kizhangu* (*Curculigo orchioides*) was washed in tap water and the soil and impurities were removed.

Nerunjil

Fruits of *Nerunjil* (*Tribulus terrestris*) was cleaned well without any dust and impurities.

Poonaikaalivithai

Seeds of *Mucuna pruriens* was washed thoroughly then boiled with 100 ml of milk and outer skin was removed.

Seendhil sarkarai

Stem of *Tinospora cordifolia* was washed thoroughly and the outer skin was peeled off .

Mul Ilavam pisin

Gums of *Mulilavu* (*Bombax malabaricum*) was washed in the running tap water and removed the soil and impurities.

Nelli vatral

Dried fruits of *Nelli* (*Phyllanthus emblica*) was first dusted with a clean cloth and then purified by gently removing the outer skin.

Preparation of *Nilapanai Kizhangu Choornam*

The ingredients after purification were grounded separately to powder. The powder was sieved through a white cloth (*Gunapadam Thathu Jeeva Vagupu*) .All these powdered ingredients were mixed thoroughly in stone mortar. The prepared test drug was stored in a clean, air tight glass container.

Labelling

Drug name	:	*Nilapanai Kizhangu Choornam*
Dosage	:	2 Gram twice a day- after food
Indication	:	Spermatorrhoea, Impotency , Nervous weakness & Sexual tonic.
Vehicle	:	Milk
Colour	:	Dark brown
Date of manufacture	:	14 March 2016
Date of expiry	:	13 June 2016
Reference	:	*Kannusamy Parambarai Vaithiyam* (part -2) page no165.

Administration of drug:

Form of the medicine	:	*Choornam* (Powder)
Route of Administration	:	Oral
Dose	:	2 grams
Anubanam (Vehicle)	:	Milk
Time of Administration	:	Twice a day;

INGREDIENTS

FiG 1.1:*Curculigo orchioides*

FIG 1.2: *Phyllanthus emblica*

FIG 1.3: *Mucuna pruriens*

FIG 1.4: *Bombax malabaricum*

FIG 1.5:*Saccharum officinarum*

FIG 1.6: *Tinospora cordifolia*

FIG 1.7: *Tribulus terrestris*

FIG 2: Image Of *Nilapanai Kizhangu Choornam*

4.2 STANDARDIZATION OF THE DRUG

Standardization of herbal formulations is essential to assess quality of drugs[28]. And also it is necessary to conduct uniform rules for preparing drug[29]. Standardization of the drug brings the validation to be used as a medicine by subjecting the drug NPKC to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus standardization brings the efficacy and potency of the drug.

4.2.1 PHYSICO CHEMICAL ANALYSIS

Organoleptic character

The organoleptic characters of the sample drug were evaluated.1gm of NPKC was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

Ph value

Potentiometrically pH value determined by a glass electrode and a suitable pH meter.

Loss on Drying

The powdered drug is dried in the oven at 100- 105°C to constant weight.

Determination of Ash Values[30]

Total Ash

Accurately weighted 3g of *Nilapanai Kizhangu Chooranam* was taken and incinerated in a crucible dish at a temperature not more than450^{o}C until free from carbon. Then it was cooled and weighted. The percentage of ash was calculated with the reference to the air-dried drug.

Preliminary phytochemical analysis

The *Nilapanai Kizhangu Chooranam* (10 g) was extracted with the solvents namely methanol, ethyl acetate and chloroform. The extracts were filtered and the concentrated under vacuum, followed by drying (40 °C). The extracts were screened for the presence of phytochemicals like alkaloids, flavonoid, carbohydrates,

glycosides, saponins, tannins and triterpenoids by standard methods. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not [31]

4.2.2 CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radicals present in the NPKC.

Preparation of extract

5g of NPKC was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Determination of Preliminary Basic and Acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the NPKC 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of NPKC extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate.

3. Test for Magnesium

To 2ml of NPKC extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium

To 2ml of NPKC extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the NPKC, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. Test for Iron (Ferrous)

The NPKC extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour.

7. Test for Zinc

To 2 ml of the NPKC extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the NPKC extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of NPKC extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate.

10. Test for Copper

a. A pinch of NPKC was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of NPKC extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate.

11. Test for Mercury

To 2m1 of the NPKC extract sodium hydroxide solution was added and noted for yellow precipitate formation.

12. Test for Arsenic

To 2 ml of the NPKC extract 2ml of sodium hydroxide solution was added and brown or redprecipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the NPKC extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The NPKC extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The NPKC extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate

The NPKC extract was treated with conc.Hcl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate

To 2ml of NPKC extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate

To 1 gm of the NPKC, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes

4.2.3 AVAILABILITY OF MICROBIAL LOAD

Enumeration of bacteria by plate count – agar plating technique[32]

The plate count technique is one of the most routinely used procedure because of the enumeration of viable cells by this method.

Principle

This method is based on the principle that when material containing bacteria is cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore are the same as the number of organisms contained in the NPKC.

Dilution

A small measured volume of NPKC are mixed with a large volume of sterile water called the diluent or dilution blank. Dilution are usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

Requirements

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)

- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter

Procedure

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the NPKC extract into a 9 ml dilution blank labelled 10^{-1} thus diluting the sample 10 times.
3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the sample have been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted NPKC extract. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidify.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 repleates)}}{\text{Amount of plated} \times \text{dilution}}$$

4.2.4 SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

Fig 3: Image Of FTIR Analyser

FT-IR spectra were recorded at SAIF, IIT Madras, India. The Perkine Elmer Spectrum One Fourier Transform Infrared (FTIR) Spectrometer was used to derive the FT IR Spectra of *Nilapanai Kizhangu Chooranam* in Potassium Bromide (KBr) matrix with scan rate of 5 scan per minute at the resolution $4cm^{-1}$ in the wave number region $450\text{-}4000cm^{-1}$. The samples were grounded to fine powder using agate motor and pestle and the mixed with KBr[33]. They were then Pelletized by applying pressure to prepare the specimen (the size of specimen about 13 mm diameter and 0.3 mm in thickness) to recorded the FT- IR Spectra under Standard conditions. FT- IR Spectra were used to determine the presence of the functional groups and bands in the *Nilapanai Kizhangu Chooranam*. The recorded spectrum shows in figure.

SEM (SCANNING ELECTRON MICROSCOPE)

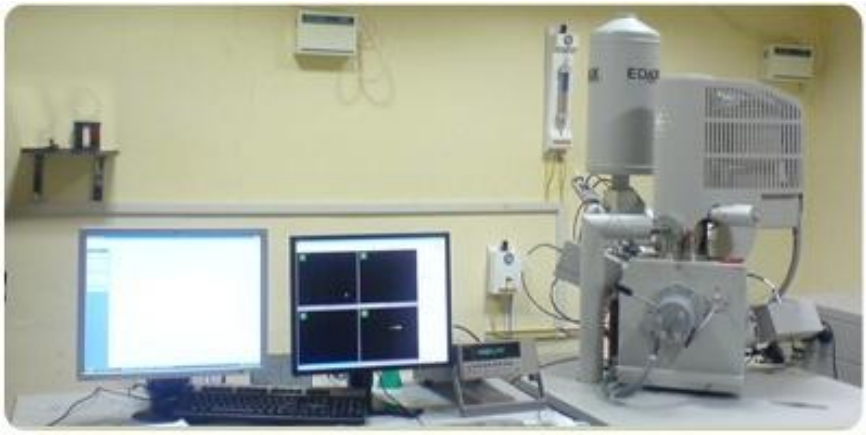


Fig 4: Image Of SEM Analyser

To evaluate the size of the particle, surface topography SEM analysis was carried out using at SAIF, IIT madras.The sample was mounted on specimen stub, placed inside the microscope's vacuum column evaporator and a beam of electrons passed from an electron gun, travelled through a series of magnetic lenses. The electrons are counted by the detector and the signals are sent to the amplifier. The number of electrons dispersed from each spot of the sample builds up the resultant image. The micrographs obtained give sufficient data about the topography of the sample.

XRD

The XRD studies was done in IIT madras by using Bruker discover D8 X ray diffractometer.

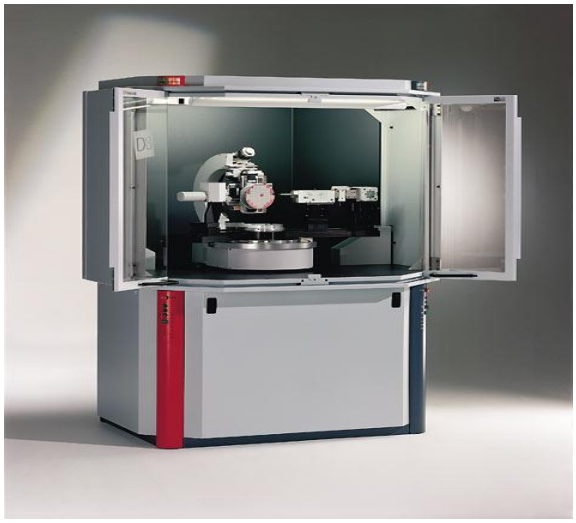


Fig 5: Image Of XRD Analyser

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig: 6 Image Of ICP-OES Analyser

The Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) analysis was done using Perkin Elmer Optima 5300 DV.

Sample preparation

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby NPKC are introduced in liquid form for analysis. 100 mg NPKCwas occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested NPKC solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

4.3 TOXICOLOGICAL STUDIES

ACUTE ORAL TOXICITY – OECD GUIDELINES - 423[34]

INTRODUCTION

The acute toxic class method was a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423.

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA .

Animal: Healthy wistar albino female rat weighing 200–220 gm

PRINCIPLE

It was the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, information was obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined doses. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing was needed − dosing of three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Studied carried out at three female rats under fasting condition, signs of toxicity was observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

METHODOLOGY

Selection of animal species

The preferred rodent species was rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat were obtained from Animal house of king's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L.BaidMetha College of pharmacy, Thuraipakkam, Chennai.

Housing and feeding conditions

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

EXPERIMENT PROCEDURE

Administration of doses

NPKC was prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavages using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 hours prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this NPKC has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study : 48 hours

Evaluation : 14 Days

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug, and
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hours following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day: -1, day 1, 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing NPKC with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline was pharmacologically inert and easy to employ for new drug development and evaluation technique.

28 DAYS REPEATED DOSE ORAL TOXICITY STUDY OF "*NILAPANAI KIZHANGU CHOORANAM*" ON RATS – (OECD-407 guidelines) [35]

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that NPKC was non-toxic and no behavioral changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route was considered to be a proposed therapeutic route.

Preparation and administration of dose

NPKC at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

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

Body Weight

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality

All animals were observed twice daily for mortality during entire course of study.

Functional Observations

At the end of the 4th week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 rpm for 10 minutes. On 28th day of the experiment, 24 hours urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from faecal contamination. Toluene was used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 hours, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations

Blood samples of control and experimental rats was analyzed for hemoglobin content, total Red Blood Corpuscles (RBC), White Blood Corpuscles (WBC) Count and Packed Cell Volume (PCV).

Biochemical Investigations

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase / Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase / Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis

Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 hours and washed in running water for 24 hours. Samples were dehydrated in an auto technique and then cleared in benzene to remove absolute

alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to Histopathological examination.

Statistical analysis

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparision test using a computer software programme GRAPH PAD INSTAT-3 version.

4.4 PHARMACOLOGICAL STUDY

4.4.1 Evaluation Of Spermatogenic Activity Of *Nilapanai Kizhangu Chooranam* in ethanol induced wistar male rats

Aim

To evaluate the spermatogenic activity of *Nilapanai Kizhangu Chooranam* (NPKC) *in Wistar* albino rats by Ethanol induced method[36]

Procurement and rearing of experimental animal

Adult male Wistar rats weighing 180-210 gms were used for this study. The inbred animals were procured from the animal house of Kings institute, Chennai and the study was conducted at C.L,Baid Metha College Of Nursing, Chennai, India. They were housed six per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C. The animals were subjected under standard photoperiodic condition of 12:12 hrs light:dark cycle. The animals were fed with standard rodent pellet and water ad libitum. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.The protocol for experimentation was approved by Institutional Animal Ethics Committee .

Experimental design

Sample Size: 24 albino rats

Experiment Duration: 60 days

Animal grouping and interventions

The animals were randomly selected and divided into four groups (I,II, III and IV) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received only 1ml milk, p.o. for 60 days. *Group II* served as negative control (Alcohol induced) , received 0.5 ml of 25% ethanol /kg /BW /day for 60 days. Experimental groups splits into group III and IV served as the treated groups and received NPKC which was grounded in mortar-pestle with milk. *Group III* was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 100mg/kg of NPKC orally once for 60 days. . *Group* IV was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 200mg/kg of NPKC orally once for 60 days Administration was done once a day by oral gavage in the morning.

Table 4 Grouping of animals for the evaluation of Spermatogenesis activity of NPKC

Groups	Intervention	No of Rats
GrouI-Normal Control	1 ml of Milk	**6**
Group II – Negative Control	25% of ethanol (0.5 ml /kg/day)	**6**
Group III – Treatment group	NPKC (100mg / kg / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	**6**
Group IV – Treatment group	NPKC (200mg / kg b.w / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	**6**

Sampling, Sacrifice and Surgical procedure

Twenty-four (24) hours after the 60th day of treatment, following over-night fasting (12 hrs), the animals were sacrificed with i.p.(intraperitonial) injection of thiopentone . The abdominal cavity was opened up through a midline abdominal incision to expose the genital organs. Testes, Prostate, seminal vesicle and epididymis were excised, trimmed of all fat and other tissues, mopped with tissue paper and then weighed. Left testicles were collected to monitor the spermatozoal characteristics and Right testicles to conduct testicular and epididymal histopathology .The sections was studied microscopically for changes in histo architecture or morphology. The left caudal epididymis were transferred into sterile bottles containing 2 ml of normal saline for semen analysis. Semen samples from caudal epididymis (left) were subjected to parameters such as count, motility, viability and abnormality. Counting was performed using a haemocytometer and light microscope with 100X.

Enumeration of sperm parameters

Semen analysis

Examinaion of sperm count, sperm motility, viability and spermatozoal abnormalities was carried out by making small cuts in the area of the cauda epididymis close to the vas deferens and apply gentle pressure to exude epididymal contents

Sperm Count

The sample was drawn into WBC pipette and diluted to the ratio of 1:100 with the modified Krebs Ringer-bicarbonate buffer containing 0.05% collagenase (pH 7.4) followed by this 1:1000 dilution was performed with 1.8% NaCl and 2% formalin). The sperm suspension was placed in the haemocytometer with improved double Neubauer ruling was used for the counting of spermatozoa. Counts for 2-4 haemocytometer chambers were averaged. The sperm suspension was evaluated for sperm count.

Total number of sperm cells in all the four chamber = X

X multiplied by 10,000 to obtain the number of cells (Y) per ml of diluted sample

Y multiplied by 100 (the dilution factor) to obtain (Z) sperm cells per ml of original semen sample.

Sperm Motility[37]:

The sample was mixed with 20mm HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid contains L-Glutamine with 5% BSA (*Bovine serum albumin*) . Final sample suspension mixed with formalin and used to assess motility. Bright field microscope magnification 100x.

$$\text{Motility (\%)} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa (Motile + Immotile)}} \times 100$$

Percentage viability[38]:

Viability was assessed by eosin Y staining (5% in saline). Forty micro litre samples of the freshly sperm suspension were placed on a glass slide, mixed with 10 µL eosin and observed under a light microscope (x400 magnification). Live sperms remained unstained following staining; whereas, those that showed any pink or red colouration were classified as dead. At least 200 sperm were counted from each sample in ten fields of vision randomly, and the percentage of live sperms was recorded

$$\text{Percentage of sperm viability} = \frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable +Dead)]}} \times 100$$

Sperm morphology[39] :

Staining

A suggested method for staining uses 1ml of sperm suspension which was transferred to a test tube. Two drops of 1% eosin Y were added to the test tube and mixed by gentle agitation. The above mixture was incubated at room temperature for approximately 45-60 minutes to allow for staining.

Slide preparation

Slides should be cleaned with detergent, washed in water followed by alcohol and dried before use. One to two drops of the stained sperm suspension were placed approximately 1cm from the frosted end of a pre-cleaned microscope slide lying on a flat surface. A second slide was held in the right hand with the Z slides's long edge gently touching across the width of the sperm slide and pulled across to produce a sperm smear. After drying the smears were fixed with formalin.

Characterization of normal and abnormal sperm

Abnormality in sperms were calculated based on the following parameter like curved tail, Tail less head, Headless tail, looped tail and coiled tail etc. Normal sperm were calculated based on the appearance and absence of above mentioned parameters.

$$\text{Percentage of normal sperm} = \frac{\text{No of Normal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

$$\text{Percentage of normal sperm} = \frac{\text{No of abnormal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

Procedure for histopathology[40]

The rats from each group were anethetized by drug with out any injury after lower pelvic region. The collected samples were washed with normal saline and fixed in 10% neutral formalin for 48 hrs for further histological observation. Paraffin section were taken at 5 µm thickness processed in alcohol-xylene series and was

stained with Haematoxylin-eosin dye. The sections were examined microscopically for histopathological changes. The magnification for low power was carried out at 10 X and for high power at 45 X.

Statistical analysis

Data collected in the study were expressed as the mean ± SEM and statistical analysis was carried out using Dunnett test. P value less than 0.05 was considered to be statistically significant.

4.4.2 Evaluation Of Aphrodisiac Activity Of *Nilapanai Kizhangu Chooranam* in ethanol treated male rats

Aim

To evaluate the aphrodisiac potential of *Nilapanai Kizhangu Chooranam* in ethanol treated wister albino male rats

Animal procurement and maintenance

Wistar Albino rats of either sex, weighing 150 g to 200 g were purchased from King Institute of Preventive medicine Animal House, Chennai, India and they were acclimatized in Animal house of C.L Baid metha college of pharmacy, Gerugambakkam, Chennai, India at 21 - 23ºC. Animal ethical guidelines of CPCSEA, Ministry of Animal Husbandry and Welfare, Govt. of India were strictly followed for the care and maintenance of procured animals. The animals were fed on standard rodent pellet and RO water was provided *ad libitum.* Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (IAEC)

IAEC No: IAEC/XLIV/28/CLBMCP/2014

Experimental Details

The Sexually Active Male Rats Were Chosen Separately And Divided Into 6 Groups; Each Group Consisting Of 6 Animals.The Animals In The Divided Groups Received The Treatment Orally.

The Sexual Behavior Of The Experimental Rats Was Observed In A Dim Light In Specially Designed Cages That Have Glasses On All The Sides And Measuring 50×30×30cm. The Male Experimental Rat Was First Placed In The Cage And Then Two Female Rats In Estrous Phase Were Introduced. An Initial Period Of 15 Minutes Was Considered As Acclimatization Period. After 15 Minutes, The Extract Or The Drug Was Introduced And The Activity Of Male Rat In Each Group Was Recorded Individually For 60 Minutes, After 30 Minutes Of Drug Administration. To Determine The Aphrodisiac Activity Of The Extracts, Several Parameters Were Observed. These Include Measuring And Observing The Mount Frequency, Mount Latency, Intromission Frequency, Intromission Latency, Genital Grooming And Anogenital Sniffing[41-44].

Definitions of individual parameters observed

Mount frequency

Mount Frequency is corresponded to the number of mounts without intromission from the time of introduction of the female until ejaculation.

Intromission frequency

Intromission is the introduction of one organ or parts into another. E.g. the penis into the vagina. Intromission Frequency is therefore defined as the number of intromissions from the time of introduction of the female until ejaculation.

Mount latency

Mount Latency is defined as the time interval between the introduction of the female and the first mount by the male.

Intromission latency

Intromission Latency is the time between the introduction of the female and the first intromission. This is usually characterized by pelvic thrusting, and springing dismounts.

Ejaculatory latency

Ejaculatory Latency is defined as the time from the first intromission to the first ejaculation.

Post-ejaculatory interval

The post-ejaculatory interval is the time between ejaculation and the forthcoming non ejaculatory intromission. The test is negative if the latency of intromission and ejaculation is greater than 20 minutes.

Sperm count (no of sperm x 10^6)

After treatment, the sperm count was carried out by using Haemocytometer (Mukherjee and Kanai, 1988). Haemocytometer is generally used for RBC as well as WBC count. It is provided with the pipettes for the dilution of the blood samples and Neaubaur's slide with special type of ruling. The counting was done in the ruled squares on the slide. The epididymis was removed and placed in a pre-chilled petri-plate. 2 ml. of 0.9% saline was added to it and the cauda epididymis was gently minced with the help of sharp razor. This sample was used for the sperm count. The sample was pipetted out with the help of pipette provided in the Haemocytometer. A clean and dry cover slip was kept on the Neaubaur's ruling. The ruling was loaded with the sample by touching the tip of the pipette to the slide.

Histopathological Analysis

At the end of 28^{th} day testis were isolated for histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 30 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5 micron thickness and stained with Haematoxylin and Eosin. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus photographing microscope. The histopathological examinations were reviewed by the pathologist.

Statistical analysis

Data collected in the study were expressed as the mean ± SEM and statistical analysis was carried out using Dunnett test. P value less than 0.05 was considered to be statistically significant. All data were summarized in tabular form Table.

Evaluation of antioxidant activity of *Nilapanai Kizhangu Chooranam* through DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of *Nilapanai Kizhangu Chooranam* was determined using the 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay[45].100µl of *Nilapanai Kizhangu Chooranam* extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates[46,47].

Free radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs of Control} - \text{Abs of Test})}{\text{Abs of Control}} \times 100$$

5. RESULTS AND DISCUSSION

ORGANOLEPTIC CHARACTER

Table 5 Organoleptic Characteristics And Physicochemical Properties Of NPKC

S.NO	Parameter	Results
1	Colour	Grey
2	Odour	Pleasant
3	Taste	Characteristic taste
4	Texture	Fine powder
5	Particle size	Completely pass through sieve no 88
6	pH at 25°C (1:10 Ratio)	3.05
7	Ash Value @ 550°C (%)	12.0%
8	Water soluble (%)	54.0%
9	Alkalinity as CaCO3 in water soluble ash (%)	1.18%
10	Acid Insoluble Ash, (%)	3.0%
11	Loss of drying @ 105°C (%)	0.21%

Interpretation

pH:

A value characteristic of an aqueous solution is its pH value, which represents conventionally its acidity or alkalinity [48].The pH scale represents the relative concentration of hydrogen ions in a solution. The concentration of Hydrogen ions is commonly expressed in terms of the pH scale. Low pH corresponds to high Hydrogen ion concentration and vice versa. In the trial drug *Nilapanai Kizhangu Chooranam* the pH is 3.05 which represents the concentration of Hydrogen ions is more when it is in the form of a solution [49].

One Research study found that acids had higher oral bioavailability and was likely to be the result of better solubility and lower clearance [50]. So, the result

concludes that the oral bioavailability of the drug *Nilapanai Kizhangu Chooram* is very high.

Ash Value

Ash is the inorganic residue left after ignition at 650-700°C. The ash content is an approximate measure of the mineral content and other inorganic matter in biomass. The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food, such as Ca, Na, K and Cl.

The quality of drugs depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability. Ash is one of the components in the proximate analysis of biological materials, consisting mainly of salty, inorganic constituents. It includes metallic salts which are important for processes requiring ions such as Na+ (Sodium), K+ (Potassium), and Ca2+ (Calcium). The ash value of *Nilapanai Kizhangu Chooranam* indicates the presence of minerals such as Sodium, Potassium and Calcium. The acid insoluble value of the drug *Nilapanai Kizhangu Chooranam* is 3.

Solubility

Solubility is the basic requirement for the absorption of the drug from GIT. Here the water soluble nature of *Nilapanai Kizhangu Chooranam* is 54 %. This nature might be helpful for the better absorption. To determine the moisture content of a sample, although occasionally it may refer to the loss of any volatile matter from the sample. Here the loss of drying value of the trial drug *Nilapanai Kizhangu Chooram* is 0.21 % @ 105°C (%) .

PHYTO CHEMICAL ANALYSIS

Table 6: Results of Phyto chemical Findings of NPKC

Phytochemicals	Test used	Chloroform	Methanol
Alkaloids	Dragendroff	-	+
Flavonoids	Shinado	+	+
Glycosides	Legal's test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride	+	-
Phytosterol	Liberman	-	-
Triterpenoids	Noller's test	+	-

Interpretation

On Phyto chemical analysis the drug *Nilapanai Kizhangu Chooranam* possess the presence of some major phytochemicals such as alkaloids, flavonoids, glycosides, tannins, triterpenoids in choloroform and methanolic extract. These phytochemicals have high range of therapeutic use. The alkaloids have more medicinal property. Some alkaloids are identified for the beneficial use for the treatment of infertility for example, a new ergot alkaloid (2-Br-alpha-ergocryptine) [51].

BIO CHEMICAL ANALYSIS

Determination of Basic Radicals

Table 7: Results of Basic radicals of NPKC

S.NO	Parameter	Observation	Result
1	Test for Potassium	Yellow colour precipitate	+ve
2	Test for Calcium	-	-ve
3	Test For Magnesium	White colour precipitate	+ ve
4	Test For Ammonium	-	- ve
5	Test For Sodium	Yellow colour	+ ve
6	Test for Iron (Ferrous)	-	-ve
7	Test For Zinc	-	-ve
8	Test For Aluminium	-	-ve
9	Test For Lead	-	- ve

10	Test for Copper	-	- ve
11	Test For Mercury	-	- ve
12	Test for Arsenic	-	- ve

Interpretation: The drug shows the presence of Potassium, Magnesium and Sodium in basic radicals determination.

Determination of Acid Radicals:

Table 8 :- Results of the Estimation of acid radicals of NPKC

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	+ ve
2	Test for Chloride	Formation of white precipitate	+ ve
3	Test for Phosphate	-	- ve
4	Test for Carbonate	-	- ve
5	Test for fluoride &oxalate	-	- ve
6	Test For Nitrate	-	- ve

Interpretation

The drug exhibits the presence of sulphide and chloride in acid radicals estimation.

AVAILABILITY OF MICROBIAL LOAD

Availability of bacterial and fungal load in *Nilapanai Kizhangu Chooranam*

Bacterial dilution

Image 7 Evaluation of Anti Bacterial Load in NPKC

Fungi dilution

Image 8 Evaluation of Anti fungal load in NPKC

Discussion

- The above results possess that the value of microbial load of bacteria and fungi of the drug Nilapanai Kizhangu Chooranam
- Here, the contamination of *NPKC* have been examined by bacterial and fungal load.
    - Total bacterial load in 10^{-4} dilution is 16 and in 10^{-6} dilution is 12.
    - Total fungal load in 10^{-2} dilution is 10 and in 10^{-3} dilution is 4.
- The load of bacteria and fungi are within the limits of WHO norms.

INSTRUMENTAL ANALYSIS

FTIR Spectrum Analysis

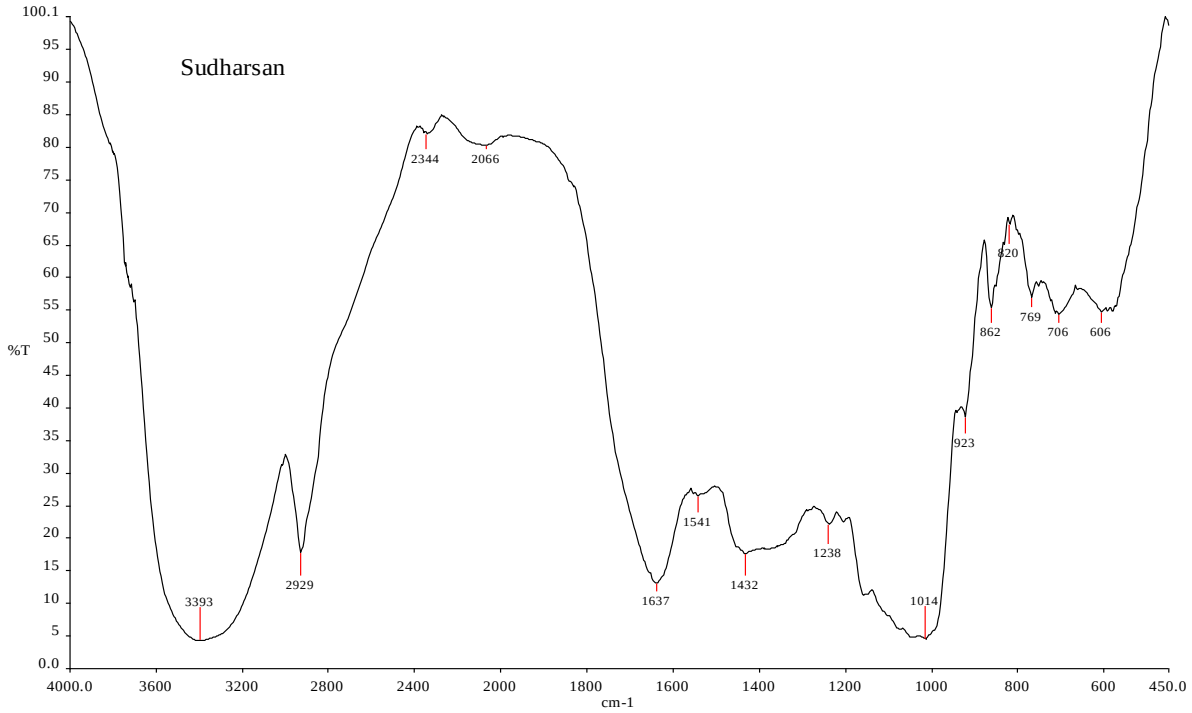


Fig 9 Image Of FTIR Spectrum

Table 9 FTIR data interpretation of NPKC

Wave number (cm-1)	Vibrational modes of NKC in IR region	Functional groups
3393	O-H Stretch, H-Bonded	Alcohols , phenols
2929	C-H stretch	alkanes
1637	N-H bend	1 amines
1541	N-O asymmetric stretch	nitrocompounds
1432	C-C stretch	Aromatics
1238	C-O stretch	Alcohols, carboxylic acids, esters, ethers
923	O-H bend	Carboxylic acids
820	C-Cl stretch	Alkyl halides
769	C-Cl stretch	Alkyl halides
606	C-Br stretch	Alkyl halides

Interpretation

In FT-IR Spectra analysis, this sample *Nilapanai Kizhangu Chooranam* exhibits the peak value at 3393, 2929, 1637, 1541, 1432, 1238, 923, 820, 769, 606 having O-H stretch, C-H stretch, N-H bend, N-O asymmetric stretch, C-C stretch, C-O Stretch, O-H bend, C-Cl stretch, C-Br stretch. This indicates the presence of some organic functional groups such as alcohols, phenols, alkanes, amines, nitro compounds, aromatics, alcohols, alkyl halides, carboxylic acids. The OH group has higher potential towards inhibitory activity against microorganisms. Sometimes the presence of Phenols in medicinal plants possess highly Anti-Oxidant property which enhances the drug effect against the disease. For example, the phenolic compound of Hypericum perforatumL[52].

SCANNING ELECTRON MICROSCOPY (SEM)

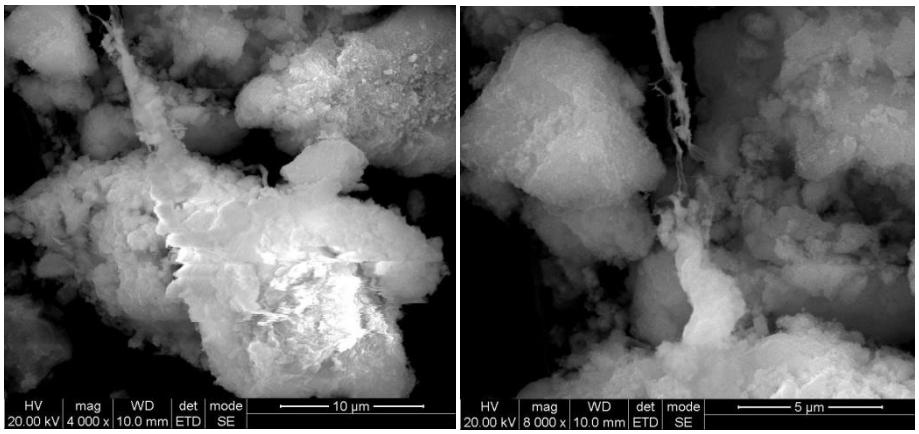


Fig 10: SEM images of NPKC

Interpretation

In the above SEM studies of the drug *Nilapanai Kizhangu Chooranam* showed objects of sizes ranging from 200nm to 500 nm. The surface of the sample grains is uniformly arranged in agglomerates. These micro sized particles of this *chooranam* helps better absorption. Thus can conclude that the therapeutic efficacy of the drug always good in nature.

XRD – X ray Diffraction Studies

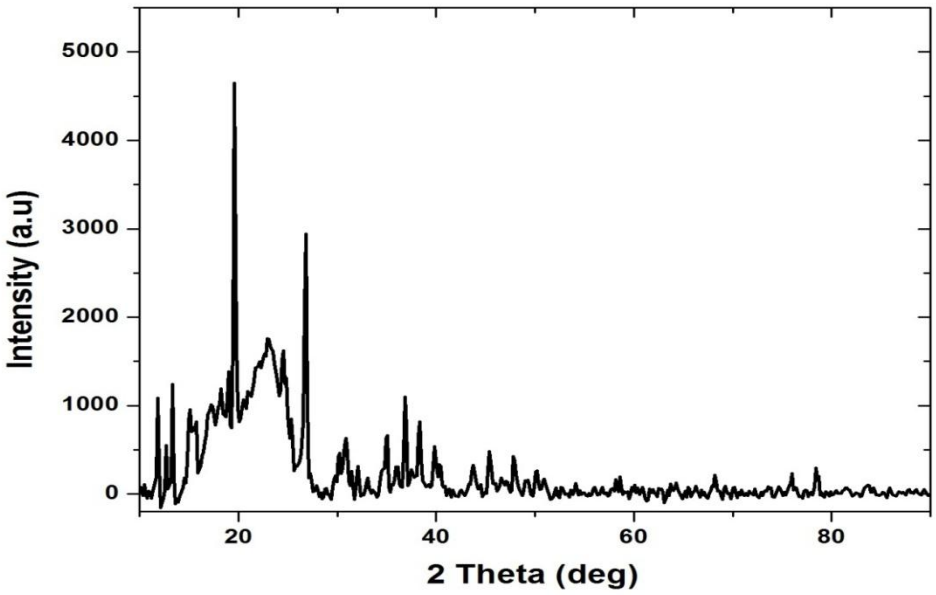


Fig 11: XRDimage of NPKC

Interpretation

XRD pattern of the trail drug *Nilapanai Kizhangu Chooranam* shows some good crystalinity. The major diffraction peaks are identified after XRD analysis. The crystaline may be due to the presence of the ingredients of the drug *Nilapanai Kizhangu Chooranam* especially due to *Karkandu* and *Seenthil Sarkarai*.

ICP-OES

Table 10: ICP-OES findings of NPKC

S. no	Elements	Detected levels
1.	Arsenic	BDL
2.	Cadmium	BDL
3.	Lead	BDL
4.	Mercury	BDL

Interpretation

From the above results, the heavy metals are observed within the permissible limits. Hence the safety of the drug *Nilapanai Kizhangu Chooranam* is ensured.

TOXICOLOGICAL RESULTS

Acute Toxicity Reports

Table 11: Findings of Acute toxicity studies of NPKC

Group	**Day**
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Dose mg/kg	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
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.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19 Respiration 20. Mortality.

Discussion

In the acute toxicity study, the rats were treated with different concentration of *Nilappanai kizhangu chooranam* from the range of 5mg/kg to 2000mg/kg which did not produce signs of toxicity, behavioural changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period. These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract. In acute toxicity test the *Nilappanai kizhangu chooranam* was found to be non toxic at the dose level of 2000mg/ kg body weight.

RESULTS OF SUB-ACUTE ORAL TOXICITY 28-DAYS REPEATED DOSE STUDY IN RATS

Body Weight:Table 12 Changes in body weight of NPKC after 28 days repeated oral dose toxicity studies.

Dose (mg/kg/day)	Days				
	0	7	14	21	28
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
100	133.99±1.16*	133.22±1.75*	139.56±1.48*	142.08±1.92*	146.92±2.03*
200	138.76±1.12**	143.05±1.48	147.29±1.72	149.35±1.62**	154.49±1.99**

Values are expressed as mean ± S.D. N=3

Organ weight

Table 13: Changes in organ weight of NPKC

Organ	Control	100 mg/kg	200 mg/kg
Liver (g)	3.07±0.20	2.73±0.61	2.48±0.33
Heart (g)	0.32±0.04	0.29±0.05	0.27±0.05
Lung (g)	0.28±0.05	0.30±0.04	0.27±0.04
Spleen (g)	0.25±0.06	0.22±0.03	0.17±0.03
Brain (g)	0.37±0.05	0.56±0.52	0.49±0.05
Kidney (g)	0.76±0.05	0.81±0.10	0.74±0.07

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=3*

Haematological parameters

Table 14: Changes Observed In Haematological Parameters Of NPKC In Rats.

Parameter	Control	100mg/kg	200 mg/kg
RBC(x 10^6/mm^3)	8.29±0.43	8.36±0.43	9.49±0.46
PCV (%)	49.66±0.77	52.45±1.61	54.52±1.62
Hb (%)	15.13±0.39	15.41±0.44	15.83±0.54
WBC(x 10^3/mm^3)	11.75±0.85	11.21±0.63	10.83±0.68
Neutrophils (%)	23.29±0.73	22.85±0.74	21.51±0.41
Lymphocytes(%)	85.5±0.46	86.07±0.49	86.80±0.29
Eosinophils(%)	4.10±0.23	3.43±0.46	2.83±0.58
Platelets(x 10^3/mm^3)	425.73±1.35	438.40±1.65	440.87±1.80

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=3*

Biochemical parameters

Table 15 : Changes Observed In Biochemical Parameters Of NPKC In Rats.

Parameters	Control	100 mg/kg	200 mg/kg
Glucose (mg/dl)	108.63±0.81	105.54±1.81	103.39±1.04
BUN (mg/dl)	22.06±1.55	22.87±1.49	24.89±0.89
Creatinine (mg/dl)	0.85±0.07	0.85±0.06	0.77±0.09
SGOT (U/L)	74.35±1.23	72.59±0.85	70.72±1.29
SGPT(U/L)	27.07±0.84	26.83±0.82	28.56±1.06
ALP (U/L)	104.63±1.14	101.17±1.71	99.84±1.50
Protein (g/dl)	8.58±0.68	7.87±0.89	7.44±0.81
Albumin (g/dl)	5.34±0.40	6.41±0.85	7.81±0.57
Total Cholesterol (mg/dl)	93.21±1.16	94.39±0.97	95.34±1.22
Triglycerides (mg/dl)	52.58±1.56	54.36±1.03	56.05±1.02

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=3*

Urine Parameter

Table 16: Changes Observed In Urine Parameters Of NPKC In Rats.

Parameter	Control	100 mg/kg	200 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Turbid
Specific gravity	1.01	1.02	1.04
pH	7.2	7.4	6.9
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelialcells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

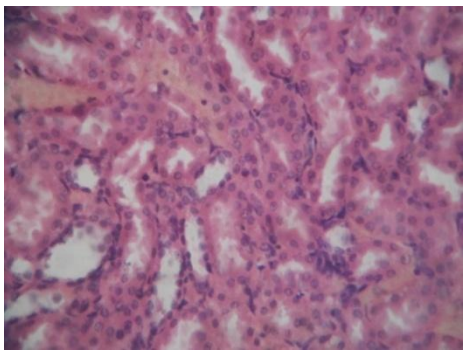
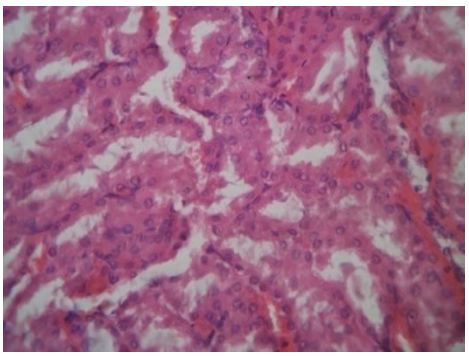
*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=3*

HISTOPATHOLOGICAL REPORTS

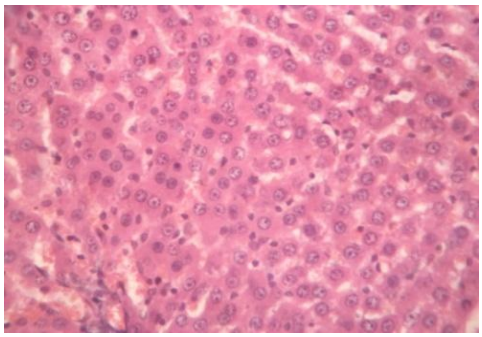
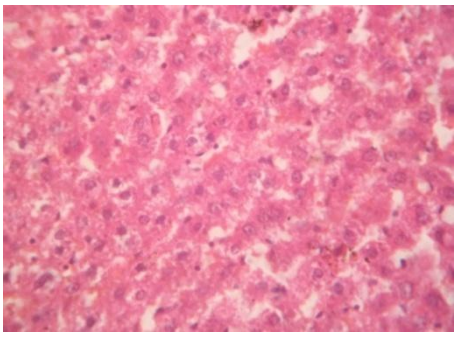
Histopathology Images Of *Nilappanai kizhangu chooranam*

(100 mg/kg) **(200 mg/kg)**

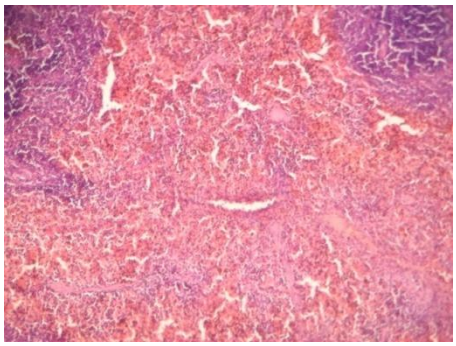
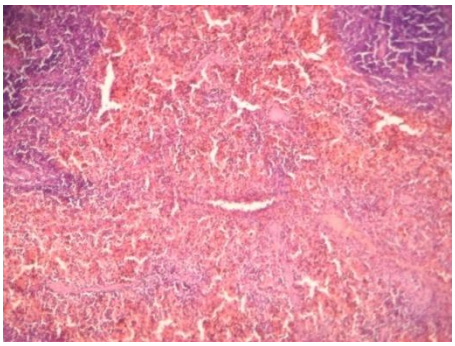
KIDNEY

LIVER

SPLEEN

DISCUSSON

In the evaluation of 28 days repeated oral dose toxicity study of the drug *Nilapanai Kizhangu Chooranam*, although the body weights were increased during treatment period, there were no significant different ($P > 0.05$) of body weights in male and female rats or between treatment and control groups. The RBC lymphocytes and neutrophils and coagulation parameters did not show any biologically or statistically significant differences between rats treated or controls. In hematological and biochemical examination, all the observations lie in the reference range. There is no change in the biochemical and haematological parameter when compared with the control groups

Furthermore no dose related histopathological changes were observed. Gross examination in necropsy and at microscopic examination revealed no changes that attribute to the administration of drug.

Compared with concurrent controls, rats fed with *trail drug Nilapanai Kizhangu Chooranam* showed no changes in clinical chemistry and hematology values at various dosages.

There were no significant different changes of organ-to-weight ratios in male and female rats or between treatment and control groups.

Treating Wister albino rats with *Nilapanai Kizhangu Chooranam* at levels of 100 and 200 mg/kg/day to male and female rats for 4 weeks did not cause death and was not associated with adverse effects in general condition, growth, body and organ weights, hematology and clinical chemistry values, nor did it cause abnormalities in necropsy and histopathology findings.

According to these results, *Nilapanai Kizhangu Chooranam* could be considered as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be of no toxicological concern.

PHARMACOLOGY STUDIES

Evaluation Of Spermatogenic Activity Of *Nilapanai Kizhangu Chooranam* in wistar albino male rats by ethanol induced method.

Spermatogenic activity

Decrease in sperm count observed after administration of ethanol was reversed by treating with the study drug *Nilapanai Kizhangu Chooranam*. In the following tables the parameters like Sperm count, Motility, Viability and Morphology of NPKC 200 mg /kg treated group shown significant increase than ethanol alone treated group(negative control).In testis histopathological of negative control (group II)animals showed germinal damage and treated group shows significant restoration. From these results it is obvious that *Nilapanai Kizhangu Chooranam* has potent spermatogenic activity.

Spermatogenic Activity Of Nilapanai Kizhangu Chooranam

Table 17 : Effect of Nilapanai Kizhangu Chooranam on sperm count and motility.

Groups	Intervention	Sperm Count	Sperm Motility(%)
Group I	1 ml of Milk	208.16±10.13	88.13±5.20
Group II	25% of ethanol (0.5 ml/kg/day)	72.66±0.52	27.09±0.53
Group III	NPKC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	124.94±0.91*	64.61±0.48*
Group IV	NPKC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	155.04±1.17**	68.82±0.38**

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Chart no: 1 Effect of *Nilapanai Kizhangu Choranam* on sperm count

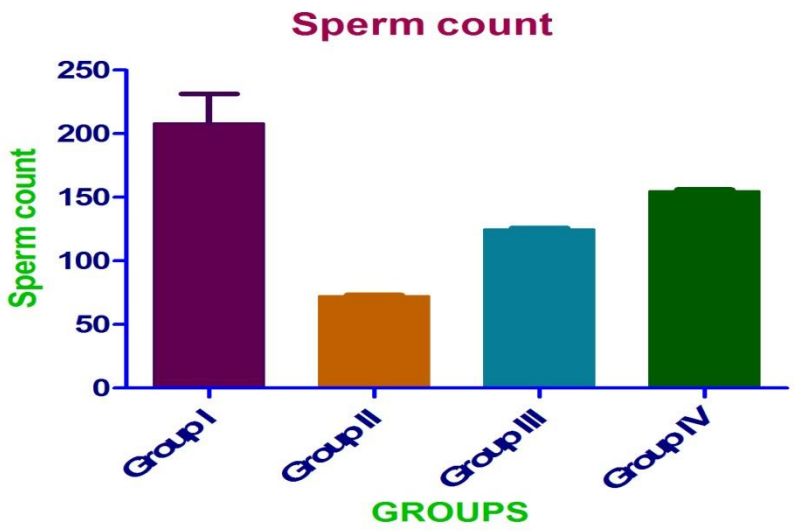


Chart no: 2 Effect of *Nilapanai Kizhangu Chooranam* on sperm motility

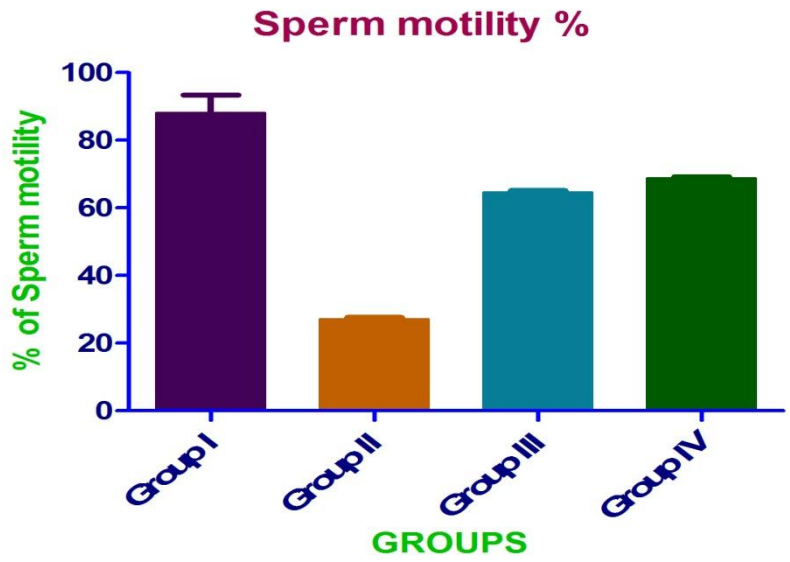


Table 18: Effect of *Nilapanai Kizhangu Chooranam* on sperm morphology and viability

Group and intervention	Morphology		Viability
	Normal	Abnormal	
Group I [C]	78.74±5.86	21.36±5.97	70.34±2.16
Group II [IG]	30.43±2.76	71.39±1.90*	47.09±0.49
Group III [TG-1]	71.49±11.48	25.67±11.54	65.33±0.72
Group IV [TG-2]	77.23±5.62	21.08±5.86	67.89±0.71

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Chart no: 3 Effect of *Nilapanai Kizhangu Chooranam* on morphology

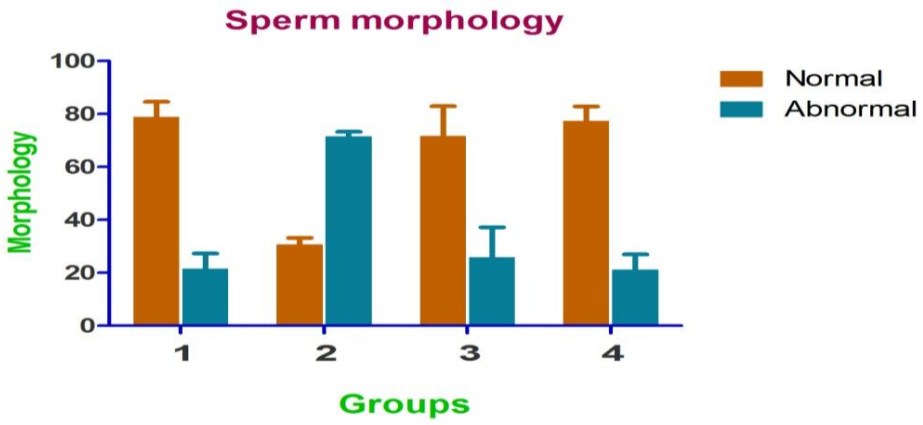


Chart no 4 Effect of *Nilapanai Kizhangu Chooranam* on sperm viability

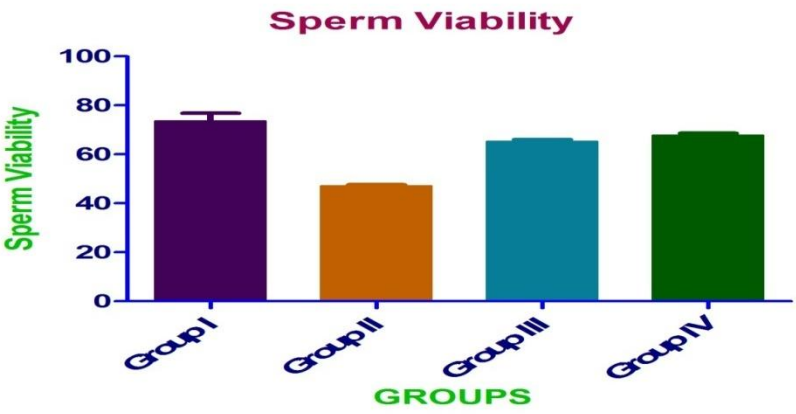


Table 19: Effect of *Nilapanai Kizhangu Chooranam* on body and testis weights

Group and intervention	Body weight(g)		Testis weight (g)
	1 st day	60 th day	
Group I [C]	197.6±3.46	280.36±5.97	13.08±1.33
Group II [IG]	198.2±2.91	223.79±1.90	10.33±1.43
Group III [TG-1]	200.12±6.48	285.47±3.54	12.01±0.89**
Group IV [TG-2]	198.58±5.62*	297.75±4.86*	13.15±1.00

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Chart no: 5 Effect of *Nilapanai Kizhangu Chooranam* on body weight

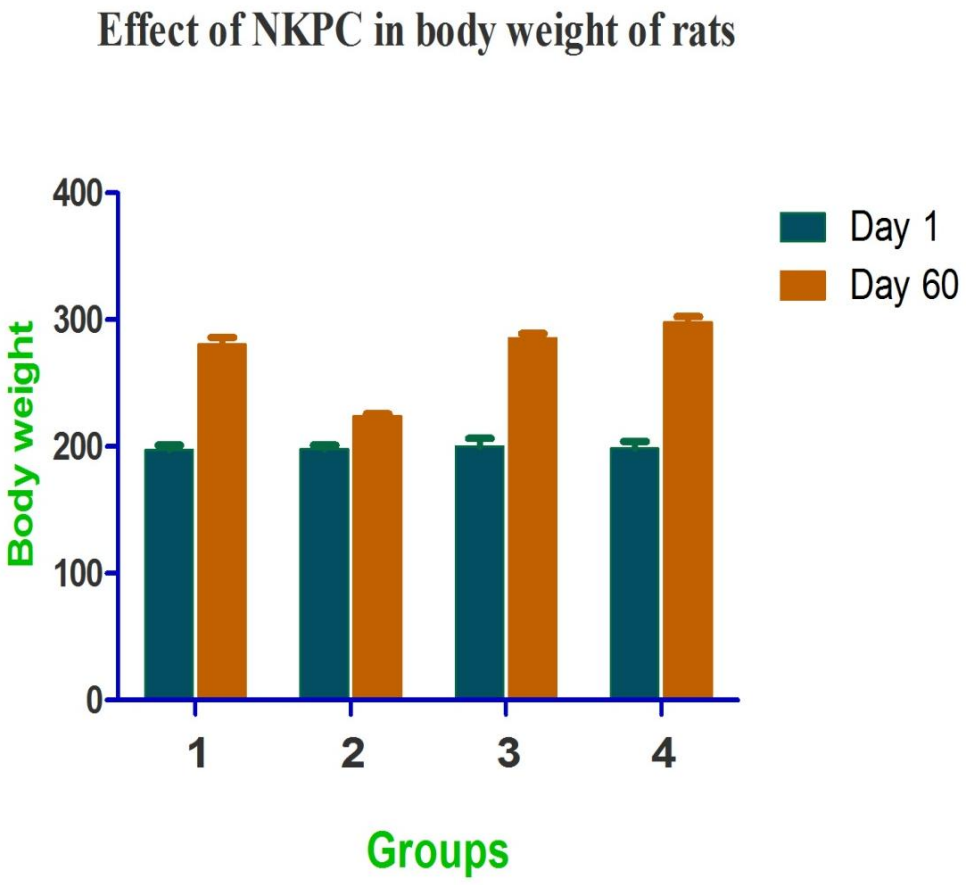
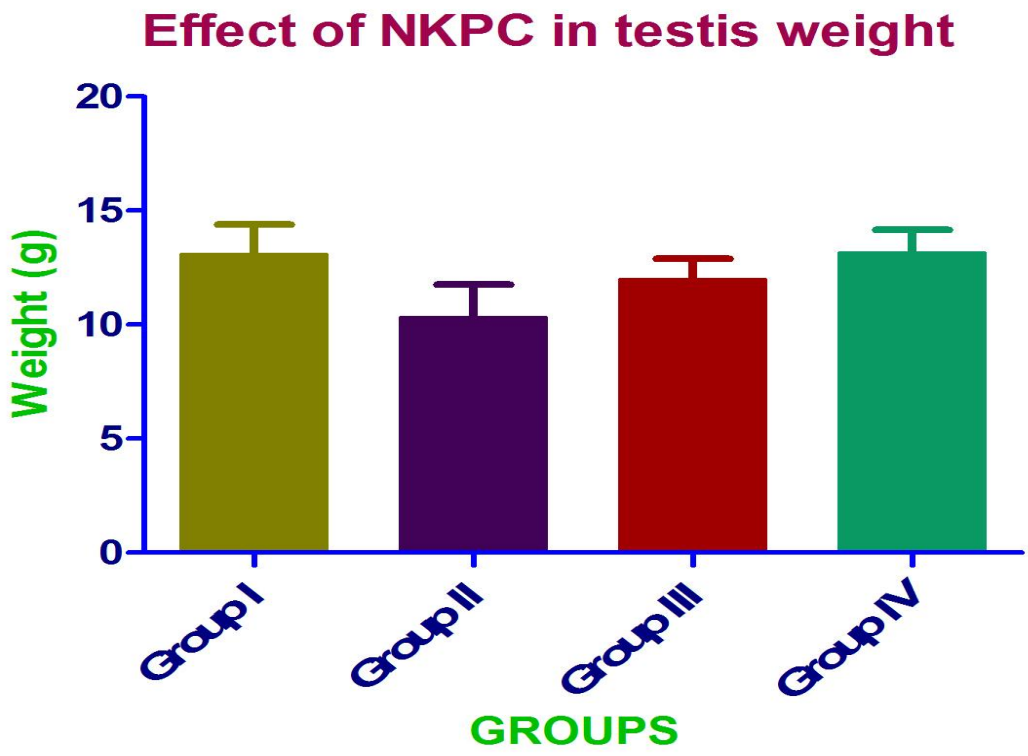


Chart no: 6 Effect of *Nilapanai Kizhangu Chooranam* on Testis weights



DISCUSSION:

From the above obtained results, can conclude that the drug *Nilapanai Kizhangu Chooranam* exhibits the spermatogenic activity by increasing the number of spermatozoa in semniferous tubules .The trial drug possess significant Spermatogenic activity at the dose levels of 100 and 200 mg/kg of body weight. usally plant products play a major role in the functioning of spermatogenesis like ashwagandha. Likewise the drug *Nilapanai Kizhangu Chooranam* also possess potent spermatogenic activity[53]. Already the ingredients of this NPKC formulation Curculigo orchioides, mucuna pruriens were proved as a potent drug for spermatogenesis[54, 55]. So, this evaluation of spermatogenic activity study will lead the way for the clinical use of the drug.

Evaluation of Aphrodisiac activity of *Nilapanai Kizhangu Chooranam* in Ethanol treated male rats.

Table 20: Grouping of Animals For The Evaluation of Aphrodisiac Activity of NPKC

Groups	**MF**	**IF**	**ML (sec)**	**IL (sec)**	**EL (min)**	**PEI (min)**
Control	3.7±0.34	1.61±0.25	282.94±4.09	655.88±3.44	2.88±0.39	5.15±0.24
Ethanol treated rats	0.76±0.11	1.09±0.29	371.46±6.38	978.29±2.07	5.63±0.37	12.73±0.28
Ethanol+ *NPKC* 200mg/kg	2.43±0.37	1.33±0.28	217.31±5.56	445.13±4.04	4.79±0.39	8.91±0.33
Ethanol+*NPKC* 400mg/kg	4.19±0.27	1.62±0.34	171.35±2.93	321.93±4.32	3.37±0.49	7.43±0.42

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Chart 7: Mount frequency observed in the evaluation of aphrodisiac activity of NPKC

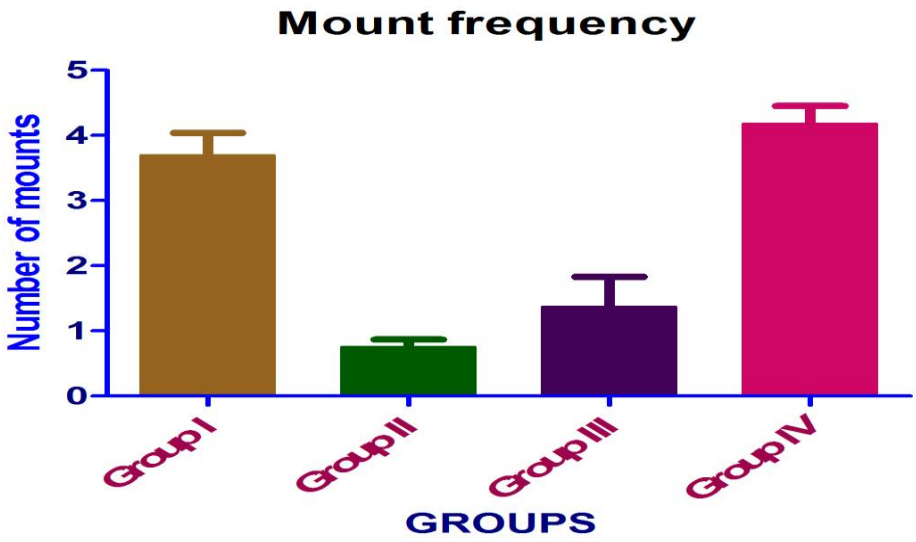


Chart 8 : Intromission frequency observed in the evaluation of aphrodisiac activity of NPKC

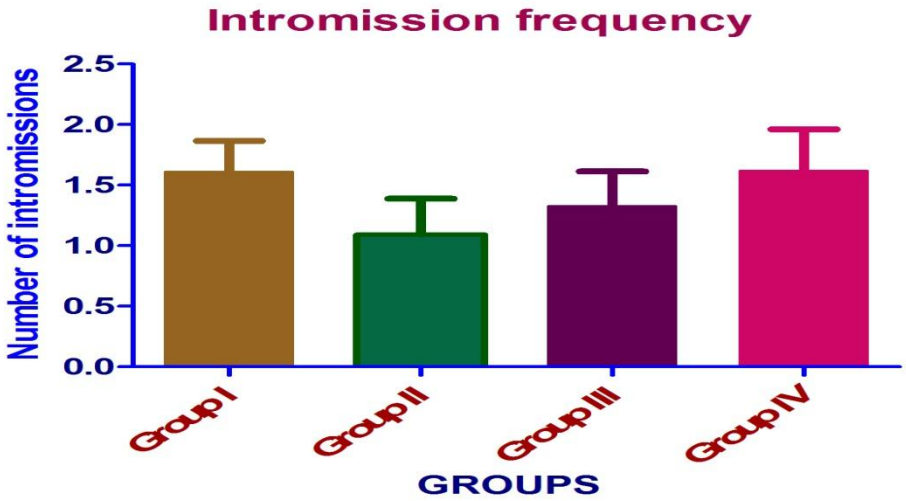


Chart 9: Mount latency observed in the evaluation of aphrodisiac activity of NPKC

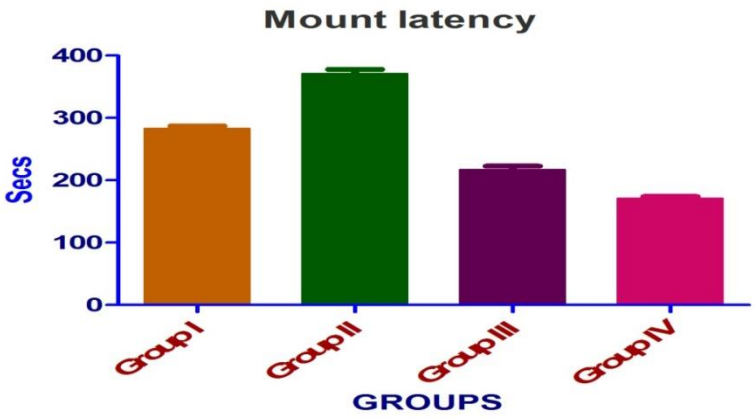


Chart 10: intromission latency observed in the evaluation of aphrodisiac activity of NPKC

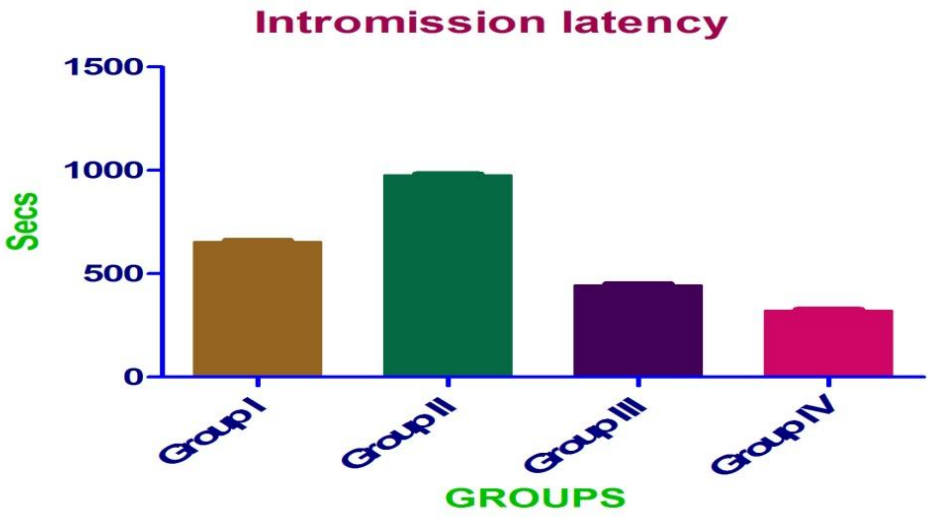


Chart 11: Ejaculation latency observed in the evaluation of aphrodisiac activity of NPKC

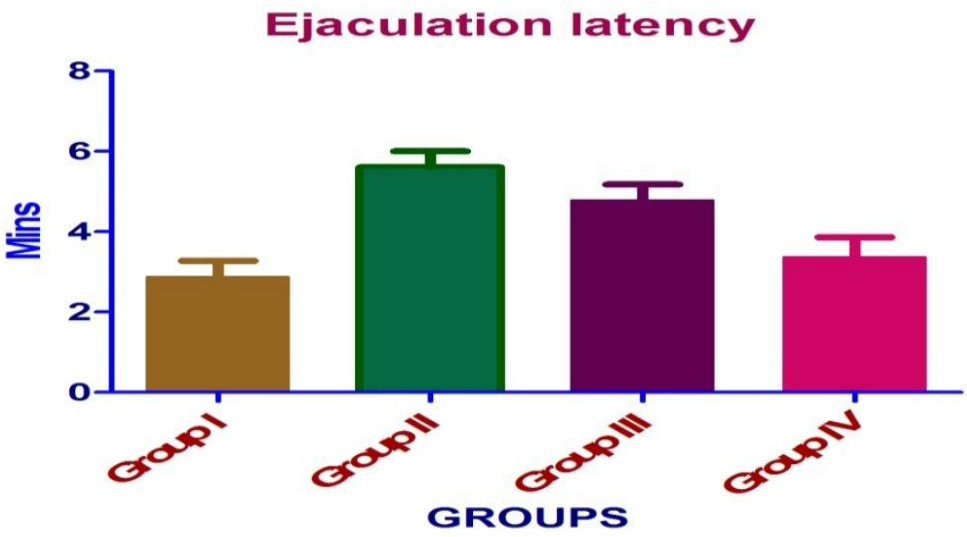
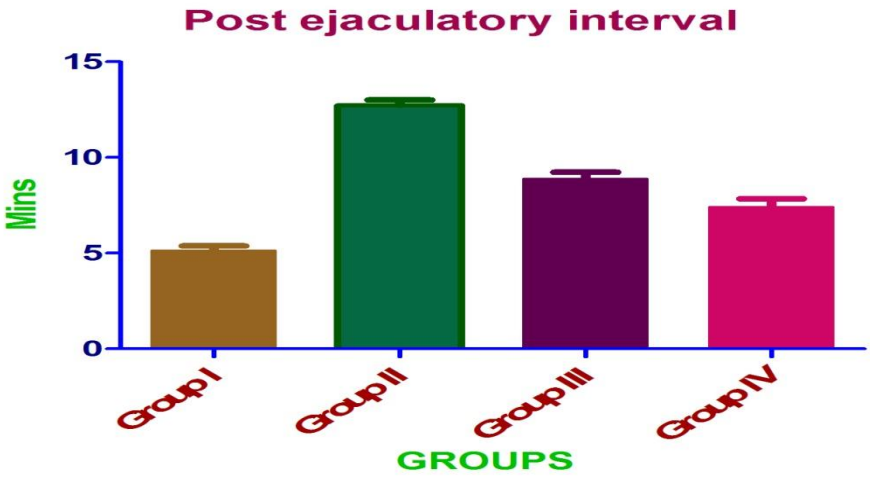


Chart 12: Post Ejaculation Interval observed in the evaluation of aphrodisiac activity of NPKC



DISCUSSION

From the observed results of mount frequency, intromission frequency, mount latency, intromission of latency, ejaculation latency and post ejaculation latency can confirmed that the trail drug *Nilapanai Kizhangu Chooranam* has the property of Aphrodisiac. The study concluded that the cumulative dose of *Nilapanai Kizhangu Chooranam* could enhance overall sexual function and performance in male rats by increasing the spermatozoa concentration and hormonal levels such as FSH, Testosterone, LH. The drug *Nilapanai Kizhangu Chooranam* showed more potent aphrodisiac activity at the dose level of 200mg/Kg BW and 400mg/Kg BW. The results suggest that the prepared *Nilapanai Kizhangu Chooranam* may be a new promising aphrodisiac combination, which can be used to improve the sex life of many troubled men. Already the scientific literatures are available regarding the evaluation of aphrodisiac nature of herbals[56].This aphrodisiac property may be due to possible synergistic action of selected plants used in this *Nilapanai Kizhangu Chooranam*.

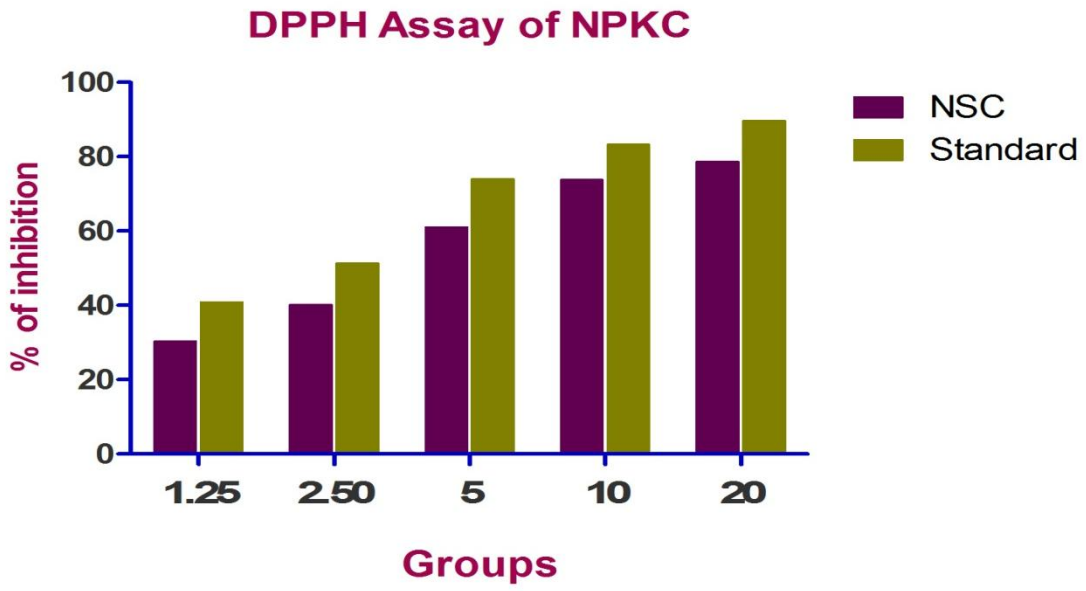
Evaluation Of The Anti Oxidant Activity Of *Nilapanai Kizhangu Chooranam* By DPPH Scavenging Assay

Table 21. Anti-oxidant activity of NPKC

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.1952	0.278	30.26	40.89
2.50	0.1678	0.202	40.22	51.25
5	0.1489	0.084	61.06	74.07
10	0.1295	0.052	73.78	83.33**
20	0.1069	0.034*	78.66*	89.62

*Values are expressed as mean ± S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control;*

Chart 13: Antioxidant Activity Of NPKC



DISCUSSION

From the investigation of DPPH radical scavenging assay of NPKC it was concluded that the test drug has shown promising antioxidant activity and exhibits significant percentage inhibition against DPPH radicals when compared to that of standard BHT. Because of this high antioxidant therapeutic nature the drug *Nilapanai Kizhangu Chooranam* will helps to treat the male infertility. Antioxidants play a major role in the treatment of male infertility [57-60]. So the presence of antioxidant property of NPKC will be highly useful fot the treatment of male infertility.

6. CONCLUSION

- The drug "*Nilapanai kizhangu chooranam*" was prepared as per the classical Siddha literature.
- It fulfills all the standardization parameters of *Chooranam* as mentioned in AYUSH guidelines.
- The results of biochemical analysis showed the presence of acid and basic radicals in the sample.
- The presence of alkaloids, flavonoids, glycosides, tannins, triterpenoids were identified in the choloroform and methanolic extract of *Nilapanai kizhangu chooranam* through Phytochemical analysis.
- FT-IR Study results exhibits the presence of some organic functional groups such as alcohols, phenols, alkanes, amines, nitro compounds, aromatics, alcohols, alkyl halides, carboxylic acids.
- SEM analysis showed the objects of sizes ranging from 200nm to 500 nm.
- XRD pattern of the trail drug *Nilapanai kizhangu chooranam* shows some good crystalinity.
- Based on OECD 423 the trail drug *Nilapanai kizhangu chooranam* is considered as non toxic up to the dose of 2000mg/kg.
- The drug *Nilapanai Kizhangu Chooranam* possess the potent Aphrodisiac activity in ethanol treated male rats.
- The drug *Nilapanai kizhangu chooranam* could be confirmed as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be of no toxicological concern.
- And the drug NPKC exhibits effective Spermatogenic activity in wistar albino male rats by ethanol induced method.

- High antioxidant therapeutic nature was evaluated in the drug *Nilapanai Kizhangu Chooranam* through DPPH Scavenging assay. Because of this anti-oxidant property, it can be used as a drug to treat the male infertility.
- Hence it is proved that the drug Nilapanai kizhangu chooranam was pharamacollogicaly evaluated for the property of aphrodisiac, spermatogenic and anti oxidant activity.
- And through the Instrumental analysis, the fingerprints was created for the standardization of the drug *Nilapanai kizhangu chooranam*.

7. SUMMARY

Herbal preparations in Siddha system of medicine always have a unique range of beneficial effect because of its wonderful therapeutic value without causing adverse effects. Here the drug *Nilapani Kizhangu Chooranam* was pharmacologically evaluated and scientifically validated and standardized.

The herbal drug *Nilapanai Kizhangu Chooranam* was prepared as per the classical Siddha text and it was subjected to many analysis like physico chemical analysis, biochemical analysis, Instrumental analysis using the modern sophisticated analytical equipments and those results were interpreted.

The drug *Nilapanai Kizhangu Chooranam* was proved that it is free from toxicity through the acute and 28 days repeated oral toxicity study as per the OECD guidelines. In acute toxicity study there was no mortality of rats observed. In 28 days repeated oral toxicity study, the obtained results of haematological, biochemical, urinary parameters were normal. The histopathological findings did not show any abnormalities.

And this herbal formulation possess more potent Aphrodisiac activity in ethanol treated male rats. This trial drug *Nilapanai Kizhangu Chooranam* exhibits high range of Spermatogenic activity in ethanol induced male rats.

Most of the research findings already reported that the role of antioxidant is essential to treat male infertility. Here the trial drug possess strong antioxidant activity.

These results conclude that the drug *Nilapanai Kizhangu Chooranam* is a best drug of choice for the treatment of male infertility.

By analyzing all those findings, it is proved that the drug *Nilapanai Kizhangu Chooranam* has high range of therapeutic value. And the safety of the drug for clinical use is ensured. So, it is confirmed that the herbal formulation *Nilapanai Kizhangu Chooranam* may never cause any adverse effects in clinical use. The preparation of the drug is cost effective too.

So, the clinical trials have to be followed on the drug *Nilapanai Kizhangu Chooranam*. Thus can treat the infertile males effectively and help them to achieve their dream of producing their own offspring.

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