SCIENTIFIC VALIDATION OF OVULATION INDUCING, GONODOTROPHIC RELEASING,AND ANTI-OXIDANT ACTIVITIES OF *"SAMBIRANI POO KULIGAI"*

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**Scientific Validation of Ovulation Inducing, Gonodotrophic releasing and Anti-Oxidant activity of *SAMBIRANI POO KULIGAI***" is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Karolin Daisy Rani M.D(S),** 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**

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Scientific Validation of Ovulation Inducing, Gonodotrophic releasing and Anti-Oxidant activity of *SAMBIRANI POO KULIGAI*"** 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 **M. Vijibala** 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 & Signature of the Guide**

Place: Chennai **Dr.R.Karolin Daisy Rani M.D(S).**

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **"Scientific Validation of Ovulation Inducing**, **Gonodotrophic releasing and Anti-Oxidant activity of *SAMBIRANI POO KULIGAI*"** is a bonafide work carried out by **M.Vijibala** under the guidance of **Dr.R.Karolin Daisy Rani M.D(S).,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

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Date: **Date:**

Place: Chennai **Place:** Chennai

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ABBREVIATIONS

Alb	Albumin
ANOVA	Analysis Of Variance
CL	Cholesterol
CMC	Carboxyl methyl cellulose
DC	Differential count
Dep	Deposits
DLS	Dynamic Light Screening
DPPH	DiphenylPicrylhydrazil
E	Eosinophil
ESR	Erythrocyte Sedimentation Rate
FPC	Few Pus Cells
FSH	Follicle stimulating hormone
FTIR	Fourier Transform Infrared Spectroscopy
GnRH	Gonodotrophin Releasing Hormone
Hb	Haemoglobin
HPTLC	High Performance Thin Layer Chromatography
IAEC	Institutional Animal Ethical Committee

L	Lymphocyte
LH	Leutinizing hormone
mg	milligram
NIH	National Institutes of Health and Human development
NTA	Nuclear Tracking Analysis
OECD	Organisation for Economic Co-Operation and Development
OGTT	Oral Glucose Tolerance Test
P	Polymorphs
PCOS	Poly Cystic Ovarian Syndrome
PCS	Pus Cells seen
SPK	Sambirani Poo Kuligai
SHBG	Sex Hormone Binding Globulin
SEM	Scanning Electron Microscope
TC	Total count
TLC	Thin Layer Chromatography
WHO	World Health Organization

1.INTRODUCTION

"WOMEN ARE THE REAL ARCHITECTS OF SOCIETY"

- "HARRIET BEECHER STOWE"

Women are the most precious and wonderful creature of God. Women play variety of significant roles in our society from their birth till the end of life. Women are considered as the goddess in the Indian society from the ancient time however it is also true that they are not treated as goddess. Nowadays she suffers from lot of health related issues. Women have to play different roles in their life such as daughter, wife, sister, mother, grand-mother, mother-in-law, etc. Women also play very important roles for continuing of life on Earth.

Women are more responsible than men in child-bearing and child-rearing. Women make home, society and country. The social role of women varies from home-makers to bread-earner of the family. In both the cases, they do not take care of their health. They spend more time to help others and they don't take time for themselves. A women being healthy throughout her life is a greatest asset for the Nation [1].

So a special attention for women's health is essential for forming a better Nation. Women health is positioned within the wider body of knowledge cited by WHO, because it places importance on gender as a social determinant of health. Apart from women's good health, reproductive health of women is considered more important within the framework of WHO.

The WHO defines reproductive health as "It is a condition in which the reproductive process is accomplished in a state of complete physical, mental, social well-being and is not merely the absence (or) disorders of the reproductive process".

Currently many health related programs are implemented and it stresses the importance of women's health. Ultimately she is the deciding factor for the country and the continuity of life on Earth. Women are facing frequently some health related concerns such as menstruation, pregnancy, birth control, menopause and post menopausal issues.

Nowadays diseases like Breast Cancer, Cervical Cancer, Ovarian Cancer etc… are also seen in women.

Out of these Polycystic Ovarian Syndrome is commonly seen in young age women. PCOS was originally described in 1935 by Stein and Leventhal as a syndrome manifested by Amenorrhoea, Hirsutism, Obesity associated with enlarged polycystic ovaries.

The exact cause of the disease is still unknown. Several factors including genetics also play a role. It is an endocrine disorder in which the hormonal cycles are interrupted [2].

PCOS is extremely prevalent and probably constitutes the most frequently encountered endocrine disorder in women of reproductive age. Primary care providers do not commonly appreciate that the syndrome is associated with significant morbidity in terms of both reproductive and non-reproductive events. Having the disorder may significantly impact the quality of life of women during reproductive years and it contributes to morbidity and mortality by the time of menopause. Most importantly there was a high prevalence of diabetes [16%] and hypertension [40%]. Between 1 in 20 women of child bearing age are affected by PCOS world wide. In India nearly 40% of women are affected by PCOS.

The symptoms for PCOS are

- Irregular periods or no periods
- Fertility problems
- Hirsutism
- Weight gain
- Thinning of hair
- Oily skin
- Skin tags

The symptoms for PCOS are not same for all. It differs from person to person according to their condition of the body. It is a growing problem of adolescent girls. Early diagnosis of PCOS in adolescent girls is very important. Several diagnostic criteria's are available for PCOS.

- NICHD in[1990]
- Rotterdam[2003]
- AE-PCOS society [2009]

Due to the lack of specific diagnostic test for PCOS the confusion remains till now. Many women are still not aware that they have PCOS. Women with PCOS are prone to many complicated risk factors. If PCOS is not treated earlier it can be a precursor to a variety of serious health conditions.

The following are the complications of PCOS

- Type2 diabetes
- Infertility
- Elevated lipids
- Increased risk of endometrial cancer
- High blood pressure
- Myocardial infarction [3].

Apart from the risk factors it totally stresses the patient. Due to obesity the total structure of the body will changes. This causes low self-esteem and depression. They are not able to conceive. If women with PCOS are able to conceive they have a higher incidence of miscarriage, gestational diabetes, pre-eclampsia and premature delivery[4] .Male type of hair growth and male type baldness all these things raises their stress level to a higher extent and pushes them in a depressive state.

Especially women with PCOS should be free from stress, because it increase the stress hormones resulting in excessive eating, weight gain, anxiety, depression. Because of stress and depression it diminishes the natural healing capacity of the body.So an effective management of stress is important to women with PCOS because they are more sensitive to stress than other women.

Prevention of PCOS is very important. Because of lack of specific etiological factor, there is no specific prevention. By conducting awareness programs among adolescents

may create the safety and prevention for the disease. In obese persons weight loss is very important to prevent some complicated risk factors in future.

Recently many advanced treatments are available for PCOS. Life style modifications play an important role by eating balanced diet and reduces their weight by doing regular exercise. Ovarian drilling, Hormonal therapies, Anti-androgens, Regulating insulin and blood glucose levels and Birth control pills are all focusing to improve the condition[5].

There are lots of treatments available for Polycystic ovarian syndrome with different pros and cons like partial cure or complete cure. All these treatments are not curing PCOS completely but they only help to manage the condition. Though they bag side effect along with the treatment.

In order to overcome all these problems, the women society needs a safe and potent medicine for this precarious problem. The traditional systems of medicines are significantly more popular because of the curative property with minimal side effects. Among the traditional systems in India, Siddha system of medicine is one of the ancient systems of medicine compiled by Siddhars. Siddhars had found and develop a unique value for the system of their own. They lived a spiritual life and they served the people to cure disease.

The history of Siddhars is not confined to a particular period. It seems to be a continuum, they know the secret of deathless life and they are still living unknown and unseen. Another possible explanation is that those who achieved “perfection” are called Siddhars. Out of the eighteen Siddhars, Thirumular one of the renowned Siddhar says that one who practices yoga to attain perfection becomes Siddhar. In his period only Siddha philosophy and doctrines attain literary expression.

The Siddha system of medicine is used not only to cure, but also to prevent diseases and in turn increase the life span of human beings. The efficacy and excellence of Siddha medicine is not only for the physical body but also for the soul.

In Siddha medicine the peculiarity is that it doesn't refer to medicine alone but it also includes several other branches of knowledge such as Pharmacology, Alchemy, and Yoga.

The system has passed different ages but its uniqueness and eternity will never change and remains as same. In Siddha system the solar rays, radiation absorption,evaporation and precipitation play a very important role in making of medicine [6].

Mostly they used herbal, mineral, and animal products as raw materials in various formulation. The herbs are dispensed in the form of *Decoctions, Karkam, Chooranam, Pills, Lehyams, Nei and Manappagu*. Other than these they prepare higher order medicines like *Chendooram , Chunnam, Pathangam, Kattu, Kalangu* etc.

They followed the Humoral pathology and explains that all diseases are caused by the mixture of three cardinal humors namely Wind, Bile and Phlegm and their relative proportion are responsible for a person's physical and mental qualities.

They preferred the type of medicine based on the severity of the disease. Another most important peculiarity is that they prepared semi synthetic drugs in that ancient days which is popular now a day. They told under the heading "*VAIPPU MURAIGAL*". Because all the raw materials are not available throughout the year. In order to overcome this non availability of certain herbals, they prepared semi synthetic drugs instead of the original drug [7].

They have plenty of medicinal preparations in their treasury. In order to secure women's health both physically and mentally, there is a need for proper and safe treatment rather than the existing treatment in modern medicine which is expensive with more complications.

According to Siddha, *Soothaga vayu* may be compared to Polycystic ovarian syndrome, which is characterized by *vayu* accumulation in the uterus may lead to Amenorrhoea, lower abdomen pain, head ache, low back ache etc [8].

Siddha medicine plays an effective role in treating PCOS. *SAMBIRANI POO KULIGAI* is one such medicine indicated for Soothaga vayu, as per the classical Siddha text

Agasthiyar Paripuranam 400. The ingredients of this medicine are *Styrax benzoin, Syzygium aromaticum, Fel bovinum purifactum* and *Piper bete*l juice. This medicine is prepared by two process. First Pathangam is obtained from sublimation of *Styrax benzoin* and then *Kuligai* is obtained from the remaining materials. Once a material under goes sublimation it converts into Nano particles and it is easily bio available to the body. The end product always has a unique chemical nature of its own. So the sublimate itself is more effective. In addition to this grounding the remaining ingredient with *Piper betle* leaf juice enhance the potency of the drug effectively. So the combination of these two processes makes the drug more valuable than the others.

Out of which the polyherbal formulation *SAMBIRANI POO KULIGAI* possessing ovulation inducing activities in many ways by inducing ovulation and maintains the hormone level have not been scientifically explored till now. So this is a right time to explore the nature of the drugs by using advanced research methodologies and techniques.

To meet the Global Standard, this is a wonderful opportunity for validating Siddha medicinal preparation scientifically. Hence validating the efficacy of *SAMBIRANI POO KULIGAI* through my work will be an eye opener for this women society. Keeping in mind in building a strong nation, the research surely bears a valuable impact with it.

2. AIM AND OBJECTIVES

Aim

The present investigation was aimed to validate the safety and efficacy of the Siddha polyherbal formulation *'**SAMBIRANI POO KULIGAI"*** for Ovulation Inducing Activity in Female Wistar Albino Rats.

Objectives

The objectives of this work were done through the following steps.

- Collection of relevant literature from classical Siddha texts as well as Modern sciences that supported this study.
- Description of pharmacognostic features of the plant in this formulation including the taxonomic identification, collection, purification of plants etc.
- Preparation of the drug according to the procedure described in Siddha literature**.**
- Standardisation of the trial drug by means of physico-chemical analysis, phyto chemical analysis.
- Revealing the anions and cations present in the drugs through proximate chemical analysis.
- Elucidation of the chemical structure, microscopical structure of the drugs by means of instrumental analysis.
- Interpreting the results of acute and repeated 28 days oral dose toxicity of **"*SAMBIRANI POO KULIGAI"*** according to OECD guidelines 423 and 407.
- Detailing the study of pharmacological activities like
  - Ovulation inducing activity
  - Estimation of FSH, LH, Estradiol and Progesterone levels
  - Anti-oxidant activity of the trial drug ***SAMBIRANI POO KULIGAI*** in female Wistar albino rats.

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. SIDDHA ASPECT OF THE DRUG

சாம்பிராணி பூ குளிகை

போமப்பா வின்னமொரு குளிகை கேளாய்
புண்ணியனே சாம்பிராணி முதற்ற ரந்தான்
நாமப்பா பளிங்காக நாலுபலம் வாங்கி
நன்றாகப் பொடிசெய்து சட்டி யிட்டு
ஓமப்பா மேற்சட்டிக் குள்ளே நீயும்
உத்தமனே காகிதத்தை யுருப்போ லொட்டி
நாமப்பா மேன் மூடிச் சீலை செய்து
நன்மையுள்ள வடுப்பேற்றித் தீபம் வையெ.....
வைத்தக்கால் பசுநெய்யாற் றீப மேற்றி
மைந்தனே யடிச்சட்டித் தூறிற் சூடு
தைத்தாக்கா னாற்சாமஞ் சென்ற பின்பு
தண்மையுடன் தானிறக்கிப் படுக்கப் போடு
செய்ததொரு சீலைமண் ணவிழ்த்து மெல்லத்
திறமான மேற்சட்டிக் காகி தத்துள்
நைத்ததொரு வப்பிரக பற்பம் போலே
நன்மையுள்ள வாசமதாய் மணக்கும் பாரே.....
பாரப்பா வப்பூவை யெடுத்து நீயும்
பாங்கான வெற்றிலைபாக் குண்ணும் போது

நேரப்பா மிளகளவு தின்று வந்தால்

நேர்மையுள்ள தாதுவிர்த்தி நிலைக்கும் பாரு

காரப்பா வப்பூவுங் கழஞ்சி ரண்டு

கனமான கிராம்புமோ கழஞ்ச மூன்று

கூறப்பா கோரோசனை கழஞ்ச தொன்று

கூட்டியரை வெற்றிலையின் சாற்றினாலே.....

சாற்றினா லிருநாழி யரைத்து மைந்தா

தண்மையுள்ள குன்றிபோற் குளிகை கட்டி

ஆற்றிநன்றாய் நிழல்தனிலே பதனம் பண்ணி

அப்பனே தேனிலொரு குளிகை யீந்தால்

போற்றியே மூன்றுநா ளாறு போதில்

பொல்லாத சூதத்தின் சுரங்கள் தீரும்;;

பார்த்திபனே நெய்மிளகி லீந்தா யானால்

பறந்துபோஞ் சூதகத்தின் சயங்க டானே.....

சயமான சர்க்கரையி லேழு நாள்தான்

தண்மையுடன் றாங்கொடுக்கத் தயவாய்க்கேளு

மயமான சூதகத்தின் வாய்வி னோடு

மைந்தனே யுதிரத்தின் கட்டுத் தீரும்

நயமான வெற்றிலையில் மென்று தின்ன

நன்மையில்லாச் சுகசந்நி நாடா தோடும்

பயமென்ன பத்தியங்கள் பயமோ யில்லை

பரமான பூரணத்தின் பதிவு தானே.....

- அகஸ்தியர் பரிபூரணம் 400

The following are the Ingredients

SAMBIRANI-Styrax benzoin

KIRAMBU-Syzygium aromaticum

KOROSANAI-Felbovinum

VETRILAI-Piper betle

***SAMBIRANI*-BENZOIN**

The tree is also a native of Siam, Java, Sumatra and Borneo.

Benzoin is the juice obtained by making incisions into the tree when it will be found deposited on the bark. Being hardened to the exposure air.

Other names:

Pen kumainjan

Dhubam

Mallaka sambirani

Vernacular names:

English	-	Benzoin, Indian olibanum tree
Telugu	-	*Sambirani*
Malayalam	-	*Sambirani*
Kanada	-	*Sambirani*
Sanskrit	-	*Deva-dhupika*
Arab	-	*Luban*
Persian	-	*Hasnlubah*
Hindhi	-	*Oud*
Duk	-	*Oud*
Part used	**-**	Resin
Taste	**-**	Pungent
Character	**-**	Heat
Division	**-**	Pungent

Actions:

Stimulant, Expectorant, Counter irritant, Diuretic

General Properties:

வாதசீ தங்கண்ணோய் மாறாத் தலைவலியும்

ஓதமுறு பீனசமும் ஓட்டுங்காண் - பூதலத்தில்

வேம்பிதுதான் என்ன மிகுகசப்பை வாய்களிக்கும்

சாம்பிராணி என்னும் சரக்கு.

-பதார்த்தகுண சிந்தாமணி மூலமும் உரையும் [9].

Indications:

It is used in Respiratory disorders, Eye related diseases, Sinusitis, Head ache and Arthritis.

Types:

There are five types

- *Mallaka sambirani*-This is common benzoin-*Styrax benzoin*
- *Parangi sambirani*-Foreign benzoin-same as true olibanum-*Boswellia serrata*
- *Madaiyan sambirani*-Gum resin-Hard-*wickia pinnata.*
- *Paal sambirani* -White dammer
- *Matti sambirani- Parkia biglandulosa.*

Uses:

- It is used as an expectorant internally
- Also used for cough, Asthma and other phlegmatic diseases.
- Its smoke is deodrant and antiseptic and drives away mosquitoes and flies.

- It is highly useful in Whooping cough.
- In the form of Fumigation, it affords great relief in eye diseases and cold.
- It also used in throat irritation and is useful for smearing on cut wounds.
- It is employed in pyrosis, irritable states of the bladder, chronic affections of the lungs etc.

PREPARATIONS

Sambirani pugai **[Benzoin smoke]**

- Benzoin smoke is obtained from by putting benzoin in the fire.
- It is an antiseptic and removes bad smell and drives away mosquitoes, and other noxions insects.
- It is therefore used in sick rooms, hospitals etc. It also serves as a fumigatory in some diseases.

Sambirani bavana thravagam **[Tincture of Benzoin]**

- It is prepared with powdered benzoin, storax, aloes, rectified spirit etc.
- It is used for cleaning the wounds and sores.

Sambirani Thylam **[Oil from Benzoin]**

- It is obtained by boiling benzoin with gingelly oil.
- It is applied for headache, cold etc.

Sambirani Pathangam **[Sublimate of benzoin]**

- Benzoic acid obtained from crude benzoin by sublimation or boiling it with an alkali.
- It is prepared in Europe and exported to India.
- It is white and occurs in the form of needles which have an agreeable odor like benzoin.
- It is sour and pungent.
- It readily dissolves in rectified spirit and burns in fire.
- It is employed with advantage in cases of Asthma, Parasitic hemoptysis, dry skin, jaundice, calculous disorders, urinary disorders etc.

- Diluted in water, it is used as a lotion for cleaning sores and ulcers.

Sambirani Kalimbu **[Benzoated lard]**

- It is an ointment prepared by mixing melted lard and powdered benzoin and allowing them to boil for some time [10].

***KIRAMBU*-CLOVES**

Other names :

Lavangam

Anjukam

Tiraili

Varangam

Chosam

Vernacular names:

Tamil	-	*Kirampu*
English	-	Cloves, Clove tree
Telugu	-	*Lavangalu*
Malayalum	-	*Karampu*
Kannadam	-	*Lavanga*
Sanskrit	-	*Lavangam*
Hindi	-	*Long*

General characters

"பித்த மயக்கம் பேதியொடு வாந்தியும்போம்
சுத்தவிரத் தக்கடுப்புந் தோன்றுமோ-மெத்த
இலவங்கங் கொண்டவருக் கேற் சுகமாகும்
மலமங்கே கட்டுமென வாழ்த்து.

சுக்கிலநட் டங்கர்ண சூர்வியங்க லாஞ்சனந்தாட்
சிக்கல்விடாச் சர்வா சியப்பிணியு- மக்கிக்குட்
டங்கப் பூவோடு தரிபடருந் தோன்றிலில்
வங்கப்பூ வோடுரைத்து வா"

-அகத்தியர் குணவகடாம்

Indications	-	It cures Giddiness, diarrhea, dysentery, vomiting, anal fissure, and Cataract
Part used	-	Dried flower buds, fruit and oil

Properties and uses

Taste	-	Acrid
Character	-	Heat
Division	-	Acrid

Action

Carminative, Antispasmodic, Appetizer [11].

Therapeutic uses

It is used in medicine and culinary preparation.

To extract oil, it must be fresh and oily.

Decoction

It is used for arresting vomiting and curing digestive disorders like indigestion, colic, flatulence, dyspepsia etc.

It is specially recommended for pregnant women in case of vomiting and rumbling noise of the stomach.

The oil distilled from this forms and esteemed remedy for tooth-ache.

***Siddha* formulations**

Kirambu thylam, Kapadamathirai, Kungmapoo mathirai [10-A].

KOROSANAI [Fel bovinum purifactum]

(Felbovinum Purifactum, Purified Ox Gall or Ox Bile)

Vernacular names:

San	-	*Gorochanam*
Arab	-	*Hajr-ul-babr*
Pers	-	*Padzehare Hawani*
Hindi	-	*Zehar mohia*
Duk	-	*Mab Kons can-Gorachana*
Guj	-	*Guruchandan*
Tamil	-	*Gorojanai*
Telugu	-	*Gorojanam*
Sinh	-	*Viragul*
Burm	-	*Goyazin*

General properties:

நீரிழிவு மேகசுரம் நீங்காக் கனல்வேகம்

கூரியஉன் மாதம் குழந்தைகள்நோய் - பாரகபம்

வீறும்ம சூரியும்போம் வேதந்தனில் இரண்டாய்க்

கூறுங்கோ ரோசனைகட்கு.

-பதார்த்தகுண சிந்தாமணி மூலமும் உரையும் *[12].*

Indications:

It is used in Diabetes, psychiatric diseases, Pediatric diseases, Chicken pox.

KOROSANUM-BENZOAR-

A drug of animal origin is zoologically equated to **BOS TAURUS LINN-GALL STONE** .The drug is prescribed for a wide range of ailments in Indian Systems of Medicine. It is reported to be highly useful to vital organs and to promote strength to the physiological system. It is also an excellent remedy in breaking up abscess and curing diseases of the heart. It is laxative, anti-spasmodic, chologogue and cooling and is given in miscarriage, cholera, convulsion and hysteria [10b].

KOROSANAI is also used as antipyretic, diuretic, and anti-inflammatory. It helps in the secretion of bile and also regulates the menstrual discharge. The siddha formulary mentions the use of this drug.

The biles of animals such as buffalo, deer, wild bear [13] , peacock, goat, cow and fish are used for medicinal purpose . Of these, cow's bile is mostly used .Since the animal's biles are costly, artificial biles are sold in markets.

The bile has got bitter, laxative, mucolytic and hypothermic properties

Dosage : For children, the dose is the size of two mustard. Adult dose ranges from 260-650 mg

FORMATION

The formation of KOROSANAI takes place in the gall bladder and bile ducts of the cow and bull, due to change in the composition of the bile.

The causes for its formation are as follows:

- An increase in the cholesterol level of the hepatic bile.
- A decrease of pH resulting in decrease stability of the bile acid cholesterol complexes.
- Stasis during which bile acid are absorbed while cholesterol concentrates in the remaining bile.
- Infection of the gall bladder wall which enhances [2] and [3] alters mucin formation and provides neuclei for stone formation.
- Occurrence of Peeled of fragment of the tissues of the gall bladder [14].

ADULTERATION OF *KOROSANAI*

Qualitative analysis of market *Korosanai* showed starch, Turmeric and other extraneous materials. On enquiry with slaughter the butchers reported that *Korosanai* is a very rare commodity and are found in one among hundreds of cattle in their bile duct.

TEST FOR *KOROSANAI*

On piercing a red hot needle into *Korosanai* it shows the deposition of yellow material and emission of yellow fumes **[15].**

PHYSIOLOGICAL ACTIONS OF THE BILE

- It assists the Pancreatic juice in neutralizing the chyme that leaves the stomach.
- It assists the absorption of fats.
- It is also a solvent of fatty acids.
- Bile helps the emulsification of fats and a chologogue.
- Its principal action is as a co adjuator to the pancreatic juice especially in the digestion of fat.
- It is said to be a natural antiseptic lessening the putrefactive process in the intestine.
- It stimulates in the large intestines.
- It stimulates Peristaltic movement in the large intestine [16].

THERAPEUTICS

- The ox bile is given with breast milk or leaf juice of *Anisochilus carnosus (karpooravalli)* for the treatment of reddish syphilis *(cenkiranthi)*
- For improvement of vision, it is dissolved in breast milk and applied on the eyes.
- It is given with betel leaf juice for dropsy.
- It is given in cow's milk, twice a day for smallpox. To control the severity of smallpox, it is mixed with a small quantity of skin burnt ash.
- Pills made by grinding the leaves of *Nyctanthes arbortistis (pavalakkaal malligai),* Pepper and ox bile are given for the treatment of intermittent fever.
- Ox bile, saffron, borneo camphor, camphor, cardamom , clove, *Costus speciosus (costum),* nutmeg and *Anacyclus pyrethrum (akkaraakaaram) (*each 16 gm*)* are taken, ground with sandal powder decoction for 12 hours, Then it is ground with *Michelia champaca (shenbaga poo)*decoction for six hours and then with C*rocus sativus (Kunkuma poo)* for six hours. Pills are made in the size of *kundri* and given in breast milk, which controls phlegmatic disease , phlegmatic fever, giddiness and delirium. Cures convulsion when it is taken with egg shell oil *(andavottu thailam.)*

- The ox bile is mixed with *Picrorhiza kurroa (katukurogini)* and *Croton tiglium (naervalam)* (each 4.2 gm) and ground by adding breast milk for 12 hours. Pills are made and dried in shadow [17]

Adjuvants	Curable diseases
Juice of *Zingiber officinale*	*Vatha* fever
Breast milk	*Pitha* fever
Adathoda vasica	*Kapha* fever
Solanum xanthocarpum	*Kapha* fever

Ichapathiyam should be observed during medication.

As the *Korosanai* pill has got purgative potency, one tablet should be taken only in the early morning.

- The oil prepared from ox bile is also effective in the treatment of thrush.
- The bile of wild goat (Goat bazoar) when given in milk at a dose of 130mg,
- counteracts with poisons and strengthens the brain and heart. It also improves spermatogenesis, gives strength to the body and controls heart diseases.
- The bile of an ox, wild boar, goat, peacock and rohitaka fish is used in medicine, either singly or in combination under the designation of Pancha pitta or the five biles.
- Bile is chiefly used in soaking powders intended for being made into pill masses.
- It is used in those cases of dyspepsia and constipation in which the natural secretion of bile is deficient [18].

VETRILLAI-BETEL LEAF

Other names:

Thaampoolam, thaampoolavalli, thiraiyal, nagavalli, mellilai, vellilai, melladagu.

Vernacular names:

Eng	-	Betel, Betel leaf vine,Betel pepper
Hin	-	*Pan,Tambuli*
Kan	-	*Villayadele*
Mal	-	*Vettilakkoti,Kotinnali*
San	-	*Tambulavalli,Tambulah*
Tam	-	*Illaikodi,Vettilai*
Tel	-	*Tamalapaku,Nagavalli*

Habitat:

It is a climb on the tree, cultivated in tropical place

Part used	-	Leaves
Taste	-	Pungent
Potency	-	Heat
Division	-	Pungent

General properties:

"ஐயம் அறுங்காண் அதன்சாரங் கொண்டக்காற்
பையச் சயித்தியம்போம் பைந்தொடியே மெய்யின்
கடியின் குணம்போகுங் காரவெற்றி லைக்குப்
படியுமுத் தோடமிதைப் பார்".

- பதார்த்த குண சிந்தாமணி.

It is effective in the treatment of *kapha* diseases, urticaria, diseases of *Tridosa*, cough and hoarseness of voice.

Actions:

- Stomachic
- Antiseptic
- Aphrodisiac
- Astringent
- Carminative
- Febrifuge
- Stomachic
- Galactagogue
- Sialogogue [18-A]

3.1.2. MODERN ASPECT

Styrax benzoin

SCIENTIFIC CLASSIFICATION

Kingdom - Plantae

Division - Angiosperms

Class - Eudicots

Subclass - Asterids

Order - Ericales

Family - Styracaceae

Genus - *Styrax*

Species - *benzoin* [19].

Description:

A moderate sized tree, with a dense spreading crown, bark brownish grey, rather smooth; young shoots densely covered with a reddish tomentum of stellate hairs.

Leaves:

Alternate, without stipules, on short petioles, 3-5 inches long, ovate, rounded below, somewhat attenuated and acuminate at the apex, irregularly denticulate or nearly entire, glabrous above when mature, with a thin fioccose covering when young, bright green above, densely and finely tomentose and rufous white beneath, with prominent veins.

Flowers:

Rather large, numerous, on short, curved, stont, floccose pedicels which are curved upwards in one direction, laxly arranged on the divaricate branches of simple, one-sided, flat, long-stalked, axillary panicles, which about equal the leaves, bracts very small, deciduous.

Calyx:

Deeply cup-shaped, truncate, with 5 obscure denticulations, finely and closely tomentose, persistent.

Corolla:

3 or 4 times the length of the calyx, with a very short tube and 5 linear-oblong subacute segments, densely hairy and white externally, dull purplish-red, except the margin on the inside, valvate in the bud.

Stamens:

10 in one row, inserted at the very base of the corolla tube, the filaments connected for a short distance upwards, afterwards free , taper in, slightly hairy, anthers linear, longer than the filaments, adnate, erect, often curved backwards, 2-celled purplish.

Ovary:

Conical, very hairy, 3-celled when quite young, usually 1-celled when mature, ovules several, ascending from the base of the axis, style -long, straight, exceeding the stamens, persistent, stigma small, 3-lobed.

Fruit:

Globular-depressed, slightly apliculate, supported on the hardened persistent flattened calyx, about ¾ inch in diameter, indehiscent, pericarp thick, very hard, reddish-brown, rather rough on the surface, and more or less covered with a scanty, yellowish-white, scurfy pubescence.

Seed:

Solitary, erect, filling the fruit, testa thick and hard, embryo straight in axis of copious endosperm.

Habitat:

This handsome tree is found wild abundantly in the island of Sumatra, especially in the hills of the interior, but plantations are made in many parts and especially near the coast. The Benzoin-tree also grows wild in Java and is found in Borneo and the Malay Peninsula, where it has been probably introduced; it is not known to occur in Siam, the source of the Benzoin from that country being probably different.

Occurrence and distribution:

Sumatra, Java, Siam, Borneo and Islands of Eastern Archipelago

Part Used:

Balsamic resin flowing from the incision of the bark, Benzoinum, Benzoin

Varieties:

There are three varieties present

- Siam Benzoin
- Sumatra Benzoin
- Karis loban

Siam Benzoin:

It is highly fragrant and occurs in masses of a dark brown color mottled with white.

Sumatra Benzoin:

It is greyish in color but inferior in quality.

Karis loban:

It is superior in quality. It contains tears which are similar in size and somewhat in shape and color to Kawri, a small shell.

Characters:

Benzoin, a Balsamic resinous exudation indurated in the air, obtained by longitudinal or somewhat oblique, incisions in the bark of the stem, is met with in separate flattened agglutinated tears or drops, yellowish or reddish brown externally and milky white within, or in masses pitch dark or amber brown, exudation adultrated with wood bark and other impurities, very brittle with a slightly waxy and transparent glossy fracture, readily softening in the mouth like mastic.

Odour: Balsamic, vanilla like and fragrant.

Taste: Slightly balsamic

Solubility: Soluble in 5 parts of warm alcohol or fixed alkaline solution.

On heating: It evolves fumes of benzoic acid.

Constituents: Three resins, Benzoic acid, Cinnamic acid, Vannilin, and Volatile oil.

Acidum Benzoicum**:** Benzoic acid, Benzoyl Hydrate.

Flowers of Benzoin**-**an organic acid obtained from Benzoin by sublimation. Artificially it is prepared from tuluol, hippuric or phthalic acid or from other organic compounds. It occurs in yellowish or white lustrous scales or friable needles; when pure it has no odor of benzoin, and a warm acid taste.

Solublity:

- Cold water-1 in 400
- Boiling water-1 in 17
- Absolute alcohol-1 in 1
- Alcohol -1 in 3
- Ether-1 in 2.5
- Chloroform- 1 in 7.

Soluble in fats, fixed, volatile oils and in alkaline solution. With alkaline solution it forms benzoates such as benzoate of ammonium, lithium, and sodium.

Resins-These are extracted from benzoin by sublimation or by adding boiling solution by caustic potash.

Vanillin- treat benzoin with caustic lime, precipitate benzoic acid with hydrochloric acid, shake the liquid with ether.

Action and uses:

- Benzoin like balsams is an antiseptic, disinfectant, stimulant and expectorant.
- It stimulates the skin, kidneys, salivary glands and bronchial mucous membrane.
- It renders the urinary acids and increases its quantity.
- It is excreted by the kidneys as hippuric acid in combination with glycol.
- If inhaled, it irritates the nose and fauces, and causes sneezing and cough.
- Taken into the stomach it causes epigastric pain.
- It is used extensively as an incense.
- Its tincture is added to a pint of hot water and the fumes inhaled in laryngeal affections and in sore throat and bronchitis.
- Often, it is used as a stimulant, expectorant in phithisis, asthma, pulmonary catarrh, chronic laryngitis, chronic diarrhea and dysentery.
- It should never be used in acute inflammatory diseases or in cases of gastric irritability.

Extraction and Commerce:

Benzoin is imported into Europe and United States from both Siam and Sumatra. Both kinds come in a great measure indirectly by way of Singapore and Penang.The botanical source of the former although commonly attributed to Styrax benzoin, has never been definitely determined; but that of the latter has been clearly ascertained to be from the plant now under description. Both are generally imported in cubical blocks, and packed in wooden cases while the resin was still soft. In some cases these blocks are marked externally when first collected, and in which they are brought to the parts of Sumatra. Siam benzoin is also very rarely imported in separate tears.

Extraction

Siam benzoin:

Derived by deeply incising the bark, when the resin exudates and hardens between the wood and bark and is afterwards collected by stripping off the latter. The appearance of commercial benzoin from Siam indicates that some has thus been obtained; but it is at the same time equally clear that Siam benzoin must also be extracted by a different mode of procedure.

Sumatra benzoin:

When the trees are from 6 to7 years old, deep incisions are made in the bark, either longitudinally or somewhat oblique and near the margin of the principal branches. The resin then exudes in a liquid state, but by exposure to the air and sun, it soon concretes and is carefully scraped off with a knife. Each tree yields about three pounds of benzoin annually, for the space of ten or twelve years, after which period the trees are cut down That which exudes during the first three years is fuller of white tears, and is therefore of best quality [20].

PREPARATION

Paste

A healing and protective paste is made and compound tincture is applied to foul and indolent ulcers, over cut surfaces, sore nipples, chaps of hands and feet, and over contused wounds.

Benzoic acid as an antiseptic and is given in genito-urinary diseases, to neutralize morbid alkaline foetid urine, in chronic cystitis, phosphatic gravel, in copious uric acid deposit, in gonorrhea and Bright's disease and also in albuminuria and incontinence of urine. A solution of [1 in 20] relieves urticarial and may used as an antiseptic application on freckles and other skin diseases.

Syzygium aromaticum

Scientific classification

Kingdom - Plantae

Class - Dicotyledons

Order - Myrtales

Family - *Myrtaceae*

Genus - Syzygium

Species - *aromaticum*

Occurrence and distribution:

A tree cultivated in many parts of the world and extent in south India Tamil Nadu and kerala. It grows in kurincitinai

Description of the part - Flower bud

Macroscopical characters:

Flower buds are measuring 10 to 17.5mm in length. Color- Dark brown to black: four sided hypanthium readily exuding oil when pressed; odor strongly aromatic; taste pungent. Aromatic followed by slight tingling of the tongue

Microscopical characters:

Small number of stone cells and prismatic crystals of calcium oxalate present in stalk. Stamens each with an oil gland in the apex of the connective triangularly centricular pollen grains. Anther walls showing a typical fibrous layer, schizolysigenous glands found in all parts.

Powder:

Dark brown; fragments of parenchyma showing large, oval, schizolysigenous oil cavities; spiral tracheids and a few rather thick walled, spindle shaped fibers; calcium oxalate crystals in rosette aggregates; fragments of anther walls with characteristic reticulated cells, pollen grains numerous.

Chemical constituents:

Caryophylleneoxide, Eugenol, Acetophenone, Benzylsalicylate, Palustrol.

Actions:

Aromatic, Stimulant, Carminative, Stomachic, Diuretic and Antispasmodic.

Medicinal Uses:

Cloves are useful in nausea, vomiting, flatulence, dyspepsia, abdominal colic etc

Piper betle

SCIENTIFIC CLASSIFICATION

Kingdom - Plantae

Class - Angiosperme

Division - Magnoliidae

Order - Piperales

Family - Piperaceae

Genus - *Piper*

Species - *betle* [19b].

Distribution:

Cultivated in the hotter and damper parts of India.

Description

A perennial dioecious root climber, stems semi-woody, much thickened at nodes. Leaves-large15-20cm long, broadly ovate, slightly cordate, shortly acuminate, acute, entire, glabrous, yellowish or bright green, shining on both sides male spikes dense, cylindrical, female spikes pendulous, brauts triangular-rotundate, rachis pilose.

Fruits: rarely produced, immersed in the fleshy spikes forming nodule like structures.

Part used:

Whole plant

Properties:

The plant is bitter

- Acrid
- Sweet
- Astringent
- Carminative
- Stomachic
- Sialagogue
- Anthelmintic
- Aromatic
- Desiccative
- Exhilarant
- Aphrodiasiac
- Expectorant
- Febrifuge
- Laxative
- Tonic

Uses:

It is useful in

- Bronchitis
- Asthma
- Catarrh
- Cough
- Dipsia
- Alcoholism
- Syncope
- Otalgia
- Fever

- Halitisis
- Impotency
- Rheumatism
- Dyspepsia
- Pharyngopathy
- Vitiated conditions of *Kapha*
- Colic
- Diarrhoea
- Laryngitis [21].

Uses in Ayurveda and Unani

The leaf has a sharp taste and good smell; improves taste and appetite; tonic to the brain, heart, liver; strengthens the teeth; lessens thirst, clears the throat; vulnerary and styptic.

The fruit is employed with honey as a remedy for cough, and in Orissa, the root is said to be used to prevent child bearing.

The juice of the leaves is dropped into the eye in painful affections of that organ; it is also used to relieve cerebral congestion and satyriasis, and to allay thirst.

The juice of the leaves is dropped into the eye in night-blindness.

The essential oil from the leaves has been successfully used in the treatment of catarrhal disorders and as an antiseptic.

In Cambodia the pounded fresh leaves are used in the preparation of lotions and baths for patients suffering from protracted fever, small-pox, enlarged glands, lymphangitis.In the Philippine Islands, he leaves are eminently the cure for the diseases of children: indigestion, colic, diarrhoea, pulmonary catarrh, and laryngitis. Applied hot to the chest they are said to act as a lactagogue [22].

Felbovinum

Ox bile is an extract of exactly what its name implies, the bile of an ox. Because it is made from the bile of an animal, it contains many of the digestive enzymes and nutrients that we need to help us properly digest our food. It can be used as supplement for fat digestion and in the treatment of nausea, diarrhoea and stomach upset.

Bile:

Bile is a yellow-greenish substance that is produced by the liver and stored in the gallbladder. When we eat substances that contain fat, it stimulates our gallbladder to release the stored bile into our digestive tract, where it mixes minerals with our food.

Because bile contains substances that promote the proper breakdown of fat, it is essential to our proper metabolism of fatty foods such as dairy products, oils and meat. In addition to its digestive function, bile contains body salts and often cholesterol. When released into the small intestine, or when taken as a supplement, bile mixes into the food in our digestive tract and helps to act on any fat there, much like a detergent. This means that it takes big fat globules and makes them into tiny balls that lipase (the fat digesting enzyme) can easily work on. Some people, who have had their gallbladder removed, use ox bile to supplement their bile production with meals since they no longer have a storage place for their own bile. With improved digestion of fats, the ability to absorb fat-soluble vitamins such as A, D, E, and K improves as well.

Sources of ox bile:

Ox bile can be found in a number of different digestive aid supplements. It is often found in combination with digestive enzymes such as lipase, protease and amylase. There is no recommended dosage associated with ox bile, so it is best to follow the label recommendations of each individual

Composition of Bile

The bile of man and carnivorous animals is of a deep orange-red color, turning to greenish-brown by decomposition of its coloring matter. In h erbivorous animals it has some shade of green when quite fresh, but turns to a muddy brown after a time. It is transparent, and more or less viscid according to the length of time it has remained in the gall bladder. It has a strong, bitter taste, a peculiar aromatic odour, and after remaining for some time in the gall bladder it has an alkaline reaction. Its specific gravity is about 1005 when taken from the bile ducts directly, but it may rise to 1030 after prolonged stay in the gall bladder, owing to the addition of mucus and the absorption of some of its fluid.

The following table gives approximately the proportions of the chief constituents of bile:

Water - 85.0 per cent

Bile salt- 10.0

Coloring matter and mucus - 3.0

Fat- 1.0

Cholesterin - 0.3

Inorganic salts - 0.7

1. The bile acids are two compound acids, glycocholic and taurocholic, which exist in the bile in combination with sodium. The amount of each varies in different animals and at different times in the same animal. The bile of the dog and other carnivora contains only taurocholate of soda. In the ox the glyco-cholate of soda is greatly in excess. In man both are present,the proportion being variable, but the glycocholate greatly preponderates. To separate these acids, bile is evaporated to one-fourth its volume, rubbed to a paste with animal charcoal to remove the pigments, and carefully dried at 100° C. The black cake is extracted with absolute alcohol, which dissolves the bile salts. From the strong alcoholic solution after partial evaporation the bile salts can

be precipitated by ether. They first appear as an emulsion, and then form glistening crystals which are soluble in water or alcohol, but insoluble in ether.

From the solution of the two salts the glycocholic acid may be precipitated by neutral lead acetate, as lead glycocholate, from which the lead may be removed by sulphuretted hydrogen, and the acid precipitated from its alcoholic solution by the addition of water. The taurocholic acid may be obtained subsequently by treating with basic lead acetate.

Glycocholic acid, when boiled with weak acids, alkalies or baryta water, takes up an atom of water, and splits into cholic acid and glycin (amido-acetic acid).

Taurocholic acid, under similar treatment, splits into cholic acid and taurin (amido-ethyl-sulphonic acid).

Cholic acid occurs free in the intestines, the bile salts being split up in digestion, and taurocholic and glycocholic acids decomposed.

The non-nitrogenous cholic acid is in a great measure eliminated with the faeces, while the taurin and glycin are reabsorbed into the blood with some of the other constituents of the bile, and are again probably utilized in the economy.

No traces of these bile acids can be detected in the blood, and there is no accumulation of them in the body after the removal of the liver; hence, it has been concluded that they are manufactured in the liver.

2. The greater proportion of the mucus contained in the bile is produced in the gall bladder, and there added to the bile. Some mucus comes from the mucous glands in the bile ducts, but, unless the bile has remained in the gall bladder. But there is an insignificant amount of mucus present, when a fistula is made from the hepatic duct. The mucus passes in an unchanged state through the intestine, and is evacuated with the faeces.

3. The bile pigment of man and carnivora is chiefly the reddish form called bilirubin. It is insoluble in water but soluble in chloroform. It can be obtained in rhombic crystals, and is easily converted by oxidation into a green pigment, biliverdin, which is the principal coloring matter in the bile of many animals, and is not soluble in chloroform,

but readily so in alcohol. Bilirubin is supposed to be identical with haematoidin, a deeply colored material found by Virchow in old extravasations of blood within the body and hence the bile pigment is said to be derived from the coloring matter of the blood. Probably the haemoglobin of some red corpuscles which have been broken up in the spleen is converted into bile pigment by the liver.

Under the influence of decomposition bilirubin undergoes a change, taking up water and forming hydro-bilirubin; this occurs in the intestine, and the bilirubin is thus eliminated as the coloring matter of the faeces (stercobilin), which is probably identical with the urobilin of the urine.

4. Fatty matters, the principal of which are lecithin, palmitin, stearin, olein and their soda soaps.

5. Cholesterin (C26HuO) is an alcohol, and crystallizes in clear rhombic plates, insoluble in water but held in solution by the presence of the bile salts. It can be obtained from gall stones, the pale variety of which are almost entirely composed of it. The cholesterin leaves the intestines with the faeces.

6. Among the inorganic salts are sodium and potassium chloride, calcium phosphate, some magnesia, and a considerable quantity of iron [23].

3.2. PHARMACEUTICAL REVIEW

3.2.1. Siddha Aspect:

Pharmaceutics is a discipline of pharmacy that deals with the process of turning a new chemical entity to be used safely and effectively by the patients. (Formulation of pure drug substance into dosage form)

Siddha pharmaceutics has very minute chemical processes in it. It has several chemical processes like purification of raw substances, grinding them with herbal juices for several days and subjecting the ground material to fire by way of *putam* process. Medicines prepared according to the above methods undergo several chemical changes.

Siddha medicines are classified into internal medicines (32) and external medicines (32). The drug taken for dissertation is in the form of *Mathirai.* Other names of *Mathirai* are *Kuligai, Urundai* and *Vattam. Mathirai* comes under the category of internal medicines [24]. The powder which is required for the preparation of *Mathirai* is *Chooranam*. It also comes under the category of internal medicines.

Purification of the drugs included:

Purification of the drugs is mainly done to remove the toxicities, impurities like soil, dust, clay present in the drugs. Also the drugs when subjected to heat like roasting or soaked in liquids undergo certain chemical reactions such as oxidation of toxic substances to non-toxic, reduction of some poisonous chemicals to non-poisonous ones, or undergo enzymatic reactions. In these ways, not only the toxicities and impurities are removed but also enhanced the potency of the drugs.

Concept and Terminology of pills:

It is a pill prepared from a finely ground paste of drugs. The term *mathirai* is the most fitting category of medicines as besides indicating the form of medicine that is pills. It also means that the minimal dosage unit is one pill (*mathirai* means 1 unit). Preparation of *mathirai* includes various processes like extraction of juices, making decoction, preparing powders, grinding pastes and rolling into pills or pressing into tablets. The raw drugs are dried in the sun or shade and the drugs which are aromatic are to be

roasted separately. The raw drugs are purified and the raw drugs are grounded separately then the compounded drug be grounded in a mortar for the prescribed period with the addition of prescribed juices and decoctions. If green drugs are to be added they should be made into fine paste before being used. Vegetable drugs which require frying are fried and powdered. However scented vegetable drugs like cinnamon leaves and cloves, cinnamon bark are dried only in shade as otherwise their volatile oil is lost by drying in the sun.

The individual drugs should be separately weighed after being powdered and then taken in the prescribed ratio. After the pill mass has been prepared by following the processes outlined in the recipe, it is convenient to roll it into long uniform pencils and then cut into bits of uniform length to give suitable pill weight and then rolling a pill from each piece. This is a fast process to prepare uniform pills as pinching and rolling every time is invariably a tiresome, tedious and time consuming messy process. The pill mass when rolled between the fingers should not stick. This is the correct consistency for rolling into pills. The pills should be always dried in a warm, dry shady place and never in the sun because volatile matters in the pills are easily lost and photochemical breakdown of active principles are faster in sunlight of the tropics, if the pill mass sticks to the fingers, a speck of ghee may be smeared on the fingers. Pills should be well dried in shade [25].

Storage and Usage:

Almost all the *mathirai* contain highly active ingredients. Hence they should be preserved in well stoppered glass vials with relevant labels and instructions. If the *mathirai* lose their natural shape, colours, smell, taste etc, it is not advisable to consume them. If properly stored, we can keep them for a year.

Preparation of *Mathirai* in Manufacturing Units:

In the manufacturing unit, *chooranam* is compressed into tablets. Tablets are unit forms of solid medicinal substances with or without suitable diluents prepared by compressing and they are mostly discoid in form. Binders like Gum acacia, lubricants like liquid paraffin and disintegrators like Talcum powder are used. *Chooranam* is first prepared

according to the above procedures. Then the ingredients are mixed with in the form of granules before compressing as tablets. Too much fine powder refuse to form satisfactory tablets and so they must be mixed with some adhesive substances or binders such as gum acacia. To prevent the sticking of the tablets to the punches and dyes a lubricant like liquid paraffin is added. If the tablet is to dissolve quickly, a disintegrator like Talcum powder is added [26].

Initial step of preparation of *Mathirai* (Preparation of *chooranam*)

Before the preparation of *mathirai*, *chooranam* is prepared. Then it is subjected to various procedures as described above before becoming *mathirai*.

Chooranam is the fine dry powder of drugs. The term *chooranam* may be applied to the powder of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed homogeneity. If the constituent drugs are dry ones, they should not be dried. If they are fresh or green drugs with moisture they should be dried. The drugs should be cleaned and foreign matter removed before powdering. The drugs enumerated in the recipe in clean and well dried state are grounded in a mortar and a pestle and sieved through a fine cloth of close mesh. The drugs which are to be used in the preparation should be taken from recently collected material. Drugs which are aged by prolonged storage change in colour, taste and scent and those which are insect infested or attacked by fungi should be positively rejected.

In general, the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic material should be removed from the drugs by close inspection. The *chooranam* should be very fine as to be called amorphous and should never damp. The fineness of the sieve should be 100 mesh or still finer. 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 card board boxes [25-A].

Purification of *Chooranam:*

The prepared *chooranam* was purified by a process called *Pittaviyal*. For this process cow's milk and water were taken in equal ratio and half filled in filled mud pot. A clean dry cloth was tied firmly around the mouth of the mud pot. *Chooranam* was placed over the tied cloth. Another mud pot of similar size was kept over the mouth of the mud pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced to the lower pot. Then the *chooranam* was taken, dried, powdered finely [27] .Using this fine powder as base for *mathirai* preparation, further steps are continued.

Shelf life of the drugs:

The shelf life of the drugs depends on the effectiveness of the preparation. The efficacy, smell, taste and appearance of the drugs gradually change as time goes on resulting in reduced potency thereby the desired effect is not attained. But some drugs appear to be good externally inspite of reduced efficacy. So they should not be considered for consumption and should be discarded. The shelf life of *mathirai* is 1 year and for *chooranam* it is 3 months. According to recently published guidelines by Ayush,the shelf life period of *chooranam* is 1 year and that of *mathirai* is 2 years. Also the following are the analytical parameters of specifications of *mathirai,*

Traditional tests for *Mathirai*

Characters:

- Non sticky on rolling
- No cracks over the surface after drying
- Shall be rolled uniformly over the plane surface

  Based on these characters the drug is assessed as the appropriate one for medication.

3.2.2. MODERN ASPECT

A tablet is a pharmaceutical dosage form. Tablets may be defined as the solid unit dosage form of medicament or medicaments with or without suitable diluents and prepared either by molding or by compression. It comprises a mixture of active substances and excipients, usually in powder form, pressed or compacted from a powder into a solid dose. The excipients can include diluents, binders or granulating agents, glidants (flow aids) and lubricants to ensure efficient tabletting; disintegrants to promote tablet break-up in the digestive tract; sweeteners or flavours to enhance taste; and pigments to make the tablets visually attractive. A polymer coating is often applied to make the tablet smoother and easier to swallow, to control the release rate of the active ingredient, to make it more resistant to the environment (extending its shelf life), or to enhance the tablet's appearance.

The compressed tablet is the most popular dosage form in use today. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets. A tablet can be formulated to deliver an accurate dosage to a specific site; it is usually administered sublingually, basically, rectally or intravaginally. The tablet is just one of the many forms that an oral drug can take such as syrups, elixirs, suspensions, and emulsions. Medicinal tablets were originally made in the shape of a disk of whatever color their components determined, but are now made in many shapes and colors to help distinguish different medicines. Tablets are often stamped with symbols, letters, and numbers, which enable them to be identified. Sizes of tablets to be swallowed range from a few millimeters to about a centimeter [28].

TYPES OF TABLETS:

Increasingly tablets are being made which are suitable for patients who cannot swallow normal tablets. These are listed below with examples provided.

Dispersible or effervescent tablets:

These tablets are designed to be added to water just prior to swallowing. They are frequently quite large and can contain large amounts of sodium. The size prevents patients from taking many of them, which is helpful for *soluble paracetamol* products

for instance however the sodium content can cause problems in patients where sodium intake is restricted.

Sub-lingual tablets:

These tablets are designed to be dissolved under the tongue, are rapidly absorbed through the tongue and therefore work quickly. This is why some tablets for the treatment of *angina pain* and others for *general pain* are formulated in this manner. The disadvantages are that they require sufficient saliva production and due to quick absorption are more likely to cause side effects and are more quickly removed from the body.

Buccal tablets:

Buccal tablets are intended to be placed on the gum or in the cheek to allow the drug absorbed. Because the medicine can be held for a longer period of time on the gum, medicines which need to be released at a slower rate than sub-lingual tablets can be given via this route. This route is used for *anti-nausea drugs* and *nicotine replacement* gums. Anti-nausea medicines are particularly suitable for buccal administration as the nausea itself can cause swallowed tablets to be vomited and therefore rendered ineffective.

Melts:

Melt tablets are placed on the tongue and are designed to dissolve directly in the mouth's saliva. The contents are then swallowed with saliva and consequently water does not have to be administered with these medicines. This is particularly useful in patients who are at risk of aspiration and therefore unable to swallow tablets with water concurrently.

Oro-dispersible tablets:

Oro-dispersible tablets are similar to melts and are designed to disperse in the mouth and to be washed down with saliva. As with sub-lingual, buccal and melts, oro-dispersible products require an adequate amount of saliva production. Some oro-dispersible tablets consist of coated granules and therefore it is not appropriate to crush the oro-dispersible product prior to dispersion [29].

3.3. DISEASE REVIEW

3.3.1. Siddha Aspect

Other names

- *Soothaga kattu*
- *Ruthuneer katti*
- *Soothaga katti*
- *Soothaga thiratchi*
- *Soothaga noi*
- *Soothaga kolaru*

***Soothaga vayu* & Pcos**

சித்தான கர்ப்பத்தில் சேர்ந்திடும் இரத்தந்தான்
வத்தாம் வுருண்டு வாயுபோல் ஓடிடும்
வற்ற பசிபோகும் உழன்றே இரைந்திடும்
வற்றாக கழிச்சலாம் வன்சூதக வாயுவே

திருமூலர் கருக்கிடை வயித்தியம் 600 [30].

Soothaga vayu refers to a condition of building up of or accumulation of *vayu* and blood in the uterine cavity. This runs around the womb and result in loss of appetite, flatulence and dysentery.

"கேளுமே சூதகத்திலக்கினி வாய்வு
கெடுத்துவிடும் மாதவிடாய் கட்டிபோகும்
ஆளுமே கருக்குழியும் தூர்ந்து தேகம்
அப்பனே யுதிரமது அடிமூலத்தில்
நீளுமே சூதகத்தில் வாய்வு தோன்றி
நேரான அடிவயிறு வலிப்புக் காணும்
பாளுமே தலைவலிக்கும் இடுப்பு ளைச்சல்
பக்குவமாய் மருந்துண்ணத் தீருந்தானே"

- **ஆவியளிக்கும் அமுதமுறைச் சுருக்கம்** [31].

Soothaga vayu may be compared to Poly cystic ovarian syndrome, which is characterized by vayu accumulation in the uterus may lead to amenorrhoea, lower abdomen pain, head ache, low back ache etc.

***Karppa vayu* & Pcos**

"பொருமிரத்தந்தனை மறித்துப்போதமிகவும் வலியுண்டாங்க
குருதிசேரா வயிறுவலிபோங் கொள்ளுங் கர்ப்பந்தனை யழிக்கும்
வருடி யிடுப்புக் குடைந்துளைக்கும் மலத்தைமிகவும் மிறுக்கிக்கி
பெருகப் பனைக்கும் எனப்பெரியோர் பேசுங்கர்ப்பவாயுவிதே"

- அகத்தியர் ஆயுள்வேதம் 1200 [32]

According to Siddha principles, the PCOS is a kind of *vayu* disorder called *karppa vayu* which is characterized by frequent miscarriages, abdominal pain, low back ache and severe constipation.

***Nirandhara maladu* & Pcos**

"நவின்றிடவே யிடுப்புவயிற் பெருத்துக் காணும்
நலமான மேனியது வூதிக் காணும்
குவின்றிடவே மும்மடிப்பு வயிற்றில் தோன்றும்
குணவதியாந் தேவதா பெண்ணா னாலும்
நவின்றிடவே சன்மத்தின் மலடே யாகும்
சதாகாலங் கருப்பமது தரியா தென்று
புவின்றிடவே யூகிமுனி சிகிச்சா சாரம்
புகன்றிட்டார் லோகத்து மாந்தற் காமே"

-யூகி வைத்திய சிந்தாமணி [33].

Siddha Yugi muni in the above lines says that the symptoms of Nirandhara maladu are obesity, increased waist hip ratio, flabby abdomen with three folds of skin. He further says that females with these features may suffer from infertility. These symptoms can be correlated to PCOS in modern terms.

Aetiology

சூதக நோய் வரும்வழி:

“தரணியில் பெண்களுக்கு கெற்பநோய்கள்
நயக்கவே வந்து தென்னவென்றால் மைந்தா
நன்மையுடன் ருதுவாகும் நாளிற்றானே
மயக்கவே மாப் பாண்டம் பால் பழத்தினாலே
வந்துதடா சூதகத்தின் வாயு தானே
தானென்ற கருக்குழியில் வாய்வு தங்கி
தளர்ந்த தொரு சோரையினால் தசைதான் மூடி
ஊனென்ற தேகமெல்லாம் மதர்த்து நல்ல
உண்மையுள்ள அடிவயிற்றில் வலி யுண்டாச்சு
பானென்ற கருக்குழிதான் விளக்க மன்றி
பரமான விந்துவங்கே அணுகாதையா
ஏனென்றால் ஆதியிலே வாய்வு கொண்டு
இருந்ததினால் கெற்பமது இல்லை தானே”

- அகத்தியர் அமுத கலை ஞானம் [34]

The above lines explain the aetiology of reproductive diseases according to Siddha science. It says consumption of high calorie diet like starchy foods, milk, fruits during menstruation results in accumulation of *Vayu* in the uterine cavity. This leads to reduced blood flow to the organ resulting in obesity, abdominal pain and finally failure of conception.

“இசைந்ததொரு பெண்மலடு எங்குமில்லை
.....னாலே மலடான சேதிகேளு
அசைந்திருக்கும் பேயாலும் பித்தத்தாலும்
அடிவயிறு நொந்துவரும் வாயுவாலும்
பிசைந்தகர்ப்பப் புழுவாலும் கிரகத்தாலும்
பிணியாலும் மேகவை சூரியாலும்
துசங்கெட்டக் கலவியினால் பூவொதுங்கித்
துலங்காமற் பிள்ளையில்லை சொல்லக்கேளே”

-பதினெண் சித்தர்கள் பாடிய வைத்திய சில்லறைக் கோவை [35].

Naadi nadai

"மாதர் கை மிடித்தபோது வந்திடும் நாடி மூன்றும்
சேதமாயிற்று நின்று சேரவே பதித்து நிற்கில்
ஓதுமே சூதகத்தில் ஓங்கிய வாய்வு நின்று
பேதமாய் வாதை பண்ணி பிணியினை விளைக்குந் தானே"

- அகத்தியர் அமுத கலை ஞானம் [34].

When the naadi is felt in women if all the 3 nadi's are found diffused and then felt together then those women is subjected to mensural troubles and those women has Soothga vaayu and has different types of physical, characteristic and mental changes and leads to problems.

3.3.2. MODERN ASPECT

Polycystic ovarian syndrome (PCOS)

Polycystic ovarian syndrome (PCOS) is a common hormonal disorder in the middle of women reproductive age. It is a complex, heterogenous disorder, containing numerous small cysts located along the outer edge of each ovary (polycystic appearance).The cysts are not harm but it leads to hormonal imbalance especially androgen, they producing slightly more androgen, this will stops ovulation, get acne and growth of extra hairs. The cause of PCOS is unknown, but genetics may be a factor. PCOS can be passed down from either your mother's or father's side.

History

It was primarily described by American Gynecologists Irving F.Stein, Sr. and Michael L. Leventhal, in 1935 that is why otherwise called as Stein – Leventhal Syndrome.

Other names

- Poly cystic ovary disease
- Functional ovarian hyper androgenism
- Ovarian hyperthecosis
- Sclerocystic ovary syndrome
- Stein – Leventhal Syndrome.

Epidemiology

8-25% - normal women having Pcos on ultra sonographic findings.

14% women who taking oral contraceptives are found to have Pcos.

Etiology

- Exact cause is unknown but hormone imbalance in brain and ovaries may cause PCOS.
- **Excess insulin:** Many women with pcos have too much insulin secretion, this excessive insulin may cause increased secretion of testosterone this will leads to some symptoms like growth of excess body hair or irregular periods.
- **Heredity:** It has an evidence of genetic involvement. Family history reveals monozygotic twins are more prone to this disease than dizygotic twins.

Classification:

The WHO criteria for classification of anovulation include the determination of oligomenorrhea (menstrual cycle >35 days) or amenorrhea (menstrual cycle > 6 months) in combination with concentration of prolactin, follicle stimulating hormone (FSH) and estradiol. Almost 80% of anovulation patients have normal serum FSH and Estradiol levels and demonstrate very heterogeneous symptoms ranging from anovulation, obesity, biochemical or clinical hyperandrogenism and insulin resistance.

PCOS is the most common cause of anovulation in women with normal serum FSH and Estradiol levels. Despite the heterogeneity in symptoms associated with PCOS, the essential feature is arrested follicular development at the stage when selection of the dominant follicle should normally occur.

The small ovarian follicles are believed to be the result of disturbed ovarian function with failed ovulation, reflected by the infrequent or absent menstruation that is typical of the condition. In a normal menstrual cycle, one egg is released from a dominant follicle essentially a cyst that bursts to release the egg. After ovulation the follicle remnant is transformed into a progesterone-producing corpus luteum, which shrinks and disappears after approximately 12–14 days. In PCOS, there is a so-called "follicular arrest", i.e., several follicles develop to a size (5–7 mm, but not further. No single follicle reaches the preovulatory size (16 mm or more)

Clinical signs and symptoms associated with PCOS

Symptom	**Frequency**
Oligomenorrhoea	29-52%
Amenorrhoea	19-51%
Hirsutism	64-69%
Obesity	35-41%
Acne	27-35%
Alopecia	3-6%
Acanthosis nigricans	<1-3%
Infertility	20-74%
Elevated Serum LH	40-51%
Elevated testosterone	29-50%

Diagnostic Criteria:

Diagnostic criteria have been established by the modified consensus of the National Institutes of Health and Child Health and Human Development (1990) and by consensus criteria established during the ESHRE/Rotterdam Conference in 2003

NIH Criteria (both required)

- Chronic anovulation
- Clinical or biochemical signs of hyperandrogenism

Minor NIH Criteria

- Insulin resistance
- Perimenarchal onset of hirsutism and obesity
- Elevated LH/FSH ratio
- Intermittent anovulation associated with hyperandrogenemia
- Ultrasound evidence of polycystic ovaries

Rotterdam Criteria-two of three required

- Oligo and /or anovulation
- Clinical or biochemical signs of hyperandrogenism
- Polycystic ovaries

Patho Physiology:

Typically, the ovaries are enlarged. The capsules are thickened and pearly white in colour. On bisection, multiple follicular cysts measuring about 5 mm in diameter are crowed around the cortex.

Histologically, there is thickening of tunica albuginea. The cysts are follicles at varying stages of maturation and regression. It should be remembered that PCOS may be unassociated with enlarged ovaries.

The pathophysiology of primary PCOS is obscure. There is abnormal pulse frequency of GnRH simultaneous with increased pituitary sensitivity to GnRH. The LH secretion is tonically elevated due to persistant high level of estrone or androgens or both. FSH

secretion remains either normal or decrease due to negative feedback effect of estrogens and inhibin.

Hormonal inervation of PCOS

Because of relative low levels FSH, there is defective ovarian folliculogenesis due to lack of aromalisation. The net effect is diminished estradiol and increased inhibin production. Due to elevated LH, there is hypertrophy of theca cells and more androgens are produced either from theca cells or stroma.

Clinical features

- **Menstrual disorders**: oligomenorrhoea, ammenorrhoea,
- **Excessive androgen**: Elevated levels of male hormones (androgens) may result in physical signs, such as excess facial and body hair (hirsutism), adult acne or severe adolescent acne, and male-pattern baldness (androgenic alopecia)
- **Polycystic ovaries:** Enlarged ovaries containing numerous small cysts can be detected by ultrasound
- Infertility
- Central obesity associated with insulin resistance, (serum insulin, high level of homocysteine)

Low-grade inflammation:

- Research has shown that women with PCOS have low-grade inflammation.

Complication

- Type 2 diabetes
- High blood pressure
- Cholesterol and lipid abnormalities, such as elevated triglycerides or low high-density lipoprotein (HDL) cholesterol, the "good" cholesterol
- Elevated levels of C-reactive protein, a cardiovascular disease marker

- Metabolic syndrome, a cluster of signs and symptoms that indicate a significantly increased risk of cardiovascular disease
- Nonalcoholic steatohepatitis, a severe liver inflammation caused by fat accumulation in the liver
- Sleep apnea
- Abnormal uterine bleeding
- Cancer of the uterine lining (endometrial cancer), caused by exposure to continuous high levels of estrogen
- Gestational diabetes or pregnancy-induced high blood pressure, if you do become pregnant

Diagnosis

- CT, MRI.

Laboratory test

- Serum values
- LH level is elevated and/or the ratio LH:FSH is > 3:1
- Reversible oestradiol : oestrone ratio (oestrone level is markedly elevated)
- SHBG level is reduced.
- Androstenedione is elevated.
- Serum testosterone and DHEA-S may be marginally elevated.
- Thyroid function tests to determine how much of the thyroid hormone your body produces
- Fasting glucose tests to measure your blood sugar levels
- Lipid level tests to assess the amount of cholesterol in your blood [37].

Radiologic Studies in PCOS

- Ultrasonographic examination of PCOS women reveals an increase in ovarian size and an increased number of immature follicles. The Rotterdam criteria include enlarged ovaries measuring >10 cm3 and more than 12 follicles measuring 2-9 mm in diameter.

- PCOS is a disorder comprising multiple clinical variants and apparent genetic propensities grouped together into the "PCOS phenotype" (Catherine J *et al*)

Differential diagnosis

CAH- congenital adrenal hyperplasia , Cushing syndrome [38].

PROGNOSIS:

PCOS maybe exist in three forms – mild, moderate and severe and the prognosis varies accordingly.In the mild forms of PCOD, the affected woman may have no abnormality of menstruations and may ovulate normally. It often takes these women longer than normal to conceive and has a higher chance of spontaneous miscarriage.

- In moderate PCOD – There are menstrual irregularities or absence of menstruation and failure of ovulation.
- The most severe form of PCOD is characterized by- Obesity, hirsutism, amenorrhea and infertility.
- Weight loss by way of reduced carbohydrate intake and exercise is the most important intervention; this step alone can restore menstrual cyclicity and fertility, and provide long-term prevention against diabetes and heart diseases. A large body of evidence has demonstrated an association between insulin resistance and polycystic ovary syndrome. Because the syndrome is also associated with lipid abnormalities, affected women could benefit from measures to prevent heart disease and the other problems associated with long-standing hypertension and diabetes that are associated with the syndrome

- Pregnancy rate with weight loss- Programs that have targeted weight loss alone have achieved pregnancy rates as high as 30 to 60 percent without medical intervention.
- Estrogen levels and Cancer risks - Long-term effects of unopposed estrogen place women with the syndrome at considerable risk for endometrial cancer, endometrial hyperplasia and breast cancer [39].

FOOD HABITS FOR PCOS

Diet and exercise are important parts of managing PCOS (Polycystic Ovary Syndrome).This is because young women with PCOS often have higher levels of insulin (a hormone) in their blood, and many have trouble maintaining a healthy weight. Knowing the right foods to eat as well as the kinds of food to limit can improve the way you feel. It will also help you lose weight. Eating well, staying active, and maintaining a healthy weight (or losing even a small amount of weight if you're overweight) can improve PCOS symptoms.

Instead of:

- Sweetened juice, canned fruit in heavy syrup or sweetened apple sauce
- Starchy vegetables such as potatoes, corn, and peas
- Refined grains made with white flour such as white bread and pasta, bagels or white rice.
- Sugared cereals such as Lucky Charms, Fruit Loops, or Frosted Flakes, and other sweetened grains such as cereal bars (Nutrigrain Bars), breakfast pastries (PopTarts), and donuts.
- Sugary drinks such as soda or juice
- Sugary foods such as cookies, cakes and candy
- Snacks such as potato chips, Frito, Doritos and tortilla chips

Choose:

- Fresh fruits or frozen/canned fruit without added sugar or unsweetened apple sauce
- Non–starchy fresh vegetables or frozen/canned vegetables such as broccoli, spinach, and carrots
- Whole grains such as whole wheat pasta, brown rice, oats and whole wheat bread
- High fiber cereals such as Kashi, shredded wheat and All Bran. (Look for cereals that have at least 5 grams of fiber per serving or sprinkle ½ cup of bran cereal or unprocessed bran on a low–fiber cereal to increase the fiber)
- Water or seltzer, flavored with fruit if desired, unsweetened iced tea
- High fiber baked goods made from whole wheat flour and oats
- Crackers and snacks with fiber such as Triscuits, Wasa or popcorn.

20 INTERESTING FACTS ABOUT PCOS

1. Women with PCOS have higher rates of anxiety and depression
2. 5-10% of women of childbearing age in the United States, or roughly 5 million, have PCOS
3. It affects 4% to 8% of women worldwide and as high as 25% in some populations, making it the most common hormone problem for women.
4. Less than 50 percent of women are properly diagnosed, leaving millions of women living with symptoms that go unsupported.
5. Elevated insulin or insulin resistance are not part of the diagnostic criteria for PCOS but are seen in the majority of women with PCOS.
6. The diagnostic criteria for PCOS is not helpful. It states that a woman has PCOS if she has at least two of the following three criteria: 1) irregular or absent periods, 2) blood tests or physical signs that show high androgens (male hormones), 3) polycystic ovaries, so this leaves many women without support.
7. Signs of hormonal imbalance are more important for PCOS such as: hair growth on the face, hair loss, acne, weight gain, irregular cycles, PMS, hot flashes, brain fog, bloating etc.

8. Women with PCOS are at a higher risk of developing obstructive sleep apnea due to the influence of androgens affecting sleep receptors in the brain.
9. Despite its name, not all women with PCOS actually have cysts on their ovaries.
10. Women with PCOS have more testosterone and can build muscle easier than women without the syndrome.
11. The prevalence of type 2 diabetes in women with PCOS at middle age is 6.8 times higher than that of the general female population.
12. A number of studies demonstrate that modest weight loss of 5-10% of initial body weight improves metabolic, physiological and psychological aspects of PCOS.
13. Women with PCOS have a higher incidence of gestational diabetes, miscarriages, preterm deliveries and stillbirths.
14. As many as 70% of women with PCOS have insulin resistance and 10% have type 2 diabetes.
15. More than 50 percent of women with PCOS will have diabetes or pre-diabetes before the age of 40.
16. The risk of heart attack is four to seven times higher and women with PCOS are also three times more likely to develop endometrial cancer than those without it.
17. Symptoms of PCOS are completely treatable and can completely resolve themselves with the right approach.
18. Science shows that it takes a 5-element process to heal from the symptoms: 1) the right nutrients to support healthy hormone and blood sugar levels, 2) using food as medicine, 3) using movement as medicine, 4) having an awareness around cravings and what to do about them and 5) a support community.
19. Over-exercising and counting calories can make PCOS symptoms much worse.
20. Healthy fats do not raise insulin so are a great food source for women with PCOS [40].

Drugs used in Pcos:

The drug therapy of infertility due to ovulatory failure was till recently empirical and disappointing.

Classification of ovulation inducing drugs

- Synthetic GnRH
- Human menopausal and chronic gonadotropins
- Bromergocriptine
- Antiestrogenic compound

GnRH

- The GnRH (then called LH-RH) was isolated by Schally in 1971 by extracting and processing one million pig hypothalami. The GnRH of sheep, pigs, cows and human origin are identical in structure.
- GnRH is decapeptide.
- It is synthesized in hypothalamus.
- It is also found human placenta.
- It also appears in high concentration in breast milk.

Therapeutic uses

- Assessment of the function of the pituitary gonadotropes.
- Induction of ovulation.
- Treatment of males and females with idiopathic, hypothalamic, hypogonadotropic hypogonadotropism (Kallman syndrome).

Adverse reactions

- Hot flushes.
- Sweating.
- Vaginal dryness.
- Headache.
- Occasionally diminished libido and depression.
- Prolonged treatment causes Osteoporosis.

Human menopausal urinary Gonadotropin (hMG, Menotropins, Pregonal)

This is prepared from the urine of the menopausal women and is available in ampules containing 75 IU of FSH and LH each.

Therapeutic uses

Induce ovulation in women with anovulation due to pituitary-hypothalamic disorders but with normal ovaries and poly cystic ovaries.

Adverse reactions

- Hyperstimulation syndrome due this the ovaries become very large and friable.
- Abdomimal pain.
- Nausea.
- Ascitis.
- Shock.

Bromorgocriptines (Bromocriptine, Parlodel, Proctinal)

This semisynthetic ergot alkaloid is a selective D2 receptors.

Therapeutic uses

- It inhibits hyper prolactinemia and stops galactorrhoea.
- It lowers the serum prolactin levels and restores potency in males.

Antiestrogenic compound

Clomiphine citrate (Clomid, Fertyl)

This is triphenyllethylene compound, the available Clomiphene preparation contains “cis” as well as “trans” forms. It is the “cis” form which is related structurally to oestrogen and is more potent than the racemic form.

Therapeutic uses

- Ovulation induction.
- In those with regular anovulatory cycles, it is started on the fifth day after onset of mensuration.
- Generally, ovulation occurs 70% of women; of these less than 50% may conceive.

Cyclofenil (ondomid)

- This compound has a structure similar to clomiphine and is used for Pcos.
- It does not exert anti-estrogenic effect.
- It is usually start on the third day of the menstrual bleeding, it can induce ovulation.

Letrozole

- This aromatase inhibitor has been used for ovulation induction in patients with PCOS.
- It acts by inhibiting aromatase and thus reducing the production of estrogen.
- Excess estrogen over-suppresses FSH production.
- Aromatase inhibitors promote the development of healthy ovarian follicle and ovulation.

Metformin

Biguanide class of anti -diabetic agents, which also includes the withdrawal of phenformin and buformin agents, comes from French lilac *(Galega officinalis),* a plant used in folk medicine for centuries.

Metformin was first described in the scientific literature in 1922 by Emil Werner and James Bell, as a product of the synthesis of N"", N""-dimethylguanidine. Anti-diabetic therapy has been proposed as a treatment for polycystic ovary syndrome (PCOS), a condition often associated with insulin resistance, since the late 1980s.

The use of metformin in PCOS was first reported in 1994 in a small study conducted at the University of the Andes. The UK National Institute for Health and Clinical

Excellence recommended in 2004 that women with PCOS and a body mass index above 25 be given metformin for anovulation and infertility treatment when other has failed to produce results.

However, two large clinical studies in most returned negative results 2006-2007, with metformin being no better than placebo and metformin-clomifene combination no better than clomiphene alone.

- It improves peripheral insulin effects, particularly on the muscle.
- It decreases hepatic glucose production and reduced FA oxidation.
- The insulin resistance is reduced by increasing the glucose consumption device, by a totally unknown muscle membrane effect.
- In vitro, there is an increase in the translocation of glucose transporters (Glut1 & 4).

Effects on obese patients with PCOS:

- Studies show after treatment for 2 months because of 3x 500 mg / day
- BMI of diminution of 7 to 10%
- decreased insulin secretion of + / - 35% during the OGTT;
- increased SHBG of 20 to 25%
- decrease in triglycerides
- decreased androgen

We also note a resurgence of ovulatory cycles in 50% of cases and pregnancy spontaneous [41].

SIDDHA DRUGS FOR PCOS

Chooranam:

- *Thratchathy chooranam*-1 to 2 gm twice a day with honey(5 ml)
- *Parangipattai chooranam*-1 to 2 gm twice a day with milk(50 ml)
- *Inji chooranam*- 1 to 2gm twice a day with water(50 ml)
- *Amukkara chooranam*-1 to 2 gm twice a day with ghee(50 ml)
- *Seenthil chooranam*-1 to 2 gm twice a day with hot water(5 ml)
- *Karisalai chooranam*-1 to 2 gm thrice a day with honey(5 ml)
- *Asoka pattai chooranam*-1 to 2 gm thrice aday with hot water(50 ml)

***Nei*:**

- *Birami nei*-10 to 15 ml at early morning
- *Senkottai nei*-15 ml twice a day
- *Venpoosani nei*-15 ml twice a day
- *Thanneervittan nei*- 10 ml twice a day

***Manapagu*:**

- *Thurunchi manapagu*-10 to 20 ml twice a day with luke warm water(50ml)
- *Madhulai manapagu*-10 to 15 ml twice a day with hot water

***Ilagam*:**

- *Vilvathy ilagam*-5 to 10gm twice a day
- *Venpoosanai ilagam*-5 to 10gm twice a day
- Nellikai ilagam-5 to 10gm twice a day
- *Impooral ilagam*-5 to 1gm twice a day
- *Karisalai ilagam*--5 to 10gm twice a day
- *Kumara ilagam*--5 to 10gm twice a day
- *Thaneervittan ilagam*-5 to 10gm twice a day

Mezhugu:

- *Gunma kudori mezhugu*-500 to 1000 mg twice a day
- *Rasagandhi mezhugu*-300 mg twice a day with 5gm palm jaggery
- *Vaan mezhugu*-50 to 100 mg twice a day with 5gm palm jaggery
- *Rasa mezhugu*- 100 to 200 mg twice a day with 5gm palm jaggery
- *Nandhi mezhugu*-- 100 to 200 mg twice a day with 5gm palm jaggery
- *Idivallathy mezhugu*-300 mg twice a day with 5gm palm jaggery

Rasayanam:

- *Gandhaga rasayanam*-1.3 to 2gm twice a day

Maathirai

- *Maha vasantha kusumaahara maathirai* (100 mg)- 1 to 2 mg twice a day with 10 ml leaf juice of *karisalai*

Parpam:

- *Kungiliya parpam*-100 to 300 mg twice a day with ghee (5 ml)
- *Muthu parpam*-30 to 60 mg twice a day with ghee (5 ml)
- *Muthuchippi parpam*-200 to 400 mg twice a day with ghee (5 ml)
- *Palagarai parpam*-65-130 mg twice a day with milk (50 ml)
- *Sangu parpam*-30 to 60 mg twice a day with ghee (5 ml)

Chendooram:

- *Vediannabedi chendooram*-100 mg twice a day with hot water(50 ml)
- *Kalluppu chendooram*-488 mg twice a day with honey(5 ml)
- *Pattu karuppu*- 65 to 130 mg twice a day with honey(5 ml)
- *Aarumuga chendooram*-65 to 130 mg twice a day with 1 to 2gm thirikadugu chooranam and honey (5 ml)
- *Ayakandgha chendooram*-65 to 130 mg twice a day with honey (5 ml)
- *Aya chendooram* -65 to 130 mg twice a day with honey (5 ml)

- *Thalaga chendooram*-30 to 65 mg twice a day with 50 ml milk
- *Kalamega narayana chendooram*-30 to 100 mg twice a day with 10 ml leaf juice of *Cassia sena*

Thylam:

- *Kalingathy thylam*-280 gm with 50 ml cold rice water from the first day of mensturation
- *Chitramutty thylam*-5 to 10 ml twice a day

Kuzhambu:

- *Kumatty kuzhambu*-130 mg with 5gm palm jaggery for 3 days from the first day menstruation [42].

3.4 PHARMACOLOGICAL REVIEW

Estradiol method

The reproductive cycles of the rats were synchronized by the following method. 100µg estradiol dissolved in 2 ml olive oil was injected subcutaneously. All rats after a 24 hr period, received intramuscular injections of 50 µg progesterone dissolved in olive oil. After few hours, vaginal smears were obtained by vaginal lavage to monitor ovulation and estrous cycle. Vaginal smears were prepared by washing vaginal opening with 0.9% w/v of sodium chloride with a glass dropper and placed in a clean glass slide and viewed under light microscope at 40X magnification. Examination of vaginal smears showed that all the animals were in the estrous stage. All the animals were weighed daily after drug administration for 10 days.

Letrozole method

A control group received 2% of CMC (carboxy methyl cellulose). Other groups for 28 days were once, daily administered, letrozole at the concentration of 1mg/kg. Vaginal smears were collected daily from all groups and studied for estrous cycle. During 28 days of administration of letrozole, the changes in estrous cycle were observed.

Prenatal androgen

Prenatal androgen (PNA) treatment in sheep and monkeys result in multiple metabolic and reproductive abnormalities. In monkeys, daily subcutaneous injections of 15 mg of testosterone propionate for 40-80 days gestation are needed to induce syndrome. In ewes, an injection of 100 mg of testosterone propionate twice a week for 60 days between days 30 and 90 of the 147-day pregnancy result in the ovarian abnormalities.

PA monkeys exhibit hyperandrogenemia, the increases are not as extreme as in PCOS women 0.3-04 ng/mL (~50-100% elevation above nomal) PCOS woamen,0.5-0.7 ng/Ml(~70-200% elevation above normal)and that although anovulation observed in PNA monkeys, its prevalence is also significantly less than that of PCOS women(PA monkeys:~40%;PCOS women ~90%).

Pre-pubertal androgen

This model exploits the association of raised androgen levels during puberty and PCOS. Immature rats(approximately 21 days old) are treated for 7-35 days with ~100 µg/days testosterone propionate or dihydrotestosterone. Similar to the PNA animal models, pre-pubertal androgen(PPA) animals models of PCOS utilize a unique window where administration of exogenous androgens results in permanent damage to the ovarian tissue and recapitulated the hallmark symptoms of PCOS in an animal model.PPA model shows a lot of same features to PCOS in women with the exception of the hallmark increase in basal LH levels. This model is realiant on artificial hyperandrogenemia and therefore does not help identify abnormalities upstream of hyperandrogenemia.

Dehydroepiandrosterone(DHEA)

Immature rats or mice (approximately 21 -22 days old) are treated with daily s.c DHEA injections (rats; 6mg/100 g body weight, mice 6 mg/kg body weight) for 15 – 20 days.

This dose of DHEA is sufficient to induce a hyperandrogenized state similar to that in PCOS women. This model is also needful on an artificial hyperandrogenemia and does not help identify abnormalities upstream of hyperandrogenemia.

RU 486

Mature cycling female rats are treated daily with RU486 (20 mg/kg body weight) for more than 8 days starting on the day of estrus. These animals exhibit elevated basal LH, polycystic ovaries, ovulation blockade and metabolic defects. This model is reversible and symptoms reduce upon cessation of the anti -progestin treatment.

Hypothalamic pro-opiomelanocortin (POMC) neuron specific leptin and insulin receptor KO

Hill and her colleagues were interested in insulin resistance and the development of type II diabetes when they developed their pomc-cre, Leprflox/floxIRflox/flox mice, effectively removing both leptin and insulin receptors specifically from the POMC. However, together with the anticipated glucose intolerance and insulin deficiency these mice suffer from hyperandrogenemia and polycystic ovaries.

A novel hyperandrogenic animal model for the study of PCOS

The unravelling of a natural pathway for PCOS and assigning etiologies to this syndrome requires the development of a novel animal model that consistently present PCOS phenotypes. We therefore launched a project to develop a mouse model of PCOS that is genetically modified and thus stably displays PCOS phenotypes. This cycle, we targeted the ovarian steriodogenic pathway to induce a preference to androgen synthesis and therefore create hyperandrogenemia.

The two cell layers act cooperatively in the production of androgen and estrogen. Theca cells produce androgens that transverse the basement membrane to the granulosa cells, where aromatase converts the androgens to estrogens. The estrogens in turn negatively feedback to the theca cells causing a decrease of androgen production. This negative feedback action of estrogen is mediated by estrogen receptor I (Esrl;Erα) in the theca cells, the prime site of Esrl expression in the ovary. Upon activation by the binding of estrogen, ESrl suppresses the expression of Cyp 17, a critical enzyme that catalyzes a rate- limiting step in androgen production, resulting in the decrease of androgen production. Therefore, deleting Esrl gene would be a logical choice for achieving sustained high levels of androgens because loss of Esr1 will exclude negative

regulation of Cyp17 gene expression by estrogen. This will ultimately result in increased production of androgens. The deletion of Esr 1 gene has to be, however, limited to theca cells because Esr1 is expressed in so many different tissues/ organs including all of the tissues of the hypothalamic-pituitary-ovary-uterus axis. Otherwise, the Esr1 deletion in non-theca cells would cause other defects, making it difficult to isolate the Esr1 deletion effect in the theca cells.

This goal was achieved by selectively knocking out Esr1 in the steroidogenic theca cells while keeping the gene intact elsewhere. In principle, this approach would result in the increased expression of Cyp17 gene that encodes a late-limiting enzyme (17apha-hydroxylase) in androgen synthesis pathway, because deletion of Esr1 will relieve Cyp17 transcription from the well-established suppression of this gene by Esr1. We acheived this aim by first generating a mouse line that expresses crerecombinase under the regulation of the Cyp17 promotes (Cyp17 iCre) and then breeding the mice successively with floxed Esr1 (Esr1flox) mice. The offspring with the genetype Esr1 flox/flox Cyp17iCre, as was expected, expresses higher level of Cyp17 mRNA expression in the ovary and maintains higher serum testosterone levels (1.5-2 folds over wild type littermates). The Esr1flox/flox Cyp17Cre mice display other phenotypic symptoms of PCOS patients (i) irregular estrous cycles, (ii) an age dependent decrease in ovulatory capacity and fertility defect and (iii) arrest of follicular development at the early antral stage. This novel animal model generated from the proof-of-principal experiment should bring a new opportunity for the study of PCOS as well as for the study of hyperandrogenemia itself.

Future direction

In contrast to the seriousness of the PCOS in women's health, the pathogenesis of this disease is poorly understood. In recent years, efforts by multiple laboratories have led to the development of novel animal models for this complicated disorder. In the next few years, use of these animal models and those to be newly developed will undoubtedly accelerate our understanding on PCOS pathogenesis and possibly lead to the development of new therapeutic protocols for treating and preventing PCOS in women [43].

3.5 LATERAL REVIEW

Styrax benzoin

Sedative and Anticonvulsant activities of Styrax after oral and Intranasal administration in Mice

Styrax was tested for sedative, hypnotic and anticonvulsant effects using locomotor activity evaluation, pentobarbital- induced sleeping time, and pentyleneterazol (PTZ)-induced convulsion, respectively.

After oral administration (25, 50, 100 mg/kg), styrax prolonged the sodium pentobarbital-induced sleeping time. In comparison with oral administration, intranasal administration (12.5, 25, 50 mg/kg) prolonged the sleeping time at lower dosage. Moreover, styrax (100 and 200 mg/kg) promoted a significant protection against PTZ-induced seizures and mortality 30 min after oral administration. In contrast, 5 min after intranasal administration, styrax promoted significant protection at lower dosages (25 and 50 mg/kg). These data show that styrax had faster onset of action (5 vs. 30 min) and better anticonvulsant efficacy (25, 50 vs. 100, 200 mg/kg) by intranasal route in comparison with that by intragastric route. Styrax decreased the spontaneous locomotor movements at 100 mg/kg during 5–60 min interval after oral administration.

Styrax has sedative and anticonvulsant activities. Furthermore, styrax has faster onset of action as well as more potent efficacy after intranasal administration at lower dosage than by intragastric route. This result illustrates that intranasal administration may act as a promising alternative to conventional routes of administration [44].

Piper betle

ANTIMICROBIAL, ANTIOXIDATIVE AND ANTIHEMOLYTIC ACTIVITY OF PIPER BETEL LEAF EXTRACTS

Piper betel L. belongs to family Piperaceae commonly known as Paan. It is extensively grown in Srilanka, India, Thailand, Taiwan and other Southeast Asian countries. The leaves are pungent, bitter, sweetish, acrid in nature. It has got larg e number of biomolecules which show diverse pharmacological activity along with car

minative, stomachic, antihelminthic, tonic, aphrodisiac, laxative activities. The leaves a re used for treating cough, foul smelling in mouth, ozoena, bronchitis, clears thro at, vulnery and styptic. In the present experiment four different extracts (water, methanolethyl acetate and petroleum ether) of Piperbetel leaves were tested against our different pathogenic bacteria namely Streptococcus pyogenes, Staphylococcus aureus, Proteus vulgaris and Escherichia coli.

Further few known and unknown metabolites were isolated from these extracts. S tructural elucidations of new metabolites were done by different analytical techniques li ke NMR, Mass and IR spectroscopy. Later on anti-oxidative and anti-
haemolytic activities were determined. Anti-
oxidative studies were done by TBARS and DPPH method.

Anti-hemolytic activity was determined using erythrocytes model and the extent of lipid peroxidation of the same was also determined [45].

Syzygium Aromaticum

Anti-Nociceptive and Anti-Inflammatory Activities of Ethanol Extract of *Syzygium Aromaticum* Flower Bud in Wistar Rats and Mice

The ethanol extracts of *Syzygium aromaticum* flower bud were tested for anti-nociceptive and anti-inflammatory effects in mice and Wistar rats which were carried out using acetic acid-induced abdominal contractions in mice and formalin-induced hind paw edema in Wistar rats. Three doses of the ethanol extract (50, 100, and 200mg/kg body weight i.p.) were used for both studies. The extract had an LD_{50} of 565.7 mg/kg body weight intraperitoneally in mice. The extracts produced significant effect ($P<0.05$) at all the three doses. Similarly, the anti-nociceptive activity produced significant effects ($P<0.05$) at all the three doses of the extract. The result supports the local use of the plant in painful and inflammatory conditions [46].

Fel bovinum(Ox Bile)

Isolation and identification of a renin inhibitor from ox bile

A renin inhibitor in ox bile was purified by ammonium sulfate fractionation, dialysis and chromatography on Bio-Gel P-2 and Amberiite XAD-2 resin. The inhibitor was shown to be taurodeoxycholic acid by means of nuclear magnetic resonance spectroscopy, gas chromatography, amino acid and elemental analyses. Further confirmation of the assignment was established by the direct comparison of its infrared spectrum with that of an authentic sample. Taurodeoxycholic acid inhibited the reaction of rabbit renin with angiotensinogen *in vitro*. Glycocholic acid, also present in ox bile, however, had no inhibitory effect on the renin angiotensinogen reaction [47].

4. MATERIALS AND METHODS

4.1. PREPARATION OF THE TRIAL DRUG:

SAMBIRANI POO KULIGAI

SELECTION OF THE DRUG:

For this thesis, the Herbal formulation *Sambirani poo kuligai* was taken as the compound drug preparation for Polycystic Ovarian Syndrome were taken from the customary Siddha literature **"*AGASTHIAR PARIPURANAM* 4OO**" Published by B. Rathina Nayakker And Sons Pg no:33.

COLLECTION OF THE DRUG:

The raw drugs like *sambirani* , *korosanai* , *kirambu* and *vetrilai* were bought from the country shop at Parrys, Chennai.

INGREIDIENTS:

Sambirani [Styrax benzoin]	-	250 g
Korosanai [Felbovinum purifactum]	-	6 g
Kirambu [Syzygium aromaticum]	-	20 g
Vetrilai [Piper betel]	-	50 ml

Identification and Authentication

All raw drugs were identified and authenticated by the Botanists and the experts of Gunapadam (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai.

The specimen samples of the identified raw drugs were preserved in the laboratory of P.G Gunapadam for future references.

PURIFICATION

Styrax benzoin [48].

The gums were purified by removing the sand, dust and odd particles.

Fel bovinum purifactum

The unwanted substances were removed.

Syzygium aromaticum [49].

The flower buds were removed and fried slightly.

Piper betle [49-A]

The stalk and the middle vein were removed.

PREPARATION OF *SAMBIRANI POO KULIGAI*

Ingredients

Styrax benzoin	-	**10.2g**
Syzygium aromaticum	-	**15.3g**
Fel bovinum purifactum	-	**5.1 g**

Associate drug

Juice of ***Piper betel*** leaf	-	**20 ml**

PROCEDURE

SUBLIMATION PROCESS

- The purified *Styrax benzoin* was powdered well.
- It was placed in a small pot.
- Then a paper was pasted on the inner surface of the big pot.
- The big pot was placed over the small pot and their mouths oppose each other.

- The gap between their mouths were covered by a seven layered muddy wet cloth and they allowed to dry.
- Then it was subjected to sublimation process for 12 hours (*4 samam*).
- After finishing sublimation process let the pot undisturbed to give away heat.
- Then the seal was opened and the sublimed product was scrapped and collected.

KULIGAI PROCESS

- *Syzygium aromaticum* was powdered well and sieved through a white cloth.
- *Felbovinum* also powdered well.
- *Syzygium aromaticum* powder and *Felbovinum* powder are added along with the sublimate.
- Then all these substances are grounded well with *Piper betle* leaf juice for 48 minutes [2 *Nazhigai*]
- The paste was made into pills in the size of seeds of *Abrus precatorius [Kundri size]* which was equivalent to **130** mg, dried in the shade and bottled up.

DOSAGE

Administration of the drug

Form of the medicine	-	pills
Route of administration	-	Enteral
Dose	-	130 mg, 7 days
Adjuvant	-	Sugar

INDICATIONS: *Soothaga vayu,* *Soothaga kattu*

PRESERVATION

The medicine was preserved in well stoppered air tight glass containers and labelled as SPK *(Sambirani Poo Kuligai)*

INGREDIENTS

Styrax benzoin

Syzygium aromaticum

Piper betle

Felbovinum

PREPARATION

SUBLIMATION PROCESS

Powdered *Stvrax benzoin*

Sublimation

Sublimation Product

KULIGAI PROCESS

Sublimate of *Styrax benzoin*

Felbovinum Purifactum

Powdered *Syzygium aromaticum*

***Piper betle* leaf juice**

Trituration with *Piper betle*

Sambirani Poo kuliaai

Figure No.1 Preparation of *Sambirani Poo kuligai*

4.2. STANDARDISATION OF THE DRUG

Standardization of drug and formulations paves the way for the establishment of consistent purification methods, consistent chemical, biological, physico-chemical profile, standardized herbal and herbo-mineral products of consistent quality containing well defined active principles responsible for exerting pharmacological actions required for reliable preclinical trials as well as clinical trials to provide consistent beneficial therapeutic effects.

4.2.1. ORGANOLEPTIC EVALUATION

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its color, odor, taste, texture etc.

Colour

A sample of ten tablets were taken in watch glasses and placed against white back ground in white tube light. The tablets were observed for its color by naked eye.

Odour

Ten tablets were smelled separately. The time intermission between two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste

A sample of about ten tablets was tasted and the taste was reported.

Size

Using the instrument Vernier Caliper the diameter of ten tablets was measured and the mean value of diameter was noted [50].

4.2.2 PHYSICO-CHEMICAL ANALYSIS

Physico-chemical studies like total ash, water soluble ash, acid Insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Central Research Institute, Chennai.

Solubility Test

A pinch of sample (SPK) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

Determination of Total Ash

About 2 to 3 g of the ground drug (SPK) was accurately weighed in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450ºC until it was free from carbon, cooled and weighed. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Water Soluble Ash

Total ash was heated up to 600^0C with 25 ml of distilled water for 10 minutes and the residue was ignited in the furnace to get a constant weight.

Determination of Acid Insoluble Ash:

The ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and insoluble matter was collected in a ash-less filter paper, washed with hot water and put up in flames to constant weight. The percentage of acid-insoluble ash with reference to the air dried drug was analyzed.

Determination of Alcohol Soluble Extractive:

The air dried drug was finely grounded, added with 100 ml of ethanol of specified strength in a closed flask for twenty-four hours, shaken frequently during the course of six hours and allowed to stand for eighteen hours. Then the mixture was filtered rapidly taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105º to constant weight. The percentage of alcohol-soluble extractive with reference to the air-dried drug was estimated.

Determination of Water Soluble Extractive

The above procedure was repeated but instead of ethanol, chloroform with water was used.

Determination of Moisture Content (Loss on Drying)

This procedure was done to determine the amount of volatile matter in the drug. A sample of about 10 gram of the drug (SPK) was placed in a tarred evaporating dish after accurately weighting without preliminary drying. The dish was dried at a temperature of 105^0 for about 5 hours and again weighed. The drying and weighing procedure was repeated again and again until the difference between two successive weights was not more than 0.25%. A constant weight was achieved only when the successive weight difference was not more than 0.01% after drying for 30 min and cooling for 30 min.

pH value

pH value of the sample (SPK) was determined potentiometrically by a glass electrode and a suitable pH meter and noted down.

Specific gravity estimation by density bottle method

Procedure

The density bottle was dried in an oven at 105^0c, weighed (M_1), sample was added and the bottle was weighed again (M_2). Then distilled water was added to the sample, shaken gently and the mass of the bottle with the contents was weighed again (M_3).

The mass of the bottle only with distilled water was taken as M_4 and the specific gravity was calculated according to the formula,

$G = \frac{M2-M1}{(M2-M1)-(M3-M4)}$ and the results were noted down.

Tablet Disintegration test

Each SPK sample was placed in six tubes individually present in the basket of disintegration apparatus. The apparatus was handled using water as the immersion fluid maintained at 35-39 °C. The basket was lifted from the fluid and the state of the tablet was observed at the end of the 30 min. The six tablets disintegrated completely in about 45 minutes [51].

Weight variation test [52].

Weight variation was carried out to make certain that, each of the tablets contain the appropriate amount of drug. The test was carried out by weighing the 20 tablets separately using analytical balance, then manipulating the average weight, and comparing the individual tablet weights to the average.

The percentage of weight variation was calculated by using the following formula.

$$\% \text{ of weight variation} = \frac{\text{Individual weight.} - \text{Average weight}}{\text{Average weight.}} \times 100$$

4.2.3. PHYTO CHEMICAL ANALYSIS

Phytochemicals are chemical compounds that are naturally present in plants. Phytochemical screening of the plant gives a vast idea about the chemical constituents present in the drug.

The SPK sample was subjected to the following phytochemical screening based on the method illustrated in Prashant Tiwari et al., 2011 and Harborne, 1973.

Preparation of the extract

5g of SPK sample 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.

Test for Alkaloids

A small portion of SPK extract was stirred separately with few drops of dilute hydrochloric acid and filtered and tested carefully with various alkaloidal reagents like **Mayer's reagent**, and observed for the appearance of coloured precipitates and noted down.

Test for Carbohydrates and Reducing Sugars

A small quantity of the SPK was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test

To the 1 ml of SPK filtrate 2-3 drops of 1% alcoholic alpha naphthol & 2ml concentrated Sulphuric acid was added along the sides of test tube and observed for the presence of violet ring at the junction of 2 layers.

b) Benedict's test

To 1ml of the SPK filtrate Benedict's reagent was added and heated gently and observed for the appearance of orange red precipitate.

Test for Glycosides:

To the SPK extract dilute. HCl was added and it was subjected to test for glycosides.

a) Modified Borntrager's test

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and dipped in boiling water for about 5 min.

The cooled mixture was extracted with equal volume of benzene.

The benzene layer was again separated and treated with ammonia solution and observed for the appearance of rose pink color in the ammoniacal layer.

b) Legal's test

The hydrolysate extract was treated with sodium nitropruside in pyridine and sodium hydroxide and observed for the presence of pink to blood red color.

Test for Saponins

0.5 ml of the SPK extract was shaken with 5 ml of distilled water and observed for the appearance of copious lather.

Test for Tannins

Gelatin test

To the SPK extract, 1% gelatin solution containing sodium chloride was added and waited till the appearance the white precipitate.

Test for Phenolic compounds

To 0.5 ml of SPK extract, 1 ml of alcoholic ferric chloride solution was added and observed for the appearance of bluish green or bluish black color.

Test for Phytosterol

Ferric chloride – acetic acid test

To 1 ml of SPK extract 1 ml of chloroform, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of concentrated. Sulphuric acid and waited for the appearance for reddish pink color.

Test for Diterpenes

Copper acetate test

To 1 ml of SPK extract water was added and 3-4 drops of Copper acetate solution was added again to see the appearance of emerald green color.

Test for Triterpenes

Salkowiski's test

To 1 ml of SPK extract 1 ml of chloroform followed by 1 ml of concentrated Sulphuric acid was added, shaken and allowed to stand for the appearance of golden yellow color.

Test for Flavonoids

a) Alkaline reagent test

To 1 ml of SPK extract, 1 ml of 10% sodium hydroxide solution was added to observe for dark yellow color.

b) Lead acetate test

To 1 ml of SPK extract, 3-4 drops of 10% lead acetate solution was added and waited for the appearance of yellow precipitate.

c) Ferric chloride test

To 1 ml of SPK extract, 3-4 drops of ferric chloride solution was added and observed for the appearance of dark green color.

d) Shinoda test

With 1ml of the SPK extract, magnesium turnings of few mg, few drops of Concentrated Hydrochloric acid was added.

The extract was boiled for 5 minutes in a boiling water bath and observed for the appearance of red color.

Test for Proteins and Free Amino Acids:

a) Xanthoproteic test

To 1 ml of SPK extract, 3-4 drops of concentrated Nitric acid was added and observed for the formation of yellow precipitate.

b) Million's test

To 0.5 ml of SPK extract, 2.5 ml of Million's reagent was added, warmed and waited for the appearance of white precipitate.

c) Biuret test

To 0.5 ml of SPK extract, 2.5 ml of diluted Biuret reagent was added and observed for the appearance of purple color or brick red precipitate.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of SPK extract, 1 ml of 10% sodium hydroxide was added to observe for the appearance of blue or green or red color [53].

TLC/ HPTLC finger print studies

HPTLC finger printing was carried out as per the reference.

Preparation of spray reagent-vanillin-sulphuric acid reagent

Vanillin (1g) was dissolved in ice cold ethanol (95ml). Add to 5ml of cooled concentrated Sulphuric acid. Ice was added and stirred well. The solution was stored in refrigerator.

Chromatographic conditions

Instrument : CAMAG (Switzerland).

Sample Applicator: Camag Linomat - IV applicator with N_2 gas flow.

Photo documentation System : Digi store - 2 documentation system with Win Cat

& video scan software.

Scanner: Camag HPTLC scanner - 3 (030618), Win Cats - IV. Development

Chamber: Camag HPTLC 10X10, 10 X 20 twin trough linear

Development chamber.

Quantity applied: 5, 10 µl for extracts and 5 µl for standards

Stationary phase : Aluminium plate pre-coated with silica gel 60(E. Merck)

Plate thickness : 0.2 mm.

Mobile Phase: For Chloroform extract - Toluene: Ethyl acetate (9:1) and ethanol extract - Toluene: Ethyl acetate (1:1).

Scanning wavelength : 254 nm

Laboratory condition : 26 ± 5^{o}C and 53 % relative humidity

The plate was developed up to a height of 8 cm, air dried, spots were observed under the UV light at 254 and 366 nm. Finally the plates were derivatized using Vanillin -Sulphuric acid reagent heated at 105° till color spots appeared [54].

4.2.4. BIO-CHEMICAL ANALYSIS

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

Preparation of extract

5g of SPK 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.

Preliminary Basic and Acidic radical studies

Test for basic radicals

1.Test for Potassium

To a pinch of the SPK 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 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 SPK extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

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

5.Test for Sodium

Hydrochloric acid was added with a pinch of the SPK sample and a paste was made and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow color.

6.**Test for Iron(Ferrous)**

The SPK extract was treated with Conc. HNO3 and ammonium thiocyanate and waited for the appearance of blood red color**.**

7. Test for Zinc

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

8. Test for Aluminium

To the 2m1 of the SPK extract sodium hydroxide was added in drops and noted for any characteristic changes.

9. Test for Lead

To 2 ml of SPK extract 2ml of potassium iodide solution was added and noted for yellow colored precipitate**.**

10. Test for Copper

a. A pinch of SPK sample was made into a paste with concentrated. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue color appearance.

b. To 2 ml of SPK extract excess of ammonia solution was added and observed for the appearance of blue colored precipitate**.**

11. Test for Mercury

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

12. Test for Arsenic

To 2 ml of the SPK extract 2ml of sodium hydroxide solution was added and brown wash red precipitate if appeared was noted.

Test for acid radicals

1. **Test for Sulphate**

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

2. Test for Chloride

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

3. **Test for Phosphate**

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

4. **Test for Carbonate**

The SPK extract was treated with concentrated Hcl and observed for the appearance of effervescence.

5. Test for Fluoride & Oxalate:

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

6. Test for Nitrate:

To 1 gm of the SPK, copper turnings was added and again concentrated H_2SO_4 was added, heated and the test tube was tilted vertically down and viewed for any characteristic changes [55].

4.2.5. AVAILABILITY OF BACTERIAL LOAD:

Enumeration of bacteria by plate count – agar plating technique

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 sample.

Dilution:

A small measured volume are mixed with a large volume of sterile water or saline 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 SPK into a 9 ml dilution blank labelled 10^{-1} thus diluting the original 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 original 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 sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c [56].

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:

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

4.2.6. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

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

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It was used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It was an excellent tool for quantitative analysis.

In FT-IR infrared was passed from a source through a sample. This infrared was absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there was no two unique molecular structures producing the same infrared spectrum. It was recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated [57].

SEM (SCANNING ELECTRON MICROSCOPE)

In scanning electron microscope high-energy electron beam was focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by a appropriate detector.

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample [58].

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer(ICP)

Principle:

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000–10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emission spectroscopy (OES), a sample solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg SPK was 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 sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106 [59].

FTIR (Fourier Transform Infrared Spectroscopy)

Figure No.2 FTIR INSTRUMENT

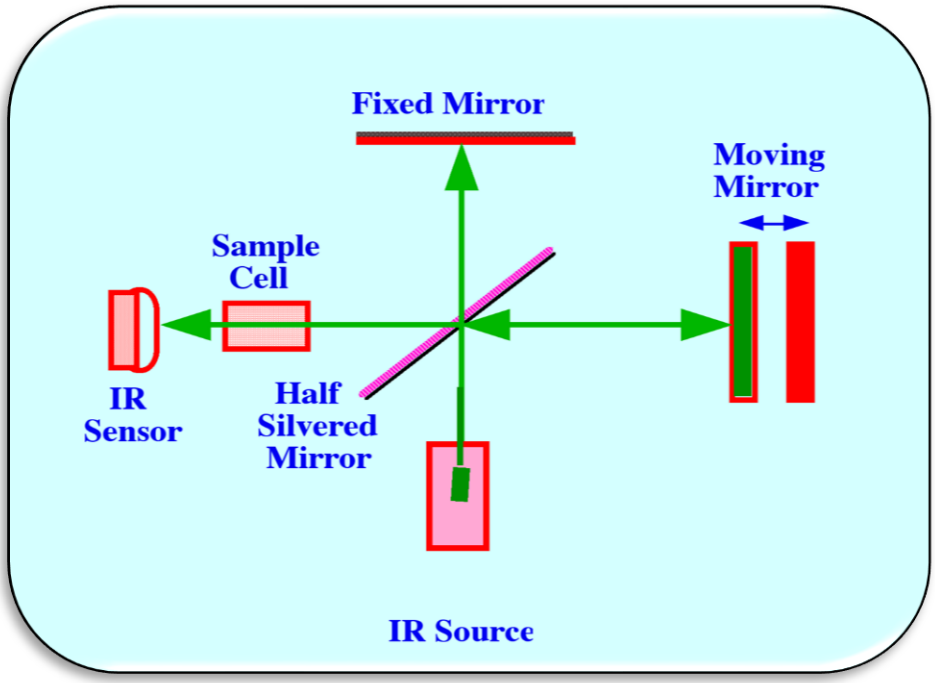


Figure No.3 FTIR MECHANISM

SEM- SCANNING ELECTRON MICROSCOPE

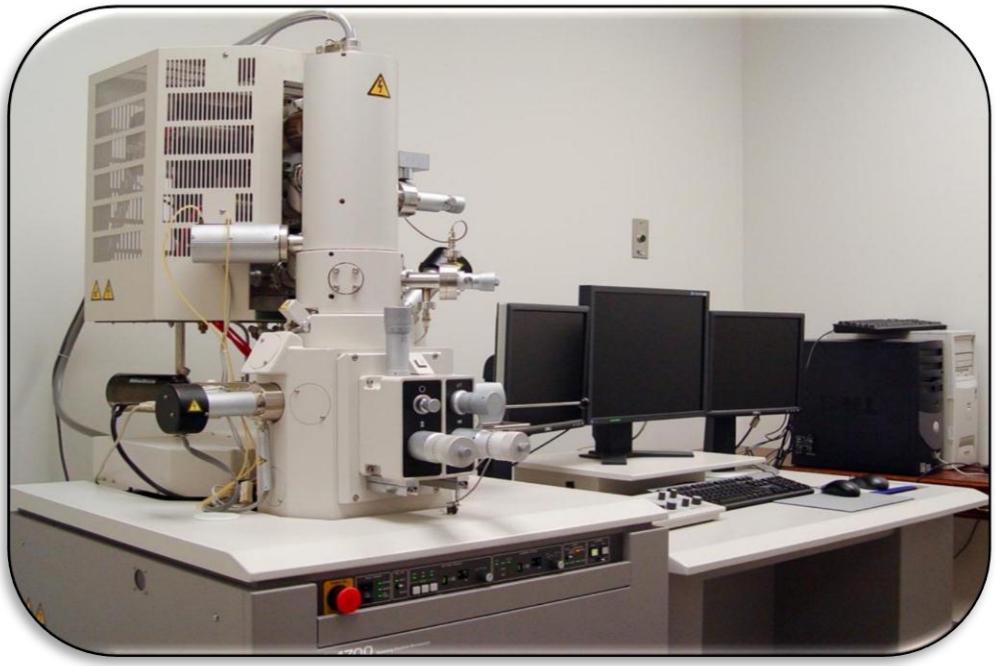


Figure No.4 SEM INSTRUMENT

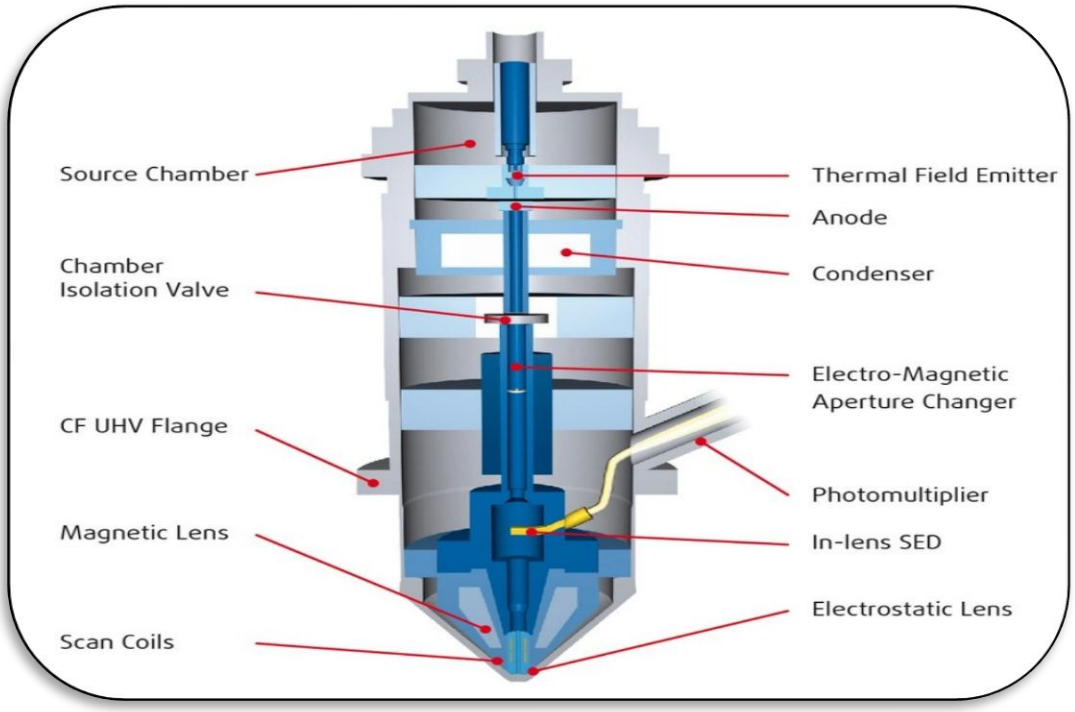


Figure No.5 SEM MECHANISM

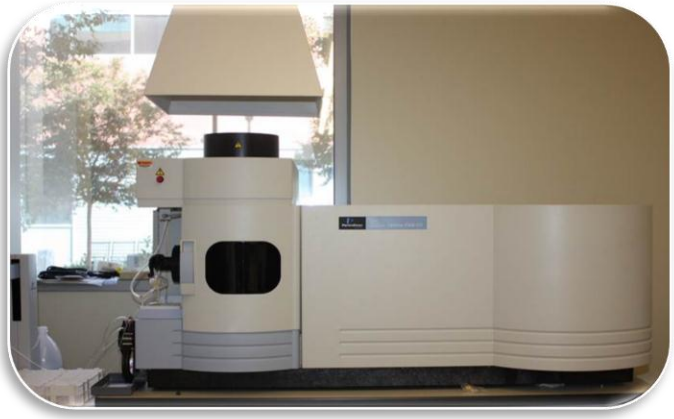


Figure No.6 ICP-OES ANALYSER (Perkin Elmer Optima 5300 DV)

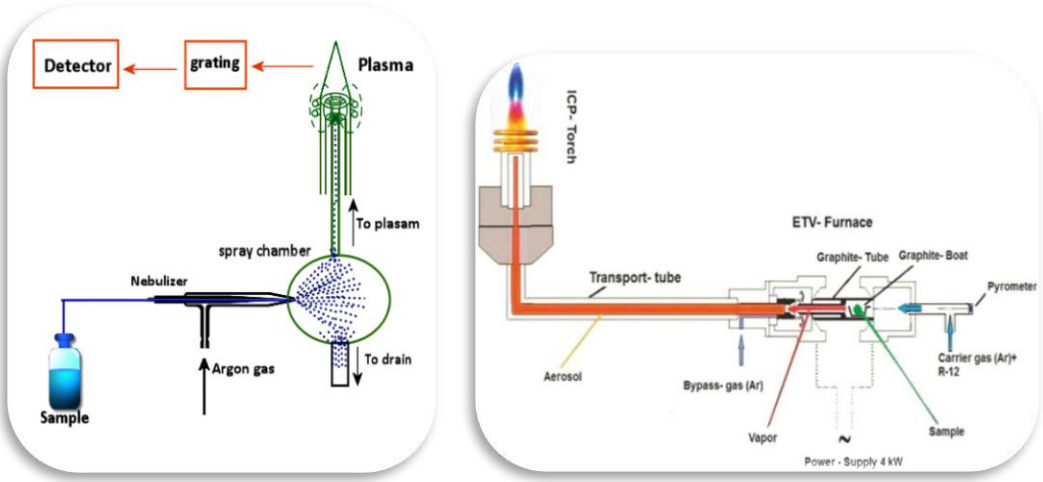


Figure No.7 Mechanism of ICP-OES

4.3 TOXICOLOGICAL STUDIES

4.3.1 ACUTE ORAL TOXICITY – OECD GUIDELINES - 423

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/or 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 [Approval no: IAEC/XLIV/27/CLBMCP/2014]

Animal: Healthy wistar albino female rats weighing 200–220 gms

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, sufficient 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.

Studies carried out at three female rats under fasting condition, signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study [60].

METHODOLOGY

Selection of animal species:

The preferred rodent species is rat, although other rodent species may be used. Healthy young adult animals of commonly used laboratory strain Swiss albino rat was 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. Baid Metha college of Pharmacy, Duraipakkam , 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 hours 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

SPK 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 gavage 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 SPK 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 is 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 SPK 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 [61].

(Schlede E., Mischke U., Diener W. and Kayser D 1992;66: 455-470)

4.3.2. REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *SAMBIRANI POO KULIGAI* ON RATS – (OECD-407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that SPK 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

SPK 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 4^{th} 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 hour 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 fecal 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 hour, 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 technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°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, hematology 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 [62].

4.4 PHARMACOLOGICAL STUDIES

4.4.1 Ovulation inducing activity in female Wister albino rat model

Method

Before starting drug treatment, the reproductive cycles of the rats were synchronized by the following method. 100µg estradiol dissolved in 2 ml olive oil was injected subcutaneously. All rats after a 24 hours period, received intramuscular injections of 50 µg progesterone dissolved in olive oil. After few hours, vaginal smears were obtained by vaginal lavage to monitor ovulation and estrous cycle. Vaginal smears were prepared by washing vaginal opening with 0.9% w/v of sodium chloride with a glass dropper and placed in a clean glass slide and viewed under light microscope at 40X magnification. Examination of vaginal smears showed that all the animals were in the estrous stage.

All the animals were weighed daily after drug administration for 10 days. The suitable sensitive rats were divided into four groups of six each as follows

Experimental design

- Group I Normal Control animals 1ml/kg of CMC solution.
- Group II rats were administered SPK 100mg/kg for 10days,
- Group III rats were administered SPK 200mg/kg for 10 days
- Group IV received Clomiphene 10mg/kg and served as standard. All the drugs were given orally.

After that 2ml of blood was collected by retro orbital puncture. Blood samples were centrifuged for 15 minutes at 4000 rpm and the separated serum samples were frozen at -20°C and kept for later estimation of LH, FSH and Estradiol by ELISA method [63].

4.4.2. Hormonal assay

Biochemical assay

The method employed was Micro well Enzyme Linked Immunosorbent Assay (ELISA) using analytical grade reagents.

Estimation of serum luteinizing hormone (LH)

The method employed was Micro well immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of LH-Enzyme reagent was added to all the wells. The micro plate was swirled for 20-30 seconds and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a micro plate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Estimation of serum follicle stimulating hormone (FSH)

The method employed was Micro well immunoassay (ELISA) using analytical grade reagents. 0.050ml of the serum was pipetted into the assigned wells. 0.001ml of FSH-Enzyme reagent was added to all the wells. The micro plate was swirled for 20-30 seconds and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for fifteen minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a micro plate reader within 30mins.The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was

constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Determination of serum progesterone levels

The method employed was Micro well immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum was pipetted into the assigned wells. 0.050ml of progesterone Enzyme reagent was added to all the wells. The micro plate was swirled for 20 seconds to mix, 0.050ml progesterone biotin reagent was added to all the wells, the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 60 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a micro plate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve.

Determination of serum Estradiol levels

The method employed was Micro well immunoassay (ELISA) using analytical grade reagents. 0.025ml of the serum reference was pipetted into the assigned wells. 0.050ml of Estradiol Biotin reagent was added to all the wells. The micro plate was swirled for 20 seconds to mix, the mixture was incubated at room temperature for 30mins,0.050ml Estradiol enzyme reagent was added to all the wells , the mixture was swirled for 20 seconds to mix and covered, this mixture was allowed to incubate for 90 minutes at room temperature. After which, the contents were discarded by decantation. 350µl of wash buffer was added and decanted for 3 times. 100µl of working substrate solution was added to all the wells and was allowed to incubate for twenty minutes. 50µl of stop solution was added to all the wells and gently mixed for 20 seconds. The optical density was read at 450nm in a micro plate reader within 30mins. The mean absorbance values for each set of standards, controls, and test samples were calculated and a standard curve

was constructed by plotting a mean absorbance obtained from each of the reference standard against its concentration from the standard curve [64].

4.4.3 Antioxidant activity:

Free radical scavenging activity:

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

The radical scavenging activity of SPK extracts was determined by using DPPH assay according to Chang et al[2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1,1-diphenyl-2-picryl hydrazyl was a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mm DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes (2.5µl - 40µl) of plant extracts were made up to a final volume of 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture

incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm. 3ml of DPPH was taken as control [65].

CALCULATION

$$\% \text{ inhibition} = \frac{control - test}{control} X100$$

`

5.RESULTS AND DISCUSSION

The trial drug *Sambirani Poo Kuligai* is indicated for *Soothaga vayu*, which is related to the modern terminology PCOS. This study deals with the scientific analysis of the trial drug with scientific parameters. To validate the traditional usage, the drug was subjected to literary review, physico-chemical and elemental analysis, toxicity studies and pharmacological studies ovulation inducing activity, estimation of hormones FSH, LH, Progesterone, Estradiol and antioxidant activity for its naturally curing property for Polycystic ovarian syndrome.

Discussion on review of literature

The extensive review on botanical aspect gave information about the microscopical, macroscopical, medicinal uses, constituents and the importance of the herbs in folklore and the details of the herbs in detail. Most of the herbs included in the formulation exerted **stimulant** action**.**

The review of the herbs in the texts depicted through the songs of Siddhars some 2000 years ago, strengthened the facts mentioned in the modern botanical aspect. Siddhars explained the medicinal properties of the plants through taste and most of the drugs included in the formulation have acrid taste and comes under the category of heat provocating medicines.

The ingredients present in this formulation will support for the activity ***Styrax benzoin*** is indicated for menstrual related problems as per the Siddha literature **[66].**

Felbovinum have the property in the regulation of menstrual discharge.

- It is used in the digestion of fatty acids
- It assists in the absorption of fatty acids
- It is a solvent for fatty acids
- Increased abdominal fat and obesity are the common complaints present in PCOS.
- It has the ability to absorb vitamins like A,D,E,K thereby improving the chances for fertility.

Piper betle leaf contains aromatic phenolic compounds which have been found to stimulate the release of catecholamines [67].

Various stimulant drugs are under Catecholamine analogues [68].

It is more important for the metabolic processes especially fat and glucose turnover.

It contains vitamins and minerals such as Calcium, Thiamine, Niacin, Riboflavin, Carotene and Vitamin C [69].

Apart from this, it is a powerful antioxidant.

Syzygium aromaticum, being an aromatic agent it has the influence in hormonal action [70].

Most of the drugs in this formulation have acrid taste.

The acrid nature drugs has the property to reduce cholesterol by digestion of fat,which is the major cause for PCOS [71].

Discussion on pharmacological aspect

- The pharmacological aspect of the drug shows the presence of various animal model availability for PCOS.
- The current pharmacological methods available for carrying out the ovulation inducing activities were explained clearly and the suitable animal for carrying out the activities were discussed to be Wistar strain of albino rats than other animals because of the parallelism with the human.
- They are also more sensitive compared to other animals and hence they were chosen for the study.
- The exact ovulogenic activity could never be better studied by analyzing the pharmacological activities like ovulation inducing activity, antioxidant activity and estimation of female hormones.
- Since the three activities could clearly explain the whether the drug effectively induce ovulation and maintains the level of hormones which gives the knowledge about the activities.

Discussion on Pharmaceutical review

- This review explained the preparation of *Mathirai* in detail including the purification of raw drugs, methods of manufacturing *Mathirai* and the Siddha parameters for the standardization of analyzing *Mathirai.*
- Roasting of aromatic substances may result in certain chemical changes and increase in antioxidants**.**
- The ingredients are cleaned, wiped with a clean cloth so as to remove the sand and other impurities.
- The powdered drugs were filtered through the white cloth so as to reduce the size of the particle in turn which enhances the bio-availability.
- The shelf life of the drug is improved by proper purification methods and preservation [72]**.**

Discussion on Materials and Methods

- The preparation of the drug was done carefully so as to achieve the highest potency.
- Sublimation of the drug *Styrax benzoin* gives additional potential to the tablet. Because sublimates are in the form of nano particles which increases the bio availability of the drug.
- So it has a unique chemical nature of its own.
- The impregnation with *Piper betle* juice is to enrich the therapeutic potentiality of the drug.

`

Standardization of the drug

The standardization of the drugs was achieved through various procedures like analyzing the organoleptic characters, physico-chemical characters, elements present in the drug and the results and discussion of standardization parameters is

Table. No.1. Organoleptic characters of *Sambirani Poo Kuligai*

Sl.no	Parameters	Results
1	Colour	Brown
2	Odour	Characteristic odour
3	State of matter	Solid
4	Consistency	Hard
5	Shape	Spherical

Interpretation of Organoleptic characters

The organoleptic characters of the drug *Sambirani Poo Kuligai* showed that the colour of the *Kuligai* is brown in colour since prepared from dry herbs, and on sight they are hard, solid in consistency, spherical in shape.

Table. No.2. Physicochemical Analysis of *Sambirani Poo Kuligai*

S.No	Parameter	Mean
1.	Loss on drying at 105^0C	13.62 (%)
2.	Total Ash	79.62 (%)
3.	Water soluble ash	16.75 (%)
4.	Acid insoluble ash	0.225 (%)
5.	Disintegration Test	25 min
6.	Average weight of 20 tablets	0.132 g
7.	Ph	5.2

Interpretation:

pH

The pH is used as a measure of whether the body is maintaining a normal Acid- Base balance.

A favorable ph is essential to the functioning of enzymes and other biochemical systems**.**

In acidic medium, lots of protons are present. Therefore, greater amount of acidic drug is unionized. Thus in acidic medium acidic drugs is present in more in unionized form, which increases its absorption. That is why acidic drugs are better absorbed from stomach[73]**.**

Total Ash

The ash content is a measure of the total amount of minerals present, whereas the mineral content is a measure of the amount of specific inorganic components such as Ca, Na , K, Cl**.** The total ash value of SPK is 79.62%, which determines the presence of inorganic content.

Acid insoluble ash

The acid insoluble ash value of the drug denotes the amount of siliceous matter (dust, sand etc,) present in that drug**.** The quality of the drug is better if the acid insoluble ash value is low. Here, acid insoluble ash value of SPK is 0.225%. Hence, it represents the superior quality of the SPK.

Water soluble ash

Water soluble ash is a part of total ash value, which denotes the colloidal or crystalline nature of the drug **.** Here, the water soluble ash value of SPK is 16.75%, which represents easy facilitation of diffusion and osmosis mechanism.

Disintegration time

According to Ayush guidelines, the disintegration time does not exist more than 45 min**.** The disintegration time of SPK is 25 min. This implies a reasonable disintegration time, thereby a better absorbability and solubility is achieved.

Loss on Drying (LOD)

It indicates the amount of volatile substance and moisture present in the drug.

This also indicates the stability and shelf life of the drug**.**

The loss on drying percentage of SPK is 13.62.

Being a tablet, the moisture content is slightly high. So the stability and shelf life of SPK is about 2 years.

Table:No:3. Results of uniformity weight test for *Sambirani Poo Kuligai*

Sample No.	Weight of each *Mathirai* (mg)	% of weight variation	Maximum weight variation with in ±7.5%	Maximum weight variation with in ±15.0%
1	126	-2.96	Yes	Yes
2	128	-1.42	Yes	Yes
3	131	0.88	Yes	Yes
4	122	-6.04	Yes	Yes
5	134	3.19	Yes	Yes
6	128	-1.42	Yes	Yes
7	131	0.88	Yes	Yes
8	128	-1.42	Yes	Yes
9	130	0.11	Yes	Yes
10	133	2.42	Yes	Yes
11	140	7.81	No	Yes
12	130	0.11	Yes	Yes
13	131	0.88	Yes	Yes
14	135	3.96	Yes	Yes
15	128	-1.42	Yes	Yes
16	127	-2.19	Yes	Yes
17	136	4.73	Yes	Yes
18	124	-4.50	Yes	Yes
19	130	0.11	Yes	Yes
20	125	-3.73	Yes	Yes
Average	129.85 mg			

Interpretation:

Average weight of the *Sambirani Poo Kuligai* was noted as 129.85 mg. Out of 20 tablets tested, 19 tablets lie within ±7.5% weight variation (1 tablet was outside the percentage limit) and 20 tablets lie within ±15% weight variation. According to the limits of weight test cited in the Indian pharmacopoeia, *Sambirani Poo Kuligai* satisfied the Uniformity Weight Test and weight among the tablets were not varied to a great extent.

Traditional test for pill

Character	**Inference**
Non sticky on rolling	+
No cracks over the surface after drying	+
Shall be rolled uniformly over the plane surface	+

`

PHYTOCHEMICAL ANALYSIS

Table No:4 . Results of phytochemical analysis of *SAMBIRANI POO KULIGAI*

Phytochemicals	Test	Result
1. Alkaloids	Mayer's test	+
2. Carbohydrates	Molisch's test	+
3. Reducing sugars	Benedicts test	-
4.Glycosides	Modified Borntrager's test	+
5. Cardiac glycosides	Legal's test	-
6. Saponins	Froth test	-
7. Tannins	Gelatin test	-
8. Phenols	Alcoholic Ferric chloride test	-
9. Phytosterols	Ferric chloride acetic acid test	+
`10. Diterpenes	Copper acetate test	-
11. Triterpenes	Salkowski's test	+
12. Flavanoids	Alkaline reagent test	-
13. Proteins and amino acid	Xanthoproteic test	+
14. Quinones	Sodium hydroxide test	-

Interpretation

From the above results the following Phytochemicals are present Alkaloids, Carbohydrates, Glycosides, Phytosterols, Triterpenes, Proteins and Amino acids.

Carbohydrates:

Carbohydrates have several roles in living organisms. Carbohydrate derivatives are actively involved in fertilization and immune systems [74] . It also boosts our immune system. Carbohydrate supports our hormonal system including female hormones, thyroid and adrenal. Without adequate starch our hypothalamus think that there is starvation and decide to stop the menstruation, thereby reducing the beneficial estrogen and progesterone. It helps to regulate the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis directs communication between the central nervous system and endocrine system. Hormonal imbalance, stress management, blood sugar regulation, are all dependent on the healthy function of the HPA axis [75].

Glycosides:

A glycoside is a molecule consisting of a sugar and a non-sugar molecule, called an aglycone. Many biologically active compounds are glycosides. The pharmacological effects are largely determined by the structure of the aglycone [76].

Phytosterols:

Phytosterols is important for the body to remove excess fat and balance the hormone levels[77].

Proteins and Amino acids:

Proteins are major constituents of every cell and body fluids.

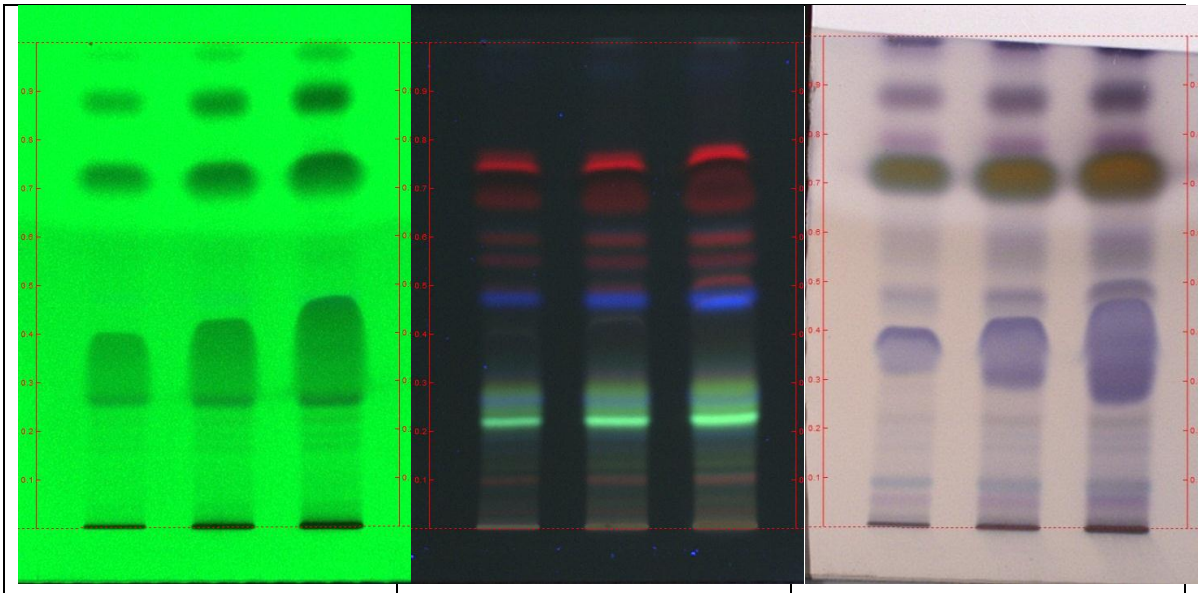
The functions of proteins are:

- It reduces body fat
- Stimulates the hormonal secretions
- Enhance oxygen and fatty acid utilization
- Stabilize blood sugar
- Produce antibodies, enzymes and hormones.
- Increase mental concentration [78].

Sample Name/ID –SPK

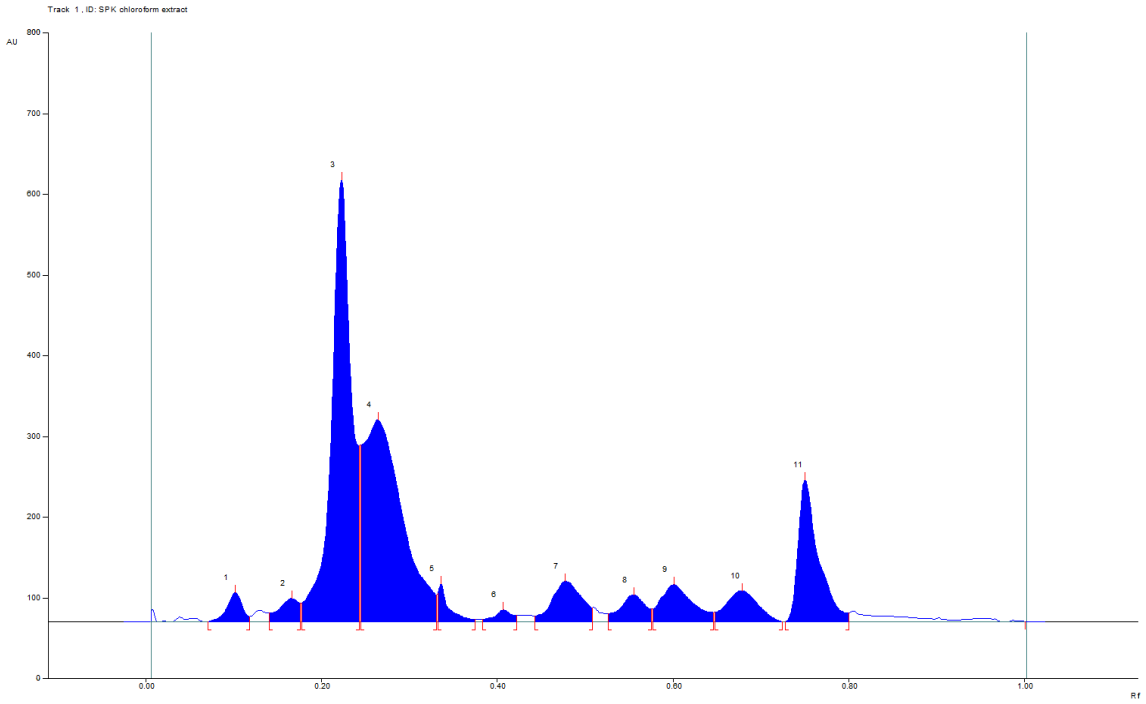
Stationary Phase - Silica Gel 60 F_{254}

Mobile Phase – Toluene : Ethyl Acetate : Acetic Acid (8 : 1.5: 2 drops v/v/v)



254 nm		**366 nm**		**575 nm (Derivatized)**	
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Green	0.17	Light Pink	0.04	Pink	0.06
Green	0.19	Pink	0.1	Violet	0.09
Green	0.22	Dark Green	0.14	Dark	0.22
Green	0.26	Fluorescent Green	0.22	Dark Blue	0.31
Green	0.38	Dark Green	0.29	Dark Blue	0.39
Green	0.73	Light green	0.32	Blue	0.47
Green	0.88	Dark Blue	0.47	Light Violet	0.56
Green	0.99	Pink	0.51	Brownish Yellow	0.71
		Pink	0.56	Violet	0.77
		Pink	0.67	Light Blue	0.87
		Dark Pink	0.77	Dark Blue	0.99

HPTLC Chromatogram @ 366 nm:



Peak Table @ 366 nm:

Track 1, ID: SPK chloroform extract

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.10 Rf	36.1 AU	2.84 %	0.12 Rf	6.4 AU	581.1 AU	1.75 %
2	0.14 Rf	10.7 AU	0.17 Rf	29.0 AU	2.29 %	0.18 Rf	23.3 AU	612.7 AU	1.84 %
3	0.18 Rf	23.4 AU	0.22 Rf	547.4 AU	43.16 %	0.24 Rf	18.3 AU	11664.1 AU	35.10 %
4	0.24 Rf	218.6 AU	0.26 Rf	250.0 AU	19.71 %	0.33 Rf	32.8 AU	10511.6 AU	31.63 %
5	0.33 Rf	35.2 AU	0.34 Rf	47.3 AU	3.73 %	0.38 Rf	2.9 AU	530.9 AU	1.60 %
6	0.38 Rf	2.8 AU	0.41 Rf	14.8 AU	1.17 %	0.42 Rf	7.8 AU	267.6 AU	0.81 %
7	0.44 Rf	7.1 AU	0.48 Rf	50.0 AU	3.94 %	0.51 Rf	17.3 AU	1577.3 AU	4.75 %
8	0.53 Rf	10.2 AU	0.56 Rf	33.2 AU	2.62 %	0.58 Rf	15.7 AU	876.3 AU	2.64 %
9	0.58 Rf	15.8 AU	0.60 Rf	46.1 AU	3.63 %	0.65 Rf	11.6 AU	1543.4 AU	4.64 %
10	0.65 Rf	11.6 AU	0.68 Rf	38.7 AU	3.05 %	0.73 Rf	0.2 AU	1299.0 AU	3.91 %
11	0.73 Rf	0.2 AU	0.75 Rf	175.8 AU	13.86 %	0.80 Rf	10.6 AU	3770.4 AU	11.34 %

A qualitative finger printing of *Sambirani Poo Kulgai* has been performed by HPTLC method, which provide qualitative insights into the bioactive Constituents present in the drug. HPTLC shows separation of components present in the Chloroform extract of *Sambirani Poo Kuligai.* The method may be applied to identify the *Sambirani Poo Kuligai* from other manufacturing process [79].

The present study revealed that *Sambirani Poo Kuligai* showed best results in Toluene : Ethyl Acetate : Acetic Acid 8 : 1.5: 2 solvent system. After scanning and visualizing the plates in absorbance mode at both 254nm, 366 nm and 575 nm and visible light range.

TLC plate showed different colour phyto constituents of chloroform extract of *Sambirani Poo Kuligai*. The bands revealed presence of dark blue, one greenish blue,violet, and dark pink, showing the presence of steroids, terpenoids, alkaloids, flavonoids, tannins, lignans and saponins.

The results from HPTLC finger print scanned at wavelength 366 nm for chloroform extract of *Sambirani Poo Kuligai*. There are eleven polyvalent phyto constituents and corresponding ascending order of Rf values start from 0.07 to 0.73 in which highest concentrations of the phyto constituents was found to be 43.16% and 19.17 % with its corresponding Rf value were found to be 0.18 and 0.24 respectively.

`

BIO CHEMICAL ANALYSIS:

Table No:5. Results of acid and basic radical studies

Parameter	Result
Test for Potassium	-ve
Test for Calcium	+ve
Test For Magnesium	+ve
Test For Ammonium	-ve
Test For Sodium	+ve
Test for Iron (Ferrous)	-ve
Test For Zinc	-ve
Test For Aluminium	-ve
Test For Lead	-ve
Test for Copper	-ve
Test For Mercury	-ve
Test for Arsenic	-ve
Test for Sulphate	-ve
Test for Chloride	-ve
Test for Phosphate	+ve
Test for Carbonate	-ve
Test for fluoride &oxalate	-ve
Test For Nitrate	-ve

Interpretation:

From the acid and basic radicals studies of *Sambirani Poo Kuligai* presents the following chemicals:

Calcium, Magnesium, Sodium, Phosphate

Calcium:

Calcium is important for insulin resistance, breast health, mood and weight loss.

Calcium is important in follicle maturation and egg development**.**

It plays a vital role in the regulation of menstrual cycle.

Maturation of the immature oocyte and the activation and fertilization of the mature egg are two separate events. Both may be synchronized by changes in intracellular calcium.

Evidence now suggests that abnormalities in calcium regulation may understandably explain the clinical presentation of PCOS, Including the reproductive abnormalities and insulin resistance. This evidence is supported by several facts.

- The significance of calcium in egg activation and in triggering meiotic resumption
- The role of calcium in LH-induced meiotic maturation
- The function of calcium and vitamin D in insulin resistance
- The clinical evidence of abnormalities in calcium homeostasis in reproductive disturbance and in women with PCOS

Antagonizing the inhibitor with changes in calcium concentration can affect continuation of meiosis. Extra cellular stimulation of the oocyte with calcium, gonadotropins and various growth factors results in changes in intracellular calcium concentrations via transmembrane movements between cytosol and internal organelles, representing are important signalling mechanism.

Calcium homeostasis in the setting of various hormones and growth factors determines the ultimate biochemical pathway selected in the phosphoinositide or adenylate cyclase-dependent protein kinase C system of reproduction and oocyte maturation.

More recently, calcium has identified as an important mineral nutrient in fertility. Vitamin D receptor knockout mice have low serum calcium concentration and are infertile [80].

Magnesium:

Magnesium is an essential mineral found in all our tissues, but mainly in our bone, muscles and brain.

It is particularly important for women with PCOS.

It enhances insulin secretion, which facilitates sugar metabolism.

Women with PCOS are 19 times more likely than the average person to have low magnesium.

To process excessive sugar in our diet it requires a great deal of Magnesium [81].

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AVAILABILITY OF BACTERIAL AND FUNGAL LOAD OF *SAMBIRANI POO KULIGAI*

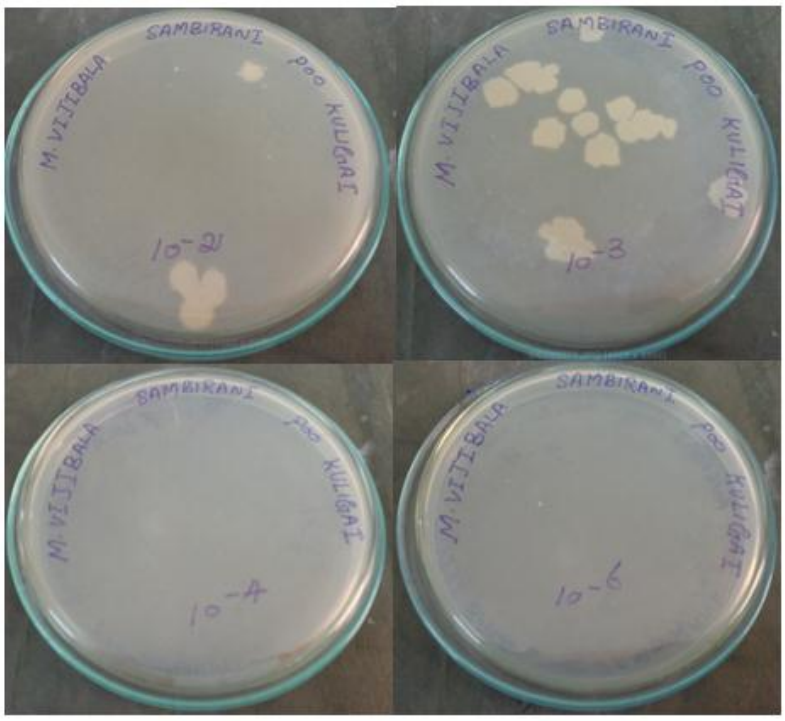

Table no:6. Availability of Bacterial and Fungi Load

MICROBES	DILUTION	RESULT
BACTERIA	10^{-4}	Nil
BACTERIA	10^{-6}	Nil
FUNGI	10^{-2}	Nil
FUNGI	10^{-3}	Nil

Interpretation:

These herbal drug are prepare from plant material they are prone to contamination. The contamination of herbal drugs by micro organism not only cause bio deterioration but also reduces the efficacy of drugs.

The toxin produce by microbes makes herbal drugs unfit for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.

No contamination present at both the bacterial and fungal load.

This determines the efficacy and purity of the drug.

Here, the contamination of *mathirai* have been examine by bacterial and fungal load. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

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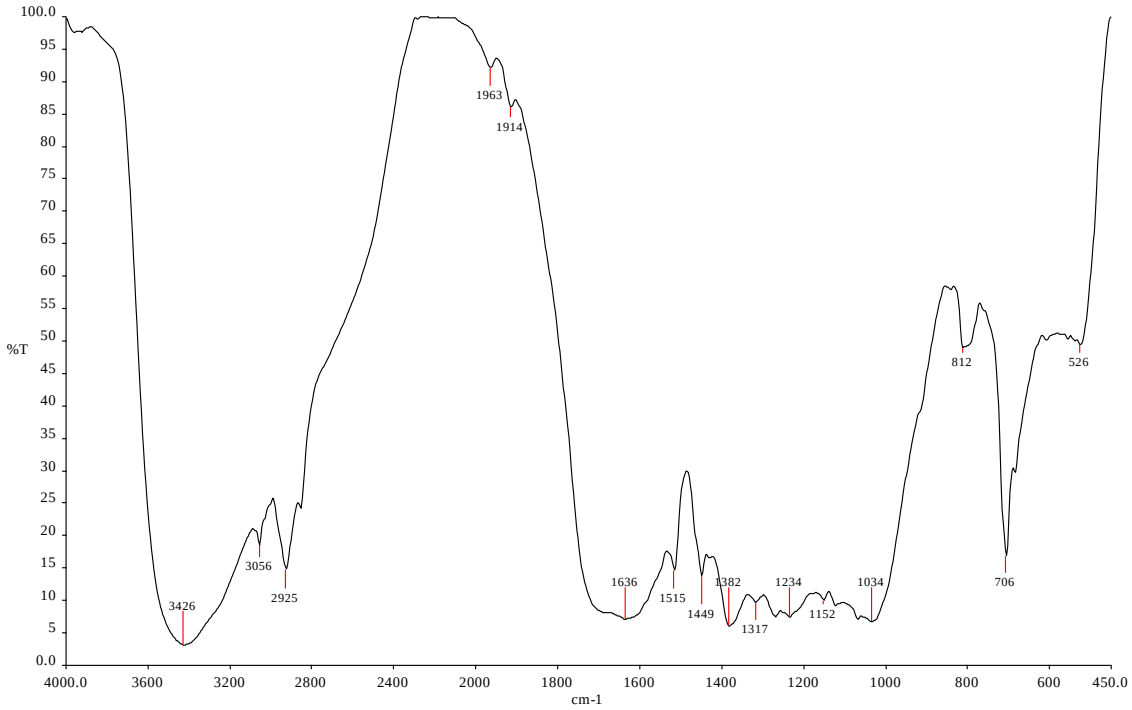
SOPHISTICATED INSTRUMENTAL ANALYSIS:

FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)



Graph showing FTIR Analysis of *Sambirani Poo Kuligai*

Table no:7. Results of FTIR Analysis

Functional Group	Type of Vibration	Characteristic Absorptions (cm^{-1})	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3426	strong, broad
Alkane			
C-H	stretch	2925	Strong
-C-H	bending	1317	Variable
Alkene			
=C-H	stretch	3056	Medium
=C-H	bending	812	Strong
C=C	stretch	1034	Variable
Alkyl Halide			
C-F	stretch	1382	Strong
C-F	stretch	1152	Strong
C-Cl	stretch	706	Strong
C-Br	stretch	526	Strong
Alkyne			
C-H	stretch	3300	strong,sharp
Amine			
C-N	stretch	1234	medium-weak
N-H	bending	1600	Medium
Aromatic			
C=C	stretch	1449	medium-weak, multiple bands
C=C	stretch	1515	medium-weak, multiple bands

`

Interpretation

The trial drug was subjected to FTIR analysis to know the functional groups of the bio molecules, to elucidate the structure and to confirm the active molecules responsible for the therapeutic effect of the drug. The study revealed the presence of functional groups Alcohol, Alkanes Alkenes, Alkyl halide, Alkyne, Amine, Aromatic.

Aromatic

It is used in the treatment and prevention of many diseases [82].

Particularly it is used in anxiety reduction, relaxation and hair loss prevention [83].

The presence of Aromatic substances has direct influence on brain, especially the limbic system through the olfactory system [84].

The pharmacological action is due the presence of essential oils [85].

So the presence of the aromatic substance helps in balancing the hormonal level.

Aromatic substances contains essential oils. These oils can mimic estrogens [86].

The presence of all these functional groups will enhance the drug action.

SEM (SCANNING ELECTRON MICROSCOPE)

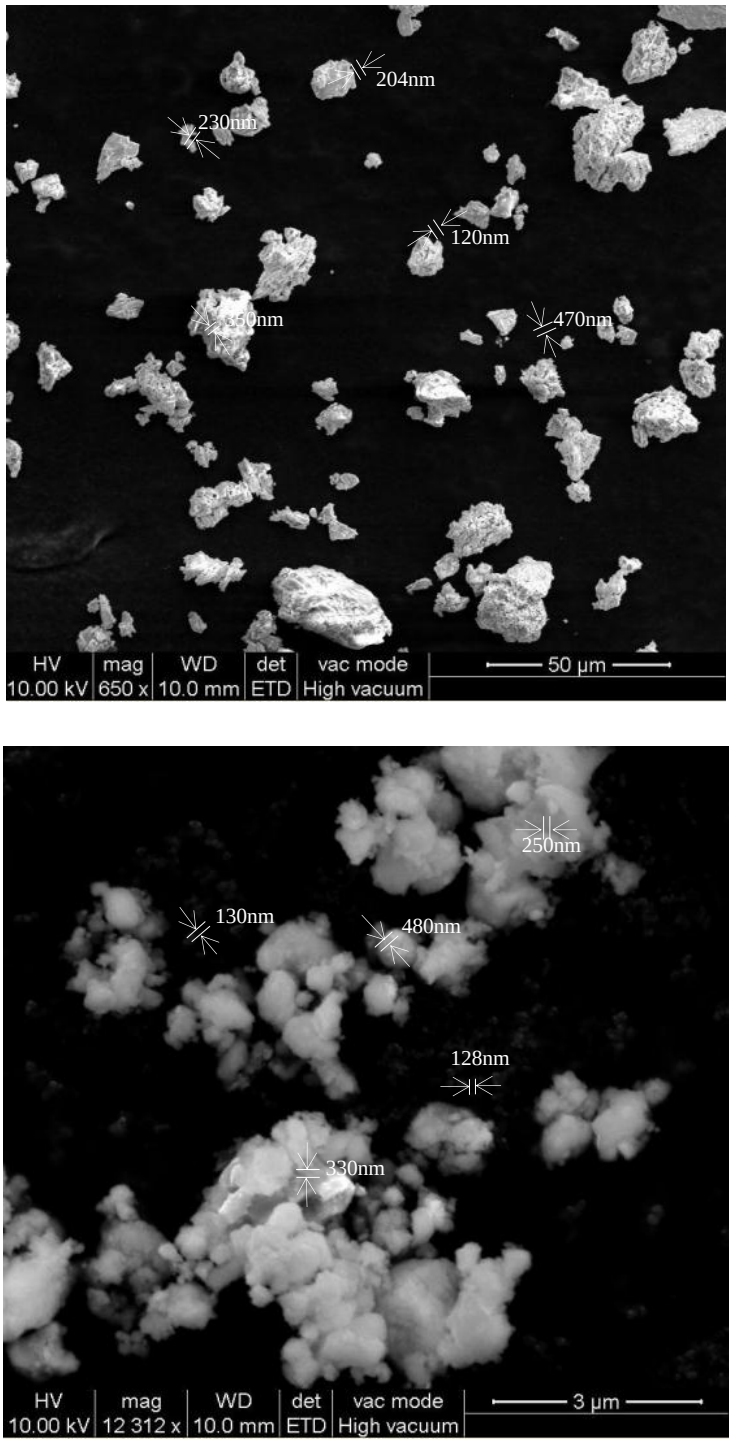


Fig.no.5. SEM images showing the shape and size of the particle size of the drug SPK

Interpretation:

Nano particles are defined as particulate dispersion or solid particles with a size in the range of 1-100nm in diameter.

They are easily

- Absorbable
- Biodegradable
- Biocompatible
- Non-antigenic in nature
- Selective/Targeted/Controlled delivery of drugs to specific site of action in the body even across the blood brain barrier
- Use to extend time window of bioavailability and to protect drug from enzymatic and chemical decomposition
- Result in reduced peripheral side effect of drugs [87].

SEM analysis of the test drug *Sambirani Poo Kuligai* revealed the presence of nano particles of size 128 nm,130 nm, 330 nm, 250 nm and 480 nm. The particles of size nano and near nano size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

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ICP-OES RESULTS OF *SAMBIRANI POO KULIGAI*

Table No. 8. Heavy Metal Analysis

S.No.	Elements	Detected levels
1	Arsenic	BDL
2	Calcium	02.130 mg/L
3	Cadmium	BDL
4	Mercury	BDL
5	Potassium	01.821 mg/L
6	Sodium	04.120mg/L
7	Nickel	BDL
8	Lead	BDL
9	Phosphorus	32.541 mg/L

Phosphorus:

The main function of phosphorus is in the formation of bones and teeth. It plays an important role in how the body uses carbohydrates and fats. It is also needed for the body to make protein for the growth, maintenance, and repair of cells and tissues**.** It balances the use vitamins such as vitamins B and D and minerals like magnesium,zinc and iodine**.**

Vitamin B helps in reversing the insulin levels and stabilizing the blood sugar levels [88]**.**

Sodium and Potassium:

Sodium and potassium are indirectly related to reproduction. Deficiency of sodium can affect the normal reproductive physiology by preventing the utilization of protein and energy. Deficiency of potassium cause muscular weakness and thereby affect the musculature of female genital tract causing impairment in the normal reproductive process. So presence of these minerals enhances the therapeutic effects [89]**.**

Heavy metals like Arsenic,Lead, Mercury are present below the detected level.

Toxicological studies- Results of Acute oral toxicity in rats

Observation done:

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	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

Table.No. 9. Dose finding experiment and its behavioral Signs of Toxicity for SPK

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.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhea 18. Writhing 19 Respiration 20. Mortality

Interpretation of Acute toxicity Studies

- The acute oral toxicity potentials of *Sambirani Poo Kuligai* in female Wistar albino rats were studied effectively.
- In the sighting study, the test substance was administered in sequential manner to one animal each at 2000 mg kg-1 body weight followed by two animals at 2000 mg kg-1 body weight.
- According to OECD guidelines, for acute oral toxicity LD_{50} dose of 2000mg/kg of *Sambirani Poo Kuligai* the drug is found to be safe.
- From the maximum tolerable dose 2000mg/kg of one-fifth and one tenth and one twentieth of the dose was taken as the therapeutic dose levels for the further pharmacological study.
- The treated animals were observed for mortality, untoward clinical/toxic signs, alterations in body weight gain and necropsy findings during the study.
- The treated animals survived throughout the study period and did not reveal any treatment related major abnormal clinical signs at the test dose levels.
- Morphological characters like changes in skin, eyes, fur, nose appeared normal. The rats did not reveal any observable signs of central nervous system.

- The rats showed signs of alertness, pile erection, grooming and touch response at the dose level of 2000mg/kg of body weight.
- The overall percentage of body weight gain in rats treated with the drug every weekly was found to be normal indicating that the test animals were in a healthy condition during the days of observation period. The changes in water and food intake recorded did not show any distinct deviations.
- On necropsy, no abnormalities were observed. In conclusion, acute oral toxicity testing of screened drug did not produce any treatment-related adverse effects. This indicates that the dosages administered were below toxic level and proves the safety of the drug**.**

SUB-ACUTE TOXICITY OF *SAMBIRANI POO KULIGAI*

Sub-acute oral toxicity 28-days repeated dose study in Wistar albino rat

Sub acute toxicity 28 days repeated dose study was executed in rats according to OECD guidelines 407. Two different dose levels of the drug i.e., 100,200mg /kg/day were administered to four groups each containing 3 rats and one group being control. The animals were observed for 28 days and the following parameters like total body weight, weight of internal organs, haematological parameters, bio chemical analysis, urine parameters were assessed.

Effect of *Sambirani Poo Kuligai* on total body weight in rats

The total body weight of the animals was weighed on 1^{st}, 7^{th}, 14^{th}, 21^{st}, 28^{th} day and is shown in the table. It was found that the test drug produced significant weight gain than control, with administration of the drug. Similarly the test drug at all dose levels induced weight gain and we could see longer the duration of administration of drug higher was the weight gain.

Table. 10. Body weight (g) changes of rats exposed to *Sambirani Poo Kuligai*

Dose (mg/kg/day)	*Days*				
	1	**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 mg	124.75±0.39	125.38±0.47	127.78±0.44	128.86±0.36	132.33±0.33
200 mg	123.12±0.10	124.0±0.44	125.93±0.21	127.30±0.19	129.46±0.15

Values are expressed as mean ± S.E.M. N=3

Effect of *Sambirani Poo Kuligai* on organ weight in rats

Administration of two different dose levels of the drug was compared with the control to examine the weight of internal organs liver, heart, lung, spleen, brain, kidney. Animals were sacrificed at the end of the dosing period and the organ weight was measured. It was found that the trial drug does not produce significant change in weight of internal organs. This is shown in the table...

Table. 11. Effect of *Sambirani Poo Kuligai* on Organ weight in Wistar albino rats

Organ	Control	100 mg	200 mg
Liver (gm)	17.50±1.86	15.83±0.73	15.76±0.70
Heart (gm)	1.83±1.73	2.39±0.28	1.87±0.29
Lung (gm)	2.24±1.67	1.81±0.33	2.13±0.17
Spleen (gm)	1.10±1.68	1.35±0.27	1.32±0.19
Brain (gm)	2.04±2.11	1.97±0.19	2.00±0.18
Kidney (gm)	1.90±1.76	1.75±0.25	1.84±0.25

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

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Effect of *Sambirani Poo Kuligai* on Haematological parameters in rats

To evaluate the sub- acute toxicity of ***Sambirani Poo Kuligai***, the haematological parameters like RBC, WBC, platelet, differential count, Hb tests were also executed. The above results showed that all parameters remained within normal limits. The rats did not reveal any observable signs.

Table No:12 Effect of *Sambirani Poo Kuligai* on Haematological parameters in rats

Parameter	Control	100 mg	200mg
RBC (x 10^6/mm^3)	8.29±0.43	6.3±0.29	7.6±0.321
PCV (%)	49.66±0.77	49.76±0.568	52.93±0.44
Hb (g/dl)	15.13±0.39	14.22±0.29	15.31±0.214
WBC (mm^3)	11.75±0.85	9.40±0.25	8.00±0.07
Neutrophils (%)	23.29±0.73	22.96±0.11	16.66±0.61
Lymphocyte %	85.5±0.46	76.40±0.41	78.69±0.26
Eosinophils (%)	4.10±0.23	2.73±0.27	3.13±0.07
Platelets (x 10^3/mm^3)	425.73±1.35	461.61±7.47	549.99±0.92

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

Table No: 13 Effect of *Sambirani Poo Kuligai* on biochemical parameters in rats

Parameters	Control	100 mg	200 mg
Protein (g/dl)	8.58±0.68	6.67 ±0.59	7.57 ±0.49
Albumin (g/dl)	5.34±0.40	3.77± 0.19	2.65 ±0.27
BUN (mg/dl)	22.06±1.55	29.32± 0.48	35.14±1.41
Blood sugar (mg/dl)	108.63±0.81	104.93± 3.14	118.48±1.42
Creatinine (mg/dl)	0.85±0.07	0.48 ±0.09	0.65±0.04
Total Cholesterol (mg/dl)	93.21±1.16	122.42± 5.29	100.42±0.97
Triglycerides (mg/dl)	52.58±1.56	53.74 ±0.98	48.75±0.31
SGOT (U/L)	74.35±1.23	139.31± 0.79	124.79± 3.44
SGPT(U/L)	27.07±0.84	121.46± 3.49	72.63±6.15
Alkaline phosphatase (U/L)	104.63±1.14	126.35± 2.52	139.35±1.19

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

The biochemical parameters are in the reference range and this showed non toxic effects on general body metabolism**.**

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Effect of *Sambirani Poo Kuligai* on Urine parameters in rats

Urine analysis was conducted with two different doses at the end of dosing period in week 4 and the results suggests that the drug did not produce any ill effect. This is shown in the table below.

Table: 14 Effect of *Sambirani Poo Kuligai* on Urine parameters in rats

Parameters	Control	100 mg	200 mg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Clear	Clear
Specific gravity	1.01	1.01	1.01
Ph	6.4	6.2	7.1
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	- ve	- ve
Ketones	-ve	- ve	- ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelial cells	Nil	Nil	Nil
Casts	Nil	Nil	Nil

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Pharmacological Studies:

Effect of *Sambirani Poo Kuligai* on weight of uterus and Ovary of Wister albino rats

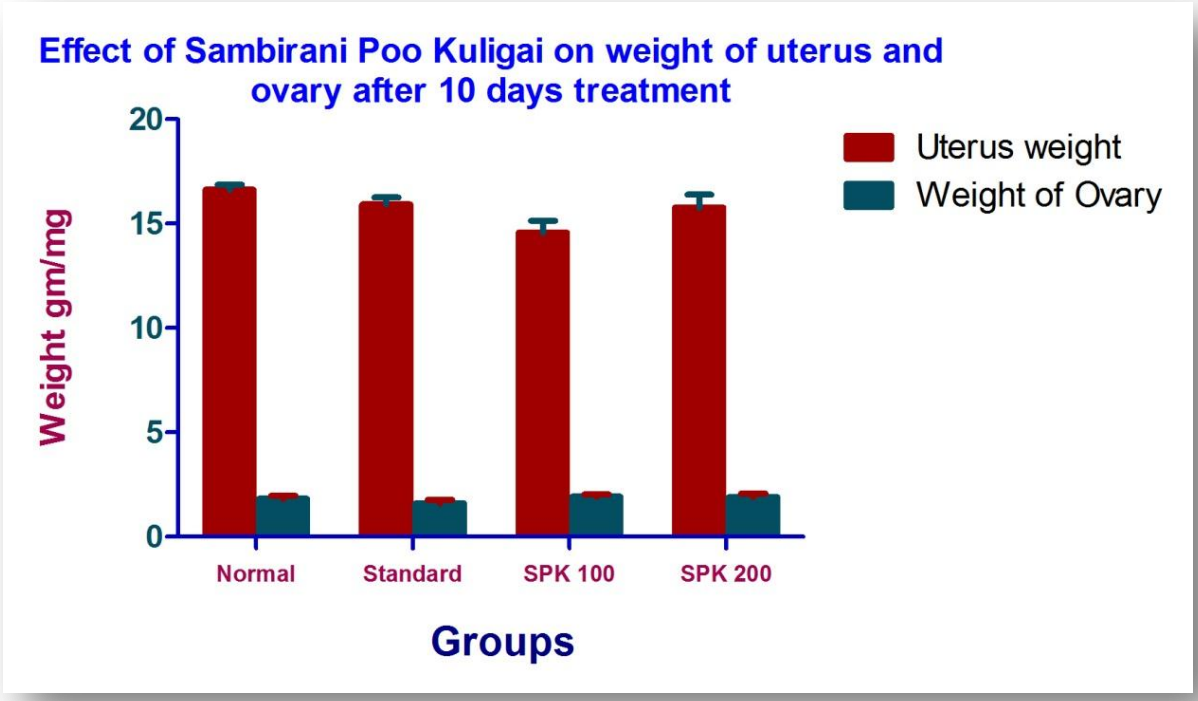
The ***Sambirani Poo Kuligai*** had no toxic effect at 2000mg/kg on rat after 48 hours of oral drug treatment. The relative weight of uterus and ovary were significantly increased ($P<0.05$) in experimental groups that received 200mg /kg at the end of the tenth day as compared with CMC treated normal female rats.

Table No:15 Effect of *Sambirani Poo Kuligai* on weight of uterus and ovary after 10 days treatment

S.No	Group	Treatment and dose	Weight of uterus (mg)	Weight of ovary (g)
1.	Normal	2ml/kg 2% CMC	16.63±0.25	1.83±0.14
2.	Standard	Clomiphene 10mg/kg	15.93±0.33	1.61±0.16
3.	Test-I	SPK 100mg/kg	14.57±0.57*	1.92±0.14*
4.	Test-II	SPK 200mg/kg	15.78±0.61*	1.91±0.17

*N = 6. Values are expressed as Mean±SEM. *P>0.05 compared to normal control*

Chart: 1. Effect of *Sambirani Poo Kuligai* on weight of uterus and ovary after 10 days treatment



Hormonal Assay

Table No: 16. Effect of *Sambirani Poo Kuligai* on Serum Concentration of reproductive hormones of female Wister albino rat

S. No	Group	Treatment and dose	LH (IU/ml)	FSH (IU/ml)	Estrodial (pg/ml)	Progesterone (pg/ml)
1.	Normal	2ml/kg 2% CMC	0.28±0.12	0.33±0.24	54.10±3.5	9.03±1.65
2.	Test-I	SPK 100 mg /kg	0.36±0.09*	0.46±0.20*	38.32±2.6**	6.6±1.14**
3.	Test-II	SPK 200 mg /kg	0.43±0.14*	0.55±0.16*	32.68±2.1**	6.4±0.46**
4.	Standard	Clomiphene 10mg/kg	0.56±0.27	0.63±0.21	27.17±1.8	5.9±0.86

*N = 6. Values are expressed as Mean±SEM. *p<0.05;**p<0.01*

Chart: 2. Effect of *Sambirani Poo Kuligai* on Serum LH and FSH in rat

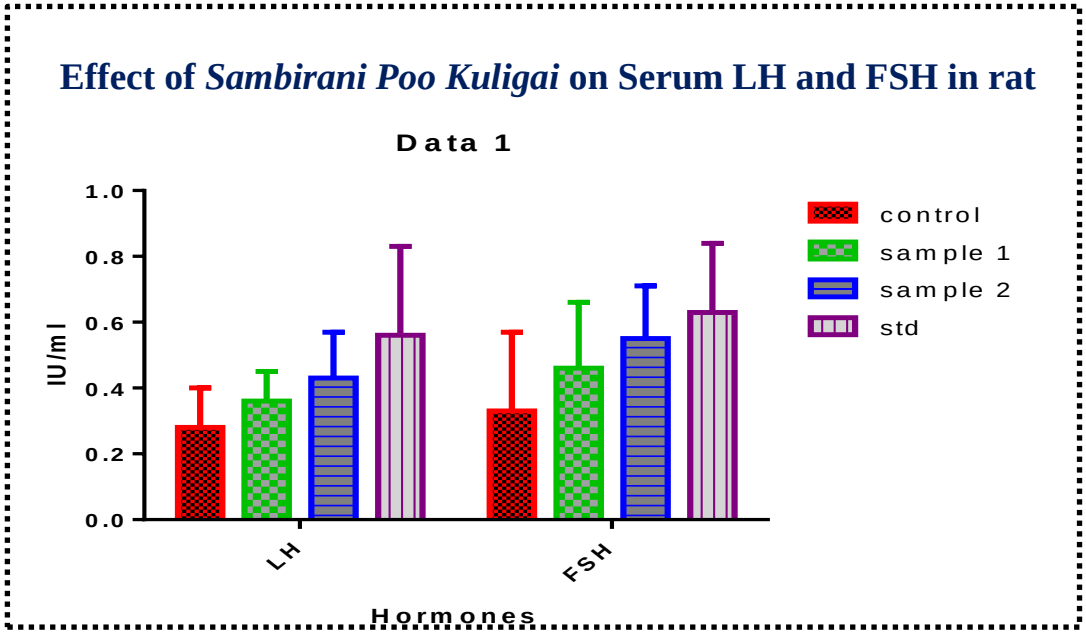
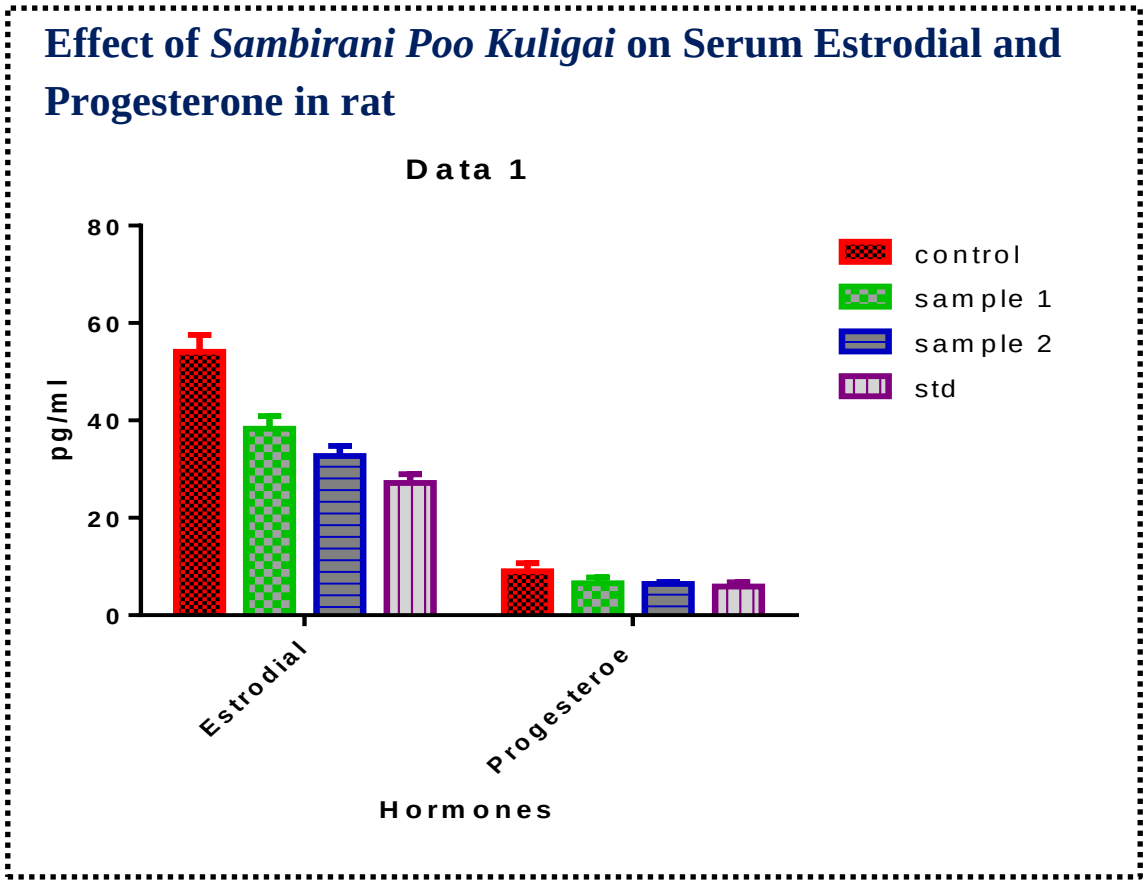


Chart: 3. Effect of *Sambirani Poo Kuligai* on Serum Estrodial and Progesterone in rat



The treatment with both doses of SPK caused an alterations in the amount FSH, which was statistically significant. SPK in the first stages of folliculogenesis strongly increased the number of primordial follicles. This increase was more pronounced at the 200mg /kg dose of the SPK acted as a stimulant, causing progression of folliculogenesis to the stage of primary follicle formation. However, at the next stage of folliculogenesis SPK caused an increase in the number of growing follicles. The SPK also caused an increase in the number of atretic follicles, which confirmed the repressing effect of the SPK on the natural growth of follicles, which seems reasonable considering the slight decrease in the level of FSH.

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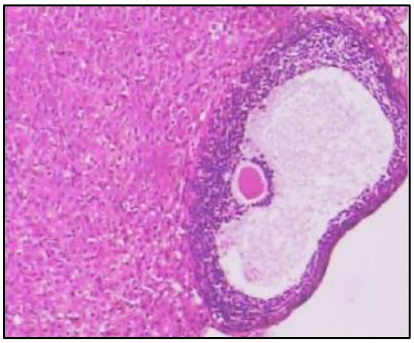
The results of ovulation effect revealed the significant influence at the dose level of 100 mg /kg and this marked effect was ensured with the histological evaluation of uterus of experimental rats also. Hence the drug ***Sambirani Poo Kuligai*** is an excellent poly-herbal medicine in the treatment for anovulatory conditions like PCOS and the effect may be attributed to the elevation of the ovulation stimulatory hormones in animal models.

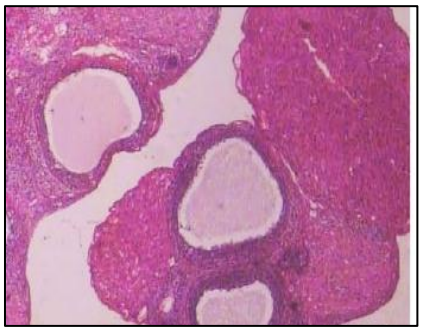
Histopathology

After sacrificing, the ovaries were collected, weighed and viewed under light microscope. Histological studies revealed that treatment with doses of 100mg/kg and 200mg /kg of SPK significantly increased the number of primordial follicles ($p<0.05$ for100mg /kg; $p<0.01$ for 200 mg /kg;). This increase was also observed in the number of primary follicles, however it was significant only in the 200mg /kg group ($p<0.01$,). Treatment with 100 and 200mg /kg dosages decreased the number of preantral and antral follicles, however, this decrease was significant only in the 200mg /kg group ($p<0.05$). Different dosages of the SPK slightly increased the number of atretic follicles, a greater increase was observed at 200mg /kg ($p>0.05$).

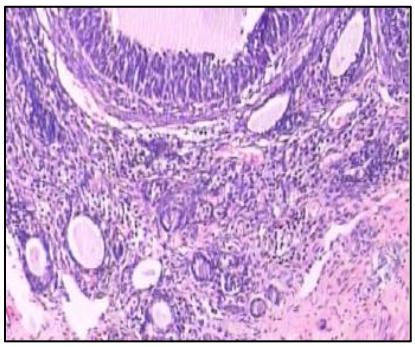
Figure: 9. Histopathology slides of ovary



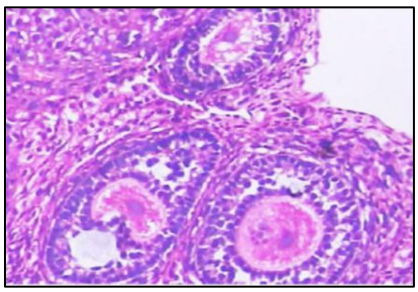
Normal control ovary with healthy follicles and corpus leutum



Induced group cystic follicles with thin granulosa layer, atretic follicles, stromal hyperplasia, and vacuolated stroma.



100mg/kg showing healthy follicles in various stages of development and with congested blood vessel



200mg/kg treated showing healthy follicle and healthy stroma.

Antioxidant Activity: (In –Vitro study)

Antioxidants are substances that can protect the body from the highly reactive free radicals and oxygen species damage by converting the free radicals into more stable substances [90]**.** Antioxidants can be generated endogenous (enzymatic), or received from foods or supplements (non-enzymatic**).**

Nowadays, the use of antioxidants in management of women with PCOS has attracted lots of interests. Some characteristics of PCOS such as obesity and abdominal adiposity, androgen excess, and insulin resistance can develop oxidative stress in these patients**.** Indeed, PCOS is a condition with significant decrease in serum antioxidant and vitamins levels and these women are in an increased risk of oxidative status [91]**.** Oxidative stress and antioxidant decrease may lead these women to increased risk of cardiovascular disease, insulin resistance, hypertension, central obesity, and dyslipidemia [92]**.**

Antioxidants that prevent or limit the damaging effects of oxygen radicals have been reported to have important roles in the female reproductive system and in the pathogenesis of female infertility [93]**.**

Oxidative stress is commonly referred as the imbalance between oxidants and antioxidants. When the imbalance favors oxidants, generation of excessive amounts of reactive oxygen species harm our body in various ways through the generation of excessive amounts of reactive oxygen species [94]**.** In other words, reproductive cells and tissues will remain stable only when antioxidant and oxidant status is in balance. Oxidative stress, which is generally known to be present in women with PCOS regardless of whether they are lean or have metabolic abnormalities, has been documented in infertile women [95]**.**

Table No 17: DPPH Assay of *SAMBIRANI POO KULIGAI*

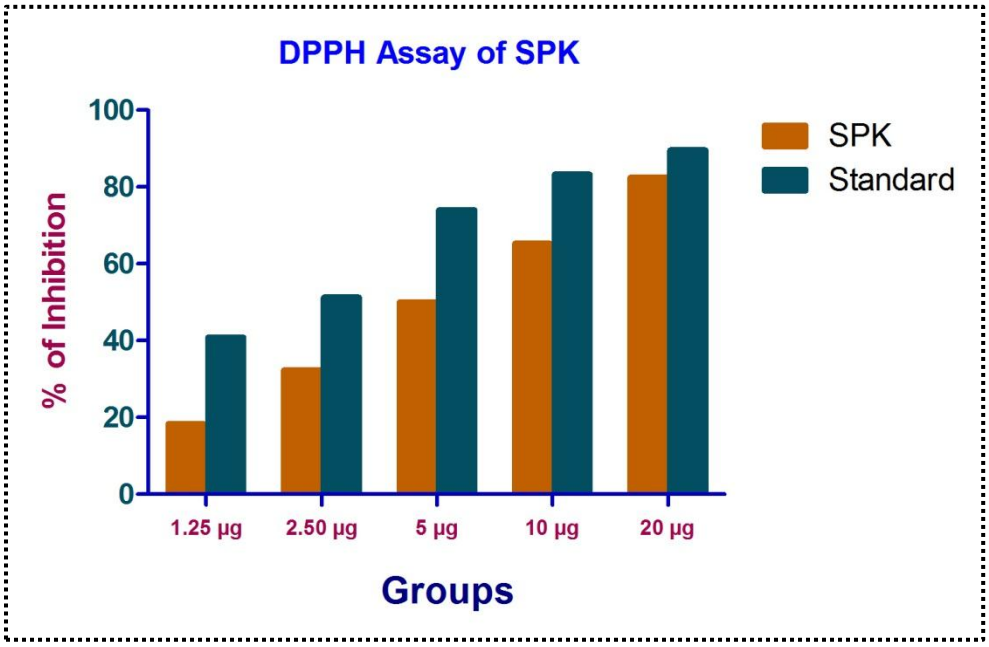
Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5271	0.312	-	-
1.25	0.4303	0.278	18.36464	40.89
2.50	0.3563	0.202	32.40372	51.25
5	0.2631	0.084	50.08537	74.07
10	0.183	0.052	65.28173	83.33
20	0.0921	0.034	82.52703	89.62

µg/ml: microgram per millilitre. Drug: SPK (1.25-20µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *SPK* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased. In the present study, the *SPK* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance.

In the present study, the extract of *SPK* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the *SPK* extract was given in (Table 19). The extract of *SPK* showed the highest DPPH scavenging activity (82.52%) at 20µg/ml and the lowest percentage of inhibition (18.36%) at 1.25µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml and the lowest percentage of inhibition (40.89%) at 1.25µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and *SPK* extract. The *SPK* extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the *SPK* extract has a marked antioxidant activity at higher concentrations.

Chart: 4. Effect of SPK on DPPH assay



6.CONCLUSION

The selection of the drug *Sambirani Poo Kuligai* from the literature "*Agasthiyar Paripuranam* 400 Published by Rathna Nayaker And Sons for the evaluation of ovulation inducing activity was done and the results supported the study. The main perspective of the study is calibered by the factors like safety, efficacy, long shelf life, bio-availability, presence of significant phytochemicals, anions and cations, minerals favouring the activity.

Physicochemical characters like ash values were signifies the low contamination, normal quality of the drugs. Loss on drying gives the total of volatile content and moisture present in the drug. The value of LOD ensures maximum stability and better shelf life. These parameters strengthen the shelf life of the drug.

The tablets have a reasonable disintegration time implicating a better bioavailability. Also the drug when subjected to SEM analysis showed the presence of nano particles. They ensure the bioavailability as well as selective targeted drug delivery on the site desired is achieved. These facts support the essentiality of the presence of such particles in the drug.

The phytochemical analysis revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, triterpenes and proteins in the drug and they may be responsible for the ovulation inducing acivity supported by some studies. Moreover the bio-chemical analysis exhibited the presence of Calcium, Magnesium, Sodium, Phosphate in the drug which might be responsible for ovulation inducing activity. The presence of these minerals may play an important role in the functioning of various enzymes in biological systems and have immunomodulatory functions

The FTIR analysis construe the results that showed the presence of functional groups like Alcohols, Alkanes, Alkenes, Alkyl halide, Alkyne, Amine and aromatic amines which might be responsible for the presence of ovulogenic action of the drug.

Further acute and subacute toxicity studies were done to evaluate the safety of the drug. The acute toxicity studies were conducted according to the OECD guidelines 423 and the LD_{50} of the drug was found to be 2000mg/kg. The toxicity helped to fix the doses as $1/5^{th}$ and $1/10^{th}$ for pharmacological activities that were carried out. The treated rats in the toxicity studies did not show any mortality, any untoward clinical sign, any behavioural signs, alterations in body weight and necropsy findings at the end of the study. This indicates that the dosages administered were below toxic level and proves the safety of the drug.

The subacute oral toxicity potentials of *Sambirani Poo Kuligai* in female Wistar albino rats was studied effectively. The treated animals survived throughout the study period of 28 days and did not reveal any treatment related major abnormal clinical signs at the test dose levels. The overall percent body weight gain in rats treated with the drug was found to be normal indicating that the test animals were in a healthy condition during the 28 days of observation period. The haematological parameters and biochemical parameters of the tested rats were not significant indicating that the drug exerted nil impact on the parameters and they were within the reference range.This strongly stress the fact of the drug having no effect on the body metabolism.Also the necropsy studies showed no remarkable changes.

Pharmacological studies had shown that the trial drug increased folliculogenesis at the dose level of 100mg /kg and this marked effect was ensured with the histological evaluation of uterus of experimental rats also. Hence it may be concluded that the *Sambirani Poo Kuligai* is an excellent poly- herbal medicine in the treatment for anovulatory conditions.

The hormonal assay of the rats was evaluated using the estimation of parameters like FSH, LH, Estradiol, Progesterone. Significant increase in the hormonal levels of FSH, LH, Estradiol, Progesterone was recorded statistically200mg/kg. The increase in the level of hormones proved to be the prime reason for ovulogenic activity.

To conclude, the drug *Sambirani Poo Kuligai* beneficiary values for the therapeutic efficacy for ovulation, it can be concluded that *Sambirani Poo Kuligai* could be a scientifically validated and had shown its sphere of action over the female reproductive system especially over the ovaries in the condition called PCOS.

7.FUTURE SCOPE

Inspite of the excellent medicinal properties referred, the drug's continuous use in folklore since antiquity, the drug remained as a traditional drug till now. This is likely due to lack of exploring it scientifically by advanced research methodologies. But the trend is changing nowadays marching towards a better research world in which elucidation of chemical, structural and biomedical aspects from modern scientific point of view is achieved.

Sufficient data are available to support the ovulation inducing activity through this work. But to make the work more fruitful, certain lacunae should be filled.

This drug should be screened for rest of the pharmacological activities like ovulogenic activity, hormonal assays, antioxidant activity to rule the effective pathways of action of the drug and development and should be taken to the next level through intensive clinical trials so that the exact benefit and aim of the study would be fulfilled.

8. SUMMARY

The trial drug for the study *Sambirani Poo Kuligai (SPK)* from the literature "Agasthiyar Paripuranam 400" published by Rathna Nayaker and Sons for evaluation of ovulation inducing activity was done and the results boosted and promised to fill the women society for a safe medicine.

The review of literature strengthened the positive facts of possessing the ovulation inducing activity by each of the single drug included in the formulation. The pharmacological review possessed all the information regarding the exertion of action of the drugs, available drugs in the market, their adverse effects

The standardization of the drug is achieved through the analysis of organoleptic and physicochemical characters. The parameters revealed a longer shelf life period of the drug. The phytochemical analysis of the drug detailed the alkaloids, carbohydrates, glycosides,proteins and terpenes present in the drug. The bio-chemical analysis of the drug revealed the anions and cations present The instrumental analysis of the drug helped in identifying the important data in SEM analysis and the presence of metals and minerals in the sample through FTIR, ICP-OES analysis. The acute and repeated oral drug toxicity study confirmed the safety of the drug which was analysed through various parameters like haematological, biochemical, organ weight, body weight, urine parameters and no abnormal changes in histopathological examination.

The results of ovulation inducing effect of SPK revealed the significant influence at the dose level of 100 mg /kg and this marked effect was ensured with the histological study of uterus and ovaries of experimental rats.

Hence the *Sambirani Poo Kuligai* is an excellent poly- herbal medicine in the treatment for anovulatory conditions like PCOS and the effect may be attributed to the elevation of the ovulation stimulatory hormones in animal models.

Thus, this current analysis authenticates that *Sambirani Poo Kuligai* has impressive ovulogenic activity over PCOS *(Soothaga vayu)* in female, which exemplifies the intelligence of the Siddha literature to reach globally for the welfare of women society.

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