PRECLINICAL EVALUATION OF SIDDHA POLY-HERBAL FORMULATION "*ASHUWATHI CHOORANAM*" FOR ITS NATURALLY CURING PCOS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical Evaluation of Siddha Poly-herbal Formulation *ASHUWATHI CHOORANAM* for its Naturally Curing PCOS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.VELPANDIAN, M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Preclinical Evaluation Of Siddha Poly-herbal Formulation *ASHUWATHI CHOORANAM* For Its Naturally Curing PCOS"** is submitted to the Tamilnadu Dr. M. G. R. Medical University in partial fulfilment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **R. Devaki** 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.

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ENDORSEMENT BY THE HOD

PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"Preclinical Evaluation Of Siddha Poly-herbal Formulation *ASHUWATHI CHOORANAM* For Its Naturally Curing PCOS"** is a bonafide work carried out by **R. Devaki** under the guidance of **Dr. V.Velpandian, M.D(S), Ph.D.,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Chennai - 106.

Signature of the HOD **Signature of the Principal**

Date: **Date:**

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ABBREVIATIONS

Alb	Albumin
ANOVA	Analysis Of Variance
AT	Adipose Tissue
CL	Cholesterol
CMC	Carboxyl methyl cellulose
CAH	Congenital Adrenal Hyperplasia
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
hMG	Human Menopausal Urinary Gonadotropin

IAEC	Institutional Animal Ethical Committee
L	Lymphocyte
LH	Leutinizing hormone
mg	milligram
MONW	Metabolically Obese Normal Weight
NIH	National Institutes of Health and Human development
NICHD	National Institutes of Health and Child 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
AC	Ashuwathi chooranam
SHBG	Sex Hormone Binding Globulin
SEM	Scanning Electron Microscope
TC	Total count
TLC	Thin Layer Chromatography
WHO	World Health Organization

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is one of the most common reproductive endocrinological disorders with a broad spectrum of clinical manifestations affecting about 6.8% of women of reproductive years[1]. A polycystic ovary is an abnormally large numbers of developing egg seen to the ovarian periphery, looking like a "String of pearls". PCOS was previously called Stein-Leventhal syndrome after the physicians who first characterized it in 1930's[2]. It is associated with oligomenorrhoea, anovulation (causing endocrine disorder such as thyroid disease, adrenal disease and pituitary tumors)[3], infertility, hirsutism and obesity in young women having bilaterally enlarged cystic ovaries. It is a condition in which women have high levels of male hormones (Androgen) and anovulation are known to interact with insulin resistance in the pathophysiology of PCOS. Hyperinsulinaemia appears to interfere with ovarian steroidogenic defects as well as anovulatory mechanisms.

Child and adolescent overweight and obesity were associated with significantly increased risk of later polycystic ovary syndrome symptoms [4].

Failure of ovulation results in prolonged, excessive endometrial stimulation by estrogens. Under these circumstances, endometrial glands undergo mild architectural changes, including cystic dilation (persistent proliferative endometrium). Thus hyperplasia occurs in most commonly around menopause or in association with persistent anovulation in younger women. Conditions leading to hyperplasia include polycystic ovarian disease including Stein Leventhal Syndrome[3a].

Globally, prevalence estimates of PCOS are highly variable, ranging from 2.2% to as high as 26%. WHO estimates that it affected 116 million women are affected Worldwide in 2012(3.4% of women).

In India the prevalence is gradually increasing. It is due to the lifestyle that people have adopted. Almost all foods are packed with chemicals that lead to hormonal imbalance. The cause of PCOS remains unclear.

The prevalence of PCOS among them was 22.5% by Rotterdam and 10.7% by Androgen Excess Society Criteria. Non-obese women comprised 71.8% of PCOS diagnosed by Rotterdam Criteria. Mild PCOS [oligomenorrhoea and polycystic

ovaries on USG] was the most common phenotype (52.6%). Hyperinsulinemia was presenting among 19.2% of diagnosed PCOS causes [5].

There is an intriguing pathophysiological frame work to support the therapeutic advantages of insulin sensitizers in the management of PCOS, including metabolic aspects, ovulatory functions and hyperandrogenemia. Metformin and thiazolineodiones are the two more widely used insulin sensitizers[6].

Obesity is clearly associated with adverse pregnancy outcomes in the lager population, and it likely increases the risk of adverse pregnancy complications with PCOS. One meta-analysis that looked at pregnancy in women with PCOS and adjusted for differences in obesity in women with PCOS did note increased rates of gestational diabetes, preterm labour and infant mortality among women with PCOS.

Medicine as everyone knows is not merely a science but an art as well. Siddha science is very ancient in origin, as old as the earliest civilization of the southern peninsula of India. Siddha system of medicine was expounded by a class of more intelligent people called *Siddhars. Siddhars* were the great scientists in the ancient period and they are the original creator of the Siddha system of medicine [7].

The term '*Siddha*' has been derived from *'Siddhi'* which means an object to be attained or achievement or perfection of heavenly bliss. *Siddhars* were the men who achieved supreme knowledge in the fields of medicine, yoga and meditation [8].

This system represents a well-established, codified and organized medical system that had been utilized and refined in India over hundreds of generations with sophisticated theoretical foundations and philosophical explanations, expressed in thousands of Tamil manuscripts dealing with curative, preventive, preservative aspects and longevity of life in the simplest way.

The Siddha system is effective in treating various diseases of the liver, gastrointestinal tract, skin diseases, allergic disorders, general fevers, chronic diseases, gynaecological disorders. Many *Siddhars* were more concentrated in treating various gynaecological disorders including *Karpavayu*.

The signs and symptoms of *Karpavayu* i.e., diseases which prevents pregnancy characterized by multiple ovarian cysts is called *Sinaippai neerkatigal, Soolaga neerkattigal, Karpapai neerkattigal* correlated with the Poly Cystic Ovarian Syndrome (PCOS) in which poly means many, cystic means water filled sac-like structure and when many are formed in the ovary called PCOS which is mentioned in the following verses,

"பொருமி இரத்தத் தனைமரித்துப் போதவுதாம் வலிஉண்டாய்
குருதியழியில் வலிதீருங் கூடுங் கெர்ப்பந் தனையழிக்கும்
வருடியிடுப்புக் குடைந்துளையு மல்த்தை மிகவுமிருக்கி நிறகும்
பெருகப் பணைக்குங் குவிமுலையாய்! பேசங் கெர்ப்பவாயுவிதே"

-தன்வந்திரி வைத்தியம் பாகம் 1

The symptoms of *Karpavayu* includes diminished menstrual flow with abdominal pain, when the flow increases the pain is relieved. Prevents conception and there is low back pain and constipation [9].

It is natural to be afflicted by disease and necessarily medicine administered. It is said that cure is effected only for those who have good sound body and those with bad constitution will not be identified.

PCOS has become a common health problem which affects women's menstrual cycle, hormone imbalance, ability to bear children, appearance, mental state and is also the main cause for infertilty. In Siddha the clinical features of PCOS are described in *"Yugi Muni Vaithya Kaaviyam", "Thirumoolar Karukidai Vaithyam"* and in *"Gnanavettiyan – 1500"* by Thiruvalluva Nayanaar discussed in detail about female reproductive problems such as PCOS, infertility, fibroids, etc.,. Prevention and cure are the basic aims of all systems of medicine whereas the *Siddha* system has in addition the transcedental motivation of what might called the immortality of the body.The basic emphasis of Siddha system is on positive health viz.to prevent diseases by carefull dieting.

Numerous single drug and compound drug formulations documented in classical *Siddha* literatures are highly efficacious and devoid of side effect or adverse

effects and are useful to the mankind. Today we are only exploring their efficacy and usefulness through advanced scientific research tools and modern techniques.

Hence, current research looked into ancient Siddha system of medicine in search of an effective alternative and number of herbs that could act at the level of hypothalamic-pituitary-axis. Various herbs are reported in Siddha system of medicine which aid in bleeding and clotting mechanisms along with potentially corrects the lipid and carbohydrate metabolism. Several herbal formulation have been developed which helps to improve PCO phenotype via directly acts on hypothalamo-pituitary-ovary-uterine axis and thereby regularize the menstrual cycles [10].

Siddha System approaches have become common for the management of such metabolic syndromes. According to Siddha, many formulations have been reported which help to restore the ovulation and minimize the PCO phenotypes. One of such effective formulation mentioned in this system is "*Ashuwathi Chooranam"* for treating '*Karpa Vayu'* (PCOS) successfully [11]. Some principal herbal ingredients of this formulation which include *Withania somnifera, Zingiber officinale, Piper nigrum, Piper longum, Myristica fragrans, Glycyrizzha glabra, Syzygiumar omaticum, Picrorrizha Scrophularia,* are known to possess various beneficial activities. *Hyoscymus niger* has a stimulatory effect on the ovarian tissue, which may produce an estrogen-like activity that enhances repair of the endometrium and stops bleeding [12]. It is found to be effective in menorrhagia and dysmenorrhea. Even though it is mentioned in Siddha system of medicine for treating PCOS, no scientific data available to confirm its effectiveness in treating various gynaecological disorder especially PCOS.

This *Siddha* drug "*Ashuwathi Chooranam"* is yet remained unexplored for its exact chemical, -pharmacological values in terms of scientific research. To fill these scientific lacunae, the present work was undertaken to standardize *Ashuwathi chooranam* to validate through physicochemical analysis, phytochemical analysis, instrumental analysis, toxicological studies and pharmacological screening in animal model.

2. AIM AND OBJECTIVES

AIM

Polycystic ovarian syndrome (PCOS) is a heterogeneous collection of signs and symptoms which together form a spectrum of the disorder with mild/severe disturbance in reproduction, endocrine and metabolic functions. The pathophysiology of PCOS appears to be multifactorial and polygenic, which is contributed by intra and extra ovarian factors. Women with PCOS demonstrate decreased sensitivity to the feedback effects of gonadal steroids on GnRH secretion[13].

This causes an accelerated frequency of LH secretion from anterior pituitary[14] resulting into abnormal steroid synthesis contributed majorly by ovarian theca cells[15]. Intrinsic anomaly of steroidogenesis reflects itself in causing follicular arrest which is evident by the presence of peripheral cysts in ovary. Biochemical alterations of ovarian function render its effect on metabolically active organs by causing insulin resistance, leading to metabolic syndrome.

With emphasis to above, current available mode of treatment for PCOS is by use of insulin sensitizers like metformin and ovulatory agent like clomiphene citrate[16]. But, these drugs have been reported for their side effects upon prolonged usage[17]. There by, in current decade, research is directed towards the ancient Siddha system of medicine to manage mainly hypoglycemia and PCOS related disorders.

As stated in Siddha classical literature, *Ashuwathi Chooranam* helps to improve menstrual irregularities in reproductive age. However, scientific evaluation of *Ashuwathi Chooranam* for treatment of female infertility has not been attempted till date. In this regard, it would be interesting to examine the **role of *Ashuwathi Chooranam* in management of Polycystic Ovarian Syndrome (PCOS) and its associated complications.**

Thereby, the present investigation was aimed to validate the safety and efficacy of the Siddha poly-herbal formulation ***"ASHUWATHI CHOORANAM"*** 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 "***ASHUWATHI CHOORANAM"*** according to OECD guidelines 423 and 407.

Detailing the study of pharmacological activities like

- Effect of *Ashuwathi Chooranam* in letrozole induced PCOS rat model.
    a) Development of PCOS rat model with the help of Letrozole (non-steroidal aromatase inhibitor).
    b) Estimation of FSH, LH, Estradiol and Progesterone levels
- Anti-oxidant activity of the trial drug ***"ASHUWATHI CHOORANAM"*** in female Wistar rats.

3. REVIEW OF LITERATURE

3.1. DRUG REVIEW

3.1.1. SIDDHA ASPECT OF THE DRUG:

அசுவாதிச் சூரணம்

“அறிவாலறிவா யகவாத சூரணந்தன் னையறையக் கேள்
பிறிவாய் வந்த பலரோகம் போகும்படிக்கு அமுக்கிறாவேர்
குறியாயிடித்து வடிகட்டி கூட்டு மருந்து திரிகடுகு
நெறியாய்ஞ்ச் சாதிக்காய் பத்திரி நீத மதுரங் கிராம்பாமே”

“ஆமே கடுகுரோகணி யஞ்சம பாகங் குறசானி
ஆமே வகைக்கு முக்கழஞ்சி யனிலில் வெதுப்பி தூளாக்கி
ஆமே யிருபத்தைங் கழஞ்சு மதிக சீனியிவை யொக்க
ஆமே ரெட்டி யமுக்கிறாவேர்ப் பொடியுஞ் சூரணமாச்சே”.

வேறு

“சூரண மாச்சதனில் வெருகடிதான் நெய்யில்
தூடர்ந்து யிருபோது மண்டலந்தானுண்ண
காரணமாய் வரண்டு வற்றியிருந்த தேகம்
கனத்த வுடல் பூரித்துப் பருக்குமப்பா
பூரணமாய் தூலித்துப் பூரித் தோர்க்கு
பூட்டடா தேனிலே ம்ண்டலந்தான் வற்றும்
வாரணமாய் பித்த்மொடு பாண்டு தீர
வாழ்மேதை புளியாத மோரிலூட்டே”

“ஊட்டடா யெலிகடிக்கும் பசுவு நீரி
லொடுங்காத சித்து விஷந் தேட்கு வேப்பெண்ணை
பூட்டடா விளக்கெண்ணை மேலும் பூசு
பொல்லாத யீளையொடு சோகை தீர
மூட்டடா வெள்ளாட்டு நீரிலேதான்
மிகுந்த பெரும்பாட்டுக்கு யெள்ளெண்ணை வெல்லம்
நீட்டடாவிரியனுக்கு வெந்நீலூட்டி
நிகழவே பூசிவிட நின்றுபோமே”

"நின்றுபோம் தலையிளைத் திருந்தாலப்பா
நேராவின் பாலிலே யூட்டுவூட்டே
நின்றுபோம் ஷட்குன்மம் வாய்வு மரோசிக்கு
நீட்டடா கச்சல் பழச்சாற்றிலே தான்
நின்றுபோஞ் சூலைகளுக்குமுரியிலே யூட்டு
நில்லாத சோகை காமாலை போக
நின்றுபோம் நிம்பத்தின் சாற்றிலுண்ண
நிலைகெட்டுப் பிள்ளையிலா தவர்க்குக் கேளே"

"பிள்ளையில்லா தவர்க்குமபா பிரமியிலைச் சாற்றில்
பிசகாமல் நல்லெண்ணை பாகலிலைச் சாறும்
தள்ளாத மிலகுடனே கூட்டியுண்ண
சந்ததிதா னுண்டாகுந் தாட்சியில்லை
கள்ளமுள்ள கைமசக்குக் காய் வெண்ணை தன்னில்
கனகமெழு கோடருந்து கனிவுண்டாகும்
தெள்ளிய நற்காய மொடு திப்பிலிச் சாற்றிலருந்த
தெரிவையர்க்கு மாதவிடை சிறக்குந்தானே".

-அகத்தியர் வைத்திய காவியம் ***1500***

Ingredients

Amukkura	-	*Withania somnifera*
Chukku	-	*Zingiber officinale (dried ginger)*
Milagu	-	*Piper nigram*
Thippili	-	*Piper longum*
Jaadhikaai	-	*Myristica fragrans*
Jaadhipathiri	-	*Myristica fragrans*
Adhimadhuram	-	*Glycirriza glabra*
Krambu	-	*Syzygium aromaticum*
Kadugurohini	-	*Picrorhiza scrophulariiflora*
Krosaniomam	-	*Hyoscymus niger*

Amukkara - Withania somnifera

The herb grows in India and Balochistan. The plant is ash in colour. Its fruit resembles tomato. When the fruit ripens it is red in colour.

Synonyms:

Amukkuri

Amukkuravi

Asuvam

Ashuvagantham

Erulichevi

Kidichevi

Varagakarni

Vernacular names:

English	-	Winter cherry
Telugu	-	*Penneru-gadda*
Malayalam	-	*Amukkuram*
Kanada	-	*Sogade-beru*
Sanskrit	-	*Aswagandha*
Arab	-	*Luban*
Persian	-	*Habdul Kaknaje*
Hindhi	-	*Habdul Kaknaje*
Urdu	-	*Asgandh*

Part used : Root, leaf, seed.

Taste : Bitter

Character: Heat

Division : Pungent

Actions :

Aphrodisiac

Tonic

Diuretic

Febrifuge

Alterative

De-obstruent

Soporific

Sedative.

General Properties:

"கொஞ்சந் துவர்ப்பாங் கொடிகயம் சூலையரி
மிஞ்சுகரப் பான்பாண்டு வெப்பதப்பு-விஞ்சி
முசுவுறு தோமும்போ மொகம்அன
நகுட வெருண்டுறுவாழ் நாள்".

-அகத்தியர் குணவாகடம்

Indications:

It is used for strength, stamina, beauty, longevity.

Therapeutic uses

It is effective in *kayam, vadha* diseases, eczema, fever and swelling.

External application

- The root tuber was ground with cow's urine and the paste was boiled. This was applied externally for tumours, swellings and for pain.
- The above paste along with *chukku* was ground in hot water, applied for swellings.

- The root tuber or leaf was ground and applied for tumours and wound.

Decoction

It is given for fever.

Amukkara podi

The root tuber was boiled in milk, washed, dried and powdered.

Dose: 2 to 4 gms.

Adjuvant : honey

Indications: *Vali iyam,* swelling, anorexia, obesity [18]

***Siddha* formulations**

Amukkara chooranam, amukkura legium.

Chukku – Zingiber officinale

Synonyms:

Arukkan

Artharagam

Upakullam

Ularntha inji

Kadupathiram

Chukku

Chundi

Chondi

Soupannam

Souvarnam

Navasuru

Vernacular names:

Tamil - *Chukku*

English - Dried ginger

Telugu - *Sonti*

Malayalum - *Chukku*

Kannadam - *ona shunti or sunti*

Sanskrit - *Nagaram*

Hindi - *Sonth*

Arab & Per - *Znagebilarataba*

Part used : Root (dried)

Taste : Acrid

Character: Heat

Division : Acrid

Action :

Stimulant

Stomachic

Carminative.

General characters

"சூலைமந்தம் நெஞ்செரிப்பு தோடமேப் பம்மழலை
மூலம் இரைப்பிருமல் மூக்குநீர்வாலகப-
தோடமதி சார்ந் தொடர்வாத குன்மநீர்த்
தோடம்ஆ மம்போக்குஇஞ் சுக்கு".

-அகத்தியர் குணவாகடம்.

Indications:

Indigestion, heart burn, belching, cough, asthma, sinusitis, haemorrhoids, gastric ulcer, bloating, anaemia, fever.

Therapeutic uses

Decoction:

The decoction prepared from it is administered for fevers.

Chukku karpam:

The chukku powder along with sugarcane juice can be given in morning for gastritis.

***Siddha* formulations**

Sowbakiya chundi, panchadepagini chooranam, panchadepagini legium [18a] .

***Kirambu* - Cloves**

Synonyms:

Lavangam

Anjukam

Tiraili

Varangam

Chosam

Vernacular names:

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

Part used : Dried flower buds, fruit and oil.

Taste : Acrid

Character: Heat

Division : Acrid

Action :

Carminative

Antispasmodic

Appetizer

General characters

"பித்தமயக்கம்பேதியொடுவாந்தியும்போம்
சுத்தவிரத்தக்கடுப்புந்தோன்றுமோ-மெத்த
இலவங்கங்கொண்டவருக்கேற்சுகமாகும்
மலமங்கேகட்டுமெனவாழ்த்து
சுக்கிலநட்டங்கர்ணசூர்வியங்கலாஞ்சனந்தாட்
சிக்கல்விடாச்சர்வாசியப்பிணியு- மக்கிக்குட்
டங்கப்பூவோடுதரிபடருந்தோன்றிலில்
வங்கப்பூவோடுரைத்துவா"

-அகத்தியர் குணவாகடம்

Indications

It cures giddiness, diarrhoea, dysentery, vomiting, anal fissure and cataract.

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**

Kumkumapoo maathira, Kirambu thylam, kapadamathirai [18b].

Jathikaai -myristica fragrans

Synonyms:

Kullakai

sathikkai

Vernacular names:

Eng	-	Nut meg
San	-	*Jatipatri*
Mal	-	*Jatipattriri*
Tel	-	*Jakikaya*
Kan	-	*Jajikayi*
Arab	-	*Jojubuva*

Pers - *Jaurzoboyah*

Hindi - *Jae-phal*

Duk - *Japhal*

Part used : Fruit

Taste : Astringent, acrid

Character: Heat

Division : Acrid

Action :

Stimulant

Stomachic

Carminative

Narcotic

Aromatic

Aphrodisiac

Tonic

General properties:

"தாது நட்டம் பேதி சருவாசி யஞ்சிர நோய்
ஓதுசுவா சங்காசம் உட்கிராணிவேதோ -
டிலக்காய் வரும்பிணிபோம் ஏற்றமயல் பிதத்ங்
குலக்காய் யருந்துவர்க் கூறு."

-அகத்தியர் குணவாகம்.

Indications:

Oligospermia, diarrhoea, headache, cough, asthma, stomach pain, bloating.

Therapeutic uses:

Sathikakai oil: The oil obtained by distillation process can be given up to 1-5 drops for tooth ache.

Sathikkai powder: It is used to cure cough, stomach pain, migraine, and menorrhagia.

Decoction: It is given in the dehydration after vomiting and diarrhoea.

Sathikkai was ground and applied around the eyes to increase the power of vision [18c].

Jathipathiri - Myristica fragrans(Arill)

Synonyms:

Jathipathiri

Vasuvasi

Vernacular names

Eng - Arillus of the nut

Tel - *Japtri*

Mal - *Jatipattriri*

Kan - *Japatri*

Sans - *Jatipatri*

Arab - *Bisbasah*

Pers - *Bazbaz*

Hind - *Javatri*

Duk - *Joutri*

Part used : Arill of the fruit

Taste : Acrid

Character: Heat

Division : Acrid

Actions

Aphrodisiac

Carminative

Stimulant

Hypnotic

General properties

"சாதிதரும் பத்திரிக்குத் தாபச் சுரந்தணியும்
ஓதுகின்ற பித்தம் உயருங்காண்தாதுவிர்த்தி -
யுண்டாங் கிரகணியோ டோதக் கழிச்சலுறும்
பண்டாங் குறையே பகர்."

-அகத்தியர் குணவாகடம்

Indications:

It is used in fever, steatorrhea, and diarrhoea. It strengthens the body.

Therapeutic uses

It is given in the dehydration after vomiting and diarrhoea.

Sathipathiri was ground and applied around the eyes to increase the power of vision[18d].

Milagu - Piper nigrum

Synonyms

Kalinai

Kari

Kayam

Kolagam

Thirangal

Miriyal

Sarumabantham

Valisam

Maasam

Vernacular names:

Eng - Black pepper

Hin - *Kali-mirch*

Kan - *Menasu*

Mal - *Kurumulaku*

San - *Maricha*

Tel - *Miriyalu*

Sans - *Maricha*

Habitat:

It is a climber. The plant is cultivated in South Indian parts of India Such as Kerala, Cochi, Kudagu Mysore. The fruit of this plant is called as *Milagu.*

Part used : Seed, shoot.

Taste : Bitter, acrid

Character: Heat

Division : Acrid

Actions :

Antidote

Carminative

Antiperiodic

Rubefacient

Stimulant

Resolvent

Antivatha

General properties:

"சீதசுரம் பாண்டு சிலேத்மங்கிராணிகுன்மம்
வாதம் அருசிபித்தம் மாமூலம்ஓதுசன்னி-
யாசமபஸ் மாரம் அடன்மேகம் காசமிவை
நாசங் கறிமிளகினால்

-தேரன் குணவாகடம்

கோணுகின்ற பக்கவலி குய்யவுரோ கம்வாத
சோணிதங்க ழுத்திற்குள் தோன்றுநோய்காணிய-
காதுநோய் மாதர்குன்மங் காமாலை மந்தமென்றீர்
ஏதுநோய் காயிருக்கில் ஈங்கு.

-அகத்தியர் குணவாகடம்.

Indications:

Fever, anaemia, diarrhoea, gastric ulcer, vatham, pitham, kapam diseases will be cured.

One of the ingredients in Trikatuku

Pills:

The pill made of this given for prolapse of pile mass.

External application:

Milagu thailam:

The oil extracted from can be given for vatha diseases, sinusitis, deaf.

Paste:

The paste made when is treated for alopecia, the hair starts to grow [18e].

Thippili - Piper nigrum

Synonyms

Aarkathi

Unsaram

Ulavainaasi

Kaman

Kudari

Kolagam

Koli

Kozhaiyaruki

Saram

Vernacular names:

Eng - Long pepper

Tel - *Pippilu*

Mal - *Thippili*

San - *Pippali*

Duk - *Pipliyan*

Kan - *Hippili*

Pers - *Daraife-fil*

Habitat:

Piper longum is a climber mainly cultivated in South India and West Bengal for the fruit. The acrid taste of it is higher than the pepper.

Types:

Arisi thippili and *yanai thippili.*

Part used: Fruit, rice

Taste: Sweet

Character: Heat

Division: Sweet

Actions:

Stimulant

Carminative

General properties:

"கட்டி யெதிர்நின்று கடுநோயல் லாம்பணியும்
திட்டி வினையகலும் தேகமெத்தபுட்டியாம்-
மாமனுக்கு மாமனென மற்றவர்க்கு மற்றவர்க்கு
மற்றவனாங்
காமமெனுந் திப்பிலிக்கும் கை.
-தேரன்வெண்பா .

Indications:

Cough, anaemia, sinusitis, anorexia, gastric ulcer, asthma, diseases of ear and nose, tuberculosis

Thippili* is one of the ingredients in *Trikatuku

Therapeutic uses:

Thippili five parts and *thettran* seed three parts are powdered well and 4gms of it given in *kaluneer*, three days in the morning gives a cure for white discharge and menorrhagia.

Thippili karpam:

The powder of it along with honey can be given for tinea about one month.

Thippili arisi:

It cures bloating, fever, tuberculosis and increases the sperm count [`18f].

Athimadhuram - Glycyrrhiza glabra

Synonyms

Athingam

Atti

Madhugam

Kundriver

Vernacular names:

Eng. - Jequitity; Indian or Jamaica liquorice.

Tel - *Ati-Madhuramu, Yashti-Madhukam*

Mal - *Ati-Madhuram, Iratti-madhuram*

San - *Yashti-Madhukam*

Arab - *Aslussus*

Pers - *Bikhe-Mahak*

Hindhi - *Jathi-Madh,Mulath*

Duk - *Mitthi-Lak*

Habitat:

The plant grows in hilly areas like the *Abrus precatorius* it is also a root. It is small or big in size, yellow in colour and externally dirty white in colour. The seeds are in different colours of black, white and red.

Part used : Root

Taste : Sweet, bitter

Character: Heat

Division : Acrid

Actions :

Emollient, Demulcent Mild, Expectorant, Laxative, Tonic.

General properties:

"கத்தியரி முப்பணியால் வருபுண்
கண்ணோய்உன் மாதம்விக்கல் வலிவெண் குட்டம்
பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த

பித்தமத மூர்ச்சை விட பாகம் வெப்ப்ந்
தத்திவரு வாதசோ ணிதங்கா மாலை
சருவவிடங் காமியங்நோய் தாது நட்டங்
குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து
குயப்புணும்போம் மதூகமெனக் கூறுங் காலே”.

- தேரன் குணவாகடம்.

Therapeutic uses:

The powder of *Atimathuram, Milagu, Kattukai* along with honey is given for cough.

Atimathuram and *Mutsangan* was ground in lime juice and given for jaundice.

Atimadhuram along with *thiratchai uppu* can be given for diarrhoea [18g].

Kadugurohini -Picrorhiza scrophulariiflora

Synonyms

Kadugurohini

Kadagarohini

Vernacular names

Eng - Picrorhiza

Tel - *Katki*

Mal - *Katukurohini, Katurohini*

San - *Katvi, Katurohini, Katuka*

Hin - *Katuka, Kuru, Kutuki*

Kan - *Katukarohini*

Habitat:

The plant grows in Himalayas from Kashmir to Sikkim. The outer surface of the root is dirty white in colour and inner it is black in colour. It is also called *Nattu kadugurohini*.

Part used : Root

Taste : Bitter, acrid

Character: Heat

Division : Acrid

Actions :

Antiperiodic

Cathartic

Stomachic

Anthelmintic

General properties:

"மாந்தஞ் சுரமையம் வாயுகரப் பானாமஞ்
சேர்ந்தமலல் கட்டு திரிதோடம்போந்தபொட்டுப் -
புண்வயிறு நோயிவைபோம் பொற்கொடியேபேதியுண்டாம்-
திண்கடுகு ரோகணிக்குத் தேர்".

-அகத்தியர் குணவாகடம்.

Indications:

Indigestion, fever, *iyya peruku*, eczema, dysentery, stomach pain, wounds.

Therapeutic uses:

Decoction:

The decoction of the root is given for dropsy [18h].

Kurosani omam – Hyoscyamus niger

Synonyms

Thippiyam

Karasavai

Karabi

Karsavai

Vernacular names

Eng - Henbaneseeds, Black henbane

Tel - *Kurasani oamamu*

Mal - *Kurasani (omum)*

San - *Parasikayavani*

Hin - *Khorasani-Ajowan*

Kan - *Kurasani voma, Kurasani Vadakki*

Habitat:

In India it grows in Himalayas at a height of 8000 to 11,000 feet. The leaf is greenish yellow in colour, it has a characteristic odour. Flowers are light yellow in colour.

Part used : Seed

Taste : Acrid, slightly bitter

Character: Heat

Division : Acrid

Actions:

Hypnotic

Sedative

Anodyne

Antispasmodic

Mild diuretic

General properties:

"வெகுமூத்திரம்வாதம் வீரியநட் டம்புண்
உகுபேதி யுட்கடுப்பி னோடேமிகுகரப்பான் -
தீராக் கபமிவைபோம் செய்யகு ரோசானியென்றால்
வாரா மயக்கமுறு மால்".

-அகத்தியர் குணவகடம்.

Indications:

Diseases of the teeth, Polycystic Ovarian Syndrome, bronchitis, glitch of memory, tremor, insomnia.

Therapeutic uses:

Decoction:

The extract of Hyoscyamus can be given for insomnia, glitch of memory [18i].

3.1.2. BOTANICAL ASPECT

Withania somnifera

Kingdom - Plantae

Class - Magnoliopsida

Order - Solanales

Family - Solanaceae

Genus - Withania

Species - somnifera

Occurrence and distribution:

Withania somnifera is an evergreen shrub growing to 1m height. It grows abundantly in India especially in Madhya Pradesh, Bangladesh, Srilanka and northern parts of Africa.

Description of the plant:

It is an upright shrub, star shaped branches covered with fine hairs, leaves alternate and ovate, up to 10cm long and up to 5cm wide, flowers yellow in colour, fruit red berries in papery protective covering. Root long fleshy tubers.

Part used:

Rhizome

Actions:

Diuretic

Antipyretic

Anthelmintic

Alterative

Aphrodisiac

De-obstruent

Chemical constituents:

Steroid lactone, withanolides, withanolide A, withanoside IV.

Uses:

Used as a sedative or calming agent and for insomnia.

Anti-inflammtory agent; it is used to treat rheumatic pain and arthritis.

General tonic and adaptogen which helps the body to adapt stress and promote strength [19].

Glycyrrizha glabra

Kingdom -Plantae

Class -Angiosperms

Order -Fabales

Family -Fabaceae

Genus -*Glycyrrizha*

Species -*glabra*

Occurrence and distribution:

A genus of perennial herbs and undershrub's distributed in the sub-tropical and warm temperate regions of the World, chiefly in the Mediterranean Countries and China. In India it grows in Himalayas and hilly districts of South India.

Description of the plant:

It is a hardy herb or undershrub attaining a height up to 6ft; leaves multifoliolate, imparipinnate; flowers in axillary spikes, papilionaceous, lavender to violet in colour; pods compressed containing reniform seeds. The underground part in some varieties consists of a rootstock with a number of long, branched stems. The dried, peeled or unpeeled underground stems and roots constitute the drug, known in the trade as Liquorice.

Part used:

Root

Actions:

Tonic

Expectorant

Demulcent

Mildly laxative

Haemolytic

Chemical constituents:

The principle constituent of liquorice to which it owes its characteristic sweet taste is glycyrrhizin which is present in different varities. Other constituents present in liquorice are glucose, sucrose, mannite, starch, asparagine, bitter principles, resins, a volatile oil and colouring matter. The yellow colour is due to the anthoxanthin glycoside; isoliquiritin. A steroid estrogen, possibly estriol is also reported to be present in liquorice.

Uses:

Useful in irritable conditions of the mucous membrane of genitourinary tract.

Liquorice extract is a constituent of cough syrups, throat lozenges and pastilles.

The extract is reported to exert a healing effect on gastric ulcers.

It is spasmolytic and stimulates hydrochloric acid secretion.

It is used in the treatment of Addison's disease [19a].

Piper longum

Kingdom - Plantae

Class - Angiosperms

Order - Piperales

Family - Piperaceae

Genus - *Piper*

Species - *longum*

Occurrence and distribution:

It is climbing plant cultivated on large scale in limestone soil 400-600 m below the Cherapunji region which receives very high rain fall. India imports a large quantity of long pepper from Malaysia and Singapore.

Description of the plant:

Long pepper is cultivated mainly by layering of the mature branches or by suckers planted at the beginning of the rainy season. The stems have nodes where the fruitful branches and leaves born. It has adventitious roots. Leaves are numerous, stalked and cordate at the base with seven nerves. The inflorescence is pedunculated flower spike that comes out from the nodes of the main stem. The long pepper fruit is a drupe small, arranged in the flower spike forming compact fruiting, welded, reminiscent of black catkins and hard, between 2 and 3(up to 5) cm long.

Part used:

Root

Actions:

Tonic

Expectorant

Demulcent

Mildly laxative

Haemolytic

Chemical constituents:

The fruit of *Piper longum* has shown the presence of the alkaloids piperine (4-5%) and piplartine and two new liquid alkaloids, one of which is designated as alkaloid A. This is closely related to pellitorine. Sesamin,

dihydrostigmasterol and e new sterol, piplasterol are also present. The oil of the fruit contains terpinolene, n-octadecane, α-thujene, p-methoxy acetophenone.

Uses:

The fruits as well as the roots are attributed with numerous medicinal uses, and may be used for diseases of the respiratory tract viz. cough, asthma, bronchitis, tuberculosis, etc.; as counter irritant and analgesic, as snuff in coma and drowsiness and internally as carminative; as sedative in insomnia and epilepsy; as general tonic and haematinic; as cholagogue in obstruction of bile duct and gall bladder; as an emmenagogue and abortifacient [19b].

Piper nigrum

Kingdom - Plantae

Class - Angiosperms

Order - Piperales

Family - Piperaceae

Genus - *Piper*

Species - *nigrum*

Occurrence and distribution:

A branching, climbing perennial shrub, mostly found cultivated in the hot and moist parts of India and other tropical countries. Pepper is one of the most ancient crops cultivated in India and ha probably originated in the hills of south-western parts of India. In India, pepper is grown mainly in Kerala, Madras and Mysore and to a very small extent in Assam.

Description of the plant:

Branches stout, trailing and rooting at the nodes; leaves entire, very variable in breadth. Flowers minute in spikes, usually dioecious, fruits ovoid or globose, bright red when ripe; seeds usually globose, testa thin, albumin hard.

Part used:

Fruit

Actions:

Stomachic

Antiperiodic

Alterative

Carminative

Chemical constituents:

The fruit of *Piper nigrum* retards the development of rancidity in oils and fats, frozen ground pork, beef and lard. This activity has been attributed to the presence of tocopherols in the oleoresin. Arginine, piperine, lysine, histidine, cystine, asparagine, serine, glutamic acid, threonine, β-alanine, γ-aminobutyric acid and pipecolic acid.

Uses:

The fruits have stimulant and carminative properties and are used in haemorrhoidal affection. They are used in tonics for languidness and after childbirth, also in digestive and other disorders. In modern Indian medicine, it is much employed as an aromatic stimulant in cholera, weakness following fevers and as an alterative in paraplegia and arthritic diseases [19c].

Picrorhiza scrophularia

Kingdom - Plantae

Class - Angiosperms

Order - Piperales

Family - Scrophulatriaceae

Genus - *Picrorhiza*

Species - *scrophularia*

Occurrence and distribution:

A small genus of perennial herbs distributed in the alpine Himalayas from Kashmir to Sikkim at altitudes of 2,700 – 4,500 m.

Description of the plant:

The leaves of the plant almost radical, spathulate sharply serrate; flowers white or pale blue-purple, in a dense terminal spicate raceme; fruit an ovoid capsule.

Part used:

Rhizome

Actions:

Antiperiodic

Cholagogue

Stomachic

Laxative

Chemical constituents:

The roots of the plant contain large proportion of bitter principles, mainly a glucoside named picrorhizin also contain glucosidal bitter principle kutkin, non-bitter product kurrin, D-mannitol, vanillic acid, kuti-sterol, sesquiterpene.

Uses:

It is used for bacterial infections [19d].

Myristica fragrans

Kingdom - Plantae

Class - Angiosperms

Order - Magnoliales

Family - Myristicaceae

Genus - *Myristica*

Species - *fragrans*

Occurrence and distribution:

In India, it is grown in Madras, Nilgiris, Coimbatore, Salem, Tirunelveli, Madurai, Ramanathapuram districts; a few trees were found in various localities in Kerala, Assam and other States.

Description of the plant:

A dioecious or occasionally monoecious evergreen, aromatic tree. Usually 9-12 m. high. Bark greyish black, longitudinally fissured in old trees; leaves elliptic or oblong-lanceolate, coriaceous; flowers in umbellate cymes, creamy yellow, fragrant; fruits yellow, broadly pyriform or globose, 6-9 cm long, glabrous, often drooping; pericarp fleshy, seed broadly ovoid, arillate, albuminous, with a shell like purplish brown testa.

Part used:

Fruit

Actions:

Stimulant

Astringent

Aphrodisiac

Carminative

Tonic

Chemical constituents:

Analysis of nutmeg contains protein, carbohydrates, fibre, minerals like calcium, phosphorous, iron, carotene.

Uses:

It is a major constituent of preparations prescribed for dysentery, stomach ache, flatulence, nausea, vomiting, malaria, rheumatism, sciatica and early stages of leprosy [19e].

Zingiber officinale

Kingdom - Plantae

Class - Angiosperms

Order - Zingiberales

Family - Zingiberaceae

Genus - *Zingiber*

Species - *officinale*

Occurrence and distribution:

Ginger has been under cultivation in India from times immemorial; the plants on the West coast of India are said to bear flowers quite frequently in October. Kerala gives much higher yield of the ginger as compared to that of the local types. Jamaican ginger is considered to be the best quality ginger and the Indian ginger comes the second.

Description of the plant:

Ginger is an herbaceous, rhizomatous perennial, reaching up to 90 cm. in height under cultivation. Rhizomes are aromatic, thick-lobed, pale yellowish,

differing in shape and size in the different cultivated types. The herb develops several lateral shoots in clumps which begin to dry when the plants matures. Leaves narrow, distichous, dark green, flowers in spikes.

Part used:

Rhizome

Actions:

Stimulant

Antioxidant

Carminative

Rubefacient

Anti-depressant

Anti-narcotic

Chemical constituents:

Analysis of nutmeg contains crude protein, crude fibre, caprylic acids, phenol, zingiberene, ar-curcumene, zingiberol, cumene, limonenephellandrene.

Uses:

Ginger is valued in medicine as a carminative and stimulant to the gastrointestinal tract. It is much in vogue as a household remedy for flatulence and colic. Ginger contains an anti-histaminic factor [19f].

Hyoscyamus niger

Kingdom -Plantae

Class - Magnoliopsida

Order - Solanales

Family - Solanaceae

Genus -*Hyoscyamus*

Species -*niger*

Occurrence and distribution:

Usually biennial, is an evergreen shrub growing to 1m height. It grows abundantly in India especially in Madhya Pradesh, Bangladesh, Srilanka and northern parts of Africa.

Description of the plant:

It is an upright shrub, star shaped branches covered with fine hairs, leaves alternate and ovate, up to 10cm long, flowers yellow in colour, fruit red berries in papery protective covering. Root long fleshy tubers.

Part used:

Seeds

Actions:

Diuretic

Antipyretic

Anthelmintic

Alterative

Abortifacient

Chemical constituents:

Hyoscyamine- N-oxide isolated from roots, leaves and stems. Total alkaloids from the aerial parts contained hyoscyamine, hyoscine, skimmianine, apoatropine, tropine, etc.

Uses:

Used as a sedative or calming agent and for insomnia.

Anti-inflammatory agent; it is used to treat rheumatic pain and arthritis.

General tonic as adaptogen helping the body to adapt stress and promote strength [19g].

3.2. DISEASE REVIEW

3.2.1. Siddha aspect of the disease

Synonyms

- *Soothaga katti*
- *Ruthuneer katti.*
- *Soothaga katti*
- *Soothaga thiratchi*
- *Soothaga noi*
- *Soothaga kolaru* [20]

***Soothaga vayu*& Pcos**

"சித்தான கர்ப்பத்தில் சேர்ந்திடும் இரத்தந்தான்
வத்தாம் வுருண்டு வாயுபோல் ஓடிடும்
வற்ற பசிபோகும் உழன்றே இரைந்திடும்
வற்றாக கழிச்சலாம் வன்சூதக வாயுவ".

-திருமூலர் கருக்கிடை வயித்தயம் 600

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, dysentery.

"கேளுமே சூதகத் திலக்கினி வாய்வு
கெடுத்து விடும்மாத விடாய்கட்டிபோகும்
ஆளுமே கருக்குழியும் தூர்ந்து தேகம்
அப்பனே யுதிர மதுஅடி மூலத்தில்
நீளுமே சூதகத்தில் வாய்வு தோன்றி
நேரான அடிவயிறு வலிப்புக் காணும்

பாளுமே தலைவலிக்கும் இடுப்பு ளைச்சல்
பக்குவமாய் மருந்துண்ணத் தீருந்தானே

-ஆவியளிக்கும் அமுதமுறைச் சுருக்கம்

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, headache, low back ache etc.

"பொருமி இரத்தத் தனைமரித்துப் போதவுதாம் வலிஉண்டாய்
குருதியழியில் வலிதீருங் கூடுங் கெர்ப்பந் தனையழிக்கும்
வருடியிடுப்புக் குடைந்துளையு மல்த்தை மிகவுமிருக்கி நிறகும்
பெருகப் பணைக்குங் குவிமுலையாய்! பேசங் கெர்ப்பவாயுவிதே"

-அகத்தியர் ஆயுள் வேதம் 1200

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, severe constipation.

"நவின்றிடவே யிடுப்பு வயிற்பெருத்துக் 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."

- யூகிவைத்தியசிந்தாமணி-

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[21].

Aetiology

“சூதகநோய்வரும்வழி:

.....

தரணியில் பெண்களுக்கு கெற்ப நோய்கள்
நயக்கவே வந்து தென்னவென்றால் மைந்தா
நன்மையுடன் ருதுவாகும் நாளிற்றானே
மயக்கவே மாப் பாண்டம் பால்பழத்தினாலே
வந்துதடா சூதகத்தின் வாயுதானே

தானென்ற கருக்குழியில் வாய்வு தங்கி
தளர்ந்த தொரு சோரையினால் தசைதான்மூடி
ஊனென்ற தேகமெல்லாம் மதர்த்து நல்ல
உண்மையுள்ள அடிவயிற்றில் வலி யுண்டாச்சு
பானென்ற கருக்குழி தான் விளக்க மன்றி
பரமான விந்துவங்கே அணுகாதையா
ஏனென்றால் ஆதியிலே வாய்வு கொண்டு
இருந்ததினால் கெற்பமது இல்லை தானே

-அகத்தியர் அமுத கலைஞானம்

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
பிசைந்த கர்ப்பப்புழுவாலும் கிரகத்தாலும்
பிணியாலும் மேகவை சூரியாலும்
துசங்கெட்டக் கலவியினால் பூவொதுங்கித்
துலங்காமற் பிள்ளையில்லை சொல்லக்கேளே

-பதினெண் சித்தர்கள் பாடிய வைத்திய
சில்லறைக் கோவை-

Causes for infertility

The causes for female infertility are increased *Pitham, Vatham,* worms in the womb, planetary positions, viral infections and increased frequency of sexual intercourse.

"நவின்றிடவே யிடுப்புவயிற் பெருத்துக்காணும்
நலமான மேனியது வூதிக்காணும்
குவின்றிடவே மும்மடிப்பு வயிற்றில்தோன்றும்
குணவதியாந்தேவதா பெண்ணானாலும்
நவின்றிடவே சன்மத்தின் மலடேயாகும்
சதாகாலங் கருப்பமது தரியாதென்று
புவின்றிடவே யூகிமுனி சிகிச்சாசாரம்
புகன்றிட்டார்லோ கத்து மாந்தற்காமே.

-யூகி வைத்திய சிந்தாமணி.

நாடி நடை

"மாதர் கைமிடித்த போதுவந்திடும் நாடிமூன்றும்
சேதமாயிற்று நின்று சேரவே பதித்துநிற்கில்
ஓதுமே சூதகத்தில் ஓங்கிய வாய்வுநின்று
பேதமாய் வாதைபண்ணி பிணியினை விளைக்குந்தானே"

-பதினென் சித்தர்களின் நாடி சாஸ்த்திரம்

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.

3.2.2 Modern aspect of the disease

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 of 5–7 mm, but not further. No single follicle reaches the preovulatory size (16 mm or more).

Fig No. 1. Poly cystic ovaries

Table No.1. Clinical signs and symptoms associated with PCOS

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)

1. Chronic anovulation

2. Clinical or biochemical signs of hyperandrogenism

Minor NIH Criteria

1. Insulin resistance
2. Perimenarchal onset of hirsutism and obesity
3. Elevated LH/FSH ratio
4. Intermittent anovulation associated with hyperandrogenemia
5. Ultrasound evidence of polycystic ovaries

Rotterdam Criteria-two of three required

1. Oligo and /or anovulation
2. Clinical or biochemical signs of hyperandrogenism
3. 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 crowded 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 un associated 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 oestrone or androgens or both. FSH secretion remains either normal or decrease due to negative feedback effect of oestrogens 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 oestradiol 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.

Fig No. 2. Difference between normal ovaries with polycystic ovaries

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.

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" [21]

Differential diagnosis

- CAH- congenital adrenal hyperplasia
- Cushing syndrome.

3.4.3 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 ampoules 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.
- Ascities.

- Shock.

Bromorgocriptines (Bromocriptine, Parlodel, Proctinal)

This semisynthetic ergot alkaloid is a selective D2 receptors.

Therapeutic uses

- It inhibits hyper prolactinemia and stops galactorrhea.
- 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 lessthan 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 antidiabetic 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. Antidiabetic 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:

- We also note a resurgence of ovulatory cycles in 50% of cases and pregnancy Studies show after treatment for 2 months because of 3x 500 mg / day or 850 mg 2x / d:

- 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 spontaneous.

POLY CYSTIC OVARIAN SYNDROME

The purpose of this chapter is to present a review of the current research on polycystic ovary syndrome (PCOS). PCOS is one of the most common endocrine disorders in women of reproductive age, affecting 5-10%of the population. Despite its prevalence, PCOS remains largely under unknown.

This review has been mainly focused on the pathogenesis, related health consequences, diagnosis as well as the prevalence and incidence of the disease. It then delves into the pathogenesis with a focus on genetics, obesity, insulin resistance and birth weight. Lastly, the health consequences related to PCOS are discussed, with a focus on insulin resistance. The health outcomes reviewed include the metabolic syndrome, cardiovascular disease, and type II diabetes mellitus.

Many of the symptoms of PCOS are solely through lifestyle modifications. Although many questions remain surrounding polycystic ovary syndrome, this chapter provides a summary of the current research.

PCOS

Polycystic Ovary Syndrome (PCOS) is a heterogeneous disorder. As one of the leading causes of anovulatory infertility, it is believed that5-

10%ofthe reproductive-aged female population is living with polycystic ovary syndrome [22]. The first description of polycystic ovary syndrome (PCOS) was performed by Stein and Leventhal in the year 1935, in which they presented to a group of patients characterized by the clinical association of amenorrhea, hirsutism and obesity [23]. The development of PCOS has been linked to hereditary and environmental factors including genetics, insulin resistance, obesity and birth weight. The presence of PCOS is associated with an increased prevalence of adverse health conditions such as the metabolic syndrome, cardiovascular disease and type II diabetes mellitus. Insulin resistance is believed to play a key role in the development of PCOS and in the development of related conditions [24]. In the past few years, research has been done to better understand the mechanisms behind the development poly cystic ovary syndrome and the impact it has on the female body, particularly in relationship to insulin resistance.

Diagnosis

Poly cystic ovary syndrome is a largely under diagnosed disorder. It was described as a clustering of symptoms including enlarged ovaries, obesity, hirsutism, and chronic anovulation [23a]. However, it took another fifty-five years before formal criterion were proposed for the diagnosis of PCOS [25]. Polycystic ovary syndrome is now diagnosed based on the presence of the following criteria: clinical and biochemical hyperandrogenism, menstrual irregularities, and the presence of poly cystic ovaries. Common features of the disease which are not part of the diagnosis include insulin resistance, luteinizing hormone (LH)/follicle stimulating hormone (FSH) concentrations, and obesity [26]. Thus, it was subsequently required for the diagnosis the plasma elevation of luteinizing hormone (LH) and the ratio of LH / FSH, being years later the visualization by ultrasound of multiple ovarian cysts (Fig.3&4).

Fig No. 3 and 4: macroscopic and ultrasonography image of polycystic ovary syndrome.

However, these criteria have been reviewed and modified recently. In the consensus held in Rotterdam in 2003, the main difference established and consisted of the inclusion of ovarian polycystosis again, defined by the presence of 12 or more follicles in each ovary between 2 and 9 mm in diameter and / or the existence of an increased ovarian volume (greater than 10 mL) as one of the criteria for diagnosis [27]. At the end of 2006, the Androgen Excess Society (AES) has defined new criteria [28], according to which the diagnosis of PCOS.

Existence of hyperandrogenism, defined by the presence of hirsutism and / or Hyperandrogenemia, together with ovulatory dysfunction, defined by the existence of oligoovulation and / or polycystic ovaries and the exclusion of secondary pathologies. (Table 1)

Table No. 2. Diagnostic Criteria for Polycystic Ovarian Syndrome.

NICHD Criteria	Criteria forRotterdam	AES Criteria
Oligoovulation	Oligo and / or Anovulation	Hyperandrogenism: Hirsutism and / or clinical Hyperandrogenemia

Hyperandrogenism And / or biochemical clinical	Hyperandrogenism And / or biochemical clinical	Ovarian anomaly: Oligo-anovulation and / or) Ovaries - Polycystic ovaries
---	Polycystic ovaries	--
Exclusion of other pathologies such as hyperprolactinemia, adrenal hyperplasia Congenital and androgen secreting tumors		

According to the NICHD and AES criteria, items 1 and 2 must be present for the diagnosis of SOP. The Rotterdam criteria require the presence of 2 of the 3 individual points. In all Secondary etiologies should be ruled out.

Clinical Hyperandrogenism

Clinical hyperandrogenism is one of the more noticeable features of PCOS. Women are often diagnosed with PCOS when they seek treatment options from their health care provider for some of the negative cosmetic outcomes associated with PCOS. Clinical androgen excess in women with PCOS manifests in the form of acne, hirsutism, and/or alopecia. Acne typically occurs on the face or the back. The presence of acne is correlated with an increase in DHEAS and it is often one of the first clinical signs of hyperandrogenismin women [29]. Hirsutism is male-pattern excess hair growth. Common areas for hair growth include the side-burns, chin, naval area, and chest. Hirsutism is ranked according to the Ferriman-gallweyscale [30].A score of 6-8 is mild, 8-15 is serious, and greater than 15 are classified as overt hirsutism. A woman is considered hirsute with as core greater than eight [31]. Alopecia, or hair loss, occurs in the form of male-pattern baldness. Alopecia is less common in women with PCOS than hirsutism and acne but it does still occur. With each of these clinical symptoms, it is important to remember that there could be another cause responsible other than PCOS. It is important to rule out other possible aetiologies.

Menstrual Irregularities

Menstrual irregularities including oligio-amenorrhea or chronic anovulation are common features of PCOS. Women are often diagnosed with PCOS when they seek treatment for menstrual irregularities or when they have difficulty becoming pregnant. A woman with oligio-amenorrhea has infrequent or very light menstruation. During the first two years post menarche, irregular cycles are common. After this time, cycles should normalize. At this time, oligiomenorrhea can be identified as less than 9 menstrual cycles per year. Amenorrhea is defined as cycles lasting more than 90 days [32]. Some women with PCOS may have normal menstruation patterns but they may not ovulate. This means ovulation cannot be determined solely by the presence of menstruation. Chronic anovulation is diagnosed based on progesterone levels on days 20-24 in the menstrual cycle [30]. Menstrual irregularities are one of the main reasons women with PCOS have difficulty becoming pregnant. Often, it is not until a woman has difficulty becoming pregnant that she will seek guidance from a medical provider.

Polycystic Ovaries

As referenced by the name, PCOS can also be identified by the presence of polycystic ovaries. Poly cystic ovaries are identified by the presence of at least one ovary greater than $10cm^3$or the presence of 12or more follicles between 2-9 mm in diameter. Follicles are fluid-filled sacs which can grow on one or both of a woman's ovaries. The presence of polycystic ovaries is typically identified with a transvaginal ultrasound. This produces better results than the transabdominal route. However, for virgin, adolescent girls, the transabdominal route is a suitable method for diagnosing the presence of polycystic ovaries [30].

National Institute of Health Criteria

From the time it was identified in 1935, until1990, there was no formal tool for diagnosing polycystic ovary syndrome. In 1990, a conference was held by the National Institute of Health (NIH) to finally establish minimal criteria. A questionnaire was sent out to 58 researchers who voted on the criteria.

Researchers agreed that PCOS would be defined as menstrual irregularity-oligomenorrhoea or anovulation, clinical or biochemical signs of hyperandrogenism, and the exclusion of other causes of these two criteria such as androgen- secreting tumours or Cushing syndrome [33]. This remained the main the diagnostic tool for PCOS until the Rotterdam conference was held in2003. (See Table1)

Rotterdam Criteria

In 2003, a conference was held by the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryologyin Rotterdam, Netherlands. The purpose of this conference was to revisit the criteria for diagnosing PCOS. Experts in Europe believed that polycystic ovarian morphology detected by ultrasound should be considered as a diagnostic criterion. The decision made in Rotterdam for the diagnosis of PCOS was that women must meet two of the following three criteria: chronic oligo-ovulation or anovulation, clinical or biochemical androgen excess with the exclusion of other etiologies, and the presence polycystic ovaries based on an ultrasound. The Rotterdam definition provided a broader set of criteria than the NIH definition, allowing for increased diagnosis. Under the Rotterdam criteria, women without androgen excess or without menstrual irregularities could still be diagnosed with PCOS [34].These criteria remained the main diagnostic tool until 2006. (See Table 1)

Adolescent girls and the better development of other diseases associated with PCOS which will be discussed later.

Prevalence and Incidence

Prevalence

Based on the current criteria, the prevalence of PCOS is between 5-10% in reproductive aged women [35]. It is difficult to determine the exact prevalence of PCOS for a variety of reasons. One issue is that health care providers do not always use the same criteria to identify PCOS. As mentioned

above, there are the National Institute of Health, Rotterdam, and Androgen Excess Society criteria which are all in use.

A retrospective birth cohort study was done on 728 women born between the years of 1973-1975 to analyze the difference between these three criteria. The 728 women were interviewed when they were between the ages of 27-34. Based on the NIH criteria, 8.7+/-2% were diagnosed with PCOS. Based on the Rotterdam criteria, the prevalence was 11.9+/-2.4%. Lastly, under the AES recommendations, PCOS prevalence came in at10.2+/-2.4%. Although the same women were analyzed for all three criteria, results varied.

A second problem with determining the prevalence of PCOS is that it remains a highly undiagnosed disease. In the study of the 728 women born in the US, 68-69% of the women suffering from PCOS had not been previously diagnosed [36]. Also, adolescents are rarely screened for the disease in studies since parental consent must be obtained. Based on an Iranian study, the current estimate for adolescents with PCOS is around 3%. This was a random cross-sectional study done in Iranian high schools [37]. One reason this value is much lower than among the adult population is that a specific set of criteria has not been identified for adolescents in diagnosing PCOS.

Pathogenesis

The cause of PCOS is currently unknown but the etiology appears to be heterogeneous. Links have been made between heritable and environmental factors.These factors include genetics, insulin resistance, obesity, and birth weightas related to the adipose tissue expandability hypothesis.

Genetics

Recent studies provide support for a genetic component of PCOS with evidence of the disorder occurring among women of the same family. A cross-sectional study was done evaluating 29 families with a history of polycystic ovary syndrome against10 control families. There was found to be nearly a 50% prevalence of PCOS among siblings [38]. In a similar study, the first degree relatives of 14 women with PCOS were screened. Female relatives were

screened for the presence of polycystic ovaries and male relatives were screened for male pattern baldness. Again, the first degree relatives of women with PCO Shad a 51% chance of being affected. The results of these two studies indicate a single gene effect or autosomal dominant inheritance [25].

Further research needs to be done to further evaluate the genetic component of PCOS. Although it appears to be of autosomal dominant inheritance, there is not enough evidence at this time to prove this theory. With continuing research on the human genome, it may be possible to someday identify the gene responsible for the development of polycystic ovary syndrome.

Insulin Resistance

Insulin resistance (IR) is a defining characteristic of polycystic ovary syndrome, occurring. In 50-70% of the PCOS population [40]. Insulin resistance is an impaired metabolic response which occurs when cells cease to respond to ordinary levels of insulin [41] .IR occurs in both lean and obese women with PCOS. In contrast, women without PCOS, insulin resistance occurs primarily in the obese. This suggests that IR is an intrinsic part of the disease. Some believe that insulin resistance may be present in all women with PCOS. However, there is a lack of consistency in measuring for IR and so some women remain undiagnosed [29].

Research suggests that levels of insulin resistance do not remain stagnant for women with PCOS. In a recent observational study, 1,212 women with PCOS were monitored for differences in hormonal, metabolic, and ultra sonographic features of PCOS between age groups. The age groups were broken down to less than 20years old, 21-30years old, and 31-39years old. The degree of insulin resistance worsened as age increased. (Panidis) This is important to keep in mind given the relationship between insulin resistance and the development of other diseases. If the presence of polycystic ovary syndrome and insulin resistance can be identified early on, perhaps the level of insulin resistance can be maintained at lower levels before it is able to worsen the symptoms of PCOS.

Obesity

Obesity is another component of PCOS which may contribute to the pathogenesis of the disorder. In patients suffering from PCOS, the incidence of obesity is somewhere between 50-75%, which is higher than in the general population [41]. Not only is obesity more common among women with PCOS, research suggests that obesity may exacerbate many of the manifestations of PCOS including androgen levels and insulin resistance.

With excess weight gain, women who were previously asymptomatic may begin to show symptoms of PCOS. There is an increased prevalence of symptoms among obese PCOS patients when compared to non-obese controls. Obese women suffering from PCOS generally have higher serum androgen concentrations and a reduced response to fertility treatments when compared to lean women with PCOS. Obese women with PCOS experience greater menstrual irregularity when compared to non-obese patients [42]. There is also an increased presence of hirsutism at 73% compared to 56% for non-obese women [43]. The same can be said for the presence of acanthosis nigricans [27].

Birth Weight and the Adipose Tissue Expandability Hypothesis

In the general population, there is a strong positive correlation between obesity and insulin resistance. In the PCOS population, this remains true. However, the degree of insulin resistance among normal-weight women is increased. These women are identified as metabolically obese, normal-weight (MONW). A study done comparing MONW and metabolically normal controls in body fat composition found an increase in total, visceral, and subcutaneous fat in the MONW group [44].

The relationship between MONW and PCOS may be explained by birth weight and the adipose tissue expandability hypothesis. According to this theory, each individual l can safely store a certain amount of excess adipose tissue (AT) before adverse metabolic effects are observed. When this level is reached, called the metabolic set point, lipotoxicity occurs. Lipotoxicity causes an increase in free fatty acids, hyper triglyceridemia and lipid deposits in non-

subcutaneous AT and in non-adipose organs. This can lead to insulin resistance**.** It is believed that women with PCOS have a lower metabolic set point than the general population. This explains why normal weight women with PCOS are insulin resistant [45].

Birth weight is a key factor in determining an individual's metabolic set point. The number of adipocytes of an adult is set early in life. When prenatal growth is stunted, the development of AT is reduced and fewer adipocytes develop. Most infants suffering from stunted pre-natal growth will experience spontaneous catch-up growth. However, this is observed as an increase in adipocyte size rather than an increase in the number of adipocytes. With fewer adipocytes, the body has a decreased ability to safely gain fat before adverse metabolic affects are observed. When AT is properly expanded in fetal and early infant life, there seems to be increased protection against obesity related insulin resistance [46].

Prenatal exposure to androgen excess may be one cause of stunted pre-natal growth, resulting in low-birth weight. If a woman has PCOS, she would have increased androgen levels which could potentially affect her developing fetus. However, data is inconclusive on this subject. Most of the research has only been done on animals and no effect has been observed in human studies.

In one study, when exposed to testosterone, neither maternal androgen a main or fetal androgenamia was seen to be elevated in girls who developed PCOS. Also, the fetus is protected from exposure to maternal androgens by the placenta [46]. This means that increased maternal androgen levels should not impact the fetus. Based on this research, it seems unlikely that maternal androgen levels have much impact on birth weight. Other factors are likely responsible.

Health Consequences

Women with polycystic ovary syndrome are at increased risk for a variety of health conditions. These conditions include the metabolic syndrome,

cardiovascular disease (CVD) and type II diabetes mellitus (T2DM). Insulin resistance is a key feature in the development of each of these diseases.

Metabolic Syndrome

Women with PCOS are at increased risk for developing the metabolic syndrome when compared to the general population. The metabolic syndrome is a clustering of symptoms associated with insulin resistance. Diagnosis is based on the presence of three of the following five criteria: elevated waist circumference (greater than 40 inches for men and 35 inches for women), elevated triglycerides (greater than ore qual to 150 mg/dl), reduced HDL cholesterol (less than 40 mg/dLin men and 50 mg/dLin women), elevated blood pressure (greater than or equal to 130/85 mm Hg), or elevated fasting glucose (greater than or equal to 10 mg/dl) [29].

Cardiovascular Disease

A cross-sectional study was done to help establish the link between PCOS and CVD. Sixty-two women with PCOS were analyzed in comparison to forty-eight healthy controls. BMI, waist circumference, and blood pressure were taken as well as plasma concentrations of glucose, triglycerides, total cholesterol, and HDL. Women with PCOS showed a significantly higher TG/HDL ratio when compared to the control group. This indicates that women with PCOS are at increased risk for cardiovascular disease. The variable which had the most influence on Tg/HDL ratio was waist circumference. This indicates that abdominal obesity remains an important feature in the development of CVD, in PCOS women and the general population alike [47].

Although PCOS is one of the most common endocrine disorders in women of reproductive age, there is currently no cure for polycystic ovary syndrome. For this reason, early diagnosis of the disease based on established criteria is important. With an early diagnosis, it is possible to manage the manifestations of PCOS. With proper management, obesity and insulin resistance can be controlled for as well as the associated diseases. The current forms of treatment for PCOS will be discussed in the next chapter. The main

focus is on drug therapy and lifestyle modifications. With the proper management of insulin resistance and obesity, it is possible to dramatically decrease the negative health outcomes associated with polycystic ovary syndrome.

A Comparison of Drug Therapies and Lifestyle Modifications Used to Treat Polycystic Ovary Syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory infertility in women of reproductive age [28]. Affecting as many as 1 in 10 women in the United States, [37] this common endocrine disorder remains largely undiagnosed. Typically, it is not until a woman has difficult becoming pregnant that her health care provider will begin to notice the clustering of symptoms associated with PCOS including clinical and biochemical signs of hyperandrogenism, menstrual irregularities, and the presence of polycystic ovaries.

The majority of women with PCOS suffer from insulin resistance. Insulin resistance and the resultant hyperinsulinaemia play a key role in the pathogenesis of PCOS. Although insulin resistance is exacerbated by weight gain, normal-weight women with PCOS are also insulin resistant.

The pathogenesis of PCOS is not completely understood; there is not yet a cure for the disease. However, with the proper management of PCOS, it is possible to restore fertility, improve menstrual regularity and reduce androgen excess. This is achieved primarily through an improvement in insulin sensitivity.

Drug Therapy

Drug therapy is an effective form of treatment for polycystic ovary syndrome. Drug therapy can improve insulin resistance and hormonal metabolic profiles, restore menstrual regularity, and decrease the clinical symptoms associated with PCOS. Common forms of drug therapy include

combined oral contraceptives, insulin-sensitizing agents, and anti-androgens. However, there are adverse side-effects associated with each of these drugs. It is important to understand the possible risks connected with each.

Combined Oral Contraceptives

Hormonal oral contraceptives are the most common form of drug therapy used in the treatment of PCOS. Oral contraceptives allow for a restoration in menstrual cycles, although ovulation does not occur. Regular menstrual cycles are associated with a decreased risk of developing endometrial cancer [48]. Oral contraceptives are also used to treat clinical symptoms of PCOS including hirsutism and acne. They work by increasing levels of sex-hormone binding globulin (SHBG) [49]. Increased levels of SHBG decrease circulating androgen levels and decrease their bioavailability [50].With an improvement in androgen levels, there is a subsequent improvement in the clinical symptoms associated with PCOS.

Based on recent studies, the use of combined oral contraceptives is an effective form of drug therapy for the treatment of PCOS. The end result is an improvement in many of the clinical and biochemical features of PCOS. Although their use is common among all women of child-bearing age, little research has been to evaluate the relationship between the use of oral contraceptives and the development of disease outcomes associated with PCOS. Some studies indicate that the use of oral contraceptives may decrease insulin sensitivity and glucose tolerance. This would increase the risk for T2DM which women with PCOS are already predisposed to develop [28].

Insulin Sensitizing Agents

Insulin Sensitizing agents are another form of drug therapy used in the treatment of PCOS. Common forms of insulin sensitizing drugs include metformin and rosiglitazone. These drugs are intended to be used in combination with diet and exercise. They are commonly prescribed to people with type II diabetes mellitus. These drugs work primarily by inhibiting hepatic

glucose production and increasing insulin sensitivity in the peripheral tissues [24].

However, fasting insulin levels only showed an improvement following the use of metformin [25]. In another 6 month trial, similar results were observed with both drugs including a decrease in fasting insulin, postprandial insulin, HOMA-IR, LH, triglyceride, LDL, and testosterone levels. A decrease in BMI was only observed with the metformin group [51].

To test its efficacy, atrial was done in India, investigating the impact of metformin when administered daily for three months. In this prospective study, there was an observed decrease in serum insulin and serum testosterone with a positive correlation between the two. This positive correlation reveals an etiological connection between insulin resistance and some of the clinical features of PCOS related to increased testosterone levels. With metformin therapy, the study subjects also experienced a fall in fasting plasma glucose to insulin ratio; a marker of insulin resistance [52].

Anti-androgens

Anti-androgens are used to improve the biochemical profiles of PCOS women. This leads to an improvement in clinical symptoms such as hirsutism and acne. The improvements seen from anti-androgens are similar to those experienced with oral contraceptive use, but the improvements are greater. Anti-androgens work by attaching to androgen-binding receptors through competitive inhibition. Anti-androgens can also decrease androgen production. Examples of anti-androgens prescribed include spironolactone, flutamide, and finasteride [24].

Once again, there are risks associated with taking anti-androgens which should be carefully considered. They can cause fatigue, dizziness, headaches, nausea, breast tenderness, weight-gain, loss of libido, hypotension and hepatoxicity. Liverand renal function should be correlated and reveals an etiological connection between insulin resistance and some of the clinical features of PCOS related to increased testosterone levels. With metformin

therapy, the study subjects also experienced a fall in fasting plasma glucose to insulin ratio; a marker of insulin resistance [53].

The benefits of metformin treatment are obvious. However, similar to other forms of drug therapy, the risks of metformin should be carefully considered. Common side-effects include nausea and diarrhoea. On rare occasion, lactic acidosis has occurred. This typically occurs in conjunction with renal or cardiovascular disease. It is unknown if metformin is safe to use during pregnancy. Despite this, medical practitioners continue to prescribe it to help prevent the development of gestational diabetes [24]. Before starting on metformin therapy, women with PCOS should carefully consider the associated risks, especially if they are pregnant or planning to become pregnant. (See Table 3)

Lifestyle Modifications

Unlike drug therapy, there are no adverse side-effects associated with lifestyle modifications in the management of PCOS [49]. Lifestyle modifications should be the first line of treatment prescribed to women with PCOS since is no evidence to suggest that drug therapy is any more effective. Lifestyle modifications include weight loss, dietary treatments, and exercise.

Caloric Restriction to Achieve Weight Loss

Mild to moderate weight loss dramatically reduces the symptoms of PCOS. Weight loss of only 2-7% of initial bodyweight leads to improved ovulation and a reduction in androgen levels [43]. An important feature of weight loss is that it leads to an improvement in insulin sensitivity. This leads to a restoration in menstrual cycles, ovulation, and fertility and an improvement in androgen levels [54].

The main goal of weight loss should be to reduce visceral adiposity which is associated with insulin resistance and the metabolic syndrome. In one study, 13 obese-women with PCOS were placed on a hypocaloric diet plan. Their mean weight loss was 12.4 kg with a reduction in truncal-abdominal skin-folds of 28% indicating that much of their weight loss was around the

abdomen. This resulted in an improvement in insulin sensitivity of 132% and an increase in SHBG of 35% [31].

Diet- Macronutrient Modifications

One proposed diet is the consumption of carbohydrates low on the glycemic index. Most women with PCOS experience compensatory hyperinsulinemia following carbohydrate ingestion. Consuming foods low on the glycemic index (GI) would help reduce this. In a randomized, prospective study, ninety-six women with PCOS were assigned to follow a reduced-energy, low-fat, low-saturated fat diet which was moderate-to-high in fibre. These women were then randomly assigned to consume foods which were either lower moderate-to-high on the glycemic index. They followed this diet until they had achieved 7% of weight loss or for 12 months.

To investigate which is more important, weight loss or carbohydrate intake, a randomized, 12-week study was done to evaluate the difference between a hypo caloric diet and a low CHO diet. Improvements were observed for both groups in BMI, waist circumference, and menstrual function. However, there was no statistical significance between groups. This indicates that weight loss and carbohydrate intake is two separate but important components in the treatment of PCOS [55].

The effect of a high-protein diet can also be observed independent or carbohydrate consumption. In a 6-month prospective trial, subjects consumed either a high protein or standard protein diet. Women on the high protein diet experienced greater weight loss (4.4 kg), body fat loss (4.3 kg), and a greater reduction in waist circumference. When adjusted for weight loss, women on the high protein diet had greater improvements in glucose metabolism [30].

Diet- Micronutrient Modification

The focus of most dietary treatments for PCOS focuses on macronutrient content. However, micronutrient content may also play an important role. Vitamin D is one of the micronutrients believed to impact PCOS. Through previous animal and human studies, it is known that vitamin D

deficiency is associated with impaired insulin secretion and glucose clearance [38].

Vitamin D supplementation may also help improve insulin resistance. Across-sectional study was done to evaluate vitamin D levels in over 500women with PCOS. Anthropometric, metabolic, and endocrine measures were taken for each of these women as well as an oral glucose tolerance test. A negative correlation was found between the degree of insulin resistance and 25(OH)D levels. A positive correlation was found between 25(OH)D levels and insulin sensitivity [47]. In contrast, in a randomized, placebo-controlled, double-blind trial of 50 women with PCOS, there was no statistical significance between groups in fasting serum insulin and insulin resistance when prescribed either a vitamin D supplement or a placebo [35]. Further studies need to be done before a conclusion can be made about the relationship between vitamin D deficiency and insulin resistance in women with PCOS.

Calcium and vitamin D are not the only micronutrients under investigation for their relationship with PCOS. In previous studies, Vitamin B12 and folate supplementation have improved insulin resistance in women with metabolic syndrome. A cross-sectional study was done to look for a similar relationship with PCOS women. Vitamin B12 levels were found to be significantly lower in obese women with PCOS compared to controls. Women with PCOS who were insulin resistant also had lower B12 levels when compared to PCOS controls who were not insulin resistant [57]. A prospective study with vitamin B12 supplementation should be done to further investigate the relationship between insulin resistance and vitamin B12. Similar to Vitamin D, no conclusions can currently be drawn about the relationship between vitamin B12 and insulin resistance. However, after speaking with a health care provider, it may be acceptable to take a Vitamin D and B12 supplement as a precautionary measure.(See Table VII)

Exercise

A final component of lifestyle modifications in the treatment of PCOS is the inclusion of exercise. Although weight loss can be achieved through diet

alone, this will result primarily in muscle loss rather than fat loss as desired. Also, weight loss through exercise is better sustained better than weight loss through diet alone [24]. With exercise, the goal should be to lose abdominal fats in cevisceral fat is closely related to insulin resistance and the development of metabolic syndrome. A similar randomized, prospective trial was done with overweight/obese women with PCOS. These women were assigned to a dietary plan, a workout plan, or both. Menstrual regularity improved in 69% of the women with are turn in ovulation for 34%. There was no statistical difference between groups. Resumption in ovulation most often occurred among women who experienced higher serum levels of insulin-like growth factor-binding protein 1 following intervention. This supports a link between insulin sensitivity and reproductive function [43]. In a similar trial, participants were assigned to diet alone, diet and aerobic exercise, or diet and aerobic-resistance exercise. Again, little variation was observed between groups with the exception of fat-free mass which decreased the most with aerobic exercise and the least with aerobic-resistance exercise [58]. This indicates that the type of exercise may also be an important factor.

Combination Therapy

Although lifestyle modifications and drug therapy are effective alone, combination therapies can also be effective in the treatment of PCOS. Metformin is one form of treatment which is often used in combination with other therapies. In a 6-month trial, girls were prescribed to a high-CHO and low-PRO diet alone and in combination with metformin. While on metformin, 10 of the 11girls resumed menstruation, 9 girls lost weight, and total plasma cholesterol and testosterone levels fell for all [59].

Metformin can also be used in conjunction with other drug therapies such as anti- androgenic drugs. In a prospective, placebo-controlled trial, PCOS women were prescribed to a hypocaloric diet and either metformin, flutamide, both metformin and flutamide, or a placebo for 12 months. The effects of flutamide and metformin were observed independently of each other suggesting that it is possible to benefit from each without adverse reactions

between the two drugs. Flutamide intake led to a decrease in visceral subcutaneous fat and a lower hirsutism score. Metformin led to an increase in menstruation, insulin sensitivity, and LDL levels. When taken together, subjects experienced all of these benefits [60].

Further research needs to be done to further evaluate the effectiveness of various combinations of therapy in treating PCOS. The major challenge with combination therapy is that as more components are measured, it becomes difficult to control for confounding variables. However, with continued research, it is possible to analyze the effectiveness of different forms of combination therapy.(SeeTableIX)

Although there are a large number of studies investigating different treatments for polycystic ovary syndrome, there remains plenty of room for continued research. This is especially true in the area of combined therapies. At this time, studies suggests that through lifestyle modifications alone, women with PCOS can improve menstrual regularity, androgen and lipid profiles, and the clinical signs of PCOS associated with androgen excess. If lifestyle modifications can improve upon all of these areas, there is no need to include drug therapy in the treatment of PCOS. Until there is evidence to suggest that drug therapy or combination therapy are significantly more effective than lifestyle modifications alone, it is safer to avoid them. At this time, therapy should focus on improving upon insulin sensitivity through weight loss, diet, and exercise.

3.3. Pharmacological activity methods

Letrozole method

A control group received 2% of CMC (carboxymethylcellulose). 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.

Estradial 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 oestrous 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 estrus stage. All the animals were weighed daily after drug administration for 10 days.

OGTT

Women with polycystic ovarian syndrome (PCOS) are at increased risk for developing glucose intolerance leading to type 2 diabetes mellitus (DM). Hence, it was necessary to evaluate the efficacy of *Ashuwathi chooranam* on glucose homeostasis. Thereby, Oral glucose tolerance test (OGTT) was performed in all groups of animals. PCOS rats exhibited high glucose tolerance compared to normal control rats (**$p<0.01$, ***$p<0.001$) at all the time points of OGTT profile.

Ashuwathi chooranam treated PCOS rats in different doses (100mg, 200mg) demonstrated significantly reduced glucose intolerance. Glucose homeostasis is governed by insulin action. Thereby, we measured serum insulin level in all group of animals wherein serum insulin levels of untreated PCOS rats were increased significantly (***$p<0.001$). Treatment of AC caused a decrease in insulin level.

ANTIOXIDANT ACTIVITY

Ashuwathi chooranam contains substantial amounts of antioxidants including phytosterols, saponins, flavonoids, tannins and it has been suggested that antioxidant action may be an important property of plant medicines used in treatment of various diseases. *Ashuwathi chooranam* contain good scavenge property wherein it able to scavenge the free radicals, DPPH. Administration of the ethanolic extract of *Ashuwathi chooranam* on tissue antioxidants have been attributed to reduction in blood glucose level in diabetic rats, which helps to prevents excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes.

3.4. PHARMACEUTICAL REVIEW

CHOORANAM

Definition

Chooranam is a fine powder s of drugs. The "Chooranam" may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity.

Method of preparation

Equipment required

1. The drug enumerated in the recipe in clean and well dried state.
2. A mortar and pestle.
3. A fine sieve or fine cloth of close mesh.

Process of preparation

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

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

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

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

Purification of the prepared chooranam

"தானென்ற சூரணத்தின் சுத்திக்கேளு
தப்பாதேசரக் கெல்லாஞ் சூரணித்து
நானென்ற வாவின் பாலாற்பிசைந்து
நலமான சட்டியிலே பாலைவிட்டு
வானென்ற சுத்த சலம்பாதிவிட்டு
வளமாக மேற்சீலை கோடுகட்டிப்
பானென்ற சூரணத்தைப் பிட்டுபோல்வைது
பதறாதே வெந்தெடுக்ச் சித்தியமே"!

- **அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்**

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

ஆமப்பா ரவியுலர்த்திப் பொடிதான் செய்து
அப்பனே சமனாய்ச் சர்க்கரையைச் சேர்த்து
நாமப்பா கொண்டு வரதோஷம் போச்சு
நன்றாக்ச் சுத்தி செய்யாச் சூரணந்தான்
தாமப்பா ரோகத்தை வெல்லா தப்பா
தளமான வியதியெல்லாம் பாரிக்கும் பார்
வேமப்பாசுத்திசெய்துகொண்டாயனால்

வெகு சுறுக்காய் தீருமாவியாதி கேளு

- அகத்தியர் வைத்திய இரத்தினச் சுருக்கம்

Then the *chooranam* is placed in the sunlight and powdered. Equal amount of sugar is added and taken internally. All type of diseases gets cured.

If the drug is taken without purification the disease does not cure. If taken after purification the disease cures easily.

Storage

The prepared *chooranam* should be allowed to cool by spreading and mixing, prior to packing.

They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cardboard boxes.

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

In industry the tablets are made, counted & packed by electronic devices.

Then *chooranam* is said to retain its potency for 2 months and then gradually deteriorate. However if properly packed & stored they keep good for a year.

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

Based on the AYUSH guidelines, the analytical specifications of Ashuwathi Chooranam were performed and standardized as follows.

Table No.3. ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM

S.NO	Test

1.	Description
	Macroscopic, Microscopic
2.	Loss on drying at 1050 C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications, TLC/HPTLC-with marker (wherever possible)
9.	Test for heavy/Toxic metals
	Lead
	Cadmium
	Mercury
	Arsenic
10.	Microbial contamination
	Total bacterial count
	Total fungal count
	Test for specific Pathogen
11.	E. coli
	Salmonella spp.
	S.aureus
	Pseudomonas aeruginosa
	Pesticide residue
12.	Organochlorine pesticides
	Organophosphorus pesticides
	Pyrethroids
13	Test for Aflatoxins (B1,B2,G1,G2)

3.5. Lateral research

Withania somnifera

Investigation the effect of an aqueous extract of *Withania somnifera* on insulin sensitivity in non-insulin dependent diabetes mellitus (NIDDM) rats. NIDDM was induced by single intraperitoneal injection streptozoin (100mg/kg) to 2 days old rat pups. *Withania somnifera* (200 and 400 mg/kg) was administered orally once a day for 5 weeks after the animals were confirmed diabetic (i.e. 75 days after streptozocin injection). A group of citrate control rats (group I) were also maintained that has received citrate buffer on the second day of their birth. A significant increase in blood glucose, glycosylated haemoglobin (HbA1c) and serum insulin levels were observed in the NIDDM control rats. Treatment with *Withania somnifera* reduced the elevated levels of blood glucose, HbA1c and insulin in the NIDDM rats. An oral glucose tolerance test was also performed in the same group, in which it was found a significant improvement in glucose tolerance in the rats treated with *Withania somnifera* [61].

Glycyrrhiza glabra

Phytoestrogens are plant constituents that possess either estrogenic activity. Although their activities are weak as compared with human endogenous estrogens, the consumption of phytoestrogens may have clinically significant consequences. A number of botanicals, or the compounds contained therein, have been identified as putative estrogenic agents, but consensus in the biochemical community has been hampered by conflicting data from various in vitro and in vivo models of estrogenic activity. Here they attempt to sort out discrepancies between various experimental models and establish whether certain herbs possess estrogenic activity [62].

Myristica fragrans

In different parts of the World, medicinal plants have demonstrated a lot of health benefits to mankind and remain an important source for the discovery of new bio-active compounds. The relative ovary weight is significantly

reduced when female rats exposed to nutmeg. The serum LH level significantly following the exposure to nutmeg and black seed [63].

4. MATERIALS AND METHODS

4.1. PREPARATION O F THE TRIAL DRUG:

ASHUWATHI CHOORANAM

Selection of the drug

The herbal formulation *Ashuwathi chooranam* was taken The preparation drug *Ashuwathi chooranam* was taken as a drug for *Soothaga vayu*, has been selected from the Siddha *literature "**AGATHIYAR VAITHYA KAAVIYAM 1500**"* first part published by Tamarai noolagam, Edition 2001, p.no 293.

Ingredients of Ashuwathi chooranam:

Amukkura	-	*Withania somnifera*
Chukku	-	*Zingiber officinale (dried ginger)*
Milagu	-	*Piper nigram*
Thippili	-	*Piper longum*
Jaadhikaai	-	*Myristica fragrans*
Jaadhipathiri	-	*Myristica fragrans*
Adhimadhuram	-	*Glycirriza glabra*
Krambu	-	*Syzygium aromaticum*
Kadugurohini	-	*Picrorhiza scrophulariiflora*
Krosaniomam	-	*Hyoscymus niger*

Collection of the drug:

The ingredients of the drug were collected from around Chennai district in Tamilnadu.

Identification and Authendification of the Drug:

The test drug was identified and authenticated by Gunapadam Experts, P.G Gunapadam branch, GSMC, Arumbakkam, Chennai-106. Sample of the ingredients kept in PG Gunapadam department for future reference. GSMCC/PGGM/0004-0013/14-17.

All the raw materials here were purified individually as per the Siddha literature

Preparation of the Trial Drug *Ashuwathi chooranam:*

Purification of the Chooranam:

The drugs that are purified by removing the sand, dust particles, and roasted are *Withania somnifera, Piper nigram, Myristica fragrans, Picrorhiza scrophulariiflora, Hyoscymus niger;Zingiber officinale (dried ginger)*-the outer skin were removed, the other drug was roasted slightly *Piper longum, Myristica fragrans, Glycirriza glabra, Syzygium aromaticum* -the flower buds were removed and fried slightly [64].

PREPARATION OF THE DRUG:

Ingredients:

Amukkura(Withania somnifera)	-35gms
Chukku(Zingiber officinale(dried ginger)	-35gms
Milagu(Piper nigrum)	-35gms
Thippili(Piper longum)	-35gms
Jaadhikaai(Myristica fragrans)	-35gms
Jaadhipathiri(Myristica fragrans)	-35gms
Adhimadhuram(Glycirriza glabra)	-35gms
Krambu(Syzygium aromaticum)	-35gms
Kadugurohini(Picrorhiza scrophulariiflora)	-35gms
Krosaniomam(Hyoscymus niger)	-15.3gms
Sugar (Saccarum officinarum)	-127.5gms

Procedure:

Take the equal quantities of all drugs *winter cherry, chukka, milagu tippili, jadhikai, jadhipathiri, adhimadhuram, krambu, kadugurogini* and *krosaniomam* were and roasted and grounded into a fine powder. The powder was sieved through a clean white cloth to get a uniform particle size of *Chooranam*.

The *Ashuwathi chooranam* was purified by pittaviyal method (steam cooking in milk) as per Siddha classical literature. For this process cow's milk and water were taken in equal ratio and half-filled in mud pot. A clean dry cloth was tied firmly around the mouth of the pot with a depression. *Chooranam* was placed over the depression on the tied cloth. Another mud pot of similar size was kept over the mouth of the mud pot. The gap between mud and pot 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 [65].

Storage of the drug:

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

Dose: 2 to 3gm, twice daily after food

Adjuvant: Perungayam (Ferula asafoetida) and thippili (Piper longum)

Route of administration: Enteral route

Indications: Soothag vaayu (PCOS)

ANALYTICAL STUDY AS PER SIDDHA LITERATURE

4.2. STANDARDIZATION OF THE DRUG:

Standardization of the this drug comes under the following categories

4.2.1. ORGANOLEPTIC EVALUATION

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

Colour

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

Odour

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

Taste

A sample of about chooranam was tasted and the taste was reported [66].

- **PHYSICO-CHEMICAL ANALYSIS**
- **CHEMICAL ANALYSIS**
- **PHYTO-CHEMICAL ANALYSIS**

4.2.2 PHYSICO-CHEMICAL ANALYSIS

Testing Physical characterization of

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.

Sample: Colour examination:

Ashuwathi chooranam was taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and wrote in results.

Odour examination:

2 gm of *Ashuwathi chooranam* were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of *Ashuwathi chooranam* was noted in results table.

Solubility Test

A pinch of sample (AC) 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 (AC) 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 (AC) 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 (AC) was determined potentiometric ally 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,

$$G = \frac{M2-M1}{(M2-M1)-(M3-M4)}$$

The results obtained from the above procedure were noted

Macroscopic Appearance of Sample:

1 g of *Ashuwathi chooranam* was taken and the colour, texture, grain size and other morphology were viewed by naked eye under sunlight.

SOPHISTICATED INSTRUMENTAL ANALYSIS

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 AC 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 AC 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 AC 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 AC 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 AC 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 AC filtrate Benedict's reagent was added and heated gently and observed for the appearance of orange red precipitate.

Test for Glycosides:

To the AC 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 colour 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 colour.

Test for Saponins

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

Test for Tannins

Gelatin test

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

Test for Phytosterol

Ferric chloride – acetic acid test

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

Test for Diterpenes

Copper acetate test

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

Test for Triterpenes

Salkowiski's test

To 1 ml of AC 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 colour.

Test for Flavonoids

a) Alkaline reagent test

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

b) Lead acetate test

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

d) Shinoda test

With 1ml of the AC 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 colour.

Test for Proteins and Free Amino Acids:

a) Xanthoproteic test

To 1 ml of AC 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 AC 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 AC extract, 2.5 ml of diluted Biuret reagent was added and observed for the appearance of purple colour or brick red precipitate.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of AC extract, 1 ml of 10% sodium hydroxide was added to observe for the appearance of blue or green or red colour [67].

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 colour spots appeared [68].

4.2.4. CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical studies:

Preparation of extract:

5g of sample was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Preliminary Basic and Acid Radical studies

Preparation of extract

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

Table: 4. Test for basic radicals

PROCEDURE	OBSERVATION	INFERENCE
Test for Potassium: A pinch of sample is treated with 2ml of sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour Precipitate	Presence of Potassium
Test for Calcium: Taken2 ml of extract in a clean test tube. Then acetic acid and potassium chromate solution were added	No Yellow precipitate	Presence of Calcium
Test For Magnesium: 2ml of extract was taken in a clean test tube, few drops of Magnason reagentwas added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test For Ammonium: 2mlof extract was taken in a test tube and added few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium
Test For Sodium: 2 pinches of drug was mixed with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): 2ml of extract was taken in a clean dried test tube and conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test For Zinc: 2 ml of the extract was taken in a test tube and Potassium ferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc

PROCEDURE	OBSERVATION	INFERENCE
Test For Aluminium: To the 2m1of the extract was taken in a test tube sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test For Lead: 2 ml of extract was taken in a test tube and added with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: To a small portion of a extract dilute hydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test For Mercury: 2m1 of the extract was taken in a test tube and treated With 2ml of sodium hydroxide solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic: 2m1 of the extract was taken in a test tube and treated with 2ml of sodium hydroxide solution.	Formation of brownish red precipitate	Presence of Arsenic

Results were noted and tabulated in Table No: 9

Table: 5. Test for acidic radical

PROCEDURE	OBSERVATION	INFERENCE
Test for Sulphate: 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.	Formation of white precipitate	Presence of Sulphate
Test for Chloride: The extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White precipitate	Presence of Cholride
Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3.	Formation of Yellow precipitate	Presence of Phoshate
Test for Carbonate: The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate
Test for fluoride &oxalate: 2ml of extract was taken in a test tube and added with 2ml of dil.acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy appearance	Presence of Fluoride & Oxalate
Test For Nitrate: 1gm of the drug was heated with copper turnings and concentrated H2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

The results were tabulated in Table No: 9

The bio-chemical analysis was done to identify the acid and basic radicals present in the AC [69]

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 procedures because of the enumeration of viable cells by this method.

Principle:

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

Dilution:

A small amount of the measured volumes are mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilutions 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 AC 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 has 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 be 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 [70].

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. SOPHASTICATED 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.

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can

be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information.

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

Applications:

Ashuwathi chooranam was a herbal drug, FT-IR study was selected to identify the unknown materials of the test drug and determine the amount of components in the sample [70].

Scanning Electron Microscopy (SEM)

SEM provides detailed high resolution images of the sample by rastering a focussed electron beam across the surface and detecting secondary or backscattered electron signal.

SEM provides images with magnifications up to ~X 50,000 allowing sub-micron-scale features to be seen i.e. well beyond the range of optical microscopes. It gives rapid, high resolution imaging with identification of elements present and

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations[71].

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) too 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, and 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 AC 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 [72].

X-Ray Powder Diffraction Method(X-RD)

Definition

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Applications:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions
- With specialized techniques, XRD can be used to:
- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
    a. determining lattice mismatch between film and substrate and to inferring stress and strain
    b. determining dislocation density and quality of the film by rocking curve measurements

c. measuring super lattices in multilayered epitaxial structures
d. determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
e. Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
- Powder less than ~10 μm(or 200-mesh) in size is preferred place into a sample holder or onto the sample surface [73].

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 [74].

Methodology

Selection of animal species:

The preferred rodent species is rat, although other rodent species may be used. A healthy young adult animal 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

AC 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 AC 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 AC 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 [75].

4.3.2. Repeated dose 28 days oral toxicity study of *Ashuwathi chooranam* on rats – (OECD-407 guidelines)

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that AC was non-toxic and no behavioural 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

AC 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 percentage body weight gain were calculated.

Clinical signs:

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

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

Functional Observations:

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

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 rpm. for 10 minutes. On 28th day of the experiment, 24 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, and haematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparison test using a computer software programme GRAPH PAD INSTAT-3 version [76].

4.4. PHARMACOLOGICAL STUDIES

4.4.1. Ovulation inducing activity in female Wistar albino rat model

Method

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 [77].

The study tried to evaluate the effect of this exercise type on reproductive dysfunction in rats with polycystic ovarian syndrome.

Materials and Methods: Female white albino rats were allocated into three groups:

- Group I: Control rats
- Group II: Letrozole induced polycystic ovarian syndrome rats (PCO) where letrozole was given orally and daily in a dose of 1 mg/kg dissolved in 0.9% NaCl solution for 21 days.
- Group III: Polycystic ovarian syndrome rats subjected to *Ashuwathi chooranam* (PCO+AC) of the lower limbs for three weeks after the induction of polycystic ovarian syndrome. After 6 weeks from the beginning of the study, final body weight, body mass and Lee indices were determined. Plasma levels of LH, free testosterone, estradiol, progesterone, prolactin, fasting glucose and fasting insulin were measured.

4.4.2. OGTT

- Women with polycystic ovarian syndrome (PCOS) are at increased risk for developing glucose intolerance leading to type 2 diabetes mellitus (DM) [97]. Hence, it was necessary to evaluate the efficacy of *Ashuwathi chooranam* on glucose homeostasis. Thereby, Oral glucose tolerance test (OGTT) was performed in all groups of animals. PCOS rats exhibited high glucose tolerance compared to normal control rats (**p<0.01, ***p<0.001) at all the time points of OGTT profile. AC formulation treated PCOS rats in different doses (5 mg, 10 mg) demonstrated significantly reduced glucose intolerance and improved cellular glucose uptake upon increasing time period of dose (30 days) of OGTT profile.

- Glucose homeostasis is governed by insulin action. Thereby, we measured serum insulin level in all group of animals wherein serum insulin levels of untreated PCOS rats were increased significantly (***p<0.001). Treatment of fresh AC caused a decrease in insulin level as compared to PCOS group (p<0.001) [78].

4.4.3. Hormonal assay

Experimental design

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

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 [79].

4.4.4 Antioxidant activity:

Free radical scavenging activity:

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

The radical scavenging activity of AC 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 [80].

Calculation

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

5. RESULTS AND DISCUSSION

The present study reveals that the polyherbal formulation "*Ashuwathi chooranam"* from the classical siddha literature had the potential for curing *"Soothaga vayu"* which in Modern is termed as PCOS. The convenience of the *chooranam* is safe and effective. To validate the trial drug, it is subjected to various scientific analysis such as literary review, physico-chemical analysis and elemental analysis, toxicity studies and pharmacological studies for promoting ovulation activity, evaluation of the hormone level FSH, LH, progesterone, estradiol and antioxidant activity for spontaneously curing Poly Cystic Ovarian Syndrome.

Discussion of review of literature:

In the review of literature the medicinal plants were identified as being used in Siddha System of Medicine from ancient period. The Siddha aspect of the herbs describes the habit, habitat, description of the plant, actions of it, phytoconstituents present in the plant, traditional and medicinal uses, preparations from the herbs such as decoction, *chooranam*, *legium*, oils (both internal and external) that are used for various diseases. Many of the herbs used here causes normal menstrual flow, regularization of menstrual cycle, induces ovulation.

Discussion of pharmacological review:

- The pharmacological aspect of the drug shows the presence availability of various animal models 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 of the pharmaceutical review:

- This review explained the preparation of *Chooranam* in detail including the purification of raw drugs, methods of manufacturing *Chooranam.*
- The ingredients are cleaned, wiped with a clean cloth so as to remove the sand and other impurities.
- The aromatic substances are roasted which may result in certain chemical changes and increase in antioxidants**.** Then the drugs are powdered well purified, as per Siddha literature.
- 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.

Discussion of materials and methods:

- The preparation of the drug was done carefully so as to achieve the highest potency.
- The drugs were ground well and filtered through a clean white cloth to get fine particles of size which in turn increases the bioavailability of the drug.
- The drug along with the Asafotida powder and *thippili* juice enhances the capability 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

Physical characterization

Table No. 6. Organoleptic characters of *Ashuwathi Chooranam*

S.No.	Parameter		Results
1	Colour		Green
2	Odour		Characteristic odour
3	State of Matter		Amorphous
4	Consistency		Fine
5	Taste		Bitter
6	Solubility		
	I	Distilled water	Soluble
	Ii	Benzene	Soluble
	Iii	Choroform	Soluble
	Iv	Carbon tetrachloride	Soluble
	V	Xylene	Soluble
	Vi	Petroleum ether	Soluble
	vii	Propylene glycol	Not Soluble

Interpretation

The organoleptic characters of the drugs, like colour, odour, pH, volatile matter, characteristic odour. The amorphous nature of the drug and its fine texture also an important factor for drug absorption.

Table No. 7. Physicochemical analysis of *Ashuwathi chooranam*

S.No.	Parameter	Mean
1.	Loss on drying at 105ºC	8.81%
2.	Total ash	2.64%
3.	Water soluble ash	1.35%
4.	Acid insoluble ash	0.185
5.	Water soluble extractive	46.37%
6.	Alcohol soluble extractive	46.92%
7.	pH	3.53

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 are present in more in unionized form, which increases its absorption. That is why acidic drugs are better absorbed from stomach.

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 AC is 2.64%, 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 AC is 0.185%. Hence, it represents the superior quality of the AC.

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 AC is 1.35%, 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 AC 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 AC is 8.81.

Being a *Chooranam*, the moisture content is high. So the stability and shelf life of AC is about three months.

PHYTOCHEMICAL ANALYSIS

Table: 8. Results of phytochemical analysis of *Ashuwathi chooranam*

Phytochemicals	Test	Result
1. Alkaloids	Mayer's test	+
2. Carbohydrates	Molisch's test	+
3.Glycosides	Modified Borntrager's test	+
4. Tannins	Gelatin test	+
5. Phenols	Alcoholic Ferric chloride test	+
6. Phytosterols	Ferric chloride acetic acid test	+
7. Flavanoids	Alkaline reagent test	+
8. Proteins and Amino Acids	Xanthoproteic test	+

Interpretation

From the above results the following Phytochemicals are present alkaloids, carbohydrates, glycosides, Phytosterols, proteins and amino acids.

Carbohydrates:

Complex carbohydrates coat on the surfaces of cells and have the potential to carry the information necessary foe cell-cell recognition. Sugar specific receptors are also present on cells, and can interact with sugars on opposing cells which causes adhesion of two cells via carbohydrate and is appears to be important in many intracellular activities including infection by bacteria and viruses, communication among cells of lower eukaryotes, specific binding of sperm to egg; and recirculation of lymphocytes, among others.

Phytosterols:

Phytosterols are the plant streols are structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and their hydrogenated forms, stanols, have attracted much attention because of their benefits in reducing LDL cholesterol levels, with vegetable oil processing being the major source in several food products.

Proteins and aminoacids:

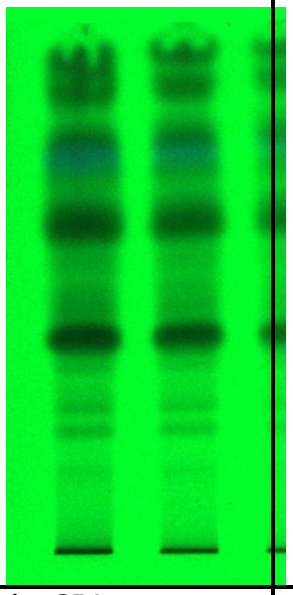
Lifestyle contributors to disease include not only calorie excess but also the dietary intake of specific nutrients. Advanced glycated end-products (AGEs) is a class of nutrients incriminated in the pathogenesis of diet-related diseases. Cooking or processing at high temperatures such as broiling, grilling, frying and roasting is the major source of AGEs. The AGEs promote oxidative stress and insulin resistance in peripheral tissues. PCOS women have increased serum AGEs levels, and these have been positively correlated with serum androgen levels. By activating protein kinase C, AGEs may impair insulin action, thereby perpetuating insulin resistance in PCOS.

HPTLC (High Performance Thin Layer Chromatography)

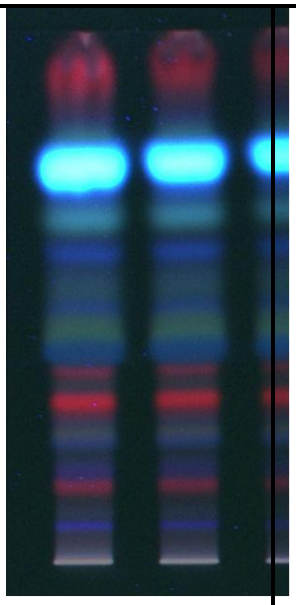
Sample Name: *Ashuwathi Chooranam*

Stationary Phase - Silica Gel 60 F_{254}

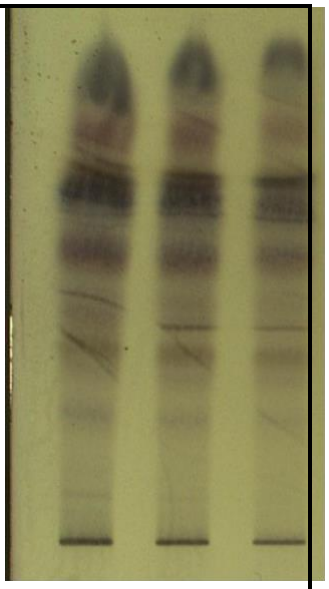
Mobile Phase – Toluene: Ethyl acetate: Acetic acid (5: 1.5: 0.15 v/v/v)



λ = 254 nm



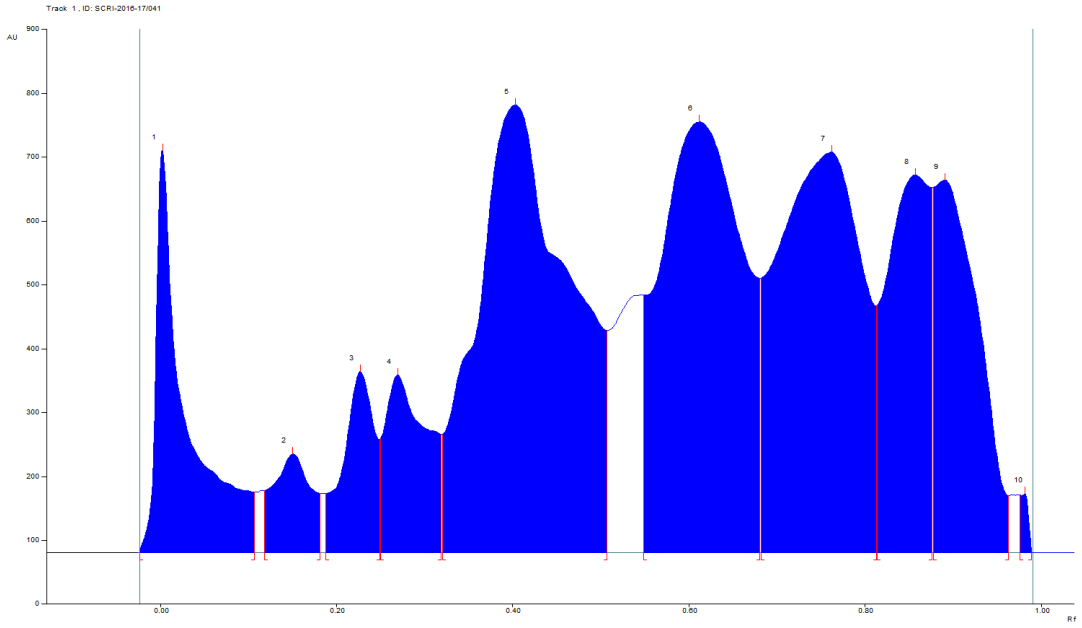
λ = 366 nm



λ = 575 nm (Derivatized)

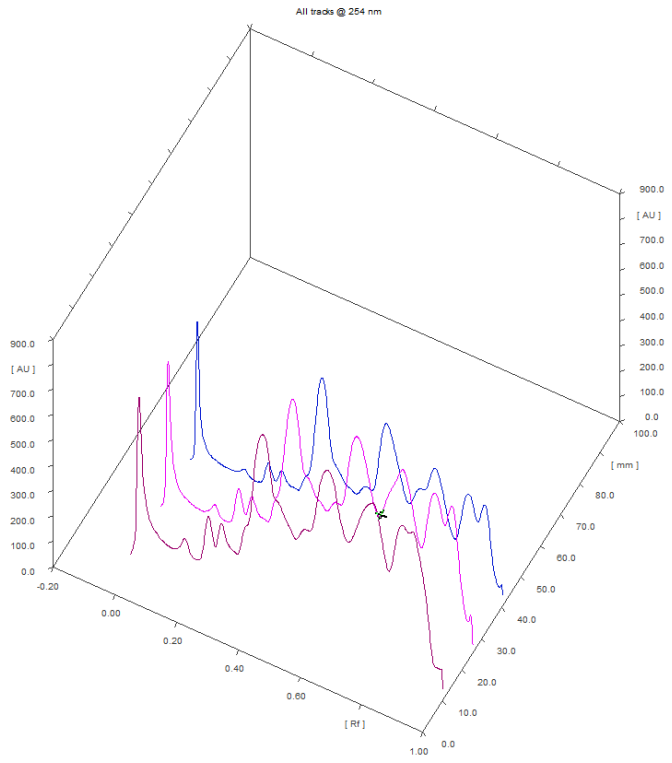
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Color	**R_f value(s)**	**Color**	**R_f value(s)**	**Color**	**R_f value(s)**
Grey	0.14	Blue	0.07	Blue	0.24
Grey	0.22	Red	0.14	Brown	0.36
Grey	0.27	violet	0.18	Brown	0.39
Grey	0.34	Blue	0.22	Yellow	0.51
Bright blue	0.40	yellow	0.25	Brown	0.52
Grey	0.53	Red	0.29	Black	0.65
Black	0.60	Red	0.35	Violet	0.75
Grey	0.70	Blue	0.38	Black	0.89
Blue	0.72	Green	0.44		
Black	0.77	Blue	0.46		
Black	0.85	Blue	0.57		
Black	0.92	Light blue	0.62		
		Bright blue	0.72		
		Red	0.88		

HPTLC Chromatogram @ 254 nm:



Peak Table @ 254 nm:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	4.1 AU	0.00 Rf	629.4 AU	13.63 %	0.11 Rf	95.1 AU	18982.5 AU	6.89 %
2	0.12 Rf	97.2 AU	0.15 Rf	154.6 AU	3.35 %	0.18 Rf	93.0 AU	5971.7 AU	2.17 %
3	0.19 Rf	92.9 AU	0.23 Rf	284.0 AU	6.15 %	0.25 Rf	77.5 AU	8934.8 AU	3.24 %
4	0.25 Rf	179.2 AU	0.27 Rf	278.2 AU	6.03 %	0.32 Rf	85.4 AU	11844.2 AU	4.30 %
5	0.32 Rf	185.6 AU	0.40 Rf	701.2 AU	15.19 %	0.51 Rf	47.7 AU	66460.6 AU	24.13 %
6	0.55 Rf	403.4 AU	0.61 Rf	674.7 AU	14.61 %	0.68 Rf	29.2 AU	56459.6 AU	20.50 %
7	0.68 Rf	429.4 AU	0.76 Rf	627.1 AU	13.58 %	0.81 Rf	86.4 AU	53919.7 AU	19.58 %
8	0.82 Rf	387.6 AU	0.86 Rf	591.4 AU	12.81 %	0.88 Rf	71.6 AU	25830.9 AU	9.38 %
9	0.88 Rf	572.1 AU	0.89 Rf	583.7 AU	12.64 %	0.96 Rf	88.9 AU	26289.8 AU	9.54 %
10	0.98 Rf	90.1 AU	0.98 Rf	92.9 AU	2.01 %	0.99 Rf	-0.0 AU	750.5 AU	0.27 %



3D Chromatogram @ 254 nm:

A qualitative finger printing of *Ashuwathi chooranam* has been performed by HPTLC method, which provides qualitative insights into the bioactive Constituents present in the drug. HPTLC shows separation of components present in the Chloroform extract of *Ashuwathi chooranam.*

The present study revealed that *Ashuwathi chooranam* showed best results in Toluene: Ethyl Acetate: Acetic Acid 5: 1.5: 0.15 solvent system. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and 575 nm and visible light range.

TLC plate showed different colour phyto constituents of chloroform extract of *Ashuwathi chooranam*. The bands revealed presence of bright blue, 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 *Ashuwathi chooranam*. There are eleven polyvalent phytoconstituents and corresponding ascending order of Rf. values start from 0.07 to

0.88 in which highest concentrations of the phytoconstituents was found to be 15.19% and 14.61 % with its corresponding Rf. value were found to be 0.32 and 0.55 respectively.

Chemical analysis of *Ashuwathi chooranam*

Table: 9. Results of basic and acidic radical studies

Parameter	Result
Test for Calcium	+ve
Test for Potassium	+ve
Test For Magnesium	+ve
Test For Sodium	+ve
Test for Phosphate	+ve

Results

The result of Acidic-Basic radical analysis shows that the trial drug has Sulphate, Chloride, Phosphate, Calcium, Sodium and Potassium.

Calcium:

Calcium is necessary for the maturation of oocyte as well as in the resumption and progression of follicular development, menstrual regularity. Polycystic ovarian syndrome (PCOS) is characterized by the hyperandrogenic chronic anovulation, theca cell hyperplasia, and arrested follicular development. The calcium dysregulation contribute to the development of follicular arrest in women with PCOS.

Magnesium:

Many women with PCOS are on the birth control pill, which also depletes magnesium and have sugar and simple carb carvings. To process excessive sugar in our diets requires a great deal of magnesium, and a refined diet that is based mostly

on white flour, meat and dairy (all of which have no magnesium). High glucose levels make the body flush magnesium from its system. So it must be added by eating magnesium rich foods.

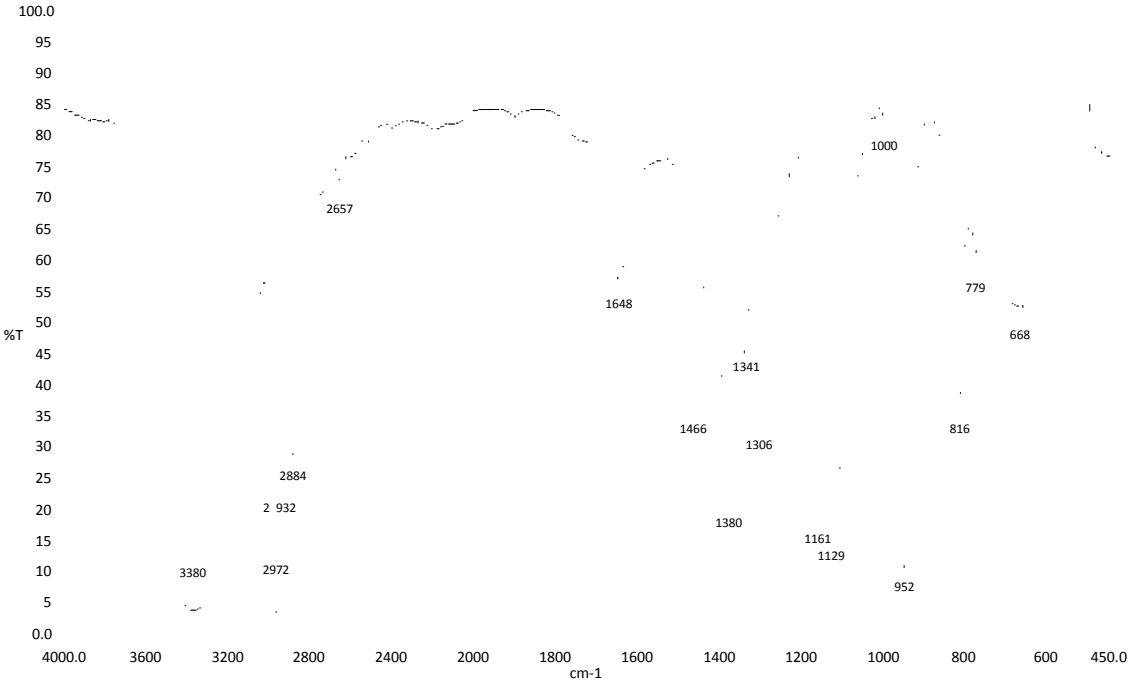
Anti- microbial load

The antibacterial and antifungal activity of *Ashuwathi Chooranam* was tested. The maximum inhibition zone was observed for the *E.coli*, *S. typhi* and *Candida albicans* 7-9mm at the concentration range of 25-100µg/ml.

Instrumental analysis

Fourier Transform InfraRed spectroscopy (FTIR)

Ashuwathi chooranam



FTIR ANALYSIS - *Ashuwathi chooranam*

Test

REF 4000 85 1300 50 700 65

3380 4 2972 3 2932 26 2884 29 2657 74 1648 57.5 1466 39 1380 23

1341 46 1306 37 1161 20 1129 17 1000 83 952 12 816 39 779 63

END: 16 PEAK(S) FOUND

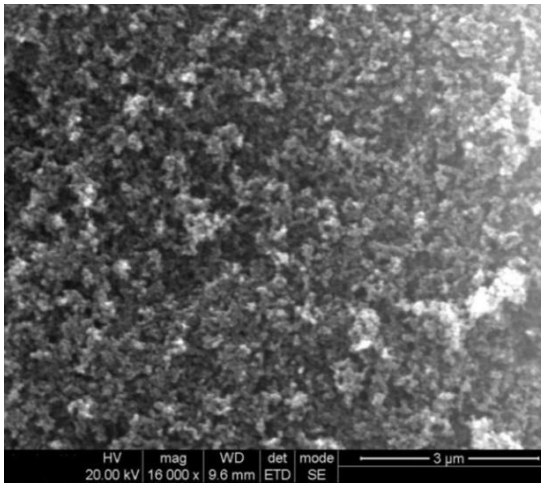
Table No. 10. FTIR interpretation

Absorbtion peak cm-1	Stretch	Functional Group
779	C-Cl Stretch C-H "oop"	Alkyl halides Aromatics
816	C-Cl Stretch C-H "oop"	Alkyl halides Aromatics
952	=C-H bend	Alkenes
1648	N-H bend	1°amine
2657	C=N	Nitriles
3380	N-H stretch	1°, 2°amines, amides, alcohol

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. It helps to understand the formation of complexes with the phytoconstituents during the processing of herbal medicines. The study revealed the presence of functional groups alkyl halides, aromatics, alkenes, 1° amine, nitriles.

SCANNING ELECTRON MICROSCOPE (SEM)



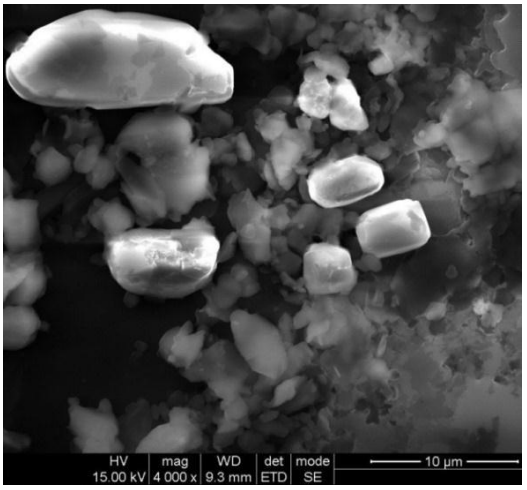


Fig. No. 5 & Fig. No.6: SEM reveals the nano size (100nm-800nm) particle of the sample.

Interpretation:

Nano particles are defined as particulate dispersion or solid particles with a size in the range of 100-800nm in diameter.

They are easily

- Absorbable
- Non-antigenic in nature
- Biodegradable
- Biocompatible
- 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 [81].

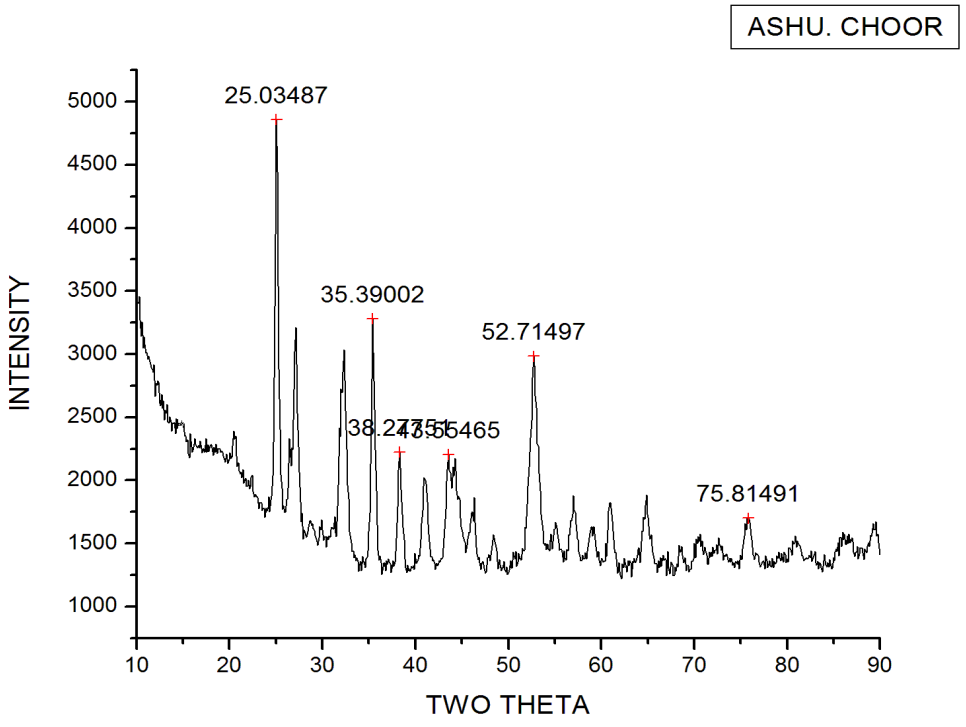
SEM analysis of the test drug *Ashuwathi chooranam* revealed the presence of nano particles of size 100-800 nm. The particles of size nano and near nano size show

that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield information about the topography (surface features of an object) morphology (shape and size of the particles making up the objects) composition (the elements and compounds that the object is composed of the relative amounts of them) and crystallographic information (how the atoms are arranged in the object).

Nanoparticles have valuable properties that can be used to improve drug delivery. Where larger particles would have been unfurnished from the body, cells take up these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, together with the ability to get drugs through cell membranes and into cell cytoplasm. Effectiveness is important because many diseases depend upon processes within the cell and can only be impeded by drugs that make their way into the cell.

X-ray Diffraction Method (XRD)



Interpretation

The structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug.

The major diffraction peaks are identified after XRD analysis of AC concluded that range 48-75nm in association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in AC act as additional supplement and possibly helps in increasing the efficacy of the formulation.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry)

Table No. 11. ICP-OES Interpretation

Ashuwathi Chooranam (wt:0.14715g)	**Element symbol** wavelength (nm)
Al396.152	BDL
As 188.979	BDL
Ca 315.807 mg/L	51.160
Cd 228.802	BDL
Cu 327	BDL
Hg 253.652	BDL
K766.491 mg/L	43.114
Mg285.213 mg/L	01.334
Na 589.592	15.310
Ni231.604	BDL
Pb220.353	BDL
P213.617	224.301
Zn206.200 mg/L	01.218

Interpretation

Potassium

A composition and method for stimulating gonadodtropin hormone production and maintaining intracellular mineral balance in the reproductive organs of mammals. The composition consists of a mineral mixture containing effective amounts of manganese, iron and zinc as amino acid chelates in appropriate ratios. Preferable the composition also contains effective amounts of one or more minerals selected from the group consisting of copper, magnesium and potassium all of which are present at least in part as amino acid chelates or complexes. When magnesium and potassium are present at least some of these are present in inorganic form. Vitamins, other minerals such as calcium and phosphorus, and fillers may also be utilized.

The composition is orally administered to female mammals to both induce estrus and bring about a stronger and more noticeable estrus [82].

Phosphorus, Calcium and Zinc

Calcium (Ca) plays an important role in gonadotropic regulation of ovarian steroidogenesis. Marginal deficiency of phosphorus cause disturbance in the pituitary-ovarian-axis including ovulation. Zinc (Zn) deficiency may reduce GnRH secretion that eventually leads to arrest of ovulation. It also suggests that Ca is involved in the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells, which contributes to the process of ovulation [83].

TOXICITY STUDIES

Preparation for Acute Toxicity Studies

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Ashuwathi Chooranam*.**

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC approved Number: 1248/AC/09/CPCSEA-9/DEC-2013/12

Test Substance : ***Ashuwathi Chooranam***

Animal Source : Kings institute, Chennai.

Animals : Wistar Albino Rats (Female-3+3)

Age : >6 weeks **Body Weight on**

Day 0 :180-300 gm. **Acclimatization** :

Seven days prior to dosing.

Veterinary examination : Prior and at the end of the acclimatization period.

Identification of animals : By cage number, animal number and individual marking by using Picric acid.

Numberofanimals : 3 Female/group,

Routeofadministration : Oral

Diet : Pellet feed supplied by Sai meera foods, Pvt Ltd, Bangalore

Water : Aqua guard portable water in polypropylene bottles.

Housing & Environment : The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature : between 22ºC $\pm$ 3ºC.

Relative humidity : between 30% and 70%,

Air changes : 10 to 15 per hour and

Dark and light cycle : 12:12 hours.

Duration of the study : 14 Days

Administration of Doses:

Ashuwathi Chooranam was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5, 50, 250 and 500 mg/kg body weight was administered stepwise. 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 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. Finally, the number of survivors was noted after 24 hrs and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe

pain or enduring signs of severe distress was humanly killed. When animals are killed for human reasons or found dead, the time of death was recorded.

Acute oral toxicity study of *Ashuwathi Chooranam*

Table 12: Dose finding experiment and its behavioural Signs of acute oral Toxicity

SL	CONTROL	Observation	SL	TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Behaviour:

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhoea, leathery, sleep and coma.

Body Weight:

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality:

Animals were observed for mortality throughout the entire period.

Results:

All data were summarized in tabular form, (Table-1-4) showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test ,description of toxic symptoms,, weight changes, food and water intake.

No of animals in each group: 3

Table No.13. Observational study Results

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

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

(+ Present, - Absent)

Table No. 14. Body weight Observation

DOSE	DAYS		
	1	7	14
CONTROL	186.6± 2.75	189.2± 3.87	194.2 ± 7.62
500 mg/kg	182.5± 4.08	184.2± 2.16	187.4 ± 2.67

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No. 15. Water intake (ml/day) of Wistar albino rats group exposed to (Ashuwathi Chooranam*):*

DOSE	DAYS		
	1	6	14
CONTROL	28.5 ± 2.74	30.0± 9.13	32.4± 3.13
500 mg/kg	30.4±2.33	36.6±1.11	38.9± 2.19

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No. 16. Food intake (gm/day) of Wistar albino rats group exposed to *Ashuwathi Chooranam*

DOSE	DAYS		
	1	7	14
CONTROL	23.56±3.36	28.60±2.42	31.61±5.46
500 mg/kg	22.42±1.64	29.31±1.22	32.22±3.24

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Subacute Oral Toxicity Study Of *Ashuwathi Chooranam*

28-Days Repeated Dose Oral Toxicity Study Of *Ashuwathi Chooranam*

Test Substance **: *Ashuwathi Chooranam***

Animal Source **:** King institute, Chennai.

Animals **:** Wistar Albino Rats (Male -24, and Female-24)

Age **:** >6 weeks **Body Weight**

: 180-300 gm. **Acclimatization** **:** Seven days prior to dose.

Veterinary examination **:** Prior and at the end of the acclimatization period.

Identification of animals : By cage number, animal number and individual marking by using Picric acid

Diet **:** Pellet feed supplied by Saimeera foods Pvt Ltd, Bangalore

Water **:** Aqua guard portable water in polypropylene bottles.

Housing & Environment : The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature : between 22ºC ±3ºC.

Relative humidity : between 30% and 70%,

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.

Duration of the study : **28 Days.**

Groups	No of Rats
Group I Vehicle control	12(6male,6 female)
Group II Ashuwathi Chooranam 50 mg/kg	12 (6male,6 female)
Group III Ashuwathi Chooranam 250 mg/kg	12 (6male,6female)
Group IV Ashuwathi Chooranam 500 mg/kg	12(6male,6female)

Methodology

Randomization, Numbering and Grouping of Animals:

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consists of 12 animals (Male -6, and Female-6). First group treated as a control and other three groups were treated with test drug (low, mid, high) for 28 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose (50 mg/kg), mid dose (250 mg/kg), high dose (500 mg/kg). X is calculated by multiplying the therapeutic dose of human (3000mg/kg) and the body surface area of the rat (0.018). i.e., X dose is 50 mg/kg/animal, 5X mid dose is 250 mg/kg, 10 X high dose is 500 mg/kg.

Preparation and Administration of Dose:

Ashuwathi Chooranam suspended in with water, it was administered to animals at the dose levels of 50, 250 and 500 mg/kg. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

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.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

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.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 29. Necropsy of all animals was carried out.

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 Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Hematological Investigations:

Hematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and 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 red.

Statistical analysis:

Findings such as body weight changes, water and food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett's test using a computer software programme – Graph pad version 5.0. All data were summarized in tabular form.

Table: 17. Body weight of Wistar albino rats group exposed to *Ashuwathi Chooranam*

Dose	DAYS				
	1	7	14	21	28
Control	165.6± 2.76	166.4 ± 3.42	167.7 ± 3.26	169.2 ± 3.73	170.7 ± 1.31
Low Dose	170.2 ± 2.12	172.7 ± 3.64	174.4± 1.51	175.2 ± 1.66	176.42± 2.76
Mid Dose	176.6± 1.64	177.3 ± 2.74	179.4 ± 8.12	182.1 ± 3.36	183.7 ± 3.12
High Dose	187.4± 6.74	189.6 ± 3.72	192.6 ± 2.46	187 ± 6.81	191.92 ± 2.49

*NS- Not Significant, ** (p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table: 18. Water intake (ml/day) of Wistar albino rats group exposed to Ashuwathi Chooranam

Dose	DAYS				
	1	6	14	21	28
Control	31.5 ± 8.95	32.0 ±6.23	28.5±6.23	29.12±8.19	31.5±3.96
Low Dose	28.5±3.31	26.4±3.62	26.7±3.02	22.2±3.29	34.9±3.13
Mid Dose	26.7±4.33	26.3±2.11	27.1±2.43	28.4±2.11	32.4±2.34
High Dose	30.1±1.32	30.2±2.13	32.7±2.13	35.2±1.73	38.4±2.65

*N.S- Not Significant, ** (p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table: 19. Food intake (gm/day) of Wistar albino rats group exposed to *Ashuwathi Chooranam*

Dose	Days				
	2	7	23	22	28
Control	37.12 ±5.37	38.5±3.22	39.5±3.37	38.5±3.37	37.12±3.12
Low Dose	43.7±2.98	45.3±1.22	45.1±1.18	45.4±2.12	45.6±2.42
Mid Dose	47.2±3.75	47.2±3.60	47.2±4.25	47.4±2.68	49.2±2.44
High Dose	46.2±2.34	46.2±2.64	49.6±2.66	48.2±3.20	48.0±3.62

*N.S- Not Significant, ** (p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table: 20. Haematological parameters of Wistar albino rats group exposed to *Ashuwathi Chooranam*

Category	Control	Low dose	Mid dose	High dose
Haemoglobin(g/dl)	14.8±1.88	13.88±1.66	14.94±0.66	15.28±0.96
Total WBC ($\times 10^3$)	10.91±2.59	11.25±3.73	11.48±3.91	12.20±3.17
Neutrophils(%)	32.65±1.06	33.23±2.14	35.61±1.36	35.40±2.20
lymphocyte (%)	69.34±2.48	72.12±3.12	72.48±2.66	73.10±3.16
Monocyte (%)	0.78±0.17	0.79±0.09	0.82±0.03	0.84±0.06
Eosinohil(%)	0.64±0.09	0.68±0.02	0.70±0.06	0.72±0.04
Platelets cells 10^3/µl	687.17±8.76	702.71±8.16	725.18±9.0	726.16±9.74
Total RBC 10^6/µl	7.99±0.12	7.82±0.57	8.82±0.59	8.38±0.72
PCV%	37.79±0.6	43.35±1.13	45.2±1.68	46.82±2.54
MCHC g/dL	33.6±2.23	35.09±1.29	35.98±1.22	36.03±1.24
MCV fL(µm^3)	49.17±3.64	50.20±1.22	52.28±1.24	53.24±1.44

*N.S- Not Significant, ** (p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table: 21. Biochemical Parameters of Wistar albino rats group exposed to *Ashuwathi Chooranam*

Biochemical Parameters	Control	Low Dose	Mid Dose	High Dose	High Dose
Glucose (R) mg/dl	76.45±13.4	78.16±8.44	78.26±11.20	78.42±11.6	78.42±11.6
Total Cholesterol (mg/dl)	115.26±1.83	115.45±1.83	116.42±1.78	116.22±1.73	116.22±1.73
Triglycerideds (mg/dl)	46.35±1.48	46.32±1.48	44.58±1.30	45.66±1.33*	45.66±1.33*
LDL	72.81±2.13	71.24±2.14	72.8±2.14	71.64±4.32	71.64±4.32
VLDL	15.2±2.44	15.42±4.64	15.44±6.64	15.64±4.36	15.64±4.36
HDL	26.66±6.88	26.86±2.24	26.68±4.66	31.78±2.22	31.78±2.22
T.Chol/HDL	4.42±2.44	4.16±3.14	4.34±8.44	4.46±2.22	4.46±2.22
LDL/HDL	2.83±4.22	2.84±2.22	2.86±2.20	2.96±6.02	2.96±6.02
Albumin(g/dl)	3.63±0.17	3.43±0.12	3.14±2.02	3.24±6.86	3.24±6.86

*NS- Not Significant, ** (p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table: 22. Renal function test of of Wistar albino rats group exposed to *Ashuwathi Chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
UREA (mg/dl)	13.35±0.99	14.31±0.16	13.06±1.08	13.48±1.12
CREATININE(mg/dl)	0.28±0.08	0.36±0.06	0.52±0.04	0.66±0.02
BUN(mg/dL)	15.02±0.10	16.10±0.60	16.22±0.44	18.10±2.12
URIC ACID(mg/dl)	5.17±0.35	5.31±0.43	5.42±1.25	5.58±0.23

*NS- Not Significant, ** (p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table: 23. Liver Function Test of of Wistar albino rats group exposed to *Ashuwathi Chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE
T.Biliribin(mg/dl)	0.48±0.07	0.53±0.06	0.51±0.08
SGOT/AST(U/L)	79.95±1.39	78.35±0.51	76.01±1.53
SGPT/ALT(U/L)	31.23±1.28	30.91±1.59	28.34±1.48
ALP(U/L)	143.25±8.70	142±16.17	145.16±24.07
T.PROTEIN(g/dL)	5.32±0.38	6.48±0.34	7.01±0.23

*NS- Not Significant, ** (p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

PHARMACOLOGICAL STUDIES

The study tried to evaluate the effect of this exercise type on reproductive dysfunction in rats with polycystic ovarian syndrome.

Experimental design: Female white albino rats were allocated into three groups:

Group I: Control rats; Group II: Letrozole induced polycystic ovarian syndrome rats (PCO) where letrozole was given orally and daily in a dose of 1 mg/kg dissolved in 0.9% NaCl solution for 21 days; Group III: Clomiphene received group. IV. Polycystic ovarian syndrome rats subjected to *Ashuwathi chooranam* (100mg) V. Polycystic ovarian syndrome rats subjected to *Ashuwathi chooranam* (200mg) orally for three weeks after the induction of polycystic ovarian syndrome. After 6 weeks from the beginning of the study, final body weight, body mass and Lee indices were determined. Plasma levels of LH, free testosterone, estradiol, progesterone, prolactin, fasting glucose and fasting insulin were measured

Fig. No.7: Effect on Ovarian structure in letrozole induced PCOS rat model.

Development of PCOS in Rat Model

Rats treated with letrozole for induction of PCOS showed a significant increase in body weight and altered estrus cyclicity as compared to control. As shown in Figure .3. PCO animals exhibited an increase in body weight and glucose tolerance as compared to control and histology of ovary revealed many peripheral small atretic cysts Figure 2. Whereas no histological abnormalities were observed in control rat.

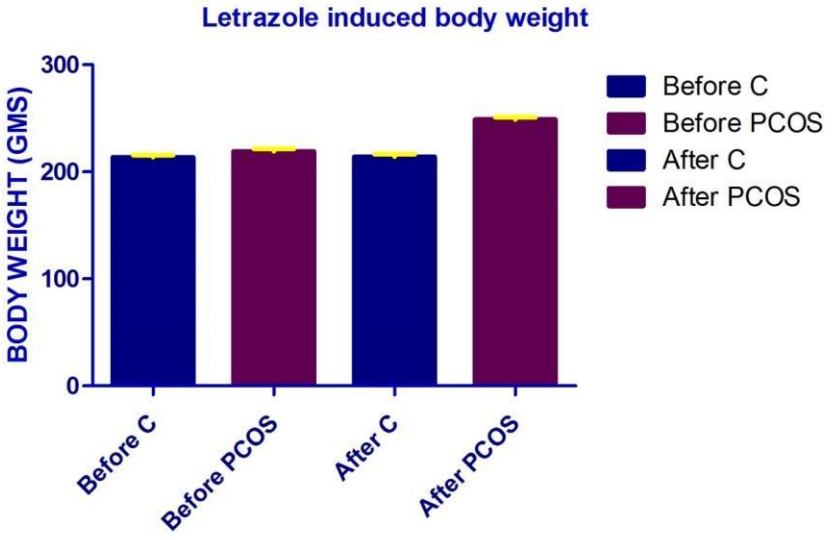


Fig. No.8. Body weight of letrozole induced PCOS in wistar albino rats

The results from the above experiments clearly demonstrate that Letrozole induced PCOS rats exhibited all the pathological characteristics similar to the clinical manifestations found in PCOS women. The main aim of the current chapter was to evaluate the efficacy of *Ashuwathi chooranam* in PCOS rodent model. Hence, dose and time dependent experiments were directed towards the understanding the most effective dose and minimum time required for management of PCOS phenotype.

Body weight

Obesity is a major feature in women with polycystic ovary syndrome (PCOS), and evidence suggests that obesity contributes to the pathogenesis of PCOS (Nestler 2000). Generally, excess abdominal adipose tissue (AT) initiates metabolic and endocrine aberrations that are central in the progression of PCOS [84]. PCOS rat model exhibited significant increase in body weight with abdominal fat as compared to normal rats. However, after treatment with *Ashuwathi chooranam*, body weight reduction was not seen.

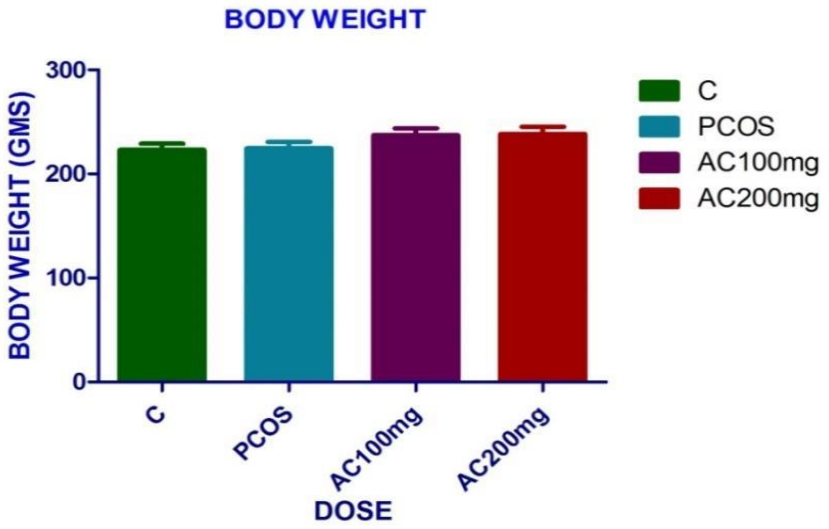


Fig. No. 9. Dose and time dependent effect of Ashuwathi Chooranam on Body weight

ORAL GLUCOSE TOLERANCE TEST (OGTT)

Women with polycystic ovarian syndrome (PCOS) are at increased risk for developing glucose intolerance leading to type 2 diabetes mellitus (DM) (Salley et al. 2007). Hence, it was necessary to evaluate the efficacy of *Ashuwathi chooranam* on glucose homeostasis. Thereby, Oral glucose tolerance test (OGTT) was performed in all groups of animals. PCOS rats exhibited high glucose tolerance compared to normal control rats (**p<0.01, ***p<0.001) at all the time points of OGTT profile. *Ashuwathi chooranam* treated PCOS rats in different doses (100mg, 200mg) demonstrated significantly reduced glucose intolerance and improved cellular glucose uptake upon increasing time period of dose (30 days) of OGTT profile.

Glucose homeostasis is governed by insulin action. Thereby, we measured serum insulin level in all group of animals wherein serum insulin levels of untreated PCOS rats were increased significantly (***p<0.001). Treatment of AC caused a decrease in insulin level as compared to PCOS group (p<0.001).

Dose and time dependent effect of *Ashuwathi chooranam* on Oral Glucose Tolerance Test (OGTT Test)

Fig. No.10. Effect of Ashuwathi Chooranam on OGTT Test.

Table No. 25. *Ashuwathi chooranam* on Insulin status

N= 6 per group, All values are represented as Mean + SEM. $^{+}p<0.05$; $^{++}p<0.01$as compared to PCOS group. Normal insulin resistance: < 3; Moderate Insulin resistance: Between 3 – 5; Severe Insulin resistance: > 5

Estrus Cyclicity

The primary clinical manifestations of polycystic ovary syndrome (PCOS) are irregular menstrual cycle and chronic anovulation, which is found to be associated with approximately 80% of PCOS women [85]. Hence, estrus cyclicity in PCOS rats was monitored, wherein PCOS rats exhibited arrested estrus cyclicity in late diestrus phase of cycle as compared to control rats. After treatment of AC at

various doses (100mg) and various time periods of (30 days), estrus cyclicity was evaluated wherein 100mg for 30 days treated group of animals exhibited reversion to normal cycle in 80% of PCOS rats. But upon increasing the doses of *Ashuwathi chooranam* (200mg) all rats showed improved cyclicity and reverted back to normal cycle.

Ovarian Histological study

Normal ovarian function relies upon the selection of a follicle that become dominant with appropriate signal FSH and ovulates with the help of LH surge during ovulation. This mechanism is disturbed in women with PCOS, resulting in multiple small cysts (or follicles), most of which contain potentially viable oocytes but within dysfunctional follicles [86]. PCOS rat model in current study also demonstrated peripheral empty follicular cysts as compared to control ovary with normal growing follicles. In dose dependent study, 100 mg dose/30 days of treatment exhibited normal growing follicles but some cysts were present in ovary. *Ashuwathi chooranam* at higher doses (100 mg and 200 mg) at 30 days of treatment, number of peripheral cysts significantly decreased and increased normal growing follicles with presence of corpus luteum was present; indicating normal ovulation due to functional ovary.

4.4. Interpretation

PCOS has many clinical manifestations, which includes oligomennorhea and hyper- androgenism, leading to metabolic dysfunction (Dickerson et al. 2010). Rat model created using letrozole exhibited an increase in ovarian androgens and thus leading to hyperandrogenism, which is a hallmark of PCOS. Also, significant weight gain was observed in letrozole treated PCO as compared to control rats, which could be attributed to deposition of abdominal fat [87]. The model created show similar characteristics of PCOS shown by [88] . It has been well documented that PCOS is positively correlated with insulin resistance [85]. Apart from, systemic level changes, ovarian steroidogenesis were also altered leading to high testosterone level in PCO phenotype [88] which could be correlated to ovarian structural changes as seen in present study [85]. Thereby, letrozole induced PCOS rat model

demonstrated increased body weight, arrested cyclicity and impaired glucose intolerance with hyperandrogenim that are key features of PCOS phenotype.

Aim of current chapter was to understand the dose and time required by *Ashuwathi chooranam* for the management of PCOS condition. Thereby, future studies were directed to evaluate minimum effective dose and time period for 30 days *Ashuwathi chooranam* treatment which would manage PCOS phenotype and restore normal ovarian function.

Thereby, experiments were carried out with various doses (100mg, 200mg,) at different time points (30 days) with *Ashuwathi chooranam* formulation.

Dose and time dependent effect demonstrated that treatment irrespective of time and dose could cause Dose and time dependent effect demonstrated that treatment irrespective of time and dose could cause a reversion to normo-glycemic condition from hyperglycemic condition as observed in PCO phenotype. *Ashuwathi chooranam* (AC) formulation treatment with higher dose (100mg, 200mg) for short period time (30 days of treatment). This could be attributed to the nutritionally rich phytosterols and phyto-phenols present in the plant [89], that helps to recover the syndrome and could be able to sensitize the insulin receptors for the glucose uptake. Also, it should be noted that *Ashuwathi chooranam* is rich in fibers that could increase transit time for diet to be get absorbed which could modulate glucose homeostasis in PCO phenotype.

In this study, PCO rats demonstrated the formation of empty cysts with follicular fluid which is similar to ovarian structural changes that was reported by [88]. PCOS rats treated with fresh AC and formulation exhibited normal follicular growth which was evident from normal estrus cyclicity as seen in higher doses (100mg, 200 mg) rats also exhibited reversion in ovarian structure. However, it should consider that with increasing dose, phyto-components content is increased. These phyto-components present in AC could be active components which would alter the steroidogenesis and expression of steroidogenic protein, which alters the PCO condition [90]. However, at 30 days of treatment caused significant change with lower dose (100mg). The reversion of estrus cyclicity upon extracts treatment could be attributed to phytochemical components present in the AC that maintains steroid status, regaining back the fertility status.

The results of ovulation effect revealed the significant influence at the dose level of 25mg/kg and this marked effect was ensured with the histological evaluation of uterus of experimental rats also. Hence it may be concluded that the *Ashuwathi Chooranam* is an excellent traditional 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.

Table: 26. Effect of *Ashuwathi Chooranam* on weight of uterus and ovary after 10 days treatment

N = 6. Values are expressed as Mean ± SEM. ^{ns}*P>0.0, *p<0.05;**p<0.01;p***<0.001 When compared to normal control.*

Fig. No.11: Effect of AC on weight of Uterus and ovary

Experimental design

- Group I Normal Control animals 1ml/kg of CMC (Carboxymethyl Cellulose) solution.
- Group II rats were administered Ashuwathi Chooranam 100mg/kg for 10days,
- Group III rats were administered Ashuwathi Chooranam 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.

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 microplate was swirled for 20-30 seconds and covered with 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 microplate 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 Microwell 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 microplate 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 microplate 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 Microwell 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 microplate 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 microplate 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 Microwell 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 microplate 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. 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 microplate 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.

Table No. 27. Effect of *Ashuwathi Chooranam* on Serum Concentration of reproductive hormones of female Wistar albino rat

*N = 6. Values are expressed as Mean±SEM. *p<0.05;**p<0.01; $^{+}$p<0.05; $^{++}$p<0.01as compared to PCOS group.*

Fig No. 12: Effect of Ashuwathi Chooranam on Serum Estrdiol and Progesterone in rats

Fig. No. 13: Effect of Ashuwathi Chooranam on Serum LH and FSH in rats

Fig. No. 14: Effect of Ashuwathi Chooranam on Serum Testosterone in rats

ANTIOXIDANT ASSAY:

DPPH ASSAY: [91]

DPPH(1,1-diphenyl-2-picrylhydrazyl)is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most fo the free radicals. The delocalisational so gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centered at about 520nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form [92] with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z\bullet + AH = ZH + A\bullet$$

Where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall

stoichiometry, that is, the number of molecules of DPPH reduced (decolourised) by one molecule of the reductant.

Chemicals

1. 1, 1 – diphenyl-2-picrylhydrazyl (DPPH)
2. Dimethylsilphoxide (DMSO)
3. BHT (standard)-1.6mg/ml in methanol
4. Samples desired concentration from 1 mg/ml–max of 5mg/ ml (in /DMSO)

Procedure:

Aliquot 3.7ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank.

Add 100µl of BHT to tube marked as standard 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30minutes.The absorbance of all samples was read at 517nm.

Table: 28. Procedure for DPPH Assay

S.NO	REAGENTS	BLANK	STANDARD	TEST
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	-	100µl	-
3	Sample	-	-	100µl
4	DPPH	200µl	200µl	200µl
Incubation at dark for30minutes				
O.D at 517 nm				

Calculation:

$$\% \text{ Antioxidant activity} = 100X \frac{\textbf{(Absorbance at blank) - (Absorbance at test)}}{\textbf{(Absorbance at blank)}}$$

Table: 29. Antioxidant activity.

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug	Standard
Control	0.5461	0.324	-	-
1.25	0.4742	0.262	13.1661	19.14
2.50	0.3863	0.198	29.2621	38.89
5	0.2545	0.113	53.3969*	65.15
10	0.1912	0.084	64.9881	74.08
20	0.1001	0.041	81.6701	87.35

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

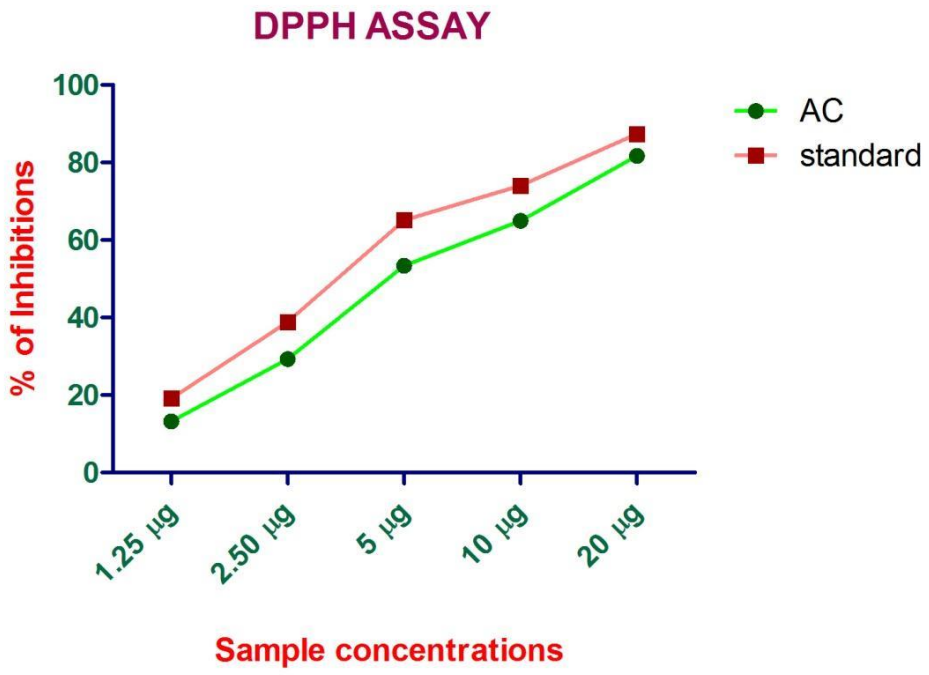


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Fig No. 15: DPPH ASSAY

6. CONCLUSION

The present dissertation is to validate the safety and efficacy of the Siddha poly-herbal formulation *Ashuwathi chooranam* for its naturally curing polycystic ovarian syndrome *(Soothaga vaayu*) in female Wistar albino rat based on the classical Siddha literature "*Agathiyar vathiyta kaaviyam 1500*".

The ingredients of the test drug are *Amukkura (Withania somnifera),Chukku (Zingiber officinale (dried ginger), Milagu Piper nigram, Thippili (Piper longum), Jaadhikaai (Myristica fragrans), Jaadhipathiri (Myristica fragrans), Adhimadhuram (Glycirriza glabra), Krambu (Syzygium aromaticum), Kadugurohini (Picrorhiza scrophulariiflora), Krosaniomam,(Hyoscymus niger).* The drugs were identified and authenticated by the experts of *Gunapadam* (Pharmacology), Government Siddha Medical College.

The drugs were purified and processed as per literature. The fine particles in it enhance the curative potential with shelf life of three months.

The trial drug *Ashuwathi chooranam* is indicated for *Soothaga vayu*, which is related to the modern terminology PCOS. To validate the traditional usage, the drug was subjected to literary review, Physico-chemical and elemental analysis, Acute and Sub-acute toxicity study, Anti-microbial load and Pharmacological studies ovulogenic activity, estimation of hormones FSH, LH, Progesterone, Estrodial and antioxidant activity for its naturally curing property for Polycystic ovarian syndrome.

The anti-microbial load shown effective control that at medium and high doses against *Streptococcus mutans, Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa.*

FTIR revealed the presence of functional groups aliphatic bromo compounds, nitro group, ketones, carbonyl group, phenols and alcohols. Phenolic group of compounds exert antioxidant activity, they are responsible for chemo-preventive properties like anti carcinogenic, antimutagenic, anti-inflammatory activity, apoptosis inducing and regulates carcinogen metabolism.

SEM revealed the presence of particles of size in which 3µm, 10µm particles have valuable properties that can be used to improve drug delivery. Where larger particles would have been unfurnished from the body, cells take up these nanoparticles because of their size.

In the pharmacological studies, Letrozole induced PCOS rats model treated with two different forms of *Ashuwathi chooranam* formulation in various doses (100 mg, 200 mg) and time (30 days) dependent manner suggested maximum effect that reduced PCO like phenotype such as decreased in peripheral cysts with growing follicles, decreased glucose intolerance. Present study also elucidated that *Ashuwathi chooranam* formulation exhibiting effect in PCO phenotype that indicates natural preservatives. *Ashuwathi chooranam* has a good efficacy for management of PCOS phenotype.

Ashuwathi chooranam is rich in various phyto-components that were analysed by several qualitative and quantitative analyses.. Further, confirmation for the presence of Phytosterols like sterols, β- sitosterol etc along with steroid derivatives has been performed by TLC, HPTLC.

The certain compounds in *Ashuwathi chooranam* suppresses the conversion of cholesterol to pregnenolone through inhibition of P_{450} cytochrome activity, thus reducing testosterone production. The modulation could be due to effect of phytosterols acting at all organ levels hyperinsulinemic condition leading to restoration of ovarian function in PCO rodent model. This proves phytosterols could be novel component which can be explored for management of PCOS.

Through the elaborate study *Ashuwathi chooranam* had shown its sphere of action over the female reproductive system especially over the ovaries in the condition called PCOS.

7. SUMMARY

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy seen in women of reproductive age. Though it is a reproductive disorder, it is associated with characteristics of metabolic syndrome including insulin resistance. Current available mode of treatment is by use of insulin sensitizers like metformin and ovulating agents like clomiphene citrate But, these drugs have been reported for their side effects upon prolonged usage.

Hence, researchers in current era are exploring alternative therapy to manage the metabolic syndrome. In this context, many scientists have demonstrated the role of medicinal plants in the management of hyperglycemic condition. *It* has gained its popularity as anti- hyperglycemic plant, wherein its phytosterols and polyphenols have been studied extensively for the above efficacy. Thereby, it would be of interest to biopropsect *Ashuwathi chooranam* for management of metabolic induced anovulatory disorder like PCOS. In this context, current work has elucidated the potentiality of *Ashuwathi chooranam* (AC).

To study the efficacy of AC, Polycystic ovarian syndrome (PCOS) rat model was developed with use of letrozole (0.5 mg/kg/body weight/21 days/orally) - a non- steroidal aromatase inhibitor in estrus cycle. Thus, letrozole induced PCOS rat model demonstrated key features of PCOS phenotype.

Further, PCOS rat model was treated with *Ashuwathi Chooranam* formulation in various dose doses (100 mg, 200mg) at different time points (30 day). After completion of experiment regime, various biochemical parameters were checked to evaluate the efficacy of *Ashuwathi chooranam*

Apart from the phytochemical analysis, *"Ex vivo"* experiments were performed with partially purified fraction to evaluate direct effect of phyto-components on ovarian steroidogenic enzymes activities using PCOS model. Further, the detailed chromatographic analysis- TLC, HPTLC, and HPLC of demonstrated the presence of various phytosterols.

In current study, treatment with both *Ashuwathi chooranam* (AC) formulation with higher dose (100mg, 200 mg) at longer period of time (30days) demonstrated more significant effect and it restored glucose sensitivity and normal insulin level. Along with the above changes, ovarian structure-function (in terms of hormone levels and presence of developing follicles) was seen upon the AC treatment. This could be attributed to the nutritionally rich phytosterols and phyto-phenols present in the plant, that helps to sensitize the insulin receptors for the glucose uptake.

Ashuwathi chooranam phyto-components independently could cause a modulation in PCO phenotype. All the treatments did not affect toxicity parameters, thus suggesting AC treatment was safe and it contributes its maximum efficacy on reproductive organs.

Current study demonstrated altered *in utero* environment like decrease in progesterone with increased testosterone levels may result in failure of implantation and prenatal defects, which is implicated in PCOS phenotype as justified by other studies.

In context of above observed physiological modulations, it was of interest to elucidate detailed phytochemistry of AC and to isolate, identify the active component responsible for mentioned efficacies. In this regard, both qualitative and quantitative analysis confirmed the presence of phyto-components namely polysaccharides, alkaloids, polyphenols, sterols, flavonoids, etc., which is similar to data reported by. Several data has suggested that phytosterols and polyphenols can have a role in modulation of steroid status.

Thus, this current analysis authenticates that *Ashuwathi chooranam* 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 mankind.

8. FUTURE SCOPE

Ashuwathi chooranam indicated for *Soothaga Vayu* as per Siddha literature is being proved that the drug has its role in the treatment of PCOS.

With reference to the in vivo studies in female Wistar albino rat, clinical trials should be conducted in future to authenticate the traditional use of AC in menstrual irregularities and fertility issues in humans.

SEM depicted the presence of nano particles, the active compound exhibiting the therapeutic efficacy should be analyzed. The composition of the compound in micro size and their percentage of presence in AC should be elucidated in future by Dynamic Light Screening and Nuclear Tracking Analysis.

More recently, with the advance of gene technology many new animal models that can aid in illumination of PCOS pathophysiology have been established.

This is first study where implication of *Ashuwathi chooranam* has shown the potential to manage PCOS, which could be considered as a novel component for future drug development and should be screened for rest of the pharmacological activities like ovulogenic activity, hormonal assays, antioxidant activity to rule the effective pathways so that the exact benefit and aim of the study would be fulfilled.

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CERTIFICATE

This is to certify that the project entitled, **Toxicological and Pharmacological study on ASHUWATHI CHOORANAM & AADUTHEENDAPALAI *(Aristolochia bracteolate)* CHOORANAM** in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2016-2017. It has been approved by the **IAEC No: IAEC/XLVIII/04/CLBMCP/2016**

C.L. Baid Metha College of Pharmacy CHENNAI-97 Thoraipakkam

(Dr.P.Muralidharan)

IAEC Member Secretary

C.L. BAID METHA COLLEGE OF PHARMACY, THORAIPAKKAM, CHENNAI - 600 097.

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20.1.2017

CERTIFICATE

Name of the student: Dr. R. Devaki, III year PG student, Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

Name of the sample: Ashuwathi Chooranam

Name of the Experiment	I	II	Mean
Loss on drying(at 105°C)	8.83 %	8.79 %	8.81 %
Total ash	2.26 %	2.62 %	2.64 %
Water soluble ash	1.25 %	1.45 %	1.35 %
Acid insoluble ash	0.20 %	0.15 %	0.18 %
Water soluble extractive	44.33 %	48.41 %	46.37 %
Alcohol soluble extractive	46.21 %	47.62 %	46.92 %
pH value (10%)	3.53	3.53	3.53
TLC/HPTLC	Report Enclosed		

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

P. Elankani 21/01/17
(Dr. P. Elankani)
Research Officer (Scientist II) (Siddha)
for Assistant Director (Siddha) I/c

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

69,Anna Salai,Guindy,Chennai - 600 032.

This Certificate is awarded to Dr/Mr/Mrs.....R...Devaki.....

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

" RESEARCH METHODOLOGY & BIOSTATISTICS "

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Organized by the Department of Siddha

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POST GRADUATE DEPARTMENT OF GUNAPADAM

(PHARMACOLOGY)

GOVERNMENT SIDDHA MEDICAL COLLEGE,

CHENNAI-106

IDENTIFICATION AND AUTHENTICATION CERTIFICATE

Name of the Student :

Department :

Batch year :

Name of the sample :

Sample description : **Dried whole plant / metal /mineral**

Date of the receipt :

Specimen Ref. No :

REPORT

This sample has been critically studied with macroscopic and organoleptic characters along with relevant literature, I declared that this plant/metal/mineral material is correctly identified as _____ and I hereby authenticate that the sample given by Dr. _____.

This certificate issued at his/her request and is given only for dissertation purpose.

Date :

Place: **Signature with Seal**