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PCOS and Cowpea Isoflavones

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Abstract:- Polycystic ovary syndrome (PCOS) is a pervasive and complex endocrine disorder. Normally, Gonadotropin releasing hormone stimulates the anterior pituitary to release Gonadotropins like Follicular Stimulating Hormone (FSH) and Luteinizing hormone (LH). LH helps in formation of corpus luteum and FSH helps in the growth and maturation of graffian follicles, the matured follicle releases estrogen. Estrogen is the key hormone in female body which is responsible for bringing about Ovulation, secondary sexual characters, bone development, fusion of epiphyseal ends of long bone, excessive vaginal secretions etc.

➢ ***What happens in women with PCOS?***

Women suffering with polycystic ovarian syndrome have excessive levels of LH secretion. Elevated levels of LH gives rise to the excessive levels of androgens (testosterone) and low FSH levels, both leads to substandard development of eggs and an impotency to ovulate. If ovulation does not occur it leads to shortage of progesterone production which inturn leads to the absence of menstrual periods. Elevated Androgen levels or also called as Hyperandrogenism is seen in women with PCOS which causes hirsutism (hair growth on face region, around nipples and in chest region), irregular ovulation or absence of ovulation which can be also termed as Anovulation, infertility etc.

➢ ***Symptoms Include:***

Anovulation or absence of menstrual periods

- **Hirsutism**
- **Infertility**
- **Obesity**
- **Multiple cysts in the ovaries**
- **Diabetes**
- **Acne**
- **Alopecia**

Causes for Polycystic Ovarian Syndrome may be due to genetic or environmental problems, abnormality in metabolism of androgens and estrogens, abnormality in production of androgen and abnormal function of hypothalamic-pituitary ovarian axis. Women with PCOS have decreased estrogen levels and increased testosterone levels, due to this they suffer from irregular menstrual cycles, pelvic pain, sometimes infertility and also hirsutism. Besides high levels of testosterone, women with PCOS show Lessened expression of the (ERβ) Beta Estrogen receptor.

Keywords:- *Phytoestrogen, ERβ, Hyperandrogenism, Isoflavones, PCOS, Anovulation.*

I. INTRODUCTION

➢ *What's the difference between PCOS and PCOD?*

- *PCOS*

PCOS is an endocrine disorder in which ovaries produce high levels of androgen which obstructs with the development of egg and its release. Some of the eggs mature into cysts. These are small sacs that are filled with fluid. These cysts are not released and instead they accumulate in the ovaries and enlarges in some of the cases.

- *PCOD*

PCOD is a hormonal imbalance which leads to collection of mature eggs in ovaries as they cannot be discharged these also may become cysts.

➢ *Drugs used in treatment of PCOS:*

Treatment goals in PCOS include inducing of ovulation, reducing of insulin resistance and inhibiting action of androgens on target tissues.

- Clomiphene- to induce ovulation
- Metformin- to decrease insulin resistance
- Gonadotropins –HMG and FSH
- Anti-Androgens like- Spironolactone, Flutamide, Finasteride and Oral contraceptives.

➢ *Herbal Drugs For PCOS:*

- *Asparagus racemosus*- Phytoestrogens
- *Tinospora cordifolia*- Hypoglycemic effect, anti-inflammatory, lowers insulin resistance.
- *Foeniculum vulgare*- Phytoestrogens
- *Ocimum tenuiflorum* (Tulasi)- excellent anti-androgenic properties.
- *Actaca racemosa* (Black cohosh)- Induce ovulation
- *Lepidium mayenii* (Maca)- natural hormone balancer.
- *Grifola frondosa (*Maitake Mushroom)- Hypoglycemic effect.
- *Taraxacum officinale (*Dandelion root)- Bile flow stimulant and liver detoxifier. Cleans the liver which may stimulate the production of SHBG (Sex Hormone Binding Globulin) as SHBG reduces the testosterone in blood.

- *Pergularia Daemia*- Normalizes menstrual irregularities and estrous cycle which decreases the development of follicular cysts.
- *Galega officinalis* (Goat's rue)- Decrease insulin resistance.
- *Glycyrrhiza glabra* (Licorice)- Potent anti-androgen, anti-inflammatory, stabilize blood sugar levels.
- *Paeonia lactiflora* (White peony)- Reduce androgen levels.
- *Saw palmetto* (Serenoa repens, Sabal serrulata) - Decrease testosterone in blood.
- *Utrica dioica* (Stinging nettle)- lowers testosterone in blood by increasing production of SHBG.
- *Linum usitatissimum* (Flax seed)- increase SHBG levels.
- *Trifolium pretense* (Red clover) - Phytoestrogens
- *Glycine max* (Soy)- Phytoestrogens

➢ *What are these Phytoestrogens and how are they useful?*

Under Phytoestrogen class Isoflavones are mostly used as food supplements as they exert beneficial effects on reproductive performance and in regulation of various hormone levels. Phytoestrogens are related to the heterogeneous group of the herbal substances and their structure is as same as estradiol-17β (E_2). Phytoestrogens are also known as estrogen-like molecules and also nonsteroidal estrogens as these are structurally same as E_2. Phytoestrogens are diphenolic compounds and are generally nonsteroidal. Phenolic ring structures of isoflavones enables them in binding with Estrogen Receptors(ERs) and hence mimics the functions of estrogen.

➢ *Objective*

As PCOS women have low estrogen levels due to which they experience irregular menstrual cycles or anovulation, by administering the isoflavones of COWPEA (*vigna unguiculata*) that micics the estrogen and bring about ovulation thereby regularizing the estrous cycle which decreases the development of follicular cysts can exert beneficial effects in PCOS women.

➢ *Cowpea Isoflavones*

Cowpea isoflavones – Diadzen and Genestein binds to the Estrogen Receptors, more possibly to ERβ rather than ERα and mimics the functions of estrogen. Cowpea has Dz and Ge in the ratio of 18:6 Dz when hydroxylated its structure will be similar to the structure of Ge. Absorption and extraction capacity of Cowpea isoflavones in rats is found to be high.

II. METHODS AND MATERIALS

A. Inducing PCOS in Rats

Female rats are to be selected as the animal model or experimental organism for this study. Blood samples should be collected from the rats and Hormone Profile Test has to be done to find out the serum levels of estrogen, progesterone, LH, FSH, LH/FSH ratio, total testosterone levels, circulating free testosterone levels and SHBG levels and the results are to be noted down. PCOS can be induced in rats by Letrozole 1mg/kg orally O.D for 21 successive days. Letrozole is an active, Nonsteroidal, selective aromatase inhibitor and hence an Antiestrogen. Letrozole can prevent aromatase from producing estrogens and hence can induce PCOS. Rats are believed to have PCOS if they exhibit irregularity in the ovarian cycles. Estrous cycle can be determined through the vaginal smears. Now after 21 days of letrozole treatment, collect the blood sample from rats and go for the Hormone Profile Test. The rats which have low estrogen levels in Hormone Profile Test are identified and are selected for the further studies.

B. Extraction of Isoflavones from Cowpeas

Cowpea (*vigna unguiculata)* isoflavones have to be extracted for the further studies. Extraction and quantification of the isoflavones have to be done by standard HPLC. HPLC-High Performance Liquid Chromatography technique is used for the separation, identification and quantification of the isoflavones from the cowpea.

The method which was used for the extraction and quantification of soy isoflavones is taken as an example and has been adopted here. Cowpeas are ground with a mill. 100mg of seed flour have to be extracted using 30ml of ethanol/water (70/30, v/v) and pH should be adjusted to 2.0 by adding Formic acid. The fat from the extract have to be removed by 15ml of n-Hexan.The ethanolic extracts are allowed to evaporate under a vacuum at room temperature and rinsed with ethanol/water (70/30, pH 2.0) to a final volume of 1.5ml. Samples are to be analyzed by HPLC-DAD (Diode Array Detector) and HPLC-MS for the qualitative and quantitative evaluation. The analyses have to be carried using a HP 1100L liquid chromatography which is equipped with a DAD.Polyphenolic compounds are to be separated using a 150 3.9mm C18 column at 26 C.The mobile phase should be a four-step linear solvent gradient system,starting from 95% water upto 100% Acetonitrile during 27-min period,and three step linear solvent system starting from 91% water upto 100% acetonitrile during 14 min period and pH have to be adjusted to 3.2 using Phosphoric acid . The flowrate in both cases is to be noted.

Many recent papers dealt with the separation of phytoestrogens in soybeans. In some of the papers the analytical methods that are used are involved with the sample hydrolisation and quantitation of the obtained aglycons. We are using a non-hydrolytic method here. The HPLC method should allow separation either of the isoflavone aglycons and the glycosides. Talking about the extraction, the choice of ethanol instead of acetonitrile as extraction solvent was made as it has lower boiling point, lower toxicity and lesser environmental problems. Notwithstanding the possibility of conversion from malonylglucoside derivative to glucoside any difference in the ratio malonylglucoside/glucoside can't be found comparing two different extraction times (3 and 24 h). The extraction yield (95%) have to be controlled adding formononetine (7- hydroxy-4¢-methoxy-isoflavone) as a

standard. The choice of this molecule is based on its absence in samples, and on its retention time which falls in an empty zone of the chromatogram (Rt = 25.08 min). The chromatographic profile of a cowpea seed flour extract is recorded at 260 nm is reported. The chromatogram have to be obtained with C18 column, using the ‘‘long’’ method which has already successfully been used in the separation of isoflavones.

C. Administering Cowpea Isoflavones to Rats

After the induction of the PCOS in rats, they are treated with cowpea isoflavones (Gn and Dz). The isoflavones are to be obtained from either any pharma company or have to be extracted using HPLC. The PCOS induced rats are to be administered with isoflavones 100mg/kg for at least 15-20 days after the induction of PCOS. Finally Hormone profile test have to be done once again and if the reports show decreased testosterone levels or increased GSH levels it gives a positive hope for the further research.

III. DISCUSSION ON EXPECTED OUTCOMES

It is expected that by administering cowpea isoflavones to PCOS subject or model may induce ovulation as isoflavones mimic the functions of estrogens. Rats (female) generally have 4-5 days of estrous cycle which comprises of proestrous, oestrous,meta estrous and diestrous phases. Notable increase in the testosterone levels in rats treated with letrozole can be seen when compared to that of normal rats. The accumulation of androgens is due to the conversion of androgens into estrogens is stopped by the letrozole which is a aromatase inhibitor. The elevated levels of testosterone in blood can be a reason for the prolongation of dioestrous phase. The rats that were treated with isoflavones may show decreased levels of testosterone when compared with untreated PCOS rats. On induction of PCOS the rats may show prolonged dioestrous days and by treatment with isoflavones the dioestrous days decrease which brings about normal estrous cycle.

Alterations in the estrous cycle of rats may be linked to the changes in the concentration of gonadotropins and sex hormones circulating in blood. There may be notable elevation in the GSH levels in rats which were treated with isoflavones when compared to untreated PCOS rats. As per the earlier reports the aglycone forms of genestin,daidzen that are Genestein and Daidzein decreases the testosterone levels by obstructing in the production of steroids in adrenals in the male rats. In this study, the rats having letrozole-induced PCOS should show reduced levels of plasma estradiol concentrations, because letrozole stops the aromatization of testosterone to oestradiol. Genistein and daidzein exert weak estrogenic effects.Treatment with cowpea isoflavones may not cause any notable changes in estradiol levels. Change in gonadotropin levels which is a typical of polycystic ovarian syndrome may offer resistance to a possible influence of cowpea isoflavones. The lack of any effect of cowpea isoflavones on the estradiol levels is one of the main discrepancies. Some studies reported that there was a decrease in the estradiol levels at variable daily dose of isoflavones which some other reports shows slight increase or no effect on this parameter in humans. Comparison is difficult to make between previous results and present study results.

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