Effect of Shatapushpa Churna in an experimental model of Polycystic ovary syndrome using rats as an animal study.

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ABSTRACT:
Poly Cystic Ovarian Syndrome (PCOS) is one of the most common metabolic and reproductive disorders among women of reproductive age. Women suffering from PCOS present with a constellation of symptoms associated with menstrual dysfunction and androgen excess, which significantly impacts their quality of life. They may be at increased risk of multiple morbidities, including obesity, insulin resistance, type II diabetes mellitus, cardiovascular disease (CVD), infertility, cancer, and psychological disorders. In the present study, the pharmacological activity of Shatapushpa Churn was evaluated against Polycystic Ovary Syndrome by oral route. The Letrozole-induced PCOS rat model was standardized in adult Wistar female rats. The rats that demonstrated irregular estrous cyclicity, glucose intolerance, increased body weight, and altered steroid status compared to vehicle control were considered as PCOS-Positive. These animals were subsequently used to evaluate the efficacy of Shatapushpa Churn. In the present study, the pharmacological activity of Shatapushpa Churn was evaluating against Polycystic Ovary Syndrome by oral route. The Letrozole-induced PCOS rat model was standardized in adult Wistar female rats. The rats that demonstrated irregular estrous cyclicity, glucose intolerance, increased body weight, and altered steroid status compared to vehicle control were considered as PCOS-Positive. These animals were subsequently used to evaluate the efficacy of Shatapushpa Churna.

KEYWORDS:
PCOS, Shatapushpa Churna, Letrozole, Wistar rats.
INTRODUCTION:

Polycystic Ovary Syndrome (PCOS) is one of the most common Endocrine Disorders affecting 4-10% of those of reproductive age. PCOS is a disorder in women in which the ovaries become enlarged with many ‘cysts,’ which are the small undeveloped follicles. Over time there is a thickening and fibrosis of the ovarian casing, which prevents any follicles which do ripen from being released. PCOS is associated with anovulation and menstrual irregularities, infertility, and insulin resistance. There may be acne, hirsutism, and weight gain. Features of PCOS may manifest at any age, ranging from teenage years (hirsutism, menstrual abnormalities), early adulthood, and middle life (infertility, glucose intolerance) to later life (diabetes mellitus and cardiovascular diseases). Shatapushpa Churn is an ayurvedic herbomineral formulation available in Powder form. The intended route of administration is oral.

AIM:

To study the therapeutic effect of Shatapushpa Churn with PCOS condition.

OBJECTIVES:

To Evaluate the therapeutic efficacy of Shatapushpa Churn, a herbomineral drug for the treatment of Polycystic Ovarian Syndrome using rat as an animal model.

MATERIALS AND METHODS:

Test System Details

Strain and Species: Wistar Rats
Sex: Female
Age of animals: Adult of the age 6-8 weeks
No. of Animals used: Total 48 Wistar rats
Source: Animal House Facility, National Institute for Research in Reproductive Health, (ICMR) Parel

Mumbai 400 012, India (Registration No.78/1999/CPCSEA dated 11/3/1999).
Experimental outlines for Shatapushpa Churn treatment to Letrozole induced PCOS rats.

<table>
<thead>
<tr>
<th>Gr No.</th>
<th>Group</th>
<th>Sex</th>
<th>Dose (mg/kg)</th>
<th>Number of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>Female</td>
<td>Vehicle</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Shatapushpa Churn low dose</td>
<td>Female</td>
<td>0.62 gm/kgbw</td>
<td>8</td>
</tr>
<tr>
<td>3.</td>
<td>Shatapushpa Churn mid dose</td>
<td>Female</td>
<td>1.24 gm/kgbw</td>
<td>8</td>
</tr>
<tr>
<td>4.</td>
<td>Shatapushpa Churn High dose</td>
<td>Female</td>
<td>1.85 gm/kgbw</td>
<td>8</td>
</tr>
<tr>
<td>5.</td>
<td>Letrozole (PCOS induced)</td>
<td>Female</td>
<td>0.5 mg/kgbw</td>
<td>8</td>
</tr>
<tr>
<td>6.</td>
<td>Metformin</td>
<td>Female</td>
<td>300 mg/kgbw</td>
<td>8</td>
</tr>
</tbody>
</table>

**PREPARATION OF DOSES:**

- 0.1457 gm of Shatapushpa Churn were dissolved in 300 µl of cow ghee (for one day one animal) (Shatapushpa Churn Low Dose 0.62 gm/kg bw).
- 0.2914 gm of Shatapushpa churn were dissolved in 300 µl of cow ghee mid dose (for one day one animal) (Shatapushpa Churn Mid Dose 1.24 gm/kg bw).
- 0.434 gm of Shatapushpa Churn were dissolved in 300 µl of cow ghee (for one day one animal) High dose 1.85 gm/kgbw were prepared.
- For Metformin 300 mg/kg B.W dose was prepared by dissolving 108 mg of metformin in 300 µl of distilled water (for one day one animal).

**TREATMENT AND DURATION:**

The test substance (Shatapushpa Churn) was administered orally for 45 days after confirming the PCOS condition. Animals from the Control Group were treated with a vehicle alone. Dose volume was adjusted based on the bodyweight of the individual animal. Except for treatment with the test item, animals in the control group were handled in an identical manner to those in the test groups. The dose-volume administered to each animal was calculated based on a constant factor of 1m 1aL/kg.
OBSERVATIONS:

1. Estrus Cyclicity

   Everyday estrus cyclicity was monitored by examination of vaginal smears.

2. Body Weight

   Body weights were recorded once before the commencement of treatment and weekly thereafter until the treatment period.

3. Feed Intake

   Daily feed intake was monitored.

4. Biochemical Parameters

   Before sacrificing animal blood, samples were taken by retro orbital puncture into different Eppendorf tubes. The blood samples were centrifuged at 3000 rpm for 15 minutes, and serum was separated for biochemical analysis. The serum was kept in a freezer at -20°C. Before analysis, all serum samples were thoroughly mixed. Total Cholesterol, Triglycerides, HDL, LDL, Total Protein, Albumin, Globulin, Albumin / Globulin ratio, Glucose, Creatinine, Urea, Uric acid, Bilirubin, ALT, and AST were determined following standard procedures described in the product kit (Erba Manheim, Germany) with automated biochemical analyzer (EM 200, Erba Mannheim).

5. Oral Glucose Tolerance Test (OGTT)

   OGTT was performed after 12 hr fasting for all rats in the experiments (Buchanan et al., 1991). Blood samples were collected by retro-orbital puncture in fluoride-coated anticoagulant vials. Next, glucose (2 g/ kg body weight) was orally fed to the rats, and blood samples were collected after time intervals of 0, 30, 60, 90, and 120 minutes. The blood was subjected to 3000 rpm for 10 min and the plasma separated. Glucose was estimated using GOD-POD based kits.

6. Serum Steroidogenic hormone and ovarian enzyme level

   Serum Testosterone estimation

   The serum concentration of testosterone was measured by using a commercially available ELISA kit (DBC, Ontario, Canada), following the immunoenzymatic methods as per the protocol given in the kit.
7. Histology of Ovary

Animals were sacrificed by CO2 asphyxiation, and necropsy was done for all animals. One ovary was fixed in Bouins Fixative. Histological examinations of ovaries from all groups were carried out using standardized histological methods. Sections of 5 μm thickness were cut in a Paraffin-embedded block and stained with Hematoxylin-Eosin.

RESULTS:

1. Estrous Cyclicity of rats between control and treatment with Shatapushpa churn group.
   
   Observation:
   
   After induction of PCOS with Letrozole rats showed irregularity in their estrous cycle; PCOS Induced the untreated group compared to vehicle control. After treatment with Shatapushpa Churn-Low, Mid and High dose for 45 days, there was an improvement in estrus cyclicity. All treatment groups, along with Metformin treated group showing a nearly normal estrus cycle. Only Group V is a PCOS group which shows a disturbed estrus cycle.

2. Weekly Body weight.

Weekly body weight of Letrozole-induced PCOS rats exhibited a significant weight gain.

3. Biochemical Parameters.

Shatapushpa Churn, Letrozole and metformin treatment biochemical parameters were found within a range compared to vehicle control.

4. Haematology

Observation:

Treatment groups (G-II, III, IV) and recovery group (G-VI) Blood parameters found within the range as compare to control group. In Group V which is PCOS group shows changes in value of PCV, MCHC & L as compare to Control group.

5. Oral Glucose Tolerance Test

OGTT profile showed the Letrozole induced PCOS groups increase glucose intolerance as compare to control group. In contrast, Shatapushpa Churn shows normal level in the OGGT profile. It is almost comparable with the Metformin is recovery group.
Observation

After induction of PCOS with Letrozole (Group II, III, IV, V and VI), rats showed high insulin Tolerance as compare to control group. But after treatment with Shatapushpa churn for 45 days, there was an improvement in insulin tolerance as compare to positive control. All treatment groups, along with Metformin treated group showing normal insulin tolerance.

6. Serum Steroidogenic hormone and ovarian enzyme level

Effect of Shatapushpa Churn was assessed by serum concentration of Testosterone, Estradiol, Progesterone, Follicle Stimulating Hormone level.

7. Serum Testosterone level and Serum Estradiol level.

Observation

Total serum Testosterone:
Letrozole-induced PCOS rats (Positive Control) showed increased in Testosterone levels as compared to vehicle control. On treatment with Shatapushpa churn (Low, Mid and High Dose) Testosterone levels were significantly decreased as compared to PCOS induced group as well with Metformin, Testosterone levels were decreased as compared to PCOS induced group (Positive Control) which is protective group.

Total serum Estradiol:
Letrozole-induced PCOS rats (Positive Control) showed decreased in Estradiol levels as compared to vehicle control. On treatment with Shatapushpa churn (Low, Mid and High Dose) Estradiol levels were significantly increased as compared to PCOS induced group as well with Metformin, Estradiol levels were increased as compared to PCOS induced group (Positive Control) which is protective group.

8. Serum Progesterone level.

Letrozole-induced PCOS rats (Positive Control) showed decreased progesterone levels as compared to vehicle control. On treatment with Shatapushpa churn (Low, Mid and High Dose) progesterone levels were nonsignificant as compared to PCOS induced group as well with Metformin, Progesterone levels were increased as compared to PCOS induced group (Positive Control) which is protective group.
9. Follicle Stimulating Hormone (FSH)

Letrozole-induced PCOS rats (Positive Control) showed decreased progesterone levels as compared to vehicle control. On treatment with Shatapushpa churn (Low, Mid and High Dose) progesterone levels were nonsignificant as compared to PCOS induced group as well with Metformin, Progesterone levels were increased as compared to PCOS induced group (Positive Control) which is protective group.

10. Ovary follicles count

Observation

Atretic follicle Count is increased in Letrazole treated PCOS rats as compared to control group. Where as primordia follicle count is also different in treated group as compared to control group.

11. Histopathology

Effect of oral administration of Shatapushpa Churn on histology of ovary in Letrozole induced PCOS rats.

Group 1. Control rat showing normal follicular development (x4)

Group 2, 3 & 4. Section of ovary from Shatapushpa Churn treated group showing normal follicle growth (x4).

Group 5. Section of ovary from Letrozole treatment leading to the formation of cysts in the ovary. (x4).

6. Section of ovary from Metformin treated group showing normal primary follicle growth (x4).

CONCLUSION

On treatment with Shatapushpa Churn decrease in weight gain was observed as compared to PCOS induced untreated group (positive control), glucose levels were significantly restored to normal level at 90 and 120 min as compared to PCOS induced untreated group which is also comparable with the recovery group (metformin group). Letrozole-induced PCOS untreated rats (positive control) shows an increase in Testosterone level compared to Vehicle control. On the contrary, though the treatment with Shatapushpa Churn was observed on testosterone levels in PCOS-induced untreated rats. As far as biochemical parameters are concerned, they remain well within the range after Shatapushpa Churn, and Metformin treatment. Histopathology of the ovary showed normal follicular growth in Shatapushpa Churn treated rats like vehicle control and recovery group (metformin group). The present study indicates that Shatapushpa Churn (low, Mid and high dose) can be used to treat PCOS.
REFERENCE:
