Biochemical and Hormonal Profile of Letrozole Induced Polycystic Ovarian Syndrome in Wistars Albino Rats treated with Cynodon dactylon.

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ABSTRACT

The polycystic ovarian syndrome is associated with increases in androgen, hyperinsulinemia, secretion of a high level of luteinizing hormone, weight gain, anovulation, cyst formation, etc. Metformin is the drug used for treating PCOS with like nausea, vomiting, etc. in order to prevent the side effect and to find a better cure, and then metformin the present drug was selected Cynodon dactylon is commonly called as Arugampul, which is used for diabetes, antioxidant, anti-hyperlipidemic, etc. Materials and method: The animals were selected based upon the weight (125-150 gm) an oestrus cycle. 36 Wistars albino rat was taken and divided into six groups with each group six animals. Control group, Induced (PCOS) group, Referral (Metformin) group, Treatment group (C.dactylon) 500mg/kg , 1,000 mg/kg, 1,500 mg/kg. The animals were induced for PCOS first 21 days by giving Letrozole excluding the control group and examined for PCOS by vaginal smear. In the second 21 days, the animal was treated with the drug and Metformin, leaving the PCOS induced group. Results: There was a significant change with control and PCOS group, PCOS and drug & Metformin group. We were able to observe very high significant changes in lipid profile and also significant changes in hormonal and glucose profiles of C. dactylon group compared metformin group. The present study shows C. dactylon treats PCOS better than Metformin and bring backs the animals for normal condition.

INTRODUCTION

The polycystic ovarian syndrome is a heterogeneous disorder with an incident of 7% and it increases every year by 0.56% to 1.14% worldwide and in India, it is about 2.2 to 26% (Nidhi et al., 2011; Christensen et al., 2013). The syndrome is associated with an increase in androgen, hyperinsulinemia, secretion of a high level of luteinizing hormone, weight gain, anovulation, cyst formation, amenorrhea leading to hirsutism and ovarian cancer (Mohan and Vignesh, 2007; Pasquali et al., 2011). LH and FSH are the hormones that control the menstrual cycle. This is regulated by the pulse frequency of hypothalamic GnRH secretion.
An abnormal change in this pulse frequency leads to polycystic ovarian syndrome (Wildt et al., 1981; Reame et al., 1984; Hayes, 1998). Metformin is the present treatment for PCOS, which is an insulin sensitizer with more side effects like nausea, vomiting, mood change, etc. (Mathur et al., 2008; Soyman et al., 2017). In order to prevent the side effect and to find a better cure, Cynodon dactylon. the present drug was selected.

Cynodon dactylon is commonly called Arugampul, Bermuda grass, or dog’s tooth grass (Figure 1). The creeping grass is mainly seen in 45 degrees north and 45 degrees south latitude (Asthana et al., 2012; Rita et al., 2012). Arugampul is used for offerings in temple and various medicinal uses like diabetes (Mahesh and Brahateeswaran, 2007; Madhankumar, 2016), antioxidants (Eskandary et al., 2017; Pawaskar and Sasangan, 2017) anti diarrheal (Rahman et al., 2015) antihyper lipidemic (Kaup et al., 2011), antimicrobial and antifungal (Rao et al., 2011; Bagewadi et al., 2014), anti-inflammatory and wound healing (Garg and Paliwal, 2011; Thakare et al., 2011), and anticancer (Venkateswarlu et al., 2015). The present study is to find alternative natural medicine to treat PCOS without the side effect of the modern drug and a better cure.

MATERIALS AND METHODS

The study was designed in Sri Lakshmi Narayana Institute of Medical Sciences Pondicherry and was done in JKK Munirajah Medical Research Foundations College of Pharmacy, Tamil Nadu, after obtaining the proper clearance from institutional animal ethical committee. 36 Wistars albino rat was taken and divided into five groups with each group six animals. Control group, Induced (PCOS) group Letrozole 1mg/kg with 0.5% Carboxymethyl cellulose, Referral (Metformin) group (100mg/kg with 0.5% Carboxymethyl cellulose (500mg/kg), Treatment group (C. dactylon) 500mg/kg, 1,000 mg/kg,1,500 mg/kg with 0.5% CMC.

Plant Material

Cynodon dactylon plant was collected from the campus of Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry. The plant was cleaned with distilled water and air-dried in the room temperature and grinded to powder in the grinding machine. The 100 gm of plant powder was mixed with 1000 ml of distilled water and heated till boiling temperature. The mixture was filtered using Whatman’s no: 1 filter paper. Then lyophilized to powder form.

Experimental design

The animals were selected based upon the weight 125-150 gm and oestrus cycle. The animals were kept in a polypropylene cage with free access to food and water. The animals were examined for the normal vaginal cycle. In 1st phase except for the control animals, all the animals are induced for PCOS by giving Letrozole with an oral gavage for 21 days and the vaginal smear was examined to confirm PCOS. In the 2nd phase (Drug) 22-42 days, the animals were treated with the drug and Metformin. The animals were divided into five groups as Induced group, treatment group with 500 mg, 1,000 mg, 1,500 mg and Referral group.

Bio-Chemical Analysis

After 24 hrs of the last dose of the drug and metformin, the animals were anesthetized with overdose as per the standard animal experimental procedure. The blood was collected in a vacutainer tube (serum, plasma, and EDTA) by direct heart puncture. The serum tube was allowed to clot and centrifuged at 3000rpm for 15 minutes and serum was separated and kept stored at-20 degrees. This serum was used for the estimation of the hormonal assay (LH, FSH, Estradiol, and Testosterone) using Enzyme-linked immunosorbent assay (ELISA). Lipid profile parameters (HDL-C, LDL-C, total cholesterol and triglyceride levels) and plasma glucose were analyzed by Merck kit method using an auto analyzer.

Statistical Analysis

Statistical analysis was done using one-way analysis of variance (ANOVA) and Tukey’s post hoc test for multiple comparisons to test the significant (P < 0.05) between groups using SPSS program v14.00. The Result values are shown with Mean±S.D.

RESULTS AND DISCUSSION

The Follicular Stimulating Hormone was significantly (P<0.05) increased in Induced group 0.148±0.013 mIU/ml compared with control 0.102±0.012 mIU/ml and was significantly (P<0.05) decreased with Treatment 500mg group 0.035±0.009 mIU/ml and Referral group 0.064±0.007 mIU/ml. (Table 1)

Luteinizing hormone was significantly (P<0.05) increased in Induced group 0.869±0.05mIU/ml compared with control 0.305±0.015 mIU/ml and was significantly (P<0.05) decreased with Treatment 500mg group 0.035±0.020 mIU/ml and Referral group 0.195±0.012 mIU/ml.

Estradiol was significantly (P<0.05) increased in Induced group 20.6±2.20 pg/ml compared with control 33.0±3.20 pg/ml and was significantly (P<0.05) increased with Treatment 500mg group 52.4±6.86
Table 1: Effect of C. dactylon in Hormonal profile

<table>
<thead>
<tr>
<th>Group</th>
<th>FSH (mIU/ml)</th>
<th>LH (mIU/ml)</th>
<th>Estradiol (pg/ml)</th>
<th>Testosterone (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.102±0.012</td>
<td>0.305±0.015</td>
<td>33.0±3.20</td>
<td>132.57±47.07</td>
</tr>
<tr>
<td>Induced</td>
<td>0.148±0.013#</td>
<td>0.869±0.05#</td>
<td>20.6±2.20#</td>
<td>200.51±13.12#</td>
</tr>
<tr>
<td>Treatment 500mg/Kg</td>
<td>0.035±0.009*</td>
<td>0.106±0.020*</td>
<td>52.4±6.86*</td>
<td>156.46±26.39</td>
</tr>
<tr>
<td>Treatment 1000mg/Kg</td>
<td>0.104±0.049</td>
<td>0.416±0.013</td>
<td>38.88±1.33</td>
<td>100.73±33.32*</td>
</tr>
<tr>
<td>Treatment 1500mg/Kg</td>
<td>0.046±0.006</td>
<td>0.160±0.030</td>
<td>37.73±6.06</td>
<td>116.01±7.43</td>
</tr>
<tr>
<td>Referral</td>
<td>0.064±0.007*</td>
<td>0.195±0.012*</td>
<td>50.60±5.99*</td>
<td>128.09±23.83*</td>
</tr>
</tbody>
</table>

Showing the hormonal profile of Control group, PCOS group, Drug group 500, 100, 1500, Metformin group. *P<0.05, #P<0.01 compared with PCOS group VS Drug&Metformin, #P<0.05 #P- compared with Control group VS PCOS group.

Table 2: Effect of C. dactylon in Lipid profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol mgs/dl</th>
<th>Triglycerides mgs/dl</th>
<th>HDL Cholesterol mgs/dl</th>
<th>LDL Cholesterol mgs/dl</th>
<th>Cholesterol mgs/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.60±8.38*</td>
<td>30.00±4.61*</td>
<td>31.00±0.89*</td>
<td>18.21±8.75</td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>120.80±1.42#</td>
<td>77.80±4.44#</td>
<td>45.80±1.24#</td>
<td>35.56±1.68#</td>
<td></td>
</tr>
<tr>
<td>Treatment 500mg/Kg</td>
<td>65.37±3.74**</td>
<td>35.00±3.24**</td>
<td>40.37±2.18**</td>
<td>20.80±4.93**</td>
<td></td>
</tr>
<tr>
<td>Treatment 1000mg/Kg</td>
<td>48.33±9.25*</td>
<td>32.20±5.27*</td>
<td>32.54±5.74</td>
<td>15.44±2.65</td>
<td></td>
</tr>
<tr>
<td>Treatment 1500mg/Kg</td>
<td>39.00±4.93</td>
<td>58.33±6.11</td>
<td>22.00±2.85</td>
<td>18.66±3.70</td>
<td></td>
</tr>
<tr>
<td>Referral</td>
<td>42.00±1.41*</td>
<td>81.00±22.93*</td>
<td>22.88±1.62*</td>
<td>16.20±2.05*</td>
<td></td>
</tr>
</tbody>
</table>

Showing the Lipid profile of Control group, PCOS group, Drug group 500, 100, 1500, Metformin group. *P<0.05,**P<0.001, #P<0.01 compared with PCOS group VS Drug & Metformin, #P<0.05 #P- compared with Control group VS PCOS group.

Table 3: Effect of C. dactylon in Glucose profile

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (mIU/ml)</th>
<th>Glucose mgs/dl</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.135±0.006*</td>
<td>110.04±30.24*</td>
<td>5.24±0.85*</td>
</tr>
<tr>
<td>Induced</td>
<td>0.201±0.019#</td>
<td>298.0±14.62#</td>
<td>10.06±0.04#</td>
</tr>
<tr>
<td>Treatment 500mg/Kg</td>
<td>0.155±0.011</td>
<td>168.35±50.10*</td>
<td>6.90±1.40</td>
</tr>
<tr>
<td>Treatment 1000mg/Kg</td>
<td>0.106±0.058**</td>
<td>114.22±23.97**</td>
<td>5.38±0.67*</td>
</tr>
<tr>
<td>Treatment 1500mg/Kg</td>
<td>0.373±0.038</td>
<td>201.56±33.92</td>
<td>7.83±0.95</td>
</tr>
<tr>
<td>Referral</td>
<td>0.138±0.105*</td>
<td>124.20±37.36*</td>
<td>5.66±1.04*</td>
</tr>
</tbody>
</table>

Showing the Glucose profile of Control group, PCOS group, Drug group 500, 100, 1500, Metformin group. *P<0.05,**P<0.001, *P&**P- compared with PCOS group VS Drug & Metformin, #P<0.05 #P- compared with Control group VS PCOS group.
pg/ml and Referral group 50.60+5.99 pg/ml

Testosterone was significantly ($P<0.05$) increased in Induced group 200.51+13.12 ng/dl compared with control 132.57+47.07 ng/dl and was significantly ($P<0.05$) decreased with Treatment 500 mg ($P<0.001$) group 65.37+3.74 mg/dl and was significantly increased in PCOS and decreased in Treatment and Referral groups, which agrees with the Muddasir Basheer study ($P<0.05$) group 40.00+1.41 mg/dl and Referral group 128.09+23.83 ng/dl.

Fasting Glucose level was significantly ($P<0.05$) increased in Induced group 298.0+14.62 mg/dl compared with control 110.04+30.24 mg/dl and was significantly ($P<0.05$) decreased with Treatment 500 mg ($P<0.001$) group 114.22+23.97 mg/dl, Treatment 500 mg ($P<0.05$) group 168.35+50.10 mg/dl and Referral group 124.20+37.36 mg/dl.

HbA1c level was significantly ($P<0.05$) increased in Induced group 124.20+37.36 mg/dl compared with control 132.57+47.07 ng/dl and was significantly ($P<0.05$) decreased with Treatment 500 mg ($P<0.001$) group 114.22+23.97 mg/dl, Treatment 500 mg ($P<0.05$) group 168.35+50.10 mg/dl and Referral group 124.20+37.36 mg/dl.

The animals were induced for PCOS using Letrozole, which is an aromatase inhibitor. This elevates the insulin, testosterone and androgen makes the animals acyclic. Aromatase inhibitor also leads to metabolic disturbances, which lead to hyper adiposity, multiple cysts in the ovary ($Çınar$ and $Eryılmaz$, 2016; Walters et al., 2012). The existing study also shows Letrozole induces PCOS similar to that of human PCOS ($Jahan$ et al., 2018). $C. dactylon$ has a potential capability in enhancing the reproductive system ($Nayanatara$ et al., 2012). The FSH and LH values were remarkably increased in the Induced group and decreased in Treatment and Referral groups, which agrees with the Muddasir Basheer study ($Basheer$ et al., 2018) and disagrees with other studies ($Vani$ et al., 2018). Estradiol significantly decreased in the Induced group and increased in the Treatment and Referral group, which is similar to the observation of the other ($Demirel$ et al., 2016; Basheer et al., 2018). Testosterone values were increased in PCOS and decreased in Treatment and Referral, which is similar to other results ($Mamata$ et al., 2013; Amoura et al., 2015). $C. dactylon$ was anti-hyperlipidemic ($Kaup$ et al., 2011), its effect were significantly increased in the treatment group compared to Referral and our finding is similar to others ($Radha$ et al., 2014; Karateke et al., 2018). Insulin was increased in the induced group compared to the Control group and there was a significant decrease in Treatment 1000 mg dose and Referral group and relatively $C. dactylon$ it an anti-diabetic medication (Mahesh and Brahateeswaran, 2007). The fasting glucose and HbA1C level increased in induced and control group and there was also a significant decrease in Treatment and Referral group which is similar to finding of others studies ($Jeong$ et al., 2012; Badawi et al., 2018).
CONCLUSION

The present study shows the medicinal effects of C. dactylon in Letrozole induced Polycystic Ovarian Syndrome in rat models. Our study proved that C. dactylon is effective in changing the lipid profile of PCOS compared to Metformin. There were also significant changes in hormonal and glucose profile compared to Metformin. It reverts the animal to better breeding conditions compared to that of Metformin and Control.

REFERENCES


Anandaramajayan Nallathambi and Rajesh Bhargavan, Int. J. Res. Pharm. Sci., 2020, 11(1), 1136-1141


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