Implication of non polar phyto-components of Aloe vera gel in management of Polycystic Ovarian Syndrome

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Introduction
- Polycystic Ovarian Syndrome (PCOS) is recognized as the most common endocrinopathy of women with increased androgen synthesis, disrupted folliculogenesis and insulin resistance.
- Current therapies are oral contraceptive pills and insulin sensitizers, which produces various side effects upon prolong usage.
- Thereby, it was worthwhile to screen herbal alternative that could manage such a complex syndrome.
- A popular herb, Aloe barbadensis Mill., reported for several medical efficacies, one being anti-diabetic property.
- In this context, we have clearly implicated that Aloe vera gel has potential to act as fertility agent (Maharjan et al., 2010, 2014, 2015) and manage PCOS associated complications (Desai et al., 2012).
- “In vitro” studies have shown that Non polar fraction of Aloe vera gel can modulate steroidogenic enzymes.
- Thereby, our interest was to elucidate the molecular targets by which non-polar fractions of Aloe vera gel (AVG) could manage PCOS phenotype.

Objectives
- Development of letrozole (0.5 mg/kg body weight) induced PCOS rat model and validation of model.
- Partially purification of Aloe vera gel based on polarity gradient method and phytochemical analysis of non polar fraction of gel extract.
- Effect of non polar fraction of Aloe vera gel on letrozole induced PCOS rat model- “In vivo” study.

Methods
A. Plan of work Rodent PCOS model
- Adult Virgin Female rats (200-225 Body Weight)
- Control (CMC Control, Vehicle Control, PCOS rats, P4 fraction of AVG (~25 µg/ml))
- (Letrozole 0.5 mg/kg body wt) (Maharjan et al., 2010)
- B. Extraction Method of Aloe vera gel
- Take Mature Aloe vera Leaf and Remove epithemis
- Make homogenous gel
- Take equal Volume of gel and Petroleum ether (1:1)
- Mix vigorously and keep in shaker for 24 hrs
- Separate organic layer and evaporate completely
- Re-suspend extract in olive oil

Results

1. Histological analysis

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Testosterone (ng/ml)</th>
<th>Estradiol (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Insulin (µIU/ml)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.41±0.08</td>
<td>0.75±0.01</td>
<td>66.0±3.0</td>
<td>7.3±1.66</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>PCOS</td>
<td>1.1±0.15**</td>
<td>0.5±0.2</td>
<td>27.6±2.4</td>
<td>17.6±0.8**</td>
<td>4.2±0.12***</td>
</tr>
</tbody>
</table>

2. Hormonal profile

HPTLC Analysis
- Track 1: P4 fraction
- Track 2: Fresh Aloe
- Track 3: Loper 50 ppm
- Track 4: β Sitosterol
- Track 5: Stigmasterol 50 ppm

<table>
<thead>
<tr>
<th>HPLC Analysis</th>
<th>β-Sitosterol (mg/ml)</th>
<th>Stigmasterol (mg/ml)</th>
<th>Lupeol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.7±0.49</td>
<td>3.38±0.48</td>
<td>1.0±0.10</td>
</tr>
</tbody>
</table>

Phytochemical analysis of non polar extract of Aloe vera gel

Role of non polar phyto-components on PCOS rat model
1. Effect of non polar phyto-components of Aloe vera gel on Histology
- Control 4x
- Vehicle Control 4x
- PCOS 4x
- P4 4x

2. Effect of non polar phyto-components of Aloe vera gel on Hormonal profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testosterone (ng/ml)</th>
<th>Estradiol (ng/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Insulin (µIU/ml)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.6±1.1</td>
<td>67±2.5</td>
<td>14.1±5.3</td>
<td>12.3±0.6</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.4±0.2</td>
<td>67.3±6.6</td>
<td>9.5±1.2</td>
<td>13.5±0.76</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>PCOS</td>
<td>12±1.5**</td>
<td>61±3.6</td>
<td>5.5±2.2**</td>
<td>21.0±1.0</td>
<td>4.5±0.3***</td>
</tr>
<tr>
<td>P4</td>
<td>4.5±1.8**</td>
<td>72±14.5</td>
<td>11.5±5.5**</td>
<td>9.1±1.2**</td>
<td>1.4±0.1**</td>
</tr>
</tbody>
</table>

Conclusion
- Letrozole treated rat exhibited no. of peripheral cysts in ovary, glucose intolerance, arrested cyclicity (data not shown) and altered hormonal profile which confirmed PCOS rat model.
- Chromatographic analysis of non polar fraction of AVG suggested that it is enriched with phy-sterols namely sitosterol, stigmasterol, lupeol and other minor sterols.
- Non polar phyto-components of Aloe vera helps to back to normal structure function of ovary by decreasing peripheral cysts, normalized hormonal profile and normal follicular growth.
- Treatment of Non polar fraction of AVG decreased transcripts of STAR, LH Receptor and Insulin Receptor compared to PCOS group; while other proteins-FSH Receptor, Aromatase did not show any significant change.
- Protein expression of STAR, 3βHSD which were altered in PCO phenotype; reverted back as similar to control values when treated with non polar fraction of Aloe vera.
- Non polar fraction of Aloe vera gel has a potential to manage PCOS phenotype, by modifying steroidogenic targets. This study serve as platform for designing drugs by exploring these novel targets.

References

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