# 6

# Role of Isolated Phytocomponents of Non-polar fraction of *Aloe vera* Gel Extract in Letrozole induced PCOS mice model

## 6.1. Rationale

Data from previous chapters have demonstrated the potential of the partially purified non-polar phytocompounds (PPNPP) of *Aloe vera* gel as a steroidogenic and metabolic modulator using *"in-silico"* and *"in-vitro"* assays. The bioactivity of the PPNPP of *Aloe vera* gel- LP1 and LP3 was evaluated in two different *"in-vitro"* PCO- like models, and they demonstrated good efficacy in terms of modulating key targets associated with PCOS phenotype at transcriptional level. Though, cell- based bioassays are the most promising tool for screening of numerous phytochemicals simultaneously (Moore et al., 2014), *"in-vitro"* studies have limitations since they can't simulate how a pharmaceutical drug interacts within a complex and dynamic ecosystem like human body, where multiple signalling pathways work in harmony to give rise to a certain phenotype. *"In-vitro"* studies may find it challenging to forecast the complexity of possible interactions as a result of this. On the other hand, *"in-vivo"* studies can provide a better understanding of the molecular targets of the drugs and their potential interactions with different organs of the body, which can improve its predictions of safety, toxicity, and efficacy.

PCOS is a multifaceted syndrome that affects multiple organ systems with significant metabolic and reproductive manifestations (Williams et al., 2016), thereby testing of efficacy of bio-isolates in an *"in-vivo"* system becomes essential. Therefore, the present study was undertaken to investigate the effects of PPNPP of *Aloe vera* gel- LP1 and LP3 in Letrozole induced PCOS mice model, with the context to hormonal and metabolic pathways for management of PCOS pathology and its comorbidities. The efficacy of letrozole (an aromatase inhibitor) in establishing PCOS in rodents is well documented (Kafali et al., 2004). It acts by inhibition of aromatase, leading to low conversion of androgens to estrogens, resulting in an excessive accumulation of androgens in the ovary (Garcia-Velasco et al., 2005). In this context, several researchers have tried to induce PCOS like characteristics in rodents, however, there is

large ambiguity in the age and species wise difference in the doses, duration and also the mode of Letrozole administration (Kafali et al., 2004; Kelley et al., 2016; Arroyo et al., 2019; Torres et al., 2019; Kauffman et al., 2015; Manneras et al., 2007). With the previous background, we sought to develop a PCOS mouse model in adult female Balb/c mice using Letrozole.

### 6.2. Materials and methods

The methods employed in this study have been discussed in detail in Chapter 2. Briefly, a letrozole induced mouse model was developed in a dose and time dependent manner. The plan of work depicting the treatment regimens is provided in Figure 6.1.

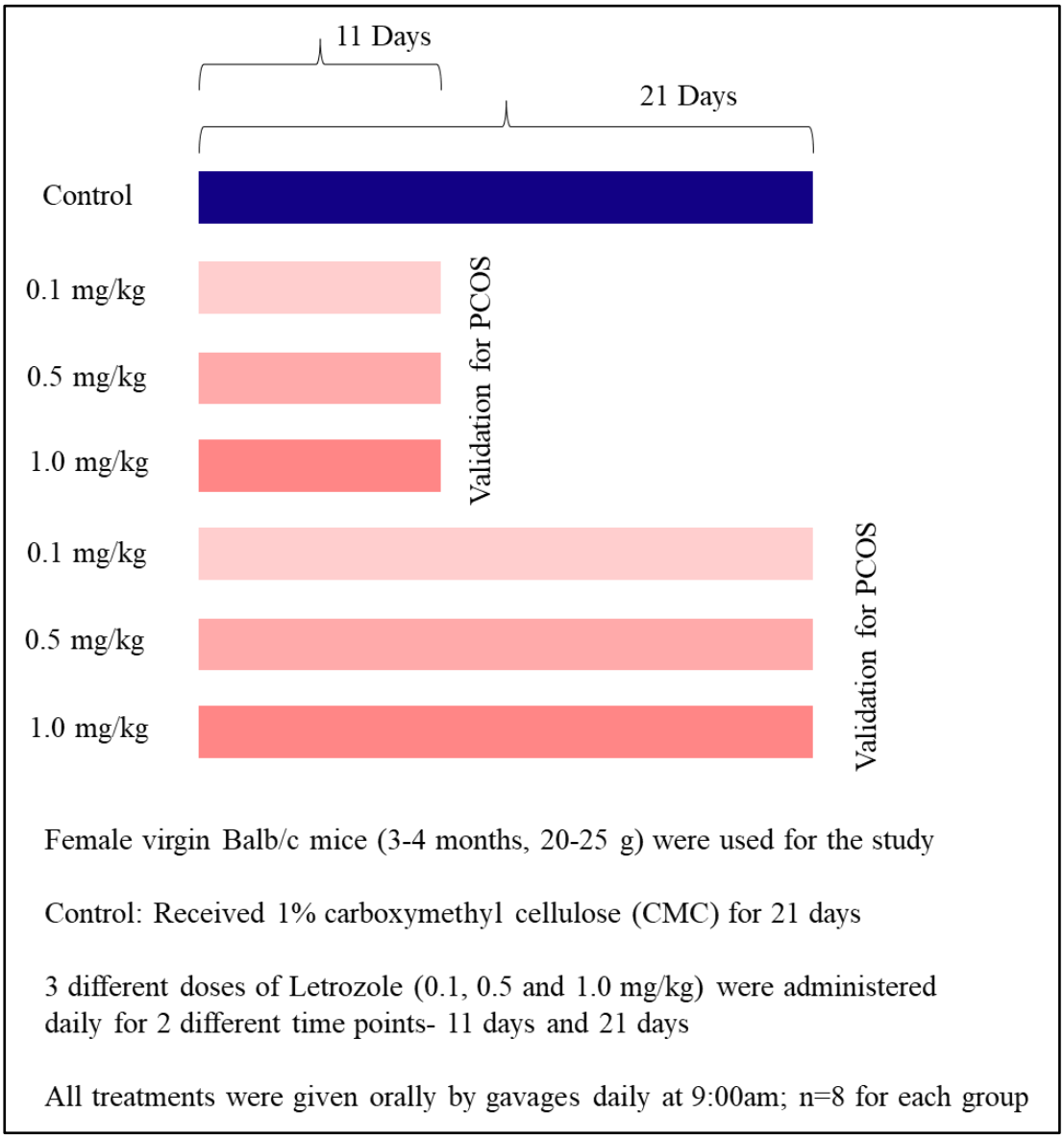
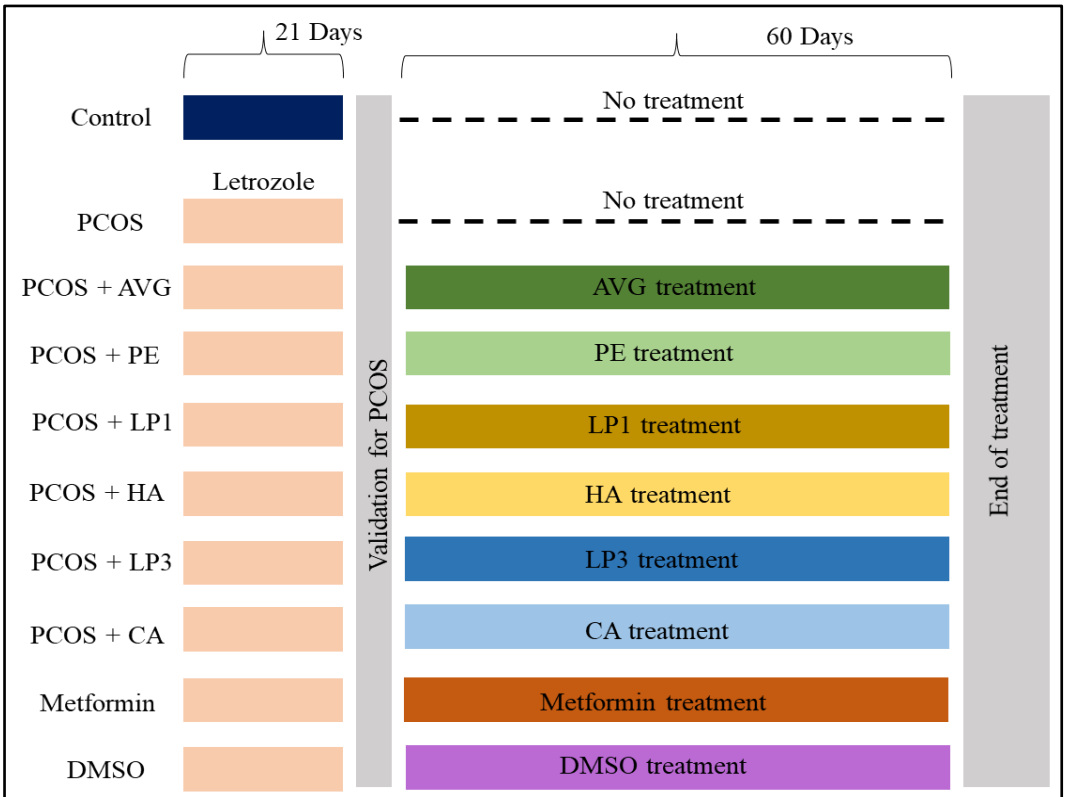



**Figure 6.1** Plan of work for development of PCOS mouse model

After validation of the model based upon Rotterdam's criteria, the animals were further divided into 10 groups as shown in Figure 6.2, for evaluating the effect of phytochemicals on various parameters as previously explained in Chapter 2. Here, metformin was used as a positive control and the efficacy of the phytochemicals were compared with it.




Female virgin Balb/c mice (3-4 months, 20-25 g) were used for the study

Control: Received 1% carboxymethyl cellulose (CMC) daily for 21 days
PCOS: 0.5 mg/kg body weight of Letrozole daily for 21 days
PCOS+AVG: PCOS animals treated with Aloe vera gel (10mg/day) for 60 days
PCOS+PE: PCOS animals treated with Petroleum ether extract of Aloe vera gel (25µg/ kg/day) for 60 days
PCOS +LP1: PCOS animals treated with LP1 (5µg/ kg/day) for 60 days
PCOS+HA: PCOS animals treated with pure compound- n-Hexadecanoic acid (5µg/ kg/day) for 60 days
PCOS +LP3: PCOS animals treated with LP3 (0.5µg/ kg/day) for 60 days
PCOS+CA: PCOS animals treated with pure compound- Campesterol acetate (0.5µg/ kg/day) for 60 days
Metformin: PCOS animals treated with Metformin (100mg/ kg/day) for 60 days and served as Positive Control
DMSO: PCOS animals treated with (1%; 0.2ml/day) for 60 days and served as Vehicle/ Negative Control

All treatments were given orally by gavages daily at 9:00am; n=8 for each group

**Figure 6.2:** Plan of work for evaluating the bioactivity of phytocompounds in Letrozole induced mouse model

## 6.3. Results

### 6.3.1. Development of Letrozole induced PCOS mouse model

For the development of PCOS mouse model, letrozole doses were given as per the plan of work provided in Figure 6.1, and validated for the PCOS phenotype based upon the Rotterdam's criteria (2003). The effect of letrozole treatment on body weight, plasma testosterone levels, estrus cyclicity and ovarian histology of the animals were evaluated.

#### 6.3.1.1. Effect of Letrozole on Body Weight

The body weight of the animals was monitored in all the groups of animals before treatment as well as after 11 days and 21 days of Letrozole treatment respectively. Results from Figure 6.3 shows that higher dose of letrozole treatment (0.5mg/kg and 1.0 mg/kg) for 21 days significantly increased the body weight ($P<0.05$) as compared to day 0 of treatment. Such observations were not seen in case of control animals.

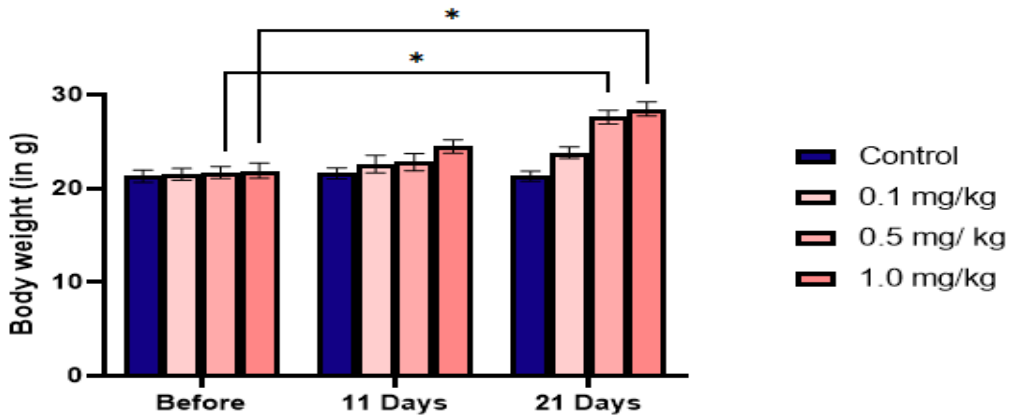



**Figure 6.3:** Dose and time- dependent effect of Letrozole on body weights of adult female Balb/c mice. All values are presented as Mean ± SEM; N=8 per group. *P<0.05 as compared to before treatment group.

#### 6.3.1.2. Effect of Letrozole on the Hormone Profile

Effect of letrozole on the hormone profile of control and treated groups were evaluated. Results from our study demonstrate that higher doses of letrozole (0.5 mg/kg and 1.0 mg/kg) significantly increased the testosterone levels ($P<0.05$) in 11 day and ($P<0.001$) in 21-day treatment, suggesting that both 0.5mg/kg and 1.0 mg/kg of letrozole for 21 days was the minimum dose and time required for development of hyperandrogenism, which is a key feature of PCOS as per Rotterdam's criteria (2003). On the contrary, similar doses of Letrozole treatment caused a significant reduction in the progesterone levels ($P<0.05$; $P<0.01$) as

compared to control (Figure 6.4 [C]). Also, there was a dose dependent decreasing trend observed in the estradiol levels (Figure 6.4 [B]), however, the results were not statistically significant.

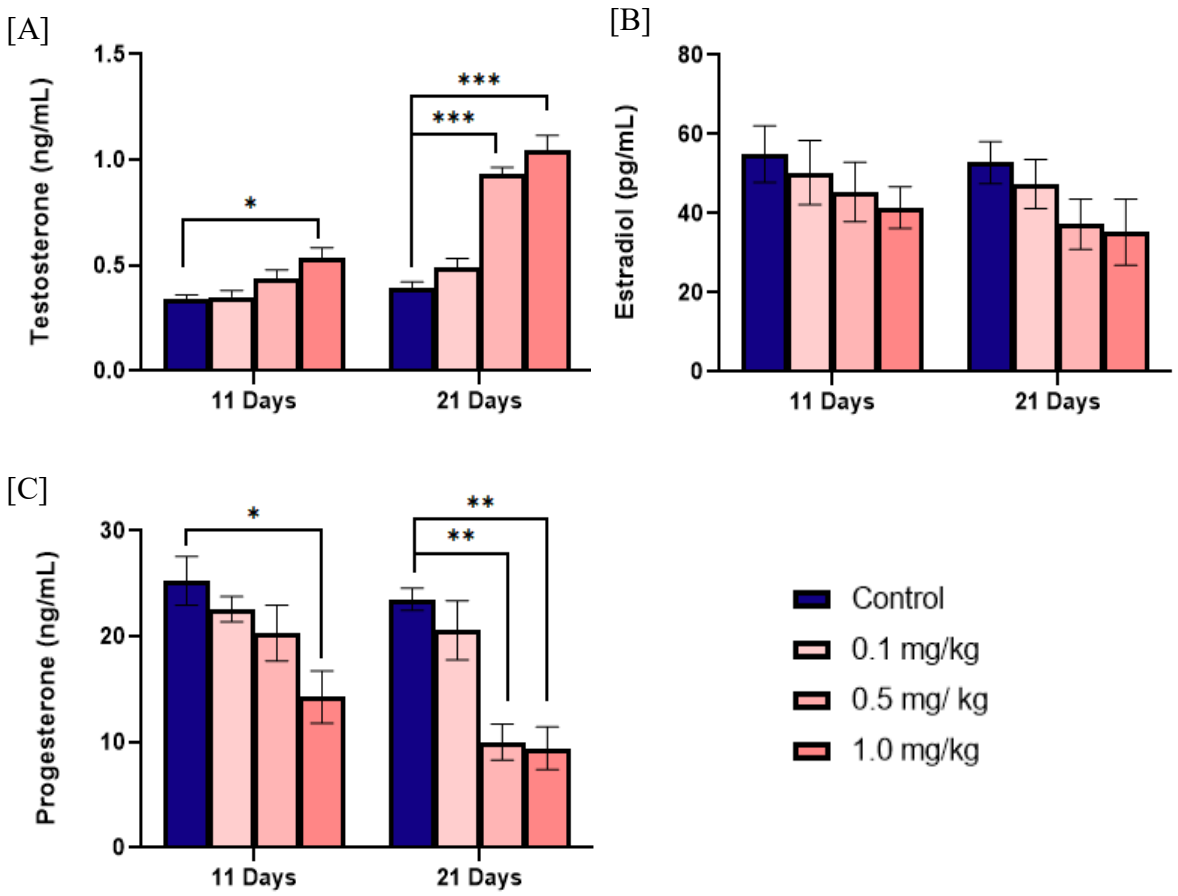



**Figure 6.4:** Dose and time- dependent effect of Letrozole on hormone profile [A] Testosterone; [B] Estradiol and [C] Progesterone levels of adult female Balb/c mice. All values are presented as Mean ± SEM; N=8 per group. *P<0.05; **P<0.01 and ***P<0.001 as compared to control group.

### 6.3.1.3. Effect of Letrozole on Estrus Cyclicity

As an indicator of ovarian function, the estrus cycle was assessed in all animal groups. Table 6.1 demonstrates that all the animals belonging to the control group exhibited a normal estrus cycle. However, treatment with letrozole led to prolonged or arrested estrus cyclicity in the animals, which could be co-related with oligo/anovulation- one of the major determinants of PCOS phenotype according to Rotterdam's criteria (2003). Higher doses of letrozole (0.5 mg/kg and 1.0 mg/kg) for 21 days had more profound impact on the estrus cyclicity of the animals.

**Table 6.1.** Dose and time- dependent effect of Letrozole on the estrus cyclicity of adult female Balb/c mice

| Stages | Control | 0.1 mg/kg body weight for 11 days | 0.1 mg/kg body weight for 21 days | 0.5 mg/kg body weight for 11 days | 0.5 mg/kg body weight for 21 days | 1.0 mg/kg body weight for 11 days | 1.0 mg/kg body weight for 21 days |
|---|---|---|---|---|---|---|---|
| Normal cycle | 100% | 70% | 5% | 20% | - | - | - |
| Extended Proestrus | - | 20% | 5% | - | - | - | - |
| Extended Estrus | - | - | - | - | - | - | - |
| Extended Metaestrus | - | - | 20% | - | 5% | 10% | - |
| Extended Diestrus | - | 10% | 70% | 80% | 95% | 90% | 100% |

### 6.3.1.4. Effect of Letrozole on Ovarian Histology

Histological section of ovaries taken from control and the letrozole treated groups are shown in Figure 6.5. Ovaries from control group exhibited follicles in various stages of development including secondary follicles, tertiary follicles, Graafian follicles and corpora lutea (Figure 6.4 [A]). On the other hand, Letrozole suppressed follicle growth in a dose-dependent and time-dependent manner in the treated groups. Small follicles, as well as follicles with indications of atresia and several huge cysts with virtually no granulosa cell layer or large cystic follicles with sparse granulosa cells, could be seen in early development (Figures 6.5 [E] to [G]). In comparison to the control group, ovaries from the letrozole (0.5 mg/kg and 1.0 mg/kg for 21 days) treated groups had a higher incidence of subcapsular peripheral cysts and capsular thickening, as well as incomplete luteinization and fewer corpora lutea (Figure 6.4 [E] and [G]). Multiple ovarian follicular cysts on the periphery of the ovary are clinical features of PCOS development. Our results demonstrate that higher doses of letrozole (0.5mg/kg and 1.0 mg/kg) for longer duration of time, i.e., 21 days was the minimum dose and time required to disrupt the ovarian structure.

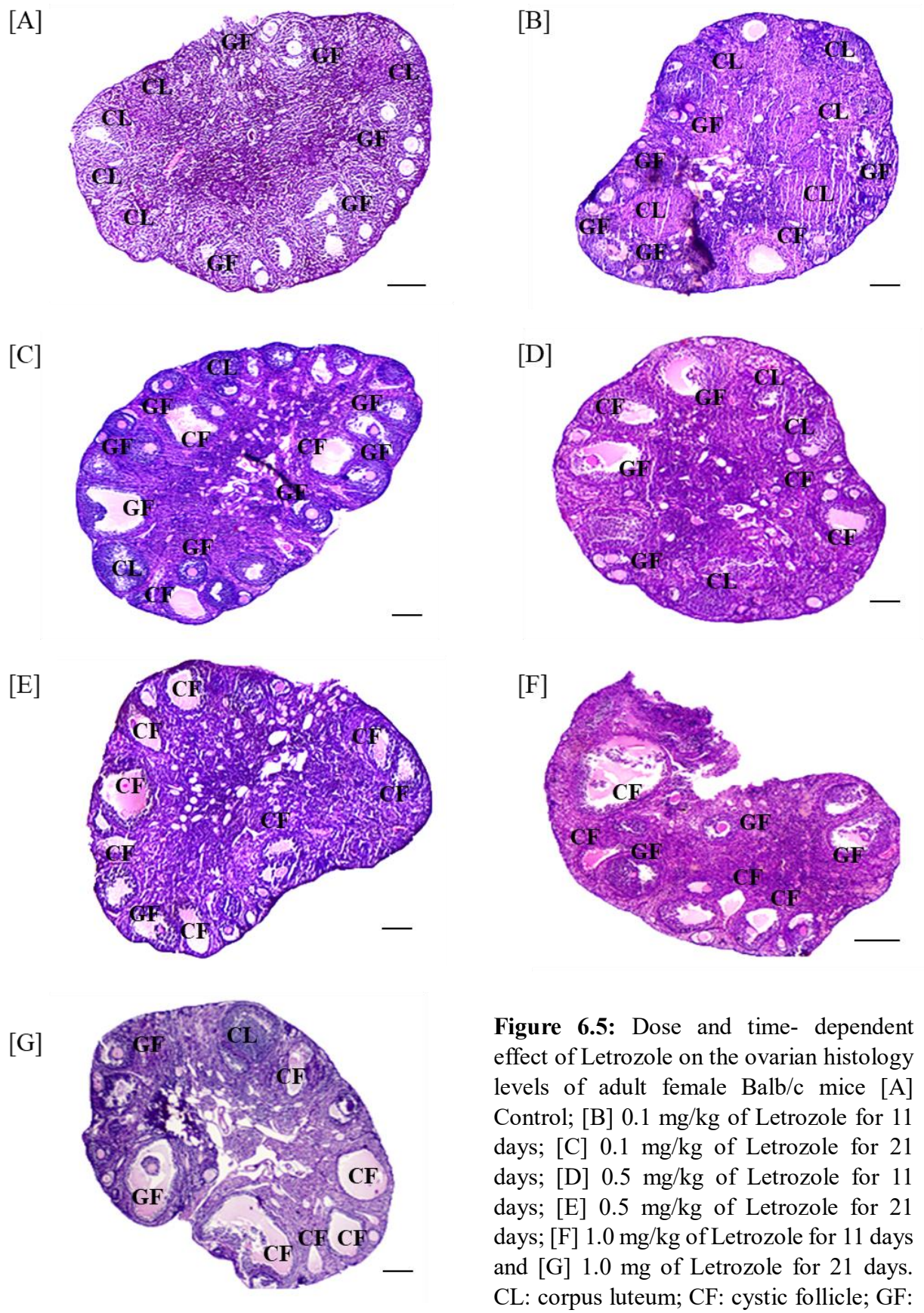



**Figure 6.5:** Dose and time- dependent effect of Letrozole on the ovarian histology levels of adult female Balb/c mice [A] Control; [B] 0.1 mg/kg of Letrozole for 11 days; [C] 0.1 mg/kg of Letrozole for 21 days; [D] 0.5 mg/kg of Letrozole for 11 days; [E] 0.5 mg/kg of Letrozole for 21 days; [F] 1.0 mg/kg of Letrozole for 11 days and [G] 1.0 mg of Letrozole for 21 days. CL: corpus luteum; CF: cystic follicle; GF: Graafian follicle. Magnification 4X. Calibration bar= 100µm

Data in the study clearly demonstrates that 0.5 mg/kg body weight of Letrozole dosage given orally daily for 21 days is the minimum effective dose to potentially develop a PCOS mouse model which is in several ways similar to human pathophysiology.

After successful development and validation of the PCOS mouse model, the next part of the chapter focused on evaluating the bioactivity of the non-polar phytochemicals of *Aloe vera* gel towards management of metabolic and reproductive abnormalities seen in pathology of PCOS.

### 6.3.2. Effects of Different Treatments on Metabolic parameters

#### 6.3.2.1. Effects of Different Treatments on Body Weight

First, the effect of different treatments on the body weight of all groups of animals were evaluated. Results from Figure 6.6 [A] demonstrate that the animal body weight before PCOS induction was similar in all groups. Following a 21-day administration of letrozole, animal body weight increased in PCOS group ($P<0.001$) as compared to the control group of animals. The administration of metformin reversed the effect of letrozole as it decreased animal body weight as compared with the PCOS group ($P<0.05$). The different phytochemical treatments also induced a similar effect, as they decreased the animal weight as compared to PCOS group ($P<0.05$), suggesting that phytochemical treatments potentially decreased the adipose tissue deposition in the abdominal and peri-ovarian regions of PCOS animals.

#### 6.3.2.2. Effects of Different Treatments on Oral Glucose Tolerance, Fasting Insulin, HOMA-IR and gene expression of Insulin Receptor

Oral Glucose Tolerance Test was performed after 60 days of different treatment procedures. Results demonstrated that the Untreated Control animals exhibited normal profile to glucose tolerance upon oral administration of glucose, whereas the letrozole induced PCOS animals showed an increase in the glucose intolerance when compared to untreated control (Figure 6.6 [B]). Blood glucose levels at 30, 60, and 120 min were higher in the PCOS group compared to the control group ($P<0.001$, $P<0.01$). AVG and PE extract of AVG treatment to the PCOS animals exhibited significant improvement in the glucose tolerance in all time point of the test except fasting ($P<0.001$). Further, PPNPP (LP1 and LP3) treated animals as well as their corresponding standards showed significant reduction in glucose levels at different time points over the period of 120 min when compared to the untreated PCOS group ($P<0.01$). The observed changes were comparable to that of Metformin treatment ($P<0.01$), which is the standard drug prescribed for management of PCOS.

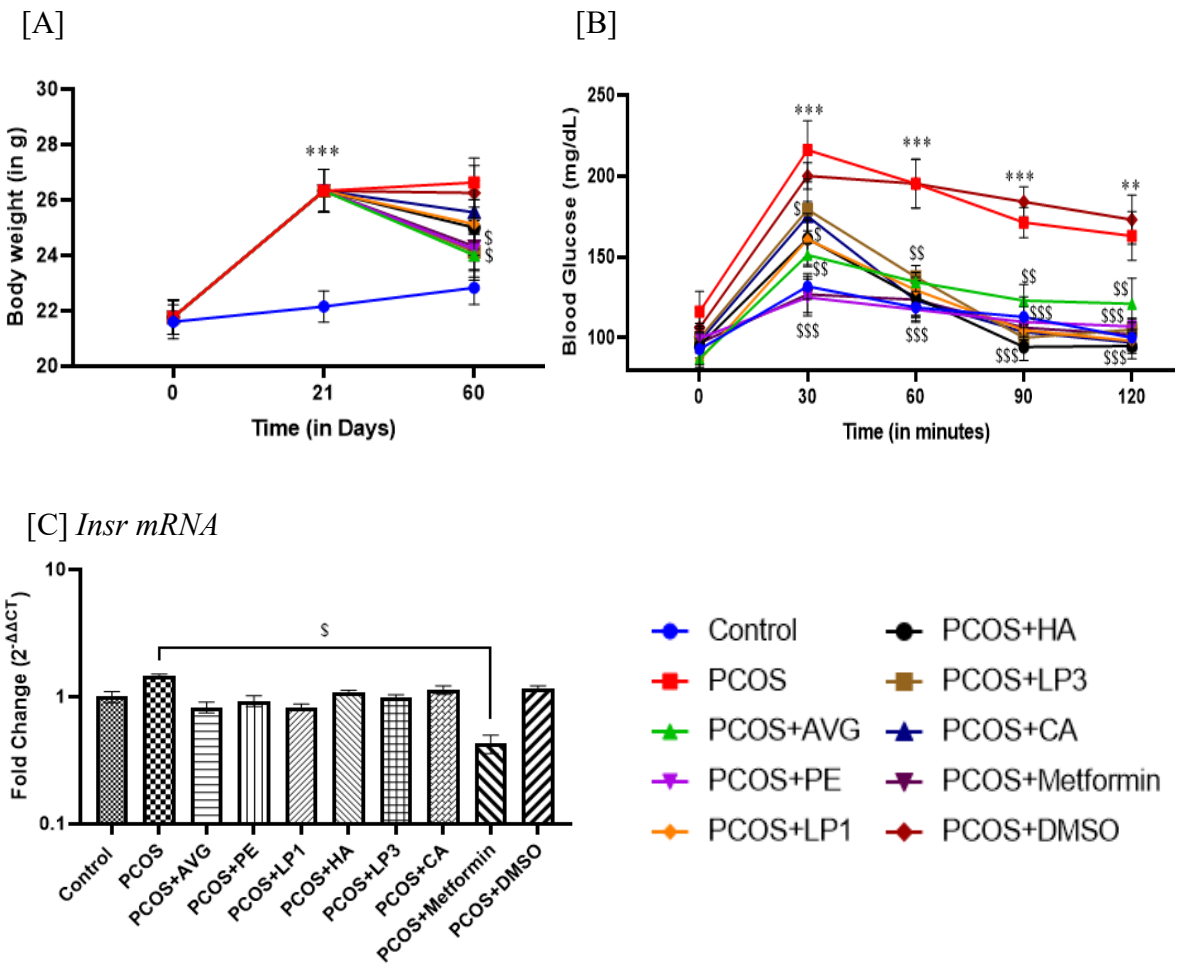



**Figure 6.6.** Effect of different treatments on the [A] Body weight, [B] Oral glucose tolerance test and [C] Fold change in gene expression levels of Insulin Receptor in ovaries of Letrozole induced PCOS mice model. All values are presented as Mean + SEM; N=8 per group. **P<0.01 and ***P<0.001 as compared to control group; $P<0.05, $$P<0.01 and $$$P<0.001 as compared to PCOS group.

Non-significant changes were observed in the fasting glucose levels amongst all the groups of animals. However, PCOS group demonstrated a significant high level of insulin (***p<0.001) as compared to control group (P<0.001), while it reverted back to normal levels after treatment with AVG, PE extract of AVG, PPNPP (LP1 and LP3) and their corresponding commercial standards as well as Metformin (P<0.01) (Table 6.2). At the molecular level, there was no significant difference in the gene expression of *Insr* mRNA in the ovaries amongst all groups of animals (Figure 6.6 [C]), in spite of improved insulin profile in the PCOS animals after different treatments, suggesting that the phytochemicals act at post-receptor level of insulin signalling.

Furthermore, HOMA-IR (an index for insulin resistance) was substantially higher in the PCOS group as compared to the control (P<0.001), metformin (P<0.05), AVG (P<0.01), PE extract of AVG (P<0.01), LP1 and HA (P<0.05) and LP3 and CA (P<0.01) groups (Table 6.2). PCOS animals exhibited insulin resistance, which reverted back to normalcy after the treatment with different phytochemicals as well as whole *Aloe vera* gel and comparable to metformin, which served as positive control. Results demonstrated that LP3, derived from *Aloe vera* gel is a potential glucose and insulin sensitizing agent.

**Table 6.2.** Effect of different treatments on Fasting Glucose, Fasting Insulin and HOMA-IR in plasma of Letrozole induced PCOS mice model

| | Fasting Glucose (mg/dL) | Fasting Insulin (μIU/mL) | HOMA-IR |
|---|---|---|---|
| Control | 93 ± 9.72 | 7.83 ± 0.14 | 1.49 ± 0.18 |
| PCOS | 106.25 ± 9.42 | 14.36 ± 0.67 *** | 4.76 ± 0.09 *** |
| PCOS+AVG | 87.25 ± 5.6 | 7.74 ± 0.09 $$ | 1.27 ± 0.11 $$ |
| PCOS+PE | 99.75 ± 7.5 | 7.68 ± 0.03 $$ | 1.3 ± 0.13 $$ |
| PCOS+LP1 | 86.33 ± 6.1 | 8.25 ± 0.06 $$ | 1.76 ± 0.14 $ |
| PCOS+HA | 95.2 ± 4.1 | 8.28 ± 0.15 $ | 1.95 ± 0.08 $ |
| PCOS+LP3 | 99.8 ± 9.13 | 7.65 ± 0.06 $$ | 1.18 ± 0.17 $$ |
| PCOS+CA | 97.8 ± 4.58 | 7.875 ± 0.10 $$ | 1.2 ± 0.06 $$ |
| PCOS+ Metformin | 96.8 ± 9.16 | 7.60 ± 0.12 $$ | 1.81 ± 0.18 $ |
| PCOS+DMSO | 111 ± 8.6 | 13.01 ± 0.90 | 3.24 ± 0.17 |

All values are presented as Mean + SEM; N=8 per group. ***P<0.001 as compared to control group; $ P<0.05 and $$ P<0.01 as compared to PCOS group.

### 6.3.2.3. Effects of Different Treatments on Lipid Profile

As seen in Figure 6.7, upon administration of letrozole for 21 days, PCOS animals demonstrated a marked increase in the plasma triglyceride levels (P<0.01), whereas plasma

Cholesterol, HDL-C and LDL-C levels remained almost the same as compared to the various control groups. Treatment of PCOS animals with AVG, PE extract of AVG, LP3 and its corresponding commercial standard- CA exhibited a reduction in the triglyceride levels ($P<0.05$), suggesting that LP3, derived from *Aloe vera* gel has excellent property to reduce the circulating elevated triglycerides (suggestive marker of metabolic syndrome).

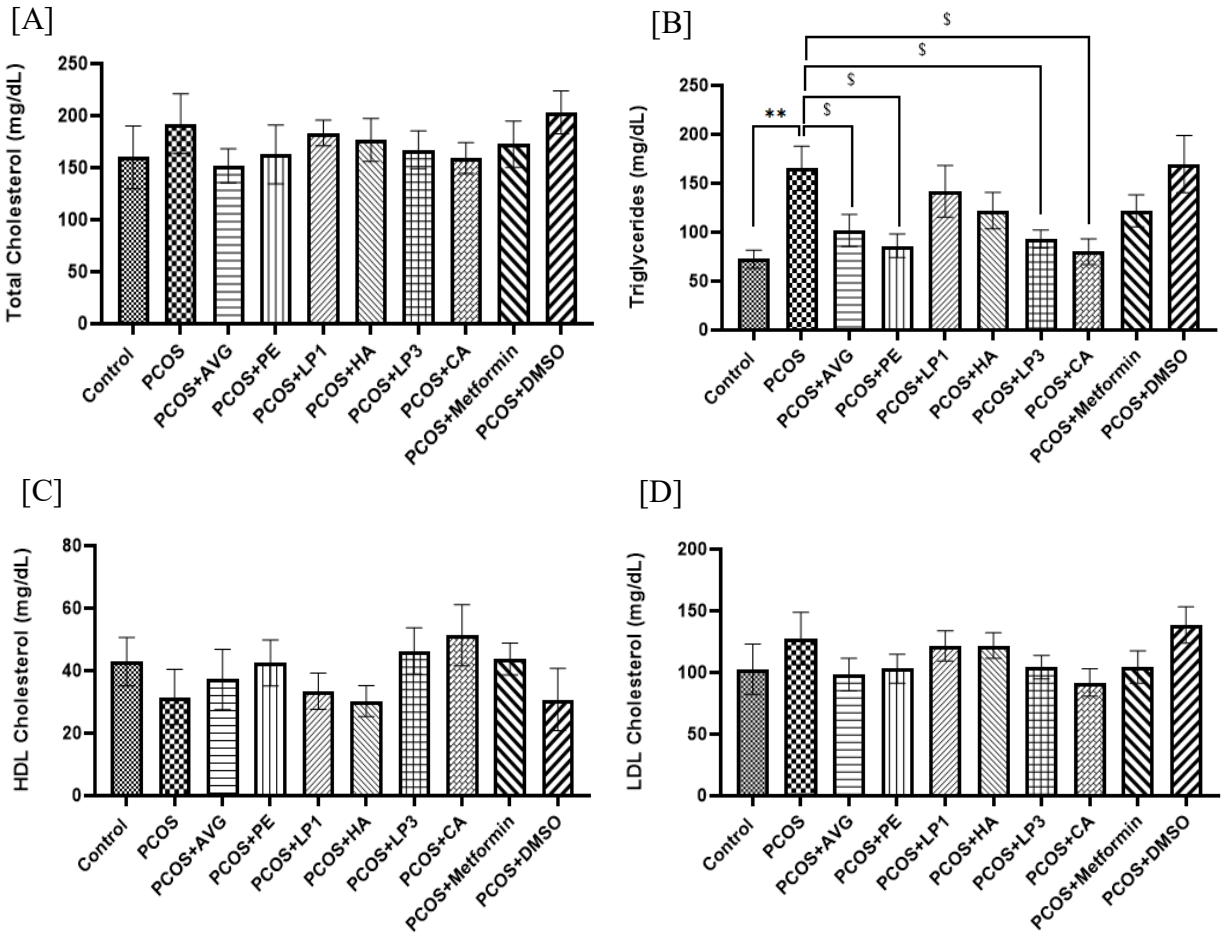



**Figure 6.7.** Effect of different treatments on the Lipid Profile [A] Total Cholesterol, [B] Triglycerides, [C] HDL-Cholesterol and [D] LDL- Cholesterol levels in Letrozole induced PCOS mice model. All values are presented as Mean + SEM; N=8 per group. **P<0.01 as compared to control group; $P<0.05 as compared to PCOS group.

### 6.3.3. Effects of Different Treatments on Reproductive parameters

#### 6.3.3.1. Effects of Different Treatments on Estrus Cyclicity

The estrus cycle was evaluated in all groups of animals as an index of ovarian function. The stage of the estrus cycle was confirmed by vaginal smear. On day 0 (before letrozole treatment), all groups had a regular estrus cyclicity. After 21 days of Letrozole treatment it was observed

that PCOS rats exhibited arrested estrus cyclicity as compared to control animals, that had regular estrus cyclicity throughout the study period (Table 6.3). Remarkably, treatment of PCOS mice with AVG and PE completely reverted the estrus cyclicity to normalcy. Similar results could be observed upon LP3 as well as CA treatment groups. However, treatment with LP1 and HA showed restoration of estrus cyclicity in only 60-75% of animals. The animals treated with Metformin had prolonged estrus and shorter metestrus and diestrus stage. The DMSO treated group demonstrated irregular and arrested estrus cycle as compared to control animals. The estrus cycle is related to alterations in the circulating steroid hormones, reflecting changes in ovarian structure and function.

**Table 6.3.** Effect of different treatments on the estrus cyclicity of Letrozole induced PCOS mice model

| | Normal cycle | Extended Proestrus | Extended Estrus | Extended Metestrus | Extended Diestrus |
|---|---|---|---|---|---|
| Control | 100% | - | - | - | - |
| PCOS | - | - | - | 5% | 95% |
| PCOS+AVG | 100% | - | - | - | - |
| PCOS+PE | 100% | - | - | - | - |
| PCOS+LP1 | 75% | - | - | 15% | 10% |
| PCOS+HA | 60% | - | - | 30% | 10% |
| PCOS+LP3 | 90% | - | 5% | 3% | 2% |
| PCOS+CA | 98% | - | - | - | 2% |
| PCOS+ Metformin | 10% | - | 80% | 5% | 5% |
| PCOS+DMSO | - | - | - | 3% | 97% |

### 6.3.3.2. Effects of Different Treatments on Ovarian Histology

Ovarian sections of the control group exhibited normal ovarian morphology with mature follicles (tertiary and Graafian follicles) and corpora lutea which is an indicator of ovulation.

On the contrary, ovarian histology of Letrozole treated mice showed extremely thin layer of granulosa cells, multiple large subcapsular peripheral cysts, fewer corpus luteum and reduced mature follicles as compared to control animals. The appearance of multiple peripheral ovarian follicular cysts represented the pivotal clinical characteristic during PCOS progression. Treatment of PCOS animals with metformin, AVG, PE and LP3 exhibited mature follicles, corpora lutea and few cystic and atretic follicles (Figure 6.8).

#### 6.3.3.3. Effects of Different Treatments on Hormone Profile

Next, we evaluated steroidogenic function in all groups of animals. The levels of testosterone, estradiol and progesterone were determined using commercially available kits. After induction of PCOS, testosterone levels were significantly increased while progesterone levels were decreased compared with control (Figure 6.9). However, there was no significant difference observed in estradiol levels between control and PCOS animals. Metformin and plant treatment (AVG, PE, LP1, LP3 and their corresponding commercial standards) in PCOS animals significantly reduced ($P<0.05$) testosterone levels. On the contrary, progesterone levels significantly improved upon Metformin ($P<0.01$) and plant treatment (AVG, PE, LP1, LP3 and their corresponding commercial standards) in PCOS animals ($P<0.05$). Both LP1 and LP3 demonstrated anti-androgenic and progestogenic potential in Letrozole induced PCOS mice.

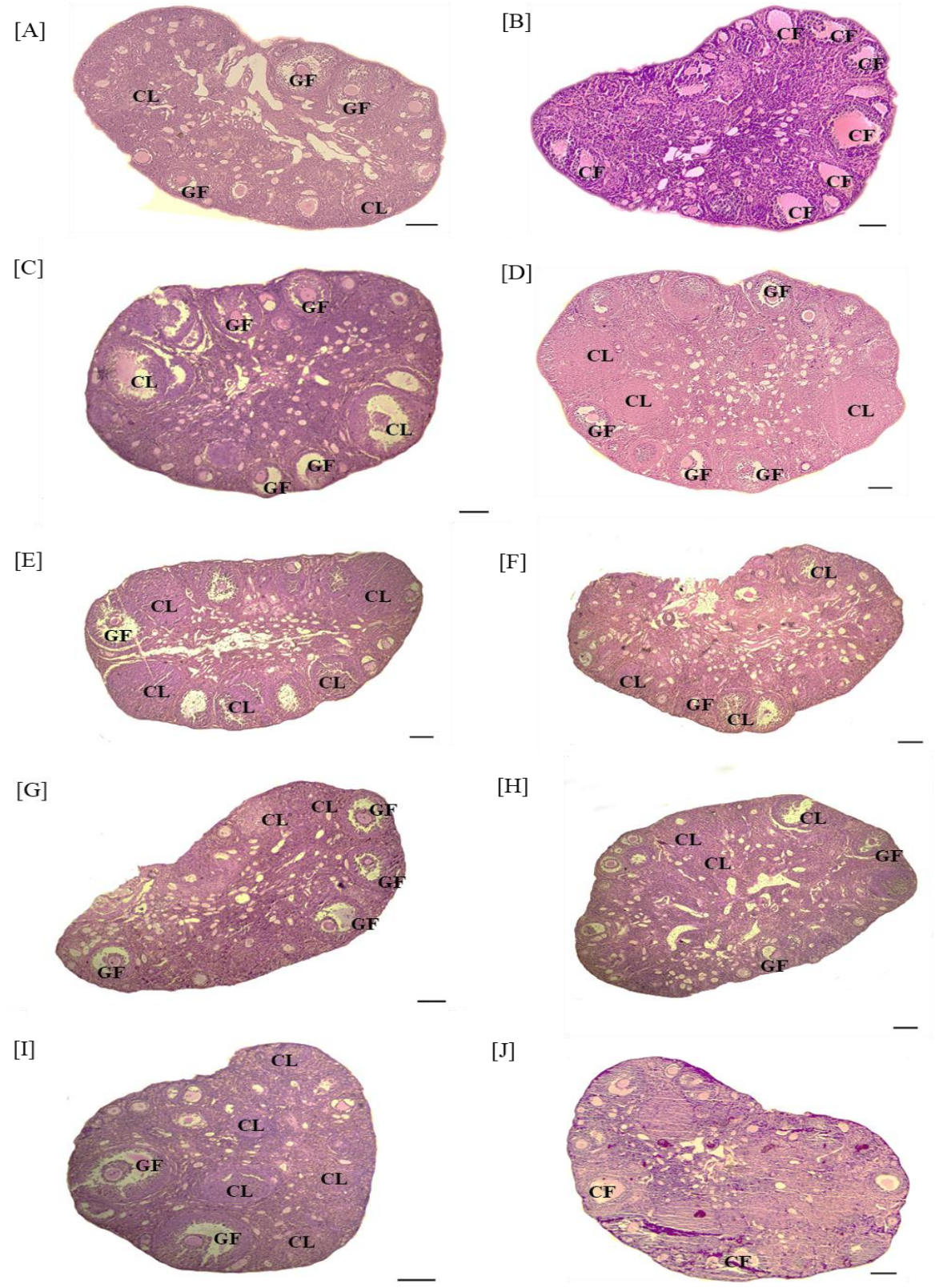



**Figure 6.8.** Effect of different treatments on ovarian histology. [A] Control; [B] PCOS; [C] PCOS+AVG; [D] PCOS+PE; [E] PCOS+ LP1; [F] PCOS+HA; [G] PCOS+ LP3; [H] PCOS+ CA; [I] PCOS+ Metformin and [J] PCOS+ DMSO. CL: corpus luteum; CF: cystic follicle; GF- Graafian follicle. Magnification 4X. Calibration bar =100 µm.

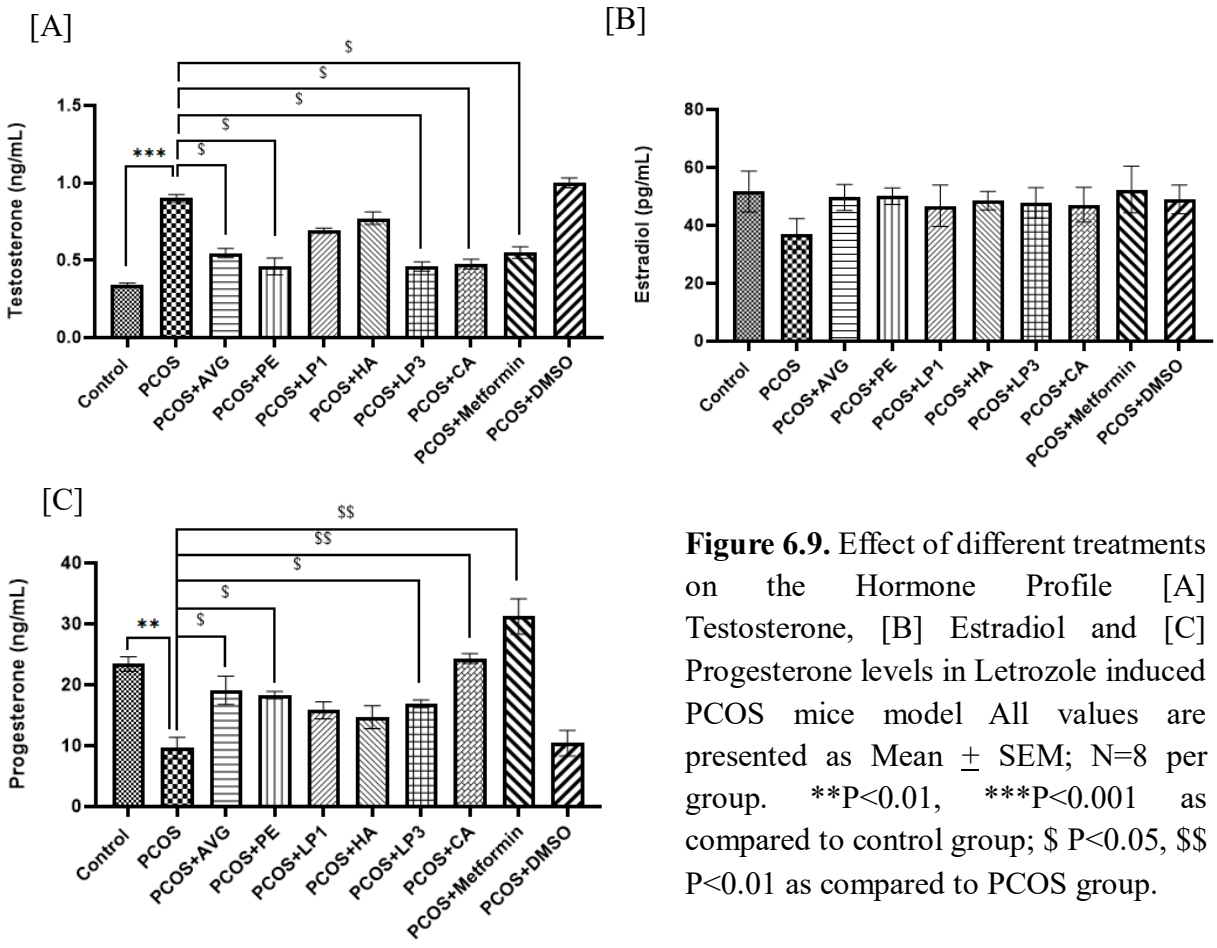



**Figure 6.9.** Effect of different treatments on the Hormone Profile [A] Testosterone, [B] Estradiol and [C] Progesterone levels in Letrozole induced PCOS mice model All values are presented as Mean ± SEM; N=8 per group. **P<0.01, ***P<0.001 as compared to control group; $ P<0.05, $$ P<0.01 as compared to PCOS group.

This study clearly demonstrates that the PPNPP of *Aloe vera* gel have the potential to restore the metabolic and reproductive parameters of Letrozole induced PCOS mice at physiological level. Further, to decipher whether changes observed is due to molecular changes, the gene expression of gonadotropin receptors, steroid receptors and key steroidogenic markers were evaluated.

### 6.3.3.4. Effects of Different Treatments on Gonadotropin Receptors

To estimate the transcript levels of gonadotropin-receptors, we performed real-time RT-PCR using specific primers for *Fshr* and *Lhr*. Data from Figure 6.10 [A] demonstrates that the mRNA levels of *Lhr* were increased in the ovaries of Letrozole-induced mice (P<0.001), however, its induction was effectively decreased upon treatments with Metformin, AVG and PE. It is to be noted that, PPNPP of AVG (LP1 and LP3) did not affect *Lhr* mRNA expression. Also, there was no significant change in the mRNA level(s) of *Fshr* amongst all the groups (Figure 6.10 [B]).

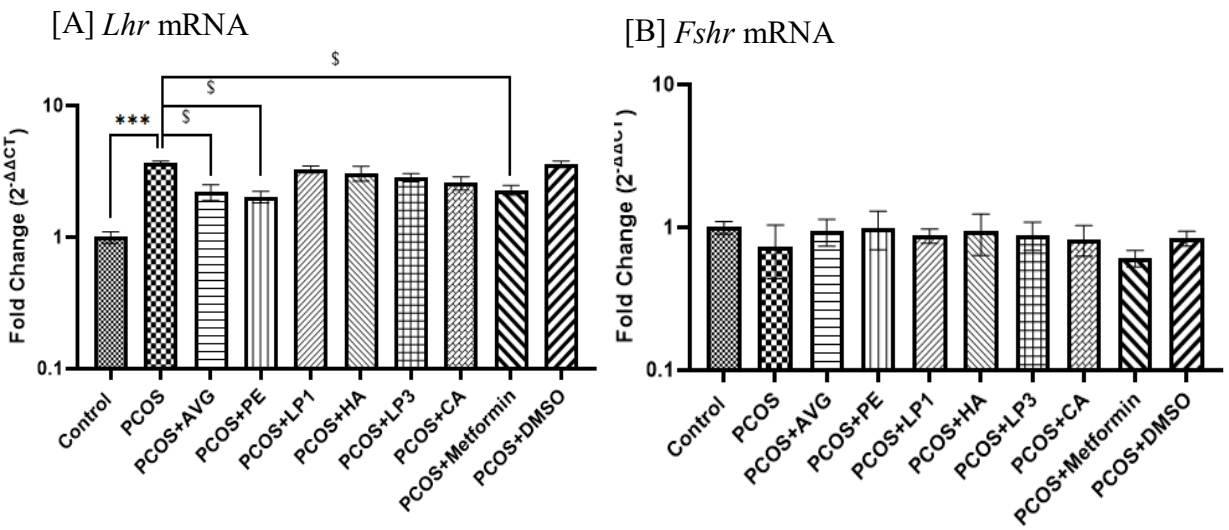



**Figure 6.10.** Comparison of the fold changes in gene expression levels of gonadotropin receptors [A] Luteinizing hormone receptor and [B] Follicle-stimulating hormone receptor in ovaries of Letrozole induced PCOS mice model upon different treatments. Values are mean fold change in gene expression. Error bars represent SEM; N=8 per group. ***P<0.001 as compared to control group; $ P<0.05 as compared to PCOS group.

### 6.3.3.5. Effect of Different Treatments on Steroid Receptors

To assess the expression levels of mRNAs encoding steroid hormone receptors, we performed real-time RT-PCR using specific primers for *Ar, Pgr, Esr-1* and *Esr-2*. Results from Figure 6.11 [A] demonstrate that the mRNA levels of ovarian Androgen Receptor (*Ar*) were significantly upregulated in Letrozole induced PCOS animals (P<0.001) as compared to control animals. However, treatment with Metformin and other phytochemicals significantly reduced the expression of *Ar* (P<0.05), suggesting anti-androgenic potential. On the other hand, the mRNA levels of Progesterone Receptor (*Pgr*), Estrogen Receptor- alpha (*Esr-1*) and Estrogen Receptor- beta (*Esr-2*) significantly decreased in Letrozole induced PCOS ovaries (P<0.01, P<0.001 and P<0.05 respectively) as compared with control group (Figure 6.11 [B], [C] and [D]). Treatment with Metformin, AVG and PE significantly increased the mRNA levels of *Pgr* (P<0.05). The PPNPP of AVG (LP1 and LP3) did not influence the mRNA levels of *Pgr*, *Esr-1* and *Esr-2*. However, administration of pure compound- CA significantly increased the mRNA expression of *Pgr* and *Esr-1* (P<0.05) in the PCOS ovaries. Metformin treatment in PCOS animals showed induced transcription of Esr-2 in the ovaries (P<0.05).

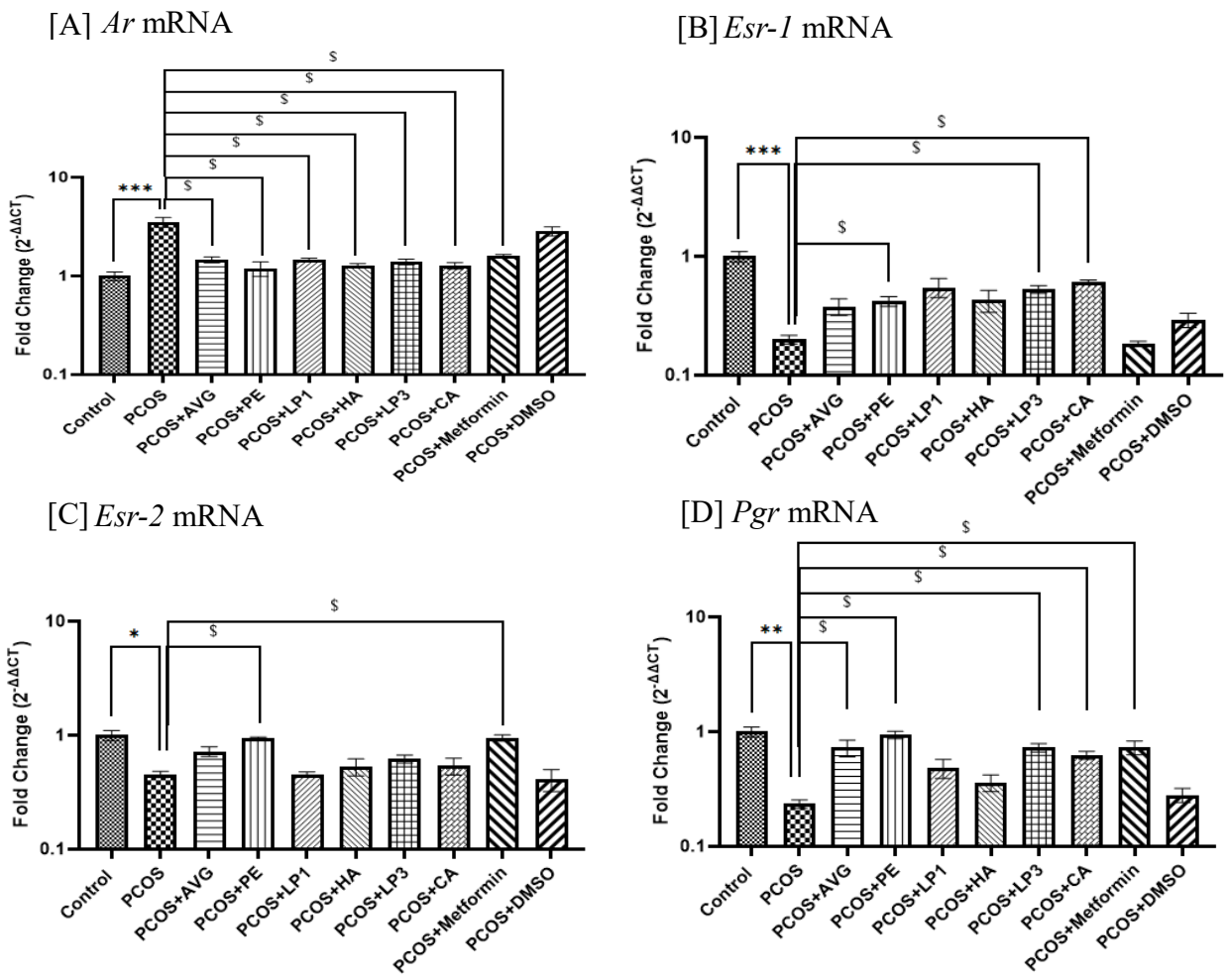



**Figure 6.11.** Comparison of the fold changes in gene expression levels of steroid receptors [A] Androgen Receptor, [B] Estrogen Receptor- alpha, [C] Estrogen Receptor- beta and [D] Progesterone Receptor in ovaries of Letrozole induced PCOS mice model upon different treatments. Values are mean fold change in gene expression. Error bars represent SEM; N=8 per group. *P<0.05, **P<0.01, ***P<0.001 as compared to control group; $ P<0.05, $$ P<0.01 as compared to PCOS group.

### 6.3.3.6. Effect of Different Treatment on Key Steroid Markers

Estimation of the expression levels of mRNA encoding enzymes/proteins involved in steroidogenesis, including anti-mullerian hormone (*Amh*), steroidogenic acute regulatory protein (*Star*), 3-beta (β)-hydroxysteroid dehydrogenase (*Hsd3b1*) and cytochrome P450, family 19, subfamily a, polypeptide 1 (*Cyp19a1*) was done using real-time PCR with their specific primers. As shown in Figure 6.12, mRNA levels of ovarian *Amh* and *Star* were induced by the Letrozole treatment (P<0.01 and P<0.001 respectively). Oral administration of Metformin, AVG and PE significantly decreased the mRNA levels of *Amh* (P<0.01, P<0.05 and P<0.05 respectively). On the other hand, treatment of PCOS animals with AVG, PE, LP3

and CA significantly reduced the mRNA levels of Star (P<0.05). Interestingly, the mRNA levels of *Hsd3b1* and *Cyp19a1* was found to be reduced in Letrozole treated PCOS ovaries (P<0.001). Oral administration of LP3 and CA to PCOS mice significantly increased the mRNA levels of *Hsd3b1* (P<0.05). On the contrary, treatment of Letrozole induced PCOS mice with AVG, PE, LP3, CA (P<0.01) and Metformin (P<0.05) significantly upregulated the transcription of ovarian *Cyp19a1*.

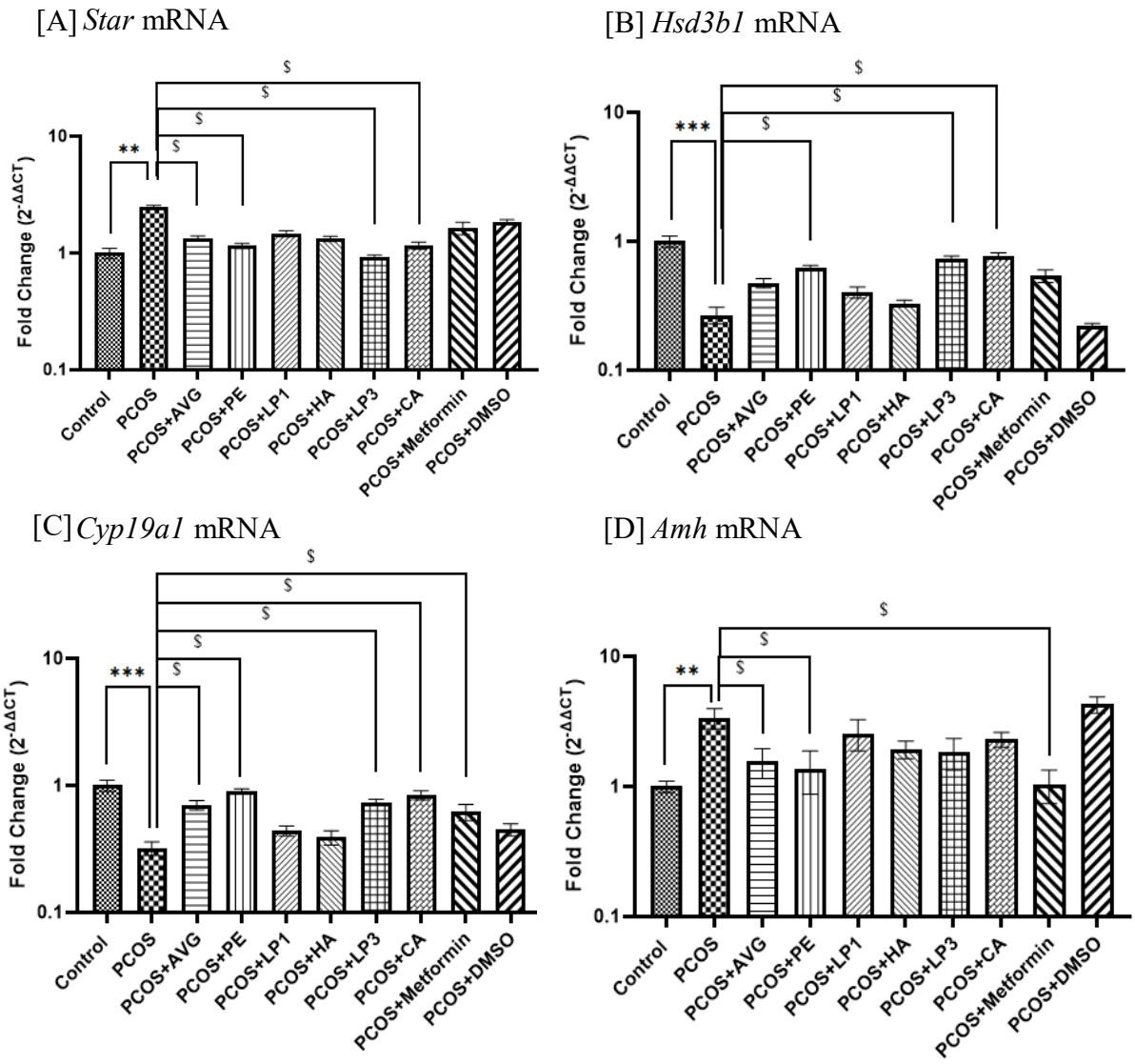



**Figure 6.12.** Comparison of the fold changes in gene expression levels of steroidogenesis regulators [A] Steroidogenic acute regulatory Protein, [B] 3-beta-hydroxysteroid dehydrogenase, [C] Aromatase and [D] Anti-Mullerian Hormone in ovaries of Letrozole induced PCOS mice model upon different treatments. Values are mean fold change in gene expression. Error bars represent SEM; N=8 per group. **P<0.01, ***P<0.001 as compared to control group; $ P<0.05, $$ P<0.01 as compared to PCOS group.

The observed bioactivity of the non-polar phytochemicals was co-related with the absorption and distribution of the compounds in plasma and ovaries of the PCOS animals.

### 6.3.4. Quantitative Analysis of LP1 and LP3 in plasma and ovaries of Letrozole induced PCOS mice

Bioaccumulation of the compound at the target site is the critical factor relating to its efficacy. Data from Table 6.4 clearly demonstrates that the concentration of LP1 and LP3 were found to be 106.22 + 7.39 (ng/mL) and 49.4 + 3.928 (ng/mL) in the plasma and 25.04 + 0.009 (ng/mg tissue weight) and 4.22 + 0.016 (ng/mg tissue weight) in the ovaries of Letrozole induced PCOS mice that have been orally administered LP1 (5 µg/kg/day) and LP3 (0.5 µg/kg/day) for 60 days respectively.

**Table 6.4** Concentration of partially purified non-polar phytochemicals (PPNPP) from *Aloe vera* gel in the plasma and ovaries of Letrozole induced mice

| PPNPP | Conc. in Plasma (ng/mL) | Conc. In Ovaries (ng/mg tissue weight) * |
|---|---|---|
| LP1 | 106.22 $\pm$ 7.39 | 25.04 $\pm$ 0.009 |
| LP3 | 49.4 $\pm$ 3.928 | 4.22 $\pm$ 0.016 |

* Mean + SD (SD is the standard deviation)

### 6.3.5 Effect of Different Treatment on Toxicity parameters

No lethal effects or mortality was observed in animals throughout the test period amongst all the treatment groups. The body weight changes serve as a sensitive indication of the general health status of animals. Data from Figure 6.6[A] demonstrates that the body weight of the animals initially increased ($P<0.001$) upon Letrozole administration for 21 days as compared to the control animals, which is a characteristic of PCOS phenotype. Upon oral administration of different phytocompounds, the body weight reduced significantly ($P<0.05$) in the PCOS animals, however, there was no statistical significance between the body weight of the treated and control animals. In addition, the food and water intakes were found to be unaltered during the entire treatment period, when compared to a control group in this study (data not shown). During the observation period, the animals' appearance did not change in any way. The morphological features (fur, skin, eyes, and nose) remained the same. Tremors, convulsions, salivation, diarrhoea, lethargy, or odd behaviours such as self-mutilation or walking backwards

were not seen in the treated animals (Table 6.5). The gross observations of the animals' anatomy disclosed that the vital organs such as the heart, liver, spleen, kidneys, lungs and brain were not adversely impacted and did not display clinical symptoms of toxicity, over the course of treatment.

Table 6.6 summarizes the results of the effect of different treatments on various biochemical parameters. No significant differences were observed in plasma glucose levels of the treated groups as compared to the control group. The letrozole induced PCOS animals showed significantly ($P<0.01$) increased triglyceride levels when compared to the control. However, upon oral administration of different treatments, the triglyceride levels of the PCOS animals significantly reduced ($P<0.05$) and were comparable with that of control animals. The was no significant difference in the total cholesterol and the HDL-cholesterol levels amongst all the different treatment regimens. AST in combination with ALT are considered as good maker of liver disease (Friedman et al., 1996). High levels of these enzymes are implicated in liver diseases or hepatotoxicity (Ramaiah, 2011). The present study reveals no significant changes in ALT as well as AST activities amongst all treated groups. Abnormally high levels of creatinine indicate kidney malfunction or renal toxicity (Davis and Bredt, 1994). No significant change in creatinine level has been observed in treated animals as compared to control. This may indicate that the different treatments did not influence the liver and renal function. All the above parameters suggest that the standardised dosage of the non-polar phytocompounds of *Aloe vera* gel are safe for oral consumption, when taken regularly for 60 days.

**Table 6.5** Effect of Different Treatment on General Appearance and Behavioural Observations

| | Skin and Fur | Eyes | Mucous membrane | Behavioural patterns | Salivation | Lethargy | Sleep | Diarrhoea | Tremors | Coma |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+ AVG | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+PE | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+ LP1 | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+HA | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+ LP3 | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+CA | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+ Metformin | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |
| PCOS+ DMSO | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Not observed | Not observed |

**Table 6.6** Effect of Different Treatments on various Biochemical parameters

| | Fasting glucose (mg/dL) | Cholesterol (mg/dL) | HDL- Chol (mg/dL) | Triglycerides (mg/dL) | ALT (IU/L) | AST (IU/L) | Creatinine (mg/dL) |
|---|---|---|---|---|---|---|---|
| Control | 93 ± 9.72 | 160.09± 30.10 | 42.86 ± 7.78 | 72.51± 9.24 | 62.4 ± 3.5 | 170.38 ± 24.3 | 0.82 ± 0.03 |
| PCOS | 106.25 ± 9.42 | 192.14± 28.80 | 31.57 ± 8.84 | 165.11± 22.60 ** | 65.39 ± 4.9 | 193.58 ± 25.39 | 0.93 ± 0.08 |
| PCOS+AVG | 87.25 ± 5.6 | 151.9± 16.4 | 37.21 ± 9.63 | 101.9± 14.3 $ | 58.48 ± 5.3 | 172.39 ± 28.2 | 0.74 ± 0.04 |
| PCOS+PE | 99.75 ± 7.5 | 162.7± 28.2 | 42.45 ± 7.37 | 86.16± 12.10 $ | 61.37 ± 4.1 | 183.57 ± 10.2 | 0.75 ± 0.07 |
| PCOS+ LP1 | 86.33 ± 6.1 | 183.36± 12.42 | 33.42 ± 5.79 | 141.9±26.4 | 55.29 ± 3.7 | 172.49 ± 26.4 | 0.83 ± 0.05 |
| PCOS+HA | 95.2 ± 4.1 | 176.67± 20.67 | 30.29 ± 4.96 | 122.21± 18.52 | 62.58 ± 5.29 | 169.39 ± 32.5 | 0.764 ± 0.1 |
| PCOS+LP3 | 99.8 ± 9.13 | 167.21± 18.13 | 46.28 ± 7.39 | 93.19± 9.28 $ | 64.38 ± 5.4 | 182.21 ± 20.6 | 0.82 ± 0.03 |
| PCOS+CA | 97.8 ± 4.58 | 159.22± 14.81 | 51.29 ± 9.79 | 80.09± 13.08 $ | 58.24 ± 3.2 | 173.59± 24.36 | 0.739 ± 0.06 |
| PCOS+ Metformin | 96.8 ± 9.16 | 172.61±22.20 | 43.74 ± 5.08 | 121.9± 16.4 | 69.38 ± 4.4 | 169.28± 30.58 | 0.89 ± 0.02 |
| PCOS+ DMSO | 111 ± 8.6 | 203.30± 20.62 | 30.78 ± 9.95 | 169.54± 29.21 | 58.28 ± 4.1 | 183.55± 22.57 | 0.78 ± 0.06 |

All values are presented as Mean ± SEM; N=8 per group. **P<0.01 as compared to control group; $ P<0.05 as compared to PCOS group.

## 4. Discussion

One of the pre-requisites for evaluation of the bioactivity of a drug/ chemical compound, requires successful development of a model. Despite the availability of several animal models for the study of human PCOS, no comprehensive model has yet been developed. Hence, the current study focused on development of a mice model of PCOS using a non-steroidal aromatase inhibitor, Letrozole. Letrozole induced (0.5 mg/kg/day orally for 21 days) mice model exhibited pathological clinical manifestation of PCOS (Dey et al., 2017). The continuous monitoring of body weight revealed a significant induction of body weight gain in the PCOS group. It has earlier been reported that an increase in accumulation of fat in the abdominal area is associated with increase in body weight and might induce adipocyte dysfunction and insulin resistance like state in PCOS (Goodarzi et al., 2011). In addition to this, letrozole treated animals exhibited glucose intolerance, elevated fasting insulin levels, increased HOMA-IR values and increased circulating levels of triglycerides. Similar indicators of metabolic dysregulation have been observed in previous studies (Kauffman et al., 2015; Kelley et al., 2016). The insulin resistance and low glucose tolerance created by letrozole are mainly due to elevated androgen concentrations as reported by Desai et al., 2012. Along with the metabolic disruptions, letrozole treatment also induced reproductive abnormalities which was evident by arrested estrus cycle at diestrus stage, elevated testosterone levels and decreased progesterone levels. Additionally, histological examination of ovarian sections showed multiple peripheral cystic follicles, which is characteristic features of PCOS. These findings are similar to other reports (Yang et al., 2018a; Yang et al., 2018b; Rajan and Balaji, 2017). Therefore, this model is suitable for studies of both reproductive and metabolic features of the syndrome and can be used effectively for better understanding of the etiopathology and for evaluating the bioactivity of drugs/phytochemicals.

*Aloe barbadensis* has been documented in Ayurveda and Siddha for treatment of female reproductive ailments (Nadkarni, 1976; Risvan et al., 2017). However, its bioactive phytochemical and molecular targets still remains to be elucidated. In this study, we systematically investigated the therapeutic potential of partially purified isolated non-polar phytocomponents (PPNPP) of *Aloe vera* gel against PCOS in Letrozole induced mouse model. The effectiveness of metformin in the treatment of PCOS by restoring ovulation, reducing weight, improving insulin sensitivity and glucose tolerance, reducing circulating androgen levels, reducing the risk of miscarriage and reducing the risk of gestational diabetes mellitus has been proven (Lashen, 2010; Johnson, 2014), nevertheless, .it is associated with the high

incidence of adverse effects including nausea, vomiting and gastro-intestinal disturbances. As reported in the literature (Jahan et al., 2016; Mvondo et al., 2020), we observed in the current study that metformin-treated PCOS animals, displayed a significantly decrease in body weight, blood glucose level, fasting insulin levels and HOMA-IR values compared with untreated PCOS animals. Remarkably, treatment of PCOS animals with AVG, PE, LP3 and CA significantly reduced the body weight, improved glucose tolerance, decreased the fasting insulin levels and HOMA-IR values. It could be suggested that non-polar phytocompounds present in *Aloe vera* gel reduces glucose resistance by controlling glucose homeostasis, improving insulin secretion and potentiating the insulin-mediated uptake of glucose. This further strengthens that isolated bioactives of *Aloe barbadensis* is a potential metabolic modulator (Desai et al., 2012). Additionally, these treatments could effectively reduce the circulating triglycerides levels in PCOS animals. These findings are similar to those of Maharjan et al., 2010; Desai et al., 2012; Alinejad-Mofrad et al., 2015). The observed efficacy can be attributed to the presence of plant sterols and fatty acids in the above-mentioned treatments. Several studies from the past have highlighted on the efficacy of phytosterols present in *Aloe vera* gel in regulating glucose and lipid metabolism in rats (Tanaka et al., 2006; Misawa et al., 2008; Misawa et al., 2012). Also, combination of omega-3 fatty acids and plant sterols was found to regulate glucose and lipid metabolism in individuals with impaired glucose regulation (Wang et al., 2019). These results suggest that LP3 derived from Petroleum ether extract of *Aloe vera* gel may be a potent therapeutic for the treatment of metabolic disturbances associated with PCOS.

Current evidence suggests that insulin-resistance and secondary hyperinsulinemia play an important role in hyperandrogenism, amenorrhoea or oligomenorrhea in both lean and obese PCOS patients (Laganà et al., 2016). In the present study, PCOS animals had irregular estrus cyclicity, elevated testosterone levels and reduced progesterone levels as compared to control animals. Interestingly, treatment with AVG and PE restored the estrus cyclicity, decreased the circulating free testosterone, increased progesterone levels. Steroid hormones in the follicular fluid play an important role in the physiology of follicular growth, oocyte maturation and ovulation (Walters et al., 2008). Treatment with LP3 and CA was found to be more potent than LP1 and HA in restoration of the estrus cyclicity. However, both LP3 and CA significantly improved the hormone milieu. Of great interest, oral administration of AVG, PE, LP3 and CA alleviated hyperandrogenism in PCOS animals and enhanced progesterone levels. The improved hormone milieu in turn might be responsible for promotion of follicular development

and induction of ovulation. Surprisingly, treatment of PCOS animals with metformin (first-line treatment for patients with PCOS), could not revert back the estrus cyclicity as efficiently as AVG, PE, LP3 and CA treatment, even though it improved the hormone levels in the animals. To substantiate our results, previous studies from our lab have demonstrated that non-polar phytochemicals of *Aloe vera* gel improved metabolic phenotype as well as ovarian function in letrozole induced PCOS rats (Radha and Laxmipriya, 2016a). Similar results were found by Demirel and his group (2016) where they demonstrated that α-tocopherol, γ-tocopherol, squalene, β-sitosterol, campesterol and stigmasterol present in Corylus avellana seed oil induced a significant reduction in testosterone level and restored ovarian function in PCOS rats. Vitex agnus-castus fruit extract also exhibited an antiandrogenic effect in PCOS rats by increasing aromatization leading to low testosterone level (Jelodar and Askari, 2012). Because abnormal increased in testosterone level contributes to the pathogenesis of PCOS, the downregulation of this hormone after LP3 treatment may have beneficial effects on reproductive disorders in PCOS.

PCOS has been reported to be associated with ovarian damage. As observed in the current study, disturbed hormone milieu in untreated PCOS rats is correlated with multiple cysts formation and low number of corpus luteum in the ovary (Dewailly et al., 2016). Moreover, the size of cystic follicles was larger than that of other follicles which can be correlated to the increase levels of intraovarian androgens (Mahesh et al., 1987). Thickness of granulosa layer was lowered while that of theca layer was increased in different follicles of untreated PCOS rats. These detrimental effects of letrozole on ovary architecture were corrected by AVG, PE, LP3 and CA probably due to antiandrogenic properties. These treatments significantly reduced the number of peripheral cysts and increased the number of graafian follicles and corpus luteum, which might be attributed to the restored hormone levels in PCOS animals upon these treatments for 60 days. Additionally, the beneficial effects of these treatments could be mediated through their anti-hyperglycaemic, anti-lipidemic and insulin sensitizing properties. Studies on similar line have demonstrated that polyunsaturated fatty acids (PUFAs) regulate the menstrual cycle, lower testosterone and insulin levels, and improve metabolic health (Prabhu and Abilash, 2021). Also, phytosterols and its metabolites may act as GnRH modulators (Arpi and Laxmipriya, 2019). Recent studies are highlighting on the implication of campesterol and stigmasterol as precursors of progesterone (Tarkowská, 2019; Janeczko, 2012), suggesting that plant sterols have the potential to regulate the hormone metabolism. The improvement in hormone levels by metformin corroborated previous studies which

demonstrated that this drug improves ovarian related markers and induces ovulation in mice with PCOS (Sander et al., 2006), although the conception rate remains disappointing (Legro et al., 2007).

Ovaries represent one of the primary steroidogenic organs, producing estrogen and progesterone under the regulation of gonadotropins during the estrus cycle. Gonadotropins fluctuate the expression of various steroidogenesis-related genes, such as those encoding gonadotropin receptors, steroid receptors and steroidogenic marker proteins. The gonadotropin receptors, *Fshr* and *Lhr* play a significant role in folliculogenesis and ovulation respectively. Although androgens and AR have known roles in the positive regulation of *Fshr* (Sen et al., 2014), its ovarian expression was not significantly modulated in any of the groups. However, the transcriptional level of *Lhr* was altered in the ovaries of PCOS animals, and these levels were restored to the normal range by AVG, PE and Metformin treatment. *Lhr* is also located on the surfaces of theca cells and granulosa cells, and *Lhr* transcript levels also influence ovulation, corpus luteum formation and the production of other steroids, i.e., estrogen, progesterone and androgen (Edson et al., 2009). The potential of AVG and PE to modulate the gene expression of *Lhr* may be attributed to the non-polar phytochemicals present in them (Nampoothiri et al., 2015). It is to be noted that LP1 and LP3 did not have any significant effect on the transcriptional regulation of gonadotropin receptors.

Ovarian steroid hormones perform several important actions related to ovarian function and their effects are mediated through interaction with specific receptors and steroidogenic enzymes (Salvetti et al. 2008). In this study we have selected most important genes that required for the developmental follicle, oocyte maturation, and regulation of steroidogenesis in the ovaries. Findings from this study provide evidence that letrozole has differential effects on the expression of all the genes studied. A significant increase in *Ar* expression was found in Letrozole treated animals. This supports the suggestion of Manneras et al. (2007), that the ovarian alteration observed in this model is mediated by the accumulation of endogenous testosterone, which also results in pronounced activation or upregulation of the Ar. Consistent with this finding, administration of testosterone propionate to prepuberal rats at 5 days of age also increased ovarian nuclear *Ar* expression (Bukovsky et al. 2002). All the different treatments exhibited reduced transcriptional levels of *Ar*, suggesting anti-hyperandrogenic effect.

On the other hand, the expression of *Pgr*, *Esr-1* and *Esr-2* were significantly decreased in PCOS animals, however, this downregulation was reversed by different treatments in the current study. *Pgr* is required specifically for LH-dependent follicular rupture leading to ovulation (Lydon et al. 1995) and transcripts of *Esr-1* and *Esr-2* have been reported to play a proliferative role during follicular development (Fitzpatrick et al., 1999). Results from our studies is in line with the results from previous studies, reporting regulation of hormonal imbalance and the expression of steroid biosynthesis-related genes and/or steroid receptor genes by herbal medicines in Letrozole induced rat model (Yang et al., 2020; Yang et al., 2018a; Yang et al., 2018b; Pyun et al., 2018).

Increasing ovarian steroidogenesis and thereby improving female reproductive function is the main treatment technique for female infertility. IVF success rates have improved in many treatment regimens that use gonadotropin or analogues to improve ovarian function (Shrestha et al., 2015). In the current study, the alteration in hormones could be correlated with changes observed in key steroidogenic markers such as *Star*, *Hsd3b1*, *Cyp19a1* and *Amh*. Also, high insulin levels have direct effect on ovarian steroidogenesis and stimulate thecal androgen production (Diamanti-Kandarakis and Dunaif, 2012). Insulin mediated modulation in steroidogenic enzymes like steroidogenic acute regulatory protein (StAR), 3b-HSD and androgen receptor (AR) are also well documented in case of PCOS. Results from the present study revealed that the expression of *Star* and *Amh* was significantly elevated whereas, expression of *Hsd3b1* and *Cyp19a1* was significantly reduced in letrozole-treated animals. Disturbed steroidogenesis was observed as a result of altered enzyme activity, which may be attributable to a shift in StAR expression profile. StAR expression was found to be high in PCOS women, possibly due to the synergistic effect of high LH and insulin levels, which increase StAR expression by co-binding to the StAR promoter region (Sekar et al., 2000). In the present study, the elevated transcript levels of Star upon Letrozole treatment, was significantly reduced when treated with AVG, PE, LP3 and CA. On the contrary, PE, LP3 and CA significantly increased the transcriptional levels of Hsd3b1, which was reduced upon Letrozole treatment. 3 beta-hydroxysteroid dehydrogenase is an important enzyme, encoded by the gene Hsd3b1, and is responsible for the biosynthesis of progesterone. The reduced capacity of PCO luteinizing granulosa cells to synthesize progesterone in vitro may be due to reduced 3 beta-HSD gene expression (Doldi et al., 2000). The non-polar phytochemicals present in *Aloe vera* gel effectively improved the hormone levels by modulating the

transcriptional levels of these key steroidogenic markers. Data from Radha and Laxmipriya (2016a) substantiate our results of present study.

While StAR and aromatase work together to control estradiol production, their effects differ in time and mode of action. An increase in StAR protein normally causes an acute increase in steroid production, while an increase in aromatase levels can cause a later increase in steroidogenesis (Huang et al., 2004). Previous studies have reported dysfunctional aromatase activity in PCOS women, and *Cyp19a1* plays a key role in the normal progression of the menstrual/estrus cycle in rats with PCOS (Kafali et al., 2004). In the present study, reduced expression of *Cyp19a1* transcript was observed in the ovaries of Letrozole treated PCOS animals, which was found to be significantly increased upon treatment with AVG, PE, LP3, CA and metformin.

Another key steroidogenic modulator, which is influenced by hyperandrogenism as well as insulin resistance is Anti- Mullerian Hormone (Wiweko et al., 2018). *Amh* mRNA is produced by the granulosa cells surrounding preantral and antral follicles and has an important role in the development and maturation of follicles. AMH production by granulosa cells in the polycystic ovary is 75 times higher compared to healthy women. AMH, a surrogate marker for hyperandrogenism, is associated with the severity of morphological and hormonal changes in PCOS patients (Wiweko et al., 2014). In the current study, elevated levels of *Amh* mRNA in the ovaries of Letrozole induced PCOS animals was significantly restored to normalcy upon treatment with AVG, PE and Metformin. The partially purified isolates of *Aloe vera* gel did not modulate the *Amh* at transcriptional level.

The present study has demonstrated that LP1 exhibited only anti-androgenic effect. On the other hand, LP3 was found to be more potent than LP1 in regulating the steroidogenic as well as metabolic parameters associated with PCOS. The possible mechanism of LP3, a partially purified non-polar phytochemical derived from *Aloe vera* gel may be due to its modulatory effect on the transcription of steroid receptors, mainly be acting as an anti-androgenic and progestenic agent. It helped in restoration of ovarian structure-function by regularizing the hormonal milieu in Letrozole induce PCOS mice. In addition to this, the metabolic dysfunction which is associated with PCOS due to glucose intolerance, insulin resistance and dyslipidaemia was improved upon treatment with LP3, AVG and PE. Remarkably, LP3 was found to be equally potent as AVG and PE towards management of reproductive and metabolic complications associated with Letrozole induced PCOS mouse, suggesting that oral

administration of LP3 (0.5 µg/kg/day) for 60 days is the adequate dose for treatment of PCOS. Interestingly, the observed therapeutic potential of LP3 was better than metformin, which is the standard prescribed drug for PCOS.

## 5. Conclusion

This study shows that Letrozole-induced (0.5mg/kg/day orally for 21 days) mice recapitulates both the reproductive and metabolic features of human PCOS, including elevated circulating androgen levels, PCO morphology and irregular/arrested estrus cycles, increased body weight, glucose intolerance, insulin resistance and elevated fasting insulin and triglyceride levels. Following which, the efficacy of LP1 and LP3, partially purified non-polar phytocompounds from *Aloe barbadensis* Mill. gel in the letrozole-induced PCOS mouse model was investigated. Body weight, glucose intolerance, HOMA-IR, triglyceride levels, estrus cyclicity, hormonal profile, ovarian histopathology, and gene expression of gonadotropin receptors, steroid receptors and key steroidogenic markers were all restored after oral administration of LP3. The results were comparable to those obtained with *Aloe vera* gel, Petroleum ether extract of *Aloe vera* gel, and Metformin therapy, suggesting that LP3 is a possible bioactive isolate of *Aloe vera* gel that can be used to treat PCOS and its comorbidities. Also, it is interesting to note that it is first time we have clearly shown that this bioactive with two potential as ovarian modulator as well as metabolic modulator and can be refined for management of PCOS pathology (Figure 6.13). Further, detailed molecular elucidation of the bioactive needs to be explored which could pave way for herbal formulation in management of the multi etiological endocrine pathology with minimum side effects. Also, the study attempts to add a new facet with scientific explanation to the *Aloe vera* that has been traditionally used in Indian system of ancient medicine.

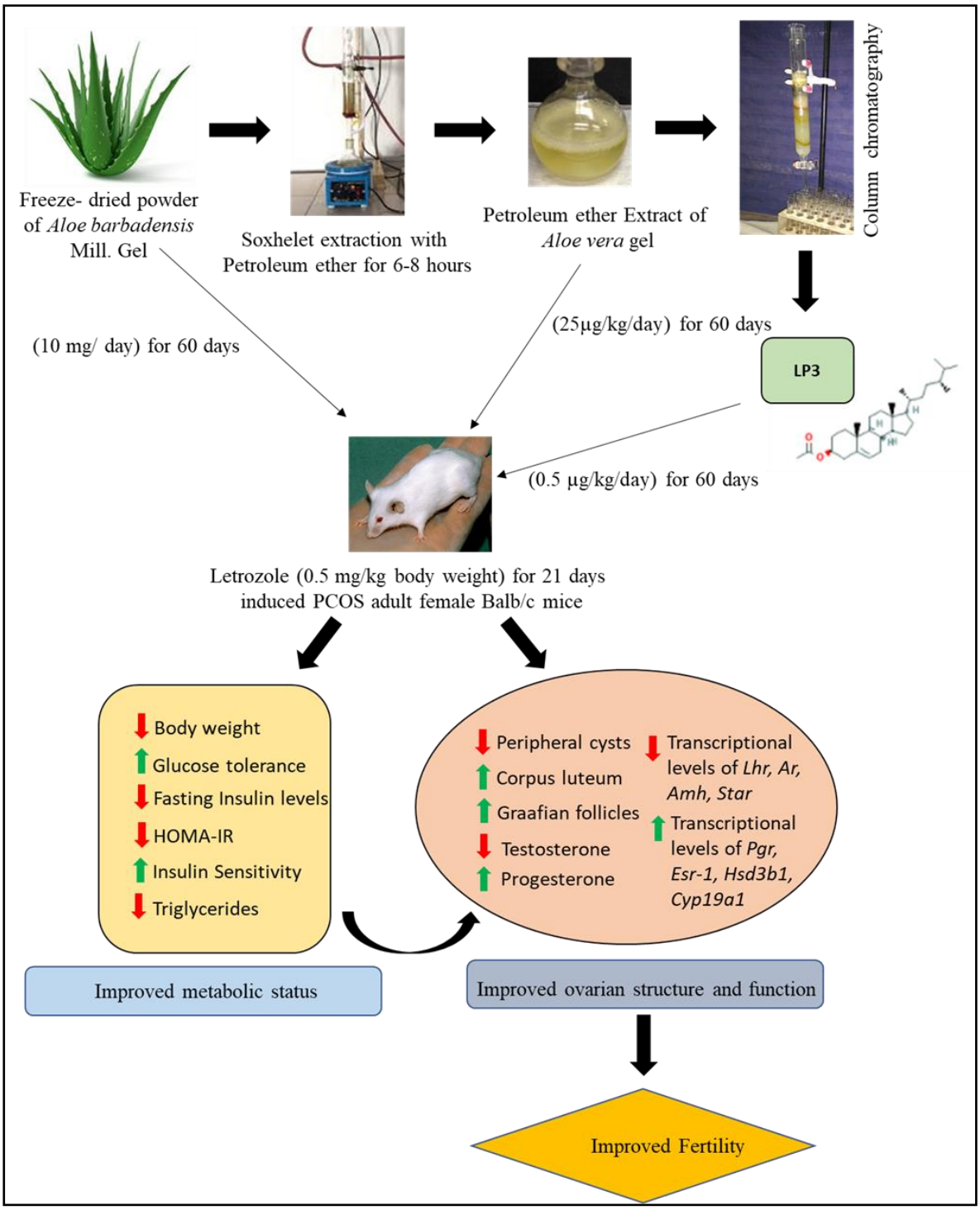



**Figure 6.13** Evaluation of therapeutic effect of partially purified non-polar phytocomponents of *Aloe vera* gel in Letrozole induced PCOS mouse model