# CHAPTER 4

# EFFECT OF *ALOE VERA* ON LETROZOLE INDUCED PCOS RAT MODEL

### 4.1 INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder among women of reproductive age (Nardo et al. 2009; Shayya and Chang 2010). The research from a past few decades has shown that PCOS is an important metabolic disorder, which is associated with an increased risk of type II diabetes (Gambineri et al. 2012). An increase in ovarian androgen production is a fundamental characteristic of PCOS and excessive androgen levels favors visceral deposition of body fat, resulting in insulin resistance and compensatory hyperinsulinism (Alpanes et al., 2012; Damir et al., 2011).

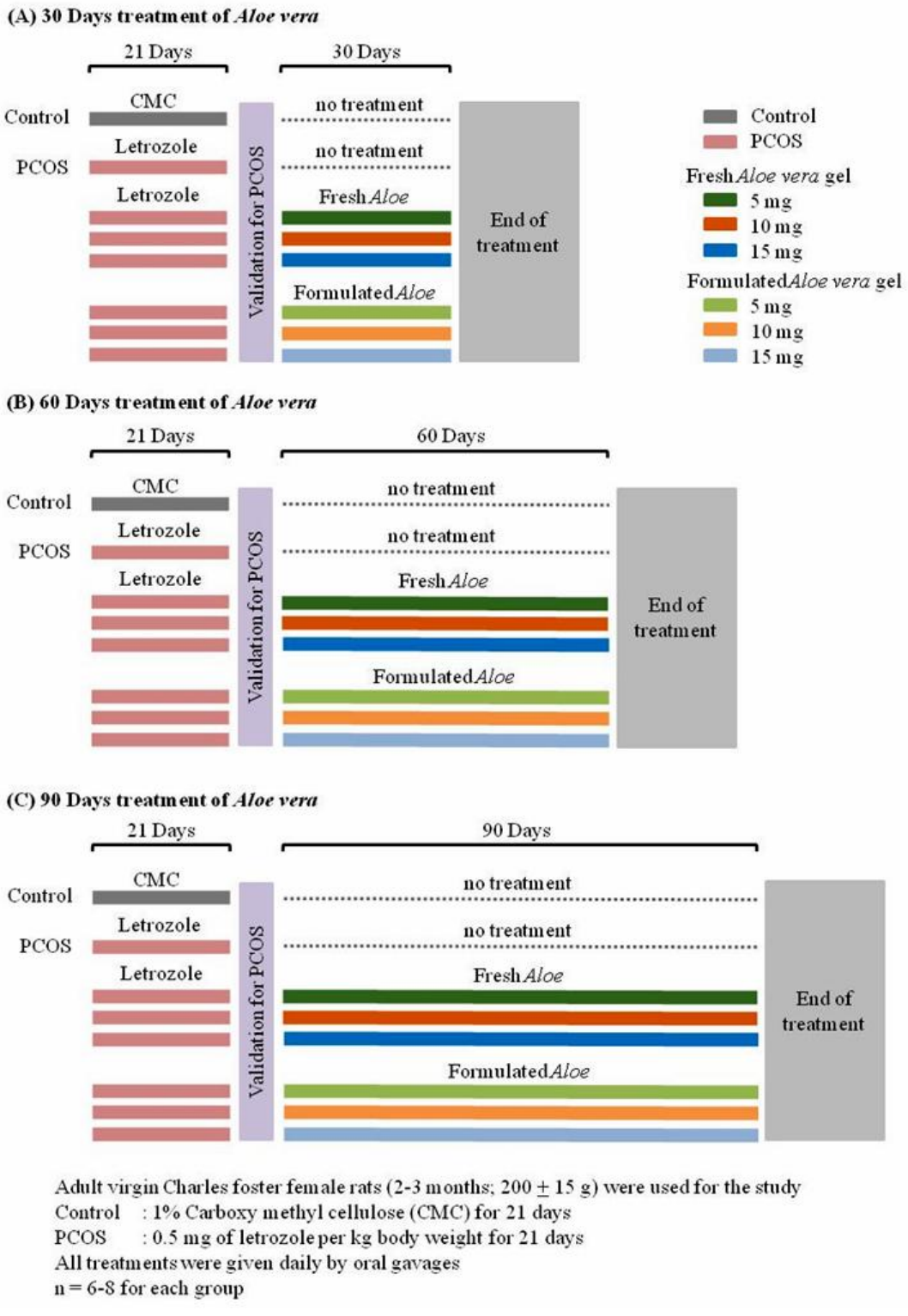
In PCOS, ovarian hyperandrogenism is mainly attributed to steroidogenic defects in theca cells of ovary. Increased Luteinizing hormone (LH) and increased insulin levels mainly amplify the intrinsic abnormality of thecal steroidogenesis (Diamanti-Kandarakis 2008). Excess androgen activity may hinder gonadotropin-induced estrogen and progesterone synthesis in PCOS follicles (Diamanti-Kandarakis 2008). Normally, testosterone and androstenedione are converted to estradiol and estrone respectively with help of $P_{450}$ aromatase. These steroids are important for the management of ovarian function. However, decreased activity of this enzyme results in an increase in ovarian androgen production; leading to development of PCOS condition (Nelson et al. 1999; Puurunen et al. 2011). As PCOS etiology is related to insulin resistance and hyperandrogenism; current available mode of treatment is with use of insulin sensitizers like metformin (De Leo et al. 1999) and steroid analogs (Prelević et al. 1990). But, these drugs have been reported with side effects upon prolonged usage (Salpeter et al. 2003).

Hence, researchers in current era are exploring alternative therapy to treat and manage this disorder (Kamat 2002; Jain et al. 2004). One such plant, which has been explored elaborately, is *Aloe vera*, which possesses hypoglycemic effect. An alcoholic extract of *Aloe vera* gel maintained glucose homeostasis in streptozotocin induced diabetes rats by controlling carbohydrate metabolizing enzymes (Rajasekaran et al. 2006). This action is ascribed to mainly phytosterols present in mixture (Tanaka et al. 2006; Pérez et al. 2007; Kim et al. 2009). As PCOS pathophysiology precipitates through insulin resistance anovulation; it was interesting to study the role of *Aloe vera* gel (AVG) in management of PCO phenotype wherein Aloe vera has reported already to have modulating properties over glucose and lipid metabolism (Pérez et al. 2007; Misawa

et al. 2012). In view of the above hypothesis, current chapter focuses on development of PCOS rat model and to study the efficacy of *Aloe vera* gel as therapeutic agent in dose and time dependent manner.

## 4.2 Materials and Methods

### 4.2.1 PLAN OF WORK




Adult virgin Charles foster female rats (2-3 months; 200 ± 15 g) were used for the study
Control : 1% Carboxy methyl cellulose (CMC) for 21 days
PCOS : 0.5 mg of letrozole per kg body weight for 21 days
All treatments were given daily by oral gavages
n = 6-8 for each group

### 4.2.2 DEVELOPMENT OF PCOS RAT MODEL

To develop PCOS rat model, adult virgin female rats weighing 180- 225 g and exhibiting regular estrus cyclicity were taken and maintained under controlled conditions of light and temperature with having free access to diet and water. Protocols for PCOS rat model development and its validation have been mentioned in materials and methods section.

### 4.2.3 ALOE VERA GEL (AVG) TREATMENT

*Aloe vera* gel treatment was done in the following ways, wherein the PCOS animals were orally fed with different dosages (5, 10 and 15 mg dry weight) of each Fresh *Aloe vera* gel (FA) and Formulated *Aloe vera* gel (FOA) daily for different time period (30, 60 and 90 days). The detailed treatment regime in mentioned in Table 1:

I. Fresh *Aloe vera* gel **(FA)** was extracted from the plant. The detailed protocol of the gel preparation is mentioned in Materials and methods section.
II. Formulation (**FOA**) was prepared by adding the natural preservatives like Turmeric [Curcuma longa L. (0.5%)], Kadaya gum [Sterculiaurens Roxb. (1%)] and lemon [Citru limon L. (0.1%)] juice to the fresh *Aloe vera* gel and was stored at 4°C.

All these groups were continuously monitored for estrus cyclicity, glucose sensitivity by OGTT test during the entire course of treatment. At the end of treatment, rats were sacrificed and assessed for various biochemical parameters along with histological examination of ovaries.

## 4.3 RESULTS

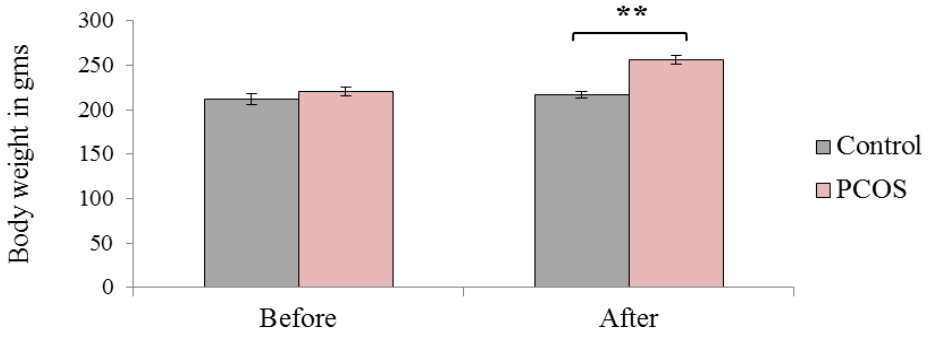
### 4.3.1 DEVELOPMENT OF PCOS RAT MODEL

Rats treated with letrozole for induction of PCOS showed a significant increase in body weight and altered estrus cyclicity as compared to control [Table 4.3.1]. As shown in Figure 4.3.1(a) and Figure 4.3.1(b), PCO animals exhibited an increase in body weight and glucose tolerance as compared to control and histology of ovary revealed many peripheral small atretic cysts [Figure 4.3.1(c)]; whereas no histological abnormalities were observed in control rats. Ovarian key steroidogenic enzymes- 3β Hydroxysteroid dehydrogenase and 17β Hydroxysteroid dehydrogenase demonstrated an increase in activities in letrozole induced

PCOS rats when compared to control rats [Figure 4.3.1(d)].

### 4.3.1 Development of PCOS rat model

**Figure 4.3.1 a Effect on body weight in letrozole induced PCOS rat model.**

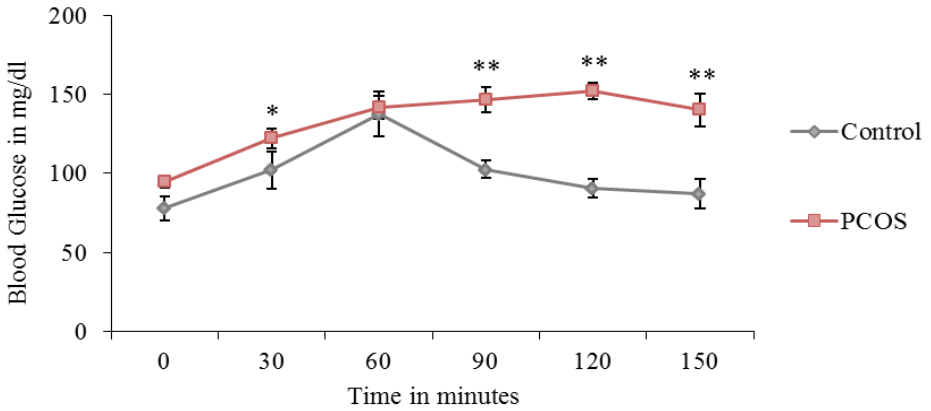



N=4-8. The values are represented as Mean + SEM.
**p<0.01 as compared to control group

**Table 4.3.1 Effect on Estrus cyclicity in letrozole induced PCOS rat model.**

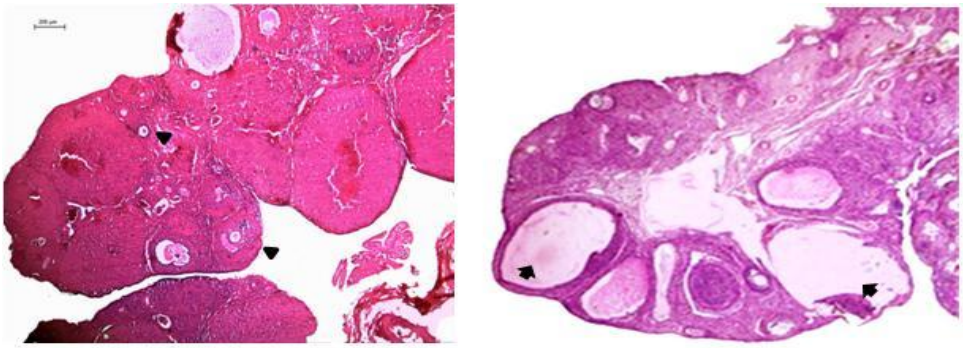
| GROUPS | Normal Animal | Extended Proestrus | Extended Estrus | Extended Metaestrus | Extended Diestrus |
|---|---|---|---|---|---|
| Control | 10/10 | - | - | - | - |
| PCOS | - | 2/10 >24 hr | - | 2/10 > 32 hr | 6/10 > 72 hr |

**Figure 4.3.1 b Effect on Oral Glucose Tolerance Test (OGTT) in letrozole induced PCOS rat model.**




N= 4-6, The values are represented as Mean ± SEM
*p<0.05, ** p<0.01as compared to control.

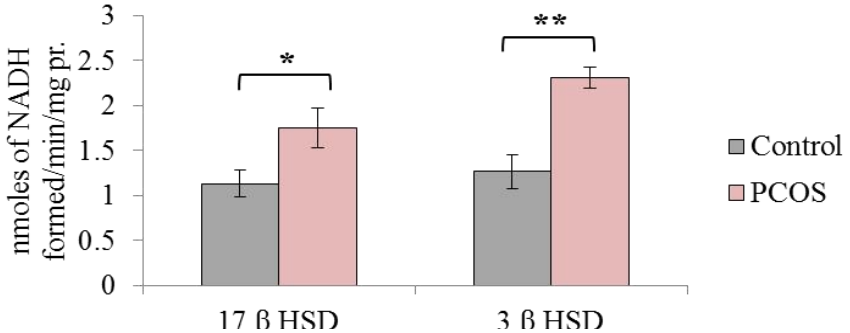
**Figure 4.3.1c Effect on Ovarian structure in letrozole induced PCOS rat model.**



Control PCOS

Sections were taken in diestrus stage of estrus cycle; Magnification = 4X
▲ : Growing follicles ◆ : Cyst

**Figure 4.3.1d Effect on Ovarian steroidal enzymes activities in letrozole induced PCOS rat model.**




N=4-8. The values are represented as Mean + SEM.
*p<0.05 **p<0.01 as compared to control group

The results from the above experiments clearly demonstrate that Letrozole induced PCOS rats exhibited all the pathological characteristics similar to the clinical manifestations found in PCOS women. The main aim of the current chapter was to evaluate the efficacy of *Aloe vera* gel in PCOS rodent model using two different preparations of *Aloe vera* gel [Fresh *Aloe vera* gel (FA) and *Aloe* formulation (FOA)]. Hence, dose and time dependent experiments were directed towards the understanding the most effective dose and minimum time required for management of PCOS phenotype.

### 4.3.2 ALOE VERA GEL TREATMENT

Fresh *Aloe* gel (FA) was prepared every alternate day during the course of experiment because the consistency of the gel changed in terms of content and texture (i.e. it started to lose water) after 2 days. However, the *Aloe* formulation (FOA) was prepared and kept at 4°C and was used for treatment. Although formulation contains

natural preservatives, *Aloe vera* phyto-components were very labile in nature and hence could degrade with time. Hence, we checked the stability of the formulation before treatment in batch wise manner (Table 4.3.2). The formulated AVG sample was analyzed at different time period (1 week, 2 week, one month, 2 months and 4 months) and qualitative analysis of the phytocomponents was performed. Data indicated that *Aloe* formulation was stable up to 4 months when stored at 4°C and upon longer period of storage, the phyto-components were degraded gradually. Thereby, all experiments were designed with to 3 months for the *Aloe vera* gel treatment.

**Table 4.3.2 Qualitative analysis for stability of *Aloe vera* gel formulation in batch wise manner**

| Test of Specific components | *Aloe* Vera Gel Formulation | 1 Week Batch | 2 weeks Batch | Batch-1 (1month) | Batch-2 (2 months) | Batch-3 (4 months) |
|---|---|---|---|---|---|---|
| **Sterols** | | | | | | |
| • Liebermann-Burhaman Test | +++ | +++ | +++ | +++ | + + | + |
| • Salkouski Test | + ++ | + ++ | + ++ | + + + | ++ + | ++ |
| **Glycosides** | | | | | | |
| • Kedde Test | + + | + + | + + | + + | + + | + |
| • Balijet Test | + | + | + | + | + | + |
| **Tannins** ( Butanol-HCl assay) | + | + | + | + | + | |
| Alkaloids | | | | | | |
| • Mayer's test | + | + | + | + | | |
| • Dragendroff | + | + | + | + | + | + |

Simultaneously, both qualitative and quantitative screening of phytocomponents present in both fresh and formulated AVG was evaluated. The detailed protocol and results of which are discussed in Chapter 3. Both Fresh and formulated AVG showed the presence of Phytosterols, flavonoids and polyphenols in abundance. Moreover, Formulated AVG did not show any significant difference in the phytosterol content as compared to the fresh AVG. On the other hand, Formulated AVG demonstrated more flavonoids ($p<0.01$) and polyphenols ($p<0.001$) as compared to the Fresh AVG. This might be due to the additional contribution of turmeric, lemon juice and Kadaya gum in formulated AVG.

Though phytochemical differences were present in both forms of AVG; experiments

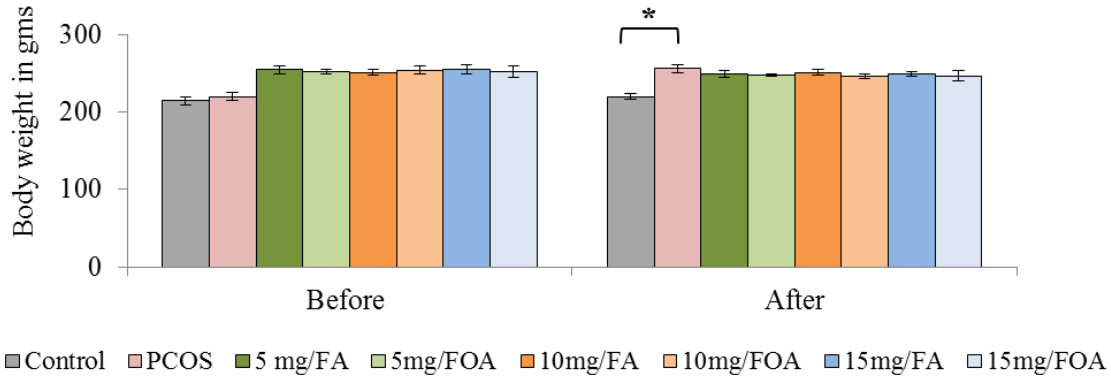
were designed to check their efficacy as a fertility agent. In this context, both fresh *Aloe vera* gel and *Aloe vera* formulation were fed to PCOS animals at different doses (5mg, 10mg, 15 mg) and were grouped according to time of treatment (30 days, 60 days, 90 days). The systemic and biochemical parameters were evaluated after the treatment was over.

### 4.3.3 BODY WEIGHT

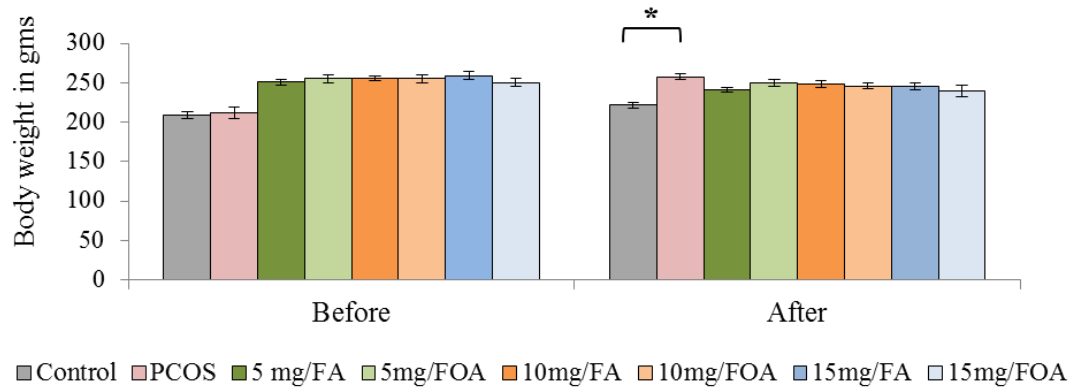
Obesity is a major feature in women with polycystic ovary syndrome (PCOS), and evidence suggests that obesity contributes to the pathogenesis of PCOS (Nestler 2000). Generally, excess abdominal adipose tissue (AT) initiates metabolic and endocrine aberrations that are central in the progression of PCOS (Escobar-Morreale and San Millán 2007). PCOS rat model exhibited significant increase in body weight with abdominal fat as compared to normal rats. However, after treatment with *Aloe* (Fresh and formulation), body weight reduction was not seen. (Figure 4.3.2 A, B and C).

**Figure 4.3.2 Dose and time dependent effect of *Aloe vera gel* (Fresh & Formulation) on Body Weight**

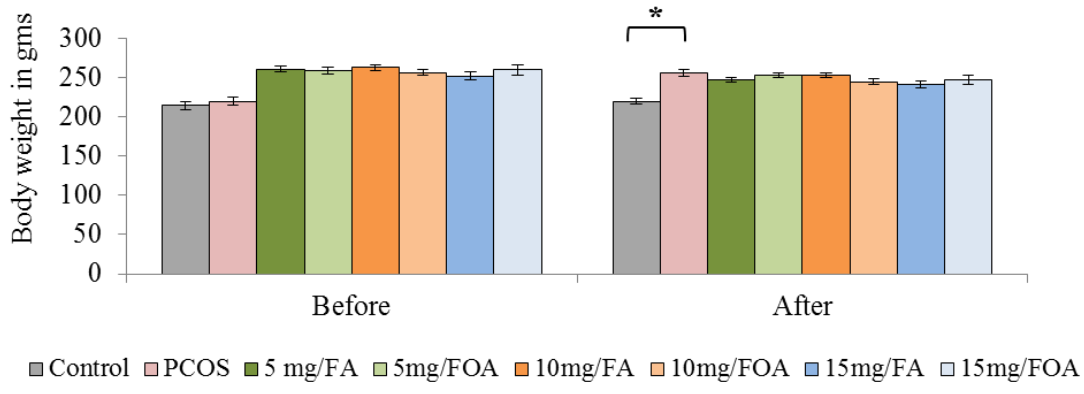
**(A) 30 days of treatment**




**(B) 60 days of treatment**




**(C) 90 days of treatment**




N=4-6. The values are represented as Mean + SEM.
*p<0.05 as compared to control group

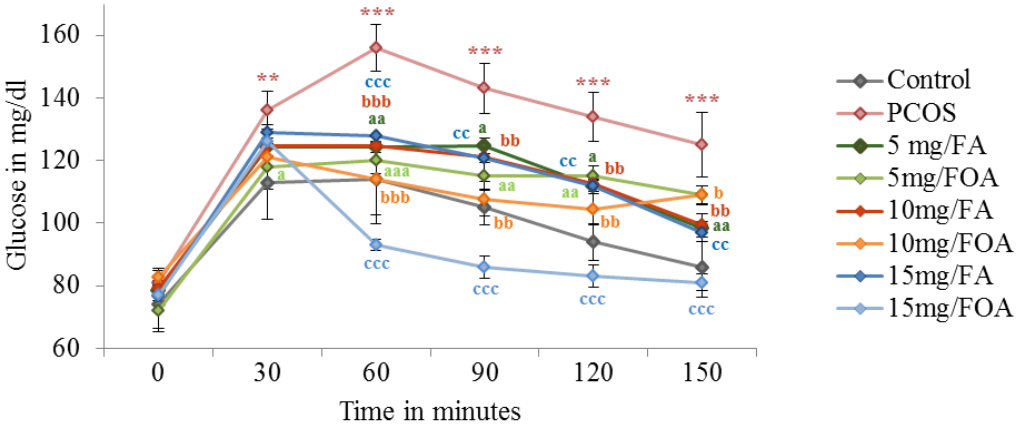
### 4.3.4 ORAL GLUCOSE TOLERANCE TEST (OGTT)

Women with polycystic ovarian syndrome (PCOS) are at increased risk for developing glucose intolerance leading to type 2 diabetes mellitus (DM) (Salley et al. 2007). Hence, it was necessary to evaluate the efficacy of *Aloe vera* gel on glucose homeostasis. Thereby, Oral glucose tolerance test (OGTT) was performed in all groups of animals. PCOS rats exhibited high glucose tolerance compared to normal control rats (**$p<0.01$, ***$p<0.001$) at all the time points of OGTT profile. Both *Aloe* fresh and formulation treated PCOS rats in different doses (5 mg, 10 mg, 15 mg) demonstrated significantly reduced glucose intolerance and improved cellular glucose uptake upon increasing time period of dose (30 days, 60 days, 90 days) at 60', 90' and 120' of OGTT profile (Figure 4.3.3 A,B and C).

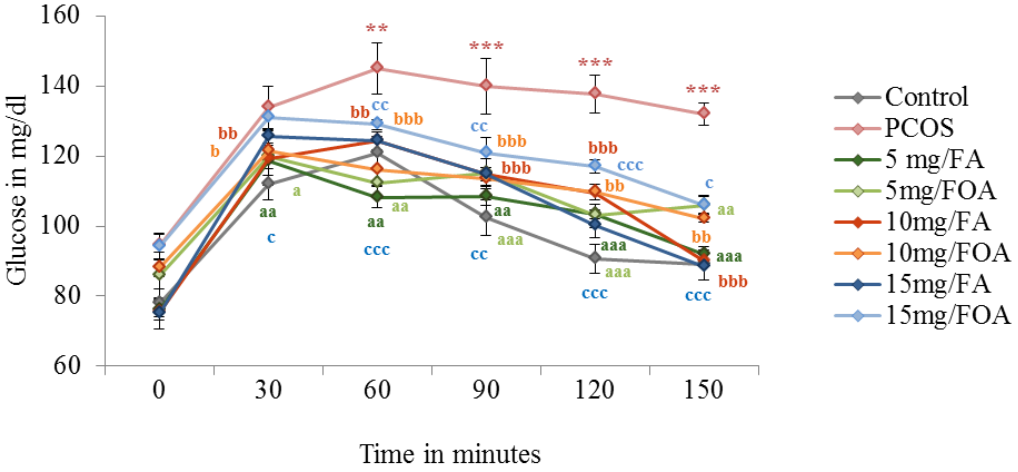
Glucose homeostasis is governed by insulin action. Thereby, we measured serum insulin level in all group of animals wherein serum insulin levels of untreated PCOS rats were increased significantly (***$p<0.001$). Treatment of fresh AVG caused a decrease in insulin level as compared to PCOS group (@@@$p<0.001$). However, decreased HOMA-IR was proportionate to the dose-time which indicates that longer period of treatment of AVG restores glucose homeostasis (Table 4.3.3).

**Figure 4.3.3 Dose and time dependent effect of *Aloe vera* gel (Fresh & Formulation) on Oral Glucose Tolerance Test (OGTT)**

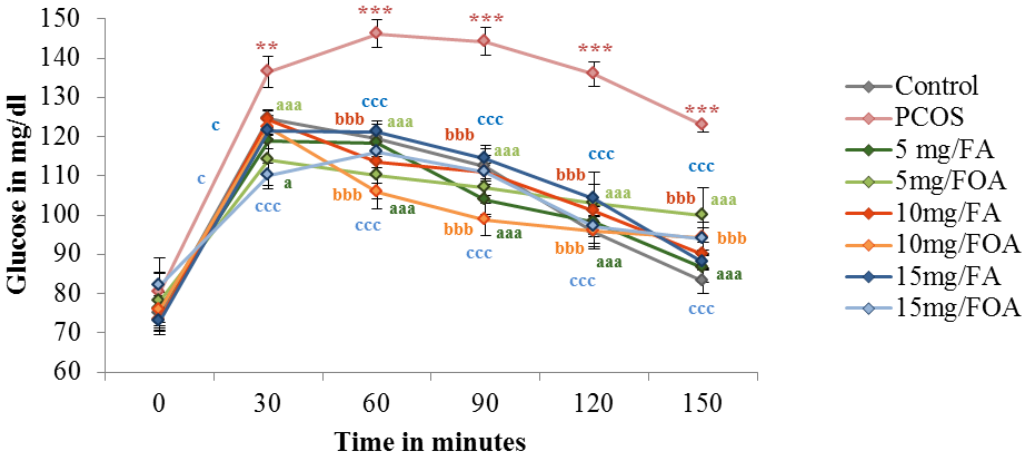
**(A) 30 days of treatment**




**(B) 60 days of treatment**




**(C) 90 days of treatment**




N= 4-6 per group; All values are represented as Mean + SEM; FA = Fresh *Aloe* gel; FOA = Formulated *Aloe* gel
**P<0.01, ***P<0.001 as compared to Control Group.;
[a] P<0.05; [aa] P<0.01; [aaa] P<0.001 for 5mg dosage compared to PCOS group
[b] P<0.05; [bb] P<0.01; [bbb] P<0.001 for 10mg dosage compared to PCOS group
[c] P<0.05; [cc] P<0.01; [ccc] P<0.001 for 15mg dosage compared to PCOS group

**Table 4.3.3 Dose and time dependent effect of *Aloe vera* gel (Fresh) on Insulin status**

| | Insulin (μIU/ml) | HOMA-IR |
|---|---|---|
| Control | 7.33±1.66 | 1.19±0.22 |
| PCOS | 17.6±0.8*** | 4.2 ±0.12*** |
| 5 mg/30 days | 5.1±0.6@@@ | 0.9±0.12@@@ |
| 5mg/60 days | 5.0±0.3@@@ | 0.8±0.04@@@ |
| 5 mg/90 days | 4.5±0.23@@@ | 0.9±0.07@@@ |
| 10mg/30 days | 3.9±0.2@@@ | 0.89±0.79@@@ |
| 10mg/60 days | 4.46±0.2@@@ | 0.87±0.02@@@ |
| 10mg/90 days | 3.7±0.4@@@ | 0.74±0.11@@@ |
| 15mg/30 days | 5.6±0.3@@@ | 1.0±0.01@@@ |
| 15mg/60 days | 4.66±0.4@@@ | 0.85±3.7@@@ |
| 15mg/90 days | 4.4±0.5@@@ | 0.8±1.2@@@ |

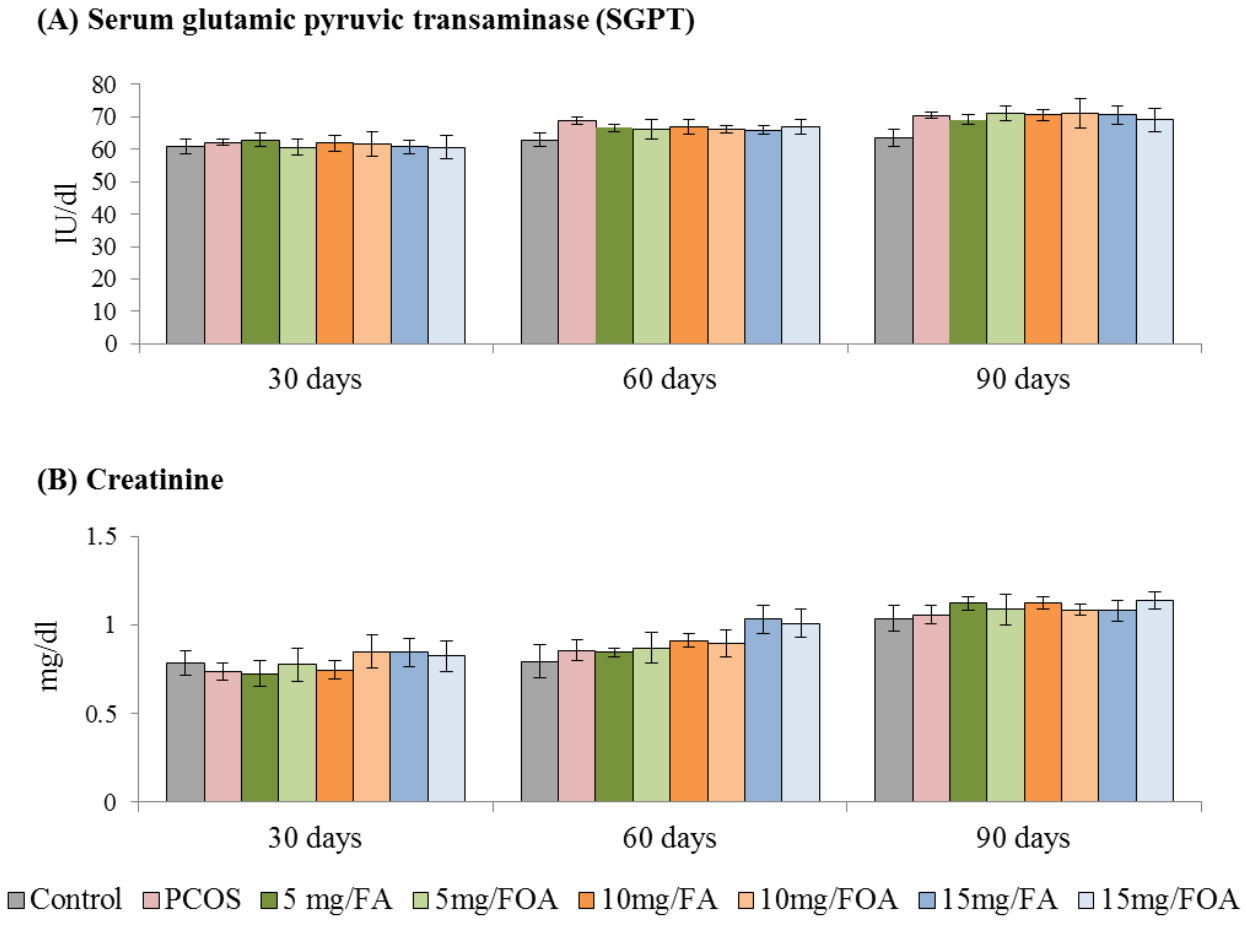
N= 4-6 per group, All values are represented as Mean + SEM.
***P<0.001 as compared to Control Group; @@@P<0.001 as compared to PCOS group.

HOMA IR = Fasting insulin x Fasting glucose / 405
Normal insulin resistance : < 3
Moderate Insulin resistance : Between 3 – 5
Severe Insulin resistance : > 5

### 4.3.5 TOXICITY STUDY

*Aloe vera* gel is a rich source of several phytocomponents. These phytocomponents or their metabolites may have a toxic effect on the animals. Hence, it was important to evaluate the toxicity parameters like Serum Glutamate Pyruvate Transaminase (SGPT) and creatinine during the experimental regime. Results demonstrate that both fresh AVG and formulation treated groups caused non-significant change in the levels of both these marker enzymes. Also, Letrozole induced PCOS rat model exhibited no significant change in the above parameters upon treatment. Hence, suggesting that all the treatment regimens were non-toxic to the animals (Figure 4.3.4 A and B). However, it is to be noted that higher doses for prolonged time of treatment may elicit toxicity.

**Figure 4.3.4 Dose and time dependent effect of *Aloe vera* gel (Fresh and Formulation) on serum toxicity markers**




N= 4-6 per group, All values are represented as Mean ± SEM

### 4.3.6 ESTRUS CYCLICITY

The primary clinical manifestations of polycystic ovary syndrome (PCOS) are irregular menstrual cycle and chronic anovulation, which is found to be associated with approximately 80% of PCOS women (Dunaif 1999). Hence, estrus cyclicity in PCOS rats was monitored, wherein PCOS rats exhibited arrested estrus cyclicity in late diestrus phase of cycle as compared to control rats. After treatment of AVG at various doses (5mg, 10mg, 15 mg of dry weight) and various time period (30 days, 60 days, 90 days), estrus cyclicity was evaluated wherein 5 mg for 30 days treated group of animals exhibited reversion to normal cycle in 80% of PCOS rats. But upon increasing the doses of *Aloe vera* (10 mg and 15 mg dry weight) for longer period of time (60 days and 90 days), all rats showed improved cyclicity and reverted back to normal cycle (Table 4.3.4 A, B and C).

**Table 4.3.4 Dose and time dependent effect of *Aloe vera gel* (Fresh and Formulation*)* on Estrus cyclicity**

**(A) 30 Days of treatment**

| GROUPS | Normal Animal | Extended Proestrus | Extended Estrus | Extended Metaestrus | Extended Diestrus |
|---|---|---|---|---|---|
| Control | 10/10 | - | - | - | - |
| PCOS | - | 2/10 >24 hr | - | 2/10 > 32 hr | 6/10 > 72 hr |
| 5 mg/FA | 4/5 | - | - | - | 1/5 >32 hr |
| 5mg/FOA | 4/5 | - | - | - | 1/5 >32 hr |
| 10mg/FA | 5/5 | - | - | - | - |
| 10mg/FOA | 5/5 | - | - | - | - |
| 15mg/FA | 5/5 | - | - | - | - |
| 15mg/FOA | 5/5 | - | - | - | - |

**(B) 60 Days of treatment**

| GROUPS | Normal Animal | Extended Proestrus | Extended Estrus | Extended Metaestrus | Extended Diestrus |
|---|---|---|---|---|---|
| Control | 10/10 | - | - | - | - |
| PCOS | - | 2/10 >24 hr | - | 2/10 > 32 hr | 6/10 > 72 hr |
| 5 mg/FA | 5/5 | - | - | - | - |
| 5mg/FOA | 5/5 | - | - | - | - |
| 10mg/FA | 5/5 | - | - | - | - |
| 10mg/FOA | 5/5 | - | - | - | - |
| 15mg/FA | 5/5 | - | - | - | - |
| 15mg/FOA | 5/5 | - | - | - | - |

**(C) 90 Days of treatment**

| GROUPS | Normal Animal | Extended Proestrus | Extended Estrus | Extended Metaestrus | Extended Diestrus |
|---|---|---|---|---|---|
| Control | 10/10 | - | - | - | - |
| PCOS | - | 2/10 >24 hr | - | 2/10 > 32 hr | 6/10 > 72 hr |
| 5 mg/FA | 5/5 | - | - | - | - |
| 5mg/FOA | 5/5 | - | - | - | - |
| 10mg/FA | 5/5 | - | - | - | - |
| 10mg/FOA | 5/5 | - | - | - | - |
| 15mg/FA | 5/5 | - | - | - | - |
| 15mg/FOA | 5/5 | - | - | - | - |

### 4.3.7 STEROIDAL ENZYME ACTIVITY

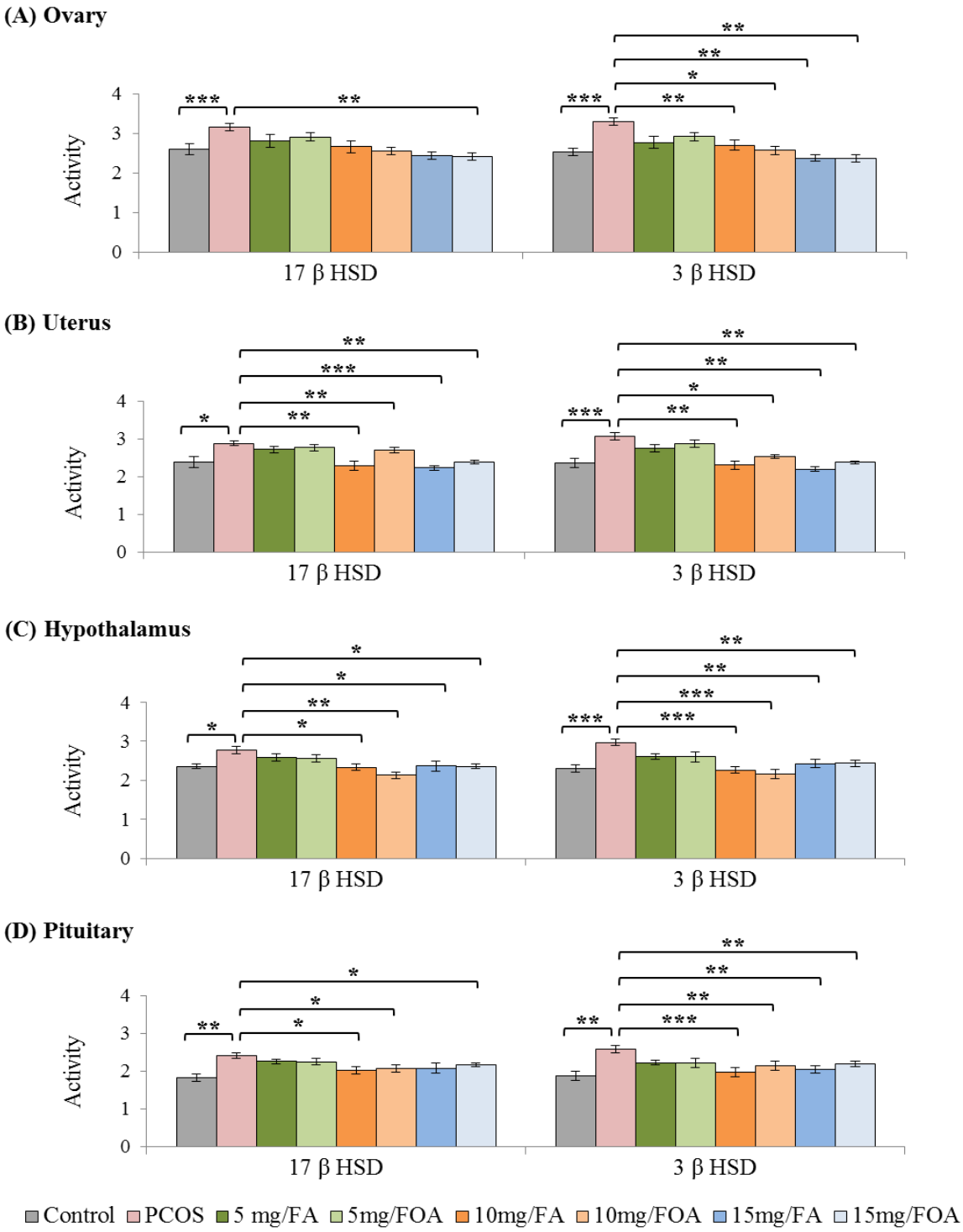
Steroidogenesis plays a crucial role in production of important steroid hormones in organs like ovaries, uterus and brain. Steroids are essential for the normal ovarian function and its regulation. Thereby, the aim was to evaluate the efficacy of *Aloe vera* gel (formulation as well as fresh AVG) in modulation of the key steroidal enzymes- 3β hydroxysteroid dehydrogenase (3β HSD) and 17β hydroxysteroid dehydrogenase (17β HSD) in reproductive tissues like ovary, uterus and brain – hypothalamus, pituitary.

There was non-significant change in steroidogenic enzymes in all tissues studied when treated at a dose of 5 mg dry weight (formulation and fresh AVG) for 30 days and 60 days. However, the animals demonstrated a significant change in steroidogenic enzyme activities as compared to the PCOS rats when the dose was continued upto 90 days. Also, it is to be noted that both fresh and formulated AVG exhibited significant reversion to the same extent. The group of animals which received 10 mg dry weight daily of both formulated as well as fresh AVG for 30 days did not show any change in 17β HSD activity in ovary and uterus, but significant modulation was observed in hypothalamus and pituitary. In case of 3β HSD, same dosage regime for 30 days could cause a significant change in ovary, uterus, hypothalamus and pituitary. It is seen that fresh AVG was more effective in bringing down the activities to normal compared to formulation especially in reproductive organs. However, maximum reversion was obtained in ovarian 3β HSD and 17β HSD enzyme activities when dose of 10 mg dry weight was given daily for 60 and 90 days. Group of animals that received 15 mg dry weight of AVG (Fresh/Formulation) for 30 days did not exhibit any modulation in 17β HSD activity in all steroidogenic organs studied (Figure-4.3.5). However, significant alteration in 17β HSD activity in ovary ($P<0.001$) was observed when the treatment was extended to 60 days and 90 days. Other tissues (uterus, hypothalamus and pituitary) demonstrated moderate alteration in 17β HSD activity. Similar status was observed in 3β HSD activity (Figure- 4.3.6 and Figure-4.3.7).

From the above data, it is evident that fresh AVG treatment at a minimum dose of 10 mg dry weight daily for 60 days was showing maximum efficacy both at systemic as well as reproductive organ level. In addition, individual phytochemical analysis proved that fresh as well as formulated *Aloe* species are rich in Phytosterols,

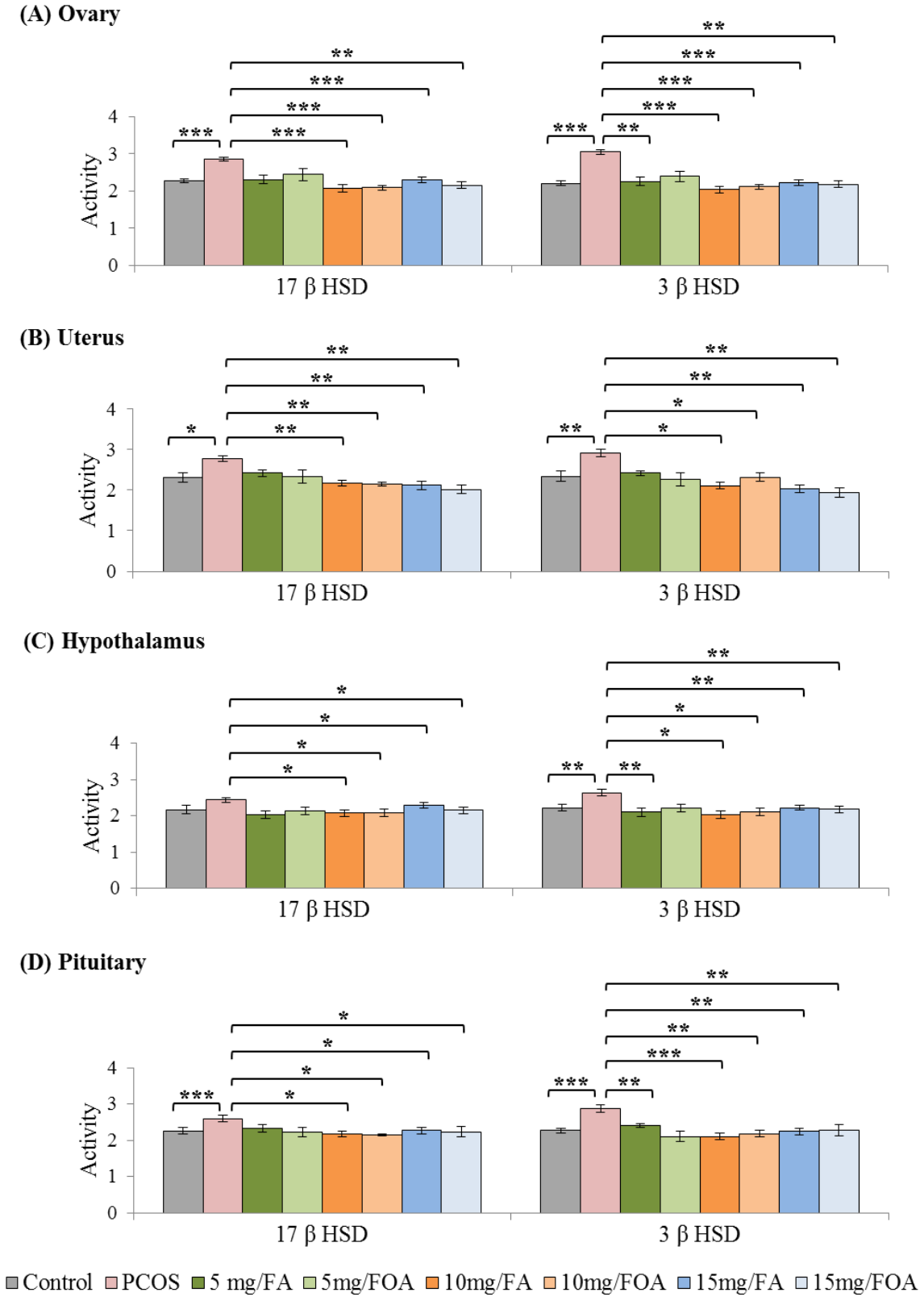
flavonoids and polyphenols. However, contribution of flavonoids and polyphenols from Turmeric and lemon present in the formulation can't be overlooked. From above, it is clear that use of fresh *Aloe vera* gel will elucidate the mechanism behind above described changes.

**Figure 4.3.5 Dose and time dependent effect of *Aloe vera* gel (Fresh and Formulation) on steroidogenic enzyme activity for 30 days of treatment**



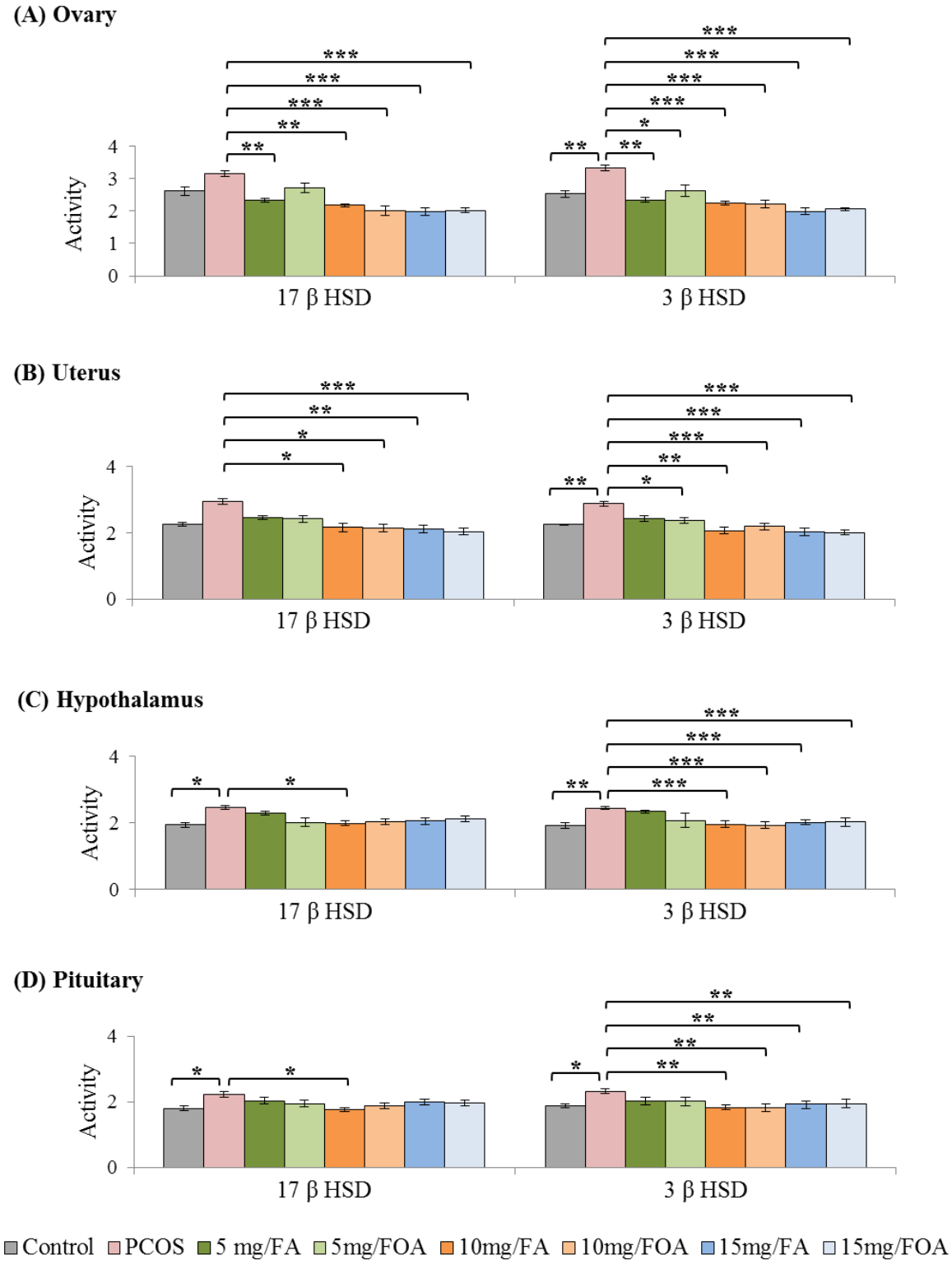

Activity = nanomoles of NADH formed/ min mg of protein
FA = Fresh *Aloe* gel; FOA = Formulated *Aloe* gel
n = 4-6 per group, All values are represented as Mean ± SEM; *P<0.05; **P<0.01; ***P<0.001

**Figure 4.3.6 Dose and time dependent effect of *Aloe vera* gel (Fresh & Formulation) on steroidal enzyme activity for 60 days of treatment**




Activity = nanomoles of NADH formed/ min mg of protein
FA = Fresh *Aloe* gel; FOA = Formulated *Aloe* gel
n = 4-6 per group, All values are represented as Mean ± SEM; *P<0.05; **P,0.01; ***P<0.001

**Figure 4.3.7 Dose and time dependent effect of *Aloe vera* gel (Fresh & Formulation) on steroidal enzyme activity for 90 days of treatment**




Activity = nanomoles of NADH formed/ min mg of protein
FA = Fresh *Aloe* gel; FOA = Formulated *Aloe* gel
n = 4-6 per group, All values are represented as Mean ± SEM; *P<0.05; **P,0.01; ***P<0.001

### 4.3.8 Hormonal profile

Data suggests that PCOS women have altered milieu of steroid hormones. Hence, an attempt was made to understand the role of fresh *Aloe vera* gel on steroid hormone profile. Group of animals treated with 5 mg dry weight of fresh *Aloe vera* gel daily for 30 days showed no change in serum testosterone levels. However, significant reduction in serum testosterone levels was observed when the dosage of fresh *Aloe vera* gel was prolonged for 60 days and 90days. In contrast, no significant alteration was observed in serum estradiol levels in all time regimes studied (30, 60 and 90 days). On the other hand, serum progesterone levels were successively elevated as the time of treatment was increased (Table-4.3.5).

Animals that received 10 mg dry weight of fresh *Aloe vera* gel for 30 days showed no modulation in serum testosterone, estradiol and progesterone levels. However, time-dependent (60 and 90 days) decrease in serum testosterone levels was observed. Additionally, the serum estrogen ($p<0.05$) and progesterone ($p<0.01$) levels were significantly elevated (60 and 90 days) as compared to PCOS.

Higher dose of 15 mg dry weight of fresh *Aloe vera* gel given at different time points (30, 60 and 90 days) showed similar hormonal profile as that of 10 mg dry weight of fresh *Aloe vera* gel given for 60 and 90 days. It is seen that alteration of hormones could be correlated with change obtained in steroidogenic enzymes when treated with *Aloe vera* gel. Hence, suggesting that 10 mg dry weight of fresh *Aloe vera* gel daily for 60 days is the minimum effective dose for reversion of the serum hormone profiles in Letrozole induced PCOS rat model.

**Table 4.3.5 Dose and time dependent effect of *Aloe vera* gel (Fresh) on steroid Hormonal profile**

| | Testosterone (ng/ml) | Estradiol (pg/ml) | Progesterone (ng/ml) |
|---|---|---|---|
| Control | 0.41±0.08 | 71.3±9.4 | 50.0±6.3 |
| PCOS | 1.13+0.15*** | 34.6±3.5* | 11.8.±1.6*** |
| 5 mg/30 days | 0.72±0.2 | 36.0±6.1 | 31±3.6@ |
| 5mg/60 days | 0.65±0.1@ | 32±6.4 | 30±2.3@ |
| 5 mg/90 days | 0.59±0.04@@ | 41±3.5 | 42.0±7.0@@ |
| 10mg/30 days | 0.86±0.11 | 63.3±14.5 | 33.0±3.3 |
| 10mg/60 days | 0.59±0.05@@ | 76.0±15.1@ | 43.3±3.70@@ |
| 10mg/90 days | 0.58±0.01@@ | 80.0±11.5@ | 46.0±7.2@@ |
| 15mg/30 days | 0.78±0.11 | 60±22.0 | 22.4±13.9 |
| 15mg/60 days | 0.64±0.06@@ | 70±20.8@ | 42.6±7.0@@ |
| 15mg/90 days | 0.69±0.03@ | 64.7±13.8@ | 32.0±3.4@ |

N= 3 per group; All values are represented as Mean ± SEM.
*P<0.5, **P<0.01, ***P<0.001 as compared to Control Group;
@P<0.05, @@P<0.01 as Compared to PCOS Group

### 4.3.9 Ovarian Histological study

Normal ovarian function relies upon the selection of a follicle that become dominant with appropriate signal FSH and ovulates with the help of LH surge during ovulation. This mechanism is disturbed in women with PCOS, resulting in multiple small cysts (or follicles), most of which contain potentially viable oocytes but within dysfunctional follicles (Webber et al. 2003; Franks et al. 2008). PCOS rat model in current study also demonstrated peripheral empty follicular cysts as compared to control ovary with normal growing follicles. In dose dependent study, 5 mg dose/30 days of treatment exhibited normal growing follicles but some cysts were present in ovary. *Aloe* at higher doses (10 mg and 15 mg) at 30 days, 60 days and 90 days of treatment, number of peripheral cysts significantly decreased and increased normal growing follicles with presence of corpus luteum was present; indicating normal ovulation due to functional ovary (Figure 4.3.8).

**Figure 4.3.8 Dose dependent effect of *Aloe vera* gel (Fresh*)* on Ovarian structure**

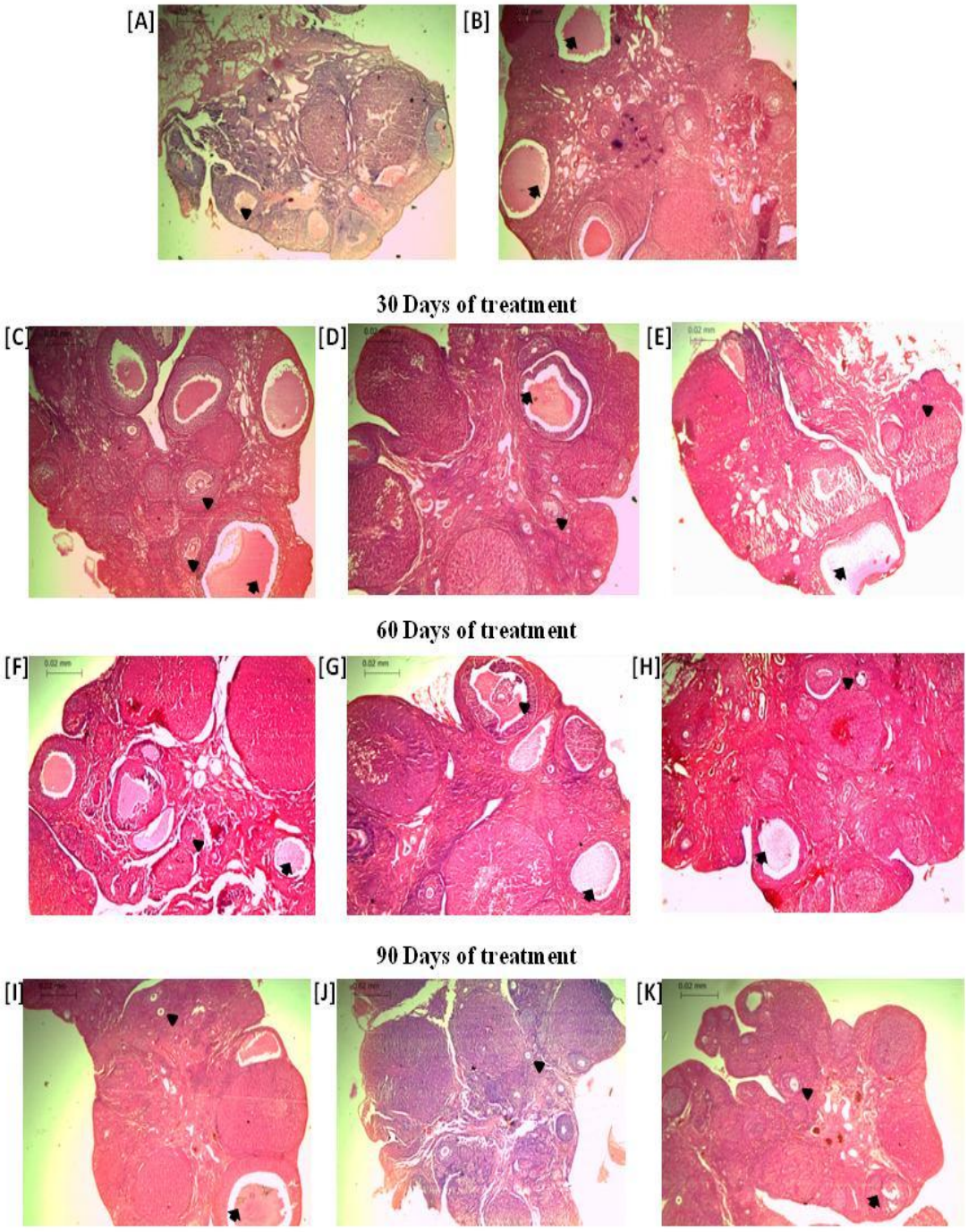



Figure 4.3.8: (A ) Control; (B) PCOS; (C-E) 5mg, 10mg, 15 mg dose of Fresh *Aloe vera* gel respectively for 30 days; (F-H) 5mg, 10mg, 15 mg dose of Fresh *Aloe vera* gel respectively for 60 days; (I-K) 5mg, 10mg, 15 mg dose of Fresh *Aloe vera* gel respectively for 90 days. Sections taken in diestrus stage of estrus cyclicity; Magnification:-4X

: Growing follicles : Cyst

### 4.4 DISCUSSION

PCOS has many clinical manifestations, which includes oligomennorhea and hyper-androgenism, leading to metabolic dysfunction (Dickerson et al. 2010). Rat model created using letrozole exhibited an increase in ovarian androgens and thus leading to hyperandrogenism, which is a hallmark of PCOS. Also, significant weight gain was observed in letrozole treated PCO as compared to control rats, which could be attributed to deposition of abdominal fat (Carmina et al. 2007; Diamanti-Kandarakis et al. 2007). The model created show similar characteristics of PCOS shown by Kafali et al., (2004). It has been well documented that PCOS is positively correlated with insulin resistance (Duanif et al., 2008). The PCOS rat model was hyperglycemic and demonstrated glucose intolerance in oral glucose tolerance test (OGTT) indicating insulin resistance (Honnma et al. 2006), which was also evident from high HOMA-IR values as observed in the current study. Apart from, systemic level changes, ovarian steroidogenesis were also altered leading to high testosterone level in PCO phenotype (Kafali et al. 2004) which could be correlated to ovarian structural changes as seen in present study (Dunaif 2008). Thereby, letrozole induced PCOS rat model demonstrated increased body weight, arrested cyclicity and impaired glucose intolerance with hyperandrogenim that are key features of PCOS phenotype.

Aim of current chapter was to understand the dose and time required by *Aloe vera* gel for the management of PCO condition. Preliminary published work suggested that *Aloe vera* gel helped to minimize PCO associated symptoms in letrozole induced rat model (Maharjan et al. 2010). Thereby, future studies were directed to evaluate minimum effective dose and time period for *Aloe vera* gel treatment which would manage PCOS phenotype and restore normal ovarian function.

Thereby, experiments were carried out with various doses (5 mg, 10 mg, and 15 mg dry weight) at different time points (30 days, 60 days, 90 days) with two different form of *Aloe vera* gel: Fresh *Aloe vera* gel and other of *Aloe* formulation with added natural preservatives.

Dose and time dependent effect demonstrated that treatment irrespective of time and dose could cause a reversion to normo-glycemic condition from hyperglycemic

condition as observed in PCO phenotype. For both, *Aloe vera* gel (AVG) and *Aloe* formulation treatment with higher dose (10, 15 mg) at longer period of time (60 and 90 days) demonstrated more significant effects as compared to low dose (5 mg) for short period time (30 days of treatment). This could be attributed to the nutritionally rich phytosterols and phyto-phenols present in the plant (Rajasekaran et al. 2006; Tanaka et al. 2006), that helps to recover the syndrome and could be able to sensitize the insulin receptors for the glucose uptake. Also, it should be noted that *Aloe vera* gel is rich in fibers that could increase transit time for diet to be get absorbed which could modulate glucose homeostasis in PCO phenotype.

In this study, PCO rats demonstrated the formation of empty cysts with follicular fluid which is similar to ovarian structural changes that was reported by Kafali et al. (2004). PCOS rats treated with fresh AVG and formulation exhibited normal follicular growth which was evident from normal estrus cyclicity as seen in higher doses (10 mg, 15 mg dry weight) at longer period of times (60 and 90 days). With low dose of 5 mg for longer period of time (60 days) rats also exhibited reversion in ovarian structure. However, it should consider that with increasing dose, phyto-components content is increased. These phyto-components present in AVG could be active components which would alter the steroidogenesis and expression of steroidogenic protein, which alters the PCO conditions (Sharpe et al. 2007).

Apart from altered steroidogenic proteins, it has been indicated that hyperinsulinemia is also positively stimulates thecal androgen production leading to hyperandrogenic phenotype (Urbanek et al. 2007; Diamanti-Kandarakis 2008) and estradiol deficiency (Gaspard 2009). As the estrogen synthesis is inhibited in letrozole induced model; the increased ovarian steroidal 3β HSD and 17β HSD activity would increase androgen concentration (Doi et al. 2006); this might affect the hormonal axis (LH: FSH ratio), which plays a crucial role for the regulation of ovarian structure-function. In present study, treatment with extracts caused a decrease in activity of 3β HSD and 17β HSD activity in PCO rats at dose (10 mg/30 days, 15mg/30) days whereas with at longer period of time (60 days and 90 days) elicited more significant changes. However, at 30 days of treatment caused no significant change with lower dose (5 mg) whereas at longer period (90 days) of time with 5mg dose caused a reversion in enzyme activity. The reversion of estrus cyclicity upon extracts treatment could be attributed to

phytochemical components present in the gel that maintains steroid status, regaining back the fertility status.

Preliminary phytochemical analysis demonstrated that gel is rich in phytosterols and polyphenols, which could be the active component to control the hyperglycemic condition and modulate steroidogenesis (Maharjan et al. 2010). In addition, Hypoglycemic potential is due to phytosterols was elucidated by Tanaka et al. (2006)**.** Also, demonstrated that Aloe poly-phenols are powerful agents for diminishing glucose absorption with modifying glucose and insulin levels in diabetic mice. In addition to this, similar report from Perez et al. (2007) indicated the hypoglycemic potential of Aloe. "In vitro" study suggested that polyphenols can down regulate the expression levels of 3β-HSD, CYP17, and CYP21 mRNA as suggested by Pieau and Dorizzi (2004) whereas other phyto-components like isoflavones inhibit the both 3β-HSD and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Keung 1995). Recent studies indicate that certain compounds in *Aloe vera*, e.g. coumaric acid, may stimulate the activity of testicular macrophages which is responsible for nitrous oxide production, and suppress the conversion of cholesterol to pregnenolone through inhibition of $P_{450}$ cytochrome activity, thus reducing testosterone production (Chrousos and Kino 2007). Also, phytoestrogens can lower the serum concentration of testosterone (Weber et al. 2001) by suppressing the secretion of LH (Malaivijitnond et al. 2004). Recent study also demonstrated that some of *Aloe* species have influence on reproductive cycle. "In vitro" production of estradiol and progesterone by ovarian cells of proestrus rat was significantly increased in the presence of increasing concentration of the plant extract containing *Aloe*. Thus, suggesting role of *Aloe* on ovarian steroids (Telefo et al., 2004). Hence, it could be possible that various phyto-components present in AVG (as discussed above) could act on various targets of HPG axis wherein they directly modulate steroidogenic key enzymes involved in steroid production in major regions of brain like hypothalamus and pituitary and also reproductive organs like ovaries and uterus. They elicit their response by improving the steroid hormone levels and hence modulate the ovarian structure and function in PCOS phenotype.

Dose and time dependent study have elucidated that with low dose of 5 mg was not sufficient to recover all symptoms of PCO phenotype. However, higher dose was

required wherein both high doses 10 mg and 15 mg demonstrated important changes in PCO phenotype upon longer time period treatment (60 and 90 days). 5 mg of both the extracts of AVG and formulation for longer period (90 days of treatment) exhibited some extent of reversion of symptoms of PCO pathology.

During AVG treatment, toxicity markers in both fresh AVG and *Aloe* formulation treated groups did not show any toxic effects during experiment. Hence, suggesting that both the extract at various doses (5 mg, 10 mg, 15 mg) are non-toxic for the animal study when treated till 90 days of treatment.

Considering all above parameters studied, 10 mg dry weight treated for 60 days was the minimum dose required for the reversion and maintenance of PCO condition. At low dose and time period (5 mg/30 days), *Aloe vera* gel is not able to modulate the steroid status completely. This could the amount of the phytosterols that is present is too less to modulate their effect. It is to be noted at high concentration of 15 mg of extracts were showing similar effect as 10 mg. This could be attributed to saturation that might have achieved in concentration of phytosterols, thus showing similar effect. However, exact quantification of phyto-components reaching ovary needs to be evaluated.

### 4.5 CONCLUSION

Thus, it can be concluded from the present study that 10 mg *Aloe vera* gel for 60 days seems to be optimum dosage to show maximum effect. However, used both the extracts Fresh *Aloe vera* gel (AVG) and of *Aloe* formulation demonstrated similar kind of effect in all parameters studied for management of PCO phenotype like reduced peripheral cysts with increasing growing follicles, decreased glucose intolerance with improved steroid status and modulate ovarian steroidogenesis. In dose (5mg, 10mg, 15mg) and time (30 days, 60 days, 90 days) experiments, increase in dose and time period of treatment successfully improved PCO phenotype and restored the ovarian structure-function with help of modulatory properties of phyto-components present in *Aloe vera* gel (AVG) and its formulation. Current chapter also studied the comparative effect of both *Aloe vera* forms (fresh gel and formulation preparation) on ovarian function wherein, fresh AVG treatment at a minimum dose of 10 mg dry weight daily for 60 days was showing maximum

efficacy both at systemic as well as reproductive organ level in non-pregnant stage. Also, Fresh AVG has proven to be more effective in regulation of steroidogenesis in reproductive organs. Thereby, further experiments were directed in understanding the potential of AVG as a fertility agent to promote conception. Also, detailed phytochemical characterization of *Aloe vera* gel phytocomponents and their identification of "in-vivo" molecular targets for management of PCOS phenotype is elucidated in future chapters.

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