CHAPTER 8

SUMMARY AND CONCLUSION

8.1 SUMMARY

Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy seen in women of reproductive age. Though it is a reproductive disorder, it is associated with characteristics of metabolic syndrome including insulin resistance (Soares et al., 2009). Current available mode of treatment is by use of insulin sensitizers like metformin and ovulating agents like clomiphene citrate (Chang, 2007). But, these drugs have been reported for their side effects upon prolonged usage (Salpeter et al., 2003).

Hence, researchers in current era are exploring alternative therapy to manage the metabolic syndrome (Kamat, 2002; West et al., 2001). In this context, many scientists have demonstrated the role of medicinal plants in the management of hyperglycemic condition (Abbott et al., 1998). An important plant that has been exploited for various efficacies in the current decade is *Aloe vera. It* has gained its popularity as anti-hyperglycemic plant (Rajasekaran et al., 2004), wherein its phytosterols (Tanaka et al., 2006) and polyphenols have been studied extensively for the above efficacy. Thereby, it would be of interest to biopropsect *Aloe vera gel* for management of metabolic induced anovulatory disorder like PCOS. In this context, current work has elucidated the potentiality of *Aloe vera* gel (AVG).

To study the efficacy of AVG, Polycystic ovarian syndrome (PCOS) rat model was developed with use of letrozole (0.5 mg/kg/body weight/21 days/orally) - a non-steroidal aromatase inhibitor (Kafali et al., 2004). The model created exhibited similar characteristics of PCOS phenotype and was in accordance with Kafali et al. (2004). Model established was hyperglycemic and glucose intolerant with high HOMA-IR values, along with the presence of atretic peripheral cysts in ovary reflecting disturbances in estrus cycle. Thus, letrozole induced PCOS rat model demonstrated key features of PCOS phenotype.

Further, PCOS rat model was treated with *Aloe vera* gel [in two different forms]: A) Fresh *Aloe vera* gel and B) *Aloe* formulation (preparation with added natural preservatives) in various dose doses (5 mg, 10 mg, 15 mg dry weight) at different time points (30 days, 60 days, 90 days). After completion of experiment regime, various biochemical parameters were checked to evaluate the efficacy of *Aloe vera* gel (in both

forms of AVG).

In current study, treatment with both *Aloe vera* gel (AVG) and *Aloe* formulation with higher dose (10, 15 mg) at longer period of time (60 and 90 days) demonstrated more significant effect as compared to lower dose (5 mg) for short period time (30 days of treatment) wherein it restored glucose sensitivity and normal insulin level along with improved HOMA IR index. Along with the above changes, ovarian structure-function (in terms of hormone levels and presence of developing follicles) was seen upon the AVG 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 sensitize the insulin receptors for the glucose uptake.

In dose-time dependent studies, it was clear that 10 mg dry weight treated for 60 days was the minimum dose and time required for the reversion and maintenance of PCO condition. At low dose and at lesser time period (5 mg/30 days), *Aloe vera* gel was not able to modulate the steroid status completely. This could be due to the fewer amounts of the phyto-components present for modulation. It is to be noted at high concentration - 15 mg dry weight of extract was showing similar effect as 10 mg. This could be attributed to saturation that might have achieved in concentration of phyto-components, thus showing similar effect. Similar effects were observed in both fresh *Aloe vera* gel and *Aloe* formulation in all studied parameters suggesting that *Aloe* phyto-components independently could cause a modulation in PCO phenotype. All the treatments did not affect toxicity parameters, thus suggesting Aloe treatment was safe. Also, it was interesting to note that reproductive organ function was restored more significantly with fresh *Aloe*. Apart from this, *Aloe* formulation having added natural preservatives (Turmeric, citric acid, kadaya gum) whose phytocomponent function in addition to *Aloe* cannot over-look. Thereby, all the further experiments were designed with Fresh *Aloe* only, as it contributes its maximum efficacy on reproductive organs.

As AVG was efficient in restoring ovarian structure-function in PCOS phenotype, it was of interest to understand role of AVG as fertility herb that could aid in healthy conception. In this context, letrozole induced PCOS rats was treated with AVG (10mg/daily/orally) for 60 days [standardized dose with time] and were further allowed to

mate with male rats. Along with this treatment regime, an additional group was considered wherein PCO rats continued to receive letrozole along with AVG till end of the experiments to understand the protective effect of *Aloe vera* gel.

Results show that PCOS model exhibited fewer implantations and decrease in live pups with retarded growth as compared to the control. Similar results are evident from literature (Bellver et al., 2011; Giudice, 2006) As steroid mileu determines the progression of pregnancy, it could suggest that altered hormonal environment could affect the development of progeny. Current study demonstrated altered *in utero* environment like decrease in progesterone with increased testosterone levels may result in failure of implantation and prenatal defects, which is implicated in PCOS phenotype as justified by other studies (Petraglia et al., 2015; Sir-Petermann et al., 2002). It is interesting to note that frequency of defects was reversed upon AVG treatment given before conception period. This is also believed that fetal development depends on pre-conceptional maternal health. PCOS model during pregnancy demonstrated altered molecular signals including up-regulation of steroidogenic regulatory proteins like StAR, AR, LHR, IR; which manifests in terms of higher steroidogenic enzymes activities in reproductive organs (3β HSD/17βHSD) leading to abnormal hormone levels. When phytochemical rich AVG was administered to PCO rats before conception, it has modulated deranged molecular events leading to normal physiology which is evidenced by increased fetal outcomes with less deformities. This implies AVG is a potential preconceptive agent that promotes healthy and successful pregnancy.

In context of above observed physiological modulations, it was of interest to elucidate detailed phytochemistry of AVG and to isolate, identify the active component responsible for mentioned efficacies. In this regard, both qualitative and quantitative analysis confirmed the presence of phyto-components namely polysaccharides, alkaloids, polyphenols, sterols, flavonoids, anthraquinones etc., which is similar to data reported by (Hamman, 2008). Several data has suggested that phytosterols and polyphenols can have a role in modulation of steroid status (Weber et al., 2001; Wu et al., 2010); thereby partial purification of AVG using polarity gradient method was attempted. Four different fractions: P1 (Petroleum ether extract), P2 (Chloroform extract), P3 (Ethyl acetate extract),

P4 (η-butanol extract) was separated and were confirmed for its phytochemical content by qualitative analysis.

Apart from the phytochemical analysis, *"Ex vivo"* experiments were performed with partially purified fraction to evaluate direct effect of phyto-components on ovarian steroidogenic enzymes activities using PCOS model. Results suggested that non polar P1 fraction of *Aloe vera* gel was most effective in modulating ovarian steroidogenesis. Further, the detailed chromatographic analysis- TLC, HPTLC, and HPLC of P1 fraction (Non polar fraction extracted with petroleum ether) demonstrated the presence of various phytosterols (β sitosterol (approx. 70 % of the content), stigmasterol and lupeol) and phytosterol glycosides. Literature has also implicated the possible role of phytosterols as modulatory agent of steroidogenesis in fish model (Sharpe et al., 2007). This implies that P1 fraction could contain the active component that may exert effect on ovarian structure-function.

With reference to above observed effects, wherein P1 (non polar) fraction of AVG could directly affect ovarian function, it would be of great interest to understand its "*in vivo*" efficacy. In this series of experiments, ~25 μg of non-polar fraction of AVG in olive oil (10 mg dry weight of AVG contained ~25 μg phytosterols) was administered to PCOS rat model and various parameters were studied. PCOS rats were hyperglycaemic, insulin resistant, hyperlipideamic with elevated levels of androgen and decreased level of gonadal steroids. This abnormal steroid level in PCOS phenotype was correlated with increased expression of steroid regulatory proteins like StAR, 3□-HSD, aromatase and LHR. Structurally, ovary had the presence of peripheral cysts.P1 fraction treated PCOS rats exhibited glucose sensitivity along normal insulin levels as mediated by improved HOMA-IR, which was contaminant with the decreased transcript of Insulin receptor, implicating phytosterols of P1 has good glucose lowering property (Tanaka et al., 2006). Also, normal lipid status was restored from dyslipidaemic condition as seen in PCOS rats.

Hormonal profile was restored in P1 fraction treated rats which indicates modulatory action has taken place in ovarian steroidogenesis by inhibiting rate limiting key protein that involved in androgen production and modulate the androgen-estrogen

flux in ovary. These changes lead to regulate the steroid hormones biosynthesis and normalized hormones level. Balanced hormone secretion by P1 treatment has reversed the existence of peripheral cysts and transformed immature follicle into developing follicle as seen in our study. Also, transcript and protein expression of cholesterol flux protein –StAR has been modulated by P1 treatment, in addition to decreased LH Receptor. Possible mechanism that can be implicated is that phytosterols may decrease in LH level that could cause a decrease LHR expression, which further regulates the key protein expression like StAR and 3□□HSD. In addition, synergism of insulin to mediate hyper androgenic effects have been also reduced on P1 treatment (as insulin level decreases in P1 treated rats) , thus suggesting insulin levels also contribute to this pathology. This can be substainted by various implicated mechanism as quoted in the literature. Data show that estrogenic effects of β-sitosterol on the pituitary and role of phytoestrogens like genistein that could decreases GnRH-induced luteinizing hormone (LH) release in rats (Recabarren et al., 2008; Walters et al., 2008). Moreover, no toxic effects were seen on P1 fraction treatment. Thus, preliminary studies suggest that P1 fraction containing phytosterols could be the active component that helps in management of PCOS symptoms. However, further mechanistic studies needs to be conducted to understand the physiological targets of non-polar phyto-components.

8.2 CONCLUSION

Following conclusions are drawn from the present study:

1) Letrozole induced PCOS rats model treated with two different forms of *Aloe*: fresh *Aloe vera* gel and *Aloe* formulation in various doses (5 mg, 10 mg, 15 mg) and time (30 days, 60 days, 90 days) dependent manner suggested that dose of 10 mg/60 day was optimum dosage to show maximum effect that reduced PCO like phenotype such as decreased in peripheral cysts with growing follicles, decreased glucose intolerance with improved steroid status and modulate ovarian steroidogenesis. Present study also elucidated that fresh *Aloe vera* gel and *Aloe* formulation both exhibiting similar kind of effects in PCO phenotype that indicates that Fresh *Aloe vera* gel is more effective in regulation of ovarian steroidogenesis rather than stored A*loe* formulation with added

natural preservatives. *Aloe* vera gel has a good efficacy for management of PCOS phenotype.

2) PCOS rats treated with *Aloe vera* gel before conception could increase implantation leading to healthier pups with few or no resorption. Treatment of AVG prior to conception also improves PCOS health status by restoration of ovarian structure implicated by improved fertility index as compared to PCOS rats. Phyto-nutrient rich AVG was successfully able to normalize the abnormal biochemistry by modulating hormonal profile as well as expression level of key proteins involved in steroidogenesis. This implies AVG is a potential pre-conceptive agent that promotes healthy and successful pregnancy.

3) *Aloe vera* gel is rich in various phyto-components that were analysed by several qualitative and quantitative analyses. Method was successfully validated as per ICH guidelines and statistical analysis proved that the method is sensitive, specific, and repeatable. Further, confirmation for the presence of Phytosterols like stigmasterol, β-sitosterol etc along with steroid deravitives has been performed by HPTLC, HPLC. Further, GC MS analysis confirmed the presence of these Phytosterols. Phytosterol component could directly act as modulator of ovarian function as depicted from "*Ex vivo*" studies.

4) Partially purified non polar P1 fraction (rich in phytosterols: β sitosterol, stigmasterol, lupeol) treatment restored glucose impairment, dyslipidemia and normalized insulin levels along with improved HOMA IR index suggesting that P1 fraction has hypoglycaemic, hypolipidemic and anti hyperinsulinemic effect in PCO condition. At organ level, P1 fraction of *Aloe vera* gel also significantly affects expression of StAR, LHR and Insulin receptor (IR) in PCOS rats, thus leading to modulated steroid levels. This modulation could be due to effect of phytosterols acting at all organ levels of HPO axis. Overall, study implies that non polar P1 fraction containing phytosterol that could be active phyto-component involved in modulation of hyperglycaemic,

hyperinsulinemic condition leading to restoration of ovarian function in PCO rodent model. This proves phytosterols (P1 fraction) could be novel component which can be explored for management of PCOS.

This is first study where implication of *Aloe vera* gel as well as phytosterol extracted from *Aloe vera gel* has shown the potential to manage PCOS, which could be considered as a novel component for future drug development.

8.3 REFERENCES

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