Synopsis of the thesis on

Bioprospecting *Aloe barbadensis* Mill. as a possible pre-conceptive agent in Polycystic Ovary Syndrome

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By

Shivani Dhadhal

(Reg No. FOS/2151)

Under the Supervision of

Dr. Laxmipriya Nampoothiri

Department of Biochemistry, Faculty of Science

The M.S. University of Baroda

Vadodara-390002

Gujrat, India

Introduction:

Polycystic Ovary Syndrome, a term of infertility has been now recognized as one of the major infertility disorders around the world (Wolf et al., 2018). Etiopathology of this disorder is mainly linked to hyperandrogenism, hyperinsulinemia, infrequent ovulation, and the presence of numerous peripheral cysts in ovaries (Robin et al., 2012). This multi etiological pathology is associated with clinical pregnancy complications, an increased rate of spontaneous abortion/early pregnancy loss, and preterm delivery (Palomba et al., 2015; Azizia & Hardiman, 2011). However, molecular alterations in PCOS pregnancy that originates from the mother, embryo, or both are still in debate.

It is known that to achieve successful conception and pregnancy, the initial move is to apposition, attachment, and invasion of the embryo to the uterine epithelium, following decidualization of the uterine stroma (Lee et al., 2007). The first step in successful pregnancy is embryo implantation which is a dynamic process that integrates numerous signaling events in which synchronized production of ovarian progesterone and estrogen hormones mediates structural and functional changes in the uterus during the implantation phase of pregnancy (Wang et al., 2013a). In the endometrium, steroid hormones mediate their effect through their receptor. Data has demonstrated abnormal expression of steroid hormone receptors, such as estrogen receptor, progesterone receptor, androgen receptor, and its cofactors in the endometrium of women with PCOS. (Li et al., 2014; Apparao et al., 2002; Tang et al., 2019). These suggest that the steroidal milieu is an important mediator for a healthy pregnancy. Further, as uterine decidualization, a key event during early pregnancy, homeobox transcription factors HOX10a, and HOX11a are known to regulate this process (Du & Taylor, 2016). The hyperandrogenaemia in patients with PCOS leads to reduced uterine HOX10a expression which may contribute to the poor endometrial function of women with PCOS (Wang et al., 2013b). In the next step, invasion starts with penetration of the embryo to the uterine wall by matrix metalloproteinases (MMP-2 and 9) in mouse uterus during early pregnancy. Activities of MMPs were tightly controlled by their endogenous inhibitors, the tissue inhibitors of MMPs (TIMPs). Imbalance in the ratio of MMP-2, 9, and their regulators TIMP-2 at the systemic level could be responsible for in women with spontaneously terminated pregnancy in the first trimester (Nissi et al., 2013). Further, invasion is followed by the differentiation/proliferation of stromal cells surrounding the implantation site of the uterus. Leukemia inhibitory factor (LIF), is a pleiotropic cytokine of

the IL-6 family that is considered to influence various stages of early pregnancy ranging from embryo adhesion to the regulation of stromal cell proliferation. LIF transduces its signal through the formation of a heterodimer with specific LIFR and the common co-receptor for the IL-6 family (gp130). The binding of the LIF to its receptor leads to activation of STAT3, its impact on modulation of embryo-uterine functions during embryo implantation (Suman et al., 2013). Further, the importance of this pathway was revealed in one of the studies in which dysregulated LIF-STAT3 pathway results in impaired implantation in mice (Wang et al., 2013a). Though all these factors are explained, the exact correlation and crosstalk that exists between the interference of steroids, receptivity markers, and fetal implantation is still not clear.

All of the underlying mechanisms that occur within the embryo and endometrium establishes an appropriate milieu that is crucial for the development and survival of the fetus in PCOS pathology. PCOS women who wish to conceive are therapeutically intervened by several pharmacological approaches to overcome the problem of infertility and improve pregnancy outcomes wherein induction of ovulation (e.g, Clomiphene citrate) and insulin-sensitizing agents (e.g, Metformin) have been used (Artini et al., 2018). However, the use of these synthetic drugs is disputable due to side effects upon prolonged usage. It is associated with an increased prevalence of pre-eclampsia and high perinatal mortality (Hellmuth et al., 2000) and gastrointestinal side effects including nausea, diarrhea & vomiting (Jorquera et al. 2020). Hence, the use of traditional medicine systems has been preferred, several medicinal plants have been explored as a therapy for PCOS (Maharjan et al., 2010; Moini Jazani et al., 2019; Khale et al., 2012; Andhalkar et al., 2021).

Among the reported medicinal plants, *Aloe barbadensis* is used as a popular folk medicine throughout the world (Baruah et al., 2016), and its application in the management of the female reproductive system has been reported (Sheba & S, 2021; Shima et al., 2011). This beneficial biological activity of *Aloe vera* gel is due to its richness in phytochemicals such as polysaccharides, glycosides, flavonoids, carbohydrates, tannins, alkaloids, anthraquinones, organic compounds, pyrones, phytosterols, anthrones, fatty acids, sterols, terpenoids, hormones, vitamins, proteins (Radha & Laxmipriya, 2015; Nalimu et al., 2021). However, there is not much scientific evidence, in this context, *Aloe vera* has been explored in our laboratory wherein, we demonstrated that *Aloe vera* gel was efficient in the management of PCOS in terms of ovarian structure-function (Maharjan et al., 2010 M. Radha et al., 2014)

and its complications (Desai et al., 2012). In addition, *Aloe vera* gel when given before conception could increase the fetal size and the number with no resorptions (Radha and Laxmipriya, 2016b). Thus, suggesting that AVG is a good pre-conceptive agent and helps in complications associated with PCOS pregnancy. Further, solvent-based extraction of AVG demonstrated that phytosterol containing a non-polar fraction of *Aloe vera* gel (25ug/kg of body wt for 60 days) could modulate steroidogenic targets in the nonpregnant letrozole induced PCOS rat model (M. Radha & Laxmipriya, 2016). Also, it is interesting to note that there are not many scientific data regarding the treatment of PCOS phenotype with indigenous plants used as a pre-conceptive therapy. In this line, preliminary data observed that a non-polar fraction of *Aloe vera* gel (25ug/kg of body wt for 30 days) could improve fetal growth and development on the day 18^{th} of pregnancy in letrozole-induced PCOS mouse model. This suggests phytosterol present in the non-polar extract of AVG could direct the early gestation events to form proper fetus development. Thereby, phytosterols might act on the molecular players that are important for the establishment of an early pregnancy window. Hence, the present study aims to understand the molecular derangement of the feto-maternal interface and their corrections by the use of active nutraceutical components. In this context, extraction of the non-polar Phyto components of AVG was carried out using petroleum ether by the soxhlet method and characterized them using GC-MS analysis. Further, to check the efficacy of the non-polar phytocomponents of AVG, pivotal molecules of early pregnancy (Day-6^{th} of pregnancy) were studied in a letrozole-induced PCOS mice model. The study also attempted to understand the possible interaction that may exist between identified phytocompounds with early pregnancy molecules using *in-silico* molecular docking.

Aim of the study

The aim of the current study was to decode the molecular cascade of embryo-uterine modulators in early pregnancy loss of letrozole-induced PCOS mouse model and to elucidate the therapeutic potential of non-polar phytocomponents of petroleum ether extract of *Aloe barbadensis* when given as a pre-conceptive agent in the improvement of embryo-uterine transmission of PCOS mouse model.

Specific Objectives:

I. To assess the key regulators of early pregnancy in the letrozole-induced PCOS mouse model.

II. To evaluate and validate the role of the non-polar extract of *Aloe vera* gel on early pregnancy in the PCOS mouse model.

III. To identify the targets of the phytocomponents of non-polar extract of *Aloe vera* gel towards modulation of early pregnancy using the *"in-silico"* approach.

Summary of work done

I. To assess the key regulators of early pregnancy in the letrozole-induced PCOS mouse model.

To study the implantation window in PCOS, a letrozole-induced PCOS mouse model, which is well established in our lab, was used (Dey et al., 2022). For PCOS induction, female Balb/c mice were orally administered 0.5µg/kg of body weight of letrozole for 21 days. After 21 days of the treatment, animals were validated for the PCOS phenotype. Next, female mice were allowed to mate with male mice of the same strain (2:1). The following morning, females were checked for the presence of a vaginal plug. The day of vaginal plug was considered day 1 of pregnancy. On the morning of day 6 (Adhesion of embryo to uterus), animals were sacrificed and further studies were carried out. In the current experiment, letrozole-induced female Balb/c mice showed elevated testosterone levels during early pregnancy. In addition, low levels of progesterone were observed, which can be associated with the disruption of corpus luteum formation in PCOS animals. The altered hormone profile in PCOS animals might influence ovarian structure. When examined for ovarian histology, the number of peripheral cysts was observed, which is one of the characteristic features of PCOS (Fox et al., 1991). Reports have shown that hyperandrogenism and low progesterone content in women with PCOS have a lower probability of childbirth, decreased pregnancy rates, and higher miscarriage rates. (Elenis et al., 2021; Su et al., 2017; Gaggiotti-Marre et al., 2019). Indistinguishable, results were noticed in our study, the number of pregnant females and the number of implanting embryos in the uterus were significantly reduced in the PCOS animals. Next, the present study attempts to further narrow down molecular deficits of pregnancy loss in PCOS pathology.

In the embryonic-uterine tissue, steroid hormones mediate their effect through their receptors in the establishment of implantation. When examined for the expression of steroid hormone receptors in the implanted region of the uterus on the day 6^{th} of pregnancy, PCOS animals exhibited declined *Pgr* gene expression. It was reported that progesterone signaling inhibited androgen receptor (*Ar)* expression, whereas estrogen dramatically elevated *Ar* abundance in

the stroma of ovariectomized mouse uteri during early pregnancy (Li et al., 2014b). In this direction, the current study revealed that altered P/*Pgr* signaling in PCOS animals did not have a prohibited influence on *Ar* expression, as the overexpression of *Ar* was observed in the implanted site of the uterus.

Further, ovarian steroid hormones have been known to regulate the cell adhesion molecules during the attachment phase of the embryo (Merviel et al., 2001). In our study, letrozole-treated animals exhibited a decrease in gene expression of integrin αv, β3, and α4 in the implanted region of the uterus. In this direction, the study reported that when ovariectomized rats were treated with a sex-steroid regime to mimic the hormonal changes of early pregnancy, their findings have shown that administration of testosterone has resulted in a marked decrease in the expression of integrin αvβ3 in the uterus. Furthermore, the upregulated expression of αvβ3 integrin in the uterus was observed following progesterone replacement suggesting that progesterone is likely responsible for the observed increase in αvβ3 integrin level (Mokhtar et al., 2018). Hence, the PCOS animals having low systemic progesterone content can lead to a decline in the expression of adhesive markers in the implanted region of the uterus.

Blastocyst attachment with the uterine epithelium is followed by the decidualization of the stromal cells. In mice, the blastocyst is the stimulus for decidualization (Deng et al., 2019), and the homeobox transcription factors are known to regulate this process (Du & Taylor, 2016). In addition, progesterone is influential in decidualization during implantation in the mouse uterus (Lim et al., 1999). When examined for the expression of the HOX10a, reduced gene expression of the HOX10a in the implanted region of the uterus was observed in the PCOS animals compared to that of control animals. Based on this, and the above-cited report, low serum progesterone concentration in the PCOS group could not induce the signals that are required for decidualization.

Matrix metalloproteases (MMPs) and their inhibitors (TIMPs) have a significant role in tissue remodeling, during early gestation (Curry & Osteen, 2001). There are reports indicating that proteases and TIMPs in the uterus have been controlled by the action of estrogen (Zhang et al., 2007; Nothnick et al., 2004). Altogether, evidence suggests the estrogenic regulation of MMP and TIMPs expression in the uterus. However, in the current study, PCOS animals did not exhibit any change in estradiol content as compared to the control animals. Also, results

revealed that the expression of MMP-9, TIMP-1 & 3 was significantly reduced in the letrozole-treated animals.

Furthermore, proper growth and development of the implanting embryo is a fundamental event in early pregnancy, and the key molecules of leukemia inhibitory factor (LIF)-STAT pathways are crucial in this process (Salleh & Giribabu, 2014). when analyzed for the key markers of the LIF signaling pathway, letrozole treated animals did not show any significant difference in the mRNA levels of LIF and LIFR in the implanted region of the uterus compared to that control animals. On the other hand, the expression of GP130 and STAT3 significantly declined in the PCOS animals. In this study, disrupted LIF-STAT signaling was observed in the PCOS animals.

Altogether, it can be concluded that the abnormal expression of key markers of early gestation in PCOS could be the reason for the early pregnancy complications/early fetal loss associated with PCOS women. As evidence of the poor fertility index and reduced number of implanted embryos were observed in the PCOS animals.

II To evaluate and validate the role of the non-polar extract of *Aloe vera* gel on early pregnancy in the PCOS mouse model.

The current study focused to elucidate the therapeutic potential of phytosterols containing petroleum ether (PE) extract of *Aloe barbadensis (Aloe vera* gel-AVG) when given as a pre-conceptive agent in the improvement of *in utero* environment for appropriate embryo maturation in letrozole induced PCOS mouse model. Treatment of PCOS animals with petroleum ether (PE) extract of AVG (25 & 75 µg/kg of body weight, orally for 30 days) before conception resulted in decreased circulating free testosterone and increased progesterone content was observed in PCOS animals which were comparable with *Aloe vera* gel and metformin. The improved hormone milieu might be responsible for the healthy follicular development in the PCOS ovary, as the reduced number of peripheral cysts and increased number of corpus luteum was observed by phytosterol containing PE extract of AVG treatment. Also, enhanced pregnancy rate, number of pregnant females, and implantation sites on the day 6^{th} of pregnancy were observed in letrozole-induced PCOS animals. This could be due to the anti-androgenic, insulin-sensitizing, and progestogenic properties of PE extract of AVG. It can be clearly seen that phytochemicals present in PE extract of AVG have the potential as an endocrine modulator in PCOS pathology. However,

their molecular targets are not defined. Thereby, the present study attempts to further narrow down molecular deficits of pregnancy loss in PCOS pathology and its management using phytochemicals extracted from *Aloe vera* gel.

Next, the effect of PE extract of AVG on steroidal receptor expression was analyzed, and the decline *Pgr* gene expression and normal expression of *Ar* was observed in the implanted region of the uterus of PCOS mice upon the treatment with Metformin, AVG, and PE extract of AVG. Hence, it could be noted that enhance progesterone signaling by the phytosterol-containing plant have a modulatory effect on the androgen receptor. To substantiate our results, the previous study from our lab has demonstrated that campesterol from partially purified non-polar phytocompounds of *Aloe vera* gel significantly modulates ovarian steroid receptor gene expression in letrozole induced PCOS mice (Dey et al., 2022).

Further, uterine decidualization, a key event during early pregnancy, homeobox transcription factors (*Hox10a*, and *Hox11a)*, are known to regulate this process (Du & Taylor, 2016) and it is evident that P/*Pgr* signaling regulates the *Hox10a* in the endometrial cells (Taylor et al., 1998). Treatment of PCOS animals with metformin and PE extract of AVG exhibit upregulating *Hox10a* expression in the implanted site of the uterus. This result also demonstrates the progestogenic potential of phytosterols of AVG, consequently engaging in the decidualization process during early pregnancy events in PCOS animals. In our experiment, the transcript level of *Mmp9, Timp1,* and *Timp3* were significantly reduced in the implanted site of the uterus in PCOS animals. Upon PE extract of AVG treatment in PCOS animals only *Mmp9* was showing upregulation, with no changes in *Timp1,3* in the implanted site of the uterus. Hence, it was observed that steroid fluctuation by phytosterol containing PE extract of AVG in PCOS animals was not responsible for the stabilization of MMP/TIMPs.

Furthermore, proper growth and development of the implanting embryo is a fundamental event in early pregnancy, and the key molecules of leukemia inhibitory factor LIF-STAT pathways are crucial to this process (Kimber, 2005). In PCOS animals declined transcript levels of *gp130* were found to be increased upon PE extract of AVG treatment. Phytochemicals present in the PE extract of AVG could be responsible for this effect. Also, declining *Stat3* in the implanted region of the uterus in PCOS animals was upregulated by PE extract of AVG treatment. Reduced levels of STAT3 in PCOS animals may lead to dysregulation of PR protein expression, because conditional ablation of STAT3 leads to dysregulation of PR mediated pathways and decreased PR protein expression *in utero*,

suggesting that STAT3 has a critical role in PR dependent pathways during implantation in mice (J. H. Lee et al., 2013). Remarkably, PE extract of AVG has been potent towards the regulation of PR mediated pathway *Stat3* in early pregnancy complications of PCOS pathology. All the studied markers were modulated upon the PE treatment at both doses when given for 30 days. It is to be noted that PE extract of AVG was equally potent as AVG and metformin towards control of pregnancy complications associated with PCOS, indicating that oral administration of PE extract of AVG in the dose of 25 µg/kg/day before conception for 30 days is adequate for treatment of PCOS pregnancy complications without causing any adverse effect to animals.

III To identify the targets of the phytocomponents of non-polar extract of *Aloe vera* gel towards modulation of early pregnancy using the *"in-silico"* approach.

The observed efficacy of the petroleum ether extract of AVG could be due to the presence of certain phytocompounds. Thereby, GC-MS analysis of petroleum ether extract of *Aloe vera* gel identified a total of 25 chemical structures. Amongst the identified phytochemicals, on the basis of a previous lab study (Dey et al., 2022) and the molecules with reported biological activities (n-Hexadecanoic acid and Campesterol) were selected for the molecular docking study. In order to elucidate the molecular targets and the magnitude of interaction with the key regulars of implantation, through which the PE extract of AVG acts, an *in silico* molecular docking experiment was performed using MGL Tools1.5.7rc1 and Autodock vina software. Protein preparation: The three-dimensional structure of all the receptors selected in this study (Androgen receptor (AR) PDB ID: 4OHA, Progesterone receptor (PR) PDB ID: 6F88, Homeobox 10A (Hox10a) PDB ID: 3K2A, Signal transducer and activator of transcription 3 (STAT3) PDB ID: 6NUQ, Glycoprotein 130 (gp130) PDB ID: 1BQU were obtained from the RCSB-Protein data bank site. The protein structures were prepared using Discovery studio 2020 in which water molecules and other small molecules were removed, and the energy was minimized using autodock 1.5.7rc1 from MGL Tools 1.5.7rc1. Ligand preparation: All the ligand structures isolated for docking studies were drawn using ChemDraw software and prepared using autodock from MGL Tools 1.5.7rc1. After ligand preparation grid generation was done using the same software autodock from MGL Tools1.5.7rc1.

From the docking studies, it has been revealed that the campesterol ligand shows the best binding affinity as compared to n-hexadecanoic acid. In the binding pocket of the androgen

receptor (PDB ID: 4OHA.pdb), n-hexadecanoic acid shows a docking score of -3.7 with alkyl and Pi-alkyl interactions with amino acids PHE A: 804, ARG A:752, TRP A:751, PRO A:801 whereas Campesterol shows a binding score -8.0 and alkyl interactions with residues LEU A: 805, PRO A:801, PRO A:682, and ALA A:748. Similarly in the active site of progesterone receptor (PDB ID: 6F88.pdb), n-hexadecanoic acid shows a docking score of -4.8 and carbon-hydrogen interaction with amino acid CYS A:340 and alkyl and pi-alkyl interactions with residues ALA A:74, VAL A:279, LEU A:162, PHE A:64, PHE A:277, VAL A:163, and campesterol shows docking score -9.5 with alkyl and Pi-Alkyl interaction interacting with PHE A:64, and ALA A:74. In the homeobox protein (PDB ID: 3K2A) active site, the n-hexadecanoic acid shows a -3.4-docking score along with the alkyl and Pi-alkyl interactions with ALA A:293, TRP A:294, ILE A:290, LEU A:312 amino acid residues similarly campesterol shows a docking score of -6.8 with alkyl and Pi-alkyl interaction with TRP A:294, LEU A:312, ILE A:290, VAL A:286. In the STAT3 protein (PDB ID: 6NUQ) binding site n-hexadecanoic acid shows alkyl interactions with ILE A: 258, ARG A:325, CYS A:251, PRO A:336, PRO A:256, ALA A:250 amino acid residue along with the docking score -3.7. Similarly, campesterol shows conventional hydrogen bonding with AMINO Acid ARG A:350 and alkyl interaction with resides PRO A:336, ILE A:258, CYS A:251 along with a -7.3-docking score. In the binding pocket of GP130 (PDB ID: 1BQU), n-hexadecanoic acid shows a docking score of -3.2 along with the Alkyl interaction with ILE A:154, VAL A:168 residue, conventional hydrogen bonding with residue GLN A:153, TYR A:143 and carbon-hydrogen bond with residue PRO A:155. Similarly, campesterol shows a -5.5-docking score along with the alkyl interaction with LYS A:189 amino acid residue.

From the molecular docking studies, it has been revealed that the non-polar phytocompounds are mainly involved in the conventional hydrogen bonding, alkyl, and Pi-alkyl interactions with the amino acid residues present in the active binding site of different receptors. Also, it has been found that campesterol has shown a higher docking score as compared to n-hexadecanoic acid. Also, the targeted receptors it has been visualized that progesterone receptors were showing good interactions with campesterol as compared to others. On the basis of the docking results validation of these markers in response to Campesterol-containing fraction administration needs to be confirmed in the future.

Conclusion

Overall, the present study demonstrated that PE extract of AVG exhibited anti-androgenic and progestogenic effects. The key molecular players of early pregnancy events were

investigated using "*in vivo*" and "*in silico*" studies. The possible mechanism of a phytosterol-containing extract derived from *Aloe vera* gel may be due to its regulatory effect on the transcription of steroid receptors and key early pregnancy marker proteins, mainly by acting as a progestogenic agent. It helped in the restoration of the majority of progesterone-responsive genes during the early phase of pregnancy in letrozole-induced PCOS mice. In addition to this, the ovarian structure was improved by normalizing the hormonal milieu in letrozole-induced PCOS animals. Remarkably, PE extract of AVG is equally potent as AVG and metformin towards control of pregnancy complications associated with letrozole-induced PCOS animals, suggesting that oral administration of PE extract of AVG before conception for 30 days is a sufficient dose for treatment of PCOS pregnancy complications without causing any adverse effect to animals. ***Altogether, the study provides a background for two important milestones for PCOS pathology: elucidation of steroid-mediated regulation and its derangement in the materno-fetal interface, and identification of a new phytochemical of Aloe vera gel with no side effects as a pre-conceptive agent. Overall, this study has successfully identified an indigenous economic important phytocompound that could be translated as a "Phyto-drug" for healthy, successful Pregnancy in multi-etiological endocrinopathy namely PCOS.***

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Abstracts published in conferences

1. **Shivani Dhadhal,** Anjana patanvdiya and Laxmipriya Nampoothiri. Association of stress mediators with early pregnancy loss in polycystic ovarian syndrome- A rodent study. Presented at an international conference on reproductive healthcare and 32nd Annual Meeting of the Indian Society for the study of reproduction and fertility. 11th-13th February 2022.
2. **Shivani Dhadhal** and Laxmipriya Nampoothiri. Remodeling of LIF-STAT signaling drives early pregnancy loss in PCOS. Presented in the National Conference on Reproduction and Endocrinology and 38th meeting of the society for reproductive biology and comparative endocrinology, Department of studies in genetics and genomics, University of Mysore, Mysuru. 28th to 30th, December 2021.
3. **Shivani Dhadhal** and Laxmipriya Nampoothiri. Status of receptivity markers and steroidogenic receptors in letrozole induced PCOS mouse model. Presented at Global Conference on Reproductive Health with Focus on Occupational, 29th Environmental and Lifestyle Factors & Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF), Jawahar Lal Nehru University, New Delhi, India. February 22-24, 2019.
4. Arpi Dey, Ishita Mehta, Priyanka Oshin, Priyanka Ghosh, Radha Maharjan, **Shivani Dhadhal** and Laxmipriya Nampoothiri. Non-polar phytocomponents of *Aloe barbadensis* Mill. act as a potential therapeutic alternative for polycystic ovarian syndrome by targeting steroidogenic and metabolic receptors. Presented at Global Conference on Reproductive Health with Focus on Occupational, 29th Environmental and Lifestyle Factors & Annual Meeting of the Indian Society for the Study of Reproduction and Fertility (ISSRF), Jawahar Lal Nehru University, New Delhi, India. February 22-24, 2019.
5. Laxmipriya Nampoothiri, Prashant Sudra, Arpi Dey, **Shivani Dhadhal**. Bioprospecting of *Garcinia indica* for its anti-obesity properties in cafeteria diet induced obese rat model. Presented at International Conference on Proteins, miRNA and exosomes in health and disease (PREHD-2018) held at The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India. December 11-13, 2018.
6. Arpi Dey, **Shivani Dhadhal** and Laxmipriya Nampoothiri. Phytosterols isolated from *Aloe barbadensis* Mill., restore reproductive and metabolic complications in Letrozole-induced PCOS mouse model. Presented at 23rd International Conference on Herbal and Alternative Remedies for Diabetes and Endocrine Disorders held at Bangkok, Thailand. November 02-04, 2017.
7. **Shivani Dhadhal** and Laxmipriya Nampoothiri - Alteration of redox status in spleen of hyperandrogenic rat model- international conference on reproductive biology and comparative endocrinology and 35th Annual meeting of the society for reproductive biology and comparative endocrinology: Hyderabad-9th-11th February, 2017.
8. Arpi Dey**, Shivani Dhadhal** and Laxmipriya Nampoothiri - Comparative evaluation of non-polar extracts of *Aloe vera* gel in management of polycystic ovarian syndrome. - international conference on bioactive chemicals for reproductive and human health and 33rd annual meeting of the society for reproductive biology and comparative endocrinology; Davangere, 26th-28th February, 2015.

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1. Laxmipriya N., Prashant Sudra, Arpi Dey, **Shivani Dhadhal**, Azazahemad A. K., Satyanshu K, Tushar, D, Raghuraj S., and Premlata, K. (2021). Fruit juice of *Garciniaindica* Choisy modulates dyslipidemia and lipid metabolism in cafeteria diet-based rat model. Annals of Phytomedicine 10(1): 1-9, 2021.
2. Dey, Arpi, **Shivani Dhadhal**, Radha Maharjan, Padamnabhi S. Nagar, and Laxmipriya Nampoothiri. . "Partially purified non-polar phytocomponents from Aloe barbadensis Mill. gel restores metabolic and reproductive comorbidities in letrozole-induced polycystic ovary syndrome rodent model-an “in-vivo” study." *Journal of Ethnopharmacology* 291 (2022): 115161.

Shivani Dhadhal

(Ph.D. student)

Dr. Laxmipriya. Nampoothiri

(Guide)

Dr. Laxmipriya P. Nampoothiri
Associate Professor
Department of Biochemistry, Faculty of Science,
The Maharaja Sayajirao University of Baroda
Vadodara - 390002. (Guj.) INDIA.

Head

Department of Biochemistry

HEAD
Biochemistry Department

Dean

Faculty of Science

DEAN
FACULTY OF SCIENCE
THE M.S. UNIVERSITY OF BARODA