

TABLE OF CONTENTS

Acknowledgements.....	i
List of Abbreviations	iv
Abstract.....	v
Chapter 1	
1 Introduction	1
1.1 Polycystic Ovary Syndrome.....	1
1.1.1 Prevalence of PCOS.....	2
1.1.2 Pathophysiology of PCOS	2
1.1.3 Hyperandrogenism	6
1.1.4 Insulin resistance and hyperinsulinemia	7
1.2 Endometrium function	9
1.3 Implantation window	9
1.3.1 Molecular signatures of early pregnancy	10
1.3.2 Steroid hormone receptors during early pregnancy	11
1.3.3 Adhesion phase of implantation.....	12
1.3.4 Decidualization during the establishment of pregnancy	13
1.3.5 Invasion of an embryo during early pregnancy.....	14
1.3.6 LIF-STAT signalling during early pregnancy.....	15
1.4 Pregnancy complications in Polycystic Ovary Syndrome	17
1.4.1 Pathogenesis of early pregnancy loss in PCOS.....	18
1.5 Endometrium abnormalities in PCOS.....	19
1.5.1 Primary endometrial function in PCOS	19
1.5.2 Secondary endometrial function related to PCOS characteristics.....	20
1.6 Letrozole-induced PCOS model	23
1.7 Lifestyle change initiatives	24

1.8	Drugs	24
1.8.1	Insulin-sensitizing drugs	24
1.8.2	Ovulation induction agents	25
1.8.3	Anti-androgens.....	26
1.9	Herbal therapy for PCOS	27
1.9.1	Classification of Aloe vera.....	29
1.9.2	Traditional Uses of Aloe vera	29
1.10	Aim of the study.....	31
1.11	Specific objectives:	32

Chapter 2

2	Materials and Methods	33
2.1	In-vivo analysis of pregnancy complications associated with PCOS	33
2.1.1	Animal housing and maintenance	33
2.1.2	Letrozole induced PCOS mouse model	34
2.1.3	Procedure/Drug administration and experimental design	34
2.2	The efficacy of the PE extract of AVG, when given as a pre-conceptive agent in the PCOS mouse model.....	37
2.2.1	Animals	37
2.2.2	Drug administration and experimental design	37
2.2.3	Establishment of pregnancy	38
2.2.4	Analysis of fertility parameters	39
2.2.5	Gene expression analysis	39
2.2.6	cDNA synthesis.....	41
2.2.7	Real-time PCR	42
2.2.8	Protein Estimation.....	43
2.2.9	Gelatin zymography	44
2.2.10	SDS-PAGE and Western blot	46

2.2.11 Western Blot.....	49
2.2.12 Drug safety parameter analysis	50
2.3 Phytochemical analysis of Petroleum ether extract of <i>Aloe vera</i> gel.....	51
2.3.1 Plant Material.....	51
2.3.2 Extraction of phytoconstituents by Soxhlet method	53
2.3.3 Quantitative analysis of Phytosterols (Kenny, 1952).....	53
2.3.4 Gas Chromatography-Mass Spectrometry (GC/MS) analysis	54
2.4 <i>In-silico</i> analysis of the non-polar phytocomponents of <i>Aloe vera</i> gel with the early pregnancy marker.....	55
2.4.1 Molecular docking study.....	56
2.4.2 Statistical analysis	56

Chapter 3

3 Assessment of the key regulators of early pregnancy in letrozole-induced PCOS mouse model	59
3.1 Rationale of the study.....	59
3.2 Material and methods.....	61
3.3 Results	62
3.3.1 PCOS induction in mice using letrozole	62
3.3.2 Fetal growth and development during the late gestation period in PCOS mice	64
3.3.3 Early pregnancy loss (Day-6 th of pregnancy) in PCOS mice.....	66
3.3.4 Altered ovarian gonadotropin receptors on the day 6 th of pregnancy in PCOS mice ..	70
3.3.5 Impairment of ovarian steroidogenesis on the day 6 th of pregnancy in PCOS mice ...	70
3.3.6 Modulation of ovarian steroid receptors on the day 6 th of pregnancy in PCOS mice	71
3.3.7 Altered steroid hormone receptor in the embryo implanted region of the uterus in PCOS mice.....	74
3.3.8 Impairment of feto-maternal interaction in the embryo-implanted region of the uterus in PCOS mice.....	76

3.3.9 Modulation in decidualization of embryo implanted region of the uterus in PCOS mice	77
---	----

3.3.10 Imbalance in the expression of matrix metalloproteinases and their endogenous inhibitor in the embryo implanted region of the uterus in PCOS mice	77
--	----

3.3.11 Disruption of the LIF-STAT3 signalling pathway in the embryo implanted region of the uterus in PCOS mice	79
---	----

3.4 Discussion	80
----------------------	----

3.5 Conclusion	87
----------------------	----

Chapter 4

4 Role of non-polar extract of <i>Aloe vera</i> gel on early pregnancy in letrozole-induced PCOS mouse model.....	88
--	-----------

4.1 Rationale of the study.....	88
---------------------------------	----

4.2 Material and methods.....	89
-------------------------------	----

4.3 Results.....	91
------------------	----

4.3.1 Phytochemical analysis of PE extract of AVG	91
---	----

4.3.2 Gas Chromatography-Mass Spectroscopy (GC/MS) analysis	91
---	----

4.3.3 Effect of PE extract of AVG on fetal growth and development during the late gestation period in PCOS mice	94
---	----

4.3.4 Effect of PE extract of AVG on early pregnancy loss (Day-6) of PCOS mice	96
--	----

4.3.5 Effect of PE extract of AVG on the ovarian gonadotropin receptors on day 6 of pregnancy in PCOS mice	105
--	-----

4.3.6 Effect of PE extract of AVG on the key markers of ovarian steroidogenesis on day 6 of pregnancy in PCOS mice.....	106
---	-----

4.3.7 Effect of PE extract of AVG on the ovarian steroid receptors on day 6 of pregnancy in PCOS mice.....	108
--	-----

4.3.8 Effect of PE extract of AVG on the steroid receptor in the embryo implanted region of the uterus in PCOS mice	111
---	-----

4.3.9 Effect of PE extract of AVG on the feto-maternal interaction in the embryo-implanted region of the uterus in PCOS mice	113
--	-----

4.3.10 Effect of PE extract of AVG on decidualization of embryo implanted region of the uterus in PCOS mice	114
4.3.11 Effect of PE extract of AVG on the MMP and TIMPs in the embryo implanted region of the uterus in PCOS mice.....	115
4.3.12 Effect of PE extract of AVG on LIF-STAT3 signalling pathway in the embryo implanted region of the uterus in PCOS mice	117
4.3.13 Effect of PE extract of AVG on toxicity parameters	119
4.4 Discussion	127
4.5 Conclusion	137

Chapter 5

5 Identification of the targets of non-polar phytocomponents of <i>Aloe vera</i> gel extract towards modulation of early pregnancy using the “<i>in-silico</i>” approach	138
5.1 Rationale of the study.....	138
5.2 Materials and methods	139
5.2.1 Molecular Docking Study	139
5.3 Results	142
5.3.1 Interaction of the identified non-polar phytocomponents of <i>Aloe vera</i> gel with the key intermediates of early pregnancy using the “ <i>in-silico</i> ” approach.	142
5.3.2 Docking studies of n-Hexadecanoic acid from the non-polar fraction of <i>Aloe vera</i> gel	143
5.3.3 Docking studies of campesterol from the non-polar fraction of <i>Aloe vera</i> gel.....	150
5.4 Discussion	159
5.5 Conclusion	165

Chapter 6

6 Summary.....	167
6.1 Overall study highlights:	175
6.2 Future Directions:.....	177

Chapter 7

7	Bibliography	178
----------	---------------------------	------------

Synopsis

Publications and citations