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Research Article

Role of *Aloe Barbadensis* Mill. as a Possible Pre-Conceptive Herb for the Management of Polycystic Ovarian Syndrome: A Rodent Model Study

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Abstract

Purpose: Current research is oriented towards identifying herbal therapeutic options for management of the complications. *Aloe vera* gel (AVG) (10 mg dry weight orally/60 days/daily) demonstrated improvement in the PCOS phenotype in non-pregnant stage and to understand the role of AVG as a pre-conceptive agent in PCOS.

Methods: Letrozole induced PCOS rat model was developed and treated with *Aloe vera* gel for 2 months (10 mg dry weight orally/60 days/daily), which was followed by induction of pregnancy. Animals were sacrificed at late gestational period (18^{th}-20^{th} day) and assayed for biosynthetic and metabolizing enzymes of steroidogenesis. Also, key steroid hormone status as well as its regulatory proteins levels was also evaluated.

Results: Results showed that reproductive performance was improved in PCOS rats after treating with AVG, suggesting that it had protective effect. AVG also altered the ovarian-placental steroid status by modulating the expression of Steroidogenic acute regulatory (StAR), Luteinizing hormone receptor (LHR), Androgen Receptor (AR) and Aromatase, which could also be correlated with a change in hormonal profile of important steroids. AVG also reduces post implantation loss during gestation period leading to increased foetal viability "*at term*".

Conclusion: These modulations could be attributed to the nutritive and active ingredients present in *Aloe vera* gel, which independently or cumulatively act to regain fertility when used prior to conception. Thus, suggesting AVG is a good pre-conceptive agent for PCOS phenotype.

Keywords: Polycystic ovary syndrome; Infertility; Insulin resistance; *Aloe vera;* Pre-conceptive agent

Introduction

Polycystic Ovarian Syndrome (PCOS) is the most common endocrine abnormality in reproductive aged women, affecting approximately 5-10% of this population [1]. The clinical characteristics include oligo or an ovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries at ultrasound [2]. In addition, PCOS women suffer from several clinical and metabolic complications like RPL (Recurrent pregnancy loss), gestational diabetes, pre-clampsia during gestation period. Studies have demonstrated that the nutritional status of foetus is hampered in females suffering from PCOS [3]. Various aetiologies have been proposed [4-6] to understand the detailed pathology. Gonadotropin abnormalities with characteristic increased GnRH pulse frequency have been recognized as a factor to cause an elevation in LH/FSH [7] ratio, which could be a risk factor for spontaneous abortions and increased early pregnancy loss [8]. Hyperinsulinemic PCOS females are more likely to produce oocytes exhibiting low fertilization rates after IVF treatment and embryos which are unable to implant [9]. It is also evident from literature that insulin affects endometrial receptivity [10]. Hence, both insulin resistance and hyperandrogenism could affect foetal development [11] and alter "*in utero*" condition during pregnancy [12].

As insulin resistance is the fundamental co-morbidity associated with PCOS, current available treatment is the use of insulin sensitizers like metformin along with an ovulatory agent like clomiphene citrate in order to manage fertility [13]. But, these drugs have profound side effects upon prolonged usage [14]. Recent studies are suggesting evidence of teratogenicity associated with metformin treatment during pregnancy [15]. Hence, currently researchers are exploring alternative therapy to treat and manage the infertility disorders [16].

In this regard, several complimentary therapies have been studied for management of PCOS that ultimately helps to regain the fertility. Several traditional Chinese medicines (TCM) and ayurvedic medicines have been reported to help in ovulation and reduce pregnancy related complications [17]. Researchers have implicated that targets of phyto-components could be steroid receptors, steroid metabolizing enzymes and proteins involved in implantation [18]. These modulatory effects might help in treatment of ovarian dysfunction and restoration of fertility [19]. Many indigenous plants have been reported to be used

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in traditional herbal remedies during pregnancy and childbirth. However, none of the previous studies demonstrate the potential of the herbal extracts towards management of PCOS pathology and its associated pregnancy related complications. In this context, *Aloe vera* was extensively evaluated for a prospective pre-conceptive herbal therapy in PCOS pathology.

A. vera has been used in folk medicine for over 2000 years, and has remained an important component in the traditional medicine of many contemporary cultures, such as China, India, the West Indies, and Japan [20]. In view of above, various *Aloe* species have gained popularity as therapeutic, botanicals and biological properties of *A. vera* [21]. Various extracts of these *Aloe* species are traditionally used and their application used to cure arthritis, skin cancer, burns, eczema, psoriasis, digestive problems, high blood pressure, and diabetes [22]. The components of gel include proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and different carbohydrates [21]. This effect would be correlated with phyto-components present in AVG.

The mechanism of phytosterol action is based on its ability to reduce cholesterol absorption and thereby, improves the hyperlipidemic condition [23,24]. In addition to this, previous work has already shown that *Aloe vera* gel (AVG) causes reversion of estrous cyclicity and improves steroid status in PCOS rat model [25] and it also acts as an anti-hyperlipidemic agent in non-pregnant stage [26]. However, there are no studies that demonstrate the potential of *Aloe vera* gel as a pre-conceptive agent in PCOS pathology. Hence, the aim of the present study was to study the role of *Aloe vera* gel as a pre-conceptive agent in PCOS phenotype, wherein, it can manage the pathophysiology and render fewer chances of miscarriages and improve the fertility.

Materials and Methods

Aloe vera gel extraction

Aloe barbadensis Mill. (Voucher no. PSN 723) was compared with the specimen (Bhatt 2486, 653, 279, JVJ 448) lying with the nationally recognized BARO Herbaria of the Department of Botany, The M.S. University of Baroda, Vadodara, Gujarat, India. Fresh mature Aloe vera leaves (3.5 years old) were taken and washed with water. Later, the leaves were incised with the sterilized knife and allowed to stand by for 2 hours in order to remove the aloin. Later, the gel was removed by separating the epidermis and was sonicated to get a homogenous gel.

Animals and treatment regime

Adult Charles foster female rats (weight 150-200 g) were used for the study. All rats were housed in cages and maintained in ambient temperature of 25±1°C and 45.5% relative humidity, with a photoperiod cycle of 12 h:12 h (light and dark) with food and free access of water. All experimental protocols were approved by the institutional animal ethical committee according to CPCSEA guidelines. After treatment regime, rats of all groups were allowed to mate with male rats. The date of copulation was determined on the basis of vaginal smears, wherein, presence of sperm in vaginal smear was considered as the first day of pregnancy. At the end of gestation period: 18th-20th day, rats were sacrificed and assessed for various biochemical, molecular parameters along with fertility index.

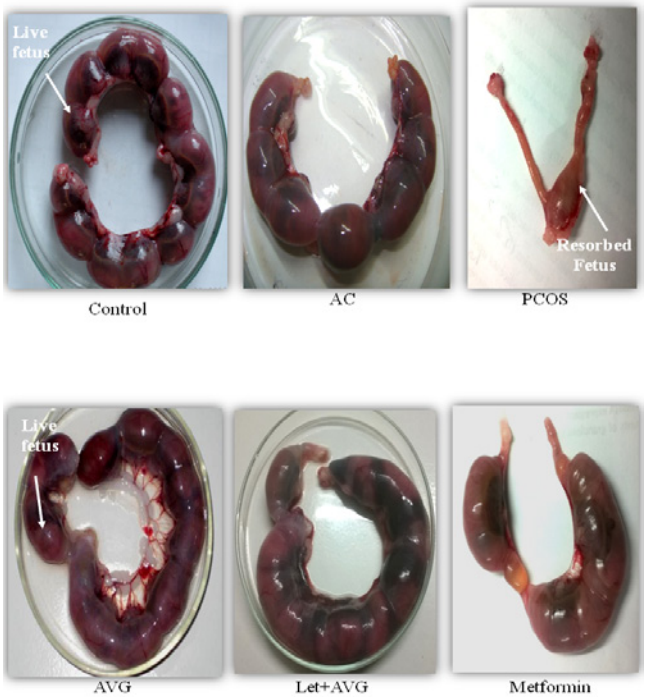


Figure 1: Effect of *Aloe vera* on fetal development at late gestation period in letrozole induced PCOS rats.

Detailed plan of work in mentioned in Figure 1.

Fertility index

At 18th-20th day of gestation, animals were sacrificed and checked for various parameters of fertility like copulation time, live fetuses, birth weight, placental weight, pups weight along with other biochemical parameters.

Histological analysis

Ovaries were removed and fixed in Bouins fixative. Histological examinations of ovary from all groups were carried out and observed in light microscope under 4x magnification.

Biochemical parameters

The key steroidogenic enzymes - 3β hydroxy steroid dehydrogenase (3 HSD) and 17 hydroxy steroid dehydrogenase (17β HSD) were assayed [27].

Hormone profile

Serum insulin and steroid hormones levels were checked in all groups of animals using ELISA kits procured from Diametra Inc, Germany.

Gene expression

RNA was isolated from the ovarian and placental tissue using TRIZOL reagent, according to standard protocol and further reverse transcribed by using a cDNA synthesis kit (Thermo Scientific) kit. Primers were designed by the Primer-BLAST software from NCBI and procured from Integrated DNA Technology (IDT). DNA was amplified by Polymerase Chain Reaction (PCR) using Sigma's 5 µl ready master mix, 10% cDNA 1 µl, Forward primer 1 µl, Reverse primer 1 µl and sterile distilled water 2 µl added into fresh PCR tube.

Table 1: Effect of *Aloe vera* gel on hormonal profile late gestation period in letrozole induced PCOS rats.

	Testosterone (nglml)	Progesterone (nglml)	Estradiol (pglml)	Insulin (pIll/m1)	HOMA-112
Control	1.185+.22	26.5+2.0	10.8+0.8	8.66+2.18	1.63+.044
AC	0.845+.52	29.0+2.1	7.3+0.3	12.33+1.74	2.25+0.28
PCOS	3.47+0.61"	13.13+3.3"	1.16+0.3"'	20+2.08"'	4.69+0.36m
AVG	0.685-F0.29@©	40-F4.61®©©	5.8-F2.1©	9.5-F1.32©©	1.89-F0.24®®®
Let+AVG	1.46+0.47	25.33+4.1@	3.4+1.2	16+0.57	3.0-F0.17©©
Metformin	0.94+0.33	23.0-F3.21g	1.5+0.2	12+0.57@	2.23+0.26©©©

Table 2: Oligonucleotide primers used in qPCR analyses.

Targeted Genes	Primer sequence	Annealing tem-perature (°C)	Reference
Arom-atase	F: 5'GCTTCTCATCGCAGAGTATCCGG 3' R: 5'CAAGGGTAAATTCATTGGGCTTGG 3'	60	NM 017085
StAR	F: 5' AGTGACCAGGAGCTGTCCTA 3' R: 5' GCGGTCCACCAGTTCTTCATA 3'	58	NM 031558.3
FSHR	F: 5' CTCATCAAGCGACACCAA 3' R: 5' GGAAAGGATTGGCACAAG 3'	54	Cavalcante, *et al.* 2013
LHR	F: 5' GCTTTTACAAACCTCCCTCGG 3' R: 5' GCGAGATTAGAGTCGTCCCA 3'	55	NM 012978
AR	F: 5' GGAAGCACTGGAACATCT 3' R: 5' GTAGTCGCGATTCTGGTA 3'	53	Suzuki M and Nishihara, 2002
GAPDH	F:51CAAGGTCATCCATGACAACTTTG 3' R:51GTCCACCACCCTGTTGCTGTAG 3'	58.	NM 017008

Table 3: Antibodies dilution used in western blot analysis.

Primary antibodies dilutions			
No.	Antibodies	Source	Dilution
1	Androgen Recepter (AR)	Rakesh Tyagi, JNU, India	1:1000
2	StAR	Stocco, Texas Tech University, Texas,USA	1:2000
3	3β-HSD	Prof. Ian Mason , Un iversity of Edin burgh, France	1:2000
4	β-actin	CST, # 4967	1:10,000
5	P450arom	CST , #8799	1:1000
Secondary antibody dilution			
	Con ju gated Anti-rabbit IgG	Pu regen e, GX1202E-3	1:2500

Temperature profile of the PCR wherein denaturation at 94°C for 30s, annealing as per mentioned in table 1 for 1 min, extension at 72°C for 1 min and the reaction was repeated for total 32 cycles. Reactions were conducted using appropriate sequenced primers as given table 2. Aliquots of each PCR reaction (10 µl) were electrophoresis through a 1.2% agarose gel stained with 0.1 µg/ml ethidium bromide. Gels were visualized on a UV transilluminator and photographed using E-Gel® imager. The photographs were scanned using Gel Quant™ Express Analysis Software, and densitometry was performed using NIH image analysis. All values were normalized to the internal control GAPDH.

Western immunoblotting

Rat ovarian and placental tissues were homogenised in lysis buffer. 40 µg of protein, along with pre-stained molecular weight markers (Bio-Rad), were separated on SDS-PAGE (10% resolving gel). Separated proteins were transferred onto nitrocellulose membranes (Genetix, 0.45 µm pore sixe) and blocked for 1 h in 5% w/v non-fat milk in Phosphate-buffered saline containing 0.05% v/v Tween 20 (PBST). The membranes were incubated overnight at 4°C with primary antibodies as per mentioned in table 3. Following washing, membranes were treated for 1 h with corresponding secondary peroxidase-conjugated (1:2500) dilution. Immunopositive bands were visualized with diaminobenzidine (DAB)-H_2O_2 substrate (Sigma-Aldrich Corp.) and subsequently scanned into a computer. Individual bands were quantified directly from membranes by densitometry using the Image J software. The signal of each protein was normalised as percentage of those of control ovaries to produced arbitrary Densitometric units of relative abundance.

Liver metabolizing enzymes assay

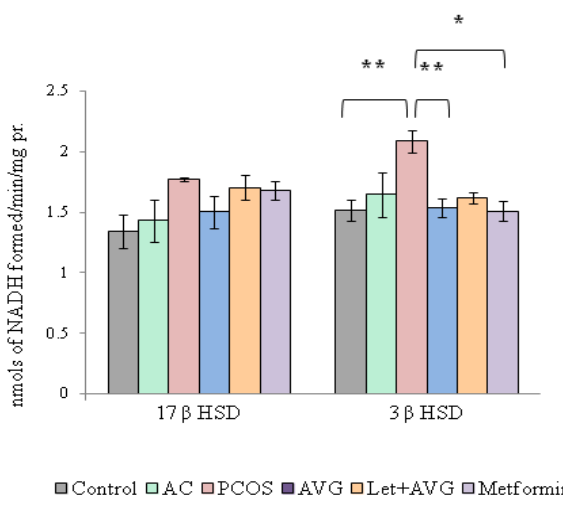
Fresh liver tissue was excised and further processed for phase I enzymes - NADH-cytochrome c reductase [28], UDP-Glucoronyl transferase (UDPGT) [29] and phase II enzymes - Glutathione-S-transferase (GST) [30], 17β hydroxy steroid oxido reductase (17β HSOR) [27].

Statistical analysis

The results were analyzed using one way analysis of variance (ANOVA) and student's t-test to determine the level of significance. P<0.05 was considered statistically significant. Results were expressed as Mean+SEM. Differences between the groups was analyzed by One-way ANOVA and subjected to Bonferroni post-test. The statistical analysis was carried out by using the Graph Pad Prism 3.0 software.

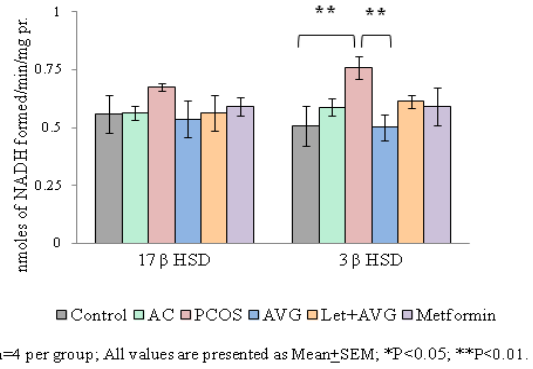
Results

Various biochemical changes were observed in late gestation period i.e. 18th-20th day wherein resorptions and retarded fetal growth were observed in PCOS rats as compared to live fetuses in control group (Figure 2). AVG treated PCOS rats demonstrated an increase in litter size and improved percent fertility growth as compared to PCOS group (Table 2). AVG group demonstrated a protective effect against letrozole and helped to improve fertility index during gestation period as compared to PCOS group. However, metformin group demonstrated lesser number of developed fetuses along with few resorptions.



n=4 per group; All values are presented as Mean±SEM; *P<0.05; **P<0.01.

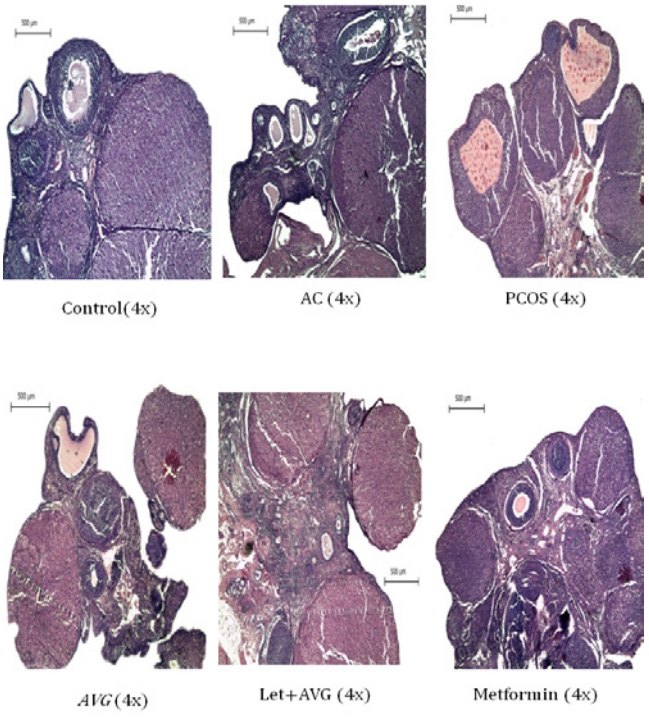
Figure 2: Effect of *Aloe vera* gel on ovarian steroidal enzyme activity at late gestation period in induced PCOS rats.



n=4 per group; All values are presented as Mean±SEM; *P<0.05; **P<0.01.

N=4, The values represented as Mean±SEM
**p<0.01 as compared to Control group.

Figure 3: Effect of *Aloe vera* gel on placental steroidal enzyme activity at late gestation period letrozole induced PCOS rats.



Group 1-Control, Group 2-*Aloe* control, Group 3-PCOS, Group 4-*Aloe*, Group 5-Let+*Aloe*, Group 6-Metformin

Figure 4: Effect of *Aloe vera* gel on ovarian histology at late gestation period in letrozole induced PCOS rats.

The effect of Aloe vera gel treatment in PCOS rats revealed that the ovarian and placental 3β HSD and 17β HSD activities were significantly altered ($p<0.01$) at 18^{th}-20^{th} day (Figure 3). PCOS control animals demonstrated high activity of 3β HSD in both ovary and placenta as compared to control group ($p<0.01$), whereas AVG treated group demonstrated modulation of the steroid enzyme activities towards normalcy. Let+AVG (Group 5) and metformin groups demonstrated improved 3β HSD enzyme activity in ovary ($p<0.05$).

Histological sections of PCOS rat ovary exhibited presence of multiple peripheral cysts as compared to control group which showed the presence of healthy growing follicles. AVG treatment in PCOS rats regained normal follicular development. Let+AVG (Group 5) animals also demonstrated partial reversion of PCO phenotype as comparable to control group (Figure 4). It is interesting to note that AVG treatment caused significant decrease in atretic follicles and reverted back ovarian structure - function to normalcy as compared to PCOS animals.

Table 3 demonstrates the hormonal profile of all the groups of animals, wherein serum insulin levels was significantly higher in untreated PCOS rats ($p<0.001$) as compared to control group, whereas AVG treated PCOS rats exhibited significantly reduced insulin levels ($p<0.01$). Apart from AVG group, metformin group also represented a decrease in insulin levels which was comparable to control group ($p<0.05$). HOMA-IR index also has been evaluated to indicate insulin resistance condition. The PCOS rats demonstrated an increased insulin resistance condition (HOMA IR- 4.2) whereas AVG treatment reduced the resistance in all group as similar to control group (HOMA IR <3) (Table 3).

Steroid hormones like Testosterone, progesterone and estradiol also have been evaluated, wherein PCOS rats demonstrated significant high level of testosterone ($p<0.01$) whereas significant decreased level of progesterone ($p<0.01$) and estradiol ($p<0.001$). AVG treated PCOS rats exhibited significant reduction in testosterone level and improved progesterone and estradiol levels as comparable to control group. Also, Let+AVG (Group 5) demonstrated reduction in testosterone level as compared to PCOS rats ($p<0.05$) whereas no significant change was observed in metformin group.

Gene expression study of key steroid regulatory proteins that play an important role in ovarian and placental structure-function were evaluated. Letrozole induced PCOS rats exhibited significant increase in key steroid regulatory protein –Steroidogenic acute regulatory (StAR) in ovary ($p<0.001$) whereas non-significant change was seen in placenta. AVG treated PCOS rats exhibited reduction in StAR expression in ovary. A significant increase in expression of key receptors namely Androgen receptor (AR) and Luteinizing hormone receptor (LHR) was observed in PCOS rats as compared to control rats ($p<0.01$). AVG treatment significantly reduced the gene expression of these receptors in PCOS rats ($p<0.05$, $p<0.01$) (Figure 5A). Both Let+AVG treated group (Group 5) and metformin groups demonstrated non-significant change in StAR and receptor genes expression as compared to PCOS group.

PCO phenotype is associated with hyperinsulinemia and insulin resistance. Hence, gene expression of insulin receptor (IR) in ovary and placenta, wherein PCO positive rats exhibited significant increase in ovarian IR gene expression as compared to control group ($p<0.05$). However, no significant change was observed in placenta. These results can be correlated with high serum insulin levels along with elevated HOMA-IR in PCOS as compared to control. This increased serum insulin and gene expression levels reverted back to normalcy after AVG treatment as compared to PCOS group ($p<0.01$). Let+AVG (Group 5) and metformin groups did not show any significant change in expression level as compared to control group.

In addition to this, gene expression of aromatase was studied,

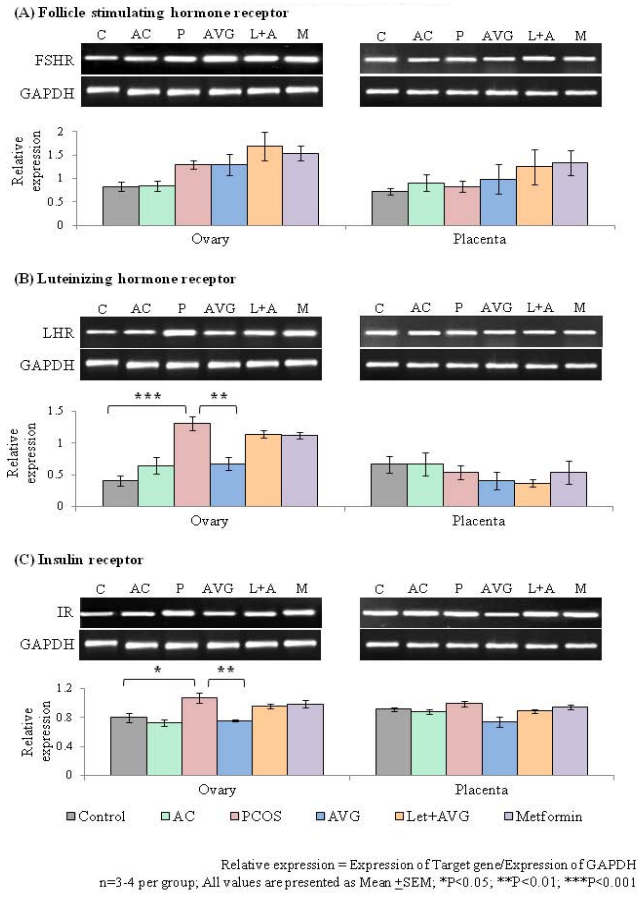


Figure 5A: Effect of *Aloe vera* gel on important steroidogenic genes in letrozole induced PCOS rats.

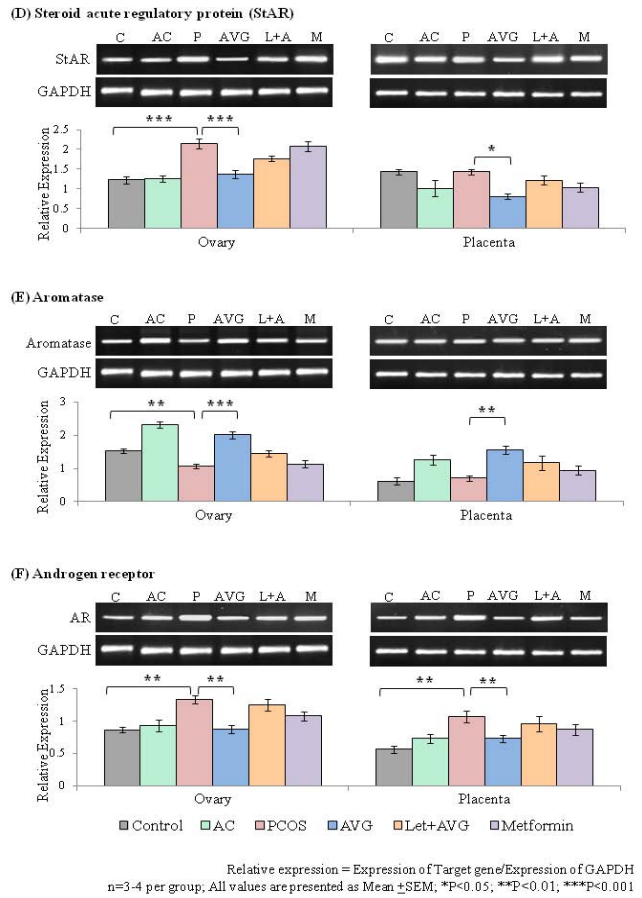


Figure 5B: Effect of *Aloe vera* gel on important steroidogenic genes in letrozole induced PCOS rats.

as it plays an important role in estrogen biosynthesis in both ovary and placental tissues. PCOS positive rats demonstrated significant decrease in gene expression of aromatase as compared to controls ($p<0.001$). AVG treatment significantly increased the expression of aromatase in ovary ($p<001$) as well as in placenta ($p<0.05$). Let+AVG (Group 5) demonstrated significant modulation of aromatase gene expression in ovary as compared to PCOS group ($p<0.05$) whereas no significant change was observed in metformin group in both the reproductive tissues (Figure 5B).

A summary of the quantitative analysis of relative protein abundance is presented in figure 6. Expression of StAR protein in placenta with no significant change in ovary of PCOS rats while AVG treatment caused a reduction in its expression ($p<0.05$). The placental StAR protein demonstrated a reduced expression in Let+AVG (Group 5) and metformin as compared to PCOS animals ($p<0.05$). Ovarian androgen receptor protein was significantly reduced in AVG treated PCOS animals ($p<0.05$) and Let+AVG (Group 5) animals as compared to PCOS positive animals ($^{*}p<0.05$) where AR expression was high but no significant change was observed in the protein expression of AR in placental tissues amongst all groups of animals (Figure 6).

An altered hormone profile as seen above could be due to the reflection of altered biotransformation. Thereby, Phase I and Phase II enzymes were also evaluated. The cytochrome P450 oxidoreductase (Cyt C) enzyme activity of phase I reaction exhibited no significant change in groups when compared (Figure 7). 17β Hydroxysteroid reductase enzyme activity (phase I) reaction demonstrated a significant reduction in its activity in AVG treated ($p<0.01$) and Let+AVG (Group 5) which could be compared to the control group ($p<0.01$); however, non-significant change was observed in metformin group. The liver steroid metabolizing enzyme UDP-Glucoronyl transferase (UDPGT) exhibited a significant increase in its activity in PCOS rats as compared to control group at 18^{th}-20^{th} day of gestational period ($P<0.05$) whose activity was reduced upon AVG treatment ($p<0.01$). The effect of AVG on liver steroid metabolizing enzyme UDP-Glucoronyl transferase (UDPGT) demonstrated significant decreased enzyme activity as compared to PCOS rats at term. Let+AVG (Group 5) and metformin group exhibited non-significant change in UDP-Glucoronyl transferase (UDPGT) activity as compared to PCOS group.

During the course of experiments, toxicity parameters like Serum Glutamate Pyruvate Transaminase (SGPT) and creatinine was evaluated. AVG treated groups exhibited non-significant change in the liver toxicity parameters. In addition to this, Letrozole treated PCOS group as well as metformin treated animals showed no change in the above parameters upon treatment.

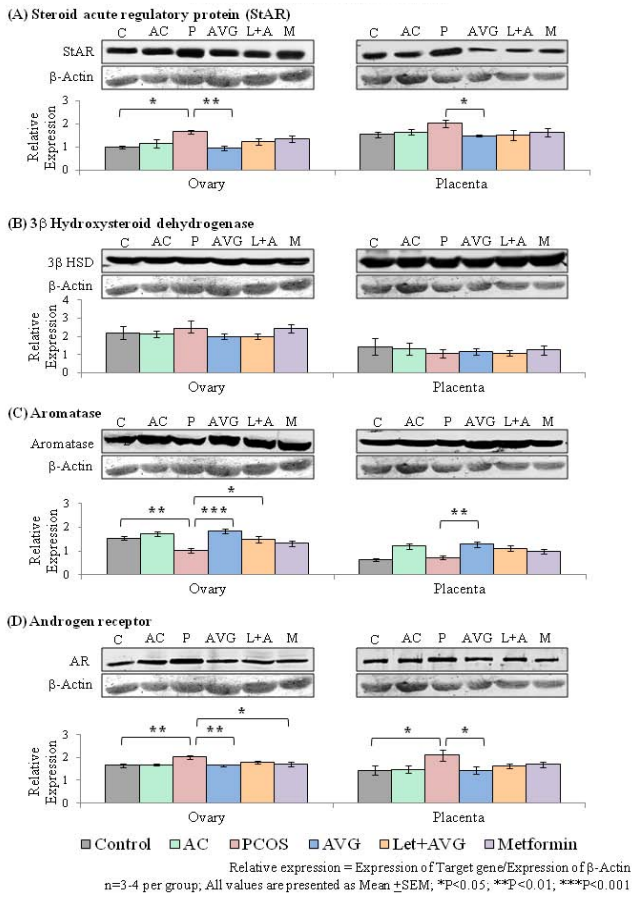


Figure 6: Effect of *Aloe vera* gel on steroid protein expression in letrozole induced PCOS rats.

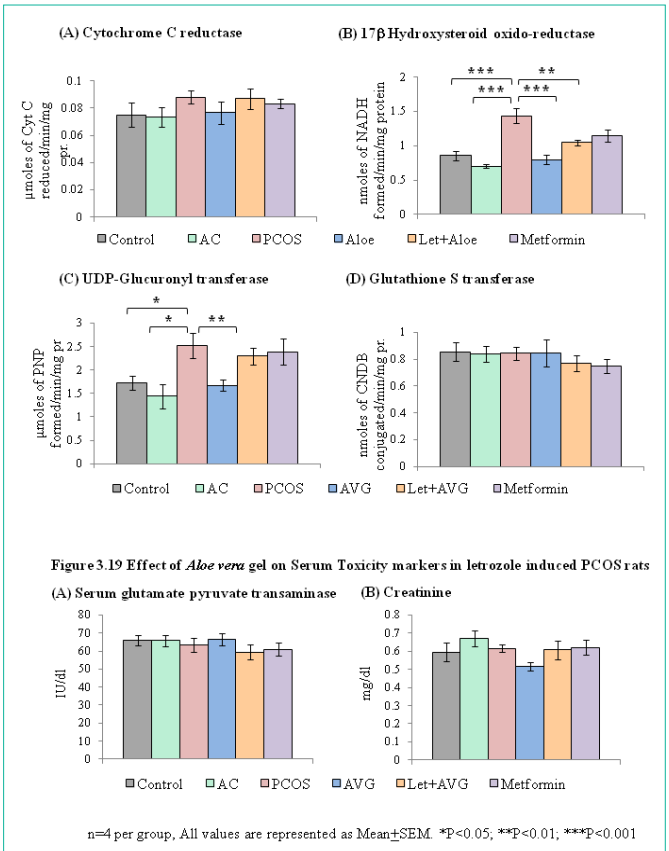


Figure 7: Effect of *Aloe vera* gel on liver steroid metabolizing enzymes I letrozole induced PCOS rats at late gestation.

Discussion

Gestation period is a crucial period for the fetal growth and development, wherein PCOS rats in current study demonstrated lesser number of live pups and presence of retarded fetal growth that may be due to high androgenic uterine microenvironment that inhibit factors involved in embryo growth and development [31]. Also, PCOS endometrium over expresses androgen receptors and fails to down regulate estrogen receptor-α in the window of early pregnancy [32]. Hence, dysregulation of steroid receptor expression and disturbed steroid hormone status also plays a crucial role as these may contribute to the lower pregnancy rates as seen in PCOS women [33]. AVG treatment decreased testosterone levels and improved progesterone levels that were useful in increasing uterine receptivity and fetal growth in PCOS rats during gestation. It has been suggested that maternal excess testosterone reduces fetal growth, placental weight and birth weight via impaired placental function [34]. Hormone profile in this study clearly demonstrates that *Aloe* has potential to sensitize the insulin receptor and reduce insulin level in PCO condition; thereby reverting insulin resistant state to sensitive status indicated by improved HOMA-IR change.

In the current study, the alteration in hormones could be correlated with changes observed in steroidogenic enzyme activities, wherein PCO rat demonstrated the altered enzyme activities of ovarian and placental steroidogenic enzymes such as 3β Hydroxysteroid dehydrogenase (3β HSD) and 17β Hydroxysteroid dehydrogenase (17β HSD) during early as well as late gestation period [35]. Also, high insulin levels have direct effect on ovarian steroidogenesis and stimulate thecal androgen production [36]. These key steroidogenic enzymes activities were significantly decreased in both ovarian and placental levels in *Aloe* treated PCOS rats. The result of altered activity could be correlated with serum steroid hormones level that regained normalcy after AVG treatment. Previous data showed that AVG has direct effect on both steroidal enzymes in the non-pregnant state of PCO condition [25,37]. This modulation may be due to presence of phytosterols which were known to have modulatory effect on key regulatory protein involved in steroidogenesis [38].

Increased insulin levels in PCOS rats directly stimulate ovarian Luteinizing Hormone receptor (LHR) gene expression leading to thecal androgens flux- Testosterone, DHEA, androsteindione rather than aromatization into estrogens in granulosa cells. This might be due to high 3β-HSD dehydrogenase enzyme activity, which is one of the key enzymes involved in ovarian androgen production. Additionally, LH pulse amplitude increases in women with PCO phenotype [39] and insulin specifically augment pituitary release of luteinizing hormone in various "*in-vitro*" studies [40].

Hence, a potential mechanism wherein insulin could enhance

ovarian androgen production is by altering LH release. The elevated levels of insulin regain normalcy after AVG treatment in PCOS rats. This may be due to the hypoglycemic effect of AVG attributed by several phyto-components. AVG reduces the hyperinsulinemic condition as well as hyper androgenic condition by modulating the steroidogenic enzyme activities in the ovary of letrozole induced PCOS rats. Disturbed steroidogenesis was observed as a result of altered enzyme activity which may due to change in expression profile of StAR in both tissues studied. High expression of StAR was observed in PCOS group which might be mainly because of synergistic effect of high LH and insulin levels that increase StAR expression by cobinding to the StAR promoter region [41]. High insulin levels also augmented LH stimulated cAMP levels that further affect StAR expression as cAMP dependent kinase A is known to be a key regulator of StAR expression. In addition to this, ovarian and placental protein content of StAR was evaluated, wherein placenta exhibited significant change in PCOS rat but no change was observed in ovary. This may be due to the fact that during the mid late gestation period of pregnancy, placenta takes up charge of major steroid production for fetal development [42]. Apart from altered steroidogenesis, PCOS rat also exhibited high gene expression of steroid receptor- Androgen receptor (AR) that plays a major role in high androgen production in PCO phenotype in ovary and placenta [43]. The hyperandrogenic condition was restored upon *Aloe* treatment, which was evident from decreasing levels of both- androgen receptor and StAR protein expressions which further minimize hyperandrogenic condition [38] previously demonstrated that phytosterols have direct effect on StAR protein. These phyto-components present in gel may act important role in modulation of ovarian steroidogenesis and H-P-G axis. This may be because of the estrogenic effects of β-sitosterol present in AVG on the ovary. In this context, studies of phytoestrogens exposure in mammals have demonstrated that genistein decreases GnRH-induced luteinizing hormone (LH) in rats [44] and that coumesterol (phytosterol) decreases the amplitude of LH pulses in ewes [45].

Present study confirms the above fact that protein expression wherein high expression of AR protein was observed in PCOS rats as compared to control. However, AVG treatment reduced the expression of androgen receptor in PCOS rats which could be compared with that of control. This may be due to the presence of flavonoids present in the gel, which are known to possess anti androgen effect by directly inhibiting the expression of androgen receptor [46]. Under normal conditions, maternal androgens or fetal adrenal androgens are rapidly converted to estrogens by the activity of the placental enzyme aromatase. In PCOS condition, the activity of this enzyme is inhibited as the bio-availability of androgens is increased. Also, high Insulin has been shown to inhibit aromatase activity in cytotrophoblasts and stimulate 3β-hydroxysteroid dehydrogenase activity [39]. In the current study, AVG treatment decreased aromatase gene expression in ovary as well as placenta in PCO condition. The gene expression study of aromatase could also be correlated with the total estradiol content.

As function is altered, it is plausible that structural alterations do occur. Hence, histological study was performed. Studies revealed that PCO rats demonstrated the presence of multiple fluid filled peripheral cysts in the ovary [47]. PCOS rats treated with AVG revealed normal follicular growth and reversal to normal cyclicity. The restoration in the ovarian structure and function can be attributed to the presence of several phyto-components that lead to modulation in the HPO axis. This modulation helped in maturation of follicles and release matured ova during ovulation. The normal follicular growth is necessary for formation and release of matured ova. Only healthy matured ova will get successfully fertilized and implanted. Apart from ovary, placenta acts as an important structural component of pregnancy. It is a mediator for both mother and fetal steroid exchange during gestation period. Reports suggest that high testosterone levels may affect placental development and function by modulating amino acid transporters [34] or by regulating the expression of enzymes and androgen/ estrogen receptors, as demonstrated in human placentas [48].

Apart from synergistic effect on steroid biosynthetic enzymes, *Aloe vera* gel also exhibited modulatory effect on steroid metabolizing enzyme, this could be attributed to the nutritionally rich phytosterols and phyto-phenols components present in the plant [39]. Also, it should be noted that *Aloe vera* gel has enriched fibers that could increase in transit time for diet to be get absorbed which could modulate glucose homeostasis. This could help to normalize hyperinsulinemic status. In current study, PCOS rats with high androgen levels demonstrated increased levels of both liver steroid metabolizing enzyme activities during late gestation period. The CYP1A1 (Cytochrome P450 1subfamily A polypeptide 1 gene encodes phase I cytochrome P450 enzyme, involved in metabolism of estrogens. In this regards, women who carry polymorphic variants in this gene confers higher CYP1A1 activity and may be at higher risk of PCOS (Wang, *et al.* 2009). AVG treated PCOS rats exhibited modulatory effects on both phases I and II steroid metabolizing enzymes activities wherein they showed reduced activities of both these enzymes during pregnant stage [49]. Previous studies have revealed that β-sitosterol containing *Aloe vera* gel causes reduction of the intestinal uptake of cholesterol and reduce the concentration of cholesterol in dietary mixed micelles via a dynamic competition mechanism [50]. In addition, there are some evidences relating to steroid metabolizing enzymes and their modulation by green tea and black tea in various rodent models [51]. Lupeol, an antioxidant rich compound present in AVG also has properties to normalize the lipid profile by decreasing LDL and total cholesterol level along with improved antioxidant status. In addition these, β-sitosterol, which is the most abundant phytosterol present in *Aloe vera* gel, was found to significantly reduce glucose levels in type 2 diabetes patients [50]. One of the studies has shown that feeding of 0.5% stigmasterol (another important phytosterol present in *Aloe vera* gel for 6 weeks to Wistar and Wistar-Kyoto (WKY) rats significantly suppressed the HMG-CoA reductase activity and resulted in approximately 11% reduction in plasma cholesterol levels [51]. Hence, it may be possible that phytosterols containing *Aloe vera* gel may restore ovarian structure-function through this possible mechanism.

In our study, *Aloe* treated PCOS animals' demonstrated modulatory effect on the steroid biosynthetic enzyme activities. It helped in restoration of the ovarian structure-function to normalcy leading to improved fertility index. Moreover, AVG treatment improved ovarian and placental steroidogenesis by modulating protein expression of key proteins, hence, improvising the steroidal milieu resulting in successful pregnancy. The observed changes could

be attributed to phyto-nutrient rich *Aloe vera* gel that mainly contains phytosterols, polysaccharides, flavonoids and polyphenols. These phyto-components can act independently or synergistically at various targets in the reproductive organs of PCOS rats and hence induce structural-functional changes in ovary as well as placenta leading to successful term pregnancy. Thereby, this study suggests that *Aloe vera* gel can act as a good herbal pre-conceptive agent in PCOS condition.

References

1. Ávila MAPd, Bruno RV, Barbosa FC, Andrade FCd, Silva ACdO, Nardi AE. Polycystic ovary syndrome: implications of metabolic dysfunction. Revista do Colégio Brasileiro de Cirurgiões. 2014; 41: 106-110.
2. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab. 2010; 95: 2038-2049.
3. Xita N, Tsatsoulis A. Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. J Clin Endocrinol Metab. 2006; 91:1660-1666.
4. Chakraborty P, Goswami S, Rajani S, Sharma S, Kabir SN, Chakravarty B, et al. Recurrent pregnancy loss in polycystic ovary syndrome: role of hyperhomocysteinemia and insulin resistance. 2013.
5. Ke RW. Endocrine basis for recurrent pregnancy loss. Obstetrics and gynecology clinics of North America. 2014; 41:103-112.
6. Usadi RS, Legro RS. Reproductive impact of polycystic ovary syndrome. Curr Opin Endocrinol Diabetes Obes. 2012; 19: 505-511.
7. Banaszewska B, Spaczynski R, Pelesz M, Pawelczyk L. Incidence of elevated LH/FSH ratio in polycystic ovary syndrome women with normo-and hyperinsulinemia. Rocz Akad Med Bialymst. 2003; 48:131-134.
8. Gürbüz B, Yalti S, Ozden S, Ficicioglu C. High basal estradiol level and FSH/LH ratio in unexplained recurrent pregnancy loss. Arch Gynecol Obstet. 2004; 270: 37-39.
9. Tandulwadkar SR, Lodha PA, Mangeshikar NT. Obstetric complications in women with IVF conceived pregnancies and polycystic ovarian syndrome. J Hum Reprod Sci. 2014; 7: 13.
10. Schulte MM, Tsai JH, Moley KH. Obesity and PCOS: the Effect of Metabolic Derangements on Endometrial Receptivity at the Time of Implantation. Reprod Sci. 2015; 22: 6-14.
11. Peters C, Geary MP, Hill NR, Mathews DR, Hindmarsh PC. Maternal hyperinsulinism and glycaemic status in the first trimester of pregnancy are associated with the development of pregnancy-induced hypertension and gestational diabetes. Eur J Endocrinol. 2013; 168: 413-418.
12. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Eng J Med. 2008, 359:61-73.
13. Yildiz BO. Oral contraceptives in polycystic ovary syndrome: risk-benefit assessment. Semin Reprod Med. 2008: 111-120.
14. Arlt W, Neogi P, Gross C, Miller WL. Cinnamic acid based thiazolidinediones inhibit human P450c17 and 3beta-hydroxysteroid dehydrogenase and improve insulin sensitivity independent of PPARgamma agonist activity. Journal of molecular endocrinology. 2004; 32: 425-436.
15. Feng L, Lin X-F, Wan Z-H, Hu D, Du Y-K. Efficacy of metformin on pregnancy complications in women with polycystic ovary syndrome: a meta-analysis. Gynecolo Endocrinol. 2015; 31: 833-839.
16. Edirne T, Arica SG, Gucuk S, Yildizhan R, Kolusari A, Adali E, et al. Use of complementary and alternative medicines by a sample of Turkish women for infertility enhancement: a descriptive study. BMC complementary and alternative medicine. 2010; 10:11.
17. Lyttleton J. Treatment of infertility with Chinese medicine. Elsevier Health Sciences: 2013.
18. Nagarathna P, Rajan PR, Koneri R. A Detailed Study on Poly Cystic Ovarian Syndrome and It's Treatment With Natural Products. Int J Toxicol Pharmacol Res. 2014; 5:109-120.
19. Kage DN, Malashetty VB, Seetharam Y, Suresh P, Patil SB. Effect of ethanol extract of whole plant of Trichosanthes cucumerina var. cucumerina L. on gonadotropins, ovarian follicular kinetics and estrous cycle for screening of antifertility activity in albino rats. Int J Morphol 2009, 27:173-182.
20. Foster M, Hunter D, Samman S. Evaluation of the nutritional and metabolic effects of Aloe vera. Chapter; 2011.
21. Hamman JH. Composition and applications of Aloe vera leaf gel. Molecules. 2008; 13:1599-1616.
22. Ahmed M, Hussain F. Chemical composition and biochemical activity of Aloe vera (Aloe barbadensis Miller) leaves. Int J Chem Biochem Sci 2013, 3:29-33.
23. Christiansen L, Lähteenmäki P, Mannelin M, Seppänen-Laakso T, Hiltunen R, Yliruusi J. Cholesterol-lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects. Eur J Nutr. 2001; 40: 66-73.
24. Misawa E, Tanaka M, Nabeshima K, Nomaguchi K, Yamada M, Toida T, et al. Administration of dried Aloe vera gel powder reduced body fat mass in diet-induced obesity (DIO) rats. J Nutr Sci Vitaminol. 2012; 58:195-201.
25. Radha M, Padamnabhi N, Laxmipriya N. Evaluation of Aloe barbadensis mill. Gel on letrozole induced polycystic ovarian syndrome (pcos) rat model-a dose dependent study. International Journal of Pharmaceutical Sciences and Research. 2014; 5: 5293-5300.
26. Desai BN, Maharjan RH, Nampoothiri LP. Aloe barbadensis Mill. formulation restores lipid profile to normal in a letrozole-induced polycystic ovarian syndrome rat model. Pharmacognosy research. 2012; 4:109.
27. Shivanandappa T, Venkatesh S. A Colorimetric Assay Method for 3β-Hydroxy-Δ 5-steroid Dehydrogenase. Analytical biochemistry. 1997; 254: 57-61.
28. Zamek-Gliszczynski MJ, Hoffmaster KA, Nezasa K-i, Tallman MN, Brouwer KL. Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. European journal of pharmaceutical sciences. 2006; 27: 447-486.
29. Starlard-Davenport A, Lyn-Cook B, Radominska-Pandya A. Identification of UDP-glucuronosyltransferase 1A10 in non-malignant and malignant human breast tissues. Steroids. 2008; 73: 611-620.
30. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat protoc. 2006; 1: 3159-3165.
31. Carlsen S, Vanky E, Fleming R. Anti-Müllerian hormone concentrations in androgen-suppressed women with polycystic ovary syndrome. Human reproduction. 2009; 24:1732-1738.
32. Apparao K, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod. 2002; 66: 297-304.
33. Gregory CW, Wilson EM, Apparao K, Lininger RA, Meyer WR, Kowalik A, et al. Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. The Journal of Clinical Endocrinology & Metabolism. 2002; 87: 2960-2966.
34. Sathishkumar K, Elkins R, Chinnathambi V, Gao H, Hankins G, Yallampalli C. Prenatal testosterone-induced fetal growth restriction is associated with down-regulation of rat placental amino acid transport. Reprod Biol Endocrinol. 2011; 9: 110.
35. Doi SA, Al-Zaid M, Towers PA, Scott CJ, Al-Shoumer KA. Steroidogenic alterations and adrenal androgen excess in PCOS. Steroids. 2006; 71: 751-759.
36. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endoc Rev. 2012; 33: 981-1030.

37. Maharjan R, Nagar PS, Nampoothiri L. Effect of Aloe barbadensis Mill. formulation on Letrozole induced polycystic ovarian syndrome rat model. J Ayurveda Integr Med. 2010, 1: 273-279.

38. Sharpe RL, Woodhouse A, Moon TW, Trudeau VL, MacLatchy DL. β-Sitosterol and 17β-estradiol alter gonadal steroidogenic acute regulatory protein (StAR) expression in goldfish, Carassius auratus. Gen Comp Endocrinol. 2007; 151: 34-41.

39. Nestler JE, Jakubowicz DJ. Lean Women with Polycystic Ovary Syndrome Respond to Insulin Reduction with Decreases in Ovarian P450c17α Activity and Serum Androgens. Journal of Clinical Endocrinology & Metabolism. 1997; 82: 4075-4079.

40. Adashi EY, Hsueh AJ, Yen SS. Insulin Enhancement of Luteinizing Hormone and Follicle-Stimulating Hormone Release by Cultured Pituitary Cells. Endocrinology. 1981; 108:1441-1449.

41. Sekar N, Garmey JC, Veldhuis JD. Mechanisms underlying the steroidogenic synergy of insulin and luteinizing hormone in porcine granulosa cells: joint amplification of pivotal sterol-regulatory genes encoding the low-density lipoprotein (LDL) receptor, steroidogenic acute regulatory (stAR) protein and cytochrome P450 side-chain cleavage (P450scc) enzyme. Molecular and cellular endocrinology. 2000; 159: 25-35.

42. Maliqueo M, Lara HE, Sánchez F, Echiburú B, Crisosto N, Sir-Petermann T. Placental steroidogenesis in pregnant women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2013; 166:151-155.

43. Zurvarra FM, Salvetti NR, Mason JI, Velazquez MM, Alfaro NS, Ortega HH. Disruption in the expression and immunolocalisation of steroid receptors and steroidogenic enzymes in letrozole-induced polycystic ovaries in rat. Reprod Fertil Dev. 2009; 21: 827-839.

44. Hughes E, Collins J, Vandekerckhove P. Gonadotrophin releasing hormone analogue as an adjunct to gonadotropin therapy for clomiphene resistant polycystic ovarian syndrome. The Cochrane Library. 2004.

45. MacLatchy DL, Vanderkraak GJ. The phytoestrogen β-sitosterol alters the reproductive endocrine status of goldfish. Toxicol Appl Pharmacol. 1995; 134: 305-312.

46. Xing EP, Jordan MI, Karp RM. Feature selection for high-dimensional genomic microarray data. In ICML. 2001; 601-608.

47. Kafali H, Iriadam M, Ozardali I, Demir N. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. Arch Med Res. 2004; 35:103-108.

48. Glueck C, Awadalla SG, Phillips H, Cameron D, Wang P, Fontaine RN. Polycystic ovary syndrome, infertility, familial thrombophilia, familial hypofibrinolysis, recurrent loss of in vitro fertilized embryos, and miscarriage. Fertil Steril. 2000; 74: 394-397.

49. Santiago LA, Mayor AB. Lupeol: An antioxidant triterpene in Ficus pseudopalma Blanco (Moraceae). Asian Pac J Trop Biomed. 2014; 4: 109-118.

50. Bicas J, Pastore GM, Maróstica Jr MR. Phytosterols: Biological effects and mechanisms of hypocholesterolemic action. Biotechnology of Bioactive Compounds: Sources and Applications 2015: 565-581.

51. Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology. 2008; 149: 3549-3558.

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