Original Research Article

Anti – Androgenic activity of *Cesalpenia bonducella* in androgen induced Polycystic ovarian syndrome rats

Abstract

Aim*:* Our goal was to use the androgen and anabolic steroid (AAS) Testosterone Propionate (T.P) to create a novel animal model to study polycystic ovaries.

Materials and Methods: Thirty albino female wistar rats weighing 200 – 250 g were split into V groups with six rats in each group. I Group was treated as normal control, II Group was treated as the PCOS induced group, III Group was treated as a standard group, Clomiphene citrate (20 mg per kg body weight), intraperitoneally (I.P) along with a 4 mg T.P injection intramuscularly (I.M) in an oily solution, IV Group was treatment control group treated with a hydro-alcoholic extract of *Cesalpenia bonducella* (CBHAE) at a dose of 200 mg per kg body weight. V Group was given CBHAE at 400 mg per kg of body weight. Blood collected from animals examined for hormonal parameters and ovaries is subjected to histopathological studies.

Results: There was a fall in testosterone volume and an increase in the efflux of female hormone constituents, which improved the ovarian development and helped to regulate menses.

Conclusion: The extract had a significant impact on reducing Testosterone production

and had improved anti-androgenic effects. However, further intervention is suggested to investigate the therapeutic effect of *Cesalpenia bonducella* seed.

Keywords- anti-androgens, Cesalpenia bonducella, *follicles,* PCOS*, tubular injury.*

1. INTRODUCTION

Androgens in normal levels is beneficial, but in abnormal range (women), it leads to serious consequences i.e. forbid ovaries from releasing egg (ovulation), excessive hair growth, acne, Insulin resistance e.t.c eventually leads to PCOS.

Luteinizing hormone (LH) is a pituitary hormone release in response to Luteinizing hormone-Releasing hormone. It regulates the length and duration of menstrual cycle, ovulation, implantation of fertilized egg in uterus, secretion of estrogen and progestron. Theca cells secreate testosterone in response to LH surge and inturn converts to estrogen by adjoining granulose cells. Ovulation is stimulated by excess LH, whereas a relative FSH deficiency affects follicular growth. LH imbalance: FSH (3:1) induces ovary theca cells to proliferate, which results in increased steroidogensis and eventually in hyperandrogenism in PCOS women. *Cesalpenia bonducella* is abundant in furanoditerpenes beneficial in management of PCOS [1]. Seeds comprises of phytochemicals includes β- caesalpin, γ- caesalpin, caesalpin, citrullin and bonducellin, as well as fatty acid; steric acid, palmitic acid, and oleic acid; amino acids such as arginine and aspartic acid [2-3]. Phytochemicals in *Cesalpenia bonducella* potent against variety of PCOS attributes [4]. There are numerous documented medicinal properties like [5] anti-oxidant [6], adaptogenic [7], anti-inflammatory [8], anti-estrogen [9], and Immunomodulatory [10]. In order to appease the consequences anti-androgens came to light. In present study by using anti-androgenic property of *Cesalpenia bonducella* to appease PCOS in rodents.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The marketed *Cesalpenia bonducella* was collected from an herbal production factory named ANNAIARAVINDHHERBALSlocatedin1, 2&3, Janaki Nagar, Maduravoyal, Chennai, and Tamil Nadu 600095.

2.2 Plant Authentification

The roots were recognized by professor P Jayaraman, Ph.D. from the plant anatomy and research center located at Tambaram (west). The authentication ID is **PARC/2019/4111** and the certificate is enclosed below in the Appendix-I

2.3 Extraction Procedure

Cesalpenia bonducella fresh fruits was collected and authenticated. After the seeds were rubbed out, the skin was left to dry in the shade. The shade-dried seeds mashed into a coarse powder and sieved through mesh no 60, were subjected to extraction.

500mg of coarsely powdered dried Grains of *Cesalpenia bonducella* was placed in a maceration flask with 70% of 2-liter Ethanol at 70^{o}C temperature for 72 hours with occasional shaking. Then it was filtered and the macerate was evaporated to attain the yield. Finally the percentage of yield was calculated.

2.4 Experimental model

2.4.1 Grouping of animal's

Female albino rats (180-220gm) were purchased from VETERINARY AND ANIMAL SCIENCES UNIVERSITY, TAMIL NADU, Laboratory Animal Medicine, and Centre for Animal Health Studies, Chennai- 51 the study was taken from an experimental facility, they were kept in a poly glycol boxes in a temperature-controlled (25+20^{0}C) environment with a 12 hours dark/light cycle with standard ad

libitum. All animals were acclimatised to the laboratory condition for a period of 15 days before the commencement of the study. The animals were divided into five groups, each of which had five animals.

2.4.2 PCOS Induction in the Animals

The treatment of PCOS is evaluated in this study using T.P-caused PCOS. [11] Thirty female virgin Wistar rats, weighing 180-220g of 10-12 weeks aged, having regular estrus cycles as determined by vaginal smear, were employed in the study. [12]. Excluding the normal control group, all the other groups were treated with 4mg TP intramuscular injection in oily solution per rat. [13, 14] each animal had subjected to vaginal smear examination daily. The absence of cyclicity was shown as criteria for induction of PCOS by prolonged cornification of vaginal smears.

2.4.3 Vaginal smears

The stage of cyclicity was determined via microscopic examination of the main cell type in vaginal smears. Estrous cycle cyclicity was assessed with 800 to 1200 hrs, and the relative components of epithelium, leukocytes, and cornified cells collect in daily vaginal lavages was studied using light microscopy. Which are known to alter at different stages of the estrous cycle. In control group or PCOS rats (proestrus, estrus, diestrus1, and diestrus2,) lasts usually about 4 days**.** [15]

2.4.4 Vaginal Cytology

Every day vaginal smears were checked, while having lumbar support, the rats were confined at the upper ventral side and thorax. Cotton soaked in a drop of physiological saline solution was used to collect vaginal secretions. The cotton swab was inserted almost one or two inches into the female rat's vagina and rotated for two to three rotations. (Allowing the cotton swab end to collect an acceptable volume of cells), and the cotton tip was rolled along the glass slide and swab has been gently removed. The dried smear was preserved by soaking it in 70% alcohol. After that, the slides

were stained with methylene blue 0.5 % solution, cleanse with tap water and inspected under a light microscope with a ×10 objective lens (without the condenser lens).[16]

2.4.5 Treatment Protocol

Except for I Group, which receives TP intramuscularly, after induction, all rats in Groups IV and V were given oral gavage for 15 days. Whereas group 3 rats were treated with Clomiphene citrate Standard drug for a period of 15 days. On16th day, Retro orbital puncture was performed to collect blood samples and serum was subjected to hormonal analysis (LH, FSH, estradiol, progesterone and testosterone). Animal in each group was selected and euthanized in carbon dioxide incubator. Finally incision was made to collect the ovaries then weighed and subjected to histopathological examination.

2.4.6 Serum Hormonal Assay

LH, FSH, Serum testosterone, progesterone and estradiol, were measured using Enzyme-Linked immunoassay kit for quantitative estimation of respective hormones.[16'

2.4.7 Histopathological examination

Bouin's solution was used to repair the removed ovaries and the ovaries were drained in a series of alcohol, and then cleansed in xylene before being immersed in heated paraffin wax at 60 degrees Celsius. They was dehydrated in a series of alcohol, and then cleaned in xylene by being embedded in paraffin wax that was melted at 60^{o}C. Consecutive slices were placed on slide with 3-aminopropyl triethsilane-coated sections were dried at 37ºC for 24 hours. Before being mounted for histology, the slides were deparaffinized, hydrated, and stained with Mayer shematoxylin& eosin dyes. Scope picture 3.0 imaging instrument (ScopeTek DCM 200) was used to examine the ovaries at a magnification of 40X (Hangzhou Scope tekOpto-Electric Co Ltd, USB 2.0). The thickness and

diameter of cystic follicles were measured. Cystic follicles have a thicker, fibrotic cortex with a visible surface theca and interior layer. [17]

2.4.8 Statistical analysis

The results are expressed as Mean ± SEM. Data were evaluated using ONE WAY ANOVA followed by Newman – Keul's multiple range test. Probability values less than ($p < 0.01$) were considered significant.

3. RESULTS AND DISCUSSION

Table 1: Percentage yield (w/w) of Plant extract

Sr No.	Extract	Color	Percentage yield (w/w)
1.	Hydroalcoholic extract of *Cesalpenia bonducella*	Brownish Black	3.70

3.1 Hormonal parameter evaluation:

3.1.1 The effect of CBHAE on LH levels in rats with T.P induced PCOS rats

When compared to normal control group (G1), T.P causes a considerable increase in LH levels & decrease in toxic control FSH (G2) levels ($P<0.001$). The levels of LH/FSH differed considerably between control groups. When compared to toxic control group, extracted treated animal's i.e, and both doses of CBHAE 200mg/kg & 400mg/kg exhibited a low levels of LH/FSH ratio and a remarkable drop in LH ($P<0.01$) &ascent in FSH levels (Tab: 2)

3.1.2 The effect of CBHAE in T.P induced PCOS rats

Estradiol levels decline markedly when T.P injection are given. Considerable rise in estradiol levels ($P<0.01$) result from simultaneous treatment for 15 days of CBHAE extracts. In standard group animals, estradiol levels are also significantly increased (Tab: 2)

3.1.3 The Effect of CBHAE on progesterone in TP induced PCOS rats

The drug induced group, which was given T.P, has considerably decreased in progesterone levels. CBHAE treatment at both dosages in accordance with T.P (200mg/kg & 400mg/kg) caused a substantial rise in progesterone ($P < 0,001$) levels to nearly normal levels. The control group found similar findings with CBHAE (Tab: 2).

3.1.4 The effect of CBHAE on ovarian morphology

There was more cystic follicle in the ovaries of the positive control group (T.P), but in 200mg/kg & 400mg/kg CBHAE shows near to normal follicles. Atretic follicles were seen and present at 200mg/kg. There were several healthy follicles in the 400 mg/kg group (Tab: 3)

3.1.5 The Effect of CBHAE on follicular diameter &thickness

The diameter and thickness of the cysts in the PCOS treatment group have increased but the standard group and extract treated group has fallen. (Tab: 3)

3.1.6 The Effect of CBHAE on ovarian weight

The T.P. control group's ovarian weight fall significantly ($P<0.01$) as compared to the other groups, but it was restored to normal levels in the treatment groups 200mg/kg & 400mg/kg. (Tab: 3)

Table 2: Effect of CBHAE on serum hormone in T.P induced PCOS

GROUP	FSH	LH	Estradiol	TSH	PRGSN
G1	8.27±0.25	6.146±0.33	54.12±2.31	0.28±0.04	14.15±0.61
G2	2.26±0.235**a	11.36±0.75**a	14.32±0.82**a	0.37±0.02**a	7.055±0.712**a
G3	7.02±0.46	5.25±0.38	46.49±1.74	0.32±0.02	12.12±0.29
G4	6.63±0.53**b	3.70±0.18**b	38.17±1.33**b	0.34±0.01**b	10.8±0.72*b
G5	6.13±0.56**b	4.57±0.21**b	41.18±0.92**b	0.32±0.01**b	11.60±0.76*b

All data's were analyzedG_1-Normal, G_2-Toxic, G3-Standard, G4-Low dose (CBHAE), G_5-High dose (CBHAE)

For each group of 6 animals, all values represented as means ± SEM.

**a- values are significantly differ from Normal control (G_1) at P<0.001

**b- values are significantly differ from PCOS control (G_2) at P<0.001

*b- values are significantly differ from PCOS control (G_2) at P<0.01

Table 3: Effect of CBHAE on ovarian morphology of PCOS rats

Dose mg.kg ovarian feature	Normal	Std control	Toxic control	Low dose	High dose
Atretic follicle	0.00±0.00	1.12±0.05	4.43±0.307	3.06±0.17**b	0.05±0.11*b

Cystic follicle	0.00±0.00	3.7±10.58	10.55±1.21	0.00±0.00	0.00±0.00
Cystic follicle diameter	0.00±0.00	71.19±2.35	87.73±2.367	0.00±0.00	0.00±0.00
Cystic follicle thickness	0.00±0.00	34.63±2.18	42.35±1.48	0.00±0.00	0.00±0.00

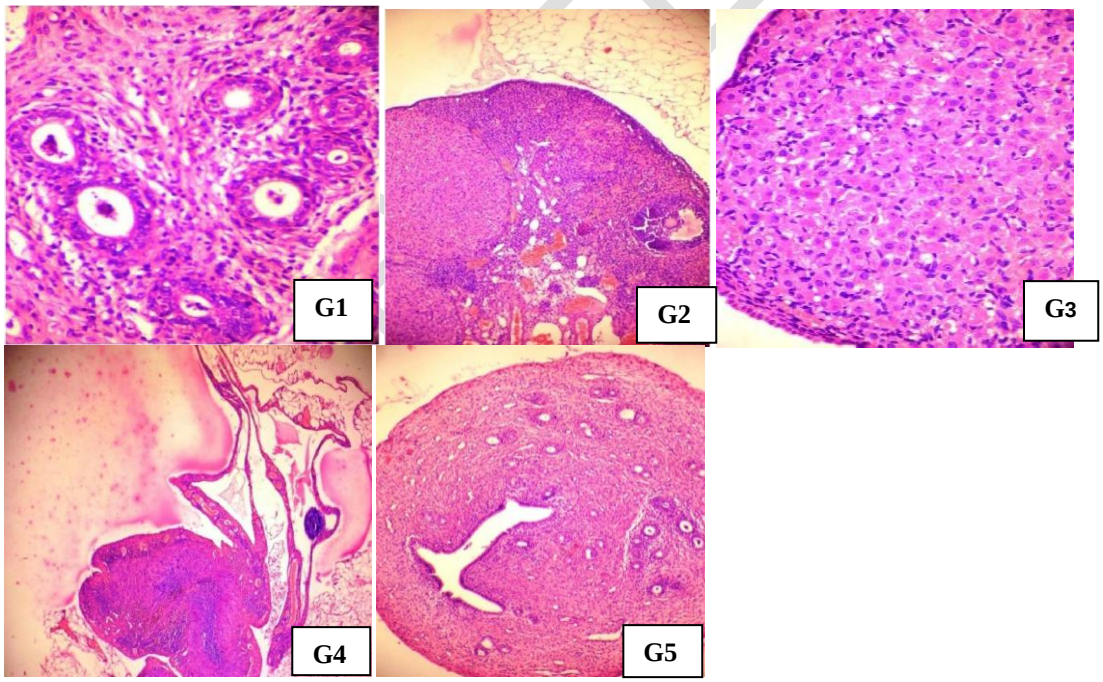
G1-Normal, G2-Toxic, G3-Standard, G4-Lowdose, G5-High dose

For each group of 6 animals, all values represented as means ± SEM.

**b- values are considerably differ from PCOS control (G2) at P<0.001

*b- values are considerably differ from PCOS control (G2) at P<0.01

3.2 Histopathalogical Examination



G1: Normal Control (10ml/kg Normal Saline) Fig.1, G2: Drug Induced Group (T.P 4mg/kg) Fig.2, G3: Standard Control (Clomiphene Citrate 20mg/ml), G4: Treatment Control (LOW DOSE of CBHAE 200mg/kg), G5: Treatment Control (High Dose of CBHAE 400mg/kg)

G1 Antral follicles, Carpus luteum, Oocyte are surrounded by granulose cells, and theca cells is visible in this section, G2 Many cystic degenerating follicle and atretic follicles with degenerated granulosa layer is observed in an ovarian section from PCOS rat, G3 A normal follicle with clear antrum, and Oocyte in granulosa layer is visible in ovarian section of PCOS rat, treated with clomiphen citrate, G4 The ovary of a PCOS rat treated with a low dose of CBHAE 200mg/kg reveals mild degenerative follicle and absence of cystic, atretic follicle, G5 The ovary of a PCOS rat treated with a low dose of CBHAE 400mg/kg reveals the existence of growing regenerating follicles and carpus luteum, as well as Oocytes with in granulosa. and thicker theca.

4. Discussion

Although many models can be used to study PCOS, induction of PCOS by Testosterone Propionate can also be considered as one of the best model for studying PCOS. Hence, in terms of exhibiting the majority of reproductive and endocrine symptoms associated with PCOS, rodent PCOS models appear to closely parallel the human condition. This study investigated the effect CBHAE on the serum levels of LH, FSH, estradiol, testosterone & progesterone in TP induced PCOS. After 30 days of PCO induction, animals were analysed both harmonically & histologically, on 16th day after treatment with hydroalcoholic extract, animals were also analysed irrespective of their estous cycle. In PCOS condition, normal gonadotropin-ovarian axis is disturbed results in hormonal imbalance reflected by the higher levels of LH, lower FSH levels and reversal of LH: FSH ratio. An elevated LH/FSH ratio and anovulation are typical findings in women with PCOS. [22,23] The extract treated

groups shows better reduction in this LH/ FSH ratio indicate that extract could reverse PCOS condition. Oestrogen similar to other steroids becomes altered in PCOS. [24] Repetitive administration of CBHAE led to significant rise in estradiol. Similarly, the reduction in the level of progesterone in the PCOS-induced animals could be responsible for the persistent oestrusphase.[104] Elevation in the concentration of serum progesterone by CBHAE may be responsible for the reversal of the luteal phase dysfunction and restoration of normalcy of the estrous cycle.

Our study showed that CBHAE induced an increase in serum estradiol implies that plant causes marked improvement in endocrine function and recovery of ovulary functions in the rats. Hyperandrogenism (as a result of high testosterone levels) which is evident in human PCOS. [25, 26] was not present in this animal model of TP induced PCOS. [27] Therefore no effect of the extract on androgen levels was observed using this model of PCOS induction. Ovarian weight in PCOS induced rats was more than the normal rats which is in accordance with earlier findings. [28-30] Treatment with HAECBE prevented further increase in ovarian weight & returned to normaly. The biochemical results are also supported by histopathological observation of light microscopy. The histomorphometry of PCOS was a suitable measurement for describing the cystic status because differences were observed in the morphological characteristic and in the presence or absence of follicular cysts.[119]It is reported that the histopathological study of PCOS induced rats shows the formation of poly cysts in the ovary. [31, 32]. Ovaries exhibited increased follicle atresia and multiple cysts with thin granulose cell layers and thickened theca cell layers. [33] After treatment with extract of *Cesalpenia bonducella* PCOS condition was reversed, number of cystic follicles reduces & found numerous healthy follicles at different stage of development. This indicates that treatment group shows marked recovery of ovarian tissue and the animals may probably be preparing for ovulation.

After treatment with CBHAE, the incidence of cystic follicles reduced and many healthy follicles at various stages of development were detected. This indicates that the therapy group has made substantial progress, and the ovarian tissue and animals are most likely preparing for ovulation. In addition, CBHAE therapy restores gonadotropin feedback inhibition (LH & FSH), resulting in a rise in estradiol and progesterone. The capability of the extract for reducing the histological, clinical, and biochemical aspects of the PCOS indicates all of these variables. The presence of flavanoids may explain the pharmacological effect of CBHAE extracts. Aside from the flavonoids discovered to have an antioxidant function in PCOS rats, the PCOS illness has been linked to a reduction in the quantity of antioxidant enzyme/molecule. Antioxidants aid in the protection of the human body from the negative effects of reactive oxygen species (ROS) (ROS). Plants with a strong antioxidant characteristic, particularly flavonoids, have shown themselves. The well-known usage of plants in the past in the management of gynaecological disorders may be responsible for these plant phytochemicals.

5. CONCLUSION

PCOS is a prevalent endocrine disorder of women, which can results in infertility. The prospective function of Herbal medicine in the treatment of PCOS is consistent with few adverse effects. Herbal medicine improves body immunity without changing hormonal level and also regularise menstrual cycle. In conclusion CBHAE reveals a considerable recovery of PCOS animals from, LH, FSH, estradiol & progesterone as well as the irregular cycle and ovarian physiology. T.P injection enhances the ovary weight and reproductive system of normal rats, which returned to normal, after treating with CBHAE. Polycyst's were seen as a group exception of the normal control group, which has no

polycysts at all. CBHAE was found to correct hormonal imbalance caused by T.P in PCOS rats, indicating that it might used to treat infertility.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research has been reviewed and approved by Institutional animal ethical committee and was carried out in accordance with CPCSEA guidelines (approval number **IAEC/219/2019**).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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