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Research Article

Evaluation of Alkaloidal Extract of *Crinum asiaticum* as a Potential Therapeutic for *Polycycstic Ovarian* Syndrome

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Abstract

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder affecting approximately 6–20% of women of reproductive age worldwide, depending on diagnostic criteria. Characterized by hyperandrogenism, chronic anovulation, and polycystic ovarian morphology, PCOS is a leading cause of infertility and metabolic dysfunction in women. The etiology of PCOS remains incompletely understood, but it is widely recognized as a complex interplay of genetic, hormonal, and environmental factors.

At the molecular level, PCOS is associated with dysregulation of the hypothalamic-pituitary-ovarian (HPO) axis, insulin resistance, and chronic low-grade inflammation. These pathophysiological features contribute to a spectrum of clinical manifestations, including menstrual irregularities, hirsutism, acne, obesity, and increased risk of type 2 diabetes mellitus and cardiovascular disease. Emerging evidence also implicates altered steroidogenesis, adipokine signaling, and oxidative stress in the progression of PCOS, highlighting the need for integrative approaches to its study. Despite its prevalence and impact, therapeutic options for PCOS remain limited and largely symptomatic. Current treatments focus on lifestyle modification, insulin sensitizers, and hormonal regulation, yet they often fail to address the underlying biological mechanisms. Consequently, there is growing interest in exploring novel compounds, cell-based assays, and targeted interventions to elucidate and modulate the molecular pathways involved in PCOS pathogenesis.

This article aims to investigate the efficacy of alkaloidal extract of Crinum asiaticum against PCOS, owing to the effect of plant extract in controlling menstrual bleeding and other effects on female reproductive system. This study will cater the need to find an alternative or supplementary therapeutics for PCOS and paving the way for more effective therapeutic strategies.

Keywords: PCOS; Crinum asiaticum; Alkaloidal Extract; Hormones, Ovary

Abbreviations

PCOS: Polycystic Ovarian Syndrome; CC: Clomiphene Citrate; KG: Kanchnar Guggulu; CAA: *Crinum asiaticum* Alkaloidal Extract; LH: Luetienizing Hormone; FSH: Follicle Stimulating Hormone; OGTT: Oral Glucose Tolerance Test

Introduction

Polycystic Ovarian Syndrome has been an issue of major concern since last two decades owing to its increasing predominance among young and middle-aged women. According to recent statistics, it has been found that at-least one among five women has PCOS. PCOS is often recognized as a reproductive endocrinopathy, as the majority of women with PCOS have anovulatory cycles, irregular/heavy bleeding, acne, hirsutism, and infertility. However, the spectrum of the syndrome is much wider. PCOS is associated with metabolic alterations related to insulin resistance, including impaired glucose tolerance, hyperinsulinemia, early onset type 2 diabetes, obesity, metabolic syndrome, non-alcoholic fatty liver disease and increased risk of cardiovascular disease. PCOS there-

fore can be characterized as a complex syndrome with reproductive, endocrine, and metabolic manifestations. Insulin resistance sets off a cascade of imbalance in a woman at a reproductive and metabolic hormone that contributes to PCOS [1-5]. Insulin resistance and Hyperinsulinemia leads to an exaggerated effect of insulin and also stimulates androgen secretion by ovarian theca cells. Typically theca cells are stimulated to produce androgens by Luteinizing Hormone (LH). Higher LH levels and stimulatory effect of insulin both leads to abnormally high level of androgens. Insulin also decreases Serum Hormone Binding Globulin (SHBG) which is a protein that is responsible for binding free testosterone. This again leads to elevated levels of androgens. The levels of FSH is lower in woman suffering from PCOS as compared to normal woman, thus higher LH/FSH ratio creates a state of hyperandrogenism. The activity of Aromatase P-450 enzyme which is present granulosa cells of follicles in reproductive age of woman and is responsible for converting androgens produced in theca cells and transported to granulosa cells of growing follicles into estradiaol is reduced in woman having PCOS. Since the treatment of PCOS is mainly focused on meliorating the symptoms associated with hyperandrogenism, variety of adverse-effect profiles have been encountered by the prevailing pharmacological therapies. It is being recognized that therapies focusing on ameliorating the comorbid relationship should also be given key importance while designing new therapeutic strategies. Thus, a search of therapeutically effective procedures with reduced side effects and the multi-targeting property is of urgent need and an utmost priority for the researchers working in this area [6-12].

Crinum asiaticum, commonly known as Lily, has reported efficacy in controlling excessive menstrual bleeding [13,14]. A lot of research work has been carried out showing efficacy of various plant extracts and various phytoconstituents, however both *Crinum asiaticum* and alkaloidal extracts remain to be least explored for their efficacy against PCOS. There are probable chances of them emerging as potential therapeutics for PCOS since their effect on female reproductive system in some or the other way has been reported. Thus the objective of current work done was to majorly assess the efficacy of alkaloidal extract of *Crinum asiaticum* through *in-vivo* studies.

Materials and Methods

Authentication of plant

All the plant materials were identified by Botany Department, The Maharaja Sayajirao University of Baroda. The voucher specimens of the herbs have been deposited in the Botany department, The Maharaja Sayajirao University of Baroda. Lily flowers were collected from local markets of Surat. The petals were allowed to open by keeping flowers in luke warm water for 3-4 hours. Whole bunch of flowers was screened and flowers carrying worms were separated. These flowers were shed dry from 15 days and then were powdered in grinder. Powder was stored in air tight container in refrigerator.

Preparation of alkaloidal extract

The powdered plant material was defatted with petroleum ether and was filtered. The residue was extracted with Methanol/water (4:1) and was filtered. The filtrate was collected and reduced to 1/10 volume at temperature less than 40 ^{0}C. Concentrated filtrate is acidified with conc. HCl to pH 2 and was extracted with chloroform thrice (same volume). Aqueous-acid layer was separated and basicity to pH 10 was adjusted with ammonia. This mixture was extracted twice with Chloroform/Methanol (3:1) and Chloroform. Chloroform/Methanol extract was evaporated in rotaevaporater and thus alkaloids were obtained through this basic extract.

In-vivo study

Grouping of animals

Nulliparous Female Wistar rats (5- 6 weeks, 130-150 g) obtained from SyncBio, Ahmedabad, were housed in cages placed in an animal room with a constant temperature of 22 °C and a fixed 12-hour light-dark cycle. The rats were given standard rat chow and water ad libitum. The Institutional Animal Ethics Committee (IAEC), Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, approved this study (protocol no. MSU/IAEC/2023-24/2303) ensuring animal welfare according to Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines. After acclimatization, rats were randomly allocated to two major groups Control (n = 6) and PCOS (n = 30). The Control animals received 1% Carboxymethyl cellulose (CMC)

orally every day for 28 days. The PCOS group were given oral dose of Letrozole (1 mg/kg body weight) daily for 28 days. Body weight measurement and prolonged diestrus phase was evaluated through vaginal smear to confirm induction of PCOS. After induction of PCOS, rats were divided in to the groups as mentioned: Group I served as normal control rats and received 1 ml of 0.5% CMC orally for 28 days. Group II served as PCOS induced model and hence received Letrozole (1 mg/kg body weight orally) till the end of the treatment. Post induction of PCOS, Group III received clomiphene citrate, that served as allopathic standard treatment. Group IV received Kanchnar guggulu (100 mg/kg b.w, p.o) for 28 days, that served as Ayurvedic Standard treatment group. Group V-VI received *Crinum asiaticum* Alkaloidal extract (5 mg and 10 mg/kg b. w. p. o.) respectively.

Body weight

To track changes in body weight throughout the experiment, all groups were weighed weekly. This data was then used to calculate appropriate drug dosages for each animal.

Estrous cyclicity

Estrus cycle staging was performed via vaginal cytology. A cotton swab moistened with saline solution was gently inserted into the vaginal canal, avoiding excessive stimulation of the cervix to prevent pseudopregnancy. The collected cells were immediately smeared onto a microscope slide and allowed to air dry. Subsequently, the slides were stained with 1% Toludene blue solution (Toludene blue dye dissolved in distilled water), rinsed with distilled water to remove excess dye and air-dried. Finally, the prepared slides were observed under a light microscope to determine the stage of the estrus cycle. The relative proportions of epithelial cells, leukocytes and cornified cells were analyzed. PCOS was considered to be induced when diestrus was prolonged.

Oral glucose tolerance test

Oral glucose tolerance test was performed after 15 h fasting at the end of the study to check glucose intolerance. Glucose (2g/kg body weight) was given orally and blood glucose levels were measured at 0, 30, 60, 90, 120, 180 minutes. using Dr. Morepen BG-03 GlucoOne Glucometer. Blood was obtained via retro orbital vein puncture.

Lipid profile

Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined by liquid reagent kits (Recombigen Laboratories Pvt Ltd.) according to manufacturer's instructions using autoanalyzer.

Hormonal assay

Serum testosterone, progesterone, estrogen, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), levels were estimated using enzyme-linked immunosorbent assay (ELISA) kits (Krishgen Biosystems).

Histopathology of ovary

Sections obtained from the ovaries were maintained in an incubator at 37 °C overnight. The paraffin sections were deparaffinized in xylene and rehydrated. Then, the specimens underwent a dehydration process with a graded series of ethanol (EtOH). The sections were then incubated in haematoxylin solution for 10 min and washed again under running water for 10 min. Next, the sections were dipped 2–3 times in a mixture of 70% alcohol plus three drops of glacial acetic acid, washed in running water for 10 min and stained with eosin solution for 5 min. Finally, the sections were washed in running water for 10 min, passed through 3 changes of 95% EtOH and 2 changes of 100% EtOH for 1 min each and cleared in 3 changes of xylene for 1 min each20. Coverslipped images were taken and evaluated on a Leica DCM 4000 computer-aided imaging system.

Statistical analysis

The data were expressed as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's Post hoc test was conducted to assess statistical significance for measured variables across groups. A P-value<0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8.4.3 for Windows.

Results and Discussion

Effect of CAA extract on body weight in Letrozole-induced PCOS

Normal group had an initial weight of 207.8 ± 5.89 gm, showed a sustainable and normal weight gain through out the study concluding experiment with 252.4 ± 2.12 gm. On the other hand, PCOS group exhibited classic feature of rapid and excessive weight gain by initiating experiment with 188.7 ± 1.52 gm, on 28th day with 250.8 ± 3.95 gm and concluding it with 303.4 ± 6.57 gm of weight. This pattern concludes a significant difference observed with respect to weight gain between PCOS and normal group. Standard treatment groups showed a substantial reduction in weight upon comparing the weight before and after the treatment. CC group showed reduction from 283 ± 5.48 gm to 260.5 ± 3.78 gm while KG group showed reduction of 273 ± 2.56 gm to 261.3 ± 6.52 gm. CAA extract was able to reduce weight to a considerable amount from 290 ± 3.66 gm to 275 ± 8.45 gm for higher dose and from 287 ± 4.42 gm and 277 gm ± 7.45 gm for lower dose of CAA.

Effect of CAA extract on the proportion and stages of estrus cycle in Letrozole-induced PCOS

The vaginal secretion is made up of three types of cells. They include leucocytes, cornified epithelial cells and nucleated epithelial cells.

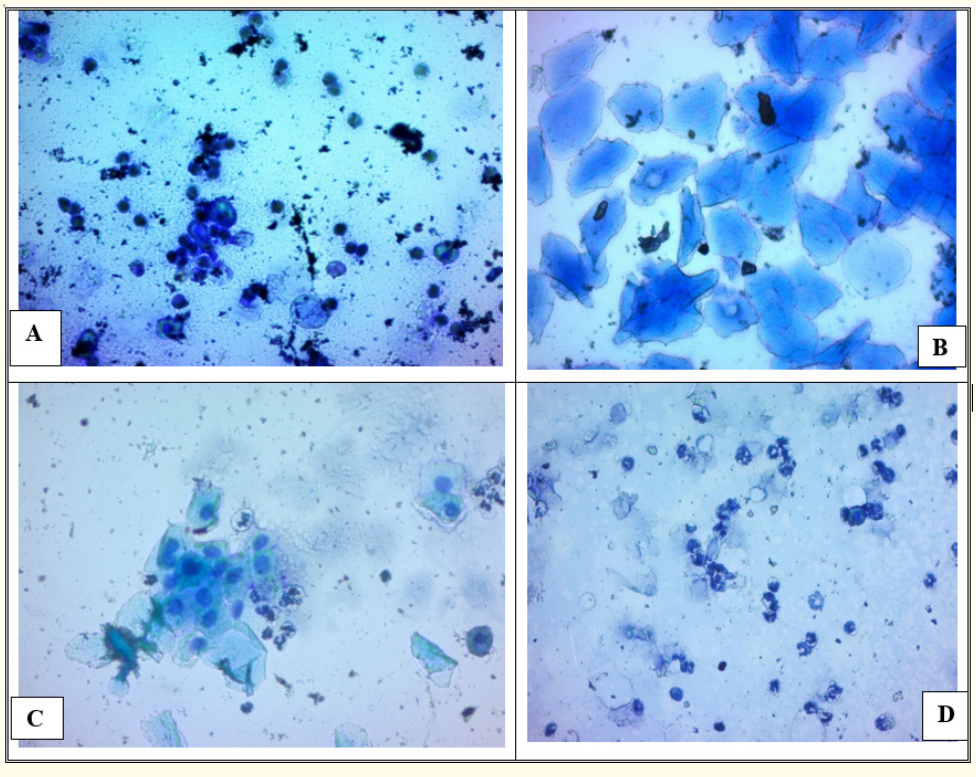


Figure 1: Various stages of estrous cycle. (A) Proestrous, (B): Estrous, (C): Metestrus and (D): Diestrus. Image magnification: 40x.

The duration of regular estrus cycle tends to be of 4-5 days consisting of Proestrus (14 hrs), Estrus (24-48 hrs), Metestrus (6-8 hrs) and Diestrus (48-72 hrs). induction of PCOS by letrozole was confirmed through arrested estrus cycle in Diestrus phase [25]. An overview of estrus cycle before and after the treatment of different groups is shown in figure 1.

The normal group followed the regular trend of estrus cycle (46.42% of Diestrus phase and 17.85% of Proestrus, Estrus and Metestrus) whereas PCOS model group showed typical characteristic of PCOS through arrested cycle. PCOS group showed a major dominance of Diestrus phase (92.85%). Clomiphene citrate and Kanchnar guggulu group after treatment showed a significant reduction in the proportion of Diestrus phase (53.57% and 57.14% respectively). Treatment groups of CAA along with Kanchnar guggulu group showed prolonged metestrus phase (21.42%). A significant reduction in the proportion of Diestrus phase was observed in both CAA_5 mg (57.14%) and CAA_10 mg (42.85%) indicating the restoration of estrus cycle after treatment. The graphical representation of proportion of various stages of estrus cycle of different groups are shown in figure 2.

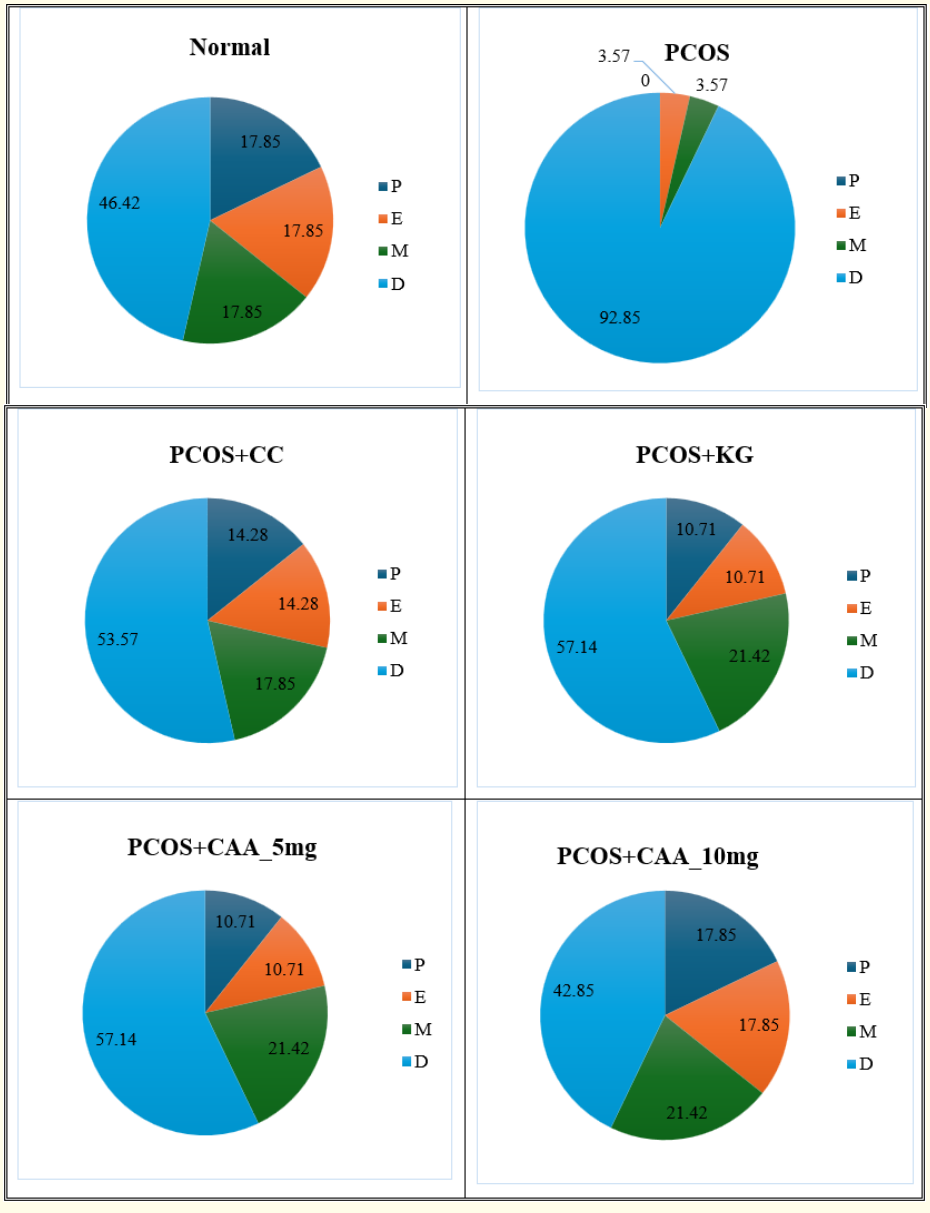


Figure 2: Effect of CAA extract on proportion of various stages of estrus cycle.

Effect of CAA extract on total number of complete estrus cycle 4 weeks (28-56 days) in Letrozole-induced PCOS

A normal rat completes one cycle in 4-5 days, depending on the stretch of individual stages of estrous cycle. Data for the completion of number of cycles of individual groups are mentioned in table 1.

Groups	No: of complete estrous cycles in 4 weeks (28-56 days)
Normal	5.5 ± 0.22
PCOS	0 ± 0$^{\#\#\#\#}$
PCOS + CC	4.5 ± 0.83*****
PCOS + KG	3.33 ± 0.81**
PCOS + CAA_5mg	3.5 ± 0.22**
PCOS + CAA_10mg	4.5 ± 0.34****

Table 1: Effect of CAA on number of complete estrus cycles.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

The data depicts a significant difference (#P < 0.0001) in the number of cycles completed between normal and PCOS group, as PCOS group showed arrested estrous cycle at diestrus stage. Treatment groups CC and CAA_10 mg (*P < 0.0001) showed a substantial restoration of the estrous cyclicity, whereas treatment groups of KG and CAA_5 mg showed significant (*P < 0.001) improvement, however were not close to normal cycle.

Effect of CAA extract on oral glucose tolerance test (OGTT) in Letrozole-induced PCOS

The data for the oral glucose tolerance test in order to understand the efficacy of extract on insulin resistance is depicted in table 2.

There was a significant difference in the baseline glucose level of PCOS group (#P < 0.01) as compared to normal group. During the entire time line of OGTT, varying glucose levels were observed and compared to to different groups, however the difference was found to be non-significant. The difference in glucose levels of CC, CAA_5 mg and CAA_10 mg at last time point of 240 min was found to be significant upon comparison to PCOS group. The graphical representation of effect of CAA on OGTT is depicted in figure 3.

Time	Normal	PCOS	PCOS+CC	PCOS+KG	PCOS+CAA_5mg	PCOS+CAA_10mg
0 min	99.17 ± 4.25	124.66 ± 5.58	100.16 ± 1.53	108.83 ± 2.94	116.5 ± 6.61	82.5 ± 2.04
30 min	133.17 ± 13.86	120.83 ± 4.51	114.16 ± 5.32	135.5 ± 3.23	126.16 ± 8.31	106 ± 3.57
60 min	112.83 ± 7.44	131.5 ± 8.02	114.67 ± 1.66	119.16 ± 6.53	115.83 ± 11.14	112.33 ± 5.38
90 min	127.66 ± 10.41	123.5 ± 6.05	117.16 ± 3.93	127.16 ± 9.66	128.5 ± 7.98	105.16 ± 5.22
120 min	128 ± 5.91	127.33 ± 4.03	127 ± 6.13	148.5 ± 5.49	140.66 ± 7.97	105.33 ± 6.11
180 min	117.5 ± 9.11	125 ± 9.75	113.83 ± 4.21	122.66 ± 7.77	130.66 ± 8.78	105.83 ± 6.51
240 min	121.16 ± 3.73	130.83 ± 6.17	104.16 ± 1.88	114.16 ± 2.91	116.5 ± 3.87	98 ± 0.96

Table 2: Effect of CAA extract on OGTT.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

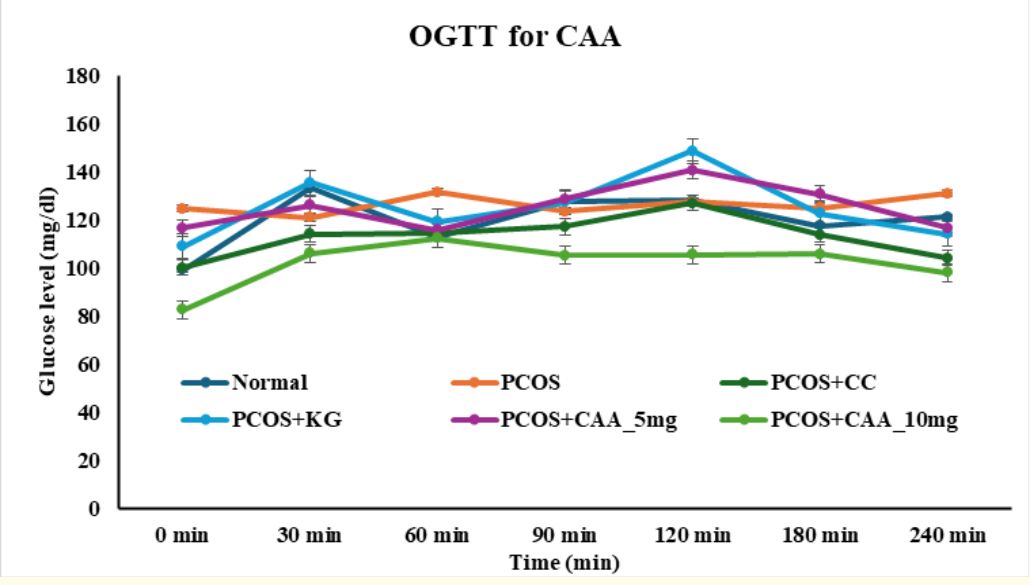


Figure 3: Graph showing effect of CAA extract on OGTT.

Effect of CAA extract on serum lipid profile in Letrozole-induced PCOS

Lipid profile was analyzed by measuring the levels of Total Cholesterol, Triglycerides, LDL-C and HDL-C. The data for lipid profile is depicted in table 3.

Elevated levels of cholesterol, TG, and LDL-C, along with a reduction in HDL-C, characterize the lipid profile of PCOS. The PCOS model showed the same tendency. Comparing the LDL, TG, and cholesterol levels to the normal group revealed a substantial rise (#P < 0.0001). A significant decrease (#P < 0.001) in HDL-C was noted in comparison to the Normal group.

When compared to the PCOS group, the treatment groups receiving KG and CAA_5 mg showed a substantial decrease in cholesterol levels of *P < 0.05, while the treatment groups receiving CC and CAA_10 mg showed a comparable degree of significance (*P < 0.01).

In comparison to the PCOS group, triglyceride levels were significantly lower (*P < 0.0001) in all therapy groups. In both the CC and CAA_10 mg treatment groups, the LDL-C level was significantly reduced (*P < 0.001). Reductions were observed in the KG and CAA_5 mg treatment groups at *P < 0.01 and *P < 0.05, respectively.

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Normal	54.59 ± 2.26	83.66 ± 2.18	13.88 ± 1.38	32.89 ± 1.12
PCOS	93.81 ± 4.33$^{\#\#\#\#}$	142.70 ± 3.53$^{\#\#\#\#}$	24.77 ± 1.98$^{\#\#\#\#}$	18.28 ± 0.75$^{\#\#\#}$
PCOS + CC	75.3 ± 2.03**	84.63 ± 2.87****	15.77 ± 0.95***	31.94 ± 1.20**
PCOS + KG	77.46 ± 2.02*	85.25 ± 1.87****	17.84 ± 1.16**	25.19 ± 1.99
PCOS + CAA_5mg	78.74 ± 2.49*	86.14 ± 2.96****	18.07 ± 0.98*	26.59 ± 3.42
PCOS + CAA_10mg	74.22 ± 4.60**	84.48 ± 1.95****	16.05 ± 0.47***	35.40 ± 2.31$^{****@}$

Table 3: Effect of CAA extract on serum lipid profile.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

HDL-C levels were considerably higher in the CC (*P < 0.001) and CAA_10 mg (*P < 0.0001) treatment groups than in the PCOS group. There was no discernible difference in HDL-C values between the PCOS group and the KG and CAA_5 mg treatment groups.

Effect of CAA extract on serum sex hormones level in Letrozole-induced PCOS

Hormonal assay profile was analyzed by measuring the levels of Testosterone, Progesterone and Estrogen. These are the main hormone that directly impact the pathogenesis of PCOS. The data for the hormonal assay of Testosterone, Progesterone and Estrogen is depicted in table 4.

GROUP	Testosterone	Progesterone	Estrogen
Normal	0.05 ± 0.01	25.26 ± 2.51	52.22 ± 1.10
PCOS	1.50 ± 0.16	5.72 ± 1.02	31.49 ± 0.90
PCOS + CC	0.50 ± 0.03****	19.56 ± 2.04****	48.13 ± 0.60****
PCOS + KG	0.60 ± 0.04****	17.47 ± 1.49**	43.55 ± 0.62****
PCOS + CAA_5mg	0.57 ± 0.07****	19.14 ± 1.97***	42.53 ± 1.14****
PCOS + CAA_10mg	0.48 ± 0.05*****	25.70 ± 1.31****	48.86 ± 1.00****

Table 4: Effect of CAA extract on serum levels of Testosterone, Progesterone and Estrogen.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

The elevated levels of testosterone is a classic hallmark of the PCOS condition indicating hyperandrogenism. A significant elevation in testosterone levels was observed in PCOS model group as compared to the Normal group (#P < 0.0001). Standard treatment groups of Clomiphene citrate and Kanchnar Guggulu and test treatment groups of CAA_5 mg and CAA_10 mg showed a significant reduction in testosterone levels (*P < 0.0001) as compared to PCOS group.

The level of Progesterone was found to be significantly reduced in PCOS model group (#P < 0.0001) as compared to normal group. Treatment groups CC and CAA_10 mg showed similar level of significant elevation (*P < 0.0001) in progesterone as compared to PCOS model group. Treatment groups of CAA_5 mg and KG were also able to increase level of progesterone at different levels of significance of *P < 0.001 and *P < 0.01 respectively in comparison to PCOS model.

Estrogen levels were found to be significantly reduced (#P < 0.0001) in PCOS group as compared to normal group. A similar level of significance (*P < 0.0001) in elevation of Estrogen level was observed in all the treatment groups in comparison to PCOS model group. A substantial difference of $P < 0.001 and $P < 0.05 was observed in the impact of treatment groups CAA_5 mg and KG in elevating estrogen level upon comparing with CC treatment group.

Apart from the above mentioned hormones, hormones like LH and FSH have indirect impact on PCOS manifestation since their impact is majorly modulated through hypothalamus gland in pituitary axis. The data for hormonal assay of LH and FSH is depicted in table 5.

Groups	LH (mIU/ml)	FSH (mIU/ml)
Normal	2.64 ± 0.68	42.05 ± 1.78
PCOS	9.90 ± 0.80	8.31 ± 2.95
PCOS + CC	3.79 ±0.99****	44.81 ± 1.94****
PCOS + KG	3.91 ± 1.01****	40.02 ± 4.50****
PCOS + CAA_5mg	4.61 ± 0.54****	42.36 ± 1.15****
PCOS + CAA_10mg	3.14 ± 0.49****	46.00 ± 1.85****

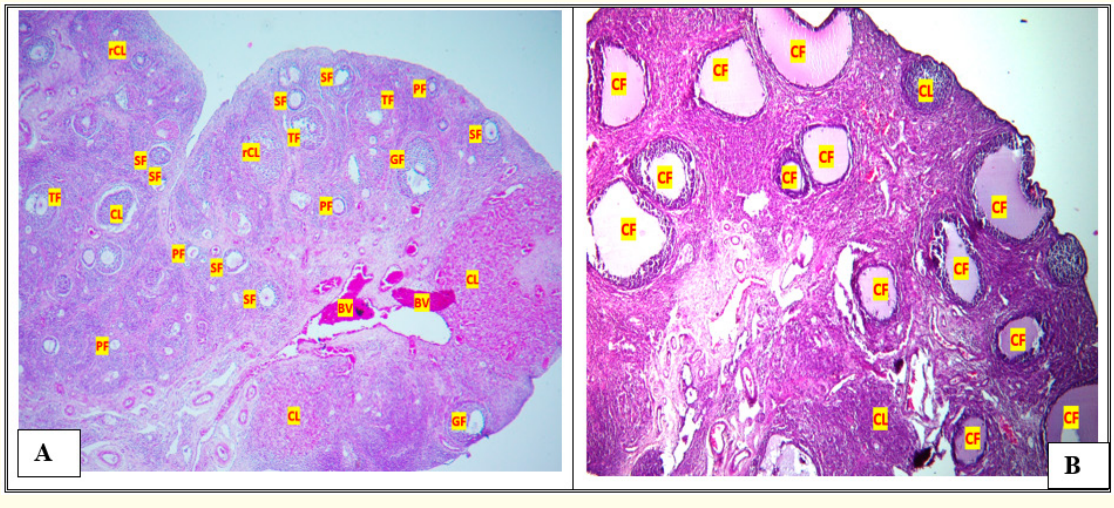
Table 5: Effect of CAA extract on serum levels of LH and FSH.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

A classic PCOS model has elevated LH levels and decreased FSH levels, which disrupts the LH:FSH ratio and harbors PCOS pathogenesis. The level of LH was found to be significantly elevated in PCOS model group (#P < 0.0001) than the normal group. All the treatment groups were able to significantly reduce (*P < 0.0001) level of LH as compared to PCOS group. PCOS model group showed significant reduction (#P < 0.0001) in FSH level as compared to normal group. All the treatment groups showed their ability to significantly increase (*P < 0.0001) FSH levels as compared to PCOS group.

Effect of CAA extract on ovarian histopathology in Letrozole-induced PCOS

The ovary of the normal group as shown in figure 4 (A) exhibited normal follicles in various stages of development including primary follicle, secondary follicles, tertiary follicle, Graafian follicles and fresh corpora lutea. Medullar area presented loose connective tissue, rich in blood and lymphatic vessels. The ovary of PCOS model (figure 4 (B)) displayed large amount of cystic follicles in inner region and peripheral region of ovary along with sparse granulosa or no granulosa cell layer. These cystic follicles indicate intense follicular atresia that led to seize the normal follicular growth and resulted in development of fluid filled cystic follicles. The ovary of CC treatment group (figure 4 (C)) showed restoration of normal ovarian morphology through the presence of primary, secondary and tertiary follicles. Appearance of cystic follicles was reduced and that of corpus luteum increased. Blood rich connective tissues were also found. The ovary of KG treatment group (figure 4 (D)) showed slight improvement as compared to CC group on the ovarian morphology in terms of amount of cystic follicles present. The amount of regressive or degenerative corpus lutea was also high indicating production of fewer corpus lutea. Also medullar region had fewer connective tissues. The ovary of CAA_5 mg treatment group amount of cystic follicles to certain extent and presence of fresh corpus lutea also in few numbers. The ovary of CAA_10 mg treatment group (figure 4 (F)) exhibited the characteristics of normal ovary through presence of fresh corpus lutea, fully developed Graafian follicles, follicles in various stages of development and reduced amount of cystic follicles.



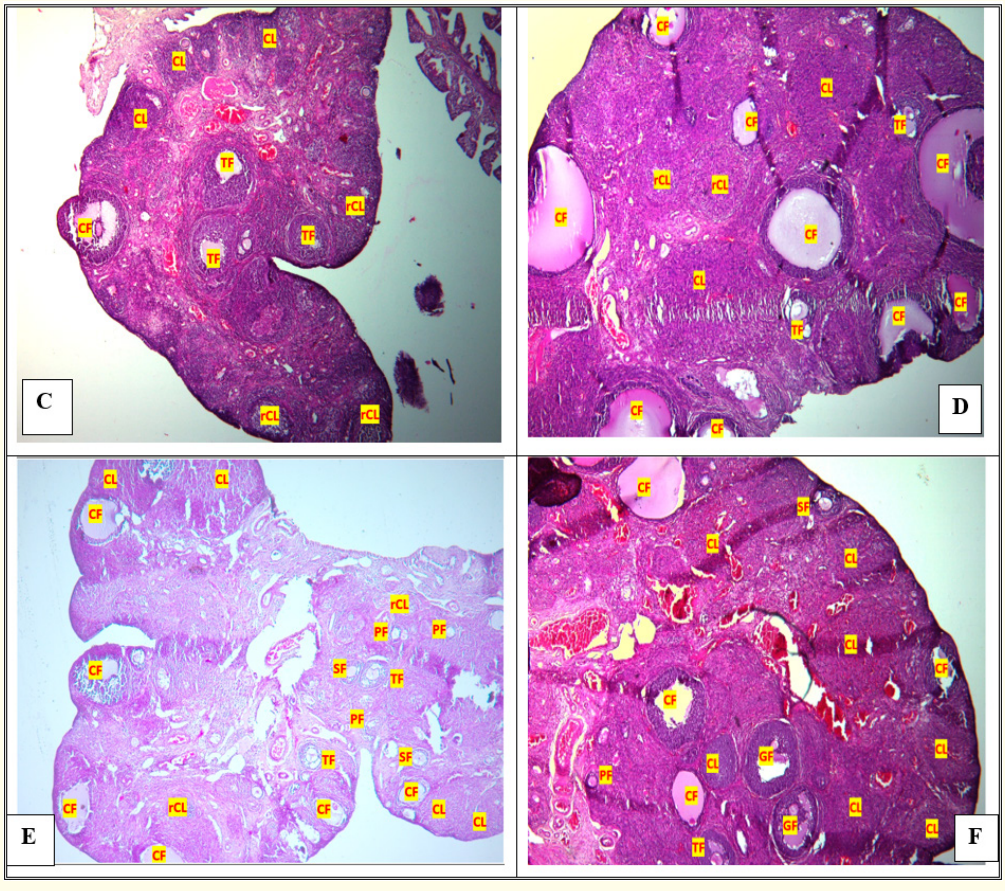


Figure 4: Effect of CAA extract on ovarian histopathology; A: Normal group; B: PCOS group; C: PCOS+CC group; D: PCOS+KG group; E: PCOS+CAA_5mg group; F: PCOS+CAA_10mg group.

Effect of CAA extract on follicle count

Primary, secondary, tertiary and Graafian follicles were counted along with corpus lutea to understand the efficacy of extracts on follicle counts. The data for various follicle counts is mentioned in table 6 and 7.

Cystic and atretic follicles are the main architectural features of PCOS ovaries. Both represent follicular atresia. PCOS model group had significantly higher number (#P < 0.0001) of cystic and atretic follicles than the normal group. Similar level of significant reduction (*P < 0.0001) in number of cystic follicles was observed for all the treatment groups. A substantial decrease in number atretic

Groups	Cystic Follicle	Atretic Follicle
Normal	1.67 ± 0.42	1.83 ± 0.47
PCOS	17.67 ± 0.76$^{\#\#\#\#}$	9.83 ± 1.35$^{\#\#\#\#}$
PCOS + CC	4.83 ± 0.70****	3.5 ± 0.22****
PCOS + KG	8.16 ± 1.88$^{\#\#****}$	4.83 ± 0.60***
PCOS + CAA_5mg	7.33 ± 0.71$^{\#\#****}$	4.83 ± 0.60***
PCOS + CAA_10mg	3.84 ± 0.47****	3.33 ± 0.33****

Table 6: Effect of CAA extract on cystic and atretic follicle count.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

Group	Graffian Follicle	Corpus Lutea	Primary Follicle	Secondary Follicle	Tertiary Follicle
Normal	3.67 ± 0.33	12.83 ± 0.94	4.33 ± 0.49	3.33 ± 0.42	3.33 ± 0.33
PCOS	0.67 ± 0.21$^{\#\#\#\#}$	0.66 ± 0.21	2.33 ± 0.21	1.16 ± 0.30	1.16 ± 0.30
PCOS + CC	2.83 ± 0.30****	9.33 ± 0.55****	4.16 ± 0.47*	2.83 ± 0.47*	3.16 ± 0.16*
PCOS + KG	2.66 ± 0.21***	7.5 ± 0.22****	3.5 ± 0.42	2.5 ± 0.22	2.83 ± 0.54
PCOS + CAA_5mg	2.67 ± 0.33***	6.83 ± 0.30****	3.5 ± 0.22	2.33 ± 0.42	2.67 ± 0.42
PCOS + CAA_10mg	3.16 ± 0.40****	10.16 ± 0.47****	4.16 ± 0.30*	2.83 ± 0.30*	3 ± 0.36*

Table 7: Effect of CAA extract on follicle count.

Values are statistically evaluated using One-way ANOVA analysis followed by Tukey's Post hoc test. Significant values were compared with Normal group vs. PCOS (##P < 0.01, ###P < 0.001, ####P < 0.0001); PCOS vs. all other groups (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001); PCOS+CC vs. all other groups ($P < 0.05, $$P < 0.01, $$$P < 0.001, $$$$P < 0.0001); PCOS+KG vs. all other groups (@P < 0.05, @@P < 0.01, @@@P < 0.001, @@@@P < 0.0001).

follicles at varying level of significance was observed for KG and CAA_5 mg (*P < 0.001) treatment groups and CC and CAA_10 mg (*P < 0.0001) treatment groups, compared to PCOS group.

PCOS model group exhibited distorted follicular morphology through a substantially lower presence of Primary, secondary, tertiary follicles (#P < 0.01) and Graafian follicles and Corpus lutea (#P < 0.0001) as compared to normal group. Similar level of significant increase (*P < 0.0001) in number of Corpus lutea and Graafian follicle was observed in all the treatment groups. In terms of primary, secondary and tertiary follicles, treatment groups CC and CAA_10 mg (*P < 0.05) showed substantial increase in the number of these follicles as compared to PCOS group. Upon comparison with PCOS group, other treatment groups like KG and CAA_5 mg showed non-significant difference in the number of these follicles.

Discussion

The Letrozole-induced PCOS model has been demonstrated to consistently produce the core features of human PCOS, including hyperandrogenism, anovulation, polycystic ovaries, oxidative stress and some metabolic disturbances like hyperglycemia and altered lipid profile. Notably, these features develop as a pathological manifestation of hyperandrogenemia, a key factor implicated in the etiology of PCOS [15-17]. Letrozole is a non-steroidal aromatase inhibitor, which acts by blocking the conversion of androgens, such as testosterone and androstenedione, into estrogens (E2 and estrone, respectively). This mechanism results in elevated androgen levels. Elevated androgens can directly suppress the release of follicle-stimulating hormone (FSH) from the pituitary gland. FSH plays a crucial role in stimulating follicular growth and development in the ovary. Consequently, suppressed FSH levels can lead to anovulation, the absence of ovulation. Furthermore, hyperandrogenemia may also disrupt the delicate balance between FSH and LH, leading to decreased FSH and increased LH levels [16].

In order to understand the efficacy of extracts on PCOS, factors like changes in body weight, estrous cyclicity, lipid profile, hormonal assay and histopathology of ovaries were analyzed in detail. Each of the factor represents a contributing or resultant factor of pathophysiology of PCOS. Genetic predisposition to PCOS, coupled with weight gain and obesity, often leads to the clinical and biochemical manifestations of the syndrome. These highlights the close link between obesity and PCOS, as evidenced by the high prevalence of overweight or obesity (38%-88%) among women diagnosed with PCOS. Supporting this association, Mannerås., *et al.* demonstrated increased body weight in a rat model of PCOS induced by Letrozole. This finding suggests that weight gain may not only be a consequence of PCOS but also potentially a contributing factor to its development [17]. The estrus cycle in female rats is characterized by distinct phases, including proestrus, estrus, metestrus and diestrus normally lasting from 4 to 5 days. Studies have shown that Letrozole-induced PCOS rats often experience a predominantly diestrus state with minimal proestrus, indicating a dysfunctional estrus cycle [18-20]. This disruption likely stems from Letrozole's suppression of estrogen production. Estrogen plays a crucial role in regulating the HPG axis, which orchestrates the cyclic release of hormones that govern estrus cyclicity. With diminished estrogen

levels and increased androgen levels, the HPG axis becomes dysregulated, leading to the observed irregularities in the estrus cycle. Hyperandrogenism (HA) is a major diagnostic feature of PCOS often combined with hormonal imbalance. Women with PCOS have decreased estrogen and progesterone levels with increased testosterone and abnormal LH:FSH ratio [23,24]. The studiesPJE-treated (400 mg/kg/day for 3 weeks also reported elevated testosterone and LH levels, decreased levels of estrogen, progesterone and FSH, as well as increased LH:FSH ratio in Letrozole-induced animal models. Letrozole blocks the conversion of testosterone to estrogen by inhibiting the aromatase enzyme contributing to abnormal levels of sex hormones. PCOS is frequently associated with IR and hyperglycemia. PCOS women are at a significantly increased risk of developing T2DM later in life. Treatment with Letrozole, as reported by Islam., *et al.* has been shown to induce insulin resistance and low glucose tolerance in rats. This effect might be associated to the increase in androgen levels. Androgens can antagonize insulin signalling pathways, hindering glucose uptake by cells and leading to hyperglycemia. Supporting this notion, research by Xu., *et al.* also observed impaired glucose tolerance in Letrozole-treated rats. PCOS is commonly associated with dyslipidemia with disturbed lipid profile such as increased TC, TG, LDL-C and decreased HDL-C levels [21,23,24]. Mannerås., *et al.* also demonstrated similar findings, increased TC, TG, LDL-C and decreased HDL-C levels in Letrozole-induced PCOS animals [15]. The current PCOS model exhibited all these conditions.

CAA extract consisted of alkaloids extracted from Lily plant. CAA extract reduced body weight, improved insulin sensitivity by reducing glucose level, lowered cholesterol, TG and LDL-C and increased HDL-C levels. It also had significant impact in restoring estrous cyclicity and reducing cystic and atretic follicles. It restored normal ovarian morphology with few evident left out cystic follicles. CAA extract was able to substantially decrease level of testosterone and LH and increase estrogen, progesterone and FSH levels. The potential of alkaloids against PCOS is not explored much. The current findings aligned with the previous studies. Alkaloids such as berberine, piperine, and trigonelline have been shown to improve insulin sensitivity by enhancing insulin signaling pathways. This includes increasing the activity of insulin receptors and promoting glucose uptake by cells. Animal studies have demonstrated that alkaloids can significantly improve insulin sensitivity and reduce blood glucose levels. For example, berberine has been shown to enhance insulin signaling and reduce insulin resistance in diabetic animal models. Alkaloids are also known to influence the levels of gonadotropins, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [26]. By modulating these hormones, alkaloids help in restoring normal ovarian function and menstrual regularity. In case of CAA extract, higher dose was found to be more efficient as compared to lower dose.

Conclusion

Polycystic Ovarian Syndrome (PCOS) can be characterized as a complex syndrome with reproductive, endocrine, and metabolic manifestations. Majority of the therapeutics target the symptoms of PCOS owing to the gaps available in the understanding and discovery of pathophysiology of PCOS, that could be seen as a limitation of therapeutics of PCOS. However, setting symptoms of PCOS as a target leads to initial screening of the therapeutic being tested, thereby serves as a stepping stone in the discovery of therapeutics for PCOS. The current study evaluated the efficacy of alkaloidal extract in terms of regulating the hormonal levels, glucose levels and weight regulation, which are the predominant symptoms of PCOS. The alkaloidal extract of *Crinum asiaticum* achieved a significant reduction in weight and was able to restore insulin resistance to normalcy. CAA showed dose dependent reduction in lipid levels. In terms of Hormonal assays, similar results were observed. Higher dose of CAA extract was able to restore estrous cyclicity. Ovarian morphology was restored to normalcy by CC and CAA by reducing the number of cystic follicles and atretic follicles. Overall it was observed that higher dose of CAA extract executed effects similar to Clomiphene citrate standard and lower dose showed harmony with Kanchnar guggulu standard. It can be concluded that alkaloidal extract of *Crinum asiaticum* has potential to emerge as a therapeutic for PCOS. Further studies to decode the underlying mechanism and targets for each factor of PCOS is required to develop this therapeutic catering to different types of PCOS and target specific therapeutic.

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Conflict of Interest

None.

Bibliography

1. World Health Organization. "Polycystic Ovary Syndrome". WHO, (2025).

2. Salari Nader., *et al*. "Global Prevalence of Polycystic Ovary Syndrome in Women Worldwide: A Comprehensive Systematic Review and Meta-Analysis". *Archives of Gynecology and Obstetrics* 310.6 (2024): 1303-1314.

3. Su Ping., *et al*. "Physiopathology of Polycystic Ovary Syndrome in Endocrinology, Metabolism and Inflammation". *Journal of Ovarian Research* 18.34 (2025): 1-15.

4. Kumar R., *et al*. "Role of Genetic, Environmental, and Hormonal Factors in the Progression of PCOS: A Review". *Journal of Reproductive Healthcare and Medicine* 3.3 (2022): 1-10.

5. Zhang Shuang., *et al*. "New Perspectives on Polycystic Ovary Syndrome: Hypothalamic-Sympathetic-Adipose Tissue Interaction". *Journal of Ovarian Research* 18.145 (2025): 1-20.

6. Wang Kai and Yifan Li. "Signaling Pathways and Targeted Therapeutic Strategies for Polycystic Ovary Syndrome". *Frontiers in Endocrinology* 14 (2023): 1191759.

7. Marshal John C and Andrea Dunaif. "All Women with PCOS Should Be Treated for Insulin Resistance". *Fertility and Sterility* 97.1 (2012): 18-22.

8. DeUgarte Catherine Marin., *et al*. "Prevalence of Insulin Resistance in the Polycystic Ovary Syndrome Using the Homeostasis Model Assessment". *Fertility and Sterility* 83.5 (2005): 1454-1460.

9. Diamanti-Kandarakis Evanthia and Andrea Dunaif. "Insulin Resistance and the Polycystic Ovary Syndrome Revisited: An Update on Mechanisms and Implications". *Endocrine Reviews* 33.6 (2012): 981-1030.

10. Chen Jie., *et al*. "The Correlation of Aromatase Activity and Obesity in Women with or without Polycystic Ovary Syndrome". *Journal of Ovarian Research* 8 (2015): 11.

11. Williams T., *et al*. "Diagnosis and Treatment of Polycystic Ovarian Syndrome". *American Family Physician* 94.2 (2016): 106-113.

12. Magoffin D A. "Ovarian Theca Cell". *International Journal of Biochemistry and Cell Biology* 37.7 (2005): 1344-1349.

13. Patel D K. "Crinum Asiaticum Linn: A Medicinal Herb as Well as Ornamental Plant in Central India". *International Journal of Environmental Sciences and Natural Resources* 6.1 (2017): 555678.

14. Mahomoodally, M. F., *et al*. "Ethnomedicinal, Phytochemistry, Toxicity and Pharmacological Benefits of Poison Bulb Crinum Asiaticum L". *South African Journal of Botany* 136 (2020): 16-29.

15. Mannerås, L., *et al*. "A New Rat Model Exhibiting Both Ovarian and Metabolic Characteristics of Polycystic Ovary Syndrome". *Endocrinology* 148 (2007): 3781-3791.

16. Osuka S., *et al*. "Animal Models of Polycystic Ovary Syndrome: A Review of Hormone-Induced Rodent Models Focused on Hypothalamus-Pituitary-Ovary Axis and Neuropeptides". *Reproductive Medicine and Biology* 18 (2018): 151-160.

17. Chen W and Y Pang. "Metabolic Syndrome and PCOS: Pathogenesis and the Role of Metabolites". *Metabolites* 11.12 (2021): 1-18.

18. Divyashree S., *et al*. "Experimental Models of Polycystic Ovary Syndrome: An Update". *Life Sciences* 237 (2019): 1-63.v

19. Walters K A., *et al*. "Rodent Models for Human Polycystic Ovary Syndrome". *Biology of Reproduction* 86 (2012): 149.

20. Liu H., *et al*. "Unraveling the Complexity of Polycystic Ovary Syndrome with Animal Models". *Journal of Genetics and Genomics* 51 (2024): 144-158.

21. Venegas B., *et al*. "In Rats with Estradiol Valerate-Induced Polycystic Ovary Syndrome, the Acute Blockade of Ovarian β-Adrenoreceptors Improves Ovulation". *Reproductive Biology and Endocrinology* 17 (2019): 1-10.

22. Stener-Victorin E., *et al*. "Animal Models to Understand the Etiology and Pathophysiology of Polycystic Ovary Syndrome". *Endocrine Reviews* 41 (2020): 1-39.

23. Kafali H., *et al*. "Letrozole-Induced Polycystic Ovaries in the Rat: A New Model for Cystic Ovarian Disease". *Archives of Medical Research* 35 (2004): 103-108.

24. Kauffman A S., *et al*. "A Novel Letrozole Model Recapitulates Both the Reproductive and Metabolic Phenotypes of Polycystic Ovary Syndrome in Female Mice". *Biology of Reproduction* 93 (2015): 1-12.

25. McLean AC., *et al*. "Performing Vaginal Lavage, Crystal Violet Staining, and Vaginal Cytological Evaluation or Mouse Estrous Cycle Staging Identification". *Journal of Visualized Experiments* 67 (2012): e4389.

26. Kumar A., *et al*. "Role of Plant-Derived Alkaloids against Diabetes and Diabetes-Related Complications: A Mechanism-Based Approach". *Phytochemistry Reviews* 18 (2019): 1277-1298.