INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Ameliorative effect of Dhatryadi Ghrita on Letrozole induced Polycystic Ovarian Syndrome in female albino Wistar rats

Sangeetha Venkatesan[1] *, Sakthi Abirami[2]

[1]*Department of Pharmacology, Student of Pharmacy, Madras Medical College, Chennai, India.*
[2]*Department of Pharmacology, Faculty of Pharmacy, Madras Medical College, Chennai, India.*

ARTICLE INFO

Received: 19 Sept 2023
Accepted: 20 Sept 2023
Published: 26 Sept 2023

Keywords:
Dhatryadi ghrita (DG); Metformin; Polycystic Ovarian Syndrome; hormone analysis; oxidative stress; hyperinsulinemia

DOI:
10.5281/zenodo.8378350

ABSTRACT

Objective: To investigate ameliorative effects of Dhatryadi ghrita on letrozole induced PCOD in female albino Wistar rats. Methods: Letrozole was given orally to rats every day for 21 days at a dose of 1 mg/kg body weight to induce PCOS. PCOS rats were then treated daily with metformin (70mg/Kg) and DG (200mg/Kg and 400mg/Kg) for 15 d. Rats which underwent none of these treatments were kept as control. Serum glucose, Lipid profile, insulin, estrogen, progesterone levels and superoxide dismutase levels were determined. Results: Rats treated with letrozole demonstrated a significant increase in body weight, serum triglycerides, cholesterol, LDL, glucose and insulin, and a significant decline in progesterone, Oestrogen, HDL and SOD levels compared to control. Rats treated with metformin, DG 200mg/Kg and DG 400mg/Kg showed a remarkable decrease in the elevated body weight, blood glucose, LDL, TC, TG and insulin levels and significant increase in the serum oestrogen, progesterone, SOD and HDL levels. Conclusion: Data indicate that DG exert potential ameliorative effects against PCOS through the modulation of hormonal and lipid profile as well as oxidative stress. These beneficial impacts of Dhatryadi ghrita make it a promising agent for the treatment and prevention of PCOS. Furthermore, these substances' beneficial effects are on par with those of metformin.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) also referred to us hyperandrogenic anovulation (HA) or Stein-Leventhal Syndrome, is one of the most common endocrine system disorders that affect 6-10% of women in their reproductive age [(1) (2)]. Since 1935, Stein and Leventhal have documented this disorder, which is characterized by an estimated 10 tiny cysts with a diameter between 2 and 9 mm develop on one or both ovaries and/or the ovarian volume in at least one ovary exceeds 10 ml [(3)].

As the name implies, ovarian dysfunction is a prominent feature of the syndrome. Due to irregular or missing ovulation, PCOS is the most

***Corresponding Author:** Sangeetha Venkatesan
Address: *Department of Pharmacology, Student of Pharmacy, Madras Medical College, Chennai, India*
Email ✉: sangeethasaran18@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frequent cause of infertility. PCOS is a frustrating condition for women, frequently challenging for clinicians managing patients, and a difficult scientific problem for researchers. According to the first community-based prevalence survey based on various diagnostic criteria, the prevalence of PCOS has increased and is now 18%. In this latest study, 70% of the women were undiagnosed, which is significant [4]. 90% to 95% of anovulatory women seeking infertility therapy have PCOS, which is the most prevalent cause of anovulatory infertility. Women could discover they have PCOS only after receiving therapy for infertility. PCOS is thought to be a complex disorder with a genetic component, approximately 20-40% of first -degree female relatives of women with PCOS go on develop PCOS, compared to an estimated 4-6% prevalence in the general population [5]. It is crucial to diagnose and treat PCOS. Clinically, PCOS may appear as a minor menstrual issue or as a serious disruption of the metabolic and reproductive systems. Hirsutism is present in the 70% of women with PCOS and is considered to be a good marker for hyperandrogenism but should be evaluated biochemically [6] [7]. Obesity exacerbates PCOS's reproductive and metabolic characteristics and promotes insulin resistance [8]. In addition, women with PCOS have higher levels of impaired glucose tolerance (IGT), DM2, and possibly CVD. The importance of PCOS for public health will grow as obesity rates rise. A crucial PCOS therapy technique that improves insulin resistance, reproductive health, and metabolic characteristics is the management of obesity through lifestyle intervention [9]. Moderate exercise (30 minutes per day), lowering psychological stressors, weight loss, calorie deficit diet of 500–1000 kcal per day, reducing caffeine intake, and low glycaemic index (GI) diet are all lifestyle changes for PCOS treatment in obese patients [10]. Current treatment for PCOS include metformin, Oral Contraceptive Pills (oestrogen progestin combinations), antiandrogens, flutamide, pioglitazone, spironolactone, cyclic progestins, and GnRH agonists. Infertility treatments like clomiphene citrate when used for longer duration causes adverse effects that limits their use. These therapies attempted to ameliorate hyperinsulinemia, hyperandrogenism, ovulatory function, and irregular menstruation in addition to increasing insulin sensitivity.

Metformin on other hand causes fatal and non-fatal lactic acidosis in 1-17/100,000 patients per year. OCPs are associated with weight gain, thromboembolism, CVS events abdominal pain, dysmenorrhoea. Antiandrogenic agents are hepatotoxic that could be very fatal [11][12].

To date, no single medication exists for PCOS, it tailors according to the symptoms. In PCOS positive subjects, alterations in the antioxidant profile have been observed [13]. Due to its connection to metabolic illnesses like cardiovascular disease, atherogenesis, diabetes mellitus, and obesity, oxidative stress its role in the etiology of PCOS cannot be ignored. [14]. It causes hyperplasia in ovarian mesenchyme which further contributes to pre-eclampsia, endometriosis, PCOS, lessened fertility and dysgenesis [15].

Currently, plant extracts are being widely used to treat female reproductive disorders. Dhatryadi ghrita which is a ghee based polyherbal formulation, contains the fruit Kusmanda which is rich in flavonoids and is found beneficial in bleeding disorders [16]. It also contains asparagus or shatavari which has fertility enhancing activity, the observed activity is due to shatavarin-L. This study proposes to evaluate the ameliorative effect of Dhatryadi ghrita on letrozole induced polycystic ovarian syndrome in female Wistar rats.

MATERIALS AND METHODS

Experimental animals

Healthy albino female Wistar rats (180 $\pm$ 20g) was procured from Animal Experimental Laboratory,

Madras Medical College, Chennai-03. The study is approved by Institutional Animal Ethical Committee which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), India. The approval No. is 1917/GO/ReBi/16/CPCSEA,20/09/2021.

Acute Toxicity Study [17]

Three healthy female adult Wistar albino rats weighing between 150-250gm were selected for the study. For all the three animals' food, but not water was withheld overnight prior to dosing. Being a traditional herbal formulation, the mortality was unlikely at the highest starting dose level (2000mg/kg). Hence a limit test one dose level of 2000mg/kg was conducted in all the three animals as per the OECD guidelines 423.

Experimental Study Design [18]

30 albino female Wistar rats were taken and were divided into 5 groups namely, Vehicle control, Disease Control, Standard Control and treatment groups consisting of DG 200mg/kg and DG400mg/kg respectively each group containing 6animals. All the animals except vehicle control group were administered with Letrozole 1mg/kg for 21 days. After 21 days group 3, 4 and 5 received Metformin (70mg/kg), Dhatryadi ghrita (200mg/kg) and Dhatryadi ghrita (400mg/kg) respectively for 15days. After completion of the experimental period (36 days), the rats were fasted overnight. On the 37st day the animals were sacrificed using isoflurane and blood was collected by cardiac puncture. The serum was separated by centrifugation at 20000 rpm for 10 minutes and was stored at -20^{o}C for biochemical and hormonal analysis.

The ovary was isolated and fixed in 10% formalin solution for the histopathological studies.

Table-1 Experimental Study Design

Group No.	Group Name	Treatment Schedule	No. Of animals
1.	Group I Vehicle control	Aqueous solution of carboxy methyl cellulose (CMC 0.5%) was given.	6
2.	Group II Disease control	Letrozole (1mg/Kg) was dissolved in CMC and was given orally for 21 days.	6
3.	Group III Standard control	Metformin (70mg/Kg) was given orally to letrozole induced PCOS rats from 22nd day to 36th day.	6
4.	Group IV Treatment control (DG 200mg/Kg)	Dhatryadi ghrita (200mg/Kg) was given orally to letrozole induced PCOS rats orally from 22nd day to 36thday.	6
5.	Group V Treatment control (DG 400mg/Kg)	Dhatryadi ghrita (400mg/Kg) was given orally to letrozole induced PCOS rats from 22nd day to 36th day	6

Evaluation Parameters [19] [20]

Physical Characteristics Assessment:

Changes in the body weight of the animals in all the groups were assessed throughout the study.

Estrous Cycle:

Estrous cycle consists of four stages, e.g., proestrus, estrous, metestrus, and diestrus. To determine these stages of the estrous cycle, the already-reported method was adopted. The proestrus stage consists of nucleated epithelial cells predominated with a small number of leukocytes. The estrus stage of the estrous cycle comprises cornified epithelial cells. Metestrus stages embraced with leukocytes, cornified epithelial cells, and nucleated cornified cells while the diestrus stage consists of mostly leukocytes with more mucus.

Biochemical Analysis:

Serum glucose, cholesterol, triglyceride and HDL-cholesterol levels was measured using commercially available kits.

Hormone Analysis:

Serum estrogen, progesterone and insulin levels were assessed using Enzyme-Linked Immunosorbent Assay-based kits.

Histopathological Studies:

Immediately after dissection, ovaries were fixed in 10% formalin. Specimens were embedded in paraffin block and sections of 4-5 µm thick was cut and stained with haematoxylin and eosin stain and visualized under light microscope.

Estimation of Antioxidant Enzymes:

Superoxide dismutase (SOD) activity in the ovarian homogenates was determined chemically using the method of Marklund and Markuland.

STATISTICAL ANALYSIS

The data will be presented as mean ± standard error of mean. Statistical significance and the difference among groups will be evaluated by analysis of the variance (ANOVA) followed by Dunnett test for multiple comparisons. Differences will be considered significant at $p<0.05$. The statistical analysis was carried out with Graph pad prism software 9.5.1.

RESULTS

Acute Toxicity Studies

Since there was no morbidity and mortality on observing the animals for 14days after the administration of Dhatryadi ghrita at the dose of 2000mg/Kg, 1/10th (200mg/kg) and 1/5th (400mg/kg) dose was selected for the low and high dose treatment groups respectively.

Body Weight Changes

The body weight of all animals was taken for every week throughout the study and is the weight of the animals in the disease control have shown a significant increase up to 36th day. The animals in the test and standard group have shown an initial increase followed by a reduction in body weight when compared to that of disease control.

Vaginal Exfoliative Cytology

The animals showing regular estrus cycle (4-5 days) were chosen for the study. After the animals were chosen, PCOS was induced by the administration of Letrozole 1mg/Kg body weight orally for 21 days. Interruption of estrus phase confirmed the induction of PCOD.

During treatment period, the reappearance of estrus phase and the regularity of estrus cycle was observed. Administration of Metformin restored the estrus cycle between 5-7 days. On treating with DG 200mg/Kg the estrus cycle restored between 7-9 days & in the dose of 400mg/Kg DG restored the estrus cycle between 6-8 days.

Biochemical Parameters

Table-2 Regularity and Duration of Estrus Cycle

Groups	Induction Period		Treatment Period	
	Regularity Of Cycle	Duration Of Cycle	Regularity Of Cycle	Duration Of Cycle
Normal control	Regular	4-5 days	Regular	4-6 days
Disease control	Irregular & estrus phase absent	-	Irregular & estrus phase absent	8-12 days
Standard control	Irregular & estrus phase absent	-	Regular	6-7 days
DG (200mg/Kg)	Irregular & estrus phase absent	-	Regular	6-7 days
DG (400mg/Kg)	Irregular & estrus phase absent	-	Regular	5-6 days

Serum Blood Glucose Level

The changes in the blood glucose levels in animals in all the groups were assessed and represented in the following table no. 3

Table-3 Serum Blood Glucose Level

Groups	Serum Blood Glucose Level (mg/dl)
Normal control	66.67 ± 0.88
Disease control	87.67 ± 0.88 ####
Standard control	71.02 ± 0.58 ***
DG (200mg/Kg)	68.66 ± 0.58 ****
DG (400mg/Kg)	67.33 ± 0.88 ****

All the Values are expressed as mean ± SEM (n=6)

Analysed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnet 't' test. #### P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control, ***P< 0.001 compared with disease control.

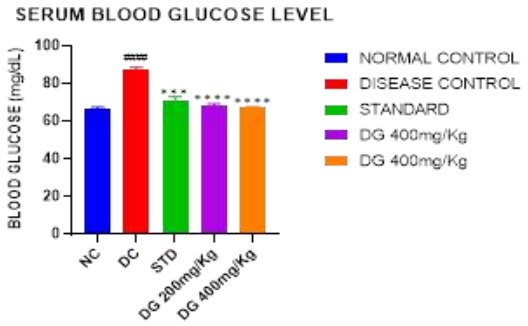


Figure-1 Graphical representation of serum blood glucose levels in various

It was seen from the above data the serum blood glucose level had increased significantly in disease control group, the standard and treatment groups have shown a decrease in the elevated blood glucose level when compared to the disease control group.

Lipid Profile

The changes in the lipid profile in animals in all the groups were assessed and represented in the following **table no.4**

Table-4 Lipid Profile

Groups	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control	36.33 ± 0.88	41± 1.86	16.00 ± 0.35	16.00 ± 0.58
Disease control	49 ± 0.58###	66 ± 1.73####	26.67 ± 0.73####	10.66 ± 0.67###
Standard control	48.33 ± 1.2**	42 ± 2.08****	17.67 ± 1.45****	15.00 ± 0.58***
DG (200mg/Kg)	37.33 ± 1.2**	43 ± 1.0****	17.00 ± 0.57****	14.67 ± 0.88***
DG (400mg/Kg)	37.33 ± 0.88***	41 ± 2.06****	17.00 ± 0.57****	15.67 ± 0.88***

All the Values are expressed as mean ± SEM (n=6)

Analyzed by One -way analysis of variance (ANOVA) followed by multiple comparison Dunnet 't' test.

TC: ###P < 0.0001 compared with normal control; ***P< 0.001 compared with disease control, **P< 0.01 compared with disease control.

TG: ####P < 0.0001 compared with normal control; ****P< 0.001 compared with disease control.

LDL: ####P < 0.0001 compared with normal control; ****P< 0.001 compared with disease control.

HDL: ###P < 0.0001 compared with normal control; ***P< 0.001 compared with disease control, **P< 0.01 compared disease control.

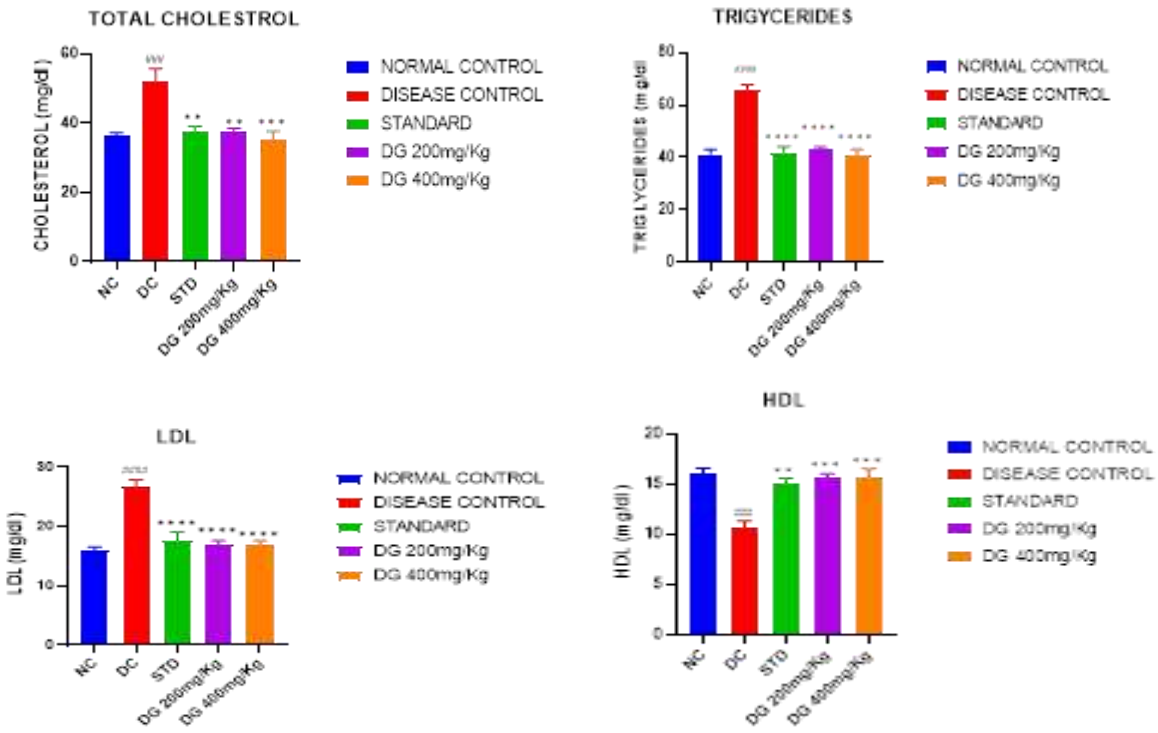


Figure-2 Graphical representation of TC, TG, LDL &HDL levels in various groups

It was seen from the above data that the Total cholesterol, Triglycerides, LDL levels had increased significantly in disease control while the HDL level was decreased when compared to that of normal group. The standard and treatment groups showed a significant decrease in the elevated levels of lipids and also showed a significant increase in HDL levels.

Hormone Analysis

The serum levels of oestrogen, progesterone and insulin in animals in all the groups were assessed and given in the **table no.5**

Table-5 Serum Hormone Analysis

Groups	Oestrogen (pg/ml)	Progesterone (ng/ml)	Insulin (uIU/ml)
Normal control	55.32 ± 2.89	45.33 ± 0.88	23.0 ± 1.43
Disease control	23.33 ± 0.89$^{\#\#\#\#}$	16.91 ± 0.50$^{\#\#\#\#}$	45.0 ± 0.08$^{\#\#\#\#}$
Standard control	53.22 ± 2.26****	43.22 ± 1.72****	22.0 ± 0.05****
DG (200mg/Kg)	51.55 ± 1.69****	42.22 ± 0.50****	21.0 ± 0.20****
DG (400mg/Kg)	53.00 ± 3.09****	44.15 ± 0.58****	23.0 ± 0.07****

All the Values are expressed as mean ± SEM (n=6)

Analyzed by Two-way analysis of variance (ANOVA) followed by multiple comparison Dunnet 't' test.

Oestrogen: $^{\#\#\#\#}$ P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control.

Progesterone: $^{\#\#\#\#}$ P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control.

Insulin: $^{\#\#\#\#}$ P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control.

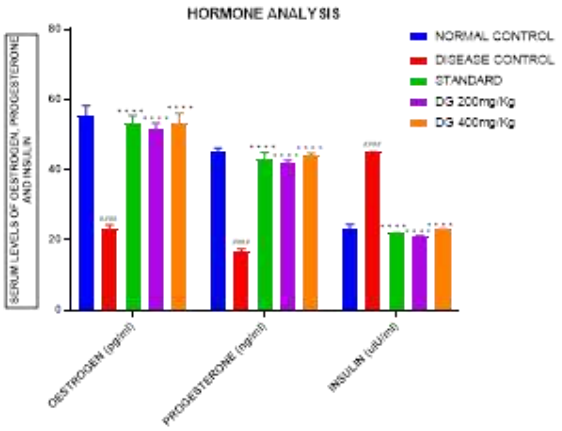


Figure-3 Graphical representation serum hormone level analysis

From the above graph the serum oestrogen and progesterone had decreased significantly while the serum insulin level had increased significantly in disease control groups. The standard, and treatment groups which were administered with Metformin(70mg/Kg), DG (200mg/Kg) and DG (400mg/Kg) respectively have shown a significant increase in serum oestrogen and progesterone levels and a significant decrease in elevated serum insulin levels when compared to that of disease control groups.

Serum SOD Level

The levels of serum SOD in animals of all the groups were assessed and represented in the following table no. 6

Table-6 Serum SOD Level

Groups	Serum Sod (pg/ml)
Normal control	444.33 ± 1.90
Disease control	251.00 ± 1.69$^{\#\#\#\#}$
Standard control	426.00 ± 2.49****
DG (200mg/Kg)	378.33 ± 0.72****
DG (400mg/Kg)	445.00 ± 2.35****

All the Values are expressed as mean ± SEM (n=6)

Analysed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnet 't' test. #### P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control.

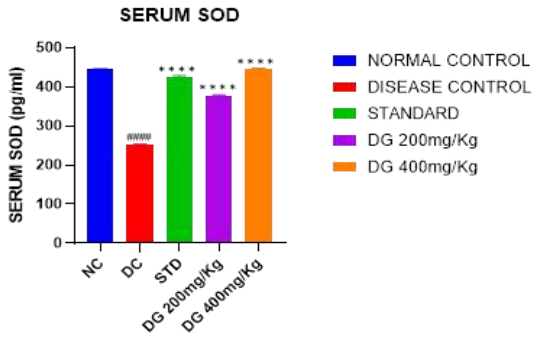


Figure-4 Graphical representation of serum SOD levels in various groups.

Changes in Ovary Weight and Uterus Weight

The changes in the weight of ovary and uterus were measured and were represented in the following **table no.7**

Table-7 Changes in Ovary and Uterus Weight

Groups	Ovary Weight (g)	Uterus Weight (g)
Normal control	0.37 ± 0.17	0.52 ± 0.02
Disease control	0.69 ± 0.03$^{\#\#}$	0.76 ± 0.02$^{\#}$
Standard control	0.35 ± 0.05**	0.49 ± 0.02**
DG (200mg/Kg)	0.36 ± 0.50**	0.50 ± 0.02*
DG (400mg/Kg)	0.36 ± 0.05**	0.50 ± 0.01*

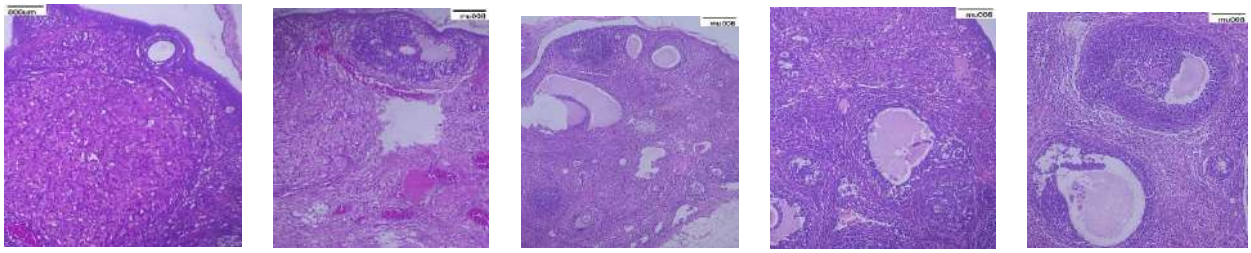
All the Values are expressed as mean ± SEM (n=6)

Analysed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnet 't' test.

Ovary weight: $^{\#\#}$ P < 0.01 compared with normal control; **P < 0.01 compared with disease control.

Uterus weight: $^{\#}$P < 0.05 compared with normal control; **P < 0.01 compared with disease control, *P < 0.05 compared with disease control.

Histopathological Examination of Ovary



A B C D E

Figure-5 Ovarian cross section obtained from Normal control (A), Disease control (B), Standard (C), DG 200mg/Kg (D) and DG 400mg/Kg (E).

Haematoxylin and eosin-stained sections of ovaries from control (**A**), PCOS (**B**), Metformin treated (**C**), DG (200mg/Kg) (**D**) and DG (400mg/Kg) (**E**). Normal histological structures could be seen in control rats, while absence of corpora lutea, few follicles and atretic follicles containing fluid filled antrum and higher incidence of pyknotic granulosa cells were seen in PCOS rats. Rats treated with metformin and DG at the dose of 200mg/Kg and 400mg/Kg showed presence of corpora lutea, absence of cysts, normal sized healthy follicles and decreased fluid filled antrum and incidence of pyknotic granulosa cells.

DISCUSSION

PCOD (polycystic ovary syndrome) is a common endocrine disorder, but its development mechanisms are not well understood. Treatment options for PCOD focus on addressing symptoms or the underlying cause. Medications like oral contraceptives, antiandrogens, GnRH agonists, and metformin are commonly used, but they have disadvantages and side effects. Tailoring treatment to individual patients can be challenging due to the complexity of PCOD. Recently, there has been a focus on natural medicines with minimal side effects. One such natural formulation is Dhatryadi ghrita, which contains eight herbal ingredients. It has been used in Siddha medicine for various conditions. Shatavari, one of the ingredients, has properties that stimulate blood cell production, increase the weight of sex glands, and promote folliculogenesis and ovulation. Dhatryadi ghrita was chosen to assess its effect on PCOD.

Phytochemical analysis revealed that Dhatryadi ghrita contains various compounds such as alkaloids, saponins, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, fats, and fixed oils. It also exhibited good antioxidant activity which was assessed by *in-vitro* methods such as DPPH radical scavenging assay and hydrogen peroxide radical scavenging assay.

To evaluate its effectiveness for PCOD, a rat model with letrozole-induced PCOD was used. Letrozole is a drug that disrupts hormone balance. The rats treated with letrozole showed PCOD-like symptoms, including irregular estrus cycles, increased ovary weight, and the presence of follicular cysts. The rats were divided into groups and treated with metformin (standard drug) and different doses of Dhatryadi ghrita. The study assessed various parameters, including body weight, estrus cycle, biochemical analysis (blood glucose and lipid profile), hormone analysis, serum superoxide dismutase (SOD) levels, ovary and uterus weight, and histopathological changes.

The results showed that Dhatryadi ghrita significantly reduced blood glucose levels and improved lipid profiles compared to the PCOD-induced group. It also increased estrogen and progesterone levels while decreasing insulin levels. Furthermore, it increased serum SOD levels, restored the estrus cycle, and reduced ovary and uterus weight. Histopathological analysis confirmed the beneficial effects, showing normal follicles, healthy corpora lutea, and the absence of cysts in the treated groups.

The positive outcomes of Dhatryadi ghrita on PCOD may be attributed to its diverse phytochemical constituents and potent antioxidant activity. Shatavari, one of the key ingredients, plays a role in improving reproductive function. Other components like saponins and flavonoids may also contribute to hormonal modulation and reproductive health.

Overall, Dhatryadi ghrita showed promising results in alleviating PCOD symptoms and improving hormonal and biochemical parameters. Its natural composition and minimal side effects make it an interesting option for PCOD treatment. However, further research is needed to validate these findings and understand the mechanisms behind its effects.

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to God for guiding and supporting me throughout the completion of my project. I owe my deep gratitude to my project guide for her gracious guidance and encouragement throughout my research work. I am immensely grateful to my parents, family and friends.

Author contributions: Concept – S.V., S.A.; Design – S.A., S.A.; Supervision – S.A.; Resources – S.V., S.A.; Materials – S.V.; Data Collection and/or Processing – S.V., S.A.; Analysis and/or Interpretation – S.A., S.V.; Literature Search – S.A.; Writing – S.V.; Critical Reviews – S.A.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

1. Samer El Hayek, Lynn H. Hamdar, Fadi G. Mirza and Georges Daoud.Polycystic Ovarian Syndome: An Updated Overview.Front.Physiol.7: 124. https://doi.org/10.3389/fphys.2016.0012.
2. Azziz, R., Woods, K. S., Reyna, R., Key, T. J., Knochenhauer, E. S., and Yildiz, B. O. (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population. J. Clin. Endocrinol. Metab. 89, 2745–2749. https://doi.org/10.1210/jc.2003-032046.
3. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ: The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010, 25:544-551. https://doi.org/10.1093/humrep/dep399.
4. H Teede, A Deeks and L Moran. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Medicine2010,8:41. http://www.biomedcentral.com/1741-7015/8/41.
5. Roe AH, Prochaska E, Smith M, Sammel M, Dokras A. Using the Androgen Excess-PCOS Society criteria to diagnose polycystic ovary syndrome and the risk of metabolic syndrome in adolescents. J Pediatr 2013;162:937–941. https://doi.org/10.1016/j.jpeds.2012.11.019.
6. Badawy A, Elnashar A. Treatment options for polycystic ovary syndrome. Int J Women's Health 2011;3:25–35. https://doi.org/10.2147/IJWH.S11304.
7. Fauser BCJM, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril 2012;97:28–38. https://doi.org/10.1016/j.fertnstert.2011.09.024.
8. Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, Jacobs HS: Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod 1995, 10:2107-2111. https://doi.org/10.1093/oxfordjournals.humrep.a136243.

9. Moran LJ, Pasquali R, Teede HJ, Hoeger KM, Norman RJ: Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. Fertil Steril 2009, 92:1966-1982. https://doi.org/10.1016/j.fertnstert.2008.09.018.
10. R. J. Norman, M. J. Davies, J. Lord, and L. J. Moran, "The role of lifestyle modification in polycystic ovary syndrome," Trends in Endocrinology and Metabolism, vol. 13, 2002. https://doi.org/10.1016/s1043-2760(02)00612-4.
11. Carrie C. Dennett and Judy Simon. The Role of Polycyctic Ovary Syndrome in Reproductive and Metabolic Health: Overview an Approaches for Treatment. https://doi.org/10.2337/diaspect.28.2.116.
12. C. M. J. Matyanga and B. Dzingirai, "Clinical pharmacology of hormonal emergency contraceptive pills," International Journal of Reproductive Medicine, vol. 2018. https://doi.org/10.1155/2018/2785839.
13. Verit FF, Erel O. Oxidative stress in nonobese women with polycystic ovary syndrome: correlations with endocrine and screening parameters. Gynecol Obstet Investig. 2008;65(4):233–9. https://doi.org/10.1159/000113046.
14. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91(3):S31–8. https://doi.org/10.1016/0002-9343(91)90281-2.
15. Goodarzi.M.O.etal. Polycycstic ovary syndrome: etiology, pathogenesis and diagnosis.NatRev Endocrinol 2011;7:219–2311. https://doi.org/nrendo2010.217.Epub2011.
16. Rashmi Saxena Pal, Amrita Mishra.A review on Dhatryadi ghrita.ijrap. https://doi.org/10.7897/2277-4343.082111.
17. OECD Acute Oral toxicity 423.,2001,[01]. https;//ntp.nieghs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
18. Amal H. Hamza, Widad M. AlBishri, Mona H. Alfaris.Effect of Vitex-castus plant extract on polycystic ovary syndrome complications in experimental rat model. apjr.2019;8(2): 63-69. http://doi.org/10.4103/2305-0500.254647.
19. Anam Younas, Liaqat Hussain, Arham Shabbir, Muhammad Asif, Musaddique Hussain, and Faiza Manzoor.Effects of Fagonia indica on letrozole-induced polycystic ovarian syndrome (PCOS) in young adult female rats. Hindawi.2022.1397060.13. https://doi.org/10.1155/2022/1397060.
20. Yasmine F. Ibrahim, Mohammed Alorabi, Walaa Yehia Abdelzaher, Nisreen DM Toni, Khaled Thabet, AbdelRahman Hegazy, Haitham Ahmed Bahaa, Gaber El-Saber Batiha, Nermeen N. Welson, Mohamed A. Myrosy, Katharigatta N. Venugopala, Asmaa Mohamed Abdel-Aziz.Diacerein ameliorates letrozole-induced polycystic ovarian syndromeinrats.Biomedicine&Pharmacotherapy.149(2022)112870. https://doi.org/10.1016/j.biopha.2022.112870.

HOW TO CITE: Sangeetha Venkatesan, Sakthi Abirami, Ameliorative effect of Dhatryadi Ghrita on Letrozole induced Polycystic Ovarian Syndrome in female albino Wistar rats, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 9, 417-426. https://doi.org/10.5281/zenodo.8378350