Evaluation of the Effect of Glutathione, an Antioxidant, with Hormonal, Metabolic and Inflammation Markers in DHEA-Induced PCOS Rat Model

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TÜBİTAK

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Introduction

NIH/ROTTERDAM/AE-PCOS

- Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in the reproductive age women.

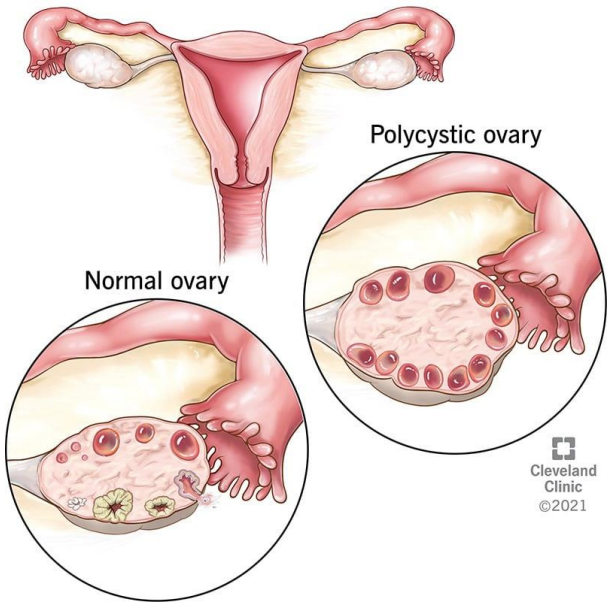

Table 1 Diagnostic Criteria for Polycystic Ovary Syndrome

Society	Criteria	
NIH Consensus 1990	1. Clinical and/or biochemical symptoms of hyperandrogenism 2. Oligo/amenorrhea, anovulation	Both criteria required
Rotterdam Consensus 2003	1. Clinical and/or biochemical symptoms of hyperandrogenism 2. Oligo/amenorrhea, anovulation 3. Polycystic ovaries on US	Two of three criteria required
AEPCOS Definition 2009	1. Clinical and/or biochemical symptoms of hyperandrogenism 2. Oligo/amenorrhea, anovulation 3. Polycystic ovaries on US	Two of three criteria required with identification of specific phenotype[a]

[a] *Four phenotypes have been identified and are influenced by genes, nutrients, physical activity, pollutants, psychological stress, and androgen excess.*

AEPCOS: Androgen Excess and PCOS Society; NIH: National Institutes of Health; US: ultrasound. Source: References 15-17.

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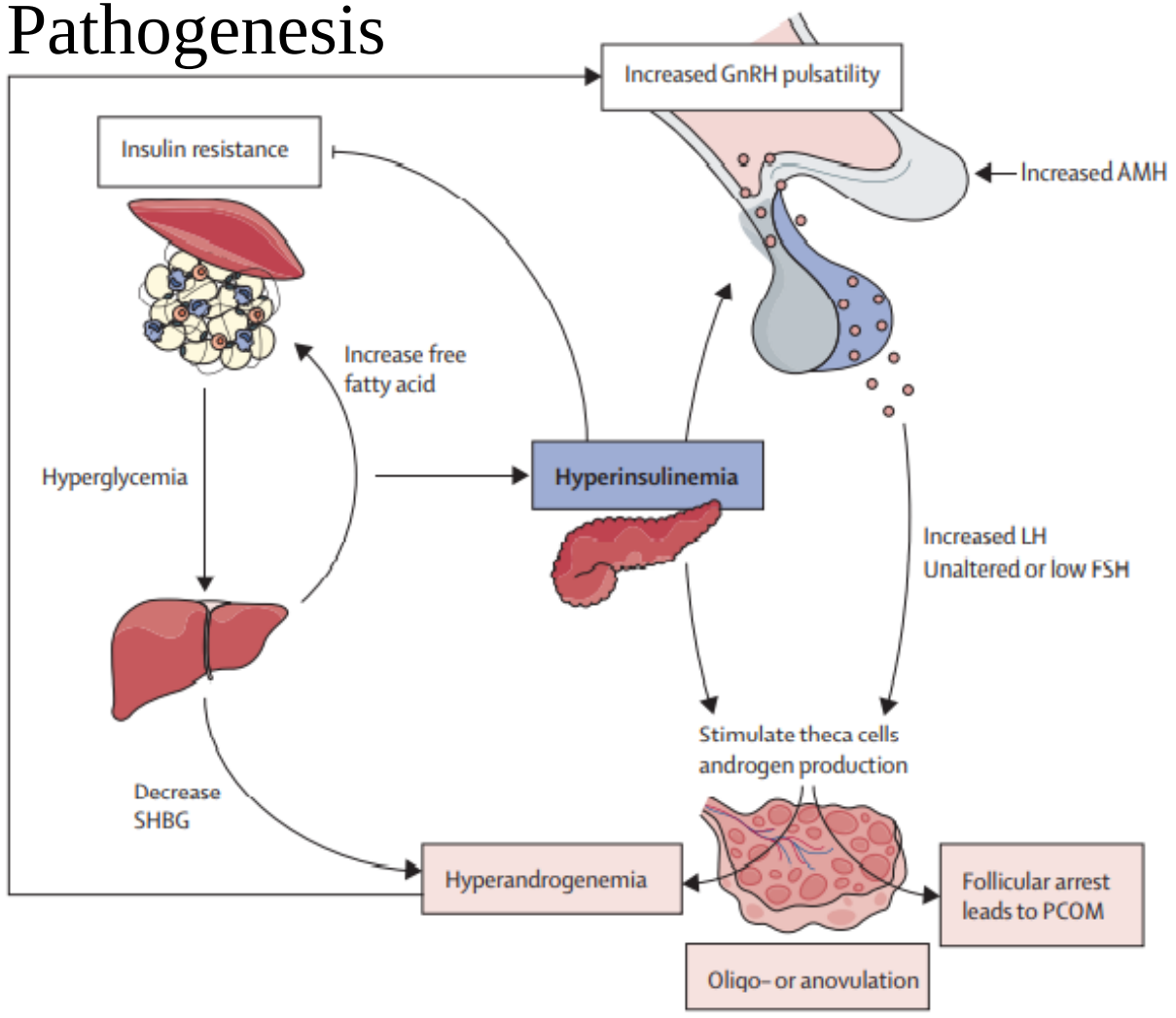
Introduction

Pathogenesis

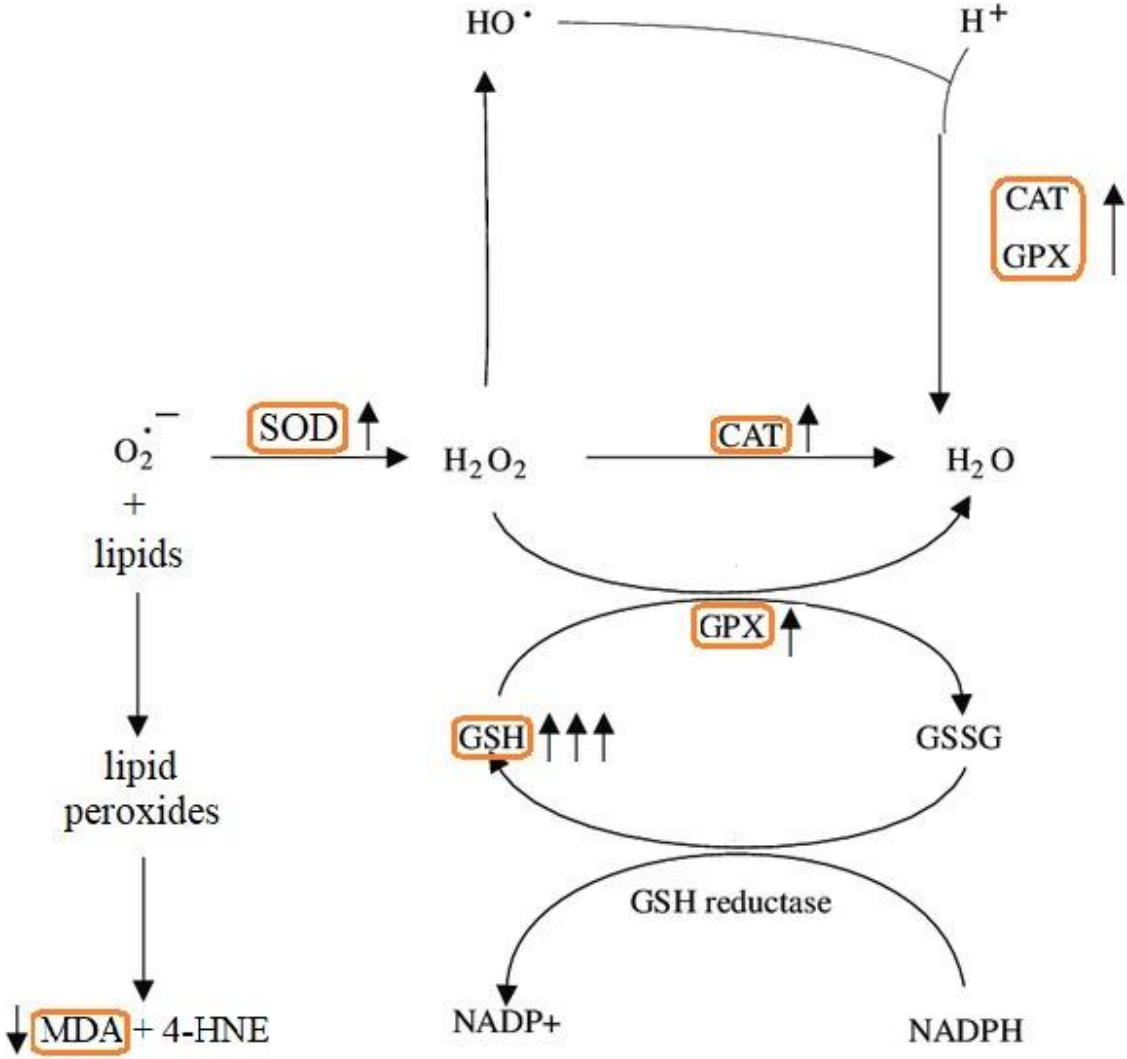


- Insulin resistance
- Obesity
- Metabolic syndrome
- Type 2 diabetes
- Dyslipidaemia
- Depression
- Anxiety
- Poor self-esteem
- Body image concerns
- Mental health disorders

- Menstrual irregularity
- Anovulation
- Hirsutism
- Infertility
- Pregnancy complications

Introduction

- The imbalance between oxidants and antioxidants, as well as the excessive generation of reactive oxygen species, is referred to as oxidative stress (ROS). According to recent research, oxidative stress may have a role in the development of PCOS via numerous mechanisms and may be a potential inducer of PCOS pathogenesis.
- Glutathione is an powerful antioxidant to handle oxidative stress by increasing metabolic detoxification. It is required for the control of disulfide bonds in proteins as well as the removal of electrophiles and oxidants.

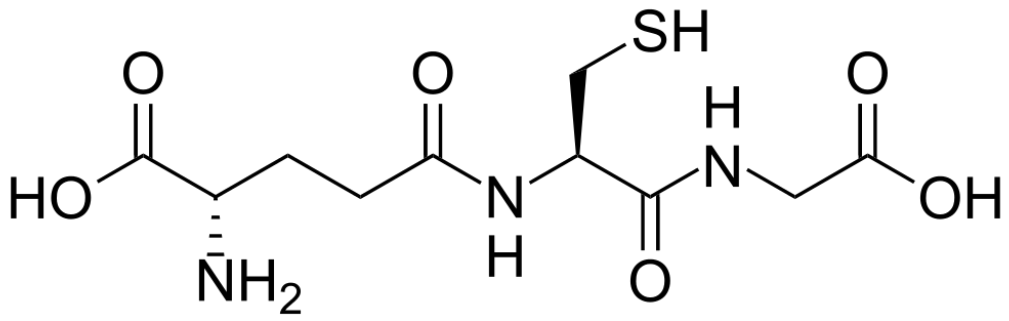


Introduction

- The combined oral contraceptive drugs (Diane-35), metformin and antiandrogens are the important widely used drugs in the clinic practice for the pharmacological treatment of PCOS to improve menstrual regulation and glucose metabolism with reducing insulin resistance and serum androgen levels. These are the pharmacological treatments that suggested by ESHRE/ASRM guidelines.

Aim

- The aim of our study is to investigate the possible positive effect of glutathione on the treatment of PCOS and also compare with Diane-35 and metformin.



Glutathione

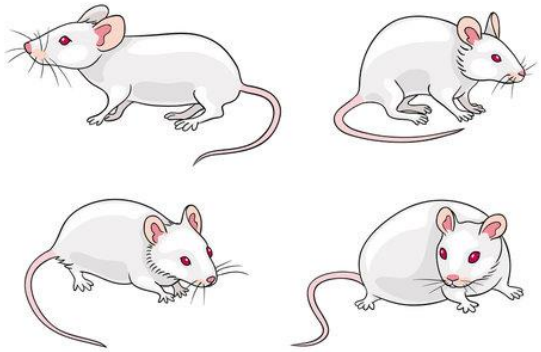
Ethical approval and Statistical power analysis

- This study was approved by the Bezmialem Vakıf University Experimental Animal Laboratory Ethics Committee. Researchers received a certificate from the animal experiments laboratory before starting the study.

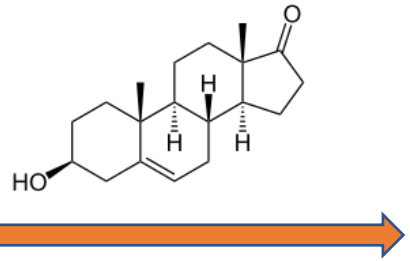
- A sample size and power calculation determined that sufficient statistical power required 5 rats for each group (power = %80, type 1 error = 0.05 and type 2 error = 0.20). Power calculation was based on serum MDA variable which was performed in a previous study (Furat Rencber et al., 2018)

Material And Method

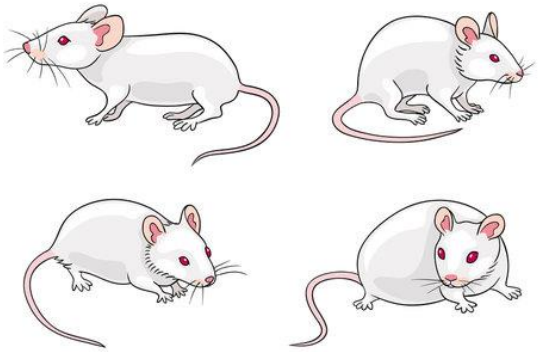
•20 female Wistar albino rats, 21 days old (weight 45-50 g), were randomly divided into 4 groups after generating PCOS model with DHEA 6 mg/100 g/day subcutaneously for 34 days.



20 female
Wistar albino rats

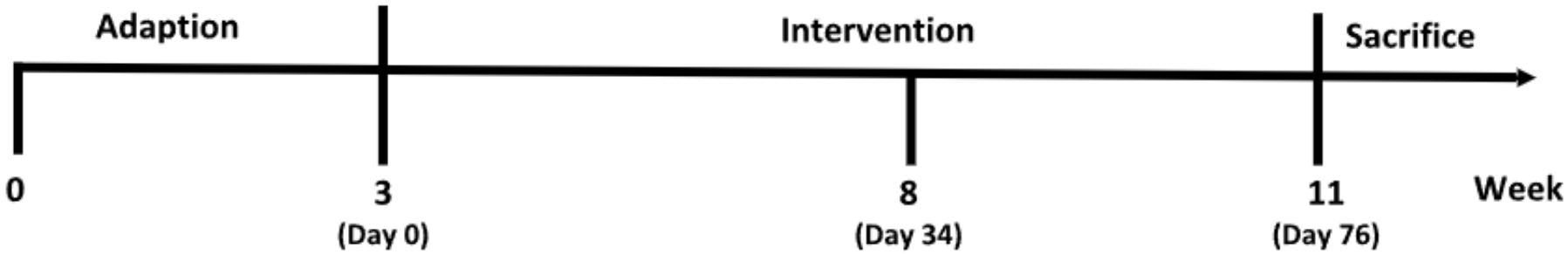


Subcutaneous
injection of DHEA



Rats with PCOS randomly
divided into 4 groups

Timeline of the study



PCOS	DHEA 6 mg/100 g/day sc.[a]	CMC %1 0,2ml/day o.[b]
Diane 35	DHEA 6 mg/100 g/day sc.	Diane 35 4,5 mg/kg/day o.
Metformin	DHEA 6 mg/100 g/day sc.	Metformin 300mg/kg/day
Glutathione	DHEA 6 mg/100 g/day sc.	Glutathione 100mg/kg ip.[c]

Abbreviation: PCOS, Polycystic ovary syndrome; DHEA, dehydroepiandrosterone; CMC, Carboxymethyl cellulose.

a: subcutaneous, b: oral, c: intraperitoneal.

Confirmation of PCOS was made by unilateral oophorectomy in two rats on day 35 and follicle evaluation in the ovaries and vaginal smear follow-up for 10 days in all rats showed that they lost their regular estrous cycles.

On day 56, rats were sacrificed by intracardiac blood sampling for evaluation of serum markers of inflammation (hs-CRP, IL-6), testosterone and insulin levels.

Results

Table 1: Serum IL-6, insulin, testosterone, hs-CRP, MDA and SHBG levels in PCOS, Diane 35, Metformin and Glutathione groups.

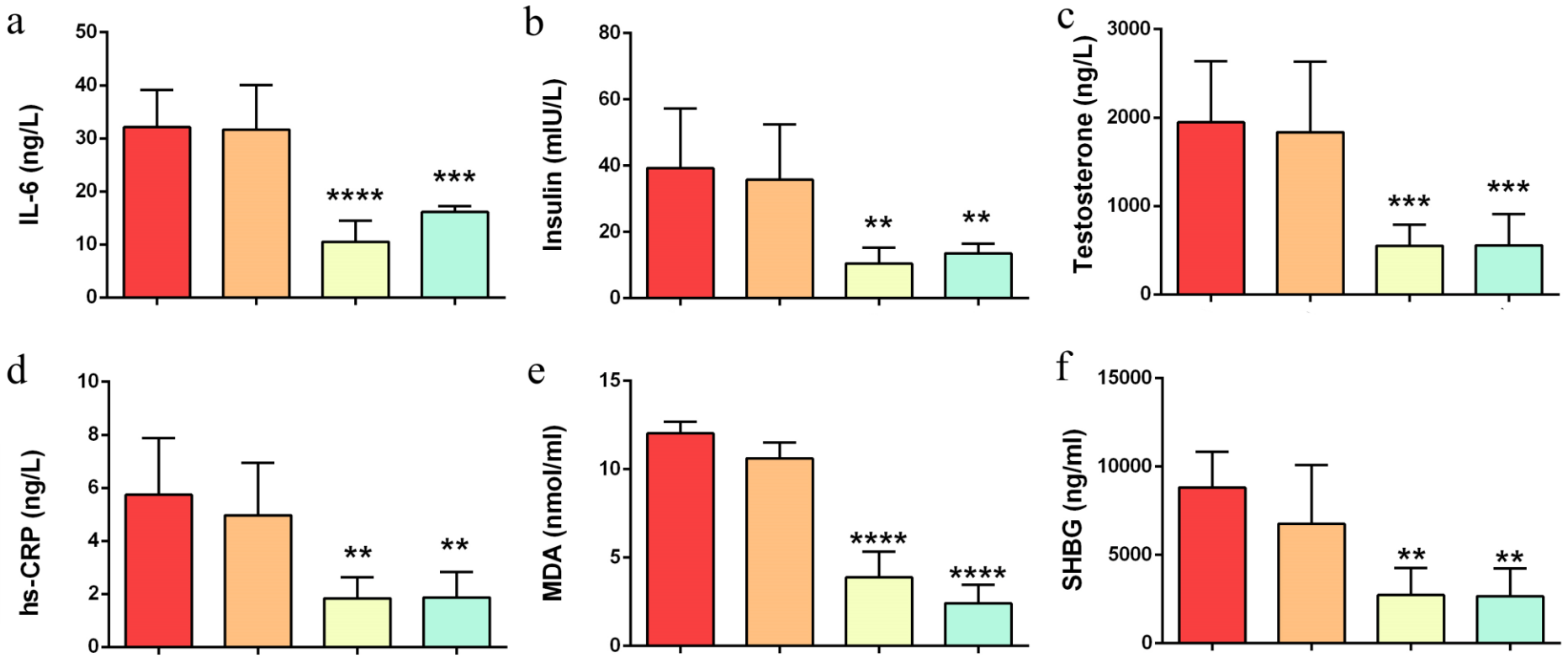
Variables	Group 1 PCOS (n=5)	Group 2 Diane 35 (n=5)	Group 3 Metformin (n=5)	Group 4 Glutathione (n=5)	p value
IL-6 (ng/L)	32.16±6.97	31.69±8.37	10.5±4.02**	16.13±1.13*	<0.0001
Insulin (mIU/L)	39.33±18	35.79±16.72	10.49±4.71*	13.59±2.84*	0.0002
Testosterone (ng/L)	1949±689.6	1837±798.6	551.9±236.8*	555.1±352.5*	<0.0001
hs-CRP (ng/L)	5.74±2.13	4.97±1.98	1.83±0.79*	1.86±0.97*	0.0002
MDA (nmol/mL)	12.03±0.66	10.62±0.89	3.88±1.45**	2.41±1.04**	<0.0001
SHBG (ng/mL)	8824±2023	6768±3321	2736±1538*	2664±1565*	0.0016

All values are expressed as mean±SD.
p <0.05 was considered statistically significant.
*,** Comparison with PCOS group; p<0.05 and p<0.001, respectively

Results

Fig. 2

a Serum concentrations of IL-6.
b Serum concentrations of insulin.
c Serum concentrations of testosterone.
d Serum concentrations of hs-CRP.
e Serum concentrations of MDA.
f Serum concentrations of SHBG.
** $p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, compared to DHEA (PCOS) group.

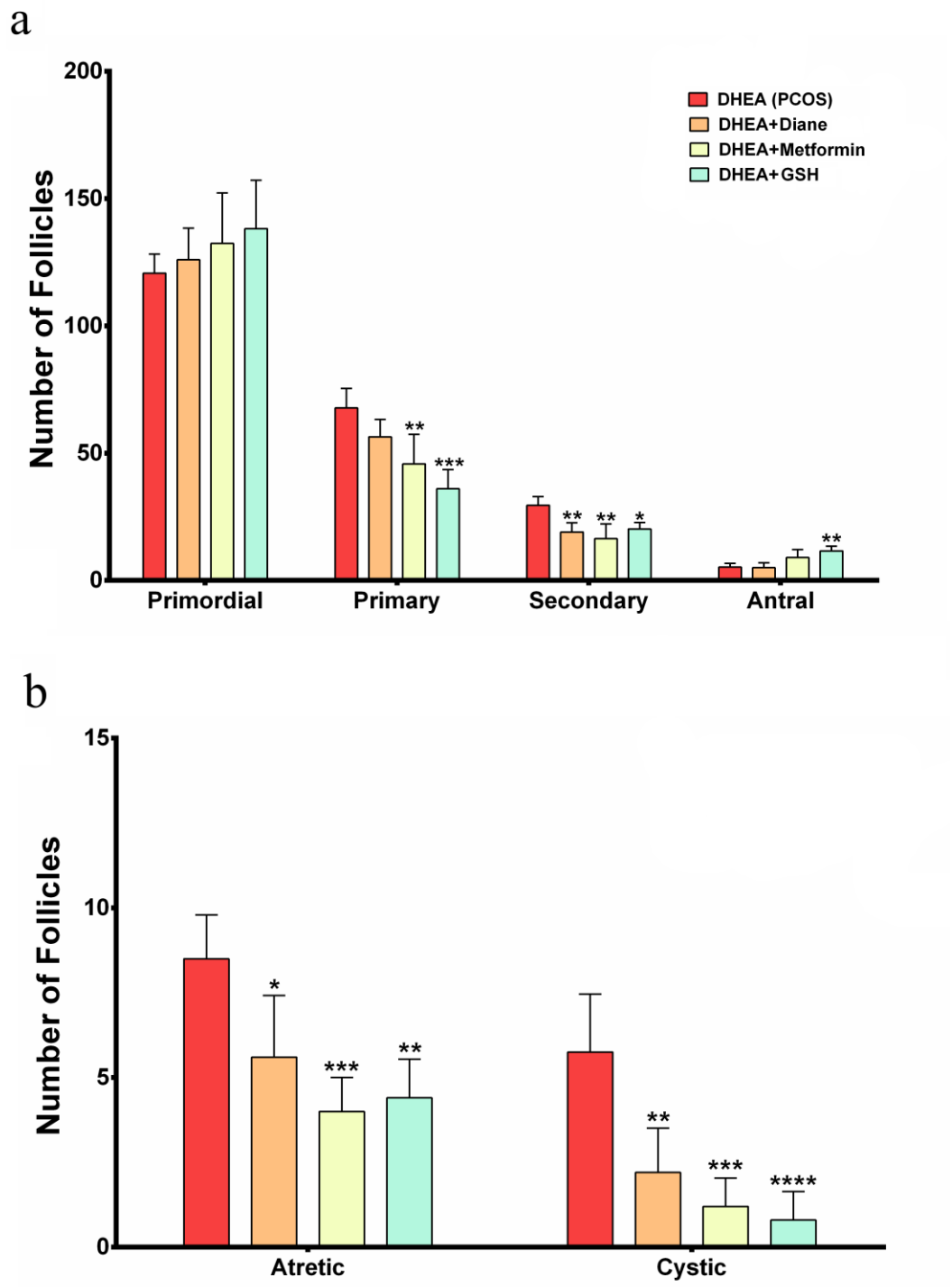


Results

Table 2: Comparison of the primordial, primary, secondary, antral, atretic and cystic follicle counts of all groups.

Variables	Group 1 PCOS (n=5)	Group 2 Diane 35 (n=5)	Group 3 Metformin (n=5)	Group 4 Glutathione (n=5)	p value
Primordial follicle counts	120.8±7.5	126±12.41	132.4±19.83	138.2±19.07	0.4
Primary follicle counts	67.75±7.63	56.4±6.87	45.8±11.61	36±7.51	0.0004*
Secondary follicle counts	29.5±3.41	19±3.67	16.4±5.77	20.2±2.58	0.0016*
Antral follicle counts	5.25±1.5	5±1.87	9±3.08	11.6±1.81	0.0007*
Atretic follicle counts	8.5±1.29	5.6±1.81	4±1	4.4±1.14	0.0008*
Cystic follicle counts	5.75±1.7	2.2±1.3	1.2±0.83	0.8±0.83	<0.0001*

Fig. 3
a Number of follicles.
Primordial, primary, secondary and antral follicle counts of ovaries in the all groups.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to DHEA (PCOS) group.
b Number of follicles.
Atretic and cystic follicle counts of ovaries in the all groups.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, compared to DHEA (PCOS) group.



Discussion

- Our study has limitation of the number of animals in the experimental and control groups is small, but this is a general limitation that we experience in animal studies. We hope that it will be instructive in terms of supporting it with clinical studies with higher sample sizes.

Conclusion

- Glutathione treatment led to the lower serum IL-6, insulin, SHBG, hs-CRP, testosterone, MDA levels in female Wistar albino rats who induced with DHEA for creating PCOS animal model. Moreover, the number of primary, secondary, atretic and cystic follicles was significantly lower in the metformin and glutathione groups, while the number of antral follicles was significantly higher in these groups.
- In conclusion, our study is the first to examine the antioxidative effect of glutathione administration on oxidative and inflammatory markers in PCOS. It will shed light on future clinical studies. If significant results are obtained in clinical studies, detailed analyzes with the dose and method of use will make important contributions to the subject.

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Thank you for coming today and thank you for your attention and consideration.

Do you have any questions?