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ORIGINAL ARTICLE

The Myokine Decorin improves Coagulation and Fibrinolytic Disturbances in Polycystic ovary Rat Model

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common metabolic and reproductive disorders affecting women during their reproductive period. PCOS patients have increased platelet aggregation and decreased fibrinolytic activity. Decorin (DCN) increases platelets activation and fibrinolytic activity. The aim of the study is to investigate the effect of DCN on hemostasis in polycystic ovary (PCO) rat model.

Methods: Thirty virgin adolescent female rats were divided in two groups: control group (I) and PCO induced group (II) (PCOS induced by single IM of estradiol valerate). The latter group was further subdivided into two equal subgroups: Subgroup IIa (PCO induced group). Subgroup IIb (DCN" 60 ug/kg" treated PCO group). In all groups, serum sex hormones, bleeding time (BT), whole blood clotting time (WBCT), prothrombin time (PT), activated partial thromboplastin time (aPTT), INR, D-dimer (DD), ovarian oxidative stress markers and steroidogenic acute regulatory protein (STAR), 3β-HSD, Cytochrome 19A1 **(**CYP 19A1), plasminogen activator inhibitor 1 (PAI-1) gene expression were estimated.

Results: There was a statistically significant prothrombotic state in the PCOS control group, as reflected by decreased bleeding, whole blood clotting times, PT, aPTT, INR and CYP 19A1 mRNA expression together with significant increases in D-dimers (DD, tissue PAI-1, STAR, 3β-HSD and PAI-1 mRNA), however, in DCN treated PCO group there was a significant increase in WBCT, PT, aPTT, INR, superoxide dismutase, CYP 19A1 mRNA expression, accompanied by a significant decrease in DD, clot retraction, and Malonaldehyde, STAR, 3β-HSD, PAI-1 gene expression in comparison to PCO control.

Conclusions: DCN can improve hemostatic profile in PCO rat model.

Keywords: Decorin (DCN), Polycystic ovary (PCO), hemostasis, D – dimer

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a quite common endocrine disorder that affects women of reproductive age **[1].** PCOS causes ovulatory dysfunction, menstrual irregularities, infertility, hyperandrogenism, insulin resistance, cardiovascular disease, and a considerable risk of type 2 diabetes mellitus **[2]**.

It is worth noting that women with PCOS have shown dysregulation of the hemostatic system with prothrombotic state, in the form of hypofibrinolysis, hypercoagulability **[3, 4],** endothelial and platelet dysfunction **[4, 5],** which cause cardiovascular, pulmonary, and renal complications **[2].**

Women with PCOS have increased fibrinogen and plasminogen activator inhibitor (PAI-1) activity

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which are associated with insulin resistance **[6]**. These disturbances are observed in the absence of hypertension, dyslipidemia, and low-grade inflammation **[7, 8].**

Decorin (DCN), an extracellular matrix protein that belongs to the small leucine-rich proteoglycan family, is widely distributed, and plays multifunctional roles in the stroma and epithelial cells. Originally, DCN was known as an effective collagen-binding partner for fibrillogenesis **[9].**

DCN affects cell differentiation, proliferation, growth, adhesion, spread and migration, and regulates inflammation and fibrillogenesis **[10]**. It acts as anti-carcinogen to many kinds of malignant tumors **[11]**.

Also, DCN expression was detected in theca cells, the corpus luteum, and follicular fluid **[12]**. In addition, it has important roles in folliculogenesis, ovulation, granulosa cell functions, and survival of corpus luteum **[13],** so it may have a role in PCO.

An effect of DCN has been shown on the thrombin inhibitor heparin cofactor II **[14],** and regulation of fibrin organization **[15],** platelets function **[16]**, insulin resistance **[17],** and PAI-1 **[18]**, Also, it can participate in hemostasis, thrombosis, and wound repair **[15].** This study was done to investigate the effects of myokine DCN on hemostatic parameters in a rat model of polycystic ovary (PCO).

METHODS

Experimental Animals

A total of thirty adolescent healthy virgin female western albino rats weighing 180-200 gm were procured from the animal house of the Faculty of Veterinary Medicine at Zagazig University.

The rats were housed in steel wire cages 19-inch length and 13-inch width (5 per cage). They were in an air-conditioned room with controlled lighting (12 hours light/12 hours dark cycle) and temperature (21-24°C), They received food and water ad libitum. To avoid the effect of different food elements on the experiments, all animals were fed the same type of food, which consisted of 25.8% protein, 62.8% carbohydrate, and 11.4% fat. **[19]**.

Ethical approval: All rats received care in accordance with the national health guidelines and the study protocol was approved by ZU-IACUC committee, approval number ZU-IACUC/3/F/370/2023 and ethics committee of faculty of medicine-Zagazig University and the study was performed in physiology department of the Zagazig University.

Drugs and chemicals:

- DCN injection: SGMA.ALDRICH, CA.3060
- Estradiol valerate injection: American regent Inc. Shirley NY 11967

Methods:

The rats were accommodated in laboratory conditions for two weeks before the experiment.

The rats were divided into:

Group I (control): (n=10): Rats were given 0.5 ml saline IM once. Then after 60 days, every other day for 21 days they were given 0.25 ml saline intraperitoneally (IP) once daily.

Group II (PCOS Induced group): (n=20): Rats were administered 4 mg/kg of estradiol valerate by single IM injection **[20],** 60 days later, after establishment of PCO model, the PCO induced group further subdivided into equal 2 subgroups (n=10 rats/group)

Subgroup IIa: Every other day for 21 days rats were given 0.25 ml saline IP once daily.

Subgroup IIb: Every other day and for 21 days, rats were given DCN 60 ug/kg IP once daily. **[21]**

Induction of PCOS:

The administration of estradiol valerate, a long-acting estrogen, interferes with hypothalamic gonadotrophin-releasing hormone release, preventing LH secretion and storage. **[22]**

A single dose of estradiol valerate in rats induces abnormal reproductive cycles, including anovulation and polycystic ovaries with a large number of atretic follicles and cysts. These ovarian alterations resemble those of PCOS in women. **[23]**

Determination of Sexual cycle:

Smears were obtained daily by vaginal washing using saline and fresh unstained samples were examined by microscope during the treatment period, cycles with duration of 4 to 5 days were considered regular **[24]**.

The four phases of the estrus cycle according to **Marcondes et al. [24]** and **Goldman et al. [25]**:

The proestrus phase: many live epithelia with smooth margins.

The estrus phase: large cornified cells with irregular margins.

The metestrus phase: many cornified cells and infiltration of leukocytes.

The diestrus phase: absence of cornified cells and presence of small leukocytes.

The presence of cornified cells in the smears during a minimum of ten consecutive days was expressed as persistent estrus, indicating

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development of follicular cysts. At the beginning of the experiments, all rats have regular cycles. **[26]**

Sampling of blood:

After the last injection at (day 82) of experiment and after 12h fasting, the blood samples were acquired from orbital sinus (sampling of controls taken during the estrus phase). A portion of the sample was put in the test tube specific for each of hemostatic parameter and the rest were allowed to clot for 2 hours at room temperature before being centrifuged for 15 minutes at 3000 rpm to determine sex steroids, LH, FSH, insulin hormones, and glucose levels. **[27]**

-Determination of serum hormonal profile

Serum estrogen, progesterone, LH, testosterone according to Tietz **[28]**, and serum FSH according to Rebar et al. **[29]**.

Determination of insulin resistance (IR) according to Matthews et al. **[30]**.

Determination of serum insulin level according to Temple et al. **[31]**.

Determination of blood glucose level according to Tietz **[28]**.

-Determination of bleeding time (BT) was estimated according to modified Martin method **[32]**.

-Determination of whole blood clotting time (WBCT) was estimated in line with method of Mayer **[33]**.

-Determination of clot retraction is expressed as amount of ml of serum extruded from the clot of 1 ml of blood after 45 minutes of sampling incubated at 37ºC **[34]**.

-Determination of prothrombin time (PT) was determined according to the method described by **Ansell [35]**, using Dade-Behring Kit.

-Determination of activated partial thromboplastin time (aPTT) was determined according to the method described by Ansell **[35]** using Dade-Behring K

- Determination of INR according to Declerck et al. **[36]**:

-Determination of D. Dimer: According to the method described by Declerck et al. **[36]**.

Tissue preparation for ovarian oxidative stress markers and Reverse transcriptase polymerase chain reaction (RT-PCR): The right ovary was excised and frozen in -80 for biochemical analysis and gene expression.

Oxidative stress/antioxidant evaluation:

Superoxide dismutase (SOD) activity and Malondialdehyde (MDA) level were measured spectrophotometrically in the ovarian homogenate using (Biodiagnostic kit) according to the instructions of the manufacturer.

Gene expression using RT-PCR:

The RNeasy Mini Kit (Qiagen) was used to isolate total RNA from tissue homogenate, following the instructions given by the manufacturer. The quality of total RNA was determined by the absorbance proportion (260/280 nm) which varied between 1.8 and 2.0 for all formulations. The QuantiTect Reverse Transcription Kit was used to generate cDNA, according to instructions from the manufacturer. The gene expression study was conducted using q RT-

PCR with 5 uL of cDNA and 10 pmol/uL of each primer listed in table (1), and 10 uL of SYBR Green 2x Master Mix Green (QuantiTect SYBR Green PCR Kits, Qiagen). Mx3005P (Stratagene, CA, USA) performed the RT-qPCR. Thermal cycling was conducted under the following conditions: Denatured at 95°C for 5 minutes, followed by forty cycles at 95°C for 15 seconds, annealing at 60°C for 30 seconds, followed by elongation at 72°C for 30 seconds. Data were normalized against β-actin transcript levels, and relative expression was calculated using the 2-ΔΔCt technique **[37].**

Statistical Analysis

Data was expressed as mean ± SD and statistically analyzed by One way ANOVA, by using SPSS program (IBM SPSS statistics 26). P value < 0.05 was considered statistically significant.

RESULTS

Table (2) represents the statistical analysis of bleeding time (Sec), whole blood clotting time (Sec), Prothrombin time PT (Sec), and activated partial thromboplastin time aPTT (Sec) among the studied groups. The mean value of BT was significantly lower in PCO group IIa when viewed alongside with that of control group I ($p < 0.05$) and non-significant change when compared to DCN treated PCO group IIb ($P > 0.05$). Also, there was a significant decrease in the mean value of BT ($p < 0.05$) in DCN treated PCO group IIb, when compared with control group I ($p < 0.05$). The mean value of the whole blood clotting was significantly lower in group IIa than DCN treated PCO group IIb ($p < 0.05$) and control group I ($p < 0.001$). Also, there was a significant decrease in the mean value of whole blood clotting time ($p < 0.05$) in group IIb when viewed alongside with that of control group I ($P < 0.01$). The mean value of PT was significantly decreased in group IIa when viewed alongside with that of group IIb (p

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<0.05), and control group I (p < 0.001). The mean PT value in group IIb decreased significantly compared to the control group I (p <0.05). The mean value of aPTT was significantly decreased in group IIa when compared with that of group IIb (p <0.05) and control group I (p <0.05). There was a non-significant change in the mean value of aPTT in group IIb when compared with group I.

Table (3) represents the statistical analysis of D-Dimer (ng/ml) level among the studied groups. The mean value of D-dimer was significantly increased in group IIa when compared with that of vit D treated PCO group IIc (p <0.05), group IIb (p <0.05), and control group (p <0.001). Also, there was a significant increase in the mean value of D-dimer in group IIb when compared with control group I (p <0.05), but no significant change (p >0.05) in comparison to group IIc. In addition, there was a significant increase (p <0.05) in the mean value of D-dimer in group IIc when compared with group I. Regarding the statistical analysis of INR among the studied groups (Table 3), the mean value of INR was significantly decreased in group IIa when compared with group IIb (0.8±0.11) and group I (both p <0.01). Also, there was a significant increase in the mean value of INR in group IIb when compared with control group I (p < 0.001). The statistical analysis of Clot retraction (ml) level was represented on table 3, showing that the mean value of clot retraction was significantly decreased in group IIb when that compared to group IIa (p <0.05, p <0.01 respectively). In addition, it was increased in group IIa as compared with group I (p <0.05), while no significant difference was noticed between group IIb, and between group IIc and group I. Moreover, no significant difference was detected between group IIb and I.

Table (4) represents the statistical analysis of serum LH levels (µIU/ml), FSH serum level (µIU/ml), Estradiol serum level (pg/ml), free testosterone serum (pg/ml) and Progesterone serum level (ng/ml) among the studied groups. The mean value of LH level was significantly increased in group IIa compared with group IIb (p <0.05), and control group I (p <0.001). Also, in group IIb, there was a non-significant change in the mean value of LH level when compared with group I. The mean value of FSH level was significantly decreased in group IIa compared with group IIb (p <0.05), and control group I (p <0.001). Also, there was a significant decrease (p <0.001) in group IIb compared with group I (p <0.05). The mean value of Estradiol level was significantly increased in group IIa as compared with group IIb (p <0.05) and control group I (p < 0.001). The group IIb showed a significant increase in estradiol levels compared to the control group I (p <0.05). The mean value of free testosterone level was significantly increased in group IIa when compared with group I, group IIb (p <0.001 for both). DCN treatment of group IIb resulted in a significant increase in free testosterone concentrations compared to control group I (p <0.001). The mean value of Progesterone level was significantly decreased in group IIa when compared with group IIb and control group I (p <0.001 for both). Also, there was a significant reduction in mean progesterone levels in group IIb compared to group I (p <0.05).

The statistical analysis of fasting serum glucose levels (mg/dL) and calculated HOMA-IR was represented on table (5) among the four studied groups. The mean value of fasting serum glucose level was significantly increased (p <0.001) in group IIa compared with group IIb, and control group I. The mean value of calculated HOMA-IR was significantly increased in group IIa when compared with that of group IIb (p <0.05) and control group I (p < 0.001). Moreover, there was a significant increase in the mean value of the calculated HOMA-IR in group IIb when compared with that group I (p <0.01). Table (5) represents the statistical analysis of serum insulin level among the studied groups. In group IIa, insulin levels were significantly elevated (p < 0.001) compared to control group I (9.43± 2.02) and group IIb. Moreover, there was a significant increase in mean value of insulin level in group IIb compared group I (p <0.05).

Table (6) shows a comparison of mean MDA and SOD values across different experimental groups. PCO group showed significant increase in the tissue level of MDA and significant decrease in the level of SOD tissue compared to control group (p < 0.05). On the hand, DCN treated group showed significant decrease in level of MDA and significant increase in SOD level compared to diseased group (p < 0.05).

PCO group showed statistically significant increase in steroidogenic acute regulatory protein (STAR) and 3β-HSD mRNA expression and significant decrease in CYP19A1 m RNA expression (p < 0.05 for both) compared to control group. Also, DCN treated group showed significant decrease in STAR and 3β-HSD mRNA expression and significant increase in CYP19A1 mRNA expression compared to diseased group (Table 1).

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Table (7) showed that PCO group revealed statistically significant increase in PAI-1 mRNA expression compared to control group. The DCN treated group showed a significant decrease in PAI-1 mRNA expression compared to the diseased group.

Table (1): Primer sequences of STAR, 3β-HSD, CYP 19A1, PAI-1 and β actin

Gene	Forward primer	reverse primer
STAR	**GCC TGA GCA AAG CGG TGT C**	**CTG GCG AAC TCT ATC TGG GTC TGT**
3β-HSD	**CCGCAAGTATCATGACAGA**	**CCGCAAGTATCATGACAGA**
CYP19A1	**CTG CTG ATC ATG GGC CTC CT**	**CTC CAC AGG CTC GGG TTG TT**
PAI-1	**GACACCCTCAGCATGTTCATC**	**AGGGTTGCACTAAACATGTCAG**
β actin	**TGACCGAGCGTGGCTACAG**	**GGGCAACATAGCACAGCTTCT**

STAR: steroidogenic acute regulatory protein, 3β-HSD: 3 beta-hydroxysteroid dehydrogenase, CYP 19A1: cytochrome 19A1, PAI-1: plasminogen activator inhibitor-1, β actin: beta actin

Table (2): The effect of Decorin administration on Bleeding time, whole blood clotting time, prothrombin time and activated partial thromboplastin time (Sec) among the studied groups.

	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
Bleeding time n=10			
Range	143-193	140-167	147-165
Mean	169.2	151.2	154.9
SD	17.2	9.02	17.7
F value		12.6	
P value		P<0.001	
P value of LSD Vs group I		P< 0.05	P< 0.05
P value of LSD Vs group II a			P> 0.05
Whole blood clotting n=10			
Range	195-235	149-186	199-221
Mean	221.4	167.5	206.5
SD	15.3	11.8	7.6
F value		49.4	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	P< 0.01
P value of LSD Vs group II a			P<0.05
Prothrombin time n=10			
Range	7.5-11.2	6.8-8.1	8.3-9.5
Mean	9.3	7.2	8.5
SD	1.3	0.47	0.97
F value		22.3	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	P<0.001
P value of LSD Vs group II a			P<0.05
Activated partial thromboplastin time n=10			
Range	16.9-32.4	13.5-19.8	19.6-25.8
Mean	24.2	17.1	23.3
SD	4.6	2.3	2.4
F value		4.3	
P value		P<0.01	
P value of LSD Vs group I		P<0.001	P>0.05
P value of LSD Vs group II a			P>0.05

P<0.05 is significant, P>0.05 is non-significant.

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Table (3): The effect of Decorin administration on D- Dimer (ng/ml), INR and Clot retraction (ml) among the studied groups.

	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
D-dimer n=10			
Range	150-220	198-220	180-210
Mean	170	206.6	189.2
SD	27.3	8.6	9.9
F value		9.4	
P value		P< 0.001	
P value of LSD Vs group I		P< 0.001	P< 0.05
P value of LSD Vs group II a			P< 0.05
INR n=10			
Range	0.75-1	0.6-0.71	0.7-0.9
Mean	0.95	0.62	0.8
SD	0.08	0.12	0.11
F value		8.99	
P value		P< 0.001	
P value of LSD Vs group I		P < 0.01	P< 0.001
P value of LSD Vs group II a			P< 0.001
Clot retraction n=10			
Range	0.2-0.5	0.5-0.6	0.3-0.5
Mean	0.37	0.54	0.42
SD	0.11	0.05	0.07
F value		12.3	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.05
P value of LSD Vs group II a			p >0.05

INR: international normalized ratio, P<0.05 is significant, P>0.05 is non-significant.

Table (4): The effect of Decorin administration on LH serum level(µIU/ml), FSH serum level (µIU/ml), estradiol serum level (pg/ml), free testosterone (pg/ml) serum level and progesterone serum level (ng/ml) of PCO rat model.

	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
LH level n=10			
Range	0.32-0.39	0.38-0.46	0.35-0.41
Mean	0.36	0.43	0.38
SD	0.02	0.02	0.01
F value		14.7	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.05
P value of LSD Vs group II a			p >0.05
FSH level n=10			
Range	2.48-2.97	0.96-1.5	2.09-2.31
Mean	2.6	1.26	2.18
SD	0.17	0.21	0.08
F value		150.8	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.001
P value of LSD Vs group II a			p >0.05
Estradiol level n=10			

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	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
Range	76-89	115-144	99-114
Mean	82.6	129.2	106.3
SD	4.6	10.2	6.1
F value		69.7	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.001
P value of LSD Vs group II a			p >0.05
Testosterone level n=10			
Range	40-60	300-400	150-250
Mean	50.00	340.5	190.62
SD	3.95	13.93	6.53
F value		24.7	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.001
P value of LSD Vs group II a			p >0.001
Progesterone level n=10			
Range	3.8-4.2	0.96-1.5	2.03-3.75
Mean	3.97	1.23	2.82
SD	0.13	0.19	0.71
F value		68.4	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.001
P value of LSD Vs group II a			p >0.05

LH: Luteinizing hormone, FSH: follicle stimulating hormone, P<0.05 is significant, P>0.05 is non-significant.

Table (5): The effect of Decorin administration on serum glucose levels (mg/dL), insulin levels (µIU/ml) and the calculated HOMA-IR of PCO rat model.

Blood glucose level n=10	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
Blood glucose level n=10			
Range	70 -85	100-120	85—95
Mean	76.10	111.70	89.90
SD	5.82	6.32	3.47
F value		89.49	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.001
P value of LSD Vs group II a			p >0.001
HOMA-IR n=10			
Range	1.36-2.47	4.35-6.76	2.15-3.45
Mean	1.85	5.50	2.70
SD	0.36	0.70	0.51
F value		83.87	
P value		P<0.001	
P value of LSD Vs group I		P<0.001	p >0.01
P value of LSD Vs group II a			p >0.001
Serum insulin n=10			
Range	6.57-12.41	17.4-22.5	8.82-14.50
Mean	9.43	19.62	11.99
SD	2.02	1.60	2.01

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Blood glucose level n=10	Group I Normal Control	Group IIa PCO	Group IIb DCN treated PCO
F value	48.88		
P value	p<0.001		
P value of LSD Vs group I		P<0.001	p >0.05
P value of LSD Vs group II a			p >0.001

P<0.05 is significant, P>0.05 is non-significant.

Table (6): Comparison among different experimental groups regarding STAR, 3β-HSD, CYP 19A1mRNA gene expression

Gene Expression	Group I (control group)	Group II (PCO group)	Group III (Decorin- treated PCO group)
STAR Mean ± SD	1.06 ± 0.06	2.86 ± 0.14	1.59 ± 0.08
3β-HSD *Mean ±SD*	1.08 ± 0.04	2.01 ± 0.14	1.64 ± 0.08
CYP19A1 Mean ±SD	1.07± 0.08	0.42 ± 0.04	0.74 ± 0.04
P value of F-test	P<0.001		
P value of LSD vs group I		P<0.001	P<0.001
P value of LSD vs group II			P<0.001

STAR: steroidogenic acute regulatory protein, 3β-HSD: 3 beta-hydroxysteroid dehydrogenase, CYP 19A1: cytochrome 19A1, PAI-1: plasminogen activator inhibitor, β actin: beta actin, P<0.05 is significant

Table (7): Comparison among different experimental groups regarding plasminogen activator inhibitor-1 mRNA gene expression

Gene Expression	Group I (control group)	Group II (PCO group)	Group III (Decorin- treated PCO group)
Plasminogen activator inhibitor-1 Mean ±SD	1.04 ± 0.08	2.6 ± 0.07	1.6 ± 0.08
P value of F-test	P<0.001		
P value of LSD vs group I		P<0.001	P<0.001
P value of LSD vs group II			P<0.001

P<0.05 is significant.

DISCUSSION

Decorin (DCN), is an extracellular matrix protein **[38].** It had a role in hemostatic control **[39].** Also, it may have a role in hemostasis in PCO. The present study showed a statistically significant prothrombotic state in the PCOS control group, as reflected by decreased bleeding, whole blood clotting times, PT, aPTT and INR together with significant increases in D-dimers (DD), plasminogen activator inhibitor 1, these results are in line with that of **Kebapcilar et al., 2009, Cignarelli et al., 2021 and Lehmann et al., 2021 [40, 41, 42].**

DD concentration expresses coagulation–fibrinolysis balance. Elevated DD concentration indicates increased coagulation activity in the presence of normal or elevated fibrinolytic activity. However, increased DD levels suggested that there is excessive stimulation of active fibrinolytic process when accompanied by prolongation of PT, aPTT **[40, 43].**

In addition, there was a significant increase in blood glucose, insulin resistance, testosterone, estrogen, and LH, with significant decrease in progesterone and FSH in comparison to normal control group.

Hypercoagulability is a symptom of hemostatic system disturbances in women with PCOS **[44].** In PCO, there was increase in the activity levels of Von-Willebrand factor (VWF), FVIII, and FX, and tissue plasminogen activator inhibitor-1 (PAI-1) concentration **[45].** These factors are released from the vascular endothelium, as insulin resistance which often accompany PCOS predisposes to endothelial injury which increases

the production of the endothelial clotting factors **[46]**.

In addition, studies have demonstrated elevated platelet volume in PCOS patients **[47, 40].** Moreover, a positive correlation between mean platelet volume and insulin resistance was observed by **Kebapcilar et al. [40].**

PAI-1 is an important endogenous fibrinolysis regulator **[41].** High testosterone levels, which are an independent risk factor for coronary atherosclerosis, have been linked to a considerable rise in PAI-1 in the PCO model **[40].** In women with PCOS, a link between hypofibrinolysis and hyperandrogenism has been proposed **[7].** likewise, transgenic female mice with a stable version of human PAI-1 have a PCOS-like phenotype with high testosterone levels and PCOS **[48].** Insulin resistance inhibits fibrinolysis by directly increasing PAI-1 production **[49],** and hence insulin resistance appears to be relevant for altered PAI-1 activity in PCO **[6].** In the present study, DCN decreased blood glucose level and insulin resistance. These results are supported by DCN knock out mice which had some improvement in insulin resistance and decreased blood glucose levels **[50, 17].**

In addition, DCN caused significant decrease in testosterone, and estrogen, and LH with significant increase in progesterone, and FSH in comparison to PCO control group, further studies are needed to clarify how DCN affects hormonal profile in PCO.

According to hemostatic parameters in group II b (DCN treated PCO group) there was a significant increase in WBCT, PT, aPTT, INR accompanied by a significant decrease in DD, PAI-1, and clot retraction, with non-significant change in bleeding time in comparison to PCO control group (IIa), DD level significantly increased in comparison to normal control group (I), However, increased DD levels suggested that there is excessive stimulation of active fibrinolytic process when accompanied by prolongation of PT, aPTT **[43, 51]**.

DCN ameliorated PCO hazardous effects on hemostatic parameters. This may be through multiple mechanisms, Dugan et al. **[15]** reported that DCN bound to the D regions of fibrinogen sterically modulates fibrin assembly that accelerated tissue-type plasminogen activator-dependent fibrinolysis.

It is worth saying that DCN in the present study decreased PAI-1, this may be through improvement of insulin resistance, decrease in testosterone (both insulin resistance and high testosterone increase PAI-1 secretion as previously discussed initially in discussion section).

Moreover**, Yamada-Nomoto et al. [52]** stated that PAI-1 is expressed in granulosa cells of ovaries, and it is stimulated by transforming growth factor B1(TGF-β1).

TGF-β1, is multifunctional cytokine that can regulate proliferation, differentiation, adhesion, migration, and apoptosis at the cellular level **[53]**.

Our results are supported by what stated by **Abdel-Wahab et al. [18]** that DCN suppresses plasminogen activator inhibitor-1 in human mesangial cells through inhibition of TGF-B 1 a mechanism that involves Ca2+-dependent phosphorylation of Smad 2/ Smad 3 signaling pathway.

In the present study, DCN caused non-significant change in bleeding time which gives an indication about platelets functions, on the contrary Guidetti et al. **[16]** found that platelets bind to DCN, causing tyrosine phosphorylation and activation. However, adding DCN did not cause aggregation. further studies are recommended to investigate the effect of DCN on platelets function.

Oxidative processes induce chemical changes (aldehydes, amino acid derivatives) causing molecular damage to cells and membranes leading to various consequences, such as loss of membrane integrity, impairment of cellular functions, activation of cell death pathways, and modulation of gene expression **[54].** Malondialdehyde (MDA) is a byproduct of lipid peroxidation and is often used as a biomarker to measure the level of oxidative stress in an organism, which is a condition where the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant systems **[55].** We found that there was a significant difference in MDA levels between the control and PCOS group. This was supported by finding a substantial difference in MDA levels in follicular fluid between the control and PCOS groups **[56].**

One of the enzymatic defensive mechanisms that reacts with reactive oxygen species is superoxide dismutase (SOD) **[57].** Upon comparing the PCOS group with the control group, it was observed that the SOD levels in the PCOS group were notably lower whereas the control group exhibited higher levels. The current findings are consistent with previous research **[58, 59, 60].**

DCN is found in a variety of connective tissues, where it acts as a key regulator in physiological processes **[61, 62],** as its expression has been connected to endometrial organization, where it declines from proliferative to secretory phase, and

it is also reduced in endometrial diseases such as PCOS patients**.**

This study found that DCN administration reduced oxidative stress in PCOS patients by decreasing serum MDA levels and increasing serum SOD levels. The levels of MDA and SOD were statistically significant different between the PCOS group and the group following DCN therapy ($p < 0.001$). After treatment by DCN the level of MDA decreased while the level of SOD increased. In line with our results, it was found that MDA level in ARPE-19 (retinal pigment epithelium) cells was significantly increased (-4.95-fold) upon H_2O_2 treatment, however this increase was significantly inhibited by DCN treatment **[63].** In addition, it was reported that administering DCN intraperitoneally prior to 60 minutes of ischemia reduced lipid peroxidation and raised SOD levels in the kidney **[64].** Furthermore, it was reported that DCN administration protected cultured rat cardiomyocytes from I/R-induced cell death **[65].** DCN possesses antioxidant characteristics that protect against traumatic brain injury **[66].**

In addition to the significant changes in metabolic indices, we conducted a comparison of the levels of steroidogenic enzymes, namely steroidogenic acute regulatory protein (STAR), aromatase (CYP19A1), and 3β-HSD, in granulosa cells obtained from both PCOS and normal rats. Through our investigation, we made an intriguing discovery. Expression of STAR and 3β-HSD mRNA was found to be upregulated in the polycystic ovaries, whereas CYP19A1 showed downregulation. This finding shed light on the potential molecular mechanisms underlying the pathogenesis of PCOS.

The aromatase gene, also known as the CYP9A1 gene, encodes an enzyme involved in the synthesis of estrogen, a crucial female sex hormone. Aromatase is the enzyme that converts androgens (male sex hormones like testosterone) into estrogens (female sex hormones like estradiol). Because PCOS is related with hormonal imbalances such as high androgen levels and impaired estrogen synthesis, the expression of the CYP19A1 gene is of special importance **[67].**

Women with PCOS are unable to keep their estrogen levels stable. Absence of aromatase is one cause of inadequate estradiol synthesis in PCOS follicles **[68].** CYP19A1 was our candidate gene because of its function in PCOS disease state.

When we compared the PCOS group to the control group, we found that CYP19A1 expression levels in the PCOS group are significantly lower whereas its level in the control group is higher as reported in various studies [**69, 70, 71, 72].**

It was reported that as compared to the control group, PCOS cumulus cells revealed significant up-regulation of the CYP19A1 gene ($p = 0.02$). They discovered tDNA genetic alterations such DNA hypomethylation, histone hyperacetylation, and hypomethylation can all contribute to chromatin remodeling, allowing the estrogen receptor easier access to DNA. Higher ER protein binding to promoter areas is expected to activate a harmful mechanism in PCOS patients by increasing CYP19A1 expression **[73].** No significant difference was found between the control and diseased groups **[74,75].**

Our findings indicated a significant difference between the PCOS group and the group that underwent DCN treatment, as evidenced by the significant heightened expression of the CYP19A1 gene ($p < 0.001$).

The DCN- treated PCO group showed higher expression of CYP19A1 expression (0.74 ± 0.04) than PCOS group (0.42 ± 0.04). The findings of the study are comparable with those of **Adam et al,** who reported that prominent levels of the extracellular matrix proteoglycan DCN are associated with testicular function suppression. They discovered that DCN becomes significantly expressed in infertile AROM+mice. As a result, our findings corroborate our hypothesis that elevated DCN levels are linked to reduced testicular function due to increased production of aromatase, which converts testosterone to estrogen **[13].**

STAR, or steroidogenic acute regulatory protein, performs the first step of steroidogenesis in the ovary and adrenal gland **[76].** Our findings demonstrated significant increase in ovarian STAR gene expression in PCOS group compared to the control group ($p < 0.001$). Among rats exhibiting EV-induced PCOS, aligning with the outcomes of our investigation **[77].** Also, an increase in STAR gene expression in the PCOS group was found **[78, 79].** However, decreased CYP19 mRNA expression in PCOS was found, but had no effect on STAR mRNA expression **[80].** Also, there was no significant difference in the relative concentration and distribution of STAR between the PCOS ovary and normal ovary **[76, 81, 82, 83].**

3β-HSD is a critical enzyme in the production of androgens as well as nearly all other physiologically active steroids **[84].** In our study we found that in rats with PCOS, there was

significant difference between Control and PCOS group ($p < 0.001$) where there was an increase in 3β-HSD in PCO group than control group. This identified an increase in ovarian and possibly adrenal androgens, which can contribute to the symptoms associated with PCOS. The particular expression and activity of 3β-HSD enzymes can affect androgen levels in the ovaries. It was discovered that 3β-HSD were enhanced in PCOS rats' granulosa cells **[85].** It has also been shown that the expression of 3β-HSD and STAR proteins, which participate in progesterone synthesis, is higher in PCOS people and animal models with polycystic ovaries **[86, 87].** Conversely, Doldi et al. found that PCOs had lowe r levels of the enzyme 3β-
HSD, which is crucial for progesterone biosynthes is. This suggests that the decreased ability of PCO luteinizing granulosa cells to synthesise prog esterone in vitro may be caused by lower levels of the 3β-HSD gene **[88].**

In our study we found there was statistically significant difference between PCO group and DCN treated PCO group as the level of STAR and 3β-HSD expression decreased in the treated group rather than in the PCOS group.

In the current study, we discovered that DCN, by decreasing steroidogenic enzyme activity, particularly STAR and 3β-HSD, and increasing aromatase activity, which is low in polycystic ovarian syndrome patients, could be useful in correcting cycle damage in these patients. However, it was demonstrated that the endometrium of PCOS women contains more small leucine-rich proteoglycans than controls; DCN and lumican **[61].**

Limitations of the study: This study could not study the direct effect of decorin on platelets aggregation, further investigations for detection of hemostatic state should be done.

Conclusions

Estradiol induced PCO rat model was associated with increased blood coagulability. DCN ameliorated PCO hazardous effect on hemostatic parameters, may be through improvement of hormonal profile and insulin resistance, decrease in PAI-1 or through direct anticoagulant effect.

Conflict of interest

The authors declared that they have no conflicts of interest with respect to the authorship and/ or publication of this article.

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