Section A-Research paper

Combined moderate exercise training and metformin treatment ameliorated rat thyroid dysfunction in polycystic ovary syndrome

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Abstract

Background: Asprosin an adipokine, is induced by fasting and targets the liver, promoting hepatic glucose release. Asprosin hormone level was found to be changed with insulin resistance. Moreover, there is an association between autoimmune thyroiditis and PCOS with insulin resistance. Metformin treatment and exercise training have been shown to be an effective treatment as insulin sensitizers.

Aim of the study: To study the changes in thyroid function and serum asprosin level in letrozole-induced PCOS in rats, and, to investigate the effect of moderate exercise training and metformin treatment on those changes.

Materials and Methods: 52 female adult albino rats were divided randomly and equally into 4 groups [control, polycystic ovary syndrome (PCOS), PCOS+ practiced exercise and PCOS+ practiced exercise+ Metformin treated]. In Control group, rats were given 1% aqueous solution of carboxy methyl cellulose (CMC) orally once daily for 11 weeks (study duration). In PCOS group, rats were administered letrozole at concentrations of 1 mg/kg BW dissolved in 1% CMC (1 ml/kg) orally once daily for 11 weeks. In PCOS+ practiced exercise group, letrozole was given as in PCOS group and the rats practiced moderate swimming training (5 days/week, for the last 8 weeks). In PCOS+ practiced exercise+ Metformin treated group, letrozole was given as in PCOS group and rats practiced exercise as in PCOS + exercise group and were given additionally metformin (50 mg/100 g BW in 0.05 ml of distilled water) orally once daily for the last 8 weeks. Serum was separated from collected blood at the end of experiment for measurement of asprosin, TSH, T3, T4, FSH, LH, estradiol, progesterone, testosterone, glucose, insulin, triglyceride, cholesterol, HDL, SOD, TNF-α and IL-6 levels. Ovarian and thyroid histopathological study were done.

Results: Moderate swimming exercise and metformin treatment resulted in a significant reduction in BMI and serum levels of asprosin, glucose, insulin, testosterone, LH, lipid profile and inflammatory cytokines, with a significant increase in serum levels of FSH, estradiol, progesterone and SOD in comparison to rats with PCOS. In addition to restoration of the thyroid functions by TSH- lowering effect in those rats to become nearly normal.

Conclusion: Letrozole-induced PCOS disturbs thyroid function and affects asprosin serum levels. Both moderate exercise training and metformin treatment have insulin sensitizing effects and thus, they could modulate disturbances in thyroid function, and in serum levels of asprosin, sex hormones, inflammatory cytokines and antioxidants.

Key words: Asprosin, Polycystic Ovary Syndrome, Thyroid Dysfunction

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality of reproductive-age women who suffer infertility, menstrual disturbances and hirsutism **(Goodarzi et al., 2011)**. PCOS is also associated with an adverse metabolic profile including obesity, insulin resistance, and dyslipidemia **(Lerner et al., 2019).** PCOS could be associated with dysregulation of adipogenic and glucogenic hormones **(Greenhill, 2016)**.

Asprosin, a white adipose tissue-derived glucogenic adipokine, is the C-terminal cleavage product of profibrillin (encoded by FBN1) **(Romere et al., 2016)**. Asprosin normally modulates hepatic glucose release through the G protein–cyclic adenosine monophosphate–protein kinase A pathway **(Romere et al., 2016)**. It can cross the blood-brain barrier and directly activate orexigenic agouti-related protein (AgRP)-positive neurons to inhibit downstream anorexigenic proopiomelanocortin (POMC)-positive neurons, leading to appetite stimulation **(Duerrschmid et al., 2017)**.

Asprosin level is also pathologically increased in humans and mice with insulin resistance which is typically defined as decreased sensitivity and responsiveness to insulin-mediated glucose disposal and inhibition of hepatic glucose production **(Duerrschmid et al., 2017)**. It is hypothesized that abnormal regulation of asprosin could be associated with some manifestation of PCOS **(Chang et al., 2019)**.

One of the common autoimmune disorders in women during childbearing period is the autoimmune thyroid diseases **(Artini et al., 2013)**. Previous studies have described that there is a connection between PCOS and thyroid disease as they are associated with insulin resistance and increase of BMI **(Romitti et al., 2018)**.

Moderate aerobic exercise enhances insulin sensitivity and blood glucose uptake. It also ameliorates lipid profile, inflammation, and adipokine production and secretion **(Yu et al., 2017**).

Metformin is a widely used insulin-sensitizing drug **(Huang et al., 2018)**. It improves PCOS's metabolic abnormalities by lowering androgen levels, improving the lipid profile, and reducing menstrual irregularities **(Macut et al., 2017)**.

Some studies addressed asprosin levels in PCOS, however, the results were controversial **(Chang et al., 2019; Rulin et al., 2020)**. Therefore, whether asprosin plays a role in PCOS has not been fully understood yet.

Hypothyroidism is associated with a broad spectrum of reproductive disorders ranging from abnormal sexual development through menstrual irregularities to infertility **(Hollowell et al., 2002)**. The hazard ratio for thyroid disease development was 2.5 times higher in patients with PCOS **(Glintborg et al., 2019)** and there is three-fold higher prevalence of AITD in patients with PCOS **(Janssen et al., 2004)**.

In women with PCOS, increased BMI can exacerbate the metabolic manifestations and insulin resistance **(Conway et al., 2014)**. Aerobic exercise promotes modest weight loss **(Swift et al., 2018)** and may affect the hormonal production of many endocrine glands **(Steinacker et al., 2005)**.

It has been demonstrated that metformin crosses the blood-brain barrier and has a central mechanism of TSH- lowering effect in hypothyroid patients with PCOS **(Rotondi et al., 2011; Hu et al., 2017)**.

Thus, this work aimed to investigate the effect of combined moderate exercise training and metformin treatment on rat thyroid dysfunction in polycystic ovary syndrome, and the possible mechanism involved.

Materials and Methods

A total number of 52 adult female Wistar albino rats weighing 160-180 g, were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University.

The animals were kept in steel cages (25 inches in length, 15 inches in width and 16 inches in height). Each group was housed per cage in the animal house of Physiology Department, Faculty of Medicine, Zagazig University under hygienic conditions.

All animals were housed under standard laboratory conditions with natural light/dark cycle at constant temperature (22 ± 2ºC) and. They were fed on a standard diet (25.8 % protein, 62.8 % carbohydrate and 11.4 % fat) **(Ahren and Scheurink, 1998)** which is obtained from nutrition department at Faculty of Agriculture with free access to water.

The experimental protocol was approved of by Physiology Department committee and by Zagazig University institutional animal care unit committee (ZU- IACUC; Sharkia; Egypt) with approval number: "ZU- IACUC/3/F/145/2020"

The experiment lasts for 11 weeks after acclimatization of the rats to the experimental conditions for 2 weeks.

Rats were divided randomly into 4 equal groups:

1) Control group (n=13): rats were given 1% aqueous solution of carboxy methyl cellulose [CMC] orally once daily for 11 weeks (along the study).

2) PCOS group (n=13): rats were administered letrozole (non-steroidal aromatase inhibitor) at concentrations of 1 mg/kg BW dissolved in 1% CMC (1 ml/kg) orally once daily for 11 weeks for PCOS rat model development **(Kafali et al., 2004)**.

3) PCOS + Exercise (PCOS + Ex) group (n=13): letrozole was given as in PCOS group and rats practiced swimming training (5 days/week, for 8 weeks) (starting from 4th week) in a swimming plastic barrel (80 cm long, 50 cm wide, and 90 cm deep) with gradually increase to a maximum of 60 min daily **(Hossein et al., 2019)**. Swimming exercise has been done individually for each rat in water maintained at 34–36°C **(Hart et al., 2010)**.

4) PCOS + Exercise + Metformin (PCOS + Ex + Met) group (n=13): letrozole was given as in PCOS group and rats practiced moderate swimming exercise (as in PCOS + Exercise group) and were treated with metformin (50 mg/100 g BW in 0.05 ml of distilled water given orally) once daily for 8 weeks (starting from 4th week) **(Elia et al., 2006)**.

• Determination of the estrous phases to assess successful induction of PCOS:

Vaginal smears of all females were taken daily at 10 a.m., collected with a plastic pipette filled with 10 uL of normal saline by inserting the tip into the rat vagina and evaluated under the light microscope without use of the condenser lens, with 10 and 40 x objective lenses. The mean frequency of diestrus, metestrus, proestrus and estrus **(Marcondes et al., 2002)** was compared between the groups and data were plotted in records for each labeled rat. Cycles with 4-5 days were considered regular **(Marcondes et al., 2002; Kafali et al., 2004)**.

At the end of the experiment, animals were put in closed plastic container to be weighed. The results were written in a record for each labeled rat **(Nascimento et al, 2008)**. Nose to anus length was measured by metal ruler graduated in centimeters **(Novelli et al., 2007)**. Then, calculation of BMI Index: by using the previous data: BMI equals body weight (g) / length^2 (cm^2) **(Novelli et al., 2007)**.

Rats' blood was collected from sinus orbitus vein of each rat, under ether anesthesia **(Yang et al., 2006)** at the end of the experimental period at 11 a.m. with animals overnight fasting. The blood was allowed to coagulate for 2 h at 4ºC and then centrifuged at 3000 rpm for 10 min. Serum was separated and stored at -20^{o}C until hormonal assessment.

Chemical analysis was done at Zagazig university, Faculty of Medicine, Biochemistry Department for estimation of serum level of asprosin, serum T3, T4, Thyroid Stimulating Hormone (TSH), Follicle-Stimulating Hormone (FSH), Luteinizing hormone (LH), estradiol, progesterone, testosterone, serum glucose, insulin, triglyceride, cholesterol, High Density Lipoprotein-Cholesterol **(**HDL), superoxide dismutase (SOD), serum Tumor Necrosis Factor- α (TNF-α), and Interleukin-6 (IL-6). Calculation of

HOMA-B= insulin (μU/mL) x glucose (mg/dl) /405 **(Sun et al., 2007)** and HOMA-IR=360 x fasting insulin (μU/mL) / (fasting glucose (mg/dL) - 63) **(Imano et al., 2014)**. LDL was calculated as follows: LDL=TC-HDL-TG/5 **(Friedewald et al., 1972)**.

• Histopathological Studies:

The rats were sacrificed by decapitation under ether anesthesia **(Yang et al., 2006)**. Thyroid gland was cut through at longest longitudinal dimension and fixed in 10% neutral formalin, serially sectioned at 4 μm, and stained with hematoxylin and eosin for histological evaluation.

Dissected ovaries were divided as follows: right ovaries were immediately frozen at -70 °C, whereas the left ovaries of each group were immediately fixed in 10% buffered formalin.

For light microscopy, fixed left ovaries were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. 5□ thick sections were mounted in slides previously treated with 3-aminopyropyltriethoxysilane and stained with hematoxylin and eosin **(Baravalle et al., 2007)**.

Commercial kits were used for asprosin (Bioassay Technology Laboratory, China; Catalog No. E1703Ra); estradiol, progesterone, cholesterol, triglycerides and HDL (Shanghai Sunred biological technology, China; Catalog No. 2011-11-0175, 2011-11-0742, 2011-11-0198, 2011-11-0250 and 2011-11-0255, respectively); FSH, LH and Testosterone (BioCheck, Inc 323 Vintage Park Dr. Foster City (Catalog No. BC-1029, BC-1031 and BC-1115, respectively); glucose (Biotechnology, Egypt); insulin (Sigma-Aldrich Chemie GmbH, USA; Catalog No. RAB0904); SOD (Egyptian Company for Biotechnology, Obour city, Cairo, Egypt); TNF-α (BioSource International Inc., California, USA; Catalog No. KRC3011); and IL-6, T3, T4 and TSH (Technical MSN service online, email:sunedrbio@mes.cn; Catalog No. 201-11-0136, 201-11-0535, 201-11-0338 and 201-11-0181, respectively).

Statistical Analysis: The obtained data were presented as mean ± standard deviation (SD). IBM SPSS Statistics Software (Version 25 for Windows) was used to perform one-way analysis of variance (ANOVA), Tukey HSD for post hoc multiple comparisons to compare means and correlation test between serum levels of asprosin and other parameters. With P value ≤ 0.05, significance was considered.

Results

A- BMI and Serum asprosin changes: (Table-1)

There was a significant increase in BMI between PCOS, PCOS +Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant decrease in BMI between PCOS + Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$).

There was a significant increase in asprosin between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant decrease in asprosin between PCOS + Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). Also, there was a significant decrease in asprosin between PCOS + Ex + Met group in comparison to PCOS+ Ex group ($p<0.05$).

B- Serum thyroid hormones changes: (Table-1)

There was a significant decrease in T3 and T4 between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant increase in T3 and T4 between PCOS + Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). There was a significant increase in T3 and T4 between PCOS + Ex + Met group in comparison to PCOS+ Ex group ($p<0.05$).

There was a significant increase in TSH between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant decrease in TSH between PCOS + Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). There was a significant decrease in TSH between and PCOS + Ex + Met groups in comparison to PCOS +Ex group ($p<0.05$).

C- Serum sex hormones changes: (Table-1)

There was a significant decrease in FSH, estradiol and progesterone between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant increase in FSH, estradiol and progesterone between PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). Also, PCOS + Ex + Met group showed a significant increase in FSH, estradiol and progesterone in comparison to PCOS + Ex group.

There was a significant increase in LH between PCOS group in comparison to control group ($P<0.05$). There was a significant increase in testosterone between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant decrease in LH and testosterone between PCOS+ Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). PCOS + Ex + Met group showed a significant decrease in testosterone in comparison to PCOS + Ex group ($p<0.05$).

D- Serum glucose and insulin changes: (Table-1)

There was a significant increase in glucose between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). There was a significant decrease in glucose between PCOS + Ex + Met group in comparison to PCOS group ($p<0.05$).

There was a significant increase in insulin and HOMA-IR between PCOS, and PCOS + Ex groups in comparison to control group ($p<0.05$). There was a significant decrease in insulin and HOMA-IR between PCOS + Ex and PCOS + Ex + Met groups in comparison to PCOS group ($p<0.05$). PCOS + Ex + Met group showed a significant decrease in glucose, insulin and HOMA-IR in comparison to PCOS+ Ex group ($p<0.05$).

There was a significant decrease in HOMA-B between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$).

E- Serum lipid profile changes: (Table-1)

There was a significant increase in cholesterol and LDL between PCOS and PCOS + Ex groups in comparison to control group ($p<0.05$). There was a significant increase in triglyceride between PCOS group in comparison to control group ($p<0.05$). PCOS + Ex + Met group showed a significant decrease in cholesterol, triglyceride and LDL in comparison to PCOS group ($p<0.05$).

There was a significant decrease in HDL between PCOS group in comparison to control group ($p<0.05$).

F- Serum inflammatory and oxidative stress markers changes: (Table-1)

There was a significant increase in TNF-α between PCOS and PCOS+ Ex groups in comparison to control group $p<0.05$). PCOS + Ex and PCOS + Ex + Met groups showed a significant decrease in TNF-α in comparison to PCOS group ($p<0.05$).

There was a significant increase in IL-6 between PCOS, PCOS + Ex and PCOS + Ex + Met groups in comparison to control group ($p<0.05$). PCOS + Ex + Met group showed a significant decrease in IL-6 in comparison to PCOS group ($p<0.05$). PCOS + Ex + Met group showed a significant decrease in IL-6 in comparison to PCOS + Ex group ($p<0.05$).

There was a significant decrease in SOD between PCOS group in comparison to control group ($p<0.05$). PCOS +Ex and PCOS +Ex+ Met groups showed a significant increase in SOD in comparison to PCOS group ($p<0.05$).

G- Correlations between all studied parameters and serum asprosin in studied groups:

There was a significant ($P < 0.05$) positive correlation between serum asprosin and BMI in PCOS group (Fig. 1).

There was a significant ($P < 0.05$) positive correlation between serum asprosin and serum LH in PCOS + Exercise group (Fig.2).

There was a significant ($P < 0.05$) negative correlation between serum asprosin and serum estradiol in PCOS + Exercise group+Metformin (Fig.3).

H- Histopathological study:

A- Ovary:

Control group showed normal follicles at different stages with a single cystic follicle (CF) in the field. Insets with higher magnifications show; primary follicle (PF) with multiple layers of follicular cells, antral follicle (AF), oocytes(O) and corpus luteum (CL) (Fig. 4a).

PCOS group showed excessive number of cystic follicles (CF) with primordial follicle (F) in the field, some are filled with eosinophilic material. There is a lack in the presence of the variety of stages of follicles and their stroma (S) show congested vessels (C) (Fig. 4b).

PCOS+ Exercise group shows multiple cystic follicles (CF) filled with eosinophilic material (EF) and an area of fatty infiltration (D) (Fig. 4c).

PCOS+ Exercise + Metformin group show multiple cystic follicles (CF) of variable sizes filled with eosinophilic material (EF) (Fig. 4d).

B- Thyroid:

Control group shows normal follicles of different sizes lined with a single layer of cuboidal follicular cells (arrow) and scattered parafollicular cells in between and in the interstitium (arrowhead) (Fig. 5a).

PCOS group show follicles surrounded with multilayer of follicular cells (M). Follicular cells' lining is flattened in wide areas (thin arrow). Exfoliated cells are numerous (zigzag arrow) with lost continuity of basement membrane (B) (Fig. 5b).

PCOS+ Exercise group show follicles surrounded with irregular follicular cells (**I**) in multiple layers. Exfoliated cells are numerous (zigzag arrow) with lost continuity of basement membrane (B) (Fig. 4c).

PCOS+ Exercise + Metformin group show some follicles with flattened follicular cells lining (thin arrow) alternating with areas of multilayers (M). (D) colloid eosinophilic material with lost continuity of basement membrane (B) (Fig. 4d).

I- Vaginal smear:

1) Control group: Three types of cells recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. We used the proportion among them for the determination of the estrous cycle phases as follow:

1-The proestrus phase: the vaginal smear consists of a predominance of nucleated epithelial cells with smooth margins.

2-The estrus phase: the vaginal smear shows large a nucleated cornified (keratinized) cells with irregular margins.

3-The metestrus phase: the vaginal smear shows many cornified cells plus infiltration of leukocytes.

4-The diestrus phase: the vaginal smear shows absence of the cornified cells and presence of small leukocytes.

2) PCOS group: the development of follicular cysts and the successful induction of PCOS were both indicated by the presence of extended cornified cells in the smears for two successive estrous cycles (estrus and metestrus).

3) PCOS +Exercise group : vaginal smear showed return of the cyclicity in treated rats with prolongations of the estrous cycle.

4) PCOS +Exercise+ Metformin group : vaginal smear showed normal estrous cycles after exercise training and metformin treatment

Table (1): Biochemical changes in the different studied groups (13rats/group).

Parameter	Control	PCOS	PCOS + Ex	PCOS + Ex+ Met
BMI (g/cm^2)	.414± .03	.522± .04 a	.477± .03 a,b	.453± .04 a,b
Serum Asprosin (pg/ml)	52.946± 8.55	367.692± 34.92 a	214.538± 22.68 a,b	144.769± 18.21 a,b,c
SerumT3 (ng/ml)	5.035± .95	.938± .10 a	1.876± .40 a,b	3.599± .48 a,b,c
SerumT4 (ug/dl)	12.176± 2.10	3.306± .60 a	5.483± .74 a,b	11.136± 1.60 a,b,c
Serum TSH (m IU/ml)	.766± .11	9.612± .51 a	6.316± .82 a,b	2.196± .67 a,b,c
Serum FSH (m IU/ml)	±2.546 .86	±.852 .08 a	1.217± .33 a	2.081± .71 a,b,c
Serum LH (m IU/ml)	±3.858 .99	±5.664 1.22 a	4.437± 1.08 b	3.823± .88 b
Serum Estradiol (Pg/ml)	474.846± 52.69	138.969± 12.68 a	144.461± 12.79 a	249.077± 31.03 a,b,c
Serum Progesterone (ng/ml)	15.263± 2.95	3.197± .67 a	3.881± .85 a	5.411± 1.09 a,b,c
Serum Testosterone (ng/ml)	.473± .11	1.019± .07 a	.780± .07 a,b	.591± .06 a,b,c
Serum Glucose (mg/dl)	96.507± 9.78	159.453± 15.98 a	147.423± 22.98 a	127.553± 18.25 a,b,c
Serum Insulin (ng/ml)	5.655± 1.38	9.597± 1.73 a	8.043± 2.05 a,b	6.026± 1.67 b,c
HOMA-IR	1.352± .36	3.775± .74 a	2.901± .75 a,b	1.901± .57 a,b,c
HOMA-B	65.383± 24.91	36.745± 8.80 a	38.289± 21.01 a	35.76± 12.20 a
Serum Total cholesterol (mg/dl)	135.677± 15.24	162.292± 18.82 a	157.469± 16.37 a	147.923± 14.86 b
Serum Triglyceride (mg/dl)	92.775± 14.76	111.833± 22.59 a	105.478± 11.76	98.310± 19.54 b
Serum HDL (mg/dl)	54.701± 5.76	50.493± 4.45 a	51.260± 4.49	53.781± 5.12
Serum LDL (mg/dl)	62.420± 13.99	89.431± 16.84 a	85.113± 16.83 a	74.479 ± 15.93 b
Serum TNF-α (Pg/ml)	175.769± 37.35	694.769± 133.76 a	274.807± 73.53 a,b	218.877± 48.78 b
Serum IL-6 (Pg/ml)	2.031± .85	9.611± 2.75 a	8.238± 2.34 a	5.933± 1.57 a,b,c

Serum SOD (U/ml)	200.961± 28.86	119.872± 22.17 a	184.538± 14.41 b	193.607± 14.39 b

Data were expressed as Mean ± SD. a: P<0.05 in comparison with control group. b: P<0.05 in comparison with PCOS group. C: P<0.05 in comparison with PCOS+ Ex group
PCOS, polycystic ovary syndrome; PCOS + Ex, polycystic ovary syndrome with exercise training; PCOS + Ex + Met, polycystic ovary syndrome with exercise training and metformin treatment.

Table (2): Correlations between studied parameters and serum asprosin (pg/ml) in different group

Group / Parameter	Control		PCOS		PCOS + Ex		PCOS + Ex+ Met	
	(r)	P value	(r)	P value	(r)	P value	(r)	P value
BMI (gm/cm^2)	**-0.115**	**0.708**	**0.707**	**0.708**	**-0.182**	**0.551**	**0.027**	**0.929**
SerumT3 (ng/ml)	**0.240**	**0.430**	**0.496**	**0.430**	**-0.221**	**0.468**	**-0.163**	**0.594**
SerumT4 (ug/dl)	**0.265**	**0.382**	**-0.287**	**0.382**	**0.115**	**0.708**	**-0.435**	**0.138**
Serum TSH (m IU/ml)	**0.274**	**0.365**	**0.096**	**0.365**	**-0.167**	**0.587**	**0.534**	**0.060**
Serum FSH (□IU/ml)	**-0.173**	**0.573**	**0.403**	**0.573**	**-0.057**	**0.853**	**-0.200**	**0.512**
Serum LH (□IU/ml)	**0.059**	**0.849**	**0.123**	**0.849**	**0.736**	**0.004***	**-0.414**	**0.160**
Serum Estradiol (Pg/ml)	**-0.089**	**0.774**	**0.144**	**0.774**	**0.207**	**0.498**	**-0.713**	**0.006***
Serum Progesterone (ng/ml)	**-0.144**	**0.638**	**0.205**	**0.638**	**-0.407**	**0.167**	**0.187**	**0.540**
Serum Testosterone (ng/ml)	**-0.294**	**0.330**	**-0.284**	**0.330**	**0.174**	**0.570**	**0.264**	**0.383**
Serum Glucose (mg/dl)	**0.152**	**0.620**	**0.276**	**0.620**	**0.246**	**0.418**	**-0.304**	**0.312**
Serum Insulin (ng/ml)	**-0.219**	**0.473**	**0.442**	**0.473**	**0.028**	**0.926**	**0.301**	**0.317**
HOMA-IR	**-0.148**	**0.629**	**0.515**	**0.629**	**0.247**	**0.416**	**0.163**	**0.594**
HOMA-B	**-0.225**	**0.459**	**0.165**	**0.459**	**-0.279**	**0.356**	**0.480**	**0.097**
Serum Total cholesterol (mg/dl)	**0.312**	**0. 299**	**-0.203**	**0. 299**	**-0.156**	**0.612**	**0.107**	**0.729**
Serum Triglyceride (mg/dl)	**-0.442**	**0.130**	**-0.207**	**0.130**	**-0.491**	**0.089**	**0.090**	**0.771**
Serum HDL (mg/dl)	**0.518**	**0.070**	**-0.488**	**0.070**	**0.182**	**0.553**	**-0.028**	**0.928**
Serum LDL (mg/dl)	**0.220**	**0.470**	**-0.043**	**0.470**	**-0.131**	**0.669**	**0.087**	**0.779**
Serum TNF-α (Pg/ml)	**0.273**	**0.368**	**-0.239**	**0.368**	**0.426**	**0.147**	**0.408**	**0.166**
Serum IL-6 (Pg/ml)	**0.242**	**0.426**	**-0.454**	**0.426**	**0.217**	**0.476**	**0.162**	**0.597**
Serum SOD (U/ml)	**-0.532**	**0.061**	**-0.053**	**0.061**	**0.126**	**0.683**	**0.083**	**0.788**

(r) represents correlation coefficient versus serum asprosin level. * correlation is significant ($p < 0.05$).

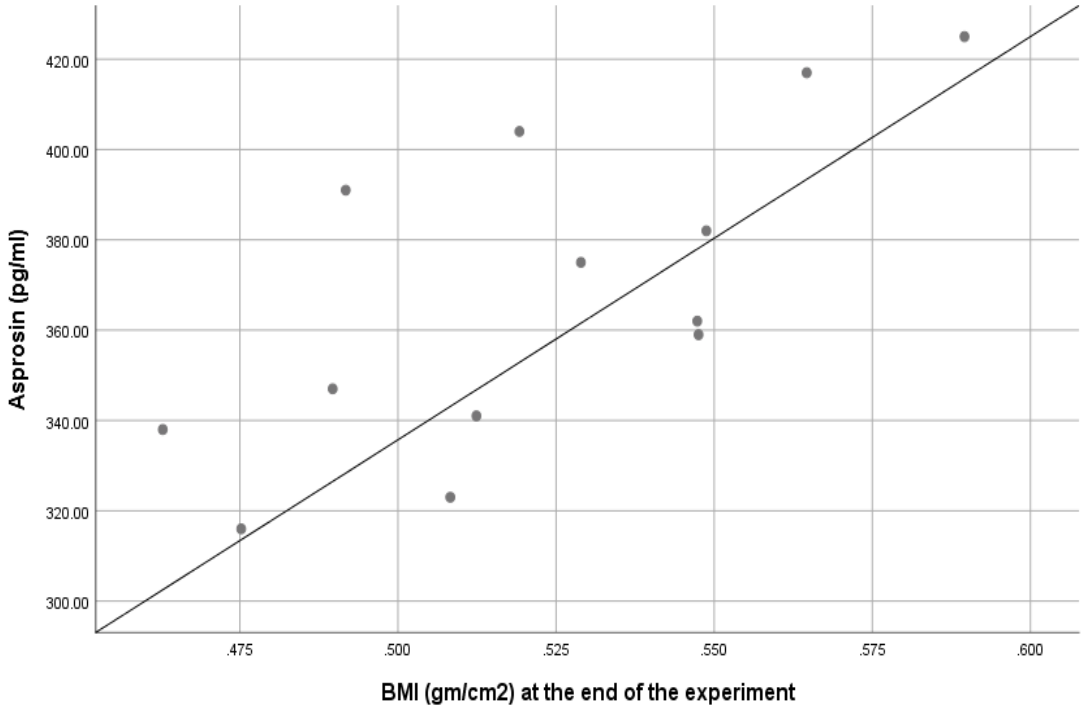

Figure 1: Correlation between asprosin (pg/ml) and body mass index (BMI) (g/cm^2) at the end of the experiment is positive (r= 0.707, p value =0.007*) in PCOS group. * correlation is significant (p value < 0.05)

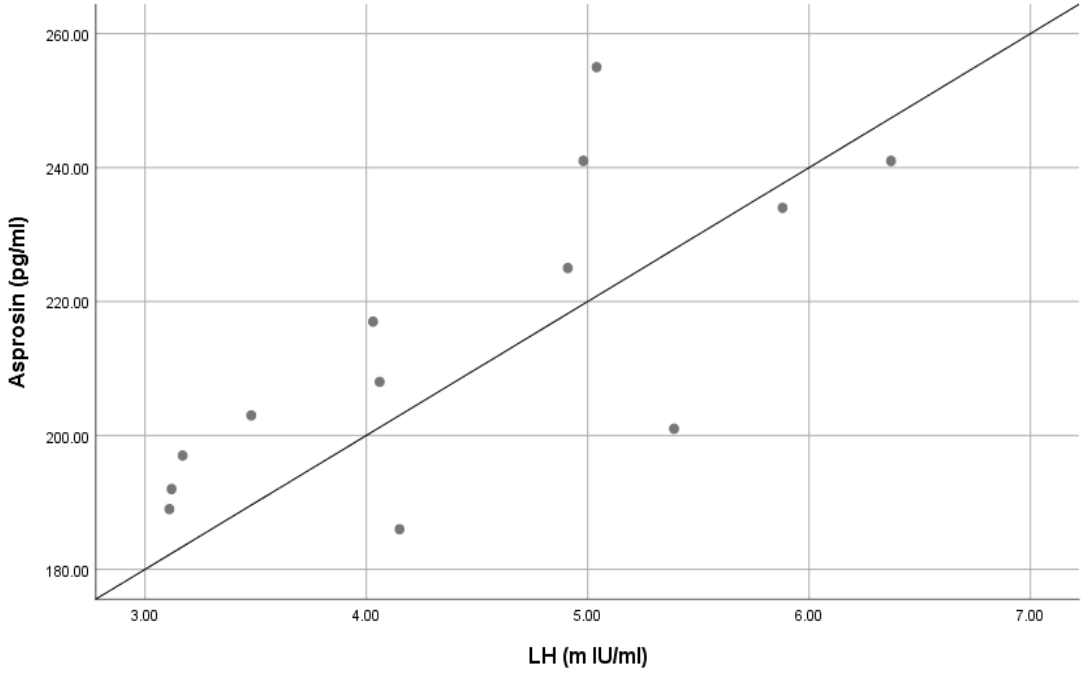

Figure 2: Correlation between asprosin (pg/ml) and LH (m IU/ml) is positive (r= 0.736, p value = 0.004*) in PCOS +Exercise group.
* correlation is significant p value < 0.05

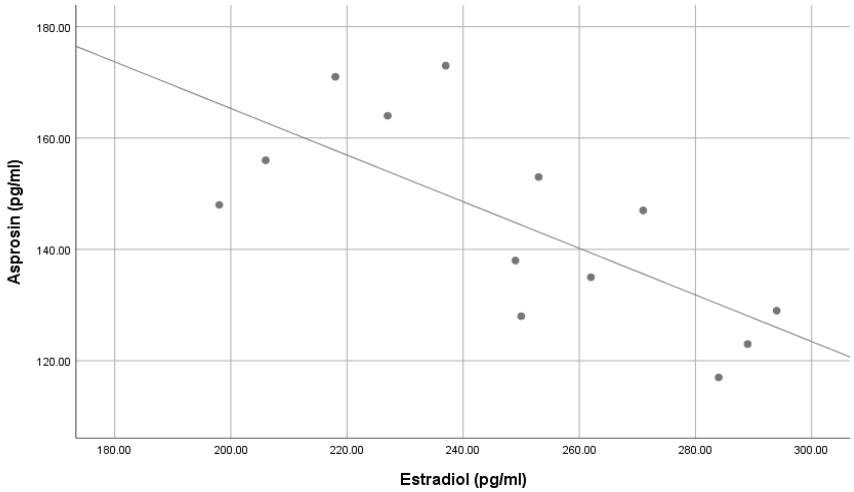

Figure 3: Correlation between asprosin (pg/ml) and estradiol (E2) (Pg/ml) is negative (r= -0.713, p value = 0.006*) in PCOS +Exercise +Metformin group. * correlation is significant (p value < 0.05)

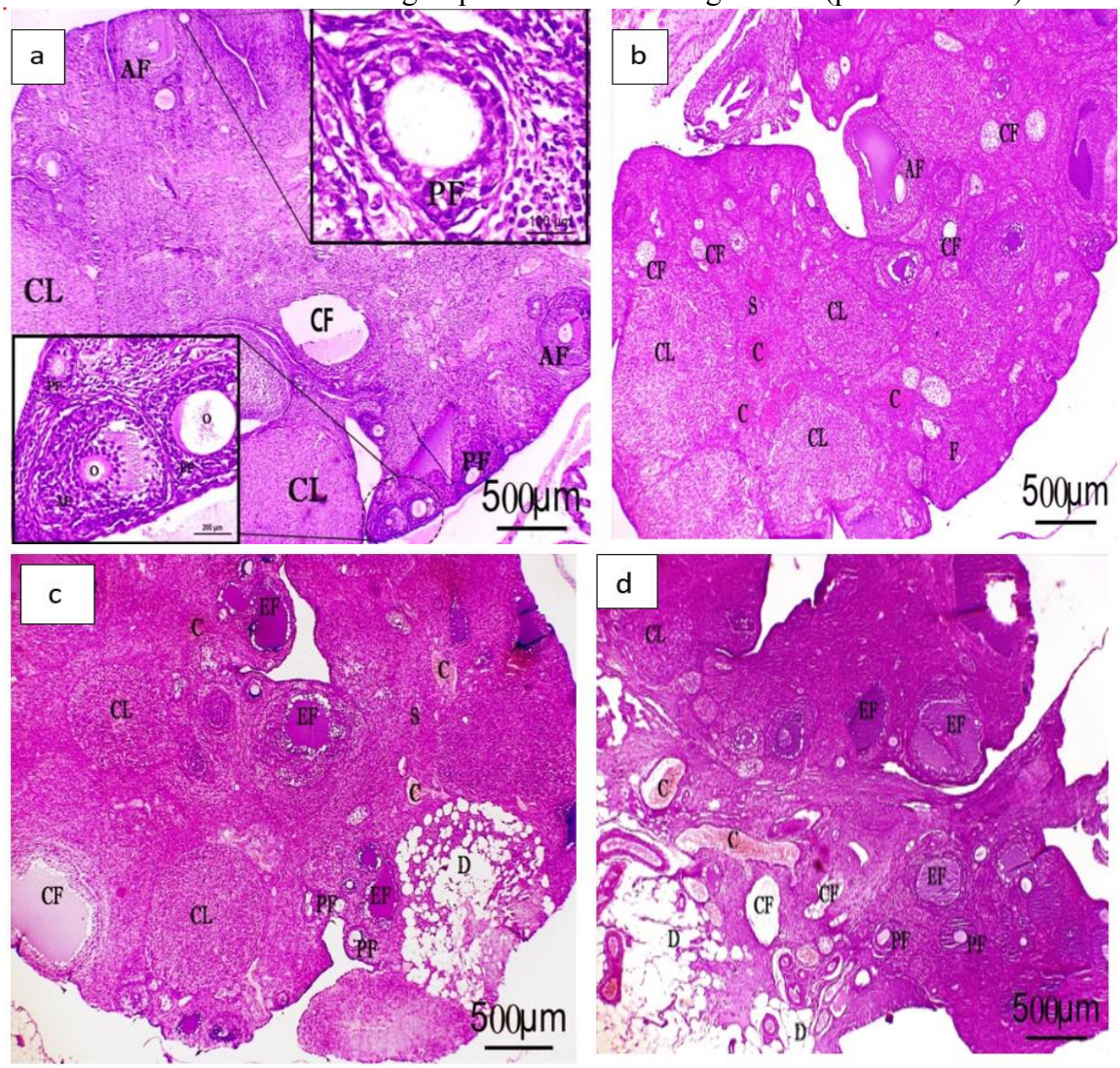

Figure 4: Ovarian histopathological examination showing a plate stained with hematoxylin and eosin of the rat ovarian tissue in all studied groups under light microscope (IHC x40). (a) Control group (b) PCOS group (c) PCOS+ Exercise group (d) PCOS+ Exercise+ Metformin group

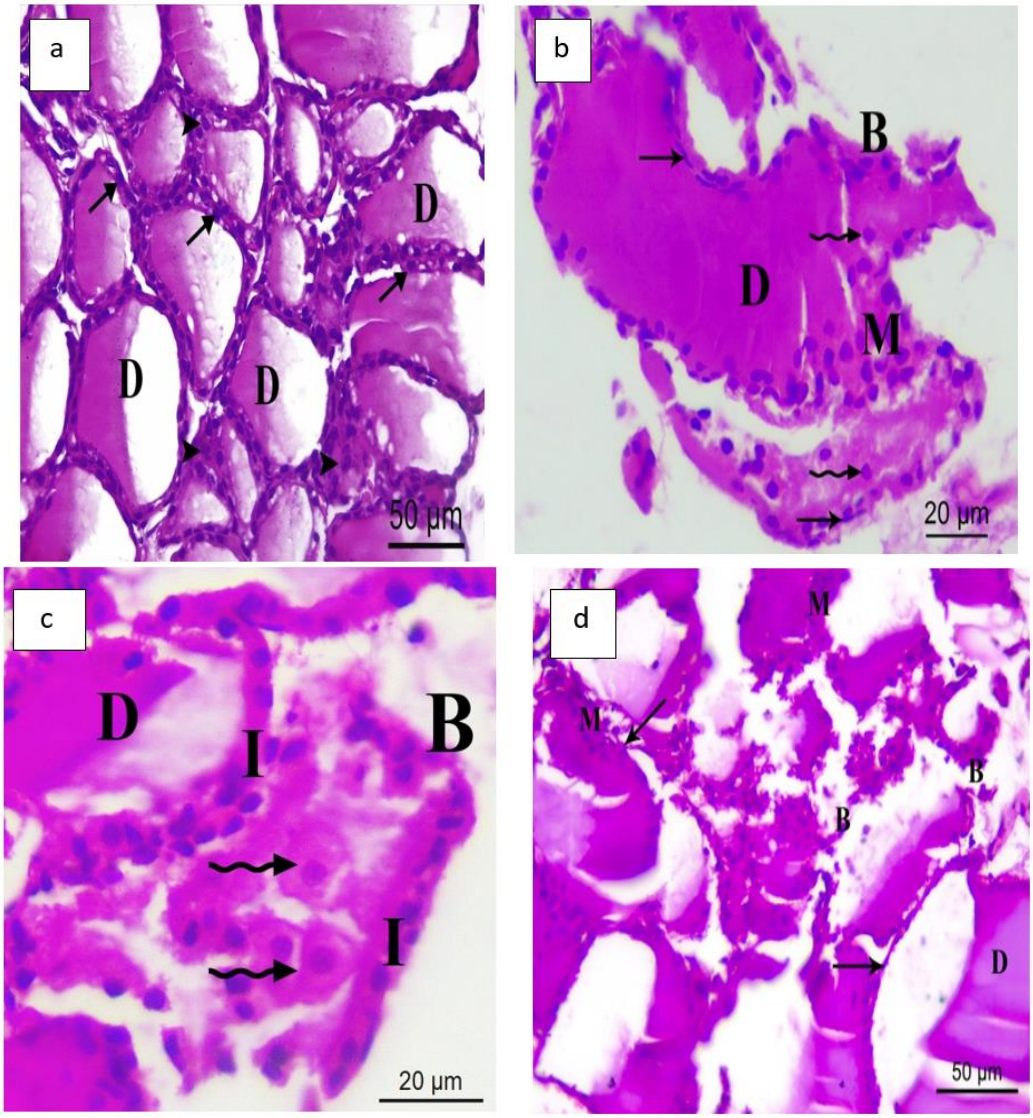

Figure 5: Thyroid histopathological examination showing a plate stained with hematoxylin and eosin of the rat thyroid tissue in all studied groups under light microscope (high magnification as shown by the scale bar). (a) Control group (b) PCOS group (c) PCOS+ Exercise group (d) PCOS+ Exercise+ Metformin group.

Discussion

Androgen excess and hyperinsulinemia are the fundamental abnormalities in PCOS patients **(Abbott et al., 2005)**. It was reported that the concentration of asprosin of white fat tissue origin increased in the presence of insulin resistance **(Zhang et al., 2019)**.

The experimentally induced PCOS in this work is similar to that of the human as letrozole administration based on 11 weeks of daily treatment with normal diet and regular assessment estrous cycle produced changes mimicking those of clinical PCOS, including elevated serum testosterone and luteinizing hormone and development of multiple ovarian cysts **(Dăneasă et al., 2016).**

The signs of PCOS induced by letrozole in rats was proved by the significant hyperandrogenism accompanied by significant reduction in both estradiol and progesterone levels in comparison to control group. In addition, persistent estrus was observed in vaginal smear, with multiple ovarian cysts were

detected in histopathological examination as letrozole blocked cytochrome P450 aromatase which is responsible for aromatization of testosterone to estradiol **(Van Voorhis et al., 1994).**

Regarding BMI, our work reveals that animals in PCOS group showed a significant progressive increase in BMI when compared with control group. This result was supported by **Polat and Şimşek (2020)**, while in PCOS rats after practicing moderate swimming exercise for 8 weeks showed a significant decrease in BMI in comparison to PCOS group.

These findings are consistent with **Wu et al. (2021)** who demonstrated that 12 weeks of aerobic exercise produced beneficial effects on BMI and concluded that the extent of BMI decline depends on the frequency, intensity, duration, type of exercise and on adherence to the exercise program.

In our study, we found that combined moderate swimming exercise and metformin treatment produced a significant reduction in BMI in PCOS rats in comparison to PCOS group.

This result was supported by **Tiwari et al. (2019)** who found that 3 months of combined marching exercise at the same place for 30 min three days per week and metformin treatment of 850 mg twice a day significally decrease the BMI in women with PCOS in comparison to PCOS group.

Helvaci et al. (2008) suggested that metformin's effect on body weight is due to its anorexigenic effect via a pathway involving neuropeptide Y in human.

Regarding serum asprosin, there was a significant increase in its levels in PCOS rats compared with controls. This result was supported by other studies like **Li et al. (2018), Alan et al. (2019),** and **Deniz et al. (2021)**. However, **Chang et al. (2019)** reported that asprosin was not associated with the etiology of PCOS. **Deniz et al. (2021)** owed these conflicting results to the differences between age, anthropometric differences, genetic and disease severity.

Furthermore, PCOS rats after performing moderate swimming exercise showed significantly lower serum levels of asprosin in comparison to PCOS group. This finding is in agreement with **Ceylan et al. (2020)** who reported that moderate aerobic exercise applied to the overweight adult men in both the morning and evening hours for 30 min by ≥3d intervals led to significant reductions in serum asprosin and insulin levels.

In addition to **Ko et al. (2019)** who found that aerobic exercise training suppresses blood glucose level through the reduction of hepatic asprosin levels in STZ-induced diabetic rats. **Romere et al. (2016)** and **Wiecek et al. (2018)** also suggested that the decrease in asprosin level after exercise might be related to blood glucose level as blood glucose plays a suppressive role on asprosin via negative feedback.

Additionally, we found that combined moderate swimming exercise and metformin treatment produced a significant reduction in serum level of asprosin in PCOS rats in comparison to PCOS group.

These results are supported by **Gozel and Kilinc (2021)** who proved that plasma asprosin levels after three months of metformin treatment decreased significantly when compared to the pre-treatment period, but they did not fall below the asprosin levels of the healthy control group patients.

The asprosin-lowering effect of metformin is suggested to be mainly by inhibiting gluconeogenesis and decreasing hepatic glucose output **(Shaw, 2013)**.

In the current study, there was a significant positive correlation between serum asprosin level and BMI in PCOS group.

These results are in line with other studies where **Alan et al. (2019)** and **Ameen et al. (2021)** found that serum asprosin levels showed a significant positive correlation with BMI in women with PCOS. Also, **Du et al. (2021)** found that asprosin levels were positively correlated with body fat mass and BMI with a significant relationship. This could be related to the orexigenic effect of asprosin **(Duerrschmid et al., 2017)**.

In this work, PCOS rats showed significant lower serum levels of T3 and T4 in addition to a significant increase in serum TSH when compared with control rats. These results reflect incidence of overt hypothyroidism, which is in line with **Sinha et al. (2013**) who reported higher occurrence of autoimmune thyroiditis and increased plasma TSH levels in patients with polycystic ovarian syndrome than those of healthy group.

Moderate swimming exercise in PCOS rats showed a significant reduction of serum TSH level, with significant elevations in serum levels of T3 and T4 when compared with PCOS group. These results reflected that moderate swimming exercise training improved thyroid function in PCOS which was supported by **Bansal et al. (2015) and Masaki et al. (2019)**.

Bansal et al. (2015) demonstrated that patients with hypothyroid disease showed that TSH level decreased but T3 and T4 levels increased after jogging (1 hour a day) for 3 months. Also, **Sultan and Rashed (2009)** reported that moderate intensity therapeutic exercise on electrical treadmill for one month every other day causes a significant increase in the level of circulating thyroid hormones and a significant decrease in TSH serum level. In addition, **Fortunato et al. (2008)** reported increased serum T4 level with moderate exercise on the treadmill in human.

However, **Onsori and Galedari. (2015)** showed that moderate-intensity aerobic exercise for 12 weeks in obese inactive women does not significantly change the plasma concentration of TSH and thyroid hormones (T3 and T4). These contradictory results may be explained by the difference in the intensity, duration and type of exercise **(Hawamdeh et al., 2012)**.

One of the mechanisms which can explain the effect of exercise on thyroid hormones is that physical stress increased the action of the plasma catecholamines which increase T3 and T4 levels **(Hawamdeh et al., 2012)**. **Baylor and Hackney (2003)** found that prolonged exercise program for 20 weeks decreased TSH level in women which was explained by lower hypothalamic pituitary signaling action.

Additionally, we found that combined moderate swimming exercise and metformin treatment showed a significant improvement in thyroid dysfunction in rats with PCOS as reflected by the significant increase in serum level of T3 and T4, with the significant decrease in serum level of TSH when compared with PCOS rats.

In accordance with our results, **Santos-Palacios et al. (2015)** declared that metformin decreases blood TSH level. **Anil et al. (2016)** reported that it may affect the size and the development of thyroid gland nodules.

The possible explanations for the TSH-reducing effect of metformin are suggested by **Cappelli et al. (2009)** who found that metformin enhanced the inhibitory feedback of thyroid hormones on TSH secretion.

In the present study, PCOS rats have significant lower serum levels of FSH, estradiol, and progesterone, with a significant increase in serum levels of testosterone and LH in comparison to control rats. These results confirmed occurrence of PCOS and are in line with **Loffler et al. (2001)** and **Bednarska and Siejka (2017)** who demonstrated that high level of LH and increased LH:FSH ratio can serve as biomarkers to diagnose PCOS in women.

The deficiency in aromatase activity is one of the major causes of the intraovarian disturbances in steroidogenesis. This effect has been observed in animals treated with letrozole compared to normal animals **(Kafali et al., 2004)**.

In this work, estrogen was decreased in letrozole-treated rats, and the reduction was correlated with early- or mid-follicular development and follicular morphology formation in the ovaries. This finding agrees with **Dewailly et al. (2016)**.

Histological analysis showed that rats with PCOS exhibited decreased follicular development and numerous subcapsular cysts. Furthermore, the follicular cysts had a flattened epithelioid cell layers and thickened hyperplastic theca interna cells in the cyst walls. This follicular dysfunction, such as atretic cysts with scant granulosa cells induced by letrozole and these results are supported with **Baravalle et al. (2006)**.

In our study, moderate swimming exercise in PCOS rats showed a significant reduction of serum LH and testosterone level when compared to PCOS group which reflected improvement of the hormonal changes associated with PCOS.

This result was supported by **Cao et al. (2017**) who found that moderate swimming exercise for 6 d/week for 3 weeks promoted loss of body weight and reduced circulating androgen and LH levels.

On the other hand, swimming exercise in PCOS rats did not significally increase serum levels of FSH, estradiol and progesterone in comparison to PCOS group. This finding is supported by **Palomba et al.**

(2008) who found that 24-week structured cycling exercise training for 30-40 min done for PCOS patients did not change serum levels of FSH, estradiol, and progesterone.

In this work, there was a significant positive correlation between serum asprosin and LH levels in PCOS + Exercise group.

This finding agrees with **Deniz et al. (2021)** who found a significant positive correlation between serum asprosin and LH levels in women diagnosed with PCOS.

Interestingly, we found that combined moderate swimming exercise and metformin treatment ameliorated ovarian dysfunction in PCOS which was evidenced by the significant increase in serum level of FSH, estradiol, and progesterone, with a significant decrease in serum level of LH and testosterone in comparison to that in PCOS group. In addition to return of the cyclicity of the estrous and little improvement of the histopathological examination.

In line with this finding, **Tiwari et al. (2019)** found that combined marching exercise at the same place for 30 min three days per week and metformin treatment of 850 mg twice a day for 6 months produced a significant decrease in the serum level of testosterone in women with PCOS in comparison to PCOS group. Also, **Xiao et al. (2022)** reported that the PCOS mice showed an increased number of corpora lutea and normal estrous cycles after metformin treatment and suggested that metformin treatment can reverse ovulatory dysfunction in PCOS mice

Metformin likely plays its role in improving ovulation induction in case of PCOS through a variety of actions, including reducing insulin level and enhancing insulin sensitivity **(De Leo et al., 2003)**. Metformin also helps in improving hyperandrogenic symptoms due to restoration of ovulation, normalization of estrogens and altering in the effect of insulin on ovarian androgen biosynthesis and theca cell proliferation (**Elnashar, 2011)**.

In this study, a significant negative correlation was found between asprosin and estradiol levels in PCOS + Exercise +Metformin group. In line with these findings, **Li et al. (2018)** and **Jiang et al. (2021)** found that asprosin is negatively correlated with estradiol in PCOS women, but the correlation was non-significant.

On the other hand, **Leonard et al. (2021)** found a significant positive correlation between plasma asprosin and estrogen levels in healthy women. **Li et al. (2018)** also found a significant positive correlation between asprosin and estradiol levels in normal-weight women with PCOS.

In our study, estradiol and LH were significantly correlated with asprosin. These results are in accordance with **Stepto et al. (2013)** who found that overweight/obesity confers IR and then worsens the reproductive and metabolic features of PCOS. Additionally, **Li et al. (2018)** found that in PCOS, asprosin can reflect the IR states which might also be mechanically related to the abnormal sex-related hormone metabolism in the pathogenesis with PCOS.

Some studies have revealed that insulin-driven androgen excess and dysfunctional lipid metabolism in the adipose tissue are causative drivers of metabolic risk in PCOS **(O'Reilly et al., 2017)**. Asprosin is derived from white adipose tissue and is correlated with sex-related hormones, in PCOS females, especially in overweight/obese subgroup of PCOS, indicating that asprosin might participate in the occurrence or development of PCOS, not only via obesity and IR but also by interacting with sex hormones **(Li et al., 2018)**.

In this study, serum level of glucose was significantly higher in PCOS group compared with control group. This finding is in agreement with **Alan et al. (2019)**. However, swimming exercise in PCOS rats did not produce a significant decrease in glucose level in comparison to PCOS group.

This finding is in accordance with **Wu et al. (2021)** who found that 30-min aerobic exercise training on a bicycle ergometer four times per week for 12-week did not lower fasting blood glucose (FBG) in trained PCOS patients in comparison to PCOS group.

In the present study, PCOS rats showed a significantly higher serum level of insulin, and HOMA-IR index when compared with control rats. While, there was a significant decrease in HOMA-B index in the same

group. These results reflected occurrence of insulin resistance which were noticed with other studies like **Hutchison et al. (2011), Alan et al. (2019),** and **Polat and Şimşek (2020)**.

Moderate swimming exercise in PCOS rats showed a significant decrease in insulin level and HOMA-IR index in comparison to PCOS group which indicated that moderate swimming exercise has an insulin sensitizing effect in PCOS.

These results are partially supported by **Hutchison et al. (2012)** who found that 12 weeks of moderate exercise training on a treadmill produced a significant improvement in insulin resistance. Also, **Ceylan et al. (2020)** reported that moderate aerobic exercise applied to the overweight adult men led to significant reductions in serum insulin levels.

Dela et al. (2004) and Li et al. (2015) found that exercise improved beta-cell function and insulin sensitivity by increasing intramyocellular triacylglycerol concentration. Improvement in insulin sensitivity could be due to more efficient lipid turnover resulting in increased muscular lipid uptake, transport, utilization, and oxidation

In addition, we found that combined moderate swimming exercise and metformin treatment produced a significant decrease in glucose and insulin serum levels and HOMA-IR in PCOS rats in comparison to PCOS group which confirmed their synergistic action on increasing insulin sensitivity.

In line with this finding, **Rajagopal et al. (2012)** found that metformin treatment for 3 months from 500mg, once daily in first week to 500mg twice daily (b.i.d.) the next week, and then to 1,000mg b.i.d. and a 500-kcal deficit diet with walking for 30–45 min daily for at least 5 days per week significally decreased fasting blood sugar level in women with PCOS in comparison to non-treated PCOS group.

Also, **Pasquali et al. (2000)** found that 7 months of combined hypo caloric diet and metformin treatment 850 mg twice per day produced a significant decrease in fasting insulin level in PCOS women when compared to PCOS group.

However, **Salama et al. (2018)** reported that adding metformin in a dose of 850 mg twice daily for 12 weeks to lifestyle modifications with a hypocaloric diet and physical activity had no superior effect to lifestyle modifications alone on the reductions in blood glucose and insulin levels and HOMA-IR in overweight women with PCOS.

Some studies owed the glucose-lowering effect of metformin to its ability to reduce hepatic glucose output and to increase insulin-stimulated glucose uptake and glycogenesis in skeletal muscle via AMPK activation **(Banerjee et al., 2016**).

Also, metformin-mediated improvements in insulin sensitivity might be associated with several mechanisms, including increased insulin receptor tyrosine kinase activity, enhanced glycogen synthesis, and produced an increase in the recruitment and activity of glucose transporter type 4 **(Wróbel et al., 2017)**.

In the present study, PCOS rats showed significantly higher serum levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL), with a significant decrease in serum level of high-density lipoprotein cholesterol (HDL) when compared with control rats.

These results confirmed occurrence of dyslipidemia in PCOS which is supported by **Li et al. (2018)** and **Alan et al. (2019)** who reported a significant increase in serum level TG in women with PCOS in comparison to control group. Also, **Chang et al. (2019)** found that the serum levels of TC, TG and LDL were significantly increased in PCOS women when compared with control women. Additionally, **Yilmaz et al. (2005), Li et al. (2018)** and **Alan et al. (2019)** declared that serum level of HDL was significantly decreased in PCOS women compared with healthy women.

Dyslipidemia observed with PCOS in the current study could be explained by the presence of insulin resistance which is in line with **Lewis et al. (2002)** who stated that insulin resistance is one of the major causes of dyslipidemia in PCOS due to metabolic abnormalities that occur since insulin has an inhibitory action on 3-hydroxy-3-methyl-glutaryl CoA (HMG-CoA) reductase, which is the rate limiting enzyme in the metabolism of cholesterol. Also, insulin activates lipoprotein lipase that hydrolyzes triglycerides.

Thereby, insulin resistance results in failure of activation of the above mentioned enzymes causing hypertriglyceridemia and resulting in impaired clearance of LDL from plasma **(Nakhaei et al., 2019)**.

In this work, moderate swimming exercise in PCOS rats showed non-significant change in serum TC, TG, HDL and LDL levels when compared to PCOS group.

These results are in line with other studies where **Dewailly et al. (2016)** found no significant effects of exercise only (without a dietary component) on lipid profile in PCOS. **Giallauria et al. (2008)** also found that exercise performed for 30 min on a bicycle for 3 months to PCOS patients did not significally change TC, TG, LDL and HDL in comparison to PCOS group without exercise.

Carroll and Dudfield (2004) indicated that interventions should be longer in duration (>20 weeks) to induce positive changes to lipid profile in people with metabolic syndrome.

In this work, we found that combined moderate swimming exercise and metformin treatment produced a significant decrease in serum levels of TC, TG and LDL in PCOS rats in comparison to PCOS group which reflected their synergistic action on improving lipid dysfunction in PCOS. This is in line with this **Rajagopal et al. (2012)** found that metformin treatment for 3 months from 500mg, once daily in first week to 500mg twice daily (b.i.d.) the next week, and then to 1,000mg b.i.d. and a 500-kcal deficit diet with walking for 30–45 min daily for at least 5 days per week produced a significant decrease in TC and TG levels in women with PCOS in comparison to non-treated PCOS group.

The possible mechanism that explain the effect of exercise on lipid profile may be increased muscle and adipose tissue nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) messenger RNA expression after exercise training **(Saghebjoo et al., 2018)**.

Leiberman and Marks (2009) reported that metformin produced phosphorylation of acetyl CoA carboxylase, thereby inhibiting its activity. As a result, malonyl CoA levels are reduced leading to a reduction in fatty acid synthesis (need for TG production) and an enhancement of fatty acid oxidation.

In this study, the serum level of TNF-α was significantly higher in PCOS group compared with control group.

This is in accordance with **Gonzalez et al (1999)** who found that serum TNF-α concentration was significally elevated in normal-weight PCOS women in comparison to normal weight control. **Gonzalez et al (1999)** reported that TNF-α originating from adipose tissue may be involved in the classic relationship between hyperinsulinemia and hyperandrogenism in PCOS as it causes functional impairment of GLUT4 leading to insulin resistance, which may in turn promotes hyperandrogenism in women with PCOS.

In this work, moderate swimming exercise in PCOS rats showed a significant reduction of serum TNF-α level when compared to PCOS group.

This finding is in agreement with **Dantas et al. (2019)** who found that moderate-intensity aerobic exercise reduced plasma and muscle TNF-α in women with PCOS compared to PCOS group without exercise. **Dantas et al. (2019)** demonstrated that the anti-inflammatory role of exercise could explain its cardiometabolic protection in PCOS.

In our study, serum level of IL-6 was significantly higher in PCOS group compared with control group. This result is supported by **Ghowsi et al. (2018)** who reported that PCOS is a chronic low grade inflammatory disorder associated with increased levels of proinflammatory cytokines, IL-6 and TNF-α

The corpus luteum secretes TNF-α and the levels of immune-reactive TNF-α vary throughout the menstrual cycle **(Brannstrom et al., 1999)**. TNF-α and IL-6 are presumed to play pivotal roles in reproductive physiology, including regulation of ovarian steroid production, follicular maturation, and the processes of ovulation, fertilization, and implantation **(Gonzalez, 2012)**. These parameters affected in women with PCOS. Moreover, variants in genes encoding several proinflammatory cytokines and their receptors associated with obesity, insulin resistance and PCOS **(Repaci et al., 2011)**.

In this work, moderate swimming exercise in PCOS rats showed non-significant reduction of IL-6 serum level when compared to PCOS group.

These results are in agreement with **Li et al. (2021)** who found that swimming exercise without load in PCOS rats 120 min/per session, 6 days/week, for 15 days showed no statistically significant decrease of IL-6 level when compared with the PCOS group.

Exercise may stimulate adipose tissue to exert anti-inflammatory effects through signal pathways such as insulin and transforming growth factor (TGF-*β)* and down-regulate the levels of TNF-*α* and IL-6 **(Suzuki, 2019)**.

Additionally, we found that combined moderate swimming exercise and metformin treatment produced a significant decrease in TNF-α and IL-6 serum levels in PCOS rats in comparison to PCOS group which confirmed their synergistic anti-inflammatory actions.

Inflammatory cytokines (IL-6 and TNF-α) are produced both by macrophages and other cells recruited to adipose tissue and in a cell-autonomous manner by adipocytes **(Olefsky & Glass, 2010)**. Metformin inhibits recruitment, migration and proliferation of macrophages through its anti-inflammatory action via AMPK activation pathway and subsequent inhibition of nuclear factor kappa B subunit 1 (NFKB) **(Kim et al., 2014)**.

In this work, a significant decrease in serum level of enzymatic antioxidant (SOD) was found in PCOS rats when compared to controls.

Our result is supported by **Zhang *et al.* (2008)** who demonstrated that the serum SOD level in PCOS patients was significantly lower than that in the control group.

Oxidative stress (OS) is considered to be one of the main causes of molecular damage to cellular and tissue structures in PCOS patient **(Murri et al., 2013)**. Insulin resistance (IR) and hyperglycemia can increase oxidant status levels **(Verit and Erel, 2008)**. OS stimulates the androgen-producing ovarian steroidogenic enzymes, while antioxidants suppress these enzymes **(Piotrowski at al., 2005)**.

In our study, moderate swimming exercise in PCOS rats showed a significant increase of serum SOD level when compared to PCOS group.

These finding is in agreement with **Wu et al. (2021)** who found that 30-min aerobic exercise on a bicycle ergometer four times per week for 12 weeks produced a significant increase of serum SOD level in women with PCOS in comparison to non-trained PCOS group.

Regular exercise training promotes the adaptation and upregulation of endogenous antioxidant defenses and/or decreased measures of systemic oxidative distress **(Morrison et al., 2015)**. Exercise decreases markers of systemic oxidative distress, often coincide with improvements in insulin and glucose regulation **(Konopka et al., 2015)**.

Interestingly, we found that combined moderate swimming exercise and metformin treatment showed a significant increase in SOD serum level in PCOS rats in comparison to PCOS group which reflected their synergistic antioxidant action.

Our results are supported by those of **Markowicz-Piasecka et al. (2017)** who demonstrated that metformin reduced oxidative stress. **Najafi et al. (2018)** also reported that metformin reduces oxidative stress via modulating intracellular NADPH oxidase activity, and by this way formation of O_2 radical is reduced indirectly.

Limitations of study

1- The duration of the exercise program should be longer to confirm if the duration of exercise could affect the degree of amelioration of different studied parameters in PCOS group.
2- Different types of exercises (rather than swimming) with different intensities could be used to explore the benefits of each type on different studied parameters.
3- The study was conducted on a small number of rats.

Conclusion

Taking the present findings together, it could be concluded that PCOS was complicated by overt hypothyroidism. Also, combined moderate swimming exercise and metformin treatment of PCOS resulted in a synergistic beneficial effect in amelioration of ovarian, metabolic, and thyroid dysfunction occurred

with PCOS. This was achieved through their anti-inflammatory, antioxidant, insulin-sensitizing, and asprosin regulating actions. Moreover, reduction of asprosin serum level appears to be a promising therapeutical target for women with PCOS.

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