Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.234154.2873 Volume 30, Issue 1.7, Oct. 2024, Supplement Issue
Manuscript ID: ZUMJ-2304-2785 (R1) Doi: 10.21608/ZUMJ.2023.205123.2785
ORIGINAL ARTICLE

Effect of Metformin Treatment on Serum Asprosin Level Changes in Rat Model of Polycystic Ovary

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Submit Date 29-04-2023
Revise Date 27-05-2023
Accept Date 11-06-2023

ABSTRACT

Background:PCOS is a common endocrine disorder that is associated with insulin resistance.Asprosin hormone level is changed with insulin resistance.Metformin is an effective treatment for the improvement of ovulation in women with PCOS.**Aim of the study:** To investigate the effect of metformin treatment on the changes in serum asprosin level in letrozole-induced PCOS in rats.**Methods:** Three groups of 39 adult female albino rats were equally distributed. 1) Control group, rats were given 1% aqueous solution of carboxy methyl cellulose orally once daily for 11 weeks. 2) 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. 3) PCOS + Metformin group, after induction of PCOS as in PCOS group for 3 weeks, rats were given metformin (50 mg/100 g BW in 0.05 ml of distilled water) orally once daily for 8 weeks. At the end of the experiment, serum was extracted from collected blood for assessment of asprosin, sex hormones, glucose, insulin, lipid profile, SOD, TNF-α and IL-6 levels. An ovarian histopathological study was done. **Results:** Metformin treatment caused a significant reduction in BMI, HOMA-IR, and serum levels of asprosin, LH, testosterone, glucose, insulin, and inflammatory cytokines with a significant increase in serum levels of FSH, Estradiol, and SOD in female albino rats with PCOS in comparison with PCOS group.
Conclusion: Metformin treatment has insulin-sensitizing; anti-inflammatory and antioxidant effects and it modulates disturbances in serum asprosin levels in PCOS.
Keywords: Asprosin, Metformin, Polycystic Ovary

INTRODUCTION

The three types of adipose tissue are white, brown, and beige. Throughout the body, white adipose tissue serves largely as a storage area for lipids that are high in energy. Additionally, it is widely known that white

adipose tissue secretes a range of adipokines, including asprosin [1]. The C-terminal cleavage product of fibrillin (encoded by FBN1) is asprosin, which Romere and his colleagues first identified in 2016. It is brought on by fasting and affects the liver by activating the G protein-cyclic adenosine monophosphate-protein kinase [2].Asprosin penetrates the blood-brain barrier and, through a cAMP-dependent mechanism, directly stimulates orexigenic Agouti-related peptide-expressing (AgRP) neurons. This signaling, which stimulates hunger and heightens the desire to accumulate body fat, suppresses anorexigenic proopiomelanocortin (POMC)-positive neurons downstream in a GABA-dependent manner [3]. Previous studies showed a close connection between plasma asprosin levels and the generation of insulin, the metabolism of glucose, body mass, and inflammation [2].The endocrine illness polycystic ovarian syndrome (PCOS) affects many women of reproductive age and is linked to an increased prevalence of major clinical issues such as insulin resistance, hypertension, dyslipidemia, and reproductive consequences [4]. Even though its molecular cause is still unknown, a widespread opinion holds that PCOS patients' primary abnormalities are androgen excess and hyperinsulinemia [5].The most significant pathological aspects of PCOS syndrome appear to be initiated by insulin resistance (IR), which appears to be a basic event. High levels of glucose and triglycerides circulate in the circulation as a result of it impairing muscle cells' capacity to absorb and store them [6].

Increases in adipokines, cytokines, and other inflammatory markers such as tumor necrosis factor (TNF-□) and interleukin 6 (IL-6) are linked to IR in obese people [7]. It was discovered that the presence of insulin resistance enhanced the concentration of asprosin from white fat tissue [8].Through improvement in the metabolic profile of women with PCOS, particularly in HDL and BMI, metformin is a successful medication for ovulation induction [9].On the other hand, the available data on changes in serum levels of asprosin in PCOS are contradictory [8,10]. Consequently, it is not yet clear whether asprosin contributes to PCOS or not. Also, scarce data were found on the effect of metformin treatment on serum level of asprosin in PCOS.Thus, this work aimed to investigate the changes in serum asprosin levels in letrozole-induced PCOS in rats and to assess the effect of metformin treatment on those changes, and the possible underlying mechanisms.

METHODS

At Zagazig University's Veterinary Medicine Faculty's Animal House, 39 adult female albino rats of local strains weighing 160–180 g were purchased. The animals were kept in steel cages that were 14 inches high, 16 inches wide, and 24 inches long. Each group was kept in a clean cage in the animal house of the Physiology Department, Faculty of Medicine, Zagazig University.

All animals were kept in standard laboratory settings with a natural light/dark cycle and a constant temperature (22±2°C). The nutrition department at the Faculty of Agriculture provided them with a regular diet (25.8% protein, 62.8% carbohydrate, and 11.4% fat) [11], and they had free access to water.

The institutional animal care unit committee of Zagazig University (ZU- IACUC; Sharkia; Egypt) and the Physiology Department committee both approved the experimental protocol, which was given the approval number "ZU- IACUC/3/F/145/2020."Three

equal groups of rats were randomly assigned: [control group (n=13), polycystic ovary syndrome (PCOS) group (n=13), and PCOS and Metformin treated (PCOS + metformin) group (n=13)].Following the rats' two-week acclimatization to the experimental circumstances, the experiment lasted for 11 weeks.

1) Control group: 1% aqueous carboxy methyl cellulose [CMC] was administered orally to rats once a day for the duration of the study (11 weeks).

2) PCOS group: For the creation of an animal model with polycystic ovaries, rats were given letrozole (a non-steroidal aromatase inhibitor) at dosages of 1 mg/kg BW dissolved in 1% CMC (1 ml/kg) orally once daily [12] for 11 weeks (through the entire investigation).

3) PCOS + Metformin group: Rats were administered metformin (50 mg/100 g BW in 0.05 ml of distilled water given orally) [13] once daily for 8 weeks (beginning from the fourth week) following PCOS induction as in the PCOS group.

Determination of the estrous phasesto assess successful induction of PCOS

Every day at 10 am, vaginal smears from all the rats were collected, examined under a light microscope, and the data from each labelled rat's records were plotted to compare the mean frequency of diestrus, metestrus, proestrus, and estrus between the groups. Cycles lasting 4-5 days were regarded as regular [14].

Rats were weighed on a digital scale at the finish of the experiment, and their length from nose to anus was measured using a ruler to determine their BMI which equals body weight (g) / length^2 (cm^2) [14]. Upon completion of the experiment, at 10 a.m., after an overnight fast for the animals. Under ether anesthesia, blood from each rat's sinus orbits vein was taken [15]. After being allowed to coagulate for two hours at 4°C, the blood was centrifuged for ten minutes at a speed of 3000 rpm. Until the time for the biochemical analysis, the separated serum was stored at -20°C.A chemical analysis was performed at the Faculty of Medicine's Biochemistry Department to determine the levels of the hormones asprosin, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and testosterone, as well as blood sugar and insulin in the bloodstream, with a calculation of HOMA-B= insulin (µU/mL) x glucose (mg/dl) /405[14] and HOMA-IR=360 x fasting insulin (µU/mL) / (fasting glucose (mg/dL) - 63) [14], serum levels of triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL), as well as tumor necrosis factor- (TNF-□), interleukin-6 (IL-6) and superoxide dismutase (SOD).

Histopathological Studies

Under ether anesthesia, the rats were decapitated as a form of sacrifice [15]. Ovaries were quickly excised, and cleaned completely. The right ovaries of each group were immediately frozen at -70°C after being dissected, whereas the left ovaries were promptly fixed in 10% buffered formalin and stained for light microscopic analysis.

The ovaries were rinsed in a phosphate buffer saline solution following fixation for 6 hours at room temperature. Fixed tissues were dehydrated in increasing concentrations of ethanol for light microscopy, cleaned in xylene, and embedded in paraffin. 5 □m thick sections were mounted on slides that had previously been stained with hematoxylin and eosin and treated with 3-aminopyropyltriethoxysilane.

Chemicals

Commercial kits were used for asprosin (Bioassay Technology Laboratory, China; Catalog No. E1703Ra); estradiol, progesterone, cholesterol, triglyceridesand 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 (Technical MSN service online, email:sunedrbio@mes.cn; Catalog No. 201-11-0136). LDL was determined using the formula: LDL=TC-HDL-TG/5[14].

Statistical Analysis: The collected information was presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), Tukey HSD for post hoc multiple comparisons to compare means, and a correlation test between serum levels of asprosin and other parameters were all carried out using the software IBM SPSS Statistics (Version 25 for Windows). P value≤0.05 was judged significant.

RESULTS

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

In comparison to the control group, there was a statistically significant rise in BMI and asprosin levels in the PCOS and PCOS + Metformin groups ($p<0.05$). When compared to the PCOS group, these alterations were considerably reduced in the PCOS + Metformin group ($p<0.05$).

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

In contrast to the control group, the FSH, estradiol, and progesterone levels were significantly lower in the PCOS and PCOS + Metformin groups ($p<0.05$). FSH and estradiol levels were significantly higher in the PCOS + Metformin group compared to the PCOS group ($p<0.05$).

When compared to the control group, the LH and testosterone levels in the PCOS group were significantly higher ($P<0.05$). In comparison to the PCOS group, the LH and testosterone levels in the PCOS + Metformin group were significantly lower ($p<0.05$).

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

In comparison to the control group, the glucose levels in the PCOS and PCOS+ Metformin groups significantly increased ($p<0.05$). Insulin levels were increased in the PCOS group compared to the control group ($p<0.05$). When compared to the PCOS group, the PCOS + Metformin group's glucose and insulin levels were significantly lower ($p<0.05$). Comparing the PCOS and PCOS + Metformin groups to the control group, there was a statistically significant rise in HOMA-IR ($p<0.05$). In contrast to the control group, the HOMA-B levels in the PCOS and PCOS + Metformin groups were significantly lower ($p<0.05$). HOMA-IR significantly decreased in the PCOS + Metformin group compared to the PCOS group ($p<0.05$).

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

When compared to the control group, the cholesterol and LDL levels in the PCOS and PCOS + Metformin groups were significantly higher ($p<0.05$). Comparing the PCOS group

to the control group, there was a statistically significant rise in triglycerides ($p<0.05$). Comparing the PCOS group to the control group, there was a statistically significant drop in HDL ($p<0.05$).

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

TNF-□ and IL-6 levels were increased in the PCOS and PCOS + Metformin groups compared to the control group ($p<0.05$). TNF-□ and IL-6 levels were significantly lower in the PCOS + Metformin group compared to the PCOS group ($p<0.05$). In comparison to the control group, SOD levels significantly decreased in the PCOS and PCOS + Metformin groups ($p<0.05$). SOD levels were elevated in the PCOS + Metformin group compared to the PCOS group ($p<0.05$).

F- Correlations between all studied parameters and serum asprosin in studied groups: (table-2)

Serum asprosin and BMI in the PCOS group showed a significant ($P<0.05$) positive association (Fig.1). Serum asprosin and serum testosterone in the PCOS + Metformin group showed a significant ($P<0.05$) negative correlation (Fig.2).

G- Histopathological study

With one cystic follicle (CF) in the field, the control group displayed normal follicles in varying stages. Insets with higher magnifications show; primary follicle (PF) with multiple layers of follicular cells, antral follicle (AF), oocytes(O) and corpus luteum (CL) (Fig.3a). 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.3b). PCOS+ Metformin groupshowed abundant cystic follicles (CF). Wide spaces appear in the stroma (V) (Fig.3c).

H- Vaginal smear

1) Control group: there are three different types of cells: leukocytes, which are small round cells, irregular non-nucleated cornified cells, and round nucleated epithelial cells. For assessment of the estrous cycle phases, we used their proportion. In the proestrus phase, nucleated epithelial cells with smooth edges predominate in the vaginal smear.The vaginal smear during the estrus phase has big, anucleated, irregularly shaped cornified (keratinized) cells.The vaginal smear during the metestrus phase reveals many cornified cells as well as leukocyte infiltration.In the diestrus phase, the vaginal smear exhibits the presence of tiny leukocytes with no cornified cells.

2) PCOS group: the appearance of prolonged cornified cells in the smears throughout two successive estrous cycles (metestrus and diestrus) suggested both the development of follicular cysts and the effective induction of PCOS.

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

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

Parameter	Control	PCOS	PCOS + Met
BMI (gm/cm^2)	.414± .03	.522± .04 [a]	.485± .04 [a,b]
Serum Asprosin (pg/ml)	52.946± 8.55	367.692± 34.92 [a]	265.538± 28.03 [a,b]
Serum FSH (m IU/ml)	±٢.٥٤٦ .86	±.852 .08 [a]	±١.٥١٢ .45 [a,b]
Serum LH (m IU/ml)	٣.٨٥٨± .99	٥.٦٦٤± 1.22 [a]	±٣.٩٦ .92 [b]
Serum Estradiol (Pg/ml)	474.846± 52.69	138.969± 12.68 [a]	183.538± 9.82 [a,b]
Serum Progesterone (ng/ml)	15.263± 2.95	3.197± .67 a	4.377± 1.06 a
Serum Testosterone (ng/ml)	.473± .11	1.019± .07 [a]	.769± .06 [a,b]
Serum Glucose (mg/dl)	96.507± 9.78	159.453± 15.98 [a]	١٣٧.٠١٥± 22.95 [a,b]
Serum Insulin (ng/ml)	5.655± 1.38	9.597± 1.73 [a]	6.597± 1.79 [b]
HOMA-IR	1.352± .36	٣.٧٧٥± .74 [a]	2.211± .62 [a,b]
HOMA-B	65.383± 24.91	36.745± 8.80 [a]	٣٦.٢٨٣± 16.67 [a]
Serum Total cholesterol (mg/dl)	135.677± 15.24	162.292± 18.82 [a]	١٥٨.٩٠٧± 21.40 [a]
Serum Triglyceride (mg/dl)	92.775± 14.76	111.833± 22.59 [a]	99.337± 13.17
Serum HDL (mg/dl)	54.701± 5.76	50.493± 4.45 [a]	52.855± 5.17
Serum LDL (mg/dl)	٦٢.٤٢٠± 13.99	٨٩.٤٣١± 16.84 [a]	86.184± 22.16 [a]
Serum TNF-α (Pg/ml)	175.769± 37.35	694.769± 133.76 [a]	٢٤٩.٤٣٨± 71.17 [a,b]
Serum IL-6 (Pg/ml)	2.031± .85	9.611± 2.75 [a]	٧.٣٦٤± 1.96 [a,b]
Serum SOD (U/ml)	200.961± 28.86	119.872± 22.17 [a]	145.684± 22.65 [a,b]

Data were expressed as Mean ± SD. a: P<0.05 in comparison with control group. b: P<0.05 in comparison with the PCOS group.
PCOS, polycystic ovary syndrome; PCOS + Met, polycystic ovary syndrome treated with metformin.

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

Group / Parameter	Control		PCOS		PCOS + Metformin	
	(r)	P value	(r)	P value	(r)	P value
BMI (gm/cm^2)	-0.115	0.708	0.707	0.007*	0.109	0.722
Serum FSH (□IU/ml)	-0.173	0.573	0.403	0.172	0.023	0.940
Serum LH (□IU/ml)	0.059	0.849	0.123	0.690	-0.271	0.370
Serum Estradiol (Pg/ml)	-0.089	0.774	0.144	0.640	0.013	0.967
Serum Progesterone (ng/ml)	-0.144	0.638	0.205	0.502	-0.439	0.134
Serum Testosterone (ng/ml)	-0.294	0.330	-0.284	0.347	-0.578	0.039*
Serum Glucose (mg/dl)	0.152	0.620	0.276	0.361	0.345	0.248
Serum Insulin (ng/ml)	-0.219	0.473	0.442	0.130	-0.084	0.786
HOMA-IR	-0.148	0.629	0.515	0.072	0.094	0.759
HOMA-B	-0.225	0.459	0.165	0.591	-0.419	0.154
Serum Total cholesterol (mg/dl)	0.312	0. 299	-0.203	0.506	0.365	0.220
Serum Triglyceride (mg/dl)	-0.442	0.130	-0.207	0.498	-0.517	0.071
Serum HDL (mg/dl)	0.518	0.070	-0.488	0.091	-0.143	0.641
Serum LDL (mg/dl)	0.220	0.470	-0.043	0.890	0.447	0.125
Serum TNF-α (Pg/ml)	0.273	0.368	-0.239	0.432	0.253	0.404
Serum IL-6 (Pg/ml)	0.242	0.426	-0.454	0.119	0.108	0.725
Serum SOD (U/ml)	-0.532	0.061	-0.053	0.864	-0.047	0.879

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

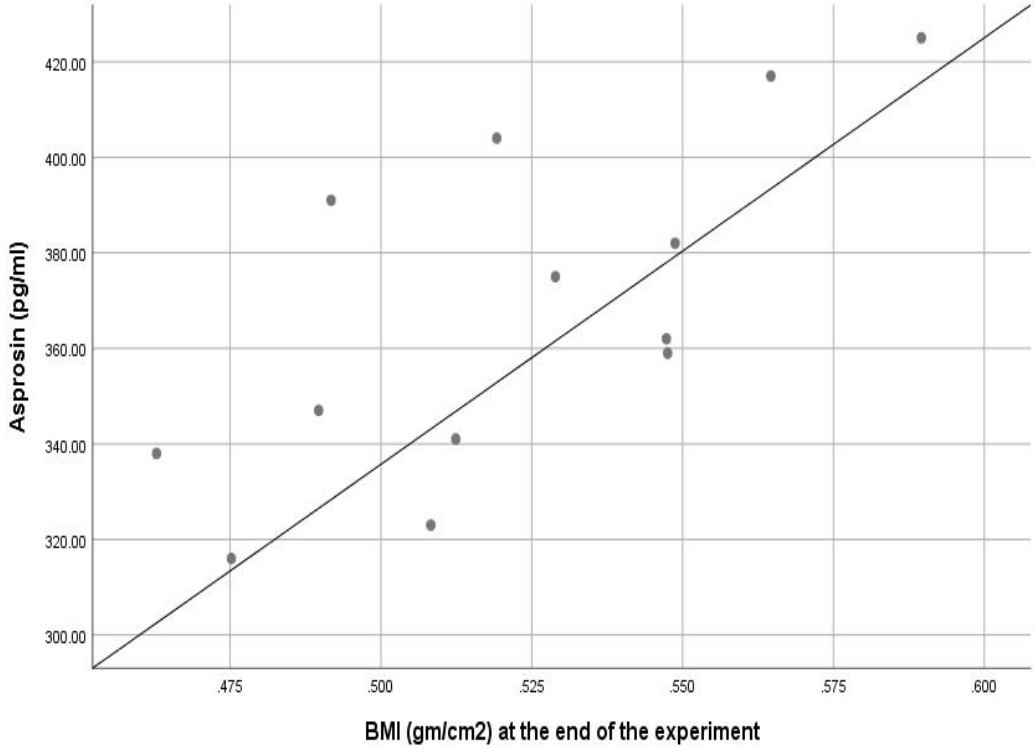

Figure 1: Correlation between asprosin (pg/ml) and body mass index (BMI) (gm/cm^2) at the end of the experiment is positive in PCOS group (r= 0.707, p value =0.007*).* correlation is significant (p value < 0.05).

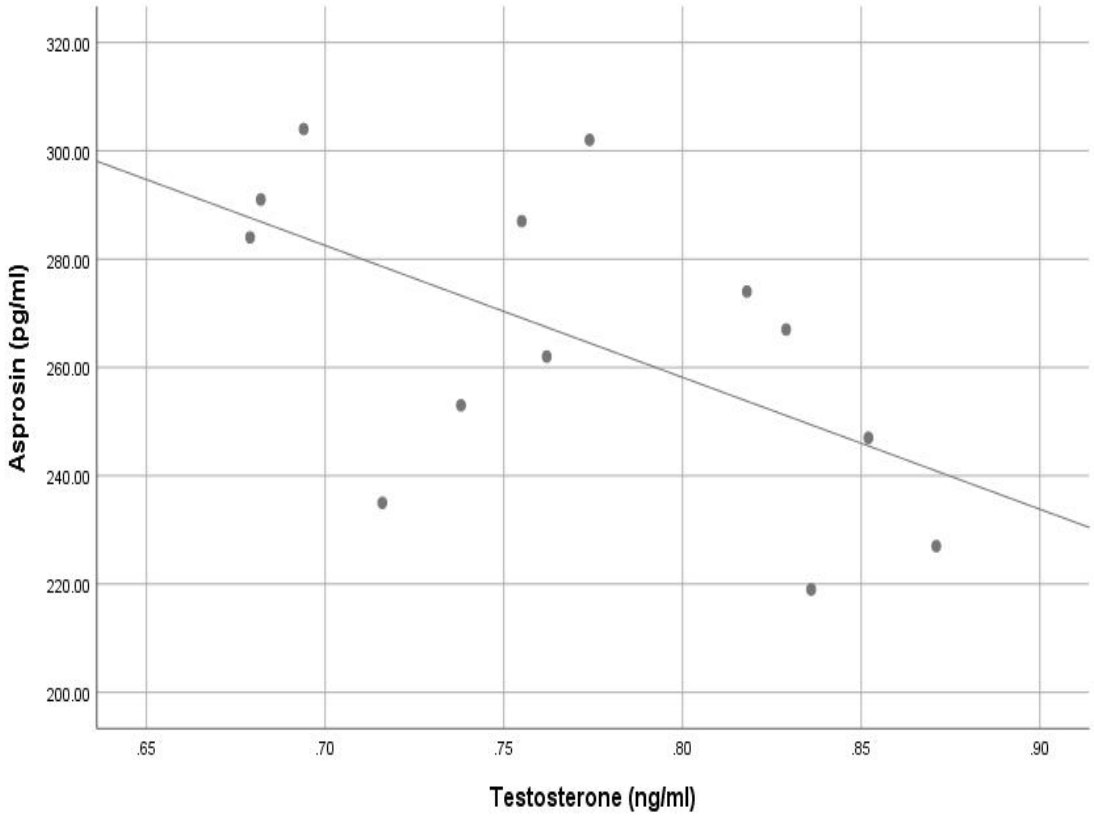

Figure 2: Correlation between asprosin (pg/ml) and testosterone (ng/ml) is negative in **PCOS + metformin group** (r= -0.578, p value = 0.039*). *Correlation is significant (p value < 0.05)

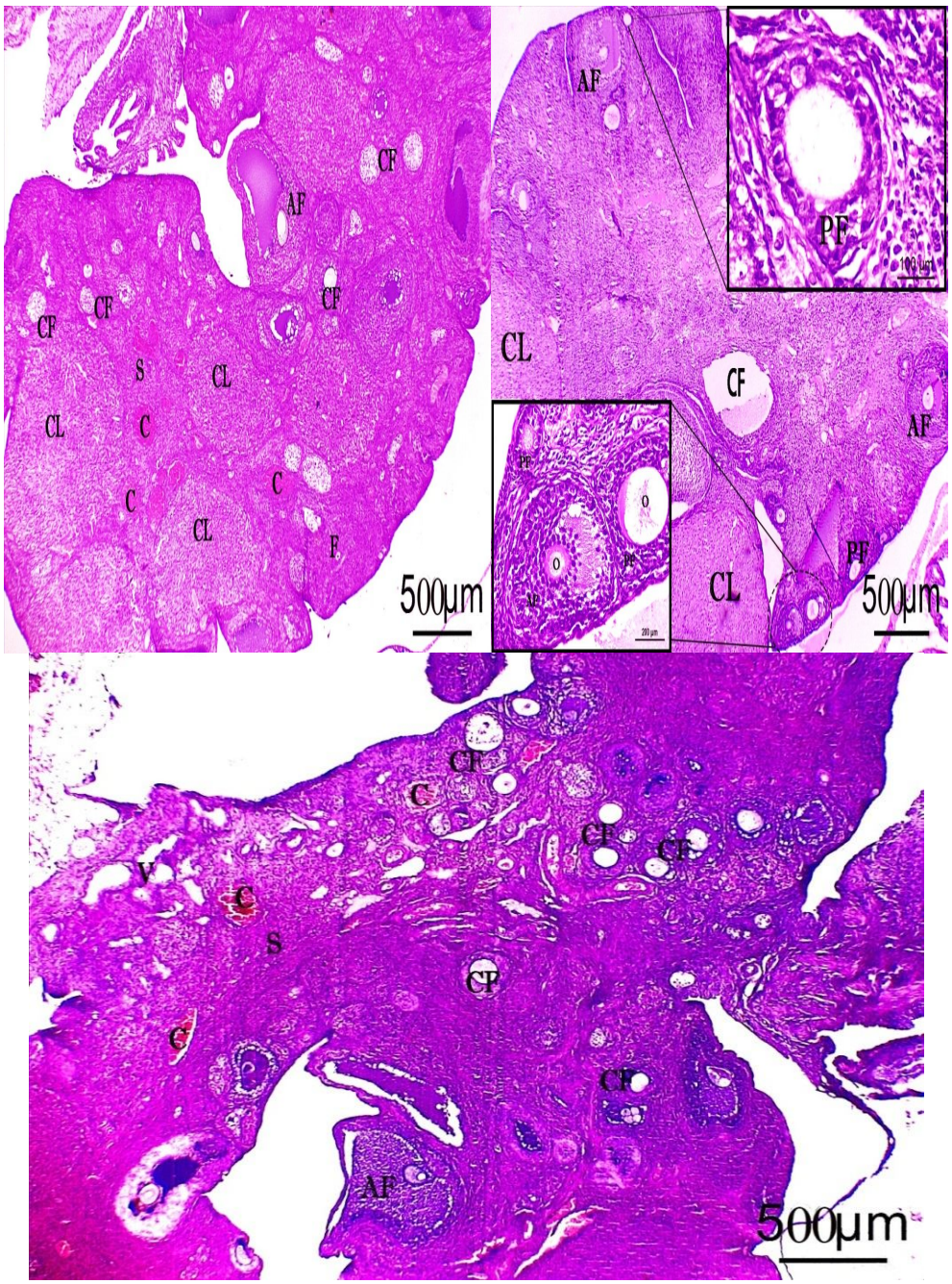

Figure 3: Ovarian histopathological examination showing a plate stained with hematoxylin and eosin of the rat ovarian tissue in all studied groups under light microscope(IHC x40). (a) Control group (b) PCOS group (c) PCOS+ Metformin group.

DISCUSSION

As letrozole administration based on 3 weeks of daily treatment created poor ovarian microenvironment, development of multiple ovarian cysts, and continuation of the estrous cycle at the metestrus and diestrus stages, the experimentally induced PCOS in this study is comparable to that of the human condition [16]. Letrozole caused considerable hyperandrogenism and a correspondingly significant decrease in the levels of estrogen and progesterone, which served as evidence that the rats had PCOS.

According to our research, animals in the PCOS group had a significantly higher BMI than those in the control group at the end of the experiment. Imşek and Polat [17] provided support for this finding.

Interestingly, we demonstrated that the PCOS group treated with metformin had a considerably lower BMI than the PCOS

group, which was previously observed by Bhandari et al. [18].

In line with Li et al. [8] and Alan et al. [10], our results showed that serum asprosin levels were significantly higher in PCOS rats when compared to controls, which could be related to obesity observed in PCOS group as reflected by increased BMI as asprosin is adipokine. However, Chang et al. [19] stated that asprosin was not linked to the causes of PCOS.

It was reported that asprosin influences insulin sensitivity and resistance, and that PCOS is linked to hyperinsulinemia. Therefore, rising asprosin levels in PCOS patients' blood may contribute to the etiopathology of this condition [8,10].

The insulin resistance reported in patients with PCOS may have been caused by a high level of asprosin in PCOS. This is because asprosin mediates release of glucose from liver into circulation, which causes accumulation of glucose in the circulation and excessive insulin secretion to bring the increased circulating glucose to physiological limits. Thus, cells cannot utilize insulin due to changes in insulin receptors after a certain threshold, and insulin resistance occurs [2,10].

In the current investigation, metformin treatment resulted in a significant drop in asprosin serum levels compared to the PCOS group. Gozel and Kilinc [20] provided evidence that plasma asprosin levels after three months of metformin treatment reduced considerably in comparison to the baseline, hence supporting our findings.

Despite having opposing effects on several routes, asprosin and metformin are crucial for maintaining the body's glucose homeostasis [20].

A significant positive association between serum asprosin levels and BMI in the PCOS group was found in the current investigation. These findings are consistent with results discovered by Alan et al. [10]. This may be connected to the idea that asprosin acts as an orexigenic hormone at the central level [3].

In the current study, PCOS rats' serum levels of FSH, estrogen, and progesterone are much lower than control rats', whereas testosterone and LH levels are significantly higher. These findings supported the presence of PCOS and are consistent with those of Bednarska and Siejka [21], who showed that high LH levels and an elevated LH:FSH ratio can be used as biomarkers to identify PCOS in female patients.

Histological analysis shows that rats with PCOS have weak granulosa cell layers, poor follicular development, and a large number of subcapsular cysts. Theca interna cells in the follicular cysts were also thicker and more hyperplastic, and the epithelioid cell layers facing the antrum were flattened. Letrozole-induced follicular dysfunction, including atretic cysts with sparse granulosa cells, and these findings are corroborated by Rajan et al. [22].

Metformin provided a notable improvement in reproductive functioning as seen by the PCOS rats' much lower serum levels of testosterone and LH and significantly higher levels of estrogen and FSH. Along with the restoration of the estrous cyclicity and the histopathological examination's modest improvement.

These results concur with those of Kocer et al. [23], who observed a significant decline in free testosterone, LH levels, and LH/FSH ratio in PCOS patients treated with metformin. Furthermore, Xiao et al. [24] found that after receiving metformin medication, the PCOS mice had an increase in the number of corpora lutea and regular estrous cycles, suggesting that metformin treatment can restore ovulatory dysfunction in PCOS animals.

Because of the restoration of ovulation, normalization of estrogen levels, and modification of the effect of insulin on ovarian androgen production and theca cell proliferation, metformin aids in the improvement of hyperandrogenic symptoms [25].

In the current research, we discovered a

strong inverse relationship between testosterone levels and serum asprosin in the PCOS + metformin group. This result is consistent with that of Jiang et al. [26].

According to Li et al.'s research [8], asprosin in PCOS may reflect insulin resistance and obesity, two factors that may be associated to the pathogenesis of PCOS' abnormal sex-related hormone metabolism.

Compared to the control group in this study, the PCOS group's serum glucose level was noticeably higherwhich is consistent with Alan et al. [10].

In this work, fasting serum glucose levels significantly dropped after taking metformin by PCOS rats compared with PCOS groupwhich reflects a potential mechanism by which metformin may improve PCOS through its blood glucose lowering effect. This finding is corroborated by Kumar et al. [27], who discovered that metformin treatment dramatically reduced fasting blood glucose in PCOS patients who had been diagnosed compared to the PCOS group who hadn't received treatment.

According to several research, metformin's capacity to lower hepatic glucose production and boost insulin-stimulated glucose uptake and glycogenesis in skeletal muscle via AMPK activation is what causes it to drop blood sugar levels [28].

In the current study, HOMA-IR and serum insulin levels in PCOS rats were both considerably higher than those in control rats. While in the same group, the HOMA-B index significantly decreased. These results reflected occurrence of insulin resistance which were noticed with other studies like Alan et al. [10] and Polat and Şimşek [17].

When compared to PCOS group, treatment of PCOS rats with metformin resulted in significantly lower serum levels of insulin, and HOMA-IR. This result is an evidence that metformin has an insulin sensitizing effect, which may be a mechanism by which it ameliorates metabolic abnormalities in PCOS. These outcomes are consistent with those of Lovvik et al. [29], who discovered that metformin dramatically improved insulin sensitivity in PCOS-affected females.

Metformin-mediated increases in insulin sensitivity may be attributed to the higher insulin receptor tyrosine kinase activity, higher glycogen synthesis, and improved recruitment and activity of glucose transporter type 4 [30].

In the current investigation, compared to control rats, PCOS rats displayed considerably elevated serum levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL), as well as a significantly lower level of HDL.

According to Li et al. [8] and Alan et al. [10], who observed a significant rise in serum TG levels in women with PCOS compared to the control group, these data validated the presence of dyslipidemia in PCOS. Also, Chang et al. [19] discovered that PCOS women had considerably higher serum levels of TC, TG, and LDL than normal women. Additionally, Yilmaz et al. [31], Li et al. [8] and Alan et al. [10] declared that PCOS women had considerably lower HDL levels in their serum than women without the condition.

According to Lewis et al. [32], insulin resistance is one of the main causes of dyslipidemia in PCOS due to metabolic abnormalities that take place 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. Dyslipidemia observed with PCOS in the current study could be explained by the presence of insulin resistance.

When compared to the PCOS group, the metformin treatment in this study's PCOS rats resulted in a non-significant drop in serum levels of TC, TG, HDL, and LDL. These results are consistent with Yilmaz et al.'s [31] finding that serum lipid profile levels, including TC, TG, LDL, and HDL, did not significantly change in PCOS patients after metformin treatment at 850 mg twice daily for 12 weeks when compared to the PCOS group before treatment.

In the present study, the PCOS group's serum TNF-□ level was considerably greater than that of the control group. This is consistent with Gonzalez [33], who discovered that normal-weight PCOS women's serum TNF-□ concentration was considerably higher than that of normal-weight controls.

Additionally, when compared to the PCOS non-treated group, metformin administration to PCOS rats resulted in a substantial drop in TNF-□ serum level. Elbandrawy et al.'s findings [34]that metformin 1,500 mg daily for 12 weeks caused a significant reduction in TNF-□ in women with PCOS compared to the PCOS group before treatment add evidence to this result.

In our investigation, the PCOS group's serum level of IL-6 was considerably greater than that of the control group. The findings of Ghowsi et al. [35], who claimed that PCOS is a chronic low grade inflammatory condition linked to elevated levels of proinflammatory cytokines, IL-6 and TNF-□, confirm this result.

The production of additional proinflammatory cytokines (TNF-□, IL-6) in adipose tissue is encouraged by excessive androgen secretion in PCOS, whereas the release of the anti-inflammatory cytokine ADP is inhibited. ADP prevents the synthesis of testosterone precursors, such as androstenedione, and increases the levels of androgens when it is depleted [36].

As compared to the PCOS group, metformin treatment resulted in a considerable drop in the serum level of IL-6 in PCOS rats. This finding is corroborated by Lin et al. [37], who discovered that PCOS women receiving metformin 500 mg three times per day for 12 weeks experienced a significant reduction in IL-6 levels.

Through its anti-inflammatory activity via AMPK activation pathway and consequent suppression of nuclear factor kappa B subunit 1 (NFKB), metformin prevents the recruitment, migration, and proliferation of macrophages that produce inflammatory cytokines (IL-6 and TNF-□) [38].

In this study, it was discovered that PCOS rats had significantly lower serum levels of SOD than controls.

Liu and Zhang's findings [39], which showed that PCOS patients' serum SOD levels were considerably lower than those in the control group, corroborate our findings.

Oxidant status levels can rise as a result of insulin resistance and hyperglycemia. The androgen-producing ovarian steroidogenic enzymes are stimulated by oxidative stress, whereas these enzymes are suppressed by antioxidants [40].

When compared to the PCOS group, metformin treatment resulted in a substantial rise in the blood level of SOD in PCOS rats. Our findings are consistent with those of Kocer et al. [23], who claimed that hyperandrogenemia contributes to the development of oxidative stress in PCOS and that metformin may lessen this stress by reducing levels of androgen.

Limitations of study

1-Immunohistochemistry of ovarian tissues with specific antibodies should be done to detect the possible underlying pathogenesis.

2-Using animals may give results that are not the same as humans. Thus, further study should be conducted on humans.

CONCLUSION

Taking the present findings together, it could be concluded that metformin treatment of PCOS resulted in a beneficial effect in amelioration of ovarian and metabolic changes that occurred with PCOS. This was achieved through its anti-inflammatory, antioxidant, insulin-sensitizing, and asprosin-regulating actions. Moreover, for PCOS patients, lowering asprosin serum levels seems like a promising treatment target.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the support from the staff members of both the Biochemistry, and Anatomy & Embryology departments, Zagazig Faculty of Medicine, for biochemical analysis, and

histopathological examination.

RECOMMENDATIONS

Additional clinical research is necessary to evaluate whether metformin combined with dietary changes can treat PCOS individuals' impaired fertility. Additionally, a deeper comprehension of asprosin's mechanism of action might make asprosin the crucial connection connecting PCOS risk variables.

CONFLICT OF INTEREST

No conflict of interest

FINANCIAL DISCLOSURES

No financial support

CONTRIBUTORS

Each author has made a significant contribution to the research concept, data collecting, processing, and discussion. The final article has been approved by all authors.

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Citation:

Abulfadle, K., Abdallah, A. B. E., Ragab, E., Mohammed, N. Effect of metformin treatment on serum asprosin level changes in rat model of polycystic ovary. *Zagazig University Medical Journal*, 2023; (4150-4164): -. doi: 10.21608/zumj.2023.205123.2785