Spexin and Metformin Comparative Ameliorated Ovarian and Liver Function Changes in Letrozole-Induced Polycystic Ovary Syndrome in Rats (Histological, Biochemical, Immunohistochemical and Morphometric Study)

Original Article

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) is characterized by polycystic ovarian follicle morphology, ovulatory dysfunction, hyperandrogenism and insulin resistance. Spexin has a role in satiety control, lipid and glucose metabolism. Metformin is an insulin sensitizing agent that has some hepato-protective functions.

Aim: This study aimed to declare and compare the effect of spexin and metformin treatment on ovarian and liver function changes in letrozole-induced PCOS in rats and the possible mechanisms involved.

Materials and Methods: Thirty-two adult female Wistar albino rats divided into control, polycystic ovary syndrome (PCOS), PCOS metformin-treated, and PCOS spexin-treated groups. In PCOS groups, rats were given oral letrozole 0.5 mg/kg dissolved in 0.9% NaCl once daily along the study. In PCOS metformin-treated group, rats were treated with metformin hydrochloride orally (300 mg/kg/day) for the last 4 weeks. In PCOS spexin-treated group, rats were treated with spexin i.p. 35 µg/kg/day, dissolved in normal saline for the last 4 weeks.

Results: In PCOS group, there was a significant increase in; BMI, relative ovarian weight, HOMA-IR, serum (LH, FSH, testosterone, insulin, glucose, TC, TG, LDL, ALT, AST, alkaline phosphatase, total & direct bilirubin) and hepatic (MDA, NO & TNF-α), with a significant decrease in; serum [estradiol, progesterone, HDL, albumin and spexin] and hepatic GPX, when compared with the control group. Histopathological results showed ovaries of PCOS marked expression of caspase-3 and P53 and decreased KI67 expression of granulosa and theca cells. Vacuolated hepatocytes, dilated and congested portal vein and positive reaction of immunostaining with αSMA, caspase and TNFα. Treatment of PCOS rats with either metformin or spexin reversed these changes significantly.

Conclusion: Spexin serum level could be used as a metabolic biomarker for polycystic ovary syndrome. Also, treatment with either metformin or spexin ameliorated ovarian and hepatic dysfunctions in rat model of PCOS through their insulin sensitizing, anti-oxidant and anti-inflammatory effects.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by polycystic ovarian follicle morphology, ovulatory dysfunction and hyperandrogenism[1]. It is the most common cause of anovulatory infertility in women during reproductive period[2]. Diamanti-Kandarakis and Dunaif[3] and Manco, Castagneto-Gissey[4] reported that PCOS was associated with glucose/insulin intolerance, obesity, dyslipidemia, endothelial dysfunction, oxidative stress and increased systemic inflammation. Liver diseases remain one of the serious health problems throughout the world associated with a high rate of morbidity and mortality[5]. Barfield, Liu[6], Targher, Solagna[7] and Vassilatou, Lafoyianni[8] had been noted that women with PCOS had an increased prevalence of liver changes as non-alcoholic fatty liver disease (NAFLD), steatosis, fibrosis and elevated liver enzymes irrespective of presence of obesity or overweight. As insulin resistance plays a key role in PCOS pathophysiology, insulin sensitizing agents were used in its treatment. Metformin acts as an insulin sensitizing agent causing a reduction of hepatic glucose secretion and increase of peripheral glucose utilization with positive effects on insulin resistance, body weight, and menstrual cycling in PCOS[9]. Loomba, Lutchman[10] reported a beneficial effect of metformin on liver function with improvement of liver enzymes and hepatic steatosis (HS). Also, Haukeland, Konopski[11], Gangale, Miele[12] and Tan, Vollmar[13] demonstrated improvement of

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liver enzymes in patients with PCOS under metformin treatment. Spexin, a peptide hormone, is expressed widely in human and rat normal endocrine and epithelial tissues including ovary, liver, pancreas and adipose tissue[14,15]. Few and contradictory data were available on changes in serum levels of spexin in cases of PCOS. Anik Ilhan and Yildizhan[16] and Guler and Demir[17] reported that women with PCOS had significant lower levels of serum spexin in comparison to the controls, while Flora, Vasiliki[18] found no significant differences in serum spexin concentrations in adolescents with PCOS in comparison with controls. Ge, Walewski[19] declared that spexin treatment in mice with HS/ NAFLD inhibited hepatocellular long-chain fatty acid uptake and reduced HS. As scarce data were available on the effect of spexin treatment on ovarian and liver function changes in rats with PCOS, thus this study was designed to investigate the possible effects of spexin and metformin on changes in ovarian and liver functions in letrozole-induced PCOS in rats and the possible mechanisms involved through evaluation of biochemical, antioxidant, anti-inflammatory and histopathological changes in ovarian and liver tissues.

MATERIALS AND METHODS

This study was performed in Physiology Department, Zagazig Faculty of Medicine, during the period between December 2019 and March 2020. Thirty-two adult female Wistar albino rats weighing 170-195 g and exhibiting normal estrous cycle were taken from Animal House, Faculty of Medicine, Zagazig University. Animals were housed in steel wire cages (50x30x20 cm), 4 rats/cage, under hygienic conditions with room temperature being maintained at 25±2°C and normal light/dark cycle for acclimation period of 2 weeks. Animals were given standard chow and water ad libitum. Rat standard chow was obtained from Faculty of Agriculture, Zagazig University, and consisted of protein 25.8%, carbohydrates 62.8% and fat 11.4%. The experimental protocol in this study was conducted according to the data guiding the use of research animals and was approved by the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC/3/F/11/2021).

After the period of acclimatization, rats were equally and randomly divided into control, polycystic ovary syndrome (PCOS), PCOS metformin-treated, and PCOS spexin-treated groups (Table 1). In the control group, rats were fed standard chow along the study (7 weeks) with free access to water and each rat was given orally 1 ml of 0.9% NaCl once daily by oral gavage. In PCOS group, rats were fed standard chow along the study with free access to water and each rat was given orally (using oral gavage) letrozole (non-steroidal aromatase inhibitor) 0.5 mg/kg dissolved in 0.9% NaCl once daily along the study[20]. Also, each rat in PCOS group was given orally 1 ml of 0.9% NaCl once daily by oral gavage for the last 4 weeks of this 7-weeks study. Letrozole was purchased from Sigma-Aldrich Co., England. In PCOS metformin-treated group, rats were managed as in PCOS group except that in the last 4 weeks of the study (from the start of 4th week to the end of 7th week) they were treated with metformin hydrochloride (obtained as Cidophage 500 mg tabs, from CID, Egypt) orally (300 mg/kg/day)[21] by oral gavage. In PCOS spexin-treated group, rats were managed as in PCOS group except that in the last 4 weeks of the study, they were treated with spexin (Phoenix Pharmaceuticals; Belmont, CA, USA), intraperitoneal (i.p.) 35 µg/kg/day, dissolved in normal saline[22]. Both the control and PCOS groups were injected intraperitoneally daily by 0.5 ml normal saline for the last 4 weeks of the study. During the experiment, phases of the estrous cycle were monitored by analysis of relative proportion of leukocytes, cornified and epithelial cells in the vaginal smear. A vaginal smear was obtained daily at 8-9 am by vaginal washing with saline, and the fresh unstained samples were evaluated microscopically to investigate the phase of the estrous cycle[20,23]. Cycles with duration of 4 to 5 days were considered regular. Estrus phases were determined according to Goldman, Murr[24] by examining the vaginal smear into proestrus (predominance of nucleated epithelial cells with smooth margins), estrus [large non-nucleated cornified (keratinized) cells with irregular margins]met estrus (many cornified cells plus infiltration of leukocytes), and diestrus (absence of the cornified cells and presence of small leukocytes). The observation of cornified cells in the smears during a minimum of 10 consecutive days was considered as a persistent estrous, indicating anovulation and development of follicular cysts[25].

Table 1: Experimental Design

	Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Duration of the study (weeks)	7	7	7	7
Number of rats	8	8	8	8
Oral letrozole treatment along the study		√	√	√
Oral saline along the study	√			
Oral saline for the last 4 weeks	√	√		
Oral metformin treatment for the last 4 weeks			√	
i.p. injection of spexin for the last 4 weeks				√
i.p. injection of normal saline for the last 4 weeks	√	√		

PCOS, polycystic ovary syndrome; i.p. intraperitoneal.

At the end of the study, rats weighed and final body mass index (BMI) were calculated according to Novelli, Diniz[26] from the following equation: BMI =body weight (g)/length2 (cm2) (from nose to anus).

Rats were anaesthetized by thiopental (50mg/kg)[27]; blood samples were collected from rat tail vein (sampling of rats in control group was taken in the estrus phase). Blood was left 30 minutes at room temperature for clotting, then, centrifuged at 3000 rpm for 15 minutes, the supernatant serum was stored at -20°C until assay. In the Biochemistry Laboratory, Zagazig Faculty of Medicine, collected serum was used to measure levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), glucose and insulin using commercial kits (MyBioSource.com, California, USA) with catalog No., MBS764675, MBS2502190, MBS7233226 and MBS289124; respectively. Also, commercial kits were purchased (Sigma-Aldrich, USA) for estimation of estradiol, progesterone and testosterone levels with catalog No., SE120084, SE120087 and SE120089; respectively. Serum spexin level was assessed using commercial kits (catalog No. DEIA10757; Creative Diagnostics, USA), with detection range 0-100 ng/ml and sensitivity 0.11 ng/ml.

Homeostasis model assessment of insulin resistance (HOMA-IR) index = Fasting serum glucose (mg/dl) x Fasting serum insulin (μIU/ml)/405[28]

Homeostasis model assessment of β-Cell function (HOMA-β) = (360 x Fasting insulin in μIU/ml)/(Fasting glucose in mg/dl-63)[29]

Estimation of lipid profile

Kits for estimation of serum levels of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) were purchased from LifeSpan BioSciences, Washington, USA (catalog No. LS-F27920, LS-F66463 and LS-F54296, respectively).

Serum low-density lipoprotein (LDL) levels (mg/dl)= (TC) – [(HDL) + (TG/5)][30]

After finishing blood sampling, rats were sacrificed by cervical dislocation, their abdominal cavities were opened, ovaries and liver were dissected and weighted. Relative ovarian weight (g/100g body weight) was calculated[31].

Assessment of oxidative/antioxidative and proinflammatory parameters

The liver specimens were removed quickly, washed with cold saline to exclude blood cells, and blotted on filter paper. The liver was divided into two parts. The 1st part (1g) was suspended in 4 ml physiological saline 0.9% Nacl for homogenization. The tissue homogenate was centrifuged at 3000 rpm for 20 minutes at 4oC. The supernatants were kept at -20oC in Eppendorf tubes until biochemical assays[32]. With commercial ELISA kits, estimation of hepatic malondialdehyde (MDA) (catalog No. ABIN6240809; antibodies-online, Schloss-Rahe-Str., Aachen, Germany), hepatic glutathione peroxidase (GPX) (catalog No. MBS744364; MyBioSource.com, 153308 San Diego, California, USA), hepatic nitric oxide (NO) (catalog No. DEIA-BJ2206; Creative Diagnostics, USA) and hepatic tumor necrosis factor-alpha (TNF-α) catalog No. EK0526; BosterBio, Pleasanton, USA) was done. The 2nd part of the liver was placed in 10% formalin solution for histopathological investigation.

Histopathological examination

Ovarian and liver histopathology

Dissected right ovaries and liver specimens were fixed in buffered formalin 10% at room temperature, processed for paraffin sections of thickness of 5 μm, sections mounted on balsam coated slides in case of Hematoxylin and Eosin (H&E) staining and poly-L-lysine coated and charged slides in case of immunostaining. The sections were subjected to the following histological stains:

- Hematoxylin and Eosin (H&E)[33].
- Immunohistochemical staining of ovarian and liver specimens:

KI67 antibodies to test for proliferation of granulosa cells according to[34].

To investigate p53 and anti-caspase 3 according to[35].

To detect α SMA according to[36].

Immunohistochemistry for TNF-alpha[37].

Avidin–biotin complex was applied to the sections. They were incubated at +4oC overnight, deparaffinized and rehydrated by a series of graded alcohols. Boiling the sections in 0.1 M sodium citrate to perform antigen retrieval then were immersed in 3 % H_2O_2 to inhibit endogenous peroxidase activity. The sections were then incubated with 10 % normal goat serum for 1 h at room temperature to block the non-specific binding of antibodies, after which they were incubated with primary antibodies targeting KI67 [Anti KI67 antibody: KI67 (Clone SP6): A rabbit monoclonal antibody (Lab Vision Corporation laboratories, USA, RM-9106-R7)], p53 (Abcam, rabbit polyclonal IgG, Cat. #ab8105), active caspase 3 (Novus, rabbit polyclonal IgG, Cat. #NB100- 56113), anti-a-SMA, (clone 1A4, at 1: 1,200 dilutions in PBS, bought from BioGenex) and Immunohistochemistry for TNF-alpha was performed by using TNF-alpha IHC Antibody (polyclonal, Abbiotec, San Diego, CA, USA) diluted at 1:80 in phosphate- buffered saline (PBS). Biotinylated anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) was added to the sections for 30 min at room temperature. Staining tissue sections without the primary antibody were negative controls. The antigen–antibody complex was detected using a streptavidin–biotin–peroxidase kit (Vector Laboratories). Finally, the sections were developed using a 3,3-diaminobenzidine substrate kit (DAB, Vector Laboratories) to visualize immunolabelling and were counterstained with Mayer's hematoxylin.

Morphometric study

In Anatomy Department in Zagazig Faculty of Medicine, the image analyzer (the Image J software plugin) was used as follow:

Sections of H&E were analyzed morphometrically by using image analyzer computer system. Data were collected by using Leica Qwin 500 Image Analyzer Computer System (England) in the image analysis unit in Human Anatomy and Embryology Department. The number of cystic follicles was estimated by using the measuring field menu in H&E slides of ovary in random microscopic areas under 100 high power fields. A mean of 15 readings was estimated from 5 serial sections from slides of each animal in each group.

To evaluate ovarian morphology and follicle number, H&E staining were applied on every 20th section of the ovary so that each section was separated by a distance of approximately 50–60 μm from the next 20th section to avoid counting the same follicle twice[38,39]. Only oocyte containing ovarian follicles were considered and follicles were classified as primordial follicle (flattened follicular cells surrounding the oocyte), unilaminar primary follicle (one layer of cuboidal follicular cells surrounding the oocyte) and multilaminar primary follicle (two or more layers of cuboidal follicular/granulosa cells surrounding the oocyte), secondary follicle (one or more cavity inside the granulosa layer with no visible antrum), Graafian follicle (a large antral cavity filled with secretory fluid)[40] and atretic follicle (presence of signs of oocyte and/or granular degeneration such as pyknosis of the nucleus, infolding of the cell wall in oocyte, ingression of granulosa cells within the antral cavity, pulling away of granulosa cells from the basement membrane, in folding or thickening of base membrane and uneven layers of granulosa cells)[38].

In addition, the area of KI67, caspase-3 and P53 positive immune reaction was measured in KI67, caspase-3 and P53 immune stained sections of ovary respectively under 400 high power fields. Moreover, the area of α SMA, TNF-alpha, and caspase-3 positive immune reaction was measured in α SMA, TNF-alpha, and caspase-3 immune stained hepatic sections respectively under 400 high power fields.

The ovarian scorings were done in 8 non-overlapping sections (×400) from each slide. A histopathological study of each parameter was done e.g. degeneration, congestion, edema, and hemorrhage based on a scoring system where none = 0, mild = +1, moderate = +2, and severe = +3. The total scores were estimated according to these parameters[41].

Histopathological alterations in liver were evaluated and scored as (0) indicated no changes, (1), (2) and (3) indicated mild, moderate, and severe changes, respectively, while the grading was determined by percentage as follows: (< 30%) showed mild changes, (30–50%) indicated moderate changes, and changes more than 50% indicated severe changes[42,43].

Statistical Analysis

The obtained data were expressed as mean ± standard deviation (SD). For statistical significance, one-way analysis of variance (ANOVA) and Tukey HSD for Post hoc multiple comparisons were used to compare means. The software, IBM SPSS Statistics (Version 26 Software for Windows), was used for that purpose. Also, GraphPad Prism (Version 8 Software for Windows) was used to analyze the Pearson's correlation between serum spexin and different studied parameters within PCOS group. Significance was considered with $P\ value \leq 0.05$. For statistical significance of ovarian and hepatic scoring, Kruskal-Wallis test and Tukey HSD for Dunn's Multiple Comparison Test was used to compare means as the data was not normally distributed.

RESULTS

Estrous cyclicity

All rats in the control group showed regular estrous cycles with a duration of 4–5 days. In the PCOS group, rats were completely acyclic and exhibited a constant estrous state when compared with that in the control group. With either metformin or spexin treatment, PCOS rats showed normal estrous cycles in comparison to rats of the control group.

Changes in final BMI and relative ovarian weight in different studied groups (Table-2)

In PCOS group, there was a significant increase in final BMI and relative ovarian weight when compared with the control group. On treatment of PCOS rats with either metformin or spexin, a significant reduction was recorded in both final BMI and relative ovarian weight as compared with the PCOS group.

Changes in serum level of gonadotrophins and sex hormones in different studied groups (Table-2)

There was a significant increase in serum level of LH, FSH and testosterone, with a significant decrease in serum level of estradiol and progesterone in PCOS group when compared with the control group. These changes were significantly reversed by treatment of PCOS rats with either metformin or spexin as compared with PCOS group.

Metabolic changes in different studied groups (Table-2)

In PCOS group, there was a significant increase in serum (insulin, glucose, TC, TG and LDL), and the value of HOMA-IR, with a significant decrease in serum level of HDL and the value of HOMA-β when compared with the control group. All these changes were significantly reversed (except for the value of HOMA-β which showed no significant change) by treatment of PCOS rats with either metformin or spexin as compared with PCOS group.

Changes in serum level of spexin in different studied groups (Table-2)

There was a significant reduction in spexin serum level in both PCOS and PCOS metformin-treated groups when

compared with control group. On treatment of PCOS rats with either metformin or spexin, significant elevation in serum spexin was recorded in comparison to PCOS group.

Liver function changes in different groups (Table-3)

In PCOS group, there was a significant increase in serum (ALT, AST, alkaline phosphatase, total & direct bilirubin) and hepatic (MDA, NO & TNF-α), with a significant reduction in serum albumin and hepatic GPX when compared with the control group. All these changes were significantly reversed on treatment of PCOS rats with either metformin or spexin as compared with PCOS group.

Correlation between serum spexin level and some studied parameters within the PCOS group (Table-4)

There was a significant negative association between serum spexin level and each of final BMI, serum testosterone, serum glucose, HOMA-IR, serum TC, serum ALT, serum AST, and hepatic TNF-α. On the other hand, there was a significant positive correlation between serum spexin and each of serum estradiol, serum HDL, serum albumin and hepatic GPX.

Histopathological analysis of the ovary

Ovarian H&E

In control rats, the ovarian section was formed from cortex and medulla (Figure 1a). The cortex is formed of multiple stages of ovarian follicles, primordial follicle, primary follicle, secondary follicle, Graafian follicle and corpus luteum (Figure 1b). The nuclei of their follicular cells were pale. The primary follicles were with its 2 types, the uni-laminar follicle and multi-laminar follicle lined with follicular cells containing the primary oocyte which was lined with zona pellucida (Figure 1c). The Graafian follicle contained antrum which was lined with granulosa cells, theca interna cells and theca externa cells (Figure 1d). The ovarian section of PCOS group was formed of cortex; had corpus luteum, multiple atretic and cystic follicles (Figure 1e), and medulla that filled with congested blood vessels (Figure 1f). The atretic follicle was lined with multiple layered vacuolated granulosa, theca interna and theca externa (Figure 1g). The cystic follicles were noted near congested blood vessels (Figure 1h). Ovarian section of PCOS metformin-treated albino rat was formed of cortex and medulla, with corpus luteum, Graafian follicle, primary, atretic and cystic follicles (Figure 2a). The cortex showed multiple primary and secondary follicles with few congested blood vessels (Figure 2b). The Graafian follicle contained antrum and lined with granulosa cells, theca interna cells and theca externa cells. (Figure 2c). Ovary of PCOS spexin-treated albino rat showed corpus luteum, Graafian follicle, atretic and cystic follicles with some areas of congestion (Figure 2d). Graafian follicle was formed from granulosa cells, secondary oocyte, surrounded by zona pellucida, corona radiata and attached by a base formed from cumulus oophorous, theca interna and theca externa and antrum (Figures 2e, 2f).

Ovarian immunostaining for KI67

Ovary of control group showed strong KI67 positive cells in all granulosa cells, theca interna and theca externa cells (Figure 3a). In PCOs group there was an apparent decrease in KI67 positive cells in all granulosa cells, theca interna and theca externa cells (Figure 3b). In PCOs metformin-treated group, ovarian specimen showed moderate immune reaction for KI67 in all granulosa cells, less in theca interna and theca externa cells (Figure 3c). On treatment of PCOS with spexin, there was strong presentation of KI67 positive cells in all granulosa cells, fewer in theca interna and theca externa cells (Figure 3d).

Ovarian immunostaining for caspase-3

Section of control group showed very weak positive cytoplasmic caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells, with minimal observable reaction in their nuclei (Figure 3e). In PCOS group, there was an apparent increase in cytoplasmic positive caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells and in their nuclei (Figure 3f). In PCOS metformin-treated group, there was moderate to severe positive cytoplasmic caspase-3 reaction in of in all granulosa cells, less in theca interna and theca externa cells and their nuclei (Figure 3g). In PCOS spexin-treated group, there was a moderate presentation of cytoplasmic positive caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells and their nuclei (Figure 3h).

Ovarian immunostaining for P53

Ovarian section of a control rat showed very weak positive P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (Figure 3i). There was an apparent increase in positive P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells of ovary of PCOS group (Figure 3j). This reaction showed mild positivity in all granulosa cells and less in theca interna and theca externa cells in PCOS rats treated with either metformin or spexin (Figures 3k, 3l).

Histopathological analysis of the liver

Hepatic H&E

In control rats, liver section showed well preserved cords of polygonal acidophilic hepatocytes with vesicular nuclei. Thin blood sinusoids radiating from thin walled central vein, between hepatocytes cords and their lining endothelium are seen (Figure 4a). The portal area contained a thin walled portal vein and bile duct lined with cuboidal cells (Figure 4e). However, in the liver of PCOS rats there was congested thick-walled central vein (CV) with highly vacuolated hepatocytes with dark nuclei, degenerated hepatocytes and areas of congestion (Figure 4b). The portal area is formed of multiple bile ducts, dilated congested portal vein had thick-walled hepatic artery with inflammatory cells (Figure 4f). These alterations decreased partially in PCOS metformin-treated

group (Figures 4c, g) and markedly in PCOS spexin-treated group (Figures 4d, h), as portal vein appeared thin walled and narrow sinusoids. Vacuolated hepatocytes were minimal with little inflammatory cells.

Immunohistochemistry of the liver

Hepatic immunostaining for α-SMA

Showing minimal reaction to anti α-SMA around the portal vein and in-between the hepatocytes in control group (Figures 5a, e). In PCOS group, there was an apparent increase in α-SMA around both central vein a, portal vein, hepatic artery and in-between the hepatocytes (Figures 5b, f). This reaction decreased markedly in PCOS spexin-treated group (Figures 5c, g) and to less extent in PCOS metformin-treated group (Figures 5d, h).

Hepatic immunostaining for TNF-α

Control group showed negative immunoreaction for TNF-α protein expression in the cytoplasm of hepatocytes (Figure 5i). In PCOS rats, there was strong positive immunoreaction for TNF-α in the cytoplasm of hepatocytes (Figure 5j). That reaction decreased to become moderate in PCOS metformin-treated group (Figure 5k) and weak positive immunoreaction for TNF-α in the cytoplasm of some hepatocytes in PCOS spexin-treated (Figure 5l).

Hepatic immunostaining for caspase-3

There was a negative immunoreaction for caspase-3 expression in the cytoplasm of hepatocytes of control group (Figure 5m). Meanwhile, PCOS group showed strong positive immunoreaction for caspase-3 in the cytoplasm and nuclei of hepatocytes (Figure 5n). Both PCOS spexin-treated and PCOS metformin-treated groups showed a weak to a moderate positive immunoreaction for caspase-3 in the cytoplasm of some hepatocytes (Figures 5o, p).

Morphometric Results

H&E staining and follicle count: in addition to morphological evaluation, analysis of the follicle counts at each stage of folliculogenesis showed a significant decrease of Graafian follicles and a significant increase of atretic follicles in PCOS group than all other groups. Graafian follicles count increased while atretic follicles count decreased significantly in PCOS metformin-treated and PCOS spexin-treated groups than PCOS group (Table 5).

Morphometric analysis of area percent of different immune staining of ovarian and liver sections in different groups

- It showed significant decreased expression to KI67 in PCOS group than all other groups, while it was increased significantly in PCOS spexin-treated group than PCOS metformin-treated group but still significantly less than control. Area percent of caspase-3 and P53 immunoreaction of PCOS group showed significant increase than all other groups. This percent was decreased significantly in PCOS spexin-treated group than PCOS metformin-treated group but still significantly higher than control (Table 6).
- Area percent of α- SMA, TNF-α and Caspase-3 immunoreaction in liver sections of different groups showed significant increase in PCOS group than all other groups. The area percent of these immunostaining showed significant decrease in PCOS spexin-treated group than PCOS metformin-treated group (Table 6).
- Scoring of both ovary and liver sections of different groups showed moderate to severe changes in PCOS group, while in PCOS metformin-treated and PCOS spexin-treated groups, there were mild to moderate changes (Table 7).
- The ovarian scorings were based on a scoring system where none = 0, mild = + 1, moderate = +2, and severe = +3.
- Liver was evaluated and scored as (0) indicated no changes, (1), (2) and (3) indicated mild, moderate, and severe changes, respectively, while the grading was determined by percentage as follows: (< 30%) showed mild changes, (30–50%) indicated moderate changes, and changes more than 50% indicated severe changes.

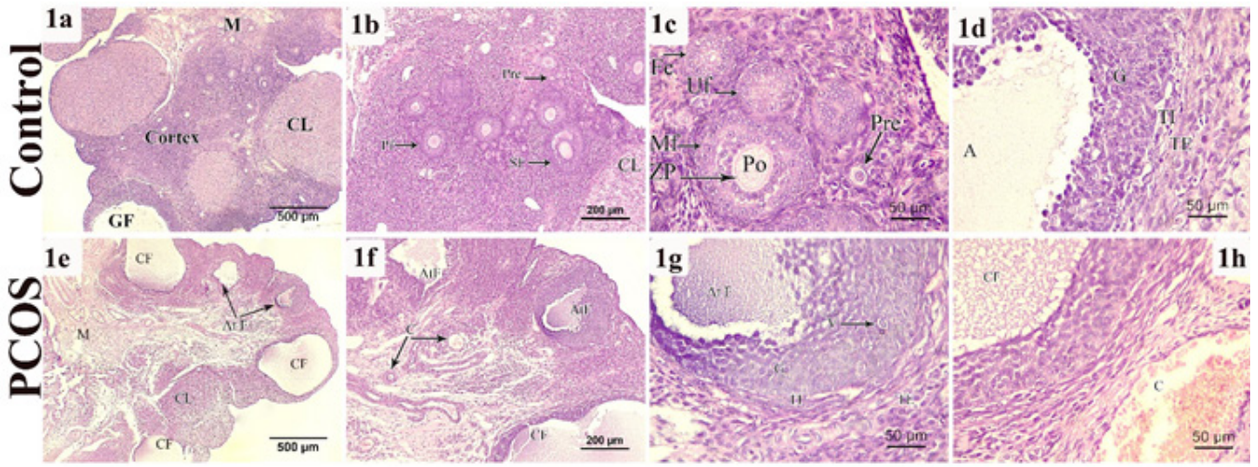

Fig. 1: Photomicrographs of sections in an ovary of control albino rat. a) The ovarian section is formed from cortex and medulla (M), with corpus luteum (CL) and Graafian follicle (GF) (H&E, x500 µm). b) The cortex is formed from multiple stages of ovarian follicles, primordial follicle (Pre), primary follicle (Pf), secondary follicle (Sf) and corpus luteum (CL) (H&E, x200 µm). c) The cortex is formed from multiple stages of ovarian follicles, primordial follicle (Pre), primary follicles are with its 2 types, the uni-laminar follicle (Uf) and multi-laminar follicle (MF) lined with follicular cells (Fc) containing the primary oocyte (PO) which is lined with zona pellucida (ZP) (H&E, x50 µm). d) The Graafian follicle containing the antrum (A) lined with granulosa cells (G), theca interna cells (TI) and theca externa cells (TE) (H&E, x50µm). Photomicrographs of sections in ovary of PCOS albino rat. e) the ovarian section is formed from cortex and medulla (M), with corpus luteum (CL), atretic (At F) and multiple cystic follicles (CF) (H&E, x500 µm). f) The cortex is formed from atretic (At F) and cystic follicles (CF) while the medulla is filled with congested blood vessels (C) (H&E, x200µm). g) The atretic follicle (At F) is lined with multiple layered granulosa cells (G) with vacuolated cytoplasm (V), theca interna (TI) and theca externa (TE) also are observed. (H&E, x50µm). h) The cystic follicles (CF) are present with nearby congested blood vessels (C) (H&E, x50µm).

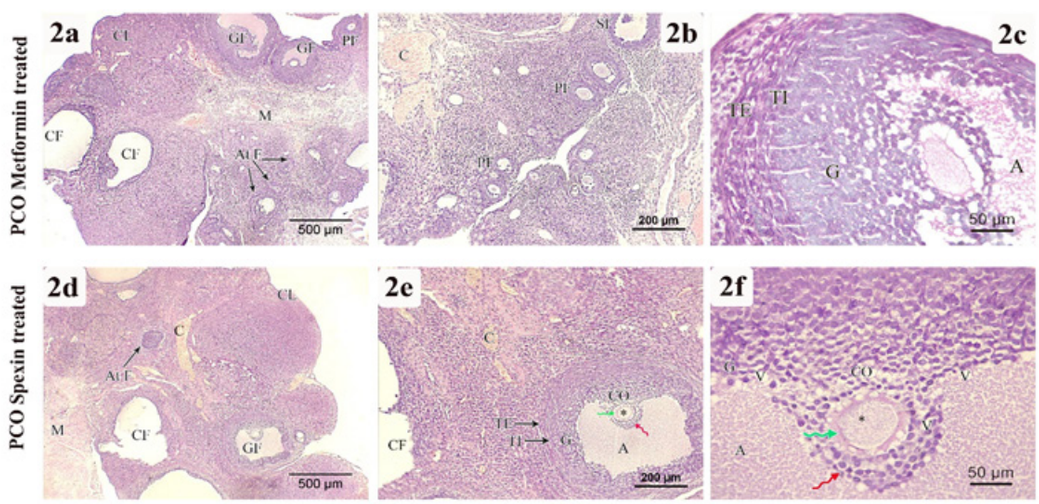

Fig. 2: Photomicrographs of sections in ovary of PCOS metformin-treated albino rat. a) The ovarian section is formed from cortex and medulla (M), with corpus luteum (CL), Graafian follicle (GF), primary (PF), atretic (At F) and cystic follicles (CF) (H&E, x500 µm). b) The cortex is formed multiple primary (PF) and secondary follicles (SF) with few congested blood vessels (C) (H&E, x200µm). c) The Graafian follicle containing the antrum (A) lined with granulosa cells (G), theca interna cells (TI) and theca externa cells (TE) (H&E, x50µm). Photomicrographs of sections in ovary of PCOS spexin-treated albino rat. d) the ovarian section is formed from cortex and medulla (M), with corpus luteum (CL), Graafian follicle (GF), atretic (At F) and cystic follicles (CF) with some areas of congestion (C) (H&E, x500 µm). e) The cortex is formed from graafian follicle formed from granulosa cells (G), secondary oocyte (Asterisk), surrounded by zona pellucida (green zigzag arrow), corona radiata (red zigzag arrow) and attached by a base formed by Cumulus oophorus (CO), theca interna (TI) and theca externa (TE) and antrum (A). Cystic follicles (CF) are also observed while the medulla is with few congested blood vessels (C) (H&E, x200µm). f) secondary oocyte (Asterisk), surrounded by zona pellucida (green zigzag arrow), corona radiata (red zigzag arrow) and attached by a base formed by cumulus oophorus (CO) (H&E, x50µm).

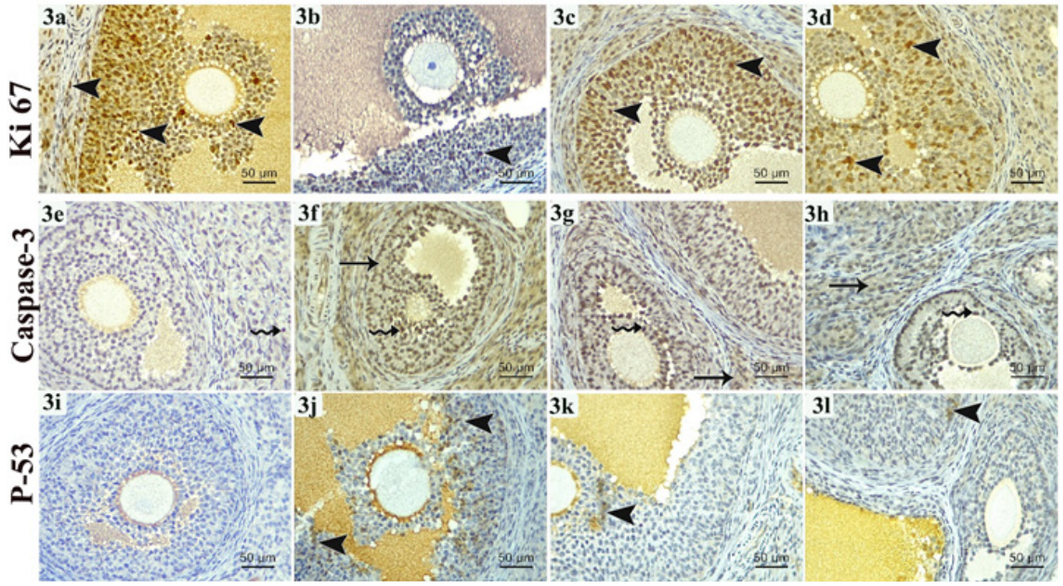

Fig. 3: Ovarian Immunostaining for KI67. (a) Strong Ki-67 positive cells (arrow heads) in all granulosa cells, theca interna and theca externa cells (control group, ×400). (b) An apparent decrease in KI67 positive cells (arrow head) in all granulosa cells, theca interna and theca externa cells. (PCOS group, ×400). (c) Moderate immune reaction for KI67 (arrow heads) in all granulosa cells, less in theca interna and theca externa cells (PCOS metformin-treated group, ×400). (d) Strong presentation of KI67 positive cells (arrow heads) in all granulosa cells, fewer in theca interna and theca externa cells (PCOS spexin-treated group, ×400). Ovarian immunostaining for caspase-3: (e)Very weak positive cytoplasmic caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells, with observable reaction in their nuclei (zigzag arrow) (control group, ×400). (f)An apparent increase in cytoplasmic positive caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (arrows) and their nuclei (zigzag arrow) (PCOS group, ×400). (g)Moderate to severe positive cytoplasmic caspase-3 reaction in of in all granulosa cells, less in theca interna and theca externa cells (arrows) and their nuclei (zigzag arrow) (PCOS metformin-treated group, ×400). (h)Moderate presentation of cytoplasmic positive caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (arrows) and their nuclei (zigzag arrow) (PCOS spexin-treated group, ×400). Ovarian immunostaining for P53. (i)Very weak positive P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (control group, ×400). (j)An apparent increase in positive P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (arrow heads) (PCOS group, ×400). (k)Mild positive P53 reaction in all granulosa cells (arrow head), less in theca interna and theca externa cells (PCOS metformin-treated group, ×400). (l)Mild presentation of positive P53 reaction in all granulosa cells (arrow head), theca interna, theca externa cells and stromal cells (PCOS spexin-treated group, ×400).

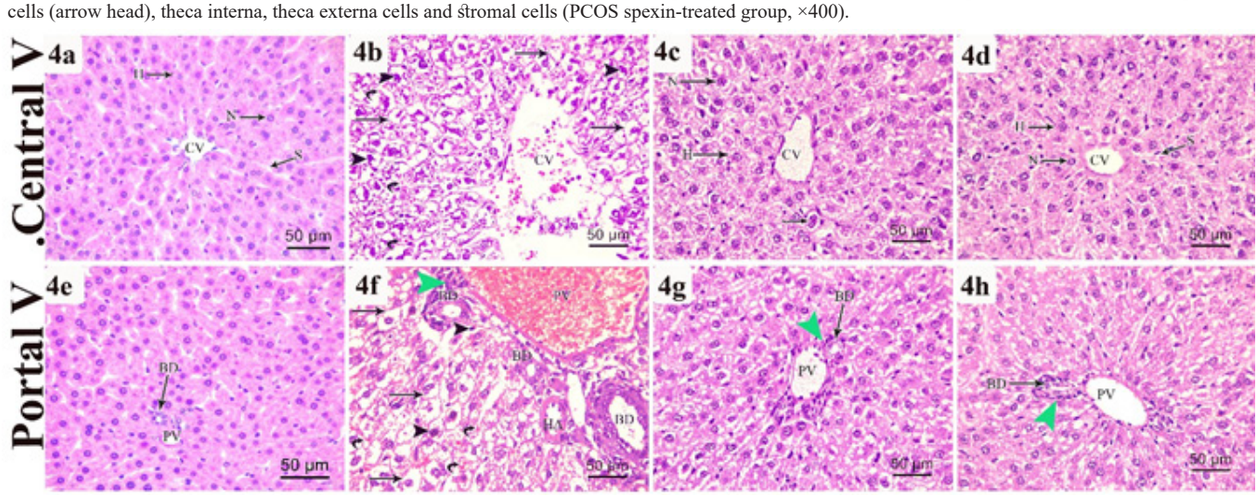

Fig. 4: Photomicrographs of sections in liver of control albino rat. a) Hepatic section is showing central vein (CV) with polygonal hepatocytes have acidophilic cytoplasm (H) and vesicular nucleus (N). Thin blood sinusoids (S) radiating between hepatocytes cords and their lining endothelium are seen (H&E, x50 µm). e) The portal area contains a thin-walled portal vein (PV) and bile duct (Bd) lined with cuboidal cells (H&E, x50µm) Photomicrographs of sections in liver of PCOS albino rat. b) Hepatic section is showing congested irregular-walled central vein (CV) with highly vacuolated hepatocytes (arrows) with dark nuclei degenerated hepatocytes (arrow heads) and areas of congestion (curved arrows). (H&E, x50µm). f) Hepatic section is showing highly vacuolated hepatocytes (arrows) with dark nuclei degenerated hepatocytes (arrow heads) and areas of congestion (curved arrows). The portal area is formed of multiple bile ducts (BD), dilated and congested portal vein (PV) and thick-walled hepatic artery (HA) surrounded with inflammatory cells (green arrow head) (H&E, x50 µm). Photomicrographs of sections in liver of PCOS metformin-treated albino rat. c) Hepatic section is showing central vein (CV) with polygonal hepatocytes have acidophilic cytoplasm (H) and vesicular nucleus (N) with some hepatocytes are binucleated (zigzag arrow). (H&E, x50 µm). g) The portal area contains a thin-walled portal vein (PV) and bile duct (Bd) lined with cuboidal cells and few inflammatory cells (green arrow head) (H&E, x50µm). Photomicrographs of sections in liver of PCOS spexin-treated albino rat. d) Hepatic section is showing central vein (CV) with polygonal hepatocytes have acidophilic cytoplasm (H) and vesicular nucleus (N). Thin blood sinusoids (S) radiating between hepatocytes cords and their lining endothelium are seen (H&E, x50 µm). h) The portal area contains a thin-walled portal vein (PV) and bile duct (Bd) lined with cuboidal cells with some inflammatory cells (green arrow head) (H&E, x50µm).

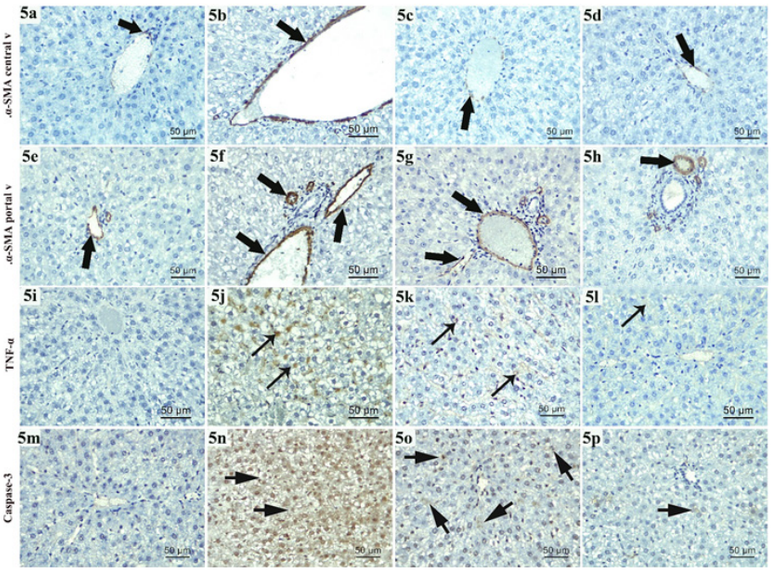

Fig. 5: Hepatic immunostaining for α-SMA. (a) Few α-SMA positive cells (thick arrow) around the central vein and in-between the hepatocytes (control group, ×400). (b) An apparent increase in α-SMA positive cells (thick arrow) around central vein. (PCOS group, ×400). (c) Moderate immune reaction for α-SMA (thick arrow) around the central vein (PCOS metformin-treated group, ×400). (d) Few α-SMA positive cells (thick arrow) around the central vein (PCOS spexin-treated group, ×400). Hepatic immunostaining for α-SMA. (e) Few α-SMA positive reaction (thick arrow) around the portal vein and in-between the hepatocytes (control group, ×400). (f) An apparent increase in α-SMA positive cells (thick arrows) around portal vein, hepatic artery and bile ducts (PCOS group, ×400). (g) Moderate immune reaction for α-SMA (thick arrow) around the portal vein and in-between hepatocytes (PCOS metformin-treated group, ×400). (h) Few α-SMA positive cells (thick arrow) around the portal vein hepatocytes (PCOS spexin-treated group, ×400). Hepatic immunostaining for TNF-α. (i) Negative immunoreaction for TNF-α protein expression in the cytoplasm of hepatocytes. (Control group, ×400). (j) Strong positive immunoreaction for TNF-α in the cytoplasm of hepatocytes (thin arrows) (PCOS group, ×400). (k) Moderate positive immunoreaction for TNF-α in the cytoplasm of some hepatocytes (thin arrow) (PCOS metformin-treated group, ×400). (l) Weak positive immunoreaction for TNF-α in the cytoplasm of some hepatocytes (thin arrow) (PCOS spexin-treated group, ×400). Hepatic immunostaining for caspase-3. (m) Negative immunoreaction for caspase-3 expression in the cytoplasm of hepatocytes. (Control group, ×400). (n) Strong positive immunoreaction for caspase-3 in the cytoplasm and nuclei of hepatocytes (short arrows) (PCOS group, ×400). (o) Moderate positive immunoreaction for caspase-3 in the cytoplasm and nuclei of some hepatocytes (short arrows) (PCOS metformin-treated group, ×400). (p) Weak positive immunoreaction for caspase-3 in the cytoplasm of some hepatocytes (short arrow) (PCOS spexin-treated group, ×400).

Table 2: Biochemical changes in different groups (number of rats in each group= 8)

	Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Final BMI (g/cm2)	0.51±0.02	0.6±0.02[a]	0.55±0.01[a&b]	0.54±0.01[a&b]
Relative ovarian weight (g/100g body weight)	0.019±0.001	0.025±0.001[a]	0.021±0.001[a&b]	0.022±0.002[a&b]
Serum LH (IU/ml)	2.24±0.07	5.15±0.06[a]	3.22±0.05[a&b]	3.31±0.0[5a,b&c]
Serum FSH (IU/ml)	3.44±0.05	4.57±0.06[a]	3.8±0.06[a&b]	3.8±0.09[a&b]
Serum Estradiol (pg/ml)	31.16±0.7	14.35±0.49[a]	26.2±0.64[a&b]	24.61±0.47[a,b&c]
Serum Progesterone (pg/ml)	7.18±0.24	5.18±0.21[a]	6.19±0.22[a&b]	5.91±0.2[a&b]
Serum Testosterone (pg/ml)	72.86±2.8	225.2±4.48[a]	160.1±2.4[a&b]	178.49±5.18[a,b&c]
Serum fasting insulin (µIU/ml)	12.06±0.13	17.77±0.22[a]	14.02±0.34[a&b]	14.11±0.3[a&b]
Serum fasting glucose (mg/dl)	79.25±3.99	185.88±5.96[a]	137.38±5.73[a&b]	138.63±6.44[a&b]
HOMA-IR	2.36±0.13	8.16±0.19[a]	4.76±0.25[a&b]	4.83±0.27[a&b]
HOMA-β	282.21±71.5	52.2±3.07[a]	68.18±5.25[a]	67.56±5.63[a]
Serum TC (mg/dl)	145.5±4.75	236.88±5.91[a]	164±8.67[a&b]	169.63±8.23[a&b]
Serum TG (mg/dl)	122.75±4.33	181.25±4.4[a]	140.38±3.11[a&b]	141.88±4.26[a&b]
Serum HDL (mg/dl)	61.63±2.67	32±3.12[a]	50.88±2.23[a&b]	49.5±2.45[a&b]
Serum LDL (mg/dl)	59.33±5.45	168..63±7.41[a]	85.05±8.71[a&b]	91.75±9.58[a&b]
Serum spexin (ng/ml)	1.72±0.05	0.67±0.05[a]	1.25±0.06[a&b]	1.72±0.06[b&c]

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 metformin-treated group. PCOS, polycystic ovary syndrome; BMI, body mass index; LH, luteinizing hormone; FSH, follicle stimulating hormone; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β homeostasis model assessment of β-cell function; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3: Liver function changes in different groups (number of rats in each group= 8)

	Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Serum ALT (IU/L)	23.63±1.07	125.5±4.54[a]	46.75±3.01[a&b]	62±2[a,b&c]
Serum AST (IU/L)	34.88±2.03	177.25±7.11[a]	73.75±5.34[a&b]	90±5.95[a,b&c]
Serum alkaline phosphatase (IU/100 ml)	8.63±1.41	40.63±4.14[a]	21±2.27[a&b]	28.25±2.12[a,b&c]
Serum albumin (g/dl)	4.38±0.04	2.71±0.07[a]	3.56±0.06[a&b]	3.23±0.05[a,b&c]
Serum total bilirubin (mg/dl)	0.53±0.05	1.16±0.05[a]	0.62±0.03[a&b]	0.69±0.04[a,b&c]
Serum direct bilirubin (mg/dl)	0.31±0.03	0.69±0.04[a]	0.39±0.02[a&b]	0.42±0.02[a&b]
Hepatic MDA (nmol/g)	254.5±7.84	384.88±5.64[a]	295±5.18[a&b]	308.75±6.78[a,b&c]
Hepatic GPX (U/g)	89.63±2.71	42.49±1.82[a]	68.05±1.8[a&b]	58.42±2.01[a,b&c]
Hepatic NO (U/g)	15.13±0.43	28.39±0.42[a]	19.16±0.72[a&b]	21.33±0.44[a,b&c]
Hepatic TNF-α (ng/g)	38.43±1.41	66.69±2.62[a]	45.03±2[a&b]	51.46±2.51[a,b&c]

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 metformin-treated group. PCOS, polycystic ovary syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase, MDA, malondialdehyde; GPX, glutathione peroxidase; NO, nitric oxide; TNF-α, tumor necrosis factor- alpha.

Table 4: Pearson's correlation coefficient (r) between serum spexin level and some studied parameters within the PCOS group (number of rats= 8)

Correlations / Parameters		Final BMI	Serum estradiol	Serum testosterone	Serum glucose	HOMA-IR	Serum TC	Serum HDL	Serum ALT	Serum AST	Serum albumin	Hepatic GPX	Hepatic TNF-α
Serum spexin	r	-0.928	0.967	-0.968	-0.98	-0.907	-0.766	0.942	-0.94	-0.868	0.948	0.912	-0.895
	P Value	<0.001	<0.001	<0.001	<0.001	<0.01	<0.05	<0.001	<0.001	<0.01	<0.001	<0.01	<0.01

BMI, body mass index; HOMA-IR, homeostasis model assessment for insulin resistance; TC, total cholesterol; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPX, glutathione peroxidase; TNFα, tumor necrosis factor alpha.

Table 5: Follicle count in rat ovaries in different groups (number of rats in each group= 8)

	Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Graafian follicles	9.625± 1.061	0.8750±0.8345[a]	4.125±0.8345[a,b]	6.750±0.7071[a,b&c]
Atretic follicles	7.875±1.458	47.25±5.120[a]	33.38±2.925[a,b]	18.00±2.000[a,b&c]

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 metformin-treated group. PCOS, polycystic ovary syndrome.

Table 6: Area Percentage of different immune reactions in different groups (number of rats in each group= 8)

		Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Ovary	KI67	57.99±5.045	16.02±2.315[a]	25.36±4.867[a&b]	34.05±2.88[a,b&c]
	Caspase-3	2.432±1.369	51.1±3.58[a]	33.64±3.849[a&b]	26.15±3.255[a,b&c]
	P53	2.066±0.6137	25.46±3.129[a]	15.72±2.899[a&b]	7.118±1.581[a,b&c]
	α-SMA	1.355±0.543	9.639±1.634[a]	4.56±0.93[a&b]	2.857±0.48[a,b&c]
Liver	TNF-α	1.126±0.666	19.73±1.82[a]	9.828±1.966[a&b]	3.568±1.438[a,b&c]
	Caspase-3	7.078±1.615	45.61±3.576[a]	16.62±2.775[a&b]	10.6±1.073[a&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 metformin treated group. PCOS, polycystic ovary syndrome, TNF-α. tumor necrosis factor- alpha.

Table 7: Scoring of ovarian and hepatic sections in different groups (number of rats in each group= 8)

	Control	PCOS	PCOS metformin-treated	PCOS spexin-treated
Ovary	0.3750± 0.5175	2.750± 0.4629[a]	1.875± 0.3536[a]	1.500± 0.5345[b]
Liver	0.2500± 0.4629	2.875± 0.3536[a]	1.750± 0.4629[a]	1.250± 0.7071[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 metformin treated group. PCOS, polycystic ovary syndrome.

DISCUSSION

This study demonstrated the effects of metformin and spexin treatment on ovarian and liver function changes in letrozole induced PCOS rats by investigating estrous cyclicity, endocrine profiles, metabolic changes, alterations in selected markers for liver function, oxidative stress, inflammation and histopathological changes in ovary and liver. The present study confirmed that both metformin and spexin ameliorated the changes in ovarian and liver function in letrozole induced PCOS in rats.

In PCOS group, rats were completely acyclic and exhibited a constant estrous state when compared with that in the control group which indicated anovulation as considered by Rezvanfar, Rezvanfar[44]. This was accompanied by a significant increase in BMI, relative ovarian weight, serum (LH, FSH and testosterone), with a significant decrease in serum (estradiol and progesterone) when compared with the control group. All these findings confirmed occurrence of PCOS and impairment of ovarian function (ovulation and steroidogenesis)[45,46]. Additionally, histopathological examination of ovary confirmed PCOS changes as manifested by corpus luteum, atretic and cystic follicles lined with multiple layered vacuolated granulosa, and medulla that filled with congested blood vessels in ovarian specimen of PCOS rat and these finding supported by significant increase in count of atretic follicles and decrease of Graafian than control. In accordance, Jahan, Abid[47] and Helal, Ismail[48] reported that rats treated with letrozole developed PCOS in their ovaries similar to women and their ovaries showed multiple cystic and atretic follicles. Goodarzi, Dumesic[49] stated that letrozole induced PCOS by reducing ovarian conversion of androgens to estrogens and caused a decrease in estradiol with an increase in testosterone serum levels which developed cystogenesis by impairing developing ovarian follicles maturation. Also, Dumesic and Richards[50] and Yang, Chou[51] reported that increased insulin caused disruption of folliculogenesis via stimulating thecal cells testosterone over production. González[52], Rezvanfar, Saeedi[53] and Cardoso, Ribeiro[54] suggested that increased testosterone was a causative agent for chronic low-grade inflammation that stimulated further ovarian testosterone production. Belgorosky, Sander[55] and Murri, Luque-Ramirez[56] declared that increased serum TNF-α levels and oxidative stress arrested follicular development through apoptosis in granulosa cells causing poor oocyte quality and progressive atresia of the follicles. These findings were confirmed by immunohistochemical study, as ovary of PCOS group showed marked decrease in KI67 expression and increase in caspase-3 and P53 in their granulosa cells, theca interna and externa. Additionally, morphometric analysis confirmed these observations. In consistence, Tadros, Mohamed[57] reported significant decrease in area percent of KI67 in the granulosa cells of rat with PCOS. Also, Lombardi, Simões[34] reported increased reactivity of caspase-3 in granulosa cells of the ovarian cysts of the PCOS group, while still low in the theca interna. In agreement with the current results, Meenakumari, Agarwal[58] and Baravalle, Salvetti[59] reported that rats with PCOS showed decreased serum progesterone, indicating possible defects in luteal phase like that noted in some women with PCOS. Zhang, Li[60] stated that in PCOS, increased LH stimulated more androgen production by the ovarian theca cells.

Results of the current study confirmed that PCOS was accompanied by liver dysfunction (increased serum ALT, AST, alkaline phosphatase, total & direct bilirubin, but decreased serum albumin) which could be owed to metabolic disturbances (increased serum insulin, glucose, TC, TG & LDL, but decreased serum HDL & value of HOMA-β, insulin resistance (increased HOMA-IR), oxidative stress (increased hepatic MDA and decreased hepatic GPX) and inflammation (increased hepatic NO and TNF-*α*) which were partly supported by Cui, Hu[61]. In line with the results of the present study, Rezvanfar, Rezvanfar[44] reported that PCOS was associated with an increase in MDA, TNF-α and reduced activity of enzymatic antioxidants. Adefegha, Oboh[62] reported that elevated serum MDA levels in PCOS rats resulted in increased lipid peroxidation and tissue damage which caused the leakage of enzymes and metabolites into the blood circulation which may explain occurrence of hepatic dysfunction and increased liver enzymes in PCOS. Moran, Norman[63] and Gao, Cheng[64] stated that pathogenesis of PCOS was associated with obesity, insulin resistance, compensatory hyperinsulinemia, dyslipidemia and NAFLD. In accordance, liver sections of PCOS group showed congested thick-walled central and portal veins with highly vacuolated hepatocytes and inflammatory cells. Also, liver specimen showed fibrosis manifested in sections stained with α SMA immunohistochemical by marked expression around portal and central veins. Sarkar, Terrault[65] found that 76% of women with PCOS showed ballooning of hepatocytes, steatosis and lobular inflammation of their liver which were some of NASH histological criteria. Moreover, they declared that some women with PCOS showed advanced fibrosis in their liver biopsy. Additionally, liver section showed marked expression of caspase-3 and TNFα in PCOS group and confirmed morphometrically by significant increase of area percent of their immunoreactivity than control group. Baranova, Tran[66] reported significant elevation of caspase-3 in PCOS patients that indicating intense pro-apoptotic environment in PCOS patients and could be due to hyperandrogenism. Targher, Solagna[7] declared that PCOS was highly associated with NAFLD diagnosed by AST elevation and/or ultrasound. Abraham[67] confirmed that the increased activities of serum liver enzymes were indicative of cellular leakage and loss of hepatic cell membrane functional integrity. The increase in total and direct bilirubin levels that was associated with a decrease in serum albumin noticed in the results of the current study could be referred to the peroxidative damage of liver as described by Bharathi, Reddy[68]. Ahmad, Arjumand[69] stated that oxidative stress released pro-inflammatory mediators such as NO through inducible nitric oxide synthase in the damaged liver causing cellular dysfunction.

As Diamanti-Kandarakis and Dunaif[3], Manco, Castagneto-Gissey[4] and Cui, Hu[61] declared that insulin resistance played a key role in PCOS pathophysiology and Tan, Hahn[9] reported that metformin acted as an insulin sensitizing agent that had positive effects on insulin resistance and menstrual cycling in PCOS, thus, this study investigated the effects of metformin treatment on ovarian and hepatic functions in rats with PCOS. The current results confirmed that metformin treatment improved both ovarian [normal estrous cycles, decreased serum testosterone and increased serum level of both estradiol & progesterone) and hepatic (decreased serum ALT, AST, alkaline phosphatase, total & direct bilirubin, but increased serum albumin) functions, possibly through; reducing both final BMI and relative ovarian weight, improving the metabolic disturbances (decreased serum insulin, glucose, TC, TG & LDL, but increased serum HDL), decreasing insulin resistance (reduced HOMA-IR), anti-oxidant (decreased hepatic MDA and increased hepatic GPX) and anti-inflammatory (decreased hepatic NO and TNF-α) effects. These results were supported by Sir-Petermann, Codner[70] found that weight reduction in women with PCOS improved their hyperandrogenism and menstrual regularity. In the same line, ovarian specimen in metformin-treated group showed follicles in different stages with little vacuolation. Moreover, there were moderate expression of KI67 and improvement in caspase-3 expression. This was supported by morphometric finding as there was a significant improvement than PCOS group. In accordance, Mahamed, Maganhin[71] declared that follicular dynamics were restored partially and number of luteal bodies were increased by metformin treatment of PCOS rats. Lemos, Peixoto[72] reported that metformin was effective against hepatic toxicity produced by PCOS, causing an improvement in its biochemical changes as it reduced serum levels of nitric oxide which confirmed that metformin had anti-inflammatory activity. Esteghamati, Eskandari[73] declared that metformin had anti-oxidant activity in diabetic individuals. This effect was manifested in liver specimen of PCOS metformin-treated group as decreased percent of hydropic degenerated cells, deposition of α SMA with moderate decrease of caspase-3 and TNFα expression. In line with the results of the present study, Huang, Chiang[74] and Miller, Chu[75] reported that metformin had hepatoprotective effects through activation of hepatic 5' adenosine monophosphate-activated protein kinase enzyme increasing glucose uptake, inhibiting mitochondrial fatty acid oxidation and blocking glucagon-dependent glucose output from liver cells. Haukeland, Konopski[11], Gangale, Miele[12] and Tan, Vollmar[13] stated that metformin treatment improved liver enzymes and body weight in patients with PCOS confirming that it positively influenced liver function.

The results of the present study declared that serum level of spexin in rats with PCOS was significantly decreased as compared to normal rats, and was negatively associated with final BMI, HOMA-IR, serum (testosterone, glucose, TC, ALT & AST), and hepatic TNF-α, but it was positively correlated with serum (estradiol, HDL & albumin) and hepatic GPX. These results were in agreement with Anik Ilhan and Yildizhan[16] and Guler and Demir[17]. Guler and Demir[17] detected a significant decrease in serum spexin levels in women with PCOS in comparison to the controls with a significant negative association between serum spexin levels and insulin resistance within PCOS group. In the same line, histology of ovarian specimen of PCOS spexin-treated group showed marked improvement in its structure and its expression of KI67 with marked decrease in apoptotic markers (caspase-3 and P53). Porzionato, Rucinski[76] detected spexin role in proliferation, differentiation, and apoptosis of cells. On the other hand, Flora, Vasiliki[18] found no significant differences in serum spexin concentrations in adolescents with PCOS in comparison with controls. This discrepancy could be related to species differences. The results of the current study indicated that spexin played a role in the pathophysiology of ovarian and hepatic dysfunctions occurred in rats with PCOS. This role was confirmed by the use of spexin in treatment of rats with PCOS. The current results confirmed that spexin treatment improved both ovarian [normal estrous cycles, decreased serum testosterone and increased serum level of both estradiol & progesterone) and hepatic (decreased serum ALT, AST, alkaline phosphatase, total & direct bilirubin, but increased serum albumin) functions, possibly through; reducing both final BMI and relative ovarian weight, improving the metabolic disturbances (decreased serum insulin, glucose, TC, TG & LDL, but increased serum HDL), decreasing insulin resistance (reduced HOMA-IR), anti-oxidant (decreased hepatic MDA and increased hepatic GPX) and anti-inflammatory (decreased hepatic NO and TNF-α) effects. These finding were supported by histopathological improvement of liver specimen in PCOS spexin-treated group. Additionally, liver of PCOS spexin-treated group showed significant decrease of caspase-3, TNF α and α SMA reactivity. These results were supported by Ge, Walewski[19] who reported that spexin administration reduced serum levels of liver enzymes, reduced insulin resistance, improved dyslipidemia and inhibited hepatocellular long-chain fatty acid uptake in mouse models of hepatic steatosis. Walewski, Ge[77] reported that spexin treatment reduced food intake and increased body weight loss in diet-induced obese rats, possibly through inhibiting the long-chain fatty acid uptake into the adipocytes. Also, Kumar, Hossain[78], Chen, Wang[79] and Guler and Demir[17] noticed a reverse association between serum levels of spexin and both BMI and insulin resistance. Al-Daghri, Al-Hazmi[80] stated that spexin indirectly affected inflammatory markers through affecting serum level of leptin. Lv, Zhou[81] suggested that spexin inhibited LH secretion.

The results of the current study also showed that serum spexin level was significantly increased with metformin treatment in rats with PCOS and this could be related to insulin sensitizing and weight lowering effects of metformin. These results may indicate that metformin performed its actions partly through regulation of spexin

formation. Also, these results could indicate that spexin may be used as a metabolic biomarker in PCOS as suggested by Anik Ilhan and Yildizhan[16].

Study limitations include that this study was designed on rats and the results may be different from that in human. Also, it was conducted on lean rats with PCOS not on obese rats with polycystic ovary. Also, the number of rats used in the current study was small.

CONCLUSION

Spexin serum level could be used as a metabolic biomarker for polycystic ovary syndrome. Also, treatment with either metformin or spexin ameliorated ovarian and hepatic dysfunctions in rat model of PCOS through their insulin sensitizing, anti-oxidant and anti-inflammatory effects. Moreover, part of the mechanism of action of metformin could be through its role in regulation of spexin formation.

ABBREVIATIONS

ANOVA: Analysis of Variance, **BMI:** Body Mass Index, **ELISA:** Enzyme-Linked Immunosorbent Assay, **FSH:** Follicle Stimulating Hormone, **HDL:** High-Density Lipoprotein, **HOMA- β:** Homeostatic Model Assessment of B-Cell Function, **HOMA-IR:** Homeostatic Model Assessment of Insulin Resistance Index, **HS:** Hepatic Steatosis, **LDL:** Low-Density Lipoprotein, **LH:** Luteinizing Hormone, **MDA:** Malondialdehyde, **NAFLD:** on-Alcoholic Fatty Liver Disease, **NO:** Nitric Oxide, **PCOS:** Polycystic Ovary Syndrome, **TC:** Total Cholesterol, **TG:** Triglycerides, **TNF- α:** Tumor Necrosis Factor-Alpha.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Moran, L.J., *et al*., Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update, 2010. 16(4): p. 347-363.
2. Dumesic, D.A., *et al*., Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. Endocr Rev, 2015. 36(5): p. 487-525.
3. Diamanti-Kandarakis, E. and A. Dunaif, Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev, 2012. 33(6): p. 981-1030.
4. Manco, M., *et al*., Insulin dynamics in young women with polycystic ovary syndrome and normal glucose tolerance across categories of body mass index. PLoS One, 2014. 9(4): p. e92995.
5. Amer, A.M., A.S. Farid, and K.M. Fararah, Hepatoprotective Effect of Allopurinol and Garlic Extract against Experimentally – Induced Hepatotoxicity. Benha Veterinary Medical Journal, 2018. 34(2): p. 66-76.
6. Barfield, E., *et al*., The prevalence of abnormal liver enzymes and metabolic syndrome in obese adolescent females with polycystic ovary syndrome. J Pediatr Adolesc Gynecol, 2009. 22(5): p. 318-22.
7. Targher, G., *et al*., Abnormal serum alanine aminotransferase levels are associated with impaired insulin sensitivity in young women with polycystic ovary syndrome. J Endocrinol Invest, 2009. 32(8): p. 695-700.
8. Vassilatou, E., *et al*., Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. Hum Reprod, 2010. 25(1): p. 212-20.
9. Tan, S., *et al*., Metformin improves polycystic ovary syndrome symptoms irrespective of pre-treatment insulin resistance. Eur J Endocrinol, 2007. 157(5): p. 669-76.
10. Loomba, R., *et al*., Clinical trial: pilot study of metformin for the treatment of non-alcoholic steatohepatitis. Aliment Pharmacol Ther, 2009. 29(2): p. 172-82.
11. Haukeland, J.W., *et al*., Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. Scand J Gastroenterol, 2009. 44(7): p. 853-60.
12. Gangale, M.F., *et al*., Long-term metformin treatment is able to reduce the prevalence of metabolic syndrome and its hepatic involvement in young hyperinsulinaemic overweight patients with polycystic ovarian syndrome. Clin Endocrinol (Oxf), 2011. 75(4): p. 520-7.
13. Tan, S., *et al*., Liver Injury Indicating Fatty Liver but Not Serologic NASH Marker Improves under Metformin Treatment in Polycystic Ovary Syndrome. Int J Endocrinol, 2015. 2015: p. 254169.
14. Liu, Y., *et al*., Spexin protects cardiomyocytes from hypoxia-induced metabolic and mitochondrial dysfunction. Naunyn Schmiedebergs Arch Pharmacol, 2019: p. 1-9.
15. Gu, L., *et al*., Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides, 2015. 71: p. 232-239.
16. Anik Ilhan, G. and B. Yildizhan, Spexin as a new metabolic biomarker in women with polycystic ovary syndrome. Fertility and Sterility, 2018. 110(4): p. e118.

17. Guler, A. and İ. Demir, Decreased levels of spexin are associated with hormonal and metabolic disturbance in subjects with polycystic ovary syndrome. Journal of Obstetrics and Gynaecology, 2020: p. 1-6.

18. Flora, B., *et al*., Serum Spexin Concentrations in Adolescent Females with Metabolic Syndrome, Polycystic Ovary Syndrome and Anorexia Nervosa. Hormone Research in Paediatrics, 2018. 82: p. 134.

19. Ge, J.F., *et al*., Regulation of Hepatocellular Fatty Acid Uptake in Mouse Models of Fatty Liver Disease with and without Functional Leptin Signaling: Roles of NfKB and SREBP-1C and the Effects of Spexin. Semin Liver Dis, 2016. 36(4): p. 360-372.

20. Kafali, H., *et al*., Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. Arch Med Res, 2004. 35(2): p. 103-8.

21. Oliveira, P.W., *et al*., Metformin ameliorates ovariectomy-induced vascular dysfunction in non-diabetic Wistar rats. Clin Sci (Lond), 2014. 127(4): p. 265-275.

22. Sassek, M., *et al*., Spexin Modulates Functions of Rat Endocrine Pancreatic Cells. Pancreas, 2018. 47(7): p. 904-909.

23. Merlo, E., *et al*., Mercury leads to features of polycystic ovary syndrome in rats. Toxicol Lett, 2019. 312: p. 45-54.

24. Goldman, J.M., A.S. Murr, and R.L. Cooper, The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. Birth Defects Res B Dev Reprod Toxicol, 2007. 80(2): p. 84-97.

25. Mohamed H. Ibrahim, et al., The Relation of Serum Irisin Level with Metabolic and Hormonal Changes in Rat Model of Polycystic Ovary. Z.U.M.J., 2018. 24(5): p. 409-419.

26. Novelli, E.L., *et al*., Anthropometrical parameters and markers of obesity in rats. Lab Anim, 2007. 41(1): p. 111-9.

27. Kanjana, K., *et al*., Laboratory studies in search of the critical hydrogen concentration. Radiation Physics and Chemistry, 2013. 82: p. 25-34.

28. Sun, G., *et al*., Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. Am J Clin Nutr, 2007. 85(2): p. 399-404.

29. Hermans, M.P., *et al*., Comparison of insulin sensitivity tests across a range of glucose tolerance from normal to diabetes. Diabetologia, 1999. 42(6): p. 678-87.

30. Friedewald, W.T., R.I. Levy, and D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 1972. 18(6): p. 499-502.

31. Zaid, S.S.M., S. Othman, and N.M. Kassim, Potential protective effect of Tualang honey on BPA-induced ovarian toxicity in prepubertal rat. BMC complementary and alternative medicine, 2014. 14: p. 509-509.

32. Yuan, H.-D., G.-Z. Jin, and G.-C. Piao, Hepatoprotective effects of an active part from Artemisia sacrorum Ledeb. against acetaminophen-induced toxicity in mice. Journal of ethnopharmacology, 2010. 127(2): p. 528-533.

33. Suvarna, S.K., C. Layton, and J.D. Bancroft, Bancroft's Theory and Practice of Histological Techniques. Churchill Livingstone, UK, 7th edition, 2013: p. 173-225.

34. Lombardi, L.A., *et al*., Immunohistochemical evaluation of proliferation, apoptosis and steroidogenic enzymes in the ovary of rats with polycystic ovary. Revista da Associação Médica Brasileira, 2014. 60(4): p. 349-356.

35. Kececi, M., *et al*., Evaluation of preventive effect of shilajit on radiation-induced apoptosis on ovaries. Archives of gynecology and obstetrics, 2016. 293(6): p. 1255-1262.

36. Mekonnen, G., J. Ijzer, and H. Nederbragt, Tenascin-C in chronic canine hepatitis: immunohistochemical localization and correlation with necro-inflammatory activity, fibrotic stage, and expression of alpha-smooth muscle actin, cytokeratin 7, and CD3+ cells. Veterinary pathology, 2007. 44(6): p. 803-813.

37. Kim, S.H., *et al*., Increased expression of tumor necrosis factor-alpha in the rat hippocampus after acute homocysteine administration. Journal of epilepsy research, 2011. 1(1): p. 6.

38. Dorostghoal, M., M.K. Mahabadi, and S. Adham, Effects of maternal caffeine consumption on ovarian follicle development in wistar rats offspring. Journal of reproduction & infertility, 2011. 12(1): p. 15.

39. Lande, Y., *et al*., Short-term exposure of human ovarian follicles to cyclophosphamide metabolites seems to promote follicular activation in *vitro*. Reproductive biomedicine online, 2017. 34(1): p. 104-114.

40. Mohammadghasemi, F., *et al*., The protective effects of exogenous melatonin on nicotine-induced changes in mouse ovarian follicles. Journal of reproduction & infertility, 2012. 13(3): p. 143.

41. Sayar, I., *et al*., Protective effects of ellagic acid and ozone on rat ovaries with an ischemia/reperfusion injury. Journal of Obstetrics and Gynaecology Research, 2016. 42(1): p. 52-58.

42. Ahmed, K., *et al*., Spirulina platensis alleviates arsenic-induced toxicity in male rats: biochemical, histopathological and immunohistochemical studies. Adv. Anim. Vet. Sci, 2019. 7(8): p. 701-710.

43. Korany, R.M., *et al*., Effect of long-term arsenic exposure on female albino rats with special reference to the protective role of Spirulina platensis. Explor Anim Med Res, 2019. 9(2): p. 125-136.
44. Rezvanfar, M.A., *et al*., Molecular mechanisms of a novel selenium-based complementary medicine which confers protection against hyperandrogenism-induced polycystic ovary. Theriogenology, 2012. 78(3): p. 620-31.
45. Boumosleh, J.M., *et al*., Metabolic Concomitants of Obese and Nonobese Women With Features of Polycystic Ovarian Syndrome. Journal of the Endocrine Society, 2017. 1(12): p. 1417-1427.
46. Shen, H.-R., X. Xu, and X.-L. Li, Berberine exerts a protective effect on rats with polycystic ovary syndrome by inhibiting the inflammatory response and cell apoptosis. Reproductive Biology and Endocrinology, 2021. 19(1): p. 3.
47. Jahan, S., *et al*., Therapeutic potentials of Quercetin in management of polycystic ovarian syndrome using Letrozole induced rat model: a histological and a biochemical study. Journal of ovarian research, 2018. 11(1): p. 26.
48. Helal, B.A.F., *et al*., Effect of vitamin D on experimental model of polycystic ovary syndrome in female rats. Life Sci, 2021: p. 119558.
49. Goodarzi, M.O., *et al*., Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol, 2011. 7(4): p. 219-31.
50. Dumesic, D.A. and J.S. Richards, Ontogeny of the ovary in polycystic ovary syndrome. Fertil Steril, 2013. 100(1): p. 23-38.
51. Yang, P.-K., *et al*., Obesity alters ovarian folliculogenesis through disrupted angiogenesis from increased IL-10 production. Molecular Metabolism, 2021. 49: p. 101189.
52. González, F., Inflammation in Polycystic Ovary Syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids, 2012. 77(4): p. 300-305.
53. Rezvanfar, M.A., *et al*., Dual targeting of TNF-alpha and free radical toxic stress as a promising strategy to manage experimental polycystic ovary. Pharm Biol, 2016. 54(1): p. 80-90.
54. Cardoso, N.S., *et al*., Polycystic ovary syndrome associated with increased adiposity interferes with serum levels of TNF-alpha and IL-6 differently from leptin and adiponectin. Archives of Endocrinology and Metabolism, 2020. 64(1): p. 4-10.
55. Belgorosky, D., *et al*., Hyperandrogenism alters intraovarian parameters during early folliculogenesis in mice. Reprod Biomed Online, 2010. 20(6): p. 797-807.
56. Murri, M., *et al*., Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update, 2013. 19(3): p. 268-88.
57. Tadros, S.J., *et al*., Effect of Adipose-derived stem cells versus clomiphen on treatment of experimental polycystic ovary in rats: Histological and Immunohistochemical study. Egyptian Journal of Histology, 2018. 41(4): p. 373-385.
58. Meenakumari, K.J., *et al*., Effects of metformin treatment on luteal phase progesterone concentration in polycystic ovary syndrome. Braz J Med Biol Res, 2004. 37(11): p. 1637-44.
59. Baravalle, C., *et al*., Microscopic characterization of follicular structures in letrozole-induced polycystic ovarian syndrome in the rat. Arch Med Res, 2006. 37(7): p. 830-9.
60. Zhang, X., *et al*., Suppression of death receptor-mediated apoptosis by 1,25-dihydroxyvitamin D3 revealed by microarray analysis. J Biol Chem, 2005. 280(42): p. 35458-68.
61. Cui, P., *et al*., Long-term androgen excess induces insulin resistance and non-alcoholic fatty liver disease in PCOS-like rats. J Steroid Biochem Mol Biol, 2021. 208: p. 105829.
62. Adefegha, S.A., *et al*., Antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of dietary clove (Szyzgium aromaticum) bud powder in a high-fat diet/streptozotocin-induced diabetes rat model. J Sci Food Agric, 2014. 94(13): p. 2726-37.
63. Moran, L.J., R.J. Norman, and H.J. Teede, Metabolic risk in PCOS: phenotype and adiposity impact. Trends Endocrinol Metab, 2015. 26(3): p. 136-43.
64. Gao, S., *et al*., The relationships of irisin with bone mineral density and body composition in PCOS patients. Diabetes Metab Res Rev, 2016. 32(4): p. 421-8.
65. Sarkar, M., *et al*., Polycystic ovary syndrome (PCOS) is associated with NASH severity and advanced fibrosis. Liver International, 2020. 40(2): p. 355-359.
66. Baranova, A., *et al*., Molecular signature of adipose tissue in patients with both non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS). Journal of translational medicine, 2013. 11(1): p. 133.
67. Abraham, P., Oxidative stress in paracetamol-induced pathogenesis: (I). Renal damage. Indian journal of biochemistry & biophysics, 2005. 42(1): p. 59-62.
68. Bharathi, P., *et al*., A Study of Certain Herbs Against Chlorpyrifos-induced Changes in Lipid and Protein Profile in Poultry. Toxicology international, 2011. 18(1): p. 44-46.

69. Ahmad, S.T., *et al*., Hesperidin alleviates acetaminophen induced toxicity in Wistar rats by abrogation of oxidative stress, apoptosis and inflammation. Toxicology letters, 2012. 208(2): p. 149-161.

70. Sir-Petermann, T., *et al*., Metabolic and reproductive features before and during puberty in daughters of women with polycystic ovary syndrome. J Clin Endocrinol Metab, 2009. 94(6): p. 1923-30.

71. Mahamed, R.R., *et al*., Metformin improves ovarian follicle dynamics by reducing theca cell proliferation and CYP-17 expression in an androgenized rat model. Journal of ovarian research, 2018. 11(1): p. 18.

72. Lemos, A.J., *et al*., Effect of the combination of metformin hydrochloride and melatonin on oxidative stress before and during pregnancy, and biochemical and histopathological analysis of the livers of rats after treatment for polycystic ovary syndrome. Toxicol Appl Pharmacol, 2014. 280(1): p. 159-68.

73. Esteghamati, A., *et al*., Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. Clin Nutr, 2013. 32(2): p. 179-85.

74. Huang, N.-L., *et al*., Metformin inhibits TNF-α-induced IκB kinase phosphorylation, IκB-α degradation and IL-6 production in endothelial cells through PI3K-dependent AMPK phosphorylation. International Journal of Cardiology, 2009. 134(2): p. 169-175.

75. Miller, R.A., *et al*., Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature, 2013. 494(7436): p. 256-60.

76. Porzionato, A., *et al*., Spexin expression in normal rat tissues. Journal of Histochemistry & Cytochemistry, 2010. 58(9): p. 825-837.

77. Walewski, J.L., *et al*., Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity (Silver Spring), 2014. 22(7): p. 1643-52.

78. Kumar, S., *et al*., Decreased Circulating Levels of Spexin in Obese Children. The Journal of clinical endocrinology and metabolism, 2016. 101(7): p. 2931-2936.

79. Chen, T., *et al*., Circulating Spexin Decreased and Negatively Correlated with Systemic Insulin Sensitivity and Pancreatic β Cell Function in Obese Children. Annals of nutrition & metabolism, 2019. 74(2): p. 125-131.

80. Al-Daghri, N.M., *et al*., Associations of Spexin and cardiometabolic parameters among women with and without gestational diabetes mellitus. Saudi Journal of Biological Sciences, 2018. 25(4): p. 710-714.

81. Lv, S.-Y., *et al*., Emerging Roles of NPQ/Spexin in Physiology and Pathology. Frontiers in pharmacology, 2019. 10: p. 457-457.

الملخص العربى

التحسين المقارن لسبيكسين و ميتفورمين لتغيرات وظائف المبيض والكبد في متلازمة تكيس المبيض التي يسببها ليتروزول في الجرذان (الدراسة الهستولوجية، البيوكيميائية المناعية الكيميائية والمورفومترية)

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خلفية البحث والهدف من الدراسة: تتميز متلازمة المبيض المتعدد الكيسات (PCOS) بتشكيل جريب المبيض المتعدد الكيسات، وخلل التبويض، وفرط الأندروجين ومقاومة الأنسولين. وعرف عن السبيكسين أنه ببتيد عصبي له دور في السيطرة على الشبع، واستقلاب الدهون والجلوكوز. كما يعد الميتفورمين عامل محسّس للأنسولين وله بعض وظائف حماية الكبد. ولذا هدفت هذه الدراسة إلى معرفة تأثير العلاج بالسبكسين والميتفورمين على التغيرات في وظائف المبيض والكبد في متلازمة تكيس المبايض التي يسببها ليتروزول في الجرذان والآليات المحتملة المعنية.

مواد وطرق البحث: تم تقسيم اثنين و ثلاثين من إناث الجرذان البيضاء البالغين بشكل عشوائي إلى أربع مجموعات متساوية كل مجموعة بها ثمان جرذان كما يلي:

المجموعة الأولى: المجموعة الضابطة.

المجموعة الثانية: المجموعة المحدث لها متلازمة تكيس المبايض باستخدام الليتروزول.

المجموعة الثالثة: المجموعة المحدث لها متلازمة تكيس المبايض باستخدام الليتروزول و عولجت بالميتفورمين.

المجموعة الرابعة: المجموعة المحدث لها متلازمة تكيس المبايض باستخدام الليتروزول و عولجت بالسبيكسين.

النتائج: في مجموعة متلازمة تكيس المبايض، كانت هناك زيادة ذات دلالة احصائية في؛ مؤشر كتلة الجسم، الوزن النسبي للمبيض، HOMA-IR، مصل (FSH، LH، هرمون التستوستيرون، الأنسولين، الجلوكوز LDL ، TG، TC AST، ALT، الفوسفاتيز القلوي، البيليروبين الكلي والمباشر) والكبد (NO، MDA وTNF-α)، مع انخفاض ذات دلالة احصائية في؛ مصل [استراديول، بروجسترون، HDL، ألبومين وسبكسين] وGPX الكبدي، بالمقارنة مع المجموعة الضابطة. كما أظهرت نتائج التشريح المرضي أن المبايض من متلازمة تكيس المبايض تعبير ملحوظ عن كاسباس 3 وP53 وانخفضت تعبير KI67 عن الخلايا الحبيبية وخلايا ثيكا. خلايا الكبد المفرغة، الوريد البابي المتوسع والمزدحم ورد فعل إيجابي لتكوين المناعة باستخدام αSMA وcaspase وTNF-α. و لقد عكست هذه التغييرات بشكل ذات دلالة احصائية بمعالجة الجرذان المحدث لها متلازمة تكيس المبايض بالميتفورمين أو السبكسين.

الخلاصة: يمكن استخدام مستوى مصل السبيكسين كمؤشر بيولوجي استقلابي لمتلازمة المبيض المتعدد الكيسات. كما أن العلاج بالميتفورمين أو السبكسين يحسن من اختلال وظائف المبيض والكبد في نموذج الفئران المصابة بمتلازمة تكيس المبايض من خلال تأثيرات الأنسولين المحسّسة للأنسولين ومضادات الأكسدة والمضادة للالتهابات. علاوة على ذلك ، يمكن أن يكون جزء من آلية عمل الميتفورمين من خلال دوره في تنظيم تكوين السبكسين.