ERYTHROPOIETIN, MYOINOSITOL AND METFORMIN MODULATE INSULIN SENSITIVITY INDICES, PANCREATIC BETA CELL MASS AND HEPATOCELLULAR CHANGES IN A RAT MODEL OF POLYCYSTIC OVARY SYNDROME

Sherine Ahmed Mohammed[1],Nesreen Gamal Abd Elhaliem[1], Youstina Lamie Fanous[2], Amany Abdelrahman[2], Hoda M moghazy [2]

[1] Histology Department, and [2]physiology Department, Faculty of Medicine, Sohag University. Sohag, Egypt

Corresponding author:
Sherine Ahmed Mohammed
Mobile: (+2) 01120301020

E.mail:
loua.sherine@yahoo.com

Received: 8/12/2022
Accepted: 10/12/2022

Online ISSN: 2735-3540

ABSTRACT:

Background: *Polycystic ovary syndrome (PCOS) is a common health problem in females throughout their reproductive age. Common symptoms of PCOS are hyperandrogenemia, menstrual disorders, and infertility. Metabolic disorders like insulin resistance and steatotic liver are also common. Metformin is most commonly used in the management of hyperglycemia. Erythropoietin and myoinositol were reported to improve insulin sensitivity.*

Objective: *This study aimed to compare the potential therapeutic role of erythropoietin, myoinositol, and the widely used metformin drug against hyperinsulinemia and hepatic injury of the letrozole-induced PCOS model in rats.*

Materials and methods: *Fifty female Wistar rats were classified equally into five groups: The control group and the PCO model group. PCO model was induced by letrozole in a dose of 0.5 mg/kg daily for 3 weeks. Then rats with PCO were administrated: erythropoietin in (EPO/PCO) group, myoinositol in (MYO/PCO) group, or metformin in (MET/PCO) group for the following 21 days. Biochemical and histological studies on the liver and pancreas were done. Immunohistochemical staining with GLUT-1 and insulin was done.*

Results: *PCO rats developed insulin resistance with pancreatic β cell degeneration, hepatocellular injury, and upregulation of hepatic GLUT-1. Metformin, erythropoietin, and myoinositol restored β-cell mass, decreased hyperinsulinemia, and attenuated hepatocellular degeneration via the reduction of stress-induced hepatic GLUT-1. However, erythropoietin was the most effective one of them.*

Conclusion: *Erythropoietin was more effective than metformin and myoinositol in decreasing hyperinsulinemia and attenuation of hepatocellular injury associated with the letrozole-induced model of PCOS.*

keywords: *polycystic ovary syndrome, metformin, insulin, GLUT-1*

Abbreviations: *PCOS; Polycystic ovary syndrome; IR; insulin* resistance LTZ; Letrozole; MET; Metformin; MYO; Myoinositol, EPO; Erythropoietin; GLUT-1; glucose transporter-1; ALT; alanine transaminase, AST; aspartate transaminase.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is mainly associated with hyperandrogenism, infertility, obesity, and insulin resistance (IR) [(1)]. The prevalence of IR is 10–25% in the general population and 60–70% in women with PCOS [(2)]. The IR and the

resulting compensatory hyperinsulinemia play pivotal roles in the evolution of PCOS [3]. Over time, most women with PCOS become diabetic after reaching 40 years old[4].

Letrozole (LTZ) was reported for the induction of PCO in rats via inhibition of aromatase enzyme. continuous prepubertal LTZ treatment disturbs the estrous cycle with anovulation. Besides, LTZ has many metabolic effects similar to PCOS signs such as IR and obesity[5].

Metformin (MET) is an insulin sensitizer. It is most commonly used in the management of type 2 diabetes and also to treat hyperglycemia associated with PCOS via the reduction of androgen levels[6]. It also has an effect on the liver by inhibiting hepatic gluconeogenesis to reduce glucose levels and consequently Insulin in the circulation[7]. Myoinositol (MYO) is an insulin second messenger which is also involved in the regulation of ovulation via follicular gonadotropin pathways[3].

Erythropoietin (EPO) is a glycoprotein hormone secreted primarily from the kidney by peritubular fibroblasts[8]. It controls erythropoiesis via binding to its receptor on progenitor cells. EPO exhibits anti-oxidative, anti-inflammatory as well as anti-apoptotic actions. It was previously reported that EPO reduces insulin resistance in metabolic syndrome via EPO receptors in insulin-sensitive tissues as muscles and the liver which augments insulin sensitivity or as a consequence of the treatment of anemia[8].

The glucose transporter-1 (GLUT-1) is expressed in most tissues of the body and acts in regulating the facilitative basal transport of glucose in these cells. GLUT-1 was reported to be highly expressed in neuronal membranes, the blood–brain barrier, and in erythrocytes. However, GLUT-1 plays an essential role in liver nonparenchymal cells as these cells obtain their own needed carbohydrates from outside, while hepatocytes store and produce glucose intracellularly[9]. Although GLUT-2 is known to be the common glucose transporter in the liver, the GLUT-1 role appears during development and stress conditions such as hypoxia and hyperglycemia[10].

AIM OF THE WORK:

This study aimed to compare the potential therapeutic role of EPO, MYO, and the widely used MET drug against hyperinsulinemia, β-cell degeneration, and hepatic disorders associated with LTZ-induced PCOS model in rats.

MATERIALS AND METHODS:

Experimental Animals

Fifty female Wister rats, 3 weeks old, 80 ±5 gm weight, were purchased and housed in the Medical Animal Laboratory in the faculty of medicine at Sohag University. Animals were allowed free access to water and food. Rats were kept in Polycarbonate cages at normal light/dark cycle as 5 rats/cage.

This study was approved by the ethical Committee of the Faculty of Medicine at Sohag University, and registration code: Soh-Med-ACUC-I: 5–17-2022-2, considering the experimental animals' care ethics.

The rats were randomly classified equally into five groups:

- The control group received: five animals recieved 2ml carboxy-methylcellulose (CMC) 0.5% by oral plastic gavage needles for 3 weeks, and another five received intraperitoneal saline for for 3 weeks .

Induction of PCO model:

The model group of PCO was induced with letrozole (LTZ) in a dose of 0.5 mg/kg dissolved in 0.5% CMC, daily for 3 weeks. Beginning from the second week of the study, the phase of the estrous cycle was determined by daily vaginal smear for each

animal to ensure that all LTZ-treated animals had developed PCO by becoming fixed at the diestrous phase.

In the next 3 weeks, these PCO rats started treatment with:

- PCO model group: received water for 3 weeks
- Group EPO /PCO: received erythropoietin in a dose of 100 IU / kg by intraperitoneal injection 3 days per week for 3 weeks [11].
- Group MYO/PCO received myoinositol orally in a dose of 420 mg/kg in 2ml H2O for 3 weeks [12].
- Group MET/PCO: received metformin orally in a dose of 150 mg/kg for 3 weeks [13].

The experimental design and animal groups are shown in Figs. 1A, 1B.

Chemicals: letrozole, myoinositol, metformin and erythropoietin were purchased from Sigma-Aldrich company.

- **The body weight** of each animal was weekly recorded for the detection of obesity.
- **vaginal smear:**

Approximately 0.2 ml of saline was withdrawn into the pipette dropper. After insertion of the tip of the dropper gently into the vaginal orifice not more than 5 – 10 mm depth, to avoid stimulating the cervix, saline was flushed 2 or 3 times into and back out of the vagina till obtaining cloudy fluid.

After the lavage, a smear was taken on the slide, allowed to dry and stained with toluidine blue for the identification of the different cell types in the four stages of the estrous cycle[14]. Proestrous stage is diagnosed by round nucleated epithelial cells. The estrous stage is diagnosed by cornified squamous epithelial cells; the metestrous stage is known by cornified squamous epithelial cells and leukocytes; and the diestrous stage is known by nucleated epithelial cells and leukocytes (Fig. 2).

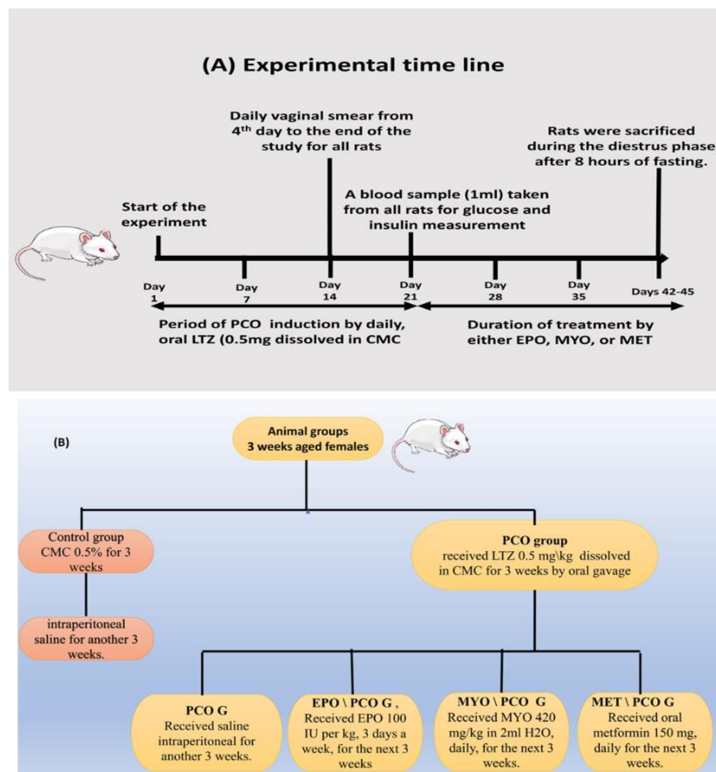

Fig. 1: (A) experimental timeline. (B) animal groups. PCOS; Polycystic ovary syndrome; IR; insulin resistance LTZ; Letrozole; MET; Metformin; MYO; Myoinositol, EPO; Erythropoietin;

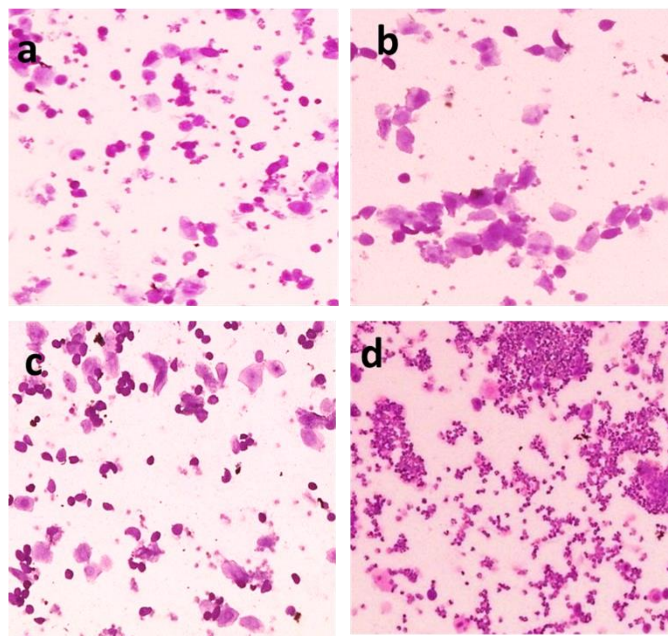

Fig. 2: Phases of the estrous cycle in a vaginal smear (a) pro-estrous phase; predominant nucleated epithelial cells (b) estrous phase, cornified cells are predominant (c) Metestrous phase, cornified cells with infiltration of leucocytes. (d) Diestrous phase, the predominance of leucocytes **(Toluidine blue stain).**

Collection of samples:

At the end of the experimental period, all animals were anesthetized with ether inhalation and blood samples were obtained from each animal by cardiac puncture and centrifuged at 3000 rpm.

A. **Biochemical assays**

1. Fasting Blood glucose level was measured by glucometer: Bionime corporation, Right test GM 300 and glucose-test strips: code556 [15] twice during the experimental period, the first one on the 21st day of the study (at the end of the duration of model induction), and the other at the end of the study. Rats were tested independent of the cycle stage after fasting for 8 hours.
2. Fasting blood insulin level was measured by enzyme-linked immune-sorbent assay (ELISA) kits: CSB-E05070r according to instructions of the manufacturer.
3. HOMA- IR and HOMA-B:
HOMA- IR is calculated for: Insulin resistance index (IRI) = fasting insulin (μIU/ml)×fasting glucose (mg/dl)/ 405.
4. Lipid profile:
Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDLC) were measured by spectrophotometry using commercial kits (Human Gesellschaft fur Biochemica and Diagnostic, ambH, Wiesbaden, Germany) according to manufacturer instructions. Low-density lipoprotein (LDLC) was calculated by the equation: LDLC= TC-HDLC-TG/5.
5. Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were estimated by using enzyme-linked immune sorbent assay by spectrophotometry apparatus (Beckman Coulter AU480)[16].

B. Light microscopy:

1. Livers and Pancreata were harvested after dissection. The liver specimens were obtained from the right lobe. The pancreas specimens were obtained from the tail. Specimens were rinsed with saline and fixed in 10% neutral buffered formalin for 24 h. Fixed tissues were

processed; dehydrated in alcohol and cleared in xylene. Then embedded in paraffin. Blocks were sliced using microtome into 5um sections. sections were stained with hematoxylin and eosin (H&E) for light microscopic examination. In the pancreas, for accurate morphometric measurements of islets of Langerhans, serial 5mm-thick were obtained with at least 3 levels per block 100–150 um apart [17].

2. Immunohistochemistry

Immunohistochemical staining for GLUT-1(glucose transporter-1) antibody in liver tissue for glucotoxicity *rabbit polyclonal GLUT-1 antibody from Abcam Inc (code ab40084; clone SPM498, Cambridge, MA, USA,1:100)* and insulin antibody for beta cells in the pancreas (Anti-Insulin Monoclonal antibody was bought from Invitrogen (INS05 (2D11-H5)), Catalog # MA5-12037. Dilution: 1:100). The reaction appeared as brown cytoplasmic staining.

Deparaffinization, rehydration and antigen retrieval was carried out by boiling the sections in 10 mmol/l citrate buffer (pH 6.2) in the microwave oven. Blocking of the endogenous peroxidase was done with 0.3 % hydrogen peroxide for 10 min. Incubation of the sections overnight at $4^{O}C$ temperature with the primary antibody. The biotinylated secondary antibody and then conjugate streptavidin were applied. Sections were counterstained with Mayer's hematoxylin, then dehydrated, and cleared in xylene. Negative control slides were made by omitting the primary antibody. Placenta specimens were the positive control for GLUT-1 and pancreas specimens were the positive control for insulin.

3. Morphometry :

The light microscope Leica ICC50 Wetzlar, Germany at the Histology Department, Faculty of Medicine, Sohag University was used. Ten nonoverlapping high power fields (x400) for each section in all animals in each group were taken and analyzed using Image J 1.51n software (National institutes of health USA Java 1.8.0_66 (32-bit) as follows:

a) **β cell percentage** was determined on insulin-immunostained sections. The percentage of **β** cell area to total islet area was measured at three serial sections which were sectioned 100um apart from the paraffin block of each animal. [18]

b) **GLUT-1 expression percentage area** was calculated[19].

C. Statistical Analysis

Data were collected and then analyzed using the Statistical package (SPSS, version 16.0). Data were presented as means± standard deviation (SD) and the values were considered significant as p-value ≤ 0.05. one way ANOVA test was used to compare results between different groups followed by post-Hoc test [20].

RESULTS:

Biochemical results:

- **Insulin resistance indices in the PCO model rats:**

The PCO group showed significantly increased body weights compared to those in the control group. Both the fasting blood glucose and the fasting insulin levels were significantly increased in the PCO group versus the control group. Furthermore, the PCOS group showed increased insulin resistance (IR) measured by HOMA-IR and decreased β cell function index versus control (table 1).

Table 1 shows that all groups: EPO\PCO, MYO\PCO, and MET\ PCO have got decreased glucose and insulin levels significantly than the PCO group. In addition, these drugs decreased insulin resistance as revealed by a decrease in

HOMA-IR and increased beta cell function index; HOMA-β. Multiple comparisons between each drug treated group, have revealed no significant difference between MYO\PCO, and the MET\ PCO groups in either of the insulin resistance indices. However, EPO decreased glucose and insulin levels more significantly than MYO and MET groups. Moreover, EPO decreased HOMA-IR and increased HOMA- β, significantly more than either MET or MYO did.

The PCO rats showed a significant rise in serum cholesterol, triglyceride, and LDL levels, while a decrease in HDL levels in comparison to the control group. This effect was minimized in the EPO\PCO, MYO\PCO, and MET\PCO groups, however, no significant difference was found between either group (Table 2).

On the other hand, the liver enzymes showed slightly significantly increased levels in PCO versus the control group. This effect was minimized in the EPO\PCO, MYO\PCO, and MET\PCO groups, however, no significant difference was found between either group (Table 2).

Table 1: Mean levels of body weight, fasting blood glucose (FBG), fasting blood insulin (FBINS), HOMA-IR, and HOMA- β in different groups

Groups	**Weight (gm)**	**FBG (mmol\L)**	**FBINS(mU\L)**	**HOMA-IR**	**HOMA- β**
Control G	195 ±8.3	4.1±0.43	4.7 ± 0.7	0.9 ± 0.2	226 ± 62
PCO G	219 ± 4.9[a]	7.2±0.37 [a]	9.5 ±0.6[a]	3.2 ± 0.5[a]	51.4 ± 7[a]
EPO\PCO G	198 ± 4.2[b]	4.6±0.7[b,c,d]	4.7 ± 1.3[b,c,d]	0.7±0.3[a,b,c,d]	219±67[a,b,c,d]
MYO\PCO G	196.4±3.02[b]	5.3±0.5[a,b,]	7.5 ± 1.6[a,b,]	1.77 ± 0.3[a,b]	90.2± 23[a,b]
MET\PCO G	203.6±3.4 [b]	5.5±0.3[a,b]	6.9 ± 1.2[a,b]	1.70 ± 0.2[a,b]	70.8 ± 19[a,b]

Data are expressed as mean ± SD (standard deviation). Data were analyzed by ANOVA test, Post hoc; Tukey's test. (N=10 rats per group). P value was considered significant when P<0.05. aP < 0.05 vs. control G.; bP<0.05 vs PCO G. cP<0.05 vs MET\PCO G. dP<0.05 vs MYO\PCO G. fasting blood glucose;FBG, fasting blood insulin; FBINS

Table 2: Mean levels of cholesterol (CHOLE), triglyceride (TRIGL), high-density (HDL), and low-density lipoprotein (LDL), aspartate transaminase (AST), and alanine transaminase (ALT) in different groups

Groups	CHOLE (mg\dl)	TRIGL (mg\dl)	HDL (mg\dl)	LDL (mg\dl)	AST (IU/L)	ALT (IU/L)
Control G	50.7 ±3.5	48± 9.07	42.4±5.4	26.2 ±0.7	147.9 ±11.6	23.6±3.2
PCO G	61.±11.7[a]	55 ±6.35[a]	25.±3.7[a]	66.2 ±6.7 [a]	168.0±26.1[a]	30.±8.6[a]
EPO\PCO G	49.9 ±3.1 [b]	50.6±7.21 [b]	43.1 ±4.7 [b]	27.0 ±1.2 [b]	148.2 ±12.4 [b]	24.6 ±5.8 [b]
MYO\PCO G	51.3 ±3.1 [b]	47.10±8.56 [b]	42.8 ±4.4 [b]	26.0 ±1.1 [b]	149.6 ±48.3 [b]	23.9 ±7.2 [b]
MET\PCO G	52.6 ±5.6 [b]	50.3± 7.02[b]	41.2 ±4.4[b]	26.6 ±1.1 [b]	147.4 ±23.5 [b]	25.0 ±6.1 [b]

Data are expressed as mean ± SD (standard deviation). Data were analyzed by ANOVA test, Post hoc; Tukey's test. (N=10 rats per group). P value was considered significant when P<0.05. [a]P < 0.05 vs. control G. ; [b]P<0.05 vs PCO G

Histological results:

- **Liver:**

1. **H&E**

Examination of H&E stained liver sections from the control group showed normal histological structure. Hepatocytes were radiating from the central vein towards the periphery. Hepatocytes had slightly vacuolated acidophilic cytoplasm with central rounded vesicular nuclei (Fig. 3a). In the PCO group, liver sections showed highly vacuolated cytoplasm in most hepatocytes and other degenerated hepatocytes with highly acidophilic cytoplasm and pyknotic nuclei. Dilated sinusoids and neutrophilic infiltration were observed (Fig. 3b). EPO/PCO and MYO/PCO livers showed more or less normal hepatocytes with slightly vacuolated cytoplasm (Figs. 3c, d). MET/PCO liver showed decreased degenerated hepatocytes; vacuolated hepatocytes less than PCO group, few apoptotic cells, less dilated sinusoids, and no inflammatory infiltration was detected (Fig. 3e).

Immunohistochemistry (glucose transporter-1; GLUT-1 antibody)

In the control liver, expression of GLUT-1 was only seen weakly expressed in Kupffer cells and endothelial cells (Fig. 4a).; however PCO group showed strong immunoreactivity of GLUT-1 in Kupffer cells and endothelial cells also in hepatocyte cell membranes especially the sinusoidal surface of most hepatocytes and cytoplasm of some hepatocytes (Fig. 4b). Erythropoietin and myoinositol showed expression of GLUT-1in Kupffer cells and endothelial cells only (Figs. 4c,d). Metformin showed strong expression of GLUT-1in Kupffer cells and endothelial cells and also in cell membranes of some hepatocytes, especially the sinusoidal surface (Fig. 4e).

Statistically, the PCO group showed a significant increase in the expression percentage area of GLUT-1 compared to the control. MET/PCO, MYO/PCO and EPO/PCO showed a significant decrease versus PCO which was significantly more pronounced in EPO/PCO versus the other two groups, but still significantly higher than in the control (Fig. 4f and Table 3).

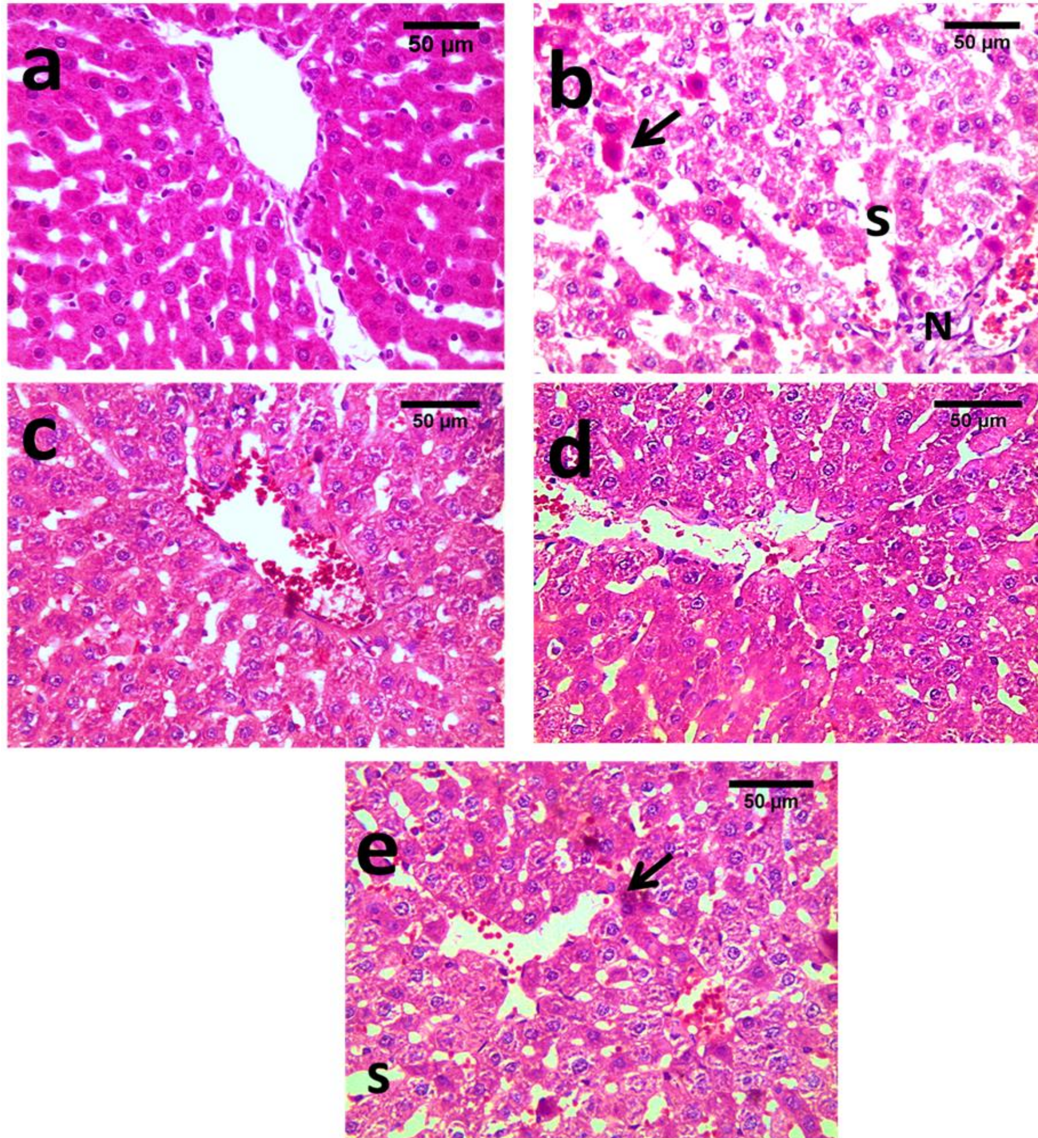

Fig.3: Photomicrographs of H&E stained liver sections of (a) control. b) group (PCO). (c)EPO/PCO (d) MYO/PCO (e) MET/PCO a) control Showing normal liver histology with normal hepatocytes radiating from the central vein**; b)** PCO showing highly vacuolated most hepatocytes and some degenerated hepatocytes with highly acidophilic cytoplasm and pyknotic nuclei(arrow).dilated sinusoids(s), neutrophilic infiltration(N); c)and d) showing more or less normal liver histology . **e)** showing less vacuolated hepatocytes and sinusoids(s) are less dilated than **b);** few apoptotic cells (arrow) **(H&Ex400, scale bar = 50 µm).**

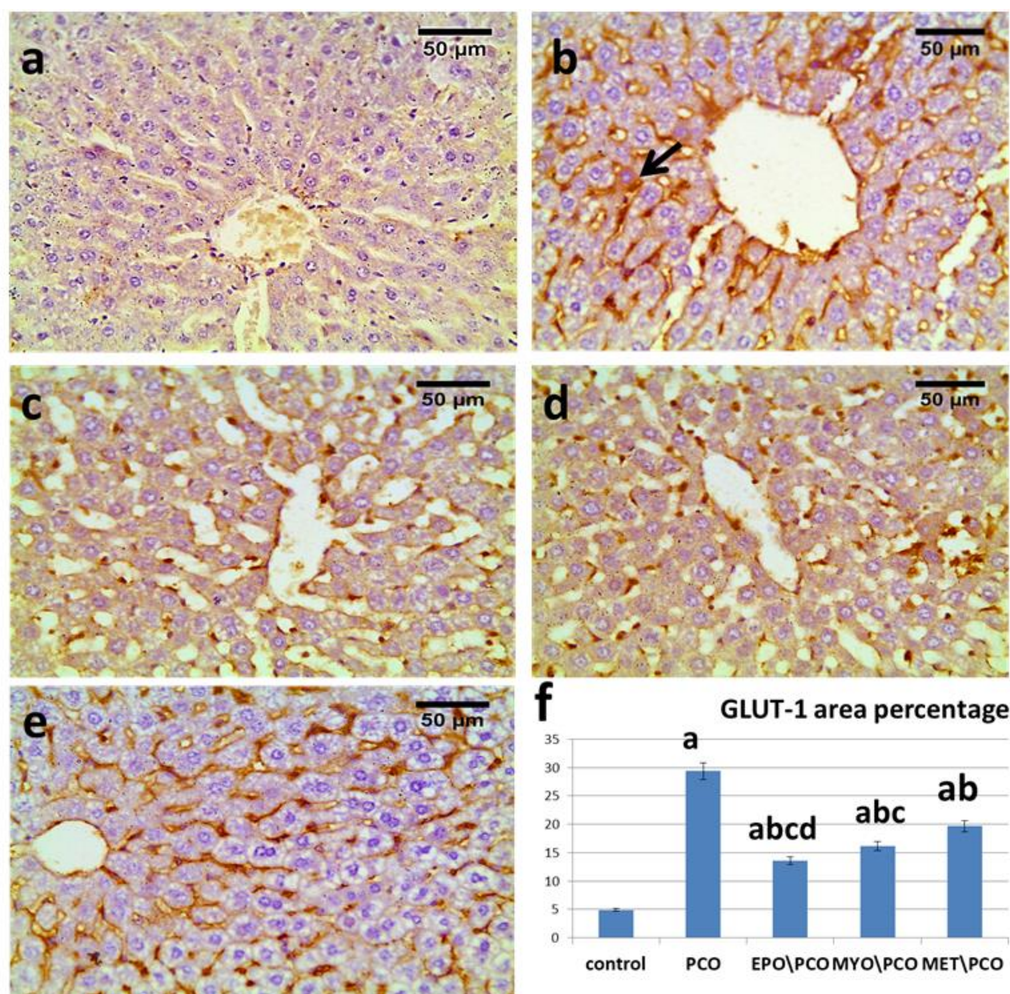

Fig. 4: Photomicrographs of GLUT-1 immunohistochemical staining of the liver. (a) control. b) group (PCO). (c)EPO/PCO (d) MYO/PCO (e) MET/PCO (a) expression of GLUT1in Kupffer cells and endothelial cells; b) showing strong immunoreactivity of GLUT-1 in Kupffer cells and endothelial cells also in cell membrane of most hepatocytes especially sinusoidal surface of and cytoplasm of some hepatocytes(arrow); (c)and d) showing expression of GLUT-1in Kupffer cells and endothelial cells. e) Expression of GLUT-1in Kupffer cells and endothelial cells also in cell membrane especially sinusoidal surface of few hepatocytes.(anti GLUT-1antibodies, scale bar =50 µm). f) histogram representing GLUT-1 expression area percentage in all groups.aP < 0.05 vs. control G.; bP<0.05 vs PCOS G. cP<0.05 vs MET\PCO G. dP<0.05 vs MYO\PCO G.

- **Pancreas:**

1. H&E

Examination of H&E-stained sections of the pancreas from the control group showed a normal histological pattern. Pancreatic lobules were separated by thin loose connective tissue. The islets of Langerhans appeared as lightly stained areas surrounded by the serous acini. The islets were formed of clusters of cells forming anastomosing cords which were separated by blood capillaries. The cells of the exocrine pancreas which form the serous acini had basal basophilic and apical acidophilic cytoplasm (Fig. 5a). PCO group showed an apparent increased surface area and irregular outline of islets of Langerhans Some Islet cells were vacuolated and few apoptotic cells appeared with highly acidophilic cytoplasm and pyknotic nuclei (Fig. 5b). EPO\PCO, MYO\PCO, and the MET\ PCO groups showed an apparent decrease in islets surface area compared to PCO which was

more pronounced in EPO\PCO, MYO\PCO groups (Figs. 5 c, d). The structure of islet cells was more or less normal in these three groups except for some vacuolated cells observed in MET\ PCO (Fig. 5e).

2. **Immunohistochemistry (insulin antibody)**

Insulin expression was in the form of brown cytoplasmic staining of islets which demonstrates β-cells. In the control, β-cells were occupying mainly the central zone of the islets (Fig. 6a). An apparent decrease in β-cells was observed in PCO as compared with the control group (Fig. 6b). MET/PCO, MYO/PCO, and EPO/PCO showed strong positive insulin expression in beta cells compared to PCO which was more pronounced in EPO/PCO group (Figs. 6 c, d, and e).

Statistically, the PCO group showed a significant decrease in the expression percentage area of β-cells compared to the control. MET/PCO, MYO/PCO, and EPO/PCO showed a significant increase versus PCO, which was significantly more pronounced in EPO/PCO versus the other two groups. EPO/PCO was non-significant versus control (Fig. 6f and Table 3).

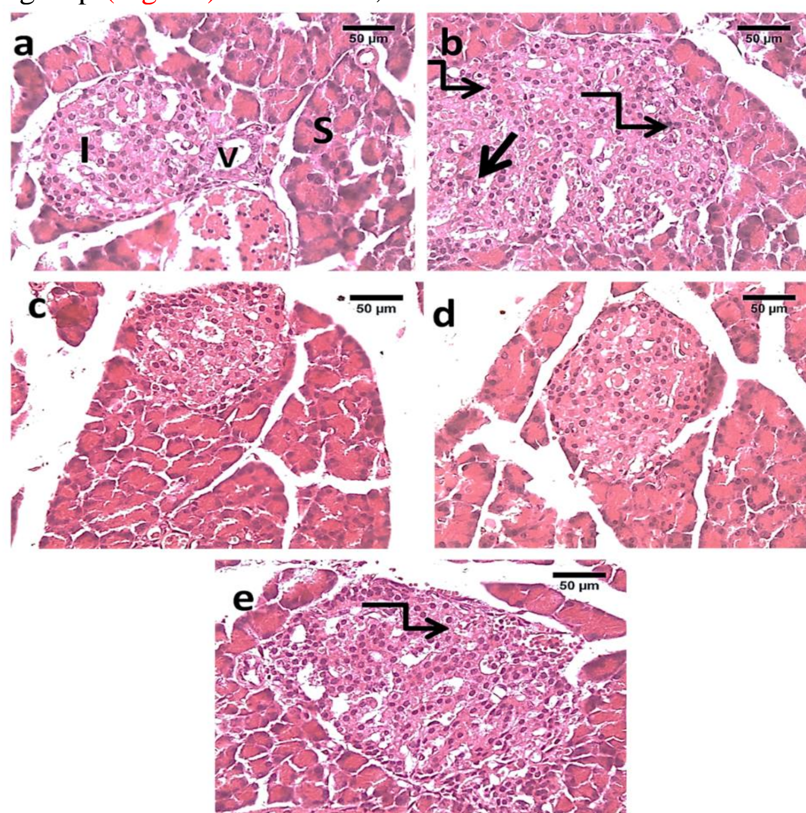

Fig.5: Photomicrographs of H&E stained pancreas sections. (a) control. (b) PCO). (c)EPO/PCO (d) MYO/PCO (e) MET/PCO (a) showing pancreatic lobules separated by loose connective tissue septa. The islet cells **(I)** are seen interspersed between the serous acinar cells **(s)**. The islets are lightly stained and formed of irregularly arranged cords of cells separated by blood vessels (v). The pancreatic acini are lined by pyramidal cells having peripheral basophilic and apical acidophilic cytoplasm with normal vesicular nuclei (b) **PCO** group showed hypertrophy of islets of Langerhans. Some Islet cells are showing vacuolations **(stepwise arrow)** and few cells appear with highly acidophilic cytoplasm and pyknotic nuclei **(arrow)**; **c), d)** showing very similar morphology to the control group. **e)** Showing some vacuolated **(stepwise arrow)** cells in the islets with decreased islet area than in the PCO group **(H&Ex400, scale bar = 50 µm)**.

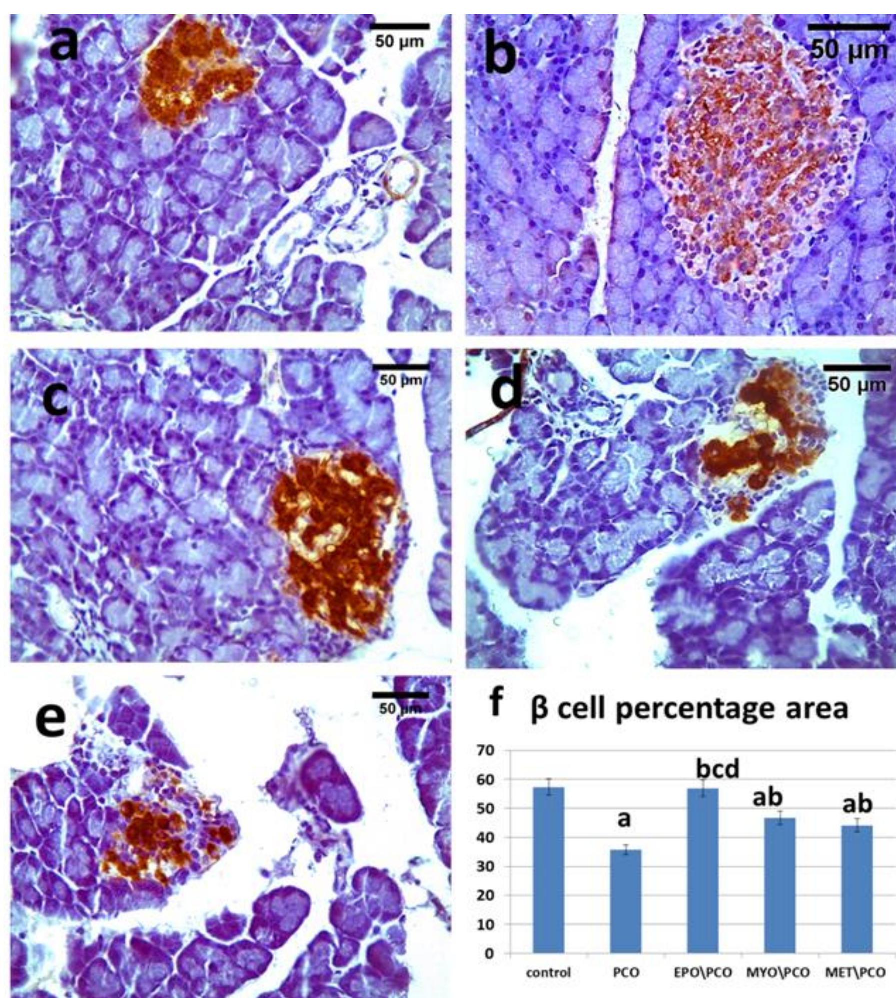

Fig. 6: Photomicrographs of insulin immunohistochemical staining of pancreatic islets. (a) control b) PCO). (c)EPO/PCO (d) MYO/PCO (e) MET/PCO (a) control is showing immunoexpression of insulin in beta cells b) weak immunoreactivity of insulin in beta-cells which occupy smaller area percentage of the islet than in control. (c), (d) and (d) showing strong expression of insulin in beta cells with an increase in beta cell area compared to (b), this is most prominent in (c). (anti-insulin antibody, scale bar =50 µm) f) histogram showing insulin expression area percentage in all groups. [a]P < 0.05 vs. control G.; bP<0.05 vs PCOS G. cP<0.05 vs MET\PCO G. dP<0.05 vs MYO\PCO G.

Table 3: The mean β cell percentage and the mean GLUT-1 expression area percentage in different groups

Groups	mean β cell percentage ± SD	mean GLUT-1 expression area percentage± SD
Control G	57.3±4	4.9±0.9
PCOS G	35.7±5 [a]	29.4±1.3[a]
EPO\PCO G	56.8±4 [bcd]	13.6±1.0 [abcd]
MYO\PCO G	46.2± 9 [ab]	16.1±0.8[abc]
MET\PCO G	45.1±4 ab	17.7±1.1[ab]

Data are expressed as mean ± SD (standard deviation). Data were analyzed by ANOVA test, Post hoc; Tukey's test. (N=10 rats per group). P value was considered significant when P<0.05. [a]P < 0.05 vs. control G.; [b]P<0.05 vs PCOS G. [c]P<0.05 vs MET\PCO G. [d.] P<0.05 vs Myo\PCO G

DISCUSSION:

PCO is a common health problem in females during their reproductive age[21&22]. Common symptoms of PCO are hyperandrogenemia, infertility, and menstrual disorders. Metabolic disorders like IR and steatotic liver are also common. This study aimed to compare the potential therapeutic role of EPO, MYO and the widely used MET drug against hyperinsulinemia, β-cell degeneration and hepatic disorders associated with LTZ-induced PCOS model in rats.

In our PCO model, LTZ induced PCO in rats by inhibiting the aromatase enzyme Aromatase enzyme converts the synthesized androgen in the female ovary into estrogen, thus leading to increased androgen level which is a benchmark in the diagnosis and pathogenesis of PCO[23]. This hyperandrogenemia is further associated with decreased estrogen, progesterone, and FSH, while increased LH in the pituitary gland. with subsequent loss of cyclicity in the estrous cycle [21,24&25].

In our PCO model, increased fasting levels of both glucose and insulin, decreased HOMA- β, and increased HOMA-IR, were observed. Similar indices of insulin resistance in PCO rats were reported previously as [24-26].

Hyperinsulinemia has a key role in the pathogenesis of PCO as it increases fat storage leading to obesity and affects cholesterol and lipoprotein metabolism. Furthermore, abdominal obesity and elevated androgen in PCO aggravate the associated insulin resistance[27].

In our study, the PCO group showed an abnormal lipid profile in form of decreased HDLc and increased total cholesterol, triglyceride, and LDLc. In consistency with our results, ***Abuelezz et al.***[26] study and ***Roy et al.***[28] study. ***Roy et al.***[28] attributed this disturbance in lipid profile to the hyperandrogenic state which disrupts genes encoding enzymes of fat metabolism. This also might be due to hyperinsulinemia which decreases lipid oxidation via controlling genes encoding enzymes of fatty acid oxidation [29].

As regards the liver enzymes in our study, these were slightly significantly elevated in the PCO group compared to the control group. Similar results were previously obtained after long-term treatment with androgen for the induction of PCO [30]. This could be attributed to degenerated hepatocytes with the release of these enzymes out of the cells to the blood.

In the current study, PCO liver showed highly vacuolated cytoplasm in most hepatocytes and other degenerated apoptotic hepatocytes. Dilated sinusoids and neutrophilic infiltration were observed. Similar results were previously reported by ***Chaiyamoon et al.***[31]. In addition, in a previous study on androgen Induced model of Polycystic Ovary, the appearance of necrotic foci were observed in the liver histology in addition to the inflammatory infiltration and dilated sinusoids as seen in our results. Thus PCO is considered a state of chronic inflammation[32].

Previous studies described the hepatocyte vacuolation observed in our results as steatosis [33, 34]. Liver fatty changes could be attributed to the increased lipolysis caused by insulin resistance in fat cells, resulting in increased free fatty acids which accumulate in the hepatic tissue [35]. ***Roy et al.***[28] study explained steatosis to be induced by the chronic high androgen level in PCO which disrupts the liver genes concerned with fat metabolism.

In the current study, H&E stained sections of the pancreatic specimens from the PCO group showed an apparent increase in surface area and irregular outline of islets of Langerhans Some Islet cells were vacuolated and few apoptotic cells appeared with highly acidophilic cytoplasm and

pyknotic nuclei. Similar results were observed in ***Peng et al.***[36] study which reported islet irregular outline and vacuolar degeneration of islet cells, especially in the center of the islet. The islet hypertrophy observed in our results could be explained by compensatory hyperplasia due to the degeneration of β-cells as reported by ***Boonnate et al.***[37]. The oxidative stress induced by hyperglycemia associated with PCO caused degeneration of islet cells [38].

In the present study, immunohistochemistry supported the histopathology results, and a significant decrease in β cell area percentage was observed in the PCO group. similar results were previously reported by ***Abuelezz et al.***[26]. PCOS exhibited a high degree of insulin resistance in the decompensated phase with compromised β-cell function[38].

Hyperinsulinemia can lead to the exhaustion of β-cells. Misfolded proteins of proinsulin accumulate in the cisternae of the endoplasmic reticulum of pancreatic β cells, resulting in ER stress, apoptosis as well as inflammation[39].

The association of PCO and pancreatic β cell degeneration was attributed to low estrogen levels as expression of estrogen receptors on β cells was reported and hence β cells are responsive to estrogen signals. Moreover, low estrogen level disturbs the metabolism of iron which represents an essential element for the proliferation of pancreatic islet cells[40].

In contrast to these mentioned studies which reported decreased insulin levels and β-cell insulin expression in PCOS compared with normal, other studies demonstrated increased insulin levels[41]. These heterogeneous findings have been observed depending on whether insulin secretion was assessed in the compensated or non-compensated state. In addition, Hyper insulinemia in PCO could be due to the reduced insulin clearance by the liver rather than the increased secretion of insulin by pancreatic islets[42].

In our study, an increase in GLUT-1 immunoexpression in the liver of the PCO model was observed and that could be attributed to hyperglycemia[43]. GLUT-1 overexpression occurs in hyperglycemia and hypoxia. GLUT-1 has a high affinity to glucose, shifting from hepatic GLUT-2 to GLUT-1 in liver injury occurs as a compensatory mechanism to the increased intracellular need for glucose[44]. GLUT1 conformational changes and cellular translocation represent an adaptive response to keep adequate cellular nutrition[45]. Moreover, GLUT-1 upregulation contributes to oxidative stress in the liver[46].

In our study, EPO, MYO, and MET all succeeded to decrease insulin resistance indices and attenuate hyperinsulinemia. They reversed the alterations in the pancreatic histopathology and hepatocellular degenerative changes. EPO significantly reduced insulin resistance more than both MET and MYO. Supporting this, as the hyperandrogenic state plays a key role in these metabolic disorders associated with PCO, ***Abdelrahman et al.***[47] study reported that hormonal changes in PCO were reversed and androgens decreased to variable extents using the same three drugs.

In our results, MET/PCO liver showed decreased degenerated hepatocytes than the PCO group and more prominent restoration of normal liver histology was observed in MYO/PCO and EPO/PCO. EPO improved liver function tests and attenuated the hepatocellular degenerative changes with decreased vacuolation. EPO has anti-inflammatory and antioxidant roles. In addition, it stimulates regeneration of the injured liver[48]. Similar to our results, it was previously reported that EPO improved fatty liver disease[49]. MYO was previuosly reported to decrease fatty changes in steatotic liver[50] . This was explained by its

ability to inhibit fatty acid synthesis enzymes[50].

In our study, EPO\PCO, MYO\PCO, and the MET\ PCO groups showed a decrease in islets surface area compared to PCO which was more pronounced in EPO\PCO, MYO\PCO groups. The structure of islet cells was more or less normal in these three groups except for some vacuolated cells observed in MET\ PCO. EPO was the most effective one of the three drugs used in our experiment which improved hyperglycemia and restored β-cell mass. Similar to our results, EPO augmented glucose metabolism and decreased pancreatic β cell damage in experimental diabetic models. EPO produces a protective effect on pancreatic β-cells and ultra-structurally restored the number of insulin secretory granules in an experimentally induced diabetic model [51]. In addition, previous studies reported that EPO improved insulin sensitivity in obesity and metabolic syndrome models [52]. This could be attributed to EPO receptors in insulin sensitive tissues as the liver. In addition, EPO upregulates GLUT-4 which regulates insulin dependent glucose utilization[47]

As for MYO/PCO, it was significantly less improved than EPO regarding β-cell mass, insulin resistance, and GLUT-1 expression. Myoinisitol makes up many secondary messengers in response to insulin secretion. However, hyperglycemia decreases its absorption and biosynthesis and triggers its degradation and excretion. In addition, Insulin resistance diminishes inositol uptake into cells [53]. Subsequently, its effect is lowered to be less effective than EPO in cases of insulin resistance and hyperglycemia as in our study.

Similarly, ***Abulfadle et al.***[54] reported that metformin ameliorated hepatic toxicity produced by PCOS, causing an improvement in its biochemical changes because metformin had anti-inflammatory activity. Moreover, ***Rababa'h et al.***[55] reported that metformin antagonizes insulin resistance and endocrine disorders in the PCOS animal model via triggering activated mitogen protein kinase . In addition, ***Tan et al.***[56] study proved that Metformin is an insulin sensitizer which decreases hepatic glucose secretion, increases peripheral glucose utilization, and alleviates insulin resistance in PCO. However, many side effects were reported for metformin.

Conclusion: EPO, MYO and MET were effective in restoring β cell mass, decreasing hyperinsulinemia associated with the LTZ-induced model of PCOS, and attenuating hepatocellular degeneration via the reduction of stress-induced hepatic GLUT-1**.** However, EPO was the most effective one of them.

Conflict of interest:

The authors report no conflicts of interest.

REFERENCES:

1. Xie, Y., Xiao, L., And Li, S. Effects Of Metformin On Reproductive, Endocrine, And Metabolic Characteristics Of Female Offspring In A Rat Model Of Letrozole-Induced Polycystic Ovarian Syndrome With Insulin Resistance. Frontiers In Endocrinology. 2021; 12: P. 701590.
2. Belani, M., Deo, A., Shah, P., Banker, M., Singal, P., And Gupta, S. Differential Insulin And Steroidogenic Signaling In Insulin Resistant And Non-Insulin Resistant Human Luteinized Granulosa Cells-A Study In PCOS Patients. J Steroid Biochem Mol Biol. 2018; 178: P. 283-292.
3. Laganà, A.S., Garzon, S., Casarin, J., Franchi, M., And Ghezzi, F. Inositol In Polycystic Ovary Syndrome: Restoring Fertility Through A Pathophysiology-Based Approach. Trends In Endocrinology & Metabolism. 2018; 29(11): P. 768-780.
4. Forslund, M., Landin-Wilhelmsen, K., Trimpou, P., Schmidt, J., Brännström, M., And Dahlgren, E. Type 2 Diabetes Mellitus In Women With Polycystic Ovary Syndrome During A 24-Year Period:

Importance Of Obesity And Abdominal Fat Distribution. Human Reproduction Open. 2020; 2020(1): P. Hoz042.

5. Hassan, N., Rashad, M., Elleithy, E., Sabry, Z., Ali, G., And Elmosalamy, S. L-Carnitine Alleviates Hepatic And Renal Mitochondrial-Dependent Apoptotic Progression Induced By Letrozole In Female Rats Through Modulation Of Nrf-2, Cyt C And CASP-3 Signaling. Drug And Chemical Toxicology. 2022: P. 1-12.
6. Ma, R.-L., Deng, Y., Wang, Y.-F., Zhu, S.-Y., Ding, X.-S., And Sun, A.-J. Short-Term Combined Treatment With Exenatide And Metformin For Overweight/Obese Women With Polycystic Ovary Syndrome. Chinese Medical Journal. 2021; 134(23): P. 2882-2889.
7. Yan, J.-B., Lai, C.-C., Jhu, J.-W., Gongol, B., Marin, T.L., Lin, S.-C., Chiu, H.-Y., Yen, C.-J., Wang, L.-Y., And Peng, I.C. Insulin And Metformin Control Cell Proliferation By Regulating TDG-Mediated DNA Demethylation In Liver And Breast Cancer Cells. Molecular Therapy - Oncolytics. 2020; 18: P. 282-294.
8. Chen, L.N., Sun, Q., Liu, S.Q., Hu, H., Lv, J., Ji, W.J., Wang, M., Chen, M.X., And Zhou, J. Erythropoietin Improves Glucose Metabolism And Pancreatic B-Cell Damage In Experimental Diabetic Rats. Mol Med Rep. 2015; 12(4): P. 5391-5398.
9. Zhou, M.Y., Cheng, M.L., Huang, T., Hu, R.H., Zou, G.L., Li, H., Zhang, B.F., Zhu, J.J., Liu, Y.M., Liu, Y., And Zhao, X.K. Transforming Growth Factor Beta-1 Upregulates Glucose Transporter 1 And Glycolysis Through Canonical And Noncanonical Pathways In Hepatic Stellate Cells. World Journal Of Gastroenterology. 2021; 27(40): P. 6908-6926.
10. Chadt, A. And Al-Hasani, H. Glucose Transporters In Adipose Tissue, Liver, And Skeletal Muscle In Metabolic Health And Disease. Pflugers Archiv : European Journal Of Physiology. 2020; 472(9): P. 1273-1298.
11. Günal, M.Y., Ozansoy, M., Kılıç, Ü., Keskin, İ., Özdemir, E.M., Aslan, İ., Eren, Z., Ersavaş, C., And Kılıç, E. Role Of Erythropoietin And Its Receptor In The Development Of Endometriosis In Rats. Journal Of The Turkish German Gynecological Association. 2019; 20(1): P. 41.
12. Bevilacqua, A., Dragotto, J., Giuliani, A., And Bizzarri, M. Myo-Inositol And D-Chiro-Inositol (40: 1) Reverse Histological And Functional Features Of Polycystic Ovary Syndrome In A Mouse Model. Journal Of Cellular Physiology. 2019; 234(6): P. 9387-9398.
13. Patel, R. And Shah, G. High-Fat Diet Exposure From Pre-Pubertal Age Induces Polycystic Ovary Syndrome (PCOS) In Rats. Reproduction. 2018; 155(2): P. 139-149.
14. Cora, M.C., Kooistra, L., And Travlos, G. Vaginal Cytology Of The Laboratory Rat And Mouse: Review And Criteria For The Staging Of The Estrous Cycle Using Stained Vaginal Smears. Toxicologic Pathology. 2015; 43(6): P. 776-93.
15. Kauffman, A.S., Thackray, V.G., Ryan, G.E., Tolson, K.P., Glidewell-Kenney, C.A., Semaan, S.J., Poling, M.C., Iwata, N., Breen, K.M., Duleba, A.J., Stener-Victorin, E., Shimasaki, S., Webster, N.J., And Mellon, P.L. A Novel Letrozole Model Recapitulates Both The Reproductive And Metabolic Phenotypes Of Polycystic Ovary Syndrome In Female Mice1. Biology Of Reproduction. 2015; 93(3): P. 69.
16. Huang, X.J., Choi, Y.K., Im, H.S., Yarimaga, O., Yoon, E., And Kim, H.S. Aspartate Aminotransferase (AST/GOT) And Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors (Basel). . 2006 ;6(7): P.:756-82. .
17. Abunasef, S., Amin, H., And Abdel-Hamid, G. A Histological And Immunohistochemical Study Of Beta Cells In Streptozotocin Diabetic Rats Treated With Caffeine. Folia Histochemica Et Cytobiologica / Polish Academy Of Sciences, Polish Histochemical And Cytochemical Society. 2014; 52: P. 42-50.
18. Laker, R., Gallo, L., Wlodek, M., Siebel, A., Wadley, G., And Mcconell, G. Short-Term Exercise Training Early In Life Restores Deficits In Pancreatic B-Cell Mass Associated With Growth Restriction In Adult Male Rats. American Journal Of Physiology. Endocrinology And Metabolism. 2011; 301: P. E931-40.

19. Sokolovska, J., Isajevs, S., Rostoka, E., Sjakste, T., Trapiņa, I., Ošiņa, K., Paramonova, N., And Sjakste, N. Changes In Glucose Transporter Expression And Nitric Oxide Production Are Associated With Liver Injury In Diabetes. Cell Biochemistry And Function. 2015; 33(6): P. 367-74.
20. Bryman, A. And Cramer, D. Quantitative Data Analysis With SPSS 14, 15 & 16: A Guide For Social Scientists. Quantitative Data Analysis With SPSS 14, 15 & 16: A Guide For Social Scientists. 2009, New York, NY, US: Routledge/Taylor & Francis Groupxxv, 381-Xxv, 381.
21. Respekta, N., Maślanka, A., Mlyczyńska, E., Billert, M., Szlaga, A., Sambak, P., Pawlicki, P., Płachno, B., Skrzypski, M., And Kotula–Balak, M. Levels Of Spexin And Its Receptors GALR2 And GALR3 In The Hypothalamus And Ovary Of Letrozole-Induced Polycystic Ovary Syndrome In Rats. Biochemical And Biophysical Research Communications. 2022; 627: P. 207-213.
22. Rodriguez Paris, V., Solon-Biet, S.M., Senior, A.M., Edwards, M.C., Desai, R., Tedla, N., Cox, M.J., Ledger, W.L., Gilchrist, R.B., Simpson, S.J., Handelsman, D.J., And Walters, K.A. Defining The Impact Of Dietary Macronutrient Balance On PCOS Traits. Nature Communications. 2020; 11(1): P. 5262.
23. Goodarzi, M.O., Dumesic, D.A., Chazenbalk, G., And Azziz, R. Polycystic Ovary Syndrome: Etiology, Pathogenesis And Diagnosis. Nature Reviews Endocrinology. 2011; 7(4): P. 219-231.
24. Azouz, A.A., Ali, S.E., Abd-Elsalam, R.M., Emam, S.R., Galal, M.K., Elmosalamy, S.H., Alsherbiny, M.A., Hassan, B.B., Li, C.G., And El Badawy, S.A. Modulation Of Steroidogenesis By Actaea Racemosa And Vitamin C Combination, In Letrozole Induced Polycystic Ovarian Syndrome Rat Model: Promising Activity Without The Risk Of Hepatic Adverse Effect. Chinese Medicine. 2021; 16(1): P. 36.
25. Wu, Y.-X., Yang, X.-Y., Han, B.-S., Hu, Y.-Y., An, T., Lv, B.-H., Lian, J., Wang, T.-Y., Bao, X.-L., Gao, L., And Jiang, G.-J. Naringenin Regulates Gut Microbiota And SIRT1/ PGC-1α Signaling Pathway In Rats With Letrozole-Induced Polycystic Ovary Syndrome. Biomedicine & Pharmacotherapy. 2022; 153: P. 113286.
26. Abuelezz, N.Z., Shabana, M.E., Abdel-Mageed, H.M., Rashed, L., And Morcos, G.N. Nanocurcumin Alleviates Insulin Resistance And Pancreatic Deficits In Polycystic Ovary Syndrome Rats: Insights On PI3K/Akt/Mtor And TNF-A Modulations. Life Sciences. 2020; 256: P. 118003.
27. Tao, X., Cai, L., Chen, L., Ge, S., And Deng, X. Effects Of Metformin And Exenatide On Insulin Resistance And Ampkα-SIRT1 Molecular Pathway In PCOS Rats. Journal Of Ovarian Research. 2019; 12(1): P. 1-8.
28. Roy, S., Abudu, A., Salinas, I., Sinha, N., Cline-Fedewa, H., Yaw, A.M., Qi, W., Lydic, T.A., Takahashi, D.L., Hennebold, J.D., Hoffmann, H.M., Wang, J., And Sen, A. Androgen-Mediated Perturbation Of The Hepatic Circadian System Through Epigenetic Modulation Promotes NAFLD In PCOS Mice. Endocrinology. 2022; 163(10): P. Bqac127.
29. Li, M., Chi, X., Wang, Y., Setrerrahmane, S., Xie, W., And Xu, H. Trends In Insulin Resistance: Insights Into Mechanisms And Therapeutic Strategy. Signal Transduction And Targeted Therapy. 2022; 7(1): P. 216.
30. Cui, P., Hu, W., Ma, T., Hu, M., Tong, X., Zhang, F., Shi, J., Xu, X., Li, X., And Shao, L.R. Long-Term Androgen Excess Induces Insulin Resistance And Non-Alcoholic Fatty Liver Disease In PCOS-Like Rats. The Journal Of Steroid Biochemistry And Molecular Biology. 2021; 208: P. 105829.
31. Chaiyamoon, A., Bunsueb, S., And Iamsaard, S. Changes Of Tyrosine Phosphorylation In Liver And Kidney Of Polycystic Ovarian Rats Induced By Letrozole. International Journal Of Morphology. 2020; 38(4): P. 919-923.
32. Krishnan, A., Muthusami, S., Periyasamy, L., Stanley, J., Gopalakrishnan, V., And Ramachandran, I. Effect Of DHT-Induced Hyperandrogenism On The Pro-Inflammatory Cytokines In A Rat Model Of Polycystic Ovary Morphology. Medicina. 2020; 56(3): P. 100.
33. Rodriguez Paris, V., Edwards, M.C., Aflatounian, A., Bertoldo, M.J., Ledger,

W.L., Handelsman, D.J., Gilchrist, R.B., And Walters, K.A. Pathogenesis Of Reproductive And Metabolic PCOS Traits In A Mouse Model. Journal Of The Endocrine Society. 2021; 5(6): P. Bvab060.

34. Weir, G.C., Gaglia, J., And Bonner-Weir, S. Inadequate Β-Cell Mass Is Essential For The Pathogenesis Of Type 2 Diabetes. The Lancet Diabetes & Endocrinology. 2020; 8(3): P. 249-256.
35. Palma, R., Pronio, A., Romeo, M., Scognamiglio, F., Ventriglia, L., Ormando, V.M., Lamazza, A., Pontone, S., Federico, A., And Dallio, M. The Role Of Insulin Resistance In Fueling NAFLD Pathogenesis: From Molecular Mechanisms To Clinical Implications. Journal Of Clinical Medicine. 2022; 11(13): P. 3649.
36. Peng, M.F., Tian, S., Song, Y.G., Li, C.X., Miao, M.S., Ren, Z., And Li, M. Effects Of Total Flavonoids From Eucommia Ulmoides Oliv. Leaves On Polycystic Ovary Syndrome With Insulin Resistance Model Rats Induced By Letrozole Combined With A High-Fat Diet. Journal Of Ethnopharmacology. 2021; 273: P. 113947.
37. Boonnate, P., Waraasawapati, S., Hipkaeo, W., Pethlert, S., Sharma, A., Selmi, C., Prasongwattana, V., And Cha'on, U. Monosodium Glutamate Dietary Consumption Decreases Pancreatic Β-Cell Mass In Adult Wistar Rats. Plos One. 2015; 10(6): P. E0131595.
38. Malin, S.K., Kirwan, J.P., Sia, C.L., And González, F. Pancreatic Β-Cell Dysfunction In Polycystic Ovary Syndrome: Role Of Hyperglycemia-Induced Nuclear Factor-Κb Activation And Systemic Inflammation. Am J Physiol Endocrinol Metab. 2015; 308(9): P. E770-7.
39. Zhu, B., Chen, Y., Xu, F., Shen, X., Chen, X., Lv, J., And Zhang, S. Androgens Impair Β-Cell Function In A Mouse Model Of Polycystic Ovary Syndrome By Activating Endoplasmic Reticulum Stress. Endocrine Connections. 2021; 10(3): P. 265-272.
40. Taneera, J., Ali, A., And Hamad, M. The Role Of Estrogen Signaling In Cellular Iron Metabolism In Pancreatic Β Cells. Pancreas. 2022; 51(2): P. 121-127. .
41. Mishra, J.S., More, A.S., And Kumar, S. Elevated Androgen Levels Induce Hyperinsulinemia Through Increase In Ins1 Transcription In Pancreatic Beta Cells In Female Rats. Biology Of Reproduction. 2018; 98(4): P. 520-531.
42. Bergman, R.N., Piccinini, F., Kabir, M., Kolka, C.M., And Ader, M. Hypothesis: Role Of Reduced Hepatic Insulin Clearance In The Pathogenesis Of Type 2 Diabetes. Diabetes. 2019; 68(9): P. 1709-1716.
43. Dalal, S., Adhikary, J., Roy, A., Biswas, S.S., Mukhopadhyay, P.K., Acharya, S., And Ghosh, A. Impact Of Hyperglycemia On The Expression Of GLUT1 During Oral Carcinogenesis In Rats. Molecular Biology Reports. 2022; 49(9): P. 8369-8380.
44. Sokolovska, J., Isajevs, S., Rostoka, E., Sjakste, T., Trapiņa, I., Ošiņa, K., Paramonova, N., And Sjakste, N. Changes In Glucose Transporter Expression And Nitric Oxide Production Are Associated With Liver Injury In Diabetes. Cell Biochemistry And Function. 2015; 33(6): P. 366-374.
45. Meireles, P., Sales-Dias, J., Andrade, C.M., Mello-Vieira, J., Mancio-Silva, L., Simas, J.P., Staines, H.M., And Prudêncio, M. GLUT1-Mediated Glucose Uptake Plays A Crucial Role During Plasmodium Hepatic Infection. Cell Microbiol. 2017; 19(2): P. E12646.
46. Zambrano, A., Molt, M., Uribe, E., And Salas, M. Glut 1 In Cancer Cells And The Inhibitory Action Of Resveratrol As A Potential Therapeutic Strategy. International Journal Of Molecular Sciences. 2019; 20(13): P. 3374.
47. Abdelrahman, A., Mahmoud, A.A., Lamie Fanous, Y., Abd Elhaliem, N.G., And Elalaf, H. Impact Of Erythropoietin And Myoinositol Versus Metformin On Insulin Resistance In A Rat Model Of Polycystic Ovary Syndrome. Archives Of Physiology And Biochemistry. 2022: P. 1-12.
48. Gul, M., Cömert, M., Çakmak, G.K., Kertis, G., Ugurbas, E., And Oner, M.O. Effect Of Erythropoietin On Liver Regeneration In An Experimental Model Of Partial Hepatectomy. International Journal Of Surgery. 2013; 11(1): P. 59-63.
49. Tsuma, Y., Mori, J., Ota, T., Kawabe, Y., Morimoto, H., Fukuhara, S., Kodo, K., Umemura, A., Nakajima, H., And Hosoi, H. Erythropoietin And Long-Acting

Erythropoiesis Stimulating Agent Ameliorate Non-Alcoholic Fatty Liver Disease By Increasing Lipolysis And Decreasing Lipogenesis Via EPOR/STAT Pathway. Biochemical And Biophysical Research Communications. 2019; 509(1): P. 306-313.

50. Shimada, M., Ichigo, Y., Shirouchi, B., Takashima, S., Inagaki, M., Nakagawa, T., And Hayakawa, T. Treatment With Myo-Inositol Attenuates Binding Of The Carbohydrate-Responsive Element-Binding Protein To The Chrebp-B And FASN Genes In Rat Nonalcoholic Fatty Liver Induced By High-Fructose Diet. Nutrition Research. 2019; 64: P. 49-55.
51. Cantarelli, C., Angeletti, A., And Cravedi, P. Erythropoietin, A Multifaceted Protein With Innate And Adaptive Immune Modulatory Activity. American Journal Of Transplantation. 2019; 19(9): P. 2407-2414.
52. González-Domínguez, Á., Visiedo-García, F.M., Domínguez-Riscart, J., González-Domínguez, R., Mateos, R.M., And Lechuga-Sancho, A.M. Iron Metabolism In Obesity And Metabolic Syndrome. International Journal Of Molecular Sciences. 2020; 21(15): P. 5529.
53. Dinicolantonio, J.J. And H O'Keefe, J. Myo-Inositol For Insulin Resistance, Metabolic Syndrome, Polycystic Ovary Syndrome And Gestational Diabetes. Open Heart. 2022; 9(1): P. E001989.
54. Abulfadle, K.A., Edrees, H., Hassan, N.H., And Mohammed, H.O. Spexin And Metformin Comparative Ameliorated Ovarian And Liver Function Changes In Letrozole-Induced Polycystic Ovary Syndrome In Rats (Histological, Biochemical, Immunohistochemical And Morphometric Study). Egyptian Journal Of Histology. 2022; 45(3): P. 774-790.
55. Rababa'h, A.M., Matani, B.R., And Ababneh, M.A. The Ameliorative Effects Of Marjoram In Dehydroepiandrosterone Induced Polycystic Ovary Syndrome In Rats. Life Sciences. 2020; 261: P. 118353.
56. Tan, S., Hahn, S., Benson, S., Dietz, T., Lahner, H., Moeller, L.C., Schmidt, M., Elsenbruch, S., Kimmig, R., And Mann, K. Metformin Improves Polycystic Ovary Syndrome Symptoms Irrespective Of Pre-Treatment Insulin Resistance. European Journal Of Endocrinology. 2007; 157(5): P. 669-676.

يحسن الايرثربيوتين والميوإينوسيتول والميتفورمين مؤشرات حساسية الأنسولين وكتلة خلايا بيتا البنكرياس وتغيرات الخلايا الكبدية في نموذج متلازمة تكيسات المبايض في الجرذان

شيرين احمد محمد[١] ، نسرين جمال الدين عبد الحليم[١] يوستينا لمعي فانوس2، أماني عبد الرحمن[٢] ,هدى محمد مغازى٢

[١] قسم علم الانسجة ، كلية الطب ، جامعه سوهاج

2قسم الفسيولوجي، كلية الطب ، جامعة سوهاج

مقدمة: تعد متلازمة تكيسات المبيض من المشاكل الصحية الاكثر شيوعا التي تصيب المرأة في سن الإنجاب و من أشهر أعراض متلازمة تكيس المبايض فرط الأندروجينية ، اضطرابات الدورة الشهرية والعقم .تتعرض النساء المصابات بمتلازمة تكيس المبايض أيضًا لمقاومة عمل الأنسولين والكبد الدهني .الميتفورمين يعتبر دواء معروف لعلاج ارتفاع السكر في الدم .الايرثربيوتين و ميوإينوسيتول يحسنان من حساسية الأنسولين

الهدف من العمل :مقارنه دور ميو اينوسيتول , الاريثروبيوتين , والميتفورمين في علاج ارتفاع الأنسولين في الدم , و الاصابة الكبدية فى نموذج متلازمه تكيسات المبايض في الجرذان.

طرق البحث: تم تقسيم خمسين أنثى من الجرذان البيضاء بالتساوي إلى خمس مجموعات: المجموعة الضابطة ومجموعة نموذج PCO: تم تحفيز نموذج PCO بواسطةالليتريزول بجرعة ٠,٥ مجم / كجم يوميًا لمدة ٣ أسابيع. ثم تم إعطاء الجرذان المصابة بالـ PCO للأيام الـ ٢١ التالية: مجموعة إرثروبويتين (EPO / PCO) ، مجموعة ميو اينوسيتول (MYO / PCO) ، و مجموعة ميتفورمين (MET / PCO). أجريت الدراسات البيوكيميائية والنسيجية على الكبد والبنكرياس. تم إجراء دراسة كيمياء نسيج مناعية باستخدام ناقلات الجلوكوز رقم١(GLUT-1) في الكبد والأنسولين فى البنكرياس.

النتائج: وقد اظهرت النتائج ارتفاع معدلات الجلوكوز والانسولين في الدم و مؤشرات مقاومة الانسولين في الجرذان المصابة بمتلازمه تكيسات المبيض مع نقص كتلة خلية بيتا فى البنكرياس وإصابة خلايا الكبد و زيادة ناقلات الجلوكوز رقم١ (GLUT-1). استعاد الميتفورمين و الايرثربيوتين و الميواينوسيتول كتلة خلية بيتا ، وخفض فرط أنسولين الدم ، وتنكس خلايا الكبد عن طريق الحد من زيادة ناقلات الجلوكوز رقم١ (GLUT-1) الناجم عن الإجهاد الخلوى . وقد كان الايرثربيوتين أكثرها فعالية .

الخلاصة: كان الإريثروبويتين أكثر فعالية من الميتفورمين و الميواينوسيتول في تقليل فرط أنسولين الدم وتخفيف إصابة الخلايا الكبدية المرتبطة بنموذج متلازمة تكيس المبايض المحدث عن طريق الليتريزول.