Original Article

Losartan Ameliorates Ovarian Ischaemia/ Revascularization Injury in Adult Albino Rats: Histological and Immunohistochemial Study

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ABSTRACT

Introduction: The ovary's partial or complete rotation around its pedicle is known as ovarian torsion. It represents around 3% of all gynecological emergencies. Treatment and diagnosis at an early stage are essential and may assist to maintain fertility. The preferred course of treatment is surgical intervention. Nonetheless, ischemia/revascularization injury (IRI) should be decreased to lessen post-torsion ovarian damage because reperfusion injury damages tissues more than ischemic injury does.
Aim of Work: This work used histological, immuno-histochemical, biochemical, and morphometric analyses to evaluate the potential protective impact of losartan on damage to the ovaries caused by ischemia revascularization (I/R) in a rat model.
Material and Methods: Twenty eight adult female albino rats were utilized. The rats were divided identically into four groups. Group I:Control(Sham). Group II:(Ischemia) undergone an ischemia lasting three hours. Group III:(Ischemia/ revascularization) similar to group II, then three hours of revascularization. Group IV:(Ischemia/revascularization and losartan) This group was subjected to ischemia for three hours accompanied by oral losartan (40 mg/kg) given 30 minutes prior to revascularization then revascularization was done for three hours. Ovaries were excised and subjected to Hematoxylin &Eosein stain, Caspase-3, TNF-α and IL-1β immuno-histolochemical, (SOD, GSH &MDA) biochemical and morphometric investigations.
Results: The ovaries in the ischemia and (I/R) groups showed degenerated follicles. Edema, dilated congested vessels, hemorrhage & hyaline degeneration in the ovarian stroma. Marked inflammatory cellular infiltration was noted. Compared to the other groups, the ischemia as well as I/R groups showed considerably higher MDA concentrations and significantly lower SOD and GSH concentrations. Also, these groups showed significant increase in positive immunoreactivity for Caspase-3, TNF-α and IL-1β compared to other groups. Losartan treatment improves histological findings, biochemical values and morphometric results.
Conclusion: Losartan ameliorates ovarian ischemia/revascularization injury via controlling inflammation, apoptosis, and oxidative stress.

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Key Words: Inflammation, losartan, ovary, oxidative stress, revascularization.

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INTRODUCTION

Approximately 3% of all gynecological emergencies are caused by torsion of the ovaries, which is defined as the ovary rotating fully or partially along its pedicle or vascular axis[1]. Ovarian torsion is thought to be associated with conditions that cause ovarian enlargement, including hyperlaxity of the ovary proprium and infundibulo pelvic ligaments, pregnancy, ovarian hyperstimulation, and adnexal cysts[2]. The most prevalent affected populations are pre-menarche girls and women of reproductive age, where ovarian torsion is frequently misdiagnosed[3]. It also can occur from early foetal life to the postmenopausal period[4].

Torsion prevents venous and lymphatic flow, which causes the development of ovarian edema that further decreased the arterial flow. Ischemia is caused by a reduction in arterial flow, and necrotic processes begin in the tissue with impairment in ovarian function. Early identification and treatment are essential because this condition may negatively affect fertility in females within the reproductive age range and could contribute to maintaining fertility[5].

Generally, abdominal surgery for ovarian torsion is performed to repair the torsion and to reestablish ovarian flow of blood. When an early diagnosis is made, the preferred course of treatment is surgical management, which includes de-torsion of the afflicted section[3].

However, this time, the oxygenation and restoration of the blood supply of ischemic tissues following detorsion, which is a necessary process for the regeneration of cells,

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causes ischemia/revascularization (I/R) damage, that is additional concern[6], this is connected to tissue neutrophil infiltration and revascularization, and the generation of reactive oxygen species (ROS) that are responsible for reperfusion damage. Owing to the peroxidation of polyunsaturated fatty acids, damages of cellular membranes occurs and these ROS cause destruction of cells[7]. Contrary to ischemia injury, reperfusion injury results in greater tissue or organ damage[8], so that, either the period of ischemia or the ischemia/revascularization injury (IRI) should be decreased to lessen post-torsion ovarian damage[9]. It has been discovered that a number of antioxidants can effectively prevent inflammation and oxidative damage in ovarian tissues that have been subjected to I/R stress[10].

Enzymatic and non-enzymatic antioxidant mechanisms within cells work in concert. Tissues are shielded from reactive oxygen species (ROS) and oxidative damage by super oxide dismutase (SOD), which is directly created in the intracellular environment[11]. Following cellular damage, catalase and glutathione (GSH) levels are lowered, which results in the inactivation of several enzymes[12]. The primary byproduct of the peroxidation of polyunsaturated fatty acids is malondialdehyde (MDA), which is a highly hazardous compound. Lipid peroxidation (LPO) can be detected by elevated MDA levels. It is therefore employed as an indirect biomarker of ROS and is commonly favored for determining the levels of both in *vitro* and in *vivo* oxidative stress[13]. Overproduction of ROS causes caspase activation, which may harm cells and ultimately cause apoptosis in the cell[14].

Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β), and Tumour Necrosis Factor alpha (TNF-α) are proinflammatory cytokines generated during I/R injury[15]. Inflammation and apoptosis rate are induced by IL-1β[13]. It is commonly recognized that TNF-α is a crucial cytokine that regulates inflammatory reactions[16]. I/R damage increases inflammation by producing TNF-α, IL-8, and IL-6. These elements contribute to inflammation, which in turn causes organ damage[17].

Losartan is considered Angiotensin II type1 receptor blocker, it is currently utilized to control blood pressure[18]. It was proved to have a protective effect opposing cerebral ischemia damage through an anti-apoptotic impact[19], and in an I/R injury model; it enhanced the expression of survival factors, preserving the heart from oxidative stress[20]. Following I/R damage, losartan blocked vascular hyperpermeability, revealing a different molecular mechanism for its cardioprotective benefits[21]. Thus, it may be proposed that losartan has therapeutic efficacy for individuals who have ovarian torsion though an antioxidant pathway.

This work used histological, immuno-histochemical, biochemical, and morphometric analyses to evaluate the potential protective impact of losartan on ovarian damage caused by ischemia /revascularization in a rat model.

MATERIAL AND METHODS

Animals

Animals were kept corresponding to the international standards. The institutional committee for the care and use of animals of Beni-Suef University authorized the study (Approval Number: 022-447). Twenty-eight adult female albino rats have been used in this investigation. They weighed from 200 to 240 grams. The animals were acquired from the animal house of faculty of veterinary Medicine, Beni-Suef University. The rats were housed in metal enclosures with appropriate temperature controls and 10–12 hours of daylight exposure. Rats were given the usual commercial pellet meal and housed for a week before the experiment started.

Drugs and Chemicals

Losartan

The tablet form was utilized. There are 50 milligrams of losartan potassium in each pill. Losartan was aquired from Amriya Pharmaceutical Industries (Alexandria - Egypt). For oral administration, the utilized dosage was dissolved in 10 milliliters of normal saline (0.9%).

Thiopental sodium

Every vial holds 20 milliliters (500 mg) of Thiopental sodium. Vials were acquired from Egypt's Sigma-Tec. Pharmaceutical Industries. For intraperitoneal injection, the estimated dose was obtained by diluting a 2 ml vial in 8 ml of distilled water.

Surgical Technique

Experimental animals were sedated with thiopental sodium following their acclimation period. A certain amount of 25 mg/kg was given intraperitoneally, and the procedure was given again as necessary. All rats were immobilized in the supine position, and then the abdominal skin was shaved and cleaned. 10 % povidone-iodine solution was used for antisepsis. A 2.5 cm midline lower abdominal longitudinal cut was made. The adnexa and uterine horns were determined, after a little peritoneal cut was made. As a control group, seven of the rats underwent a sham procedure, underwent laparotomy only with no extra intervention. The incision was closed with 4/0 nylon sutures. In the other groups, vascular clamps were placed beneath the female rats' ovaries to induce bilateral adnexal (ovarian) ischemia. It was demonstrated that the histological as well as biochemical alterations of torsion of the ovaries and the use of vascular clamps were extremely similar[3], and then after three hours of ischemia; the surgical removal of both ovaries was done in the ischemia group for histological and biochemical examination. After three hours of ischemia in the Ischemia/ revascularization and Ischemia – revascularization and losartan groups, the clamps were removed to allow for three hours of revascularization. Subsequently, both ovaries of each rat were excised for histological as well as biochemical analyses[8]. After the experiment is finished, using ether inhalation the rats were anesthetized and executed.

The experimental animals were randomly split into four groups, each one has 7 rats:

Group I: Control (sham) group (I) (7 rats): The female rats were just exposed to laparotomy but no procedure was carried out.

Group II: Pure ischemia group (II) (7 rats): The ovaries were exposed to 3 hours of ischemia (using vascular clamps). After the 3 hours of ischemia, both ovaries were surgically excised.

Group III: Ischemia - revascularization (I/R) group (III) (7 rats): The ovaries were exposed to 3 hours of ischemia, followed by 3 hours of revascularization (during which vascular clamps were removed), and then surgical excision of both ovaries was performed.

Group IV: Ischemia – revascularization (I/R) and losartan group (IV) (7 rats): This group underwent 3 hours of ischemia, accompanied by oral administration of losartan (40 mg/kg) thirty minutes before the revascularization. After the three hours of revascularization, the two ovaries were excised[8].

After the experiment is finished, using ether inhalation the rats were anesthetized and executed. Ovaries, both left and right, were removed and dissected from each animal. The following investigations were conducted on the separated ovarian tissues:

Histological study

For every female rat, the right ovary was removed and preserved in 10% formal saline solution. |Paraffin blocks were prepared. Each block was cut into sections that were 5 µm thick, and the ovarian general architecture was examined using hematoxylin and eosin stains[22].

Immuno-histochemical study

Tissue of the ovary that was previously formalin-fixed was placed within paraffin blocks for immunohistochemical analysis. Absolute alcohol was used to deparaffinize the sections. Blocking endogenous peroxidase activity with 100 liters of hydrogen peroxide and 0.5% absolute methanol and 0.4% hydrochloric acid (1M) for forty minutes at environment temperature. After being cleaned with water, the sections were kept in 1% trypsin and then 0.05 M Tris-buffered saline. Then pieces were cleaned using cold water.

a. Caspase-3 immunostaining, using a peroxidase-conjugated rabbit monoclonal antibody IgG (Cell Signaling Technology, Ipswich, MA) at a dilution of 1:200, Caspase-3 activation was evaluated. Caspase-3 is an apoptotic marker, its positive reaction appeared as cytoplasmic with some nuclear brown coloration.

b. Tumor necrosis factor-alpha (TNF-α) (Novus Biological, Cat. No: NBP1-19532, Dilution: 1/100)[23].

c. Interleukin-1beta (IL-1β) (Bioss, Cat. No: bs-0812R, Dilution: 1/100)[13], were applied as the primary antibody

TNF-α and IL-1β are inflammatory markers. Positive reaction appeared as cytoplasmic brown coloration.

For one hour, biotinylated secondary antibodies were used[24]. After being exposed to chromogen and streptovidin peroxidase, slides were cleaned with PBS. A counterstain using Mayer's hematoxylin was applied to the slides. The levels of immunopositivity were assessed as follows: mild (+), moderate (++), strong (+++), and none (−).

Biochemical study

Left ovarian specimens for this investigation were kept freeze at -80º C till the time of the chemical test. Ovarian tissue in cold 0.9% NaCl was homogenized using a glass homogenizer to create tissue homogenate. The SOD, GSH, and MDA enzymatic activity was measured by centrifuging tissue homogenates and using the supernatant. At room temperature, all enzymatic assays were estimated[25]. The kinetic colorimetric technique was employed to estimate the SOD activity, with measurements taken at 25C and 460 nm. Readings of its absorbance were taken after 0 and 8 minutes of light[26]. Using a spectrophotometer and Sedlak and Lindsay's technique, absorbance at 412 nm was measured for tissue (GSH)[27]. Thiobarbituric acid was boiled with the ovary homogenate to perform colorimetric measurement of MDA in the homogenate. At 532 nm, the resultant colored material was gathered and measured[28].

Morphometric study

The mean area percentage (%) of Caspase-3, TNF-α and IL-1β immunoreactivity was measured using an image analysis system at Faculty of Medicine, Al-Azhar University, Cairo (Leica Qwin 500 C Image analyzer computer system, Leica Imaging system LTD., Cambridge, UK). A 400x magnification was utilized to assess the mean area% of positive reaction. Each rat has been investigated in five serial sections, with ten high-power, non-overlapping fields measured in each section.

Statistical analysis

The quantitative data related to Caspase-3, TNF-α and IL-1β immune-staining were summed up as mean and SD, and the one-way analysis of variance (ANOVA) test was used to compare them. Furthermore, the biochemical data were reported as mean ± SD for all of them. To evaluate variations in means, analysis of variance was applied. The statistical study was carried out utilizing IBM's Armonk, New York, USA SPSS (version 9). *P-values* below 0.05 were regarded as statistically significant, *p*-less than 0.001 as very significant, and *P*-more than 0.05 as irrelevant[29].

RESULTS

Histological Results

The control group's ovarian sections demonstrated that the ovarian cortex had ovarian follicles in various

developmental stages primary and secondary follicles. Several layers of granulosa cells with multiple antral cavities were present in secondary follicles. The stroma between the follicles of the ovary was normal. It was also observed that the corpus luteum had pale nuclei and acidophilic cytoplasm (Figure 1A). The huge antrum, oocyte surrounded by zona pellucida, the cumulus oophorous, multiple layers of granulosa cells, and thecal cells were all visible in the mature graffian follicle (Figure 1B). Sections of the ischemia group displayed follicles that were deteriorated and distorted, along with a lack of normal ovarian architecture showing exfoliation desquamation of granulosa cells with many cells exhibiting dark pyknotic nuclei with degenerated oocyte. Additionally, edema in the stroma of the ovary and dilated, congested blood vessels were observed (Figures 2 A-C). Massive cellular infiltrate was noted in ovarian stroma with vacuolations in the corpus luteum's granulosa and theca lutetin cells. (Figure 2D). Ischemia revascularization group showed the same histological findings of ischemia group, degenerated follicles, ovarian stroma edema and dilated congested blood vessels, massive cellular infiltrate and hemosiderin deposits (Figures 3 A-D). An aberrant secondary follicle with desquamated and exfoliated granulosa cells into the follicular cavity was detected (Figure 3 E). Ischemia revascularization losartan group showed partial improvement than ischemia (Torsion) and ischemia revascularization groups. Although the stroma and follicles of the ovary were almost normal, some deteriorated follicles were still visible. There were still a few vacuolations. Also, few congested blood vessels were observed but without hemorrhage (Figures 4 A-C).

Immunohistochemical results: mild (+), moderate (++), strong (+++), and none (−)

Caspase-3 immunostaining

The control group exhibited a mild positive Caspases 3 immunoreaction expression in the granulosa cell nuclei of the follicles of the ovary. The majority of the ovarian follicle-lining cells in the ischemia group had strong positive immunological caspase-3 expression. Strong positive caspase-3 immuno-expression was detected in many granulosa cells of the ovarian follicle in the ischemia revascularization group. Ischemia revascularization losartan group indicated a mild positive caspase-3 immunoreaction (Figures 5 A-D).

TNF-α immunostaining

In the control group, lutein and interstitial cells showed a negative expression of TNF-α immunoreaction. TNF-α had a strong positive immunological expression in the ischemia group. Ischemia revascularization group expressed strong positive immune reaction of TNF-α in lutein and interstitial cells. Ischemia revascularization losartan group expressed mild positive immune reaction of TNF-α in lutein and interstitial cells (Figures 6 A-D).

IL-1β immunostaining

Negative expression of the IL-1β immunoreaction was noticed in the control group in lutein and interstitial cells. The ischemia group had strong IL-1β positive immune expression. Strong positive expression of IL-1β was detected in the ischemia revascularization group in lutein and interstitial cells. Losartan group expressed mild positive immunoreactivity of IL-1β in lutein and interstitial cells (Figures 7 A-D).

Biochemical Results

In both the ischemic and ischemia revascularization groups, levels of SOD were lowered. However, losartan administration (40 mg/kg) before reperfusion in group IV increased the levels in ovarian tissue. Ischemia (G II) and ischemia revascularization (G III) groups displayed an extremely significant decrease (P<0.001) of SOD activity in the rat's ovary comparable to the control group (GI) and losartan treated (IV) groups with no significant difference between the two groups (G II& GIII). No significant difference between the Control (GI) and Losartan treated groups (G IV) was detected (Table 1, Figure 8).

The tissue homogenates of ovarian specimens showed a highly significant decrease (P<0.001) in the reduced GSH levels in ischemia and ischemia revascularization groups when compared with the other groups which increased in the losartan treated group (G IV) with no significant difference between the two groups (G II& GIII). No significant difference between the Control (GI) and losartan treated groups (G IV) was detected (Table 1, Figure 8).

Furthermore, comparative analysis between the ischemia and ischemia revascularization groups revealed an extremely significantly (P<0.001) rise in MDA concentrations in the homogenate contents. The MDA levels decreased in the group treated with losartan (G IV) with no significant difference between the two groups (G II& GIII). No significant difference was noted between control group (GI) and Losartan treated group (G IV) (P<0.05) (Table 1, Figure 8).

Morphometric and statistical analysis

Mean Area Percentage of Caspase-3 immune-reaction

The mean area% of the positive immune-reaction of Caspase-3 showed highly significant increase (p<0.001) in (group II &group III) in comparison to the other experimental groups, with no significant difference between the both groups (group II &group III). There was no significant difference (p<0.05) between control group (GI) and Losartan treated group (G IV) (Table 2, Figure 9).

Mean Area Percentage of TNF- α immune-reaction

The mean area% of the positive immune-reaction of TNF- α showed highly significant increase (p<0.001) in (group II &group III) in comparison to the other experimental groups with no significant difference between both groups (group II &group III). There was no significant difference (p<0.05) between control group (GI)

and Losartan treated group (G IV) (Table 2, Figure 9).

Mean Area Percentage of IL-1β immune-reaction

The mean area% of the positive immune-reaction of IL-1β showed highly significant increase (p<0.001) in (group II &group III) in comparison to the other experimental groups with no significant difference between both groups (group II &group III). There was no significant difference (p<0.05) between control group (GI) and Losartan treated group (G IV) (Table 2, Figure 9).

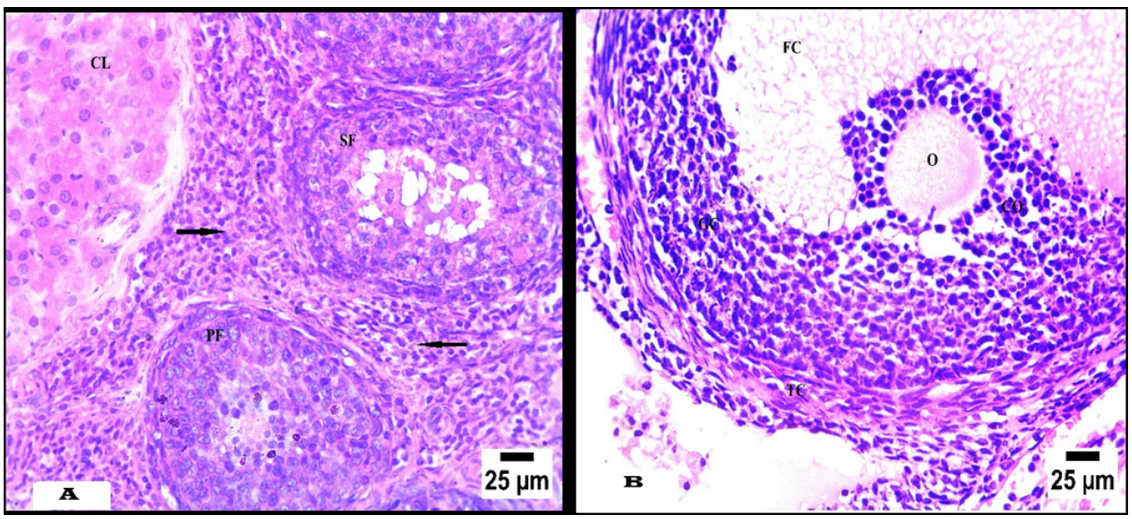

Fig.1: Control group I (A) Demonstrating normal ovarian follicle development, primary follicle (PF) and secondary follicles (SF) with normal appearance of the ovarian stroma (black arrows). Observe the cells of the corpus luteum (CL) have pale nuclei and acidophilic cytoplasm (H&E x400). (B) Displaying a fully developed graffian follicle with granulose cells (GC), thecal cells (TC), cumulus oophorous (CO), follicular cavity (FC), and oocyte (O) (H&E x400).

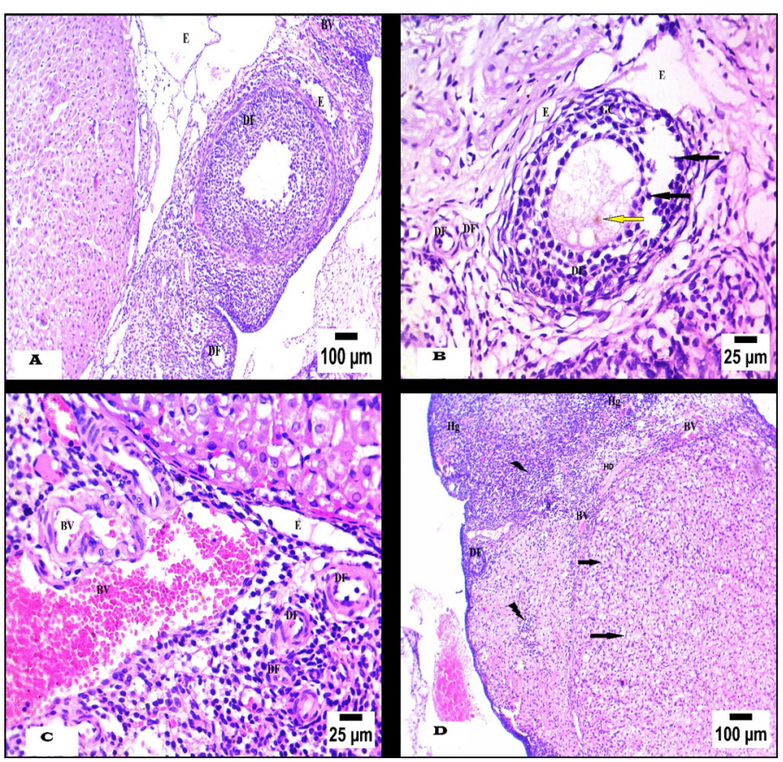

Fig. 2: Ischemia group II (A) Displaying edema in the stroma of the ovary (E), dilated and congested blood vessels (BV), and degenerated follicles (DF) (H&E x100). (B) Demonstrating a lack of the typical ovarian structure and degenerated follicles (DF). A distorted and degenerated follicle (DF) displaying granulosa cells (GC) exhibiting desquamation and exfoliation with many cells exhibiting dark pyknotic nuclei (black arrows) the oocyte nucleus is degenerating (yellow arrow). Observe the oedema in ovarian stroma (E) (H& E x400). (C) Showing multiple degenerated follicles (DF), markedly dilated and congested blood vessels (BV). Additionally, stromal edema is observed (E) (H& E x400). (D) Demonstrating the corpus luteum's granulosa and theca lutetin cells' vacuolation (arrows), hyaline degeneration (HD), congested blood vessels (BV) and hemorrhage in stroma (Hg). Marked inflammatory cell infiltration is noted (corrugated arrows). Degenerated follicle (DF) (H& E x100).

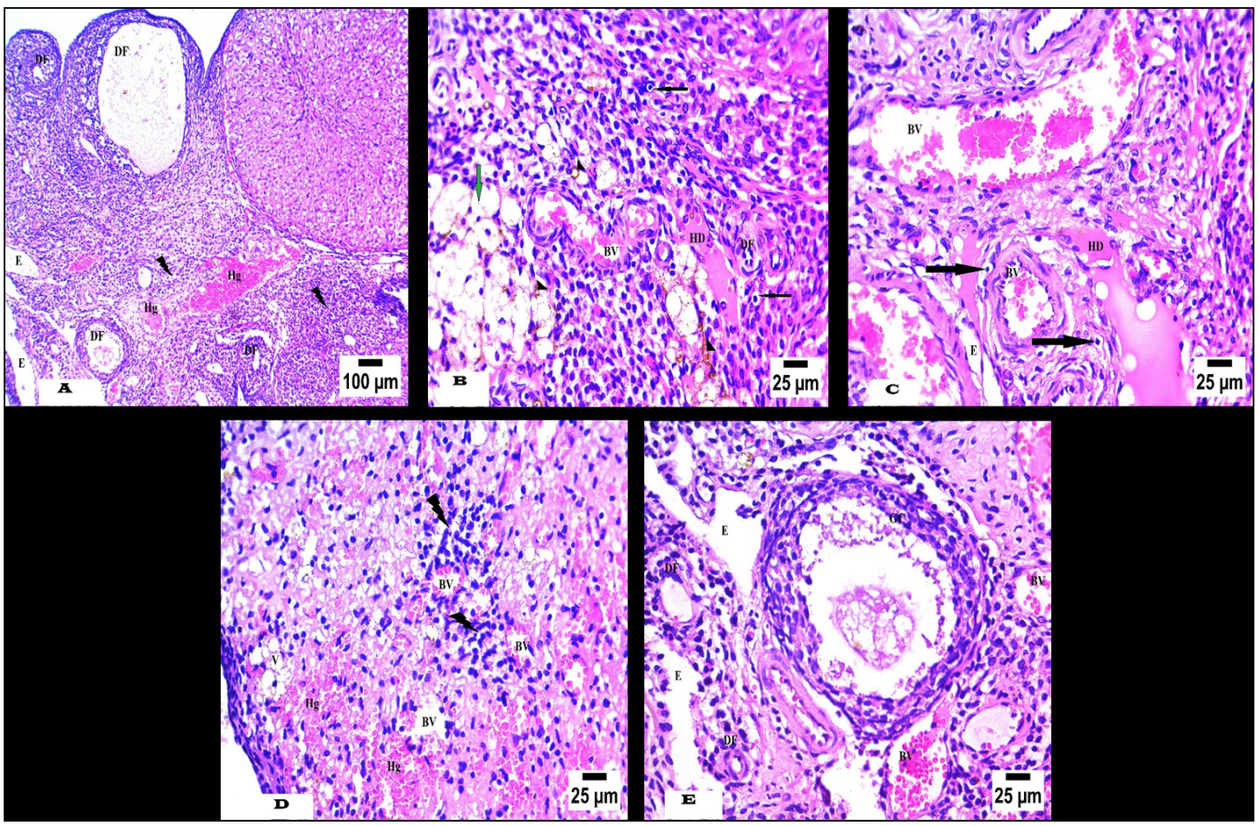

Fig.3: Ischemia / Revascularization group III (A) Displaying loss of normal ovarian histological architecture; degenerated follicles (DF), Hemorrhage in ovarian stroma (Hg), Inflammatory cell infiltration (corrugated arrows). Edema is also noted (E) (H&E x100). (B) Demonstrating the corpus luteum's granulosa and theca lutetin cells' vacuolation (green arrow). Dilated, Congested blood vessels (BV), ovarian stroma hyaline degeneration (HD) and hemosiderin deposits (arrow heads). Degenerated follicles (DG) and cells with Pyknotic dark nuclei (black arrows) are also noted (H &E x400). (C) Displaying a large number of dilated, congested blood vessels (BV), acidophilic hyaline degeneration (HD) and pyknotic dark nuclei (black arrows). Edema of the stroma is also present (E) (H& E x400). (D) Showing multiple vacuolations (V) in the ovarian stroma, congested blood vessels (BV) with noted hemorrhage (Hg) & red blood cells extravasation. Marked inflammatory cellular infiltration (corrugated arrows) is noted (H&E x 400). (E) Exhibiting an aberrant secondary follicle that has granulose cells (GC) that have been desquamated and exfoliated into the follicular cavity. Dilated, congested blood vessels (BV), edema (E) (H& E x400).

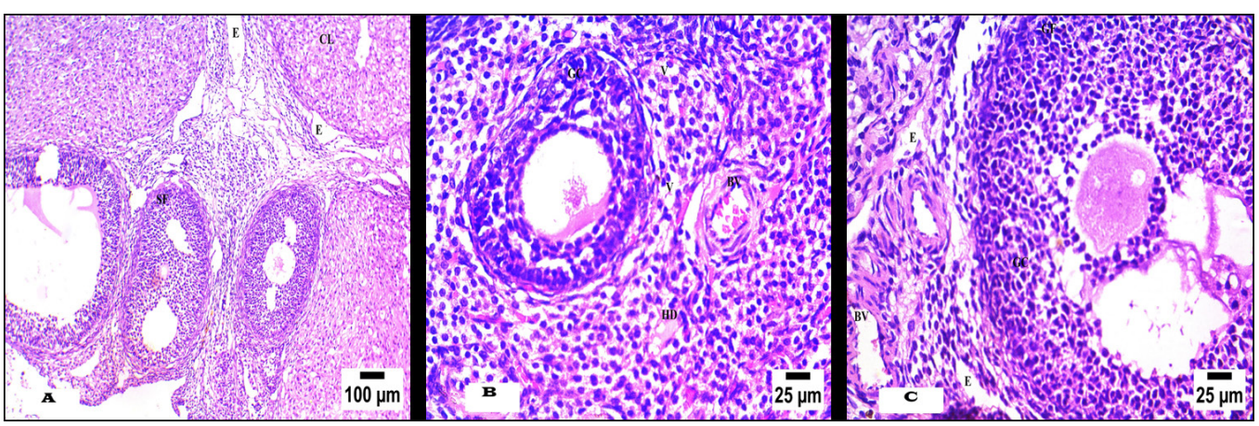

Fig.4: Ischemia / Revascularization losartan group IV (A) Displaying ovarian stroma that is almost normal, a developing secondary follicle (SF) and corpus lutem (CL). Note the edema (E) in the ovarian stroma (H& E x100). (B) Showing developing follicle with some restoration of the surrounding granulosa cells (GC). Some vacuolation (V), and widened, congested blood vessels (BV) are still denoted (H& E x400). (C) Showing a mature graffian follicle (GF), Restored granulosa cells (GC) encircle the oocyte. Note that the blood vessels (BV) are not congested. Edema is still noted (E) (H& E x 400).

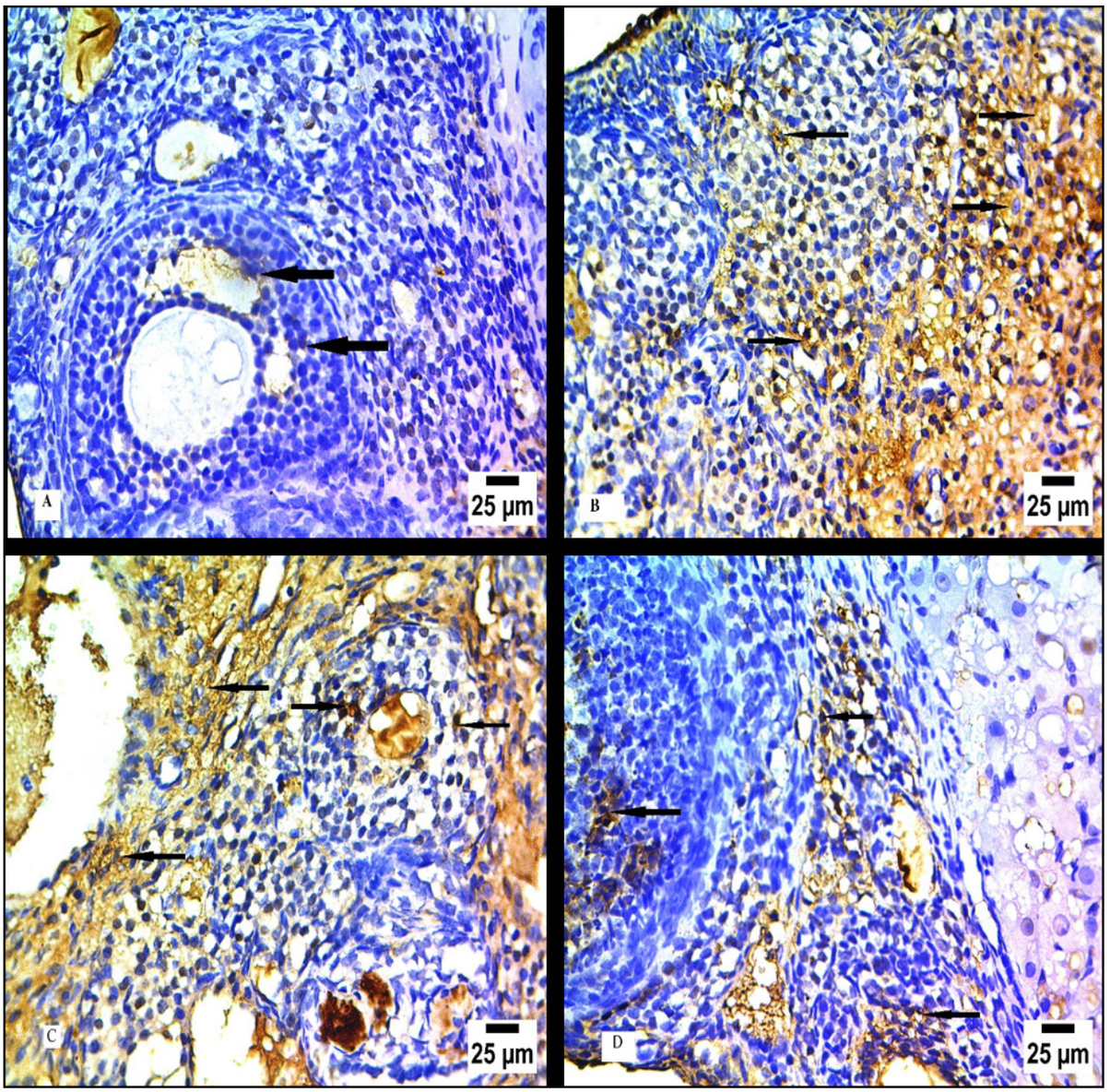

Fig.5: A- Control group I showing mild positive Caspases 3 immunoreaction expression in ovarian follicular granulosa cells (arrows). B- Ischemia group II showing strong positive immunoreaction to caspase-3 expression in granulosa cells of ovarian follicles (arrows). C- Ischemia / Revascularization group III demonstrating a strong positive caspase-3 immunoreactivity expression in ovarian follicle granulosa cells (arrows). D- Ischemia / Revascularization losartan group IV showing mild caspase-3 immunoreactivity in ovarian follicle granulosa cells (arrows). (Caspase-3 immunostaining, x 400)

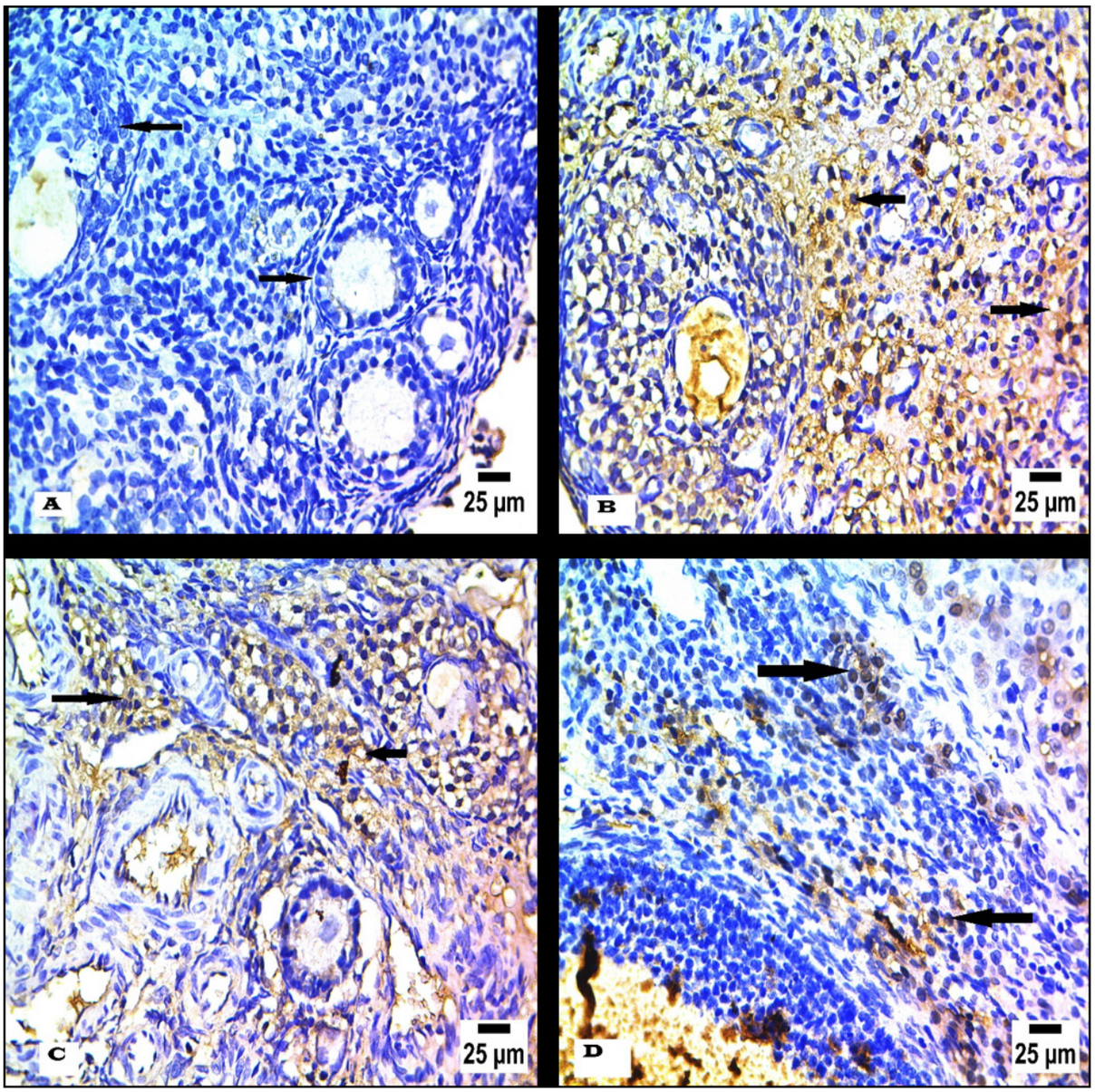

Fig.6: A- Control group I demonstrating a negative immunoreaction to TNF-α expression in the lutein and interstitial cells (arrows). (TNF-α immunostaining, x 400). B- Ischemia group II demonstrating a strong positive reaction of TNF-α in the lutein and interstitial cells (arrows). C- Ischemia / Revascularization group III displaying a strong positive immunoreaction of TNF-α in the lutein and interstitial cells (arrows). D- Ischemia / Revascularization losartan group IV showing some cells exhibiting positive expression of TNF-α immunoreaction in the lutein and interstitial cells (arrows). (TNF-α immunostaining, x 400)

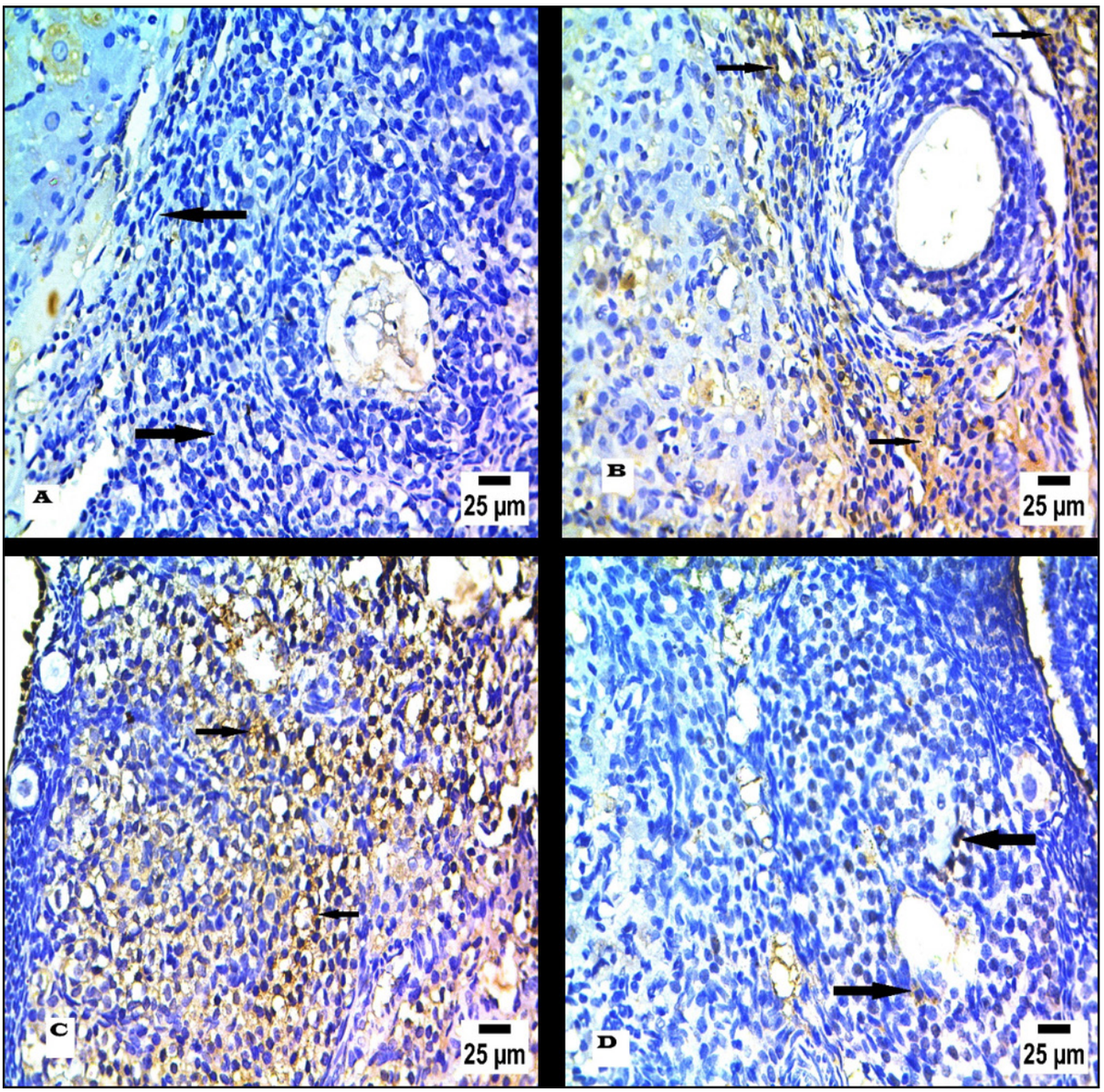

Fig.7: A- Photomicrograph of adult rat ovary of control group I showing negative expression of IL-1β immunoreaction in the lutein and interstitial cells (arrows). B- Ischemia II demonstrating a strong positive immunoreaction to IL-1β expression in the lutein and interstitial cells (arrows). C- Ischemia / Revascularization group III showing intense positive immunoreactivity of IL-1β in the lutein and interstitial cells (arrows). D- Ischemia / Revascularization losartan group IV showing mild positive expression of IL-1β immunoreaction in the lutein and interstitial cells (arrows). (IL-1β immunostaining, x 400)

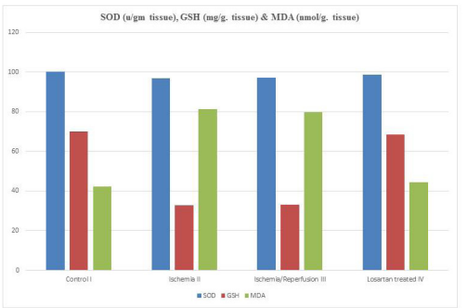

Fig.8: Analysis of the differences in SOD, reduced GSH, and MDA levels in the studied groups.

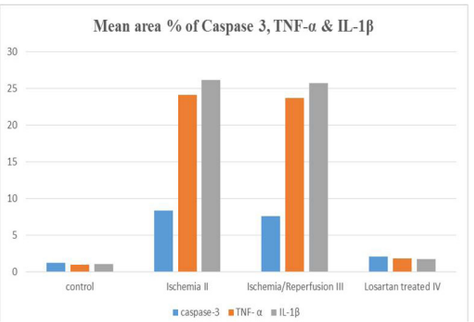

Fig.9: comparison of mean area % of Caspase 3, TNF- α and IL-1 β immune-positive reaction in the studied groups.

Table 1: Analysis of the differences in SOD, reduced GSH and MDA levels among the studied groups.

Experimental groups (n=7)	SOD (u/gm. Tissue)	GSH (mg/ g tissue)	MDA(nmol/g.tissue)
Sham (control) (GI)	100.26 ± 1.73	70.11 ±1.6	42.11 ± 1.78
Ischemia (GII)	96.86 ± 1.03^{ab}**	32.86 ± 0.94^{ab}**	81.17 ± 1.65^{ab}**
Ischemia/Reperfusion (GIII)	97.03 ± 1.63^{ab}**	33.17 ± 1.3^{ab}**	79.82 ± 1.82^{ab}**
Losartan Ischemia/Reperfusion (GIV)	98.76 ± 0.96	68.53 ± 1.8	43.34 ± 1.23

Table 2: Mean area% of Caspase-3, TNF- α and IL-1 β immune-positive reaction (±SD) among various rat groups.

Experimental groups	Mean area % of caspase-3	Mean area % of TNF- α	Mean area % of IL-1 β
Control (sham) (I)	1.21 ± 0.51	0.94 ± 0.76	1.02 ± 0.51
Ischemia (II)	8.27 ± 0.74^{ab}**	24.05 ± 0.41^{ab}**	26.12 ± 0.46^{ab}**
Ischemia/Reperfusion (III)	7.56 ± 1.11^{ab}**	23.69 ± 0.82^{ab}**	25.67 ± 0.77^{ab}**
Losartan Ischemia/Reperfusion (IV)	2.01 ± 1.25	1.75 ± 0.95	1.72 ± 0.91

DISCUSSION

Ovarian torsion affects all females, but especially those who are of reproductive age. So, early detection and treatment are critical for preserving the affected ovary and, thus, fertility. Ovarian torsion has 2 pathological phases: the ischemia phase, which begins with torsion and ends with detorsion, and the reperfusion phase, which begins with detorsion and is characterized by blood re-circulation and formation of ROS[30].

ROS produced upon reperfusion result in enhanced lipid peroxidation and cytokine release from activated neutrophils, which significantly damage DNA, cell membranes, and mitochondria. This ultimately results in tissue damage[31].

Earlier, numerous studies documented how various agents could prevent ovarian I/R injury[8].

Therefore, the purpose of this research was to assess if losartan may prevent ovarian damage caused by ischemia and ischemic reperfusion and to determine its possible mechanistic pathway; utilizing histological, immune-histochemical, biochemical and morphometric investigation in rat model.

In the present study, ischemia group rats stained with H&E sections recruited loss of normal ovarian histoarchitecture, degenerated follicles, dilated and congested blood vessels and edema in the ovarian stroma. The degenerated follicles indicated granulosa cell desquamation and exfoliation with many cells exhibiting dark pyknotic nuclei with degeneration of the oocyte nucleus. Hyaline degeneration, hemorrhage in ovarian stroma and marked inflammatory cell infiltration were detected. These results were consistent with those of earlier histology investigations[32]. They reported histological changes in ischemic ovarian tissue featuring many dark nuclei and vacuolations in the corpus luteum, desquamated follicular cells are found in the antral cavity of secondary follicles, and different deformed and atretic follicles without oocytes. The generation of ROS led to the prior findings[33].

Due to partial and total twisting of the mesovarium, the venous and lymphatic flow is interfered with, causing ovarian oedema, but not the arterial blood flow[34].

The primary hallmark of an atretic follicle is the alteration of the oocyte and granulosa cell separation from surrounding cells, which is indicative of zona pellucida apoptosis accompanied by vacuolation of theca interna[35].

Previous study reported that, the blood supply to the ovary was compromised by torsion, leading to venous congestion and hemorrhaging. This can cause ovarian tissue necrosis and a localized acute inflammatory reaction at the damage location[36]. The hemorrhage causes iron-overload resulting in hemosiderin deposits[37].

The appearance of eosinophilic homogenous exudate in the ovarian stroma might indicate structural changes with accumulation of protein secondary to cellular degeneration and ovarian dysfunction as denoted by[38]who documented histopathological changes in alveolar wall and interstitium associated with hyaline necrosis[39], also observed acidophilic hyaline material development within the renal tubules of male rats.

Current work revealed that in ischemia / revascularization group there were loss of normal ovarian histological architecture; degenerated follicles, hemorrhage in ovarian stroma, edema and an invasion of inflammatory cells. Within the corpus luteum, cytoplasmic vacuolation was observed in the follicular granulosa and theca luetin cells. Many dilated congested blood vessels, acidophilic hyaline degeneration and pyknotic dark nuclei were also detected. Atypical secondary follicles with granulose cell exfoliation and desquamation within the follicular cavity have been identified. These outcomes were consistent with the conclusions of recent studies that ovarian I/R caused a rise in follicular degeneration as indicated by a significant decline in follicular numbers and a rise in the quantity of atretic follicles, in addition to inducing ovarian damage as proved by distortion of the ovarian histoarchitecture[40].

Others reported that the ovarian I/R damage group showed necrotic and apoptotic alterations, vascular dilatation, hemorrhage and significant inflammatory cell infiltration[41].

Oxygen deprivation is the initial stage of ovarian I/R injury, which then advances to the over production of free radicals, exacerbates inflammation, and terminates in apoptosis and cell death[42]. Following diagnosis, a variety of surgical procedures can be safe and helpful in treating the patient, nevertheless, studies on non-surgical therapy are continuously being conducted[43].

Recent work stated that many antioxidants have been tried to minimize ovarian tissue loss with reperfusion injury[44].

In the present study ischemia / revascularization losartan group detected close to a typical ovarian stroma, developing secondary follicle and corpus lutem. Some vacuolation and dilated congested blood vessel were still noted. Mature graffian follicle contained oocyte which was surrounded by restored granulosa cells were present. Edema was still observed.

The advantages of anti-inflammatory and antioxidant drugs for I/R-induced injuries have been the subject of an increasing number of research[45]. Additionally, the advantages of proven medicinal compounds like losartan when taken off-label[8] on ovarian torsion/detorsion -induced I/R injuries have been documented.

Angiotensin II type 1 receptor blocker losartan may protect against ovarian I/R damage through an anti-apoptotic effect[19], and an antioxidant pathway[20].

Losartan might maintain the tubular structure following renal I/R. When was given to the rats during reperfusion it provided improving various renal function parameters and morphology[46]. Another study recruited that losartan played a cardioprotective role against IR injury[47].

As regard the immunohistochemical results, Caspase-3 immunostaining showed mild positive caspase-3 immunoreactivity in the granulosa cells of the control group's ovarian follicle. The majority of the ovarian follicle-lining cells in the ischemia group had strong positive immunological reaction of caspase-3. This reaction was found in many granulosa cells of the follicle in the ischemia revascularization group. Ischemia revascularization losartan group showed mild positive expression of caspase-3 immunoreaction. Morphometric results showed that the mean area% of Caspase-3 immune-reaction demonstrated a statistically significant rise in the ischemia and ischemia revascularization (I/R) groups when compared to the other studied groups with no significant difference between control and losartan treated groups. These results were consistent with[48] who reported that there was a substantial difference between the I/R group and all other groups with regard to caspase-3 expression in the ovarian tissues. They relied on caspase activation and subsequent apoptosis to explain their findings. Caspase-3 is therefore a potential indicator of apoptosis.

Pathophysiological mechanisms have been suggested for the apoptotic induction caused by ischemia-damaged mitochondrial proteins, the chemotaxis activation, and the endothelial adherence of leukocytes caused by defective membrane proteins and phospholipids as a result of ROS on lipid peroxidation[49].

Ovarian damage and decreased ovarian functional capability are caused by apoptosis, which performs a vital role in post-I/R injury. By using the TUNEL technique to measure apoptosis, they discovered that it was higher in the ischemia and IR groups[5].

Earlier study proved that Losartan may prevent the death of cardiomyocytes after reperfusion and ischemia. It's possible that the process increased the bcl-2/bax ratio by inhibiting the expression of the bax gene[50].

IL-1β, and TNF-α are pro inflammatory cytokines generated in I/R damage[15]. IL-1β increases the rate of apoptosis and inflammation[51]. One of the main cytokines that mediates inflammatory reactions is TNF-α, as is widely recognized. Reperfusion-related tissue damage is mostly dependent on TNF-α and IL-1β[52]. In a prior study, the use of Ura resulted in a reduction in IL-1β and TNF-α levels, hence supporting the reduction in inflammation[13].

TNF-α, IL-1β are rapidly produced by different types of cells in response to inflammatory and apoptotic signals. They have important functions in cellular growth, differentiation, proliferation, inflammatory response, angiogenesis, and inflammation[30].

Activated leukocytes, triggered by reperfusion, cause nuclear transcription factors to become active and proinflammatory cytokines like TNF-α and IL-1β to be synthesized[5].

In the present study negative expression of TNF-α and IL-1β immunoreaction were noticed in lutein and interstitial cells of control rats. Ischemia group exhibited strong positive immune reaction of TNF-α and IL-1β. The revascularization group revealed strong positive immune expression of TNF-α and IL-1β in lutein and interstitial cells. The revascularization losartan group exhibited mild positive expression of TNF-α and IL-1β immunoreaction in lutein and interstitial cells. Morphometric findings indicated that the mean area% of TNF- α and IL-1β immune-reaction demonstrated an extremely large increase in the groups experiencing ischemia and ischemia revascularization (I/R) comparable to the other experimental groups with no significant difference between control and Losartan treated groups.

The binding of TNF-α to TNF receptor (TNFR) and subsequent TNF R recruitment of adaptor proteins to the intracellular domain and homotrimerization are likely facilitated by I/R intermediated stimulation of TNF-α, which in turn causes inflammation, oxidative stress, and apoptosis[53].

TNF-α controls hematopoietic, inflammatory, and immunological responses[54]. Ovarian tissue may have experienced oxidative-inflammatory reaction leading to DNA breakage and apoptosis in response to TNF-'s pleiotropic biological effects[55]. TNF-α can exacerbate tissue and organ damage by inducing the synthesis of additional inflammatory markers, such as IL-1β[56].

The group with ovarian I/R injury exhibited elevated levels of TNF-α and IL-1β. These findings are in line with earlier studies that discovered ovarian I/R damage raises cytokine levels, such as TNF-α and IL-1β[41].

In mice suffering from antigen-induced arthritis (AIA), losartan treatment reduced migration and the levels of TNF-α, IL-1β, and chemokine ligand 1. Apart from lowering the generation of cytokines, losartan also directly decreased leukocyte adhesion and rolling. These results offer probable explanations for losartan's anti-inflammatory properties and support its usage in the treatment of arthritic patients in humans[57].

The present work detected that the levels of both SOD and reduced GSH concentrations were diminished in the ischemia and ischemia revascularization (I/R) groups. However, administration of losartan before revascularization in group IV, the rat's ovary's levels were reversed. Groups (II & III) showed extremely significant decrease of both SOD and reduced GSH concentrations activity in the rat’s ovary compared to the control and losartan treated groups with no significant difference between control group and losartan treated group. In previous studies, a decrease in SOD activity was observed in the revascularization group as opposed to the sham operated group[58].

Also there was highly significant increase in MDA concentrations between the Ischemia /Revascularization and ischemia groups, and the other group. However, were decreased in the losartan treated group, with no significant difference between control group and Losartan treated group. Previous studies came to the conclusion that MDA is a hazardous byproduct of ROS that builds up in ischemia/ revascularization injury and is indicative of compromised cell wall integrity and permeability. It is a useful indicator of lipid peroxidation[59].

Tissues are protected against reactive oxygen species (ROS) and oxidative damage by SOD, which is directly formed in the intracellular environment. In general, MDA is favored as an indirect ROS indication[13].

Prior research revealed that the groups treated with losartan exhibited increased levels of SOD activation compared to the DM group. Conversely, MDA levels in the groups receiving losartan were considerably lower than those in the diabetic group. Additionally, data indicate that losartan suppresses NF-κB activation and removes products of lipid peroxidation in the retina[60] and pancreatic cells[61].

In the present study, the losartan-treated group's ovarian tissue showed reduced damage. It displayed almost normal follicles together with a small number of deteriorated follicles. Losartan's enhancement of follicular growth due to ROS inhibition is likely the cause of these changes. Losartan medication prior to I/R also dramatically decreased the mean area% of caspase-3 immunoreaction in comparison to the control group. It was established that losartan reduced oxidative stress and increased ovarian granulosa cell proliferation, which in turn enhanced the growth of follicular cells. So, by lowering the expression of caspase-3, the mechanism of apoptosis was inhibited.

CONCLUSION

The data obtained in this study strongly imply that tissue injury caused by ischemia and I/R in the ovaries can be effectively reduced by conservative therapy with losartan. Histological, immunohistochemical, morphometric, and biochemical investigations in torsion and detorsion injury in rat model demonstrated that losartan administration decreased ovarian damage. Losartan's protective impact is mostly mediated by its antioxidant function. Losartan would therefore be beneficial in preserving the ovaries from damage caused by torsion-detorsion, most likely as a result of antioxidant down-regulation abilities. Losartan's protective impact on the ovaries will be valuable not only in the treatment and prevention of ovarian torsion but also in other ovary-related disorders where oxidative stress plays a direct or indirect role. It is advised to conduct more research to find the ideal dosage and timing for losartan. Future successful trials using antioxidant chemicals such as losartan may help sustain surgically untwisted ovaries.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Karakas S., Kaya C., Guraslan H., Sakiz D., Çayplnar S. S., Cengiz H., Ekin M.,Yasar L. (2020): Effect of metformin and detorsion treatment on serum anti-Müllerian hormone levels and ovarian histopathology in a rat ovarian torsion model. Turkish Journal Medical Sciences. 50 (2): 455-463. doi: 10.3906/sag-1803-196.
2. Tsafrir Z., Azem F., Hasson J. *et al*., "Risk factors, symptoms, and treatment of ovarian torsion in children (2012): The twelve year experience of one center," Journal of Minimally Invasive Gynecology, vol. 19, no. 1, pp. 29–33. https://doi.org/10.1155/2019/9701874.
3. Bashandy M and Khair N. (2018): Ischemia–reperfusion induced injury of rat ovary and the possible protective effect of amlodipine: Histological, immunohistochemial and biochemical study. Egyptian Journal of Histology (EJH) Vol. 41, No. 4. Page 487-502. DOI: 10.21608/EJH.2018.3445.1006.
4. Vijayalakshmi K, Reddy G, Subbiah V N, Sathiya S. and Arjun B (2014): The evaluation of clinical, radiological, pathological profile of ovarian torsion cases. Journal of Clinical & Diagnostic Research. Vol. 8 (6): OC04–OC07. doi: 10.7860/JCDR/2014/8167.4456.
5. Çaltekin M. D., Özkut M. M., Çaltekin I., Kaymak E., Çakır M., Kara M., and Yalvaç E. S., (2021): The protective effect of JZL184 on ovarian ischemia reperfusion injury and ovarian reserve in rats Journal of Obstetrics and Gynaecology Research, Vol. 47, No. 8: 2692–2704. doi: 10.1111/jog.14859.
6. Balsak D., Togrul C., Ekincil C., Seckin K., Karsli M., Ekinci A., Tahaoglu A., Bademkiran H., Acar Z., Tunc S., Deveci E., and Kirman G., (2015): Effects of melatonin on ischemia-reperfusion injury in rat ovary: Histo-pathologic and immunohistochemical study Open Journal of Obstetrics and Gynecology. Vol. 5, No (11); 639 - 645. DOI: 10.4236/ojog.2015.511090.
7. Melekoglu R., Ciftci O., Eraslan. S, Alan S., and Basak N., (2018): The Protective Effects of Glycyrrhetinic Acid and Chrysin against Ischemia-Reperfusion Injury in Rat Ovaries. BioMed Research International, Volume 2018, Article ID 5421308, 7 pages. doi: 10.1155/2018/5421308.
8. Hortu I., Ilgen O., Sahin C., Akdemir A., Yigitturk G. and Erbas O. (2020): Losartan ameliorates ovarian ischaemia/reperfusion injury in rats: an experimental study. Journal of Obstetrics and Gynaecolgy Volume 40, Issue 8, 1148–54. doi: 10.1080/01443615.2019.1701639.
9. Kirmizi DA, Baser E, Okan A, Kara M, Yalvac ES, Doganyigit Z. (2020): The effect of a natural molecule in ovary ischemia reperfusion damage: does lycopene protect ovary? Experimental Animals, 70 (1):37–44. https://doi.org/10.1538/expanim. 20-0080.
10. Prieto-Moure B., Lloris-Cars´J. M., C. Barrios-Pitarque *et al*., (2016): "Pharmacology of Ischemia–Reperfusion. Translational Research Considerations," Journal of Investigative Surgery, vol. 29, no. 4, pp. 234–249. doi: 10.3109/08941939.2015.1119219.
11. Meštrović J., Pogorelić Z., Drmić-Hofman I., Vilović K., Todorić D., Popović M. (2017): Protective effect of urapidil on testicular torsion–detorsion injury in rats. Surgery Today; 47:393-398. DOI: 10.1007/s00595-016-1388-3.
12. Yalin S., Balli E. and Berköz M. (2012): Ovariectomy decreases biomechanical quality of skin via oxidative stress in rat. Turkish Journal Medical Science, 42 (2):201–209. DOI 10.3906/sag-1011-1237.
13. Güler M. C., Tanyeli A., Erdoğan D. G., Eraslan E., Çomaklı S., Polat E., Doğanay S. (2021): Urapidil alleviates ovarian torsion detorsion injury via regulating oxidative stress, apoptosis, autophagia, and inflammation. Iranian Journal of Basic Medical Sciences. 24 (7):935-942. doi: 10.22038/ijbms.2021.57196.12736.
14. Lee JY, Baw C-K, Gupta S, Aziz N, Agarwal A. (2010): role of oxidative stress in polycystic ovary syndrome Current Women's Health Reviews. 6 (2):96-107. DOI: https://doi.org/10.2174/157340410791321336.
15. Munoz M., Lopez-Oliva M.E., Pinilla E., Martinez M.P., Sanchez A., Rodriguez C., *et al*. (2017): CYP epoxygenase-derived H2O2 is involved in the endothelium-derived hyperpolarization (EDH) and relaxation of intrarenal arteries. Free Radical Biology& Medicine. 106:168-183. doi: 10.1016/j.freeradbiomed.2017.02.031.
16. Hashmp SF, Sattar MZA, Rathore HA, Ahmadi A, Johns EJ. (2017): A critical review on pharmacological significance of hydrogen sulfide (h2s) on nf-kappab concentration and icam-1 expression in renal ischemia reperfusion injury. Acta Poloniae Pharmaceutica; 74 (3):747-752. ISSN 0001-6837.
17. Peng J., Ren X, Lan T., Chen Y, Shao Z., Yang C. (2016): Renoprotective effects of ursolic acid on ischemia/reperfusion induced acute kidney injury through oxidative stress, inflammation and the inhibition of STAT3 and NFkappaB activities. Molecular Medicine Reports; 14 (4):3397-3402. doi: 10.3892/mmr.2016.5654.
18. Zhao Y., Cao J., Melamed A., Worley M., Gockley A., Jones D., Nia H. T., Zhang Y., Stylianopoulos T., Kumar A. S., Mpekris F., Datta M., Sun Y., Wu L., Gao X., Yeku O., Carmen M. G. D., Spriggs D. R., Jain R. K. and Xua L. (2019): Losartan treatment enhances chemotherapy efficacy and reduces ascites in ovarian cancer models by normalizing the tumor stroma. Proceedings of the National Academy of Sciences of United States of America (Proc Natl Acad Sci U S A.);116 (6): 2210–2219. doi: 10.1073/pnas.1818357116.

19. Chen L., Ren Z., Wei X., Wang S., Wang Y., Cheng Y., Gao H., Liu H. (2017): Losartan protects against cerebral ischemia/reperfusion-induced apoptosis through β-arrestin1-mediated phosphorylation of Akt. European Journal of Pharmacology, Volume 815, Pages 98-108. https://doi.org/10.1016/j.ejphar.2017.08.028.

20. Klishadi M.S., Zarei F., Hejazian S. H., Moradi A., Hemati M., Fatemeh Safari F. (2015): Losartan protects the heart against ischemia reperfusion injury: sirtuin3 involvement. Journal of Pharmacy and Pharmaceutical Sciences; 18 (1):112-23. doi: 10.18433/j3xg7t.

21. Li Y., Yao Y., Li J., Chen Q., Zhang L., Wang Q.K. (2019): Losartan protects against myocardial ischemia and reperfusion injury via vascular integrity preservation. Official Puplication of the Federation of the American Societies for Experimental Biology (FASEB J.); 33(7):8555-8564. doi: 10.1096/fj.201900060R.

22. Bancroft D, Uvarna S and Layton C (2018): Bancroft's Theory and practice of Histological Techniques E-Book, 8th edition, Elsevier Health Science Publishing (pp. 126-138). https://doi.org/10.1016/C2015-0-00143-5.

23. Çelik H, Kandemir FM, Caglayan C, Özdemir S, Çomaklı S, Kucukler S, *et al*. 2020: Neuroprotective effect of rutin against colistin-induced oxidative stress, inflammation and apoptosis in rat brain associated with the CREB/BDNF expressions. Molecular Biology Reports; 47:2023-2034. https://doi.org/10.1007/s11033-020-05302-z.

24. Lee, j.; Jeng, S. and Lee, T. (2006): Increased activated caspase-3 expression in testicular germ cells of varicocele-induced rats. JTUA. 17(3):8185. IP: 154.176.43.126.

25. Yanardag R., Ozsoy-Sacan O., Ozdil S. and Bolkent S. (2007): Combined effects of vitamin C, vitamin E, and sodium selenate supplementation on absolute ethanol-induced injury in various organs of rats. International Journal of Toxicology 26 (6); 513–523. doi: 10.1080/10915810701707296.

26. Bashandy M., ZanatyA., El-Seidy A. and El-Shafie A., (2012): Combined effects of vitamins C and E on acute ethanol toxicity of the liver and jejunum of albino rats: histological, immunohistochemical, and biochemical study. The Egyptian Journal of Histology, 35 (3):496-508. DOI: 10.1097/01.EHX.0000418551.51683.ec.

27. Hassanin K M A and Hashem k S (2013): Hepatoprotective effects of vitamin C and micronized vitamin C against paracetamol induced hepatotoxicity in rats: a comparative study. Journal of Veterinary Medical Research, 22(1): 46-52. doi: 10.21608/JVMR.2013.77678.

28. 28- Liu Y., Shimizu I., Omoya T., Ito S., Gu, S. and Zuo J., (2002): Protective effect of estradiol on hepatocytic oxidative damage. World Journal of Gastroentrology. 8(2):363 -366. doi: 10.3748/wjg.v8.i2.363.

29. Emsley, R., Dunn, G., & White, I. R. (2010): Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. Statistical Methods in Medical Research, 19(3), 237-270. doi: 10.1177/0962280209105014.

30. kbaş N., Gürbüzel M., Sayar I., Bakan N. (2023): The Effect of Resveratrol on Ischemia–Reperfusion Induced Oxidative Rat Ovary Injury: A Biochemical, Histopathological, and Genetic Evaluation. Archives of Basic Clinical Research; 5(2): 230-239. DOI: 10.5152/ABCR.2023.22046.

31. Sun M.S., Jin H., Sun X., Huang S., Zhang F., Guo Z. Yang Y. (2018): Free radical damage in ischemia-reperfusion injury: an obstacle in acute ischemic stroke after revascularization therapy. Oxidative Medicine and Cellular Longevity; 2018: 3804979. doi: 10.1155/2018/3804979.

32. Sak ME, Soydinc HE, Sak S, Evsen MS, Alabalik U, Akdemir F, Gul T. (2013): The protective effect of curcumin on ischemia–reperfusion injury in rat ovary. International Journal of Surgery; 11(9):967-70. doi: 10.1016/j.ijsu.2013.06.007.

33. Kara M, Daglioglu YK, Kuyucu Y, Tuli A, Tap O. (2012): The effect of edaravone onischemia–reperfusion injury in rat ovary. European Journal of Obstetrics, Gynecology and Reproductive Biology; 162(2):197–202. doi: 10.1016/j.ejogrb.2012.02.026.

34. Varma A., Chakrabarti P. R., Gupta G., and Kiyawat P. (2016): Massive ovarian edema: A case report presenting as a diagnostic dilemma. Journal of Family Medicine and Primary Care, 5 (1): 172–174. doi: 10.4103/2249-4863.184658.

35. Alchalabi A. S.H., Rahim H., Aklilu E., Al-Sultan I. I., Aziz A., Malek M. F., Ronald S. H., Khan M. A. (2016): Histopathological changes associated with oxidative stress induced by electromagnetic waves in rats' ovarian and uterine tissues. Asian Pacific Journal of Reproduction, Volume 5, Issue 4, Pages 301-310. https://doi.org/10.1016/j.apjr.2016.06.008.

36. Kart C., Aran T., Guven S., Karahan S.C., Yulug E. (2011): Acute increase in plasma D-dimer level in ovarian torsion: an experimental study. Human Reproduction; 26(3):564-8. doi: 10.1093/humrep/deq378.

37. Mori M., Ito F., Shi L., Wang Y., Ishida C., Hattori Y., Niwa M., Hirayama T., Nagasawa H., Iwase A., Kikkawa F., Toyokuni S. (2015): Ovarian endometriosis-associated stromal cells reveal persistently high affinity for iron. Redox Biology, Volume 6, Pages 578-586. doi: 10.1016/j.redox.2015.10.001.

38. Moftah, O. M. Y., Gadallah, A. A., Elemam, S. I. A., & El-Sayyad, H. I. H. (2020). Impairment of Hepatic, Cardiac and Lung Tissues in Aspartame Treated Male Wistar Albino Rats. East African Scholars Journal of Medical Sciences, (3)2: 41-46. DOI:10.36349/EASMS.2020.v03i02.009.

39. El Haliem, N. G., & Mohamed, D. S. (2011). The effect of aspartame on the histological structure of the liver and renal cortex of adult male albino rat and the possible protective effect of Pimpinella anisum oil. Egyptian Journal of Histology, 34(4): 715-726. DOI:10.1097/01.EHX.0000406589.05585.8d.

40. Afolabi O. A., Hamed M. A., Anyogu D. C., Adeyemi D. H., A. F. Odetayo A. F. and Akhigbe R. E. (2022): Atorvastatin-mediated downregulation of VCAM-1 and XO/UA/caspase 3 signaling averts oxidative damage and apoptosis induced by ovarian ischaemia/reperfusion injury. REDOX REPORT, VOL. 27, NO. 1, 212–220. doi: 10.1080/13510002.2022.2129192.

41. Yuksel T. N., Halici Z., Cadirci E., Toktay E., Ozdemir B. and Bozkurt A. (2023): Effect of trimetazidine against ovarian ischemia/reperfusion injury in rat model: A new pathway: JAK2/STAT3. Iranian Journal of Basic Medical Sciences; 2023; 26(11): 1370–1379. doi: 10.22038/IJBMS.2023.72544.15776.

42. Chang ZP, Deng GF, Shao YY, Xu D, Zhao YN, Sun YF, *et al*. (2021): Shaoyao-gancao decoction ameliorates the inflammation state in polycystic ovary syndrome rats via remodeling gut microbiota and suppressing the TLR4/NF-kappa B pathway. Frontiers in Pharmacology; 12:670054. doi: 10.3389/fphar.2021.670054.

43. Koc K, Erol HS, Colak S, Cerig S, Yildirim S, Geyikoglu F (2019): The protective effect of propolis on rat ovary against ischemia-reperfusion injury: Immunohistochemical, biochemical and histopathological evaluations. Biomedicine and Pharmacotherapy; 111(2019): 631–637. https://doi.org/10.1016/j.biopha.2018.12.113.

44. Kula H, İlgen O, Kurt S, Yılmaz F (2023): Effects of allium cepa on ovarian torsion-detorsion injury in a rat model. Turkish Journal of Obstetrics and Gynecology; 20(2):137-41. doi: 10.4274/tjod.galenos.2023.41763.

45. Mogilner JG, Lurie M, Coran AG, *et al* (2006): Effect of diclofenac on germ cell apoptosis following testicular ischemia-reperfusion injury in a rat. Pediatric Surgery International; 22(1):99–105. doi: 10.1007/s00383-005-1580-9.

46. Srisawat U, Kongrat S, Muanprasat C and Chatsudthipong V (2015): The Pharmaceutical Society of Japan Regular Article Losartan and Sodium Nitroprusside Effectively Protect against Renal Impairments after Ischemia and Reperfusion in Rats. Biological & Pharmaceutical Bulletin; Vol. 38, No. 5 38, 753–762. doi: 10.1248/bpb.b14-00860.

47. Kilic A, Ustunova S, Usta C, Bulut H, Meral I, Tansel CD and Gurevin EG (2019): Angiotensin II type 2 receptor blocker PD123319 has more beneficial effects than losartan on ischemia–reperfusion injury and oxidative damage in isolated rat heart. Canadian Journal of Physiology and Pharmacology Volume 97 (12):1124-1131. doi: 10.1139/cjpp-2019-0076.

48. Ergenoglu M, Erbaş O, Akdemir A, Yeniel AO, Yildirim N, Oltulu F, *et al*. (2013): Attenuation of ischemia/reperfusion-induced ovarian damage in rats: does edaravone offer protection? European Surgical Research; 51(1-2):21–32. doi: 10.1159/000353403.

49. Halestrap A. P., Clarke S. J., and Javadov S. A. (2004): "Mitochondrial permeability transition pore opening during myocardial reperfusion - A target for cardioprotection," Cardiovascular Research, vol. 61, no. 3, pp. 372–385. https://doi.org/10.1016/S0008-6363(03)00533-9.

50. Zhang Dongqing Z, Liming Y, Zhengxiang L & Shizan M (2000): Study on the effects of losartan on cardiomyocyte apoptosis and gene expression after ischemia and reperfusion in *vivo* in rats. Journal of Tongji Medical University volume 20, pages49–52. doi: 10.1007/BF02887675.

51. Hasturk A, Atalay B, Calisaneller T, Ozdemir O, Oruckaptan H, Altinors N. (2009): Analysis of serum pro-inflammatory cytokine levels after rat spinal cord ischemia/reperfusion injury and correlation with tissue damage. Turkish Neurosurgery; 19(4):353-359. PMID: 19847755.

52. Yang Q., Zheng F.P., Zhan Y.S., Tao J., Tan S.W., Liu H.L., *et al*. (2013): Tumor necrosis factor-alpha mediates JNK activation response to intestinal ischemia-reperfusion injury. World Journal of Gastroenterology; 19(30):4925-4934. doi: 10.3748/wjg.v19.i30.4925.

53. Kalliolias G.D. and Ivashkiv L.B. (2016): TNF biology, pathogenic mechanisms and emerging therapeutic strategies. Nature Reviews, Rheumatology; 12(1): 49–62. doi: 10.1038/nrrheum.2015.169.

54. Kong D.H., Kim Y.K., Kim M.R., Jang J.H., Lee S. (2018): Emerging roles of vascular cell adhesion molecule-1 (VCAM-1) in immunological disorders and cancer. International Journal of Molecular Sciences; 19(4): 1057–1072. doi: 10.3390/ijms19041057.

55. Akhigbe R. and Ajayi A. (2020): Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up-regulation of NO/TNF-alpha and caspase 3 activities. PLoS One; 15(3): e0224052. DOI: 10.1371/journal.pone.0224052.

56. C., Li Y., Guan S., Han F., Zhang S. (2013): Catalpol improves cholinergic function and reduces inflammatory cytokines in the senescent mice induced by D-galactose. Food Chemical Toxicology; 58: 50–55. doi: 10.1016/j.fct.2013.04.006.

57. Silveira K.D., Coelho F.M., Vieira A.T., Barroso L.C., Queiroz-Junior C.M., Costa V.C., Sousa L.F., Oliveira M.L., Bader M., Silva T.A., Santos R.A., Silva A.C., Teixeira M.M. (2013) :Mechanisms of the anti-inflammatory actions of the angiotensin type 1 receptor antagonist losartan in experimental models of arthritis. Peptides; 46:53-63. doi: 10.1016/j.peptides.2013.05.012.

58. Banerjee O , Singh S , Bose A, Kundu S,Banerjee M, Ray D, Maji BK, Mukherjee S (2021): Therapeutic potential of L-arginine in a rat model of ovarian ischemia-reperfusion injury Environmental and Experimental Biology; 19: 81–88. http://doi.org/10.22364/eeb.19.08.

59. Garden, D. and Granger, D. (2000): Pathophysiology of ischaemia – reperfusion injury. Journal of Pathology; 190(3): 225-266. doi: 10.1002/(SICI)1096-9896(200002).

60. Nasiri A., Ziamajidi N., Abbasalipourkabir R., Goodarzi M.T., Saidijam M. (2017): Beneficial effect of aqueous garlic extract on inflammation and oxidative stress status in the kidneys of type 1 diabetic rats. Indian Journal of Clinical Biochemistry; 32(3):329 -336. doi: 10.1007/s12291-016-0621-6.

61. Lee MY, Shim MS, Kim BH, Hong SW, Choi R, Lee EY, Nam SM, Kim GW, Shin JY, Shin YG, Chung CH (2011): Effects of spironolactone and losartan on diabetic nephropathy in a type 2 diabetic rat model. Diabetes & Metabolism Journal; 35(2):130-7. doi: 10.4093/dmj.2011.35.2.130.

الملخص العربى

اللوسارتان يخفف اصابة وقف تدفق الدم وإعادة ضخ الدم المبيض في الجرذان البيضاء البالغة: دراسة نسيجية وهستوكيميائية مناعية

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الخلفية: يُعرف الدوران الجزئي أو الكلى للمبيض حول عنقه بالتواء المبيض. وهو يمثل حوالي ٣٪ من جميع حالات الطوارئ المتعلقة بأمراض النساء. يعد العلاج والتشخيص في مرحلة مبكرة ضروريين وقد يساعدان في الحفاظ على الخصوبة. المسار المفضل للعلاج هو التدخل الجراحي. ومع ذلك، يجب تقليل إصابة وقف تدفق الدم /إعادة الارواء لتقليل تلف المبيض بعد الالتواء لأن إصابة إعادة الارواء تدمر الأنسجة أكثر من الإصابة بوقف تدفق الدم.

الهدف من العمل: استخدم هذا العمل التحليلات النسيجية والكيميائية النسيجية المناعية والكيميائية الحيوية والمورفومترية لتقييم التأثير الوقائي المحتمل للوسارتان على تلف المبيضين الناجم عن إعادة ضخ الدم بعد وقف التدفق (I / R) في نموذج الجرذان.

المواد والطرق: تم استخدام ثمانية وعشرين جرذا بيضاء بالغة. تم تقسيم الجرذان بشكل متساوى إلى أربع مجموعات. المجموعة الأولى: الضابطه. المجموعة الثانية: (نقص تدفق الدم) أصيبت بنقص تدفق الدم لمدة ثلاث ساعات. المجموعة الثالثة: (نقص تدفق الدم/إعادة ضخ الدم) مشابهة للمجموعة الثانية، ثم ثلاث ساعات من إعادة ضخ الدم و المجموعة الرابعة: (نقص تدفق الدم/إعادة ضخ الدم ولوسارتان) تم تعريض هذه المجموعة لنقص تدفق الدم لمدة ثلاث ساعات ثم أعطيت اللوسارتان عن طريق الفم (٤٠ ملغم / كغم) قبل ٣٠ دقيقة من إعادة ضخ الدم ثم تم إعادة ضخ الدم لمدة ثلاث ساعات. بعد ذلك تم إجراء استئصال المبيض وإخضاعها لصبغة الهيماتوكسيلين والإيوسين، وCaspase-٣، و TNF-α وIL-β١، والكيمياء المناعية النسيجية (SOD، GSH &MDA) والفحوصات البيوكيميائية والمورفومترية.

النتائج: أظهرت المبايض في مجموعتي نقص تدفق الدم و(نقص تدفق الدم/إعادة ضخ الدم) بصيلات متدهورة. وذمة، وتوسع الأوعية الدموية المحتقنة، ونزيف، و تنكس زجاجي في سدى المبيض. ولوحظ وجود تسلل خلوي التهابي ملحوظ. بالمقارنة مع المجموعات الأخرى، أظهرت مجموعة نقص تدفق الدم وكذلك مجموعة نقص تدفق الدم/إعادة ضخ الدم تركيزات MDA أعلى بكثير وتركيزات SOD وGSH أقل بكثير. كما أظهرت هذه المجموعات زيادة ذات دلاله احصائيه في النشاط المناعي الإيجابي لـ Caspase-٣ وTNF-α وIL-β١ مقارنة بالمجموعات الأخرى. يحسن علاج اللوسارتان النتائج النسيجية والقيم البيوكيميائية والنتائج المورفومترية.

الاستنتاج: اللوسارتان يخفف اصابة نقص تدفق الدم بالمبيض/اعادة ضخ الدم عن طريق السيطرة على الالتهاب، وموت الخلايا المبرمج، والإجهاد التأكسدي.