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Original Article

The Rat Ovaries after U-74389G Process

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ABSTRACT

Background: This study co-evaluated the 4 quoted histologic variables after U-74389G (L) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating two respective histologic variables of Ovarian Epithelium Edema (OE), Oophoritis (OO), Ovarian Epithelium Karyorrhexis (OK), Ovarian Congestion (OC); in an induced ischemia reperfusion animal experiment. Furthermore, the Cytokine (TNFα) and the marker foroxidative stress Malondialdehyde (MDA) were also calculated. **Materials and Methods:** The 2 main experimental endpoints at which the OE, OO and OK, OC and TNFα, MDA scores were evaluated was the 60th reperfusion min (for the groups A and C) and the 120th reperfusion min (for the groups B and D). Specially, the groups A and B were processed without drugs, whereas the groups C and D after L administration. **Results:** The first preliminary study showed that L has a very significant recessing potency for OE and OC together (p-values=0.0157) within the "without lesions" alterations 0.1772727 [-0.3191054 - -0.03544]. The second preliminary study showed that L had a non-significant recessing potency for OK and OC within the "without lesions" grade 0.1772727 [-0.3716027 - +0.0170573] together (*p*-values=0.0726). These 2 studies were co-evaluated since they came from the same experimental setting. This study co-evaluated the combined diagnostic values of the four variables together. Separate calculations were performed for TNF-α and MDA scores. **Conclusion:** L administration significantly suppressed the 4 histologic variables within the "without lesions alterations" score 0.1772727 [-0.3208232 - -0.0337223] (*p*-value=0.0169), non-significantly reduced the TNF-α levels by 4.69% [+8.40%] (*p*-value=0.5749) and significantly reduced the MDA levels by 9.85%+2.66% (*p*-value=0.0005).

Keywords: Ischemia, U-74389G, Ovarian epithelium edema, Ovarian congestion, Ovarian epithelium karyorrhexis, Oophoritis, Reperfusion.

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INTRODUCTION

U-74389G is a novel Lazaroid (L) antioxidant factor implicating just only 264 published studies. The Ischemia Reperfusion (IR) type of experiments is noted in 19.69% of these studies. A tissue protective feature of U-74389G is obvious in these IR studies. The U-74389G chemically known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant complex, which prevents the lipid peroxidation either iron-dependent, or arachidonic acid-induced one. Animal kidney, liver, brain microvascular endothelial cells monolayers and heart models are protected by U-74389G after IR injury. U-74389G also attenuates the leukocytes; down-regulates the proinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents antishock property. 4 histologic variables in a Ovarian Ischemia Reperfusion (OIR) experiment were tested for this purpose. The two variables were those of Ovarian Epithelium Edema (OE) and Oophoritis (OO) which were very significantly recessed together (p-values=0.0157) within the "without lesions" alterations 0.1772727 [-0.3191054 - -0.03544].[1] The other variables were those of Ovarian Epithelium Karyorrhexis (OK) and Ovarian Congestion (OC) which were non-significantly recessed within the "without lesions" grade 0.1772727 [-0.3716027 - +0.0170573] together (*p*-values=0.0726).[2] Furthermore, the cytokine (TNFα) and the marker foroxidative stress Malondialdehyde (MDA) were also calculated. The present experimental work tried to co-evaluate these OE, OO, OK and OC variables together and to end up to their outcome totally, from the same rat induced OIR protocol.

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MATERIALS AND METHODS

Animal preparation

The study received 2 ethics committee approvals under the 3693/12-11-2010 and 14/10-1-2012 numbers fully following the tenants of the Declaration of Helsinki. The granting company, the experiment location and the Pathology Department are mentioned in preliminary references.[1,2] The human animal care of Albino female Wistar rats, the 7 days pre-experimental *ad libitum* diet, the non-stop intra-experimental anaesthesiology techniques, the acidometer, the electrocardiograms, the oxygen supply and the post-experimental euthanasia are also described in preliminary references. Rats were 16–18 weeks old. They were randomly assigned to four (4) groups consisted in n=10. The stage of 45 min ischemia was common for all 4 groups. Afterwards, reperfusion of 60 min was followed in group A; reperfusion of 120 min in group B; immediate L Intravenous (IV) administration and reperfusion of 60 min in group C; immediate L IV administration and reperfusion of 120 min in group D. The dose height assessment was described at preliminary studies as 10 mg/Kg body mass.

Ischemia was caused by laparotomic clamping the inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reperfusion. After the blood flow interruption, the protocol of OIR was applied, as described above for each experimental group. L was administered at the time of reperfusion; through inferior vena cava catheter. The OE, OO and OK, OC and TNFα, MDA scores were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups). Relation was rise between animals' mass with neither OE scores (p-value=0.0855), nor with OO ones (p-value=0.3991), nor with OK scores (p-value=0.9317), nor with OC ones (p-value=0.1016), nor with TNFα levels (p-value=0.6004) nor with MDA levels (p-value=0.4672). The pathologic score grading was maintained the same as in preliminary studies: (0-0.499) without lesions, (0.5-1.499) for mild lesions, (1.5 -2.499) for moderate lesions and (2.5-3) for serious lesions damage.

Model of ischemia-reperfusion injury

Control groups: The 20 control rats were the same for preliminaries and this study

Group A: Reperfusion which lasted 60 min concerned 10 controls rats of combined OE, OO and OK, OC ovarian score (cOS) as the mean of OE&OO scores and the OK&OC ones (Table 1).

Group B: Reperfusion which lasted 120 min concerned 10 controls rats of combined OE, OO and OK, OC ovarian score (cOS) as the mean of OE&OO scores and the OK&OC ones (Table 1).

L group: The 20 L rats were the same for preliminaries and this study.

Group C: Reperfusion which lasted 60 min concerned 10 L rats of combined OE, OO and OK, OC ovarian score (cOS) as the mean of OE&OO scores and the OK&OC ones (Table 1).

Group D: Reperfusion which lasted 120 min concerned 10 L rats of combined OE, OO and OK, OC ovarian score (cOS) as the mean of OE&OO scores and the OK&OC ones (Table 1).

Statistical analysis

Every cOS groups score was compared with each other from 3 remained groups applying Wilcoxon signed-rank test (Table 2). Then, the generalized linear models (glm) were applied with dependent variable the cOS scores, and independent variables the L administration or no, the reperfusion time and their interaction.

RESULTS

L administration non significantly restored the 4 histologic variables within the "without lesions alterations" score 0.1375 [-0.38362415-+0.10862415] (p-value=0.1174) as outcome by both glm and Wilcoxon signed-rank tests (Tables 3 and 6). However, reperfusion time non-significantly accentuated the 4 histologic variables within the "without lesions alterations" score 0.175 [-0.06579545 - +0.41579545] (p-value=0.1533), after co-calculation by the same methods (Tables 3 and 6). Totally, L administration and reperfusion time together non significantly restored the 4 histologic variables within the "without lesions alterations" score 0.1772727 [-0.3208232 - +0.0337223] (p-value=0.0169) (Tables 3 and 6). Similar calculations were performed for TNFα (Table 4) and MDA (Table 5) variables. A concise form of the above findings is depicted at Table 6.

DISCUSSION

Sevim *et al.* counted higher primordial follicle but decreased vascular endothelial growth factor immunoreactivity in treated groups and proliferating cell nuclear antigen immunoreactivity in the secondary follicles in all transplant groups; documenting that both benfotiamine and N-acetylcysteine are equal and effective agents in protection of ovarian tissue against ischemic neovascularization injury in rat autologous intraperitoneal ovarian autotransplants.[3] Nuri Yildirim *et al.* evaluated that Ginkgo Biloba (GB) supplementation significantly decreased scores for follicular degeneration, vascular congestion, edema, hemorrhage, and leukocyte infiltration in an experimental tissue malondialdehyde and plasma pentraxin-3 levels based ovarian torsion/de-torsion IR injury rat model.[4] Mohammed Al-Sadawi *et al.* concluded that women of a relatively younger age may be at 83.3% risk for developing myocardial infarction and cardiac thrombosis approximately 23 days after starting ovarian stimulation medications although 16.67% of women may be pregnant at presentation.[5] Chest pain

Table 1: Ovarian Epithelium Edema (OE), Oophoritis (OO), Ovarian Epithelium Karyorrhexis (OK) and Ovarian Congestion (OC) and their mean and SD scores.

	Mean OE&OO score +/-SD	Mean OK&OC score +/-SD	Mean OE&OO&OK&OC score +/-SD
Group A	Without lesions 0.35 ±0.4116363	Mild lesions 0.85 ±0.5797509	Mild lesions 0.6 ±0.4281744
Group B	Mild lesions 0.55±0.4972145	Mild lesions 1.05±0.4972145	Mind lesions 0.8±0.4048319
Group C	Without lesions 0±0.0	Mild lesions 0.5±0.4082483	Without lesions 0.25±0.2041241
Group D	Without lesions 0.15±0.2415229	Mild lesions 0.65±0.4743416	Mild lesions 0.4±0.3162278

Table 2: The values Difference for Groups (DG) after Wilcoxon signed-rank test for all histologic variables mean scores.

DG	Difference	*p*-value
A-B	+0.2	0.2810
A-C	-0.35	0.1206
A-D	-0.2	0.2769
B-C	-0.55	0.0072
B-D	-0.4	0.0259
C-D	+0.15	0.3259

Table 3: The ameliorating influence of L for all histologic variables in connection with reperfusion time.

Alteration	95% c. in.	Reperfusion time	Wilcoxon	Glm
Without lesions alterations -0.35	-0.7560696 - +0.0560696	1 hr	0.1206	
Without lesions alterations +0.025	-0.3273353 +0.3773353	1 hr		0.8832
Without lesions alterations -0.375	-0.600439 - -0.149561	1.5 hr		0.0017
Without lesions alterations +0.1	-0.1668093 +0.3668093	1.5 hr	0.2332	
Without lesions alterations -0.05	-0.4088007 - +0.3088007	2 hr		0.7730
Without lesions alterations -0.4	-0.7286838 - -0.0713162	2 hr	0.0259	
Without lesions alterations +0.175	-.0753714 - +0.4253714	Reperfusion		0.1652
Without lesions alterations +0.175	-0.0562195 - +0.4062195	Reperfusion	0.1414	
Without lesions alterations -0.1772727	-0.3208232 -+0.0337223	Interaction		0.0169

Table 4: The attenuating influence of L on TNF-α levels in connection with reperfusion time.

Alteration	+/-SD	Reperfusion time	*p*-value
+13.94%	±131.17%	1 hr	0.7167
+3.40%	±67.05%	1.5 hr	0.8147
-7.13%	±92.34%	2 hr	0.8174
-29.86%	±54.52%	Reperfusion	0.0254
-4.69%	±8.40%	Interaction	0.5749

Table 5: **The attenuating influence of L on MDA levels in connection with reperfusion time.**

Alteration	+/-SD	Reperfusion time	*p*-value
-13.39%	±20.37%	1 hr	0.0663
-17.71%	±16.97%	1.5 hr	0.0001
-22.03%	±12.64%	2 hr	0.0003
+3.54%	±16.10%	Reperfusion	0.4103
-9.85%	±2.66%	Interaction	0.0005

Table 6: **Concise form of the Table 3.**

Alteration	95% c. in.	Reperfusion time	*p*-value
Without lesions alterations -0.1625	-0.54170245 +0.21670245	1 hr	0.5019
Without lesions alterations -0.1375	-0.38362415 +0.10862415	1.5 hr	0.1174
Without lesions alterations -0.225	-0.56874225 - +0.11874225	2 hr	0.3994
Without lesions alterations +0.175	-0.06579545 - +0.41579545	Reperfusion	0.1533
Without lesions alterations -0.1772727	-0.3208232 -+0.0337223	Interaction	0.0169

is the most common presenting symptom ~ 66.67%, whereas 16.67% are presented with stroke or abdominal distention. 25% of patients underwent coronary angiography were treated with percutaneous coronary intervention, since Ovarian Hyperstimulation Syndrome (OHSS) is characterized by increased vascular permeability and hypercoagulable states resulting in strokes and peripheral ischemia. Meryem Kurek Eken *et al.* invented that tissue 8-OHdG, caspase-3 activity and reduction of AMH were significantly lower; whereas the primordial, preantral and small antral follicle numbers were also significantly higher in the I/R + etanercept group than the I/R group.[6] Etanercept which is widely used in autoimmune diseases for blocking Tumor Necrosis Factor α (TNF-α), as an inflammatory cytokine; attenuated inflammation and related oxidative stress and also helped to preserve ovarian reserve following ovarian I/R damage. Fatma Beyazit *et al.* evaluated the effects of an antitumor necrosis factor-α antibody. Inflammation, AI, vascular congestion and hemorrhagia were significantly lower in adalimumab-treated group.[7] Adalimumab - an antitumor necrosis factor-α antibody-therapy attenuated I/R induced ovarian injury, possibly suppressing inflammation, inhibiting oxidative stress and altering apoptotic pathways in a rat model of induced ovarian torsion. Atilla Topcu *et al.* observed infiltration and diffuse edematous areas in addition to diffuse vascular congestion and hemorrhage findings.[8] MDA and TNF-α concentrations were decreased in the Metformin (MET) treatment groups, while GSH and E_2 levels were increased. The findings showed that MET application was effective in preventing damage in ovarian tissue by reducing levels of reactive oxygen species, proinflammatory cytokines caspase-3 and NF-κβ and apoptosis in female Sprague Dawley rats. Cenk Nayki *et al.* found that rutin (a flavonoid glycoside) significantly decreased MDA levels, the expressions of TNF-α and IL-1β, and also the activity of COX-2 while it increased significantly tGSH levels and the activity of COX-1 in the RG group than the IRG group; ameliorating the I/R-induced ovarian injury via its possible antioxidative and anti-inflammatory effects in Wistar albino female rats.[9] C Turkler *et al.* invented that lutein ameliorates the I/R-induced ovarian injury by its antioxidative and anti-inflammatory activities increasing antioxidant enzymes.[10] Total glutathione and cyclooxygenase-1 were decreased whereas these ratios were reversed in the LIRG group ($p < 0.05$) in ovarian tissues of animals after I/R. Marwa M M Refaie *et al.* attributed the protective effect of Pioglitazone (PIO) to its PPARγ agonist effect, anti-inflammatory, anti-apoptotic and antioxidant properties; since PIO non significantly reduced the induced increased ovarian tissue levels of MDA, NOx, gene expressions of p53, TNF-α, iNOS, GSH, HO-1 levels, PPARγ, eNOS gene expressions and the marked ovarian edema, hemorrhage and congestion associated with cell injury than control group in an Ovarian Ischemia Reperfusion (OIR) experiment.[11] Banu Güleç *et al.* made the assumption that preoperative Progesterone (PG) treatment might exert protective effects on ovarian I/R injury through its anti-apoptotic and antioxidative properties; since serum and tissue TOS levels, histologic score including vascular congestion, hemorrhage, polymorphonuclear neutrophils, number of apoptotic cells and interstitial edema were significantly lower and tissue TAS levels were higher in treated group ($p<0.001$).[12] However, the PG pre-treatment decrease was not statistically significant.

Table 7: The L influence (+SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time.[13]

35 Variables	1hr rep+/-SD	*p*-value	1.5 hr rep+/-SD	*p*-value	2hr rep+/-SD	*p*-value	Interaction of Epo and rep+/-SD	*p*-value
Mean	+2.03%±27.26%	0.2168	+0.19%±29.41%	0.1836	-1.63%±33.15%	0.2389	-0.33%±16.23%	0.2016

The present experiment shows that L attenuates the cytokine TNFα levels which is involved in systemic inflammation, is one of the cytokines that make up theacute phase reaction, stimulates phagocytosis and the production of IL-1 oxidants and thus attenuates all of these procedures by 4.69% [+8.40%] (p-value=0.5749). Also, L significantly attenuates the MDA levels, which is a marker foroxidative stress and specifically of lipid peroxidation in tissues; thus improving since assess the membrane damage by 9.85%+2.66% (*p*-value=0.0005). A numeric evaluation of the L efficacies was provided by a meta-analysis of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion time, coming from the same experimental setting (Table 7).[13]

CONCLUSION

L administration significantly suppressed the 4 histologic variables within the "without lesions alterations" score 0.1772727 [-0.3208232 - -0.0337223] (*p*-value=0.0169), having clear antioxidant capacity from the results on TNF-α and MDA levels. Hence, it challenges for further research about its beneficial usage in protection of ovarian tissue against ischemic neovascularization injury in autologous intraperitoneal ovarian auto transplants, preservation of ovarian reserve following ovarian I/R damage induced or in ovarian torsion/de-torsion IR injury and ovarian stimulation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

COX-2: Cyclooxygenase-2; **E_2:** Oestradiol; **eNOS:** Endothelial nitric oxide synthase; **GSH:** Glutathione; **HO-1:** Heme oxygenase-1; **IL-1β:** Interleukin-1 beta; **iNOS:** Inducible nitric oxide synthase; **I/R:** Ischemia/reperfusion; **LIRG:** Lutein Ischemia/reperfusion group; **MDA:** 3,4-Methylenedioxyamphetamine; **MET:** Metformin; **NF-κβ:** Nuclear factor kappa B; **NO:** Nitric oxide; **O(~):** Ovarian(~); **OC:** Ovarian congestion; **OE:** Ovarian epithelium edema; **8-OHdG8:** Hydroxyguanosine; **OK:** Ovarian epithelium karyorrhexis; **OO:** Oophoritis; **p53:** tumor protein 53; **PG:** Progesterone; **PIO:** Pioglitazone; **PPAR:** Peroxisome proliferator-activated receptors; **SD:** Standard deviation; **TAS:** Total antioxidant status; **tGSH4:** 4′-Sulfonylbis[2-(2-propenyl) phenol/3,3′-Diallyl-4,4′-dihydroxybiphenyl Sulfone; **TNFα:** Tumor necrosis factor; **TOS:** Total oxidant status; **U-74389G:** 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate.

REFERENCES

1. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos GC, Tsarea K, *et al*. The co-evaluation of ovarian epithelium edema and oophoritis after the antioxidant Drug" U-74389G" effect on ovarian ischemia reperfusion injury. Open J Surg. 2018;1(2):8-14.
2. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos GC, Tsarea K, *et al*. The Co evaluation of Ovarian epithelium Karyorrhexis and Ovarian Congestion after the Antioxidant Drug" U-74389G" Effect on Ovarian ischemia-reperfusion Injury. Clin J Surg. 2018;1(2):1-6.
3. Sevim T, Remzi A, Şehmus P, Tuncay K, Gökhan A, Süleyman A. N-acetylcysteine and Benfotiamine protect autotransplanted ovarian tissue from ischemia-reperfusion injury: an experimental study. Exp Clin Transplant. 2018.
4. Yildirim N, Simsek D, Kose S, Yildirim AGS, Guven C, Yigitturk G, *et al*. The protective effect of Gingko biloba in a rat model of ovarian ischemia/reperfusion injury: improvement in histological and biochemical parameters. Adv Clin Exp Med. 2018;27(5):591-7. doi: 10.17219/acem/68896, PMID 29790691.
5. Al-Sadawi M, Chowdhury R, Asun S, Ray J, Soni L, Bahtiyar G, *et al*. Ovarian hyperstimulation syndrome and myocardial infarction: A systematic review. Int J Clin Endocrinol Metab. 2019;5(1):009-12.
6. Eken MK, Ersoy GS, Kaygusuz EI, Devranoğlu B, Takır M, Çilingir ÖT, *et al*. Etanercept protects ovarian reserve against ischemia/reperfusion injury in a rat model. Arch Med Sci. 2019;15(4):1104-12. doi: 10.5114/aoms.2017.72406, PMID 31360205.
7. Beyazit F, Büyük B, Turkon H, Elmas S, Uzun M. Adalimumab mitigates ovarian ischemia-reperfusion injury in rats by regulating oxidative stress, apoptosis and resolution of inflammation. J Obstet Gynaecol Res. 2019;45(2):358-67. doi: 10.1111/jog.13846, PMID 30358007.
8. Topcu A, Balik G, Atak M, Mercantepe T, Uydu HA, Tumkaya L. An investigation of the effects of metformin on ovarian ischemia-reperfusion injury in rats. Eur J Pharmacol. 2019;865(865):172790. doi: 10.1016/j.ejphar.2019.172790, PMID 31730761.
9. Nayki C, Nayki U, Keskin Cimen FK, Kulhan M, Yapca OE, Kurt N, *et al*. The effect of rutin on ovarian ischemia-reperfusion injury in a rat model. Gynecol Endocrinol. 2018;34(9):809-14. doi: 10.1080/09513590.2018.1450378, PMID 29564969.
10. Turkler C, Kulhan NG, Ata N, Kiremitli T, Cimen FK, Suleyman H. The ameliorative effect of lutein on ovarian ischemia-reperfusion injury in rats. Bratisl Lek Listy. 2018;119(11):713-7. doi: 10.4149/BLL_2018_127, PMID 30686005.
11. Refaie MMM, El-Hussieny M. Protective effect of pioglitazone on ovarian ischemia reperfusion injury of female rats via modulation of peroxisome proliferator activated receptor gamma and heme-oxygenase 1. Int Immunopharmacol. 2018;62:7-14. doi: 10.1016/j.intimp.2018.06.037, PMID 29966944.
12. Başer BG, Taşkın MI, Adalı E, Öztürk E, Hısmıoğulları AA, Yay A. Does progesterone have protective effects on ovarian ischemia-reperfusion injury? J Turk Ger Gynecol Assoc. 2018;4;19(2):87-93.
13. Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The antioxidant Drug" U-74389g" effect on alanine aminotransferase levels. J Anal Pharm Res. 2017;4(2);00095.

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