Arrival Date: 18.08.2021 | Published Date: 25.11.2021 | 2021, Vol: 6, Issue: 15 | pp: 45-51 | Doi Number: http://dx.doi.org/10.46648/gnj.276

Effects Of Melatonin And Curcumin Treatments On The Ovarium In Kidney Ischemia-Reperfusion Injury: A Histopathological Investigation

Ayşe KÖSE VURUŞKAN[1], Nur ELAGÜL[2,3], Tansel SAPMAZ[4], Sude TOPKARAOĞLU[5,6]

ABSTRACT

Aim: We aimed to investigate how bilateral renal ischemia-reperfusion (I/R) damage affects the ovaries as a distant organ and the effects of melatonin (MEL), curcumin (CUR) and melatonin+curcumin (MEL+CUR) treatments on I/R damage.

Material and Method: 35 female Wistar rats were used in the study. Rats were divided into 5 groups and study was designed as follows: Control group (G1) – opening and closing the abdomen only (sham surgery group) –, I/R group (G2) – 45 min ischemia followed by 2 h reperfusion –, I/R+MEL group (G3) – 45 min ischemia, intraperitoneal (i.p) 20 mg/kg MEL injection 5 min before reperfusion, followed by 2 h reperfusion –, I/R+CUR group (G4) – 45 min ischemia, 5 min before reperfusion i.p 200 mg/kg CUR injection and then 2 hours reperfusion –, I/R+MEL+CUR group (G5) – 45 min ischemia, 5 min before reperfusion i.p 20 mg/kg MEL and 200 mg/kg CUR injection, followed by 2 hours reperfusion –. At the end of the reperfusion period, the rats were sacrificed. Right ovaries were removed from the peritoneum and fixed. After fixation and follow-up, tissue sections were stained with hematoxylin&eosin (H&E), Periodic acid-Schiff (PAS)+Hematoxylin (PAS+H) and Masson's trichrome stains. Pathological changes were scored and statistically evaluated.

Results: Compared to the control group, there was a decrease in hemorrhage, vascular congestion, follicular degeneration, inflammation, interstitial edema, vasodilation and growing follicle numbers in all groups; these changes were severe in the G2 group; Mild to moderate severity was observed in the G3, G4 and G5 groups.

Conclusion: Renal I/R damage significantly affects the ovaries histopathologically. MEL, CUR, and MEL+CUR partially preserve the histological structure, but MEL treatment seems to be more effective than CUR treatment.

Keywords: Curcumin, Distant Organ Damage, Ischemia-Reperfusion, Melatonin, Ovary

INTRODUCTION

Ischemia-reperfusion injury is a condition that causes pathological problems associated with surgical procedures such as myocardial infarction, organ transplantation, coronary angioplasty and an unavoidable (Zang et al., 2020). It is a process characterized by the temporary interruption of blood flow to an organ, followed by reperfusion and oxygenation. The level of tissue damage is related to the amount and duration of the decrease in blood flow, and the situation is exacerbated by reperfusion (Tang-Zhuang, 2019). After reperfusion, migration of polymorphonuclear leukocytes (PML) and platelets into the tissue is stimulated. PML, on the other hand, increases the formation of inflammatory cytokines and free oxygen radicals, and lipid peroxidation occurs in mitochondria and cellular membranes (Karaçor et al., 2020). As a result of these changes, inflammation and organ dysfunction may develop in the tissues (Tang-Zhuang, 2019).

Melatonin is a hormone secreted by the pineal gland, discovered by Aaron Lerner in 1958 (Arendt, 2007). While its synthesis and secretion increase in the dark, its production is inhibited by light, and it regulates the circadian rhythm

[1] Asst. Prof. Dr., University of Health Sciences, Faculty of Medicine, Department of Histology and Embryology, Istanbul/Turkey, ayse.kose@sbu.edu.tr, ORCID: 0000-0002-4136-3014
[2] Res. Asst., Istanbul Yeni Yuzyil University, Faculty of Medicine, Department of Histology and Embryology, Istanbul/Turkey
[3] University of Health Sciences, Health Sciences Institute, Department of Histology and Embryology, Istanbul/Turkey, n.elagul1@hotmail.com, ORCID: 0000-0001-5962-5935
[4] Asst. Prof. Dr., University of Health Sciences, Faculty of Medicine, Department of Histology and Embryology, Istanbul/Turkey, tansel.sapmaz@sbu.edu.tr, ORCID: 0000-0002-7820-5837
[5] Res. Asst., University of Health Sciences, Faculty of Medicine, Department of Histology and Embryology, Istanbul/Turkey
[6] University of Health Sciences, Health Sciences Institute, Department of Histology and Embryology, Istanbul/Turkey, sude.topkaraoglu@sbu.edu.tr, ORCID: 0000-0002-7680-7156

with this function (Tordjman et al., 2017). As a result of studies, it has been reported that melatonin has many cell protective effects such as anti-inflammatory, anti-oxidant and anti-apoptotic (Amaral - Cipolla-Neto, 2018; Cipolla-Neto - Amaral, 2018; Ma et al., 2017), and it has been shown that melatonin reduces I/R damage thanks to these effects (Han et al., 2020; Ma et al., 2017; Palareti et al., 2016; Shi et al., 2019).

Curcumin is a bioactive polyphenolic compound identified in the turmeric (Curcuma longa) (Kotha - Luthria, 2019). Curcumin, which has come to the fore in recent years, has been shown to have anti-inflammatory, anti-diabetic, anti-cancer and anti-aging effects (Esatbeyoglu et al., 2012; Gupta et al., 2013; Kunnumakkara et al., 2017; Moghaddam Aghili et al., 2018). However, it has been reported that it may have a protective role in I/R injury, thanks to its biological functions (Hongtao et al., 2018; Liu et al., 2017; Mokhtari-Zaer et al., 2018; Zhang et al., 2018).

It has been reported in the literature that acute kidney injury, which may occur due to I/R or other reasons, causes damage by activating various signaling pathways on tissues and organs in distant regions such as the brain, heart, lung, liver and gastrointestinal system (Grams - Rabb, 2012). However, we could not find any study examining the ovary as a distant organ damage after kidney I/R injury. In this study, we aimed to investigate the effects of melatonin and curcumin on the ovary as distant organ damage by causing kidney I/R damage.

MATERIAL AND METHOD

Animals and Establishment of Experimental Model

The animals were housed in plastic cages at 21-22 °C, 12 hours dark and 12 hours bright light period and fed with normal pellet feed and water without being subjected to any diet. Our study was modified according to the experimental design of Aydin et al. (2014). Thirty-five (35) female Wistar rats weighing 170-200 grams were randomly divided into 5 groups two weeks before the experiment and the groups were formed as follows: G1 (Control group) (n=6) – opening and closing the abdomen only (sham surgery group) –, G2 (I/R group) (n=7) – 45 min ischemia, then 2 h reperfusion –, G3 (I/R+MEL group) (n=7) – 45 min ischemia, 20 mg/kg MEL (Sigma- Aldrich, Germany) as i.p injection 5 min before reperfusion, followed by 2 h reperfusion –, G4 (I/R+CUR group) (n=7) – 45 min ischemia, 200 mg/kg CUR (Sigma-Aldrich, Germany) injection 5 min before reperfusion as i.p and then 2 h of reperfusion –, G5 (I/R+MEL+CUR group) (n=7) – 45 min of ischemia, i.p injections of 20 mg/kg MEL and 200 mg/kg CUR 5 min before reperfusion, followed by 2 h of reperfusion. Right ovaries of all animals were taken into 10% buffered formaldehyde for histopathological investigation.

Surgical Procedures

All procedures were carried out according to the guidelines of the Council of Europe (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes). This study was found ethically appropriate by the University of Health Sciences Hamidiye HADYEK.

After anesthesia of 90 mg/kg ketamine and 10 mg/kg xylazine to all rats, the lower peritoneum was opened by midline laparotomy. After determining the right and left renal arteries entering the organ from the hilum of both kidneys, microvascular clamps were placed, and the peritoneum was closed by suturing immediately and ischemia was applied for 45 minutes. Injections were administered 40 minutes after the onset of ischemia. 5 minutes after the injection, the clamps were removed, the kidneys were waited for blood circulation again, and then the peritoneal areas were closed by suturing again. All rats whose reperfusion period was completed were sacrificed by exsanguination method and their right ovaries were dissected.

Histopathological Evaluation

The ovaries were fixed in 10% buffered formaldehyde for 48 hours. Then, histological tissue processing was performed with an automatic tissue processor (ThermoScientific, USA), and then all tissues were embedded in paraffin blocks, 5-µm semi-thin sections were taken on microtome (ThermoScientific, USA). After dewaxing the tissue sections, they were stained with routine H&E, PAS+H and Masson's trichrome kit (ChemBio, Turkey). The stained sections were examined under x10 objective with a light microscope (Zeiss, Germany). At least five microscopic fields were analyzed and scored semi-quantitatively. Ovarian injury criteria included hemorrhage, vascular congestion, follicular degeneration (granulosa cells), inflammation (PML infiltration), edema, vasodilation, and follicle counts including corpus luteum. Samples were scored on a scale of 0 to 3 (0: none; 1: mild; 2: moderate; 3: severe). Evaluations were done blindly by the same pathologist.

Statistical Analysis

Continuous data were presented as mean ± standard deviation. Statistical analyzes were performed using GraphPad Prism 8.1.0 (GraphPad Inc., California, USA). Data suitable for normal distribution were searched with the Shapiro Wilk's test. One-way ANOVA and Tukey's test were performed for parametric data, Kruskal Wallis analysis was performed for non-parametric data and $p<0.05$ values were considered statistically significant.

RESULTS

The results of all variables scored histopathologically are presented in **Table 1** and **Table 2**. Micrographs showing the

histopathological findings of the groups are shared in **Figure 1**. It was determined that the hemorrhage in the G2 (I/R) increased significantly compared to the control group (p=0.0093) and no significant difference was observed between the other groups for hemorrhagic profile. When all groups were compared to the control group, it was observed that the vascular congestion of the G4 (I/R+CUR) increased (p=0.0045); follicular degeneration of the G2 increased (p=0.0281); the inflammation levels of the G2 and G3 (I/R+MEL) were significantly increased (p=0.0106; p=0.0490, respectively); the edema levels of the all groups [G2, G3, G4, G5 (I/R+MEL+CUR)] showed a significant increase (p=0.0062; p=0.0204; p=0. 0153; p=0.0010, respectively); vasodilation levels of the G4 and G5 increased (p=0.0132; p=0.0321, respectively). The difference in primordial, primary, secondary follicle and corpus luteum counts of the groups was not statistically significant [p=ns (not significant)]. However, the number of tertiary follicles in the G2 decreased significantly compared to the control group (p=0.0299). Also, the level of vacuolization in cytoplasm of corpus luteum's lutein cells is severe in G2, moderate in G4; mild in G3 and G5.

Table 1. Comparison of the histopathological conditions of the groups.

	G1	G2	G3	G4	G5	*p* value
Hemorrhage	0,60±0,55	1,67±1,03**	1,17±0,75	1,43±0,53	1,00±1,15	0,107
Vascular Congestion	0,60±0,55	2,00±0,89	1,67±0,82	2,43±1,13**	1,86±1,35	0,009*
Follicular Degeneration	0,40±0,55	1,83±1,47**	1,17±0,41	1,14±0,69	0,71±1,11	0,031*
Inflammation	0,40±0,55	2,00±1,10**	1,67±0,82**	1,43±0,79	1,14±1,21	0,020*
Edema	0,00±0,00	2,00±1,10**	1,83±0,75**	1,86±0,69**	2,29±0,95**	0,007*
Vasodilation	0,40±0,55	1,67±0,82	1,33±1,03	2,14±1,07**	1,86±1,35**	0,004*

*Statistically significant, ** Statistically significant compared to the control group.

Table 2. Comparison of follicle and corpus luteum counts of the groups.

	G1	G2	G3	G4	G5	*p* value
Primordial Follicle Count	16,80±9,71	8,00±6,23	5,83±2,40	7,43±4,12	7,71±5,77	0,185
Primer Follicle Count	5,40±3,91	4,83±2,40	5,17±4,79	5,86±3,76	7,57±5,88	0,916
Secondary Follicle Count	4,80±2,28	2,50±2,88	2,67±3,39	4,43±3,05	4,86±2,54	0,247
Tertiary Follicle Count	2,80±1,48	0,83±0,98**	2,50±1,52	2,14±1,77	2,00±1,53	0,286
Count of Corpus Luteum	9,60±3,65	8,00±3,74	11,33±4,46	8,29±3,25	8,43±4,65	0,444

*Statistically significant, ** Statistically significant compared to the control group.

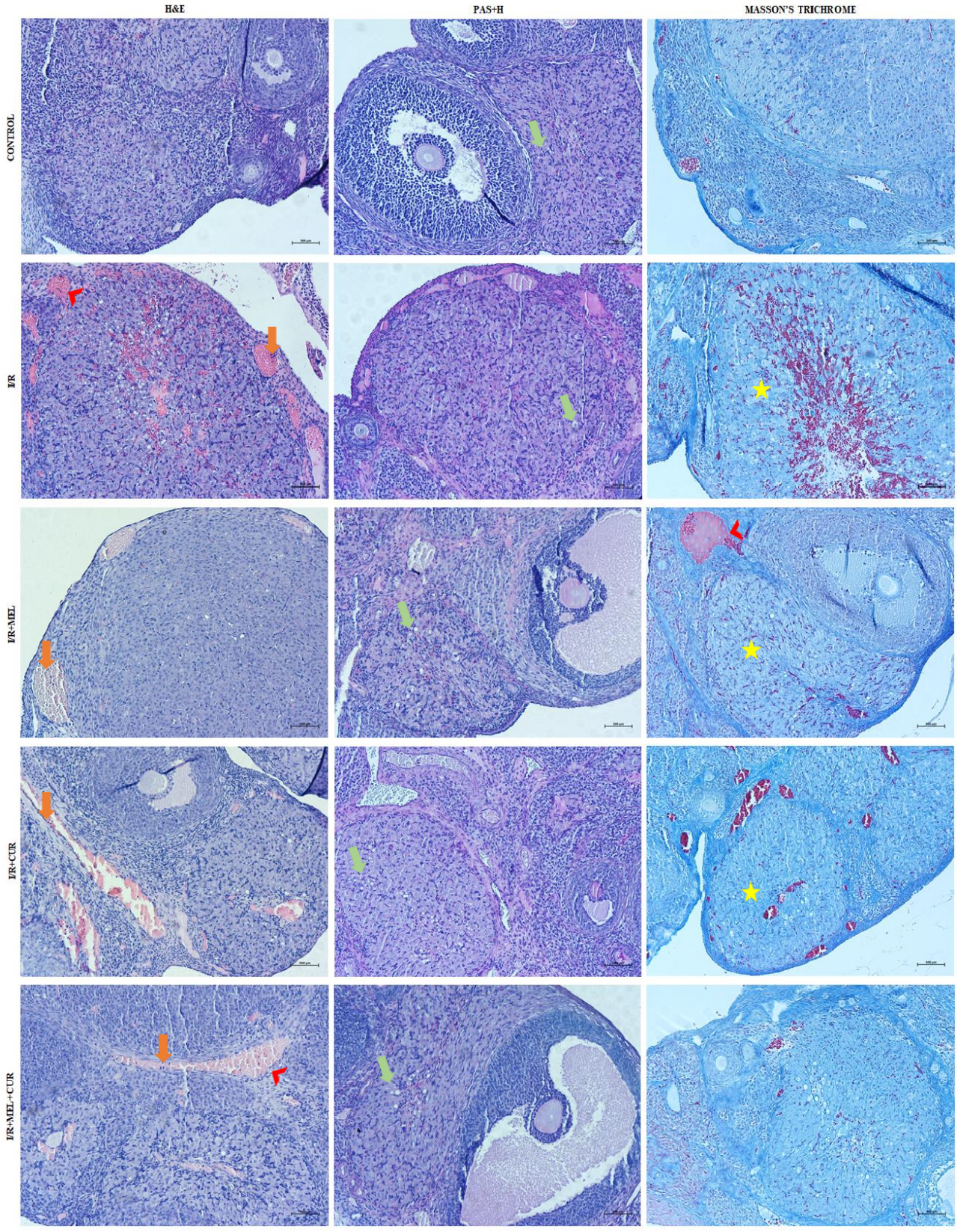

Figure 1. Histopathological status in ovarian samples (Vascular congestion: red arrowhead, PML infiltration: orange arrow, Vacuolization: green arrow, Hemorrhagic areas: yellow star, Scale bar: 500 µm).

DISCUSSION

Due to interventions such as organ transplantation or other surgical interventions, blood pressure changes, inflammation occurs with PML infiltration, free oxygen radicals increase, and for these reasons other organs are also affected by these changes. It has been demonstrated by studies that I/R damage causes damage to distant tissues or organs, and studies that try to eliminate this damage have taken their place in the literature (Aydin et al., 2014; Grams - Rabb, 2012).

MEL, which is associated with the circadian rhythm; it has been reported that it can be used as a therapeutic agent in cases where membrane structures are disrupted, and situations such as increased mitochondrial redox and mitophagy due to I/R damage (Ma et al., 2017). In addition, studies have shown that MEL treatments have a wide application area for treatment purposes such as stroke, hypertension, cancer, and reproduction (Cipolla-Neto - Amaral, 2018; Esteban-Zubero et al., 2016; Tordjman et al., 2017). CUR, which is found in large amounts in turmeric, has been researched in preclinical studies since 1949 for reasons such as its high biological safety and tolerance, and modulation of many signaling molecules, and CUR has continued to be used as a therapeutic agent against several human diseases with many aspects, especially its anti-inflammatory, antioxidant and antimicrobial effects (Gupta et al., 2013). CUR may be a protective agent due to such as its anti-inflammatory effect on kidney I/R injury, its antioxidant effect protecting neurons by reducing the levels of reactive oxygen derivatives that are emerged at high levels with I/R injury, and titration of microglia/macrophage polarization in ischemic damage in brain tissue demonstrated by studies (Esatbeyoglu et al., 2012; Liu et al., 2017; Zhang et al., 2018). However, since we have not encountered a study showing how the ovaries are affected by kidney I/R damage, we aimed to investigate the effect of bilateral kidney I/R damage on the ovaries and how this damage is affected by the injection of MEL and CUR agents via the i.p route.

Based on the findings of our study, we observed that the renal I/R application also causes damage to the ovaries, MEL and CUR applications are effective in mitigating I/R damage for histological structure, but MEL provides more effective protection than CUR. On the other hand, we found that there was no significant difference between the G5 and G3 groups.

We evaluated the histological findings of the control group as normal in terms of all variables, we did not detect any pathological conditions. In the study of Karaçor et al. (2020), it was reported that the levels of hemorrhage, follicular degeneration, vascular stasis, inflammation, interstitial edema, and vacuolization increased significantly in the I/R group compared to the control group (Kirmizi et al., 2021; Koc et al., 2019; Tokgoz et al., 2018). We found a significant increase in the levels of hemorrhage, follicular degeneration, inflammation with PML infiltration, edema, vasodilation, vacuolization and cellular degeneration in lutein cells of the corpus luteum in the we applied I/R (G2). In the ovarian torsion-detorsion model performed by Turkoz et al. (2004), they reported that the acute findings of the I/R group turned into partial PML infiltration, edema, and vascular dilatation findings in the group in which I/R+MEL was applied, but they said that the MEL application did not completely preserve the histological structure. M. Hemadi et al. (2011), demonstrated that MEL application reduced the levels of mixed oocyte- granulosa complex, shedding and scattered interstitial appearance of granulosa cells. Finally, in the study of Kalyoncu et al. (2020), it was reported that MEL significantly reduced hemorrhage, follicular degeneration, and general tissue damage. In our study, MEL reduced the levels of all pathological findings, especially corpus luteum vacuolization, compared to the G2 in terms of all parameters, the severity of the damage in the G3 was mild compared to the G2, but these findings were not statistically significant; when the G3 was compared with the control group, pathological findings continued. In studies examining the effect of CUR on I/R damage in the ovarian tissue, it was reported that pathological conditions (hemorrhage, vascular congestion, cellular degeneration, PML infiltration, interstitial edema) decreased to a mild level (Behroozi-Lak et al., 2018; Eser et al., 2015; Sak et al., 2013). The pathological changes were found in the G4 compared to the control group, but the severity of these findings was moderate compared to the G2. Among the groups, the G5 was closest to the control group for hemorrhage, follicular degeneration, and inflammation. G3 was closest to the control group for vascular congestion and vasodilation.

M. Hemadi et al. (2011) found that, in general, the number of growing follicles increased with MEL treatment. In another study by M. Hemadi et al. (2012), they reported that primordial follicles that underwent apoptosis after ovarian transplantation reappear as of the 7th day of transplantation with the effect of MEL. Kalyoncu et al. (2020) found that there was a significant increase in the number of primordial follicles in the 10 mg/kg MEL group compared to the I/R group, but there was no statistically significant difference between the groups for other follicle counts. On the contrary, in the study of Noori Hassanvand et al. (2019), the group that received 20 mg/kg MEL showed a significant increase in follicle numbers compared to the transplant group. In our study, we did not find a significant difference between the groups for primordial, primary, secondary follicle and corpus luteum counts. However, we obtained the highest number of follicles in the G5 for primordial, primary, and secondary follicles. We found the highest number of tertiary follicles in the G3, and we found a significant decrease in the G2 compared to the control group. On the other hand, the level of vacuolization in the cytoplasm of corpus luteum's lutein cells was severe in the G2, but in the other groups was less compared the G2. At this point, we determined that MEL, CUR and MEL+CUR treatments generally increase the count of growing (primordial, primary, and secondary) follicles after I/R injury.

In this study, we observed that especially melatonin significantly improved the histological structure against I/R damage for both groups (G3 and G5), while curcumin partially reduced I/R damage, although not as much as

melatonin. However, with the decrease in pathological severity, it is possible to say that the follicle structure and number were preserved in the groups.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our findings revealed that renal I/R intervention can significantly affect the ovaries as distant organ damage, and that applying melatonin, curcumin or both agents together for organ transplantation or another surgical intervention can be used to alleviate or minimize the side effects that may occur. Since there has not been any study on this subject in the literature yet, we can recommend advanced dosage studies, biochemical, immunohistochemical and molecular tests to have a deeper level of knowledge.

REFERENCES

Amaral, F. G. do, & Cipolla-Neto, J. (2018). A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metab.*, *62*(4), 472–479. https://doi.org/10.20945/2359-3997000000066

Arendt, J. (2007). Aaron Lerner, who discovered melatonin. *Journal of Pineal Research*, *43*(1), 106–107. https://doi.org/10.1111/j.1600-079X.2007.00457.x

Aydin, M. S., Caliskan, A., Kocarslan, A., Kocarslan, S., Yildiz, A., Günay, S., Savik, E., Hazar, A., & Yalcin, F. (2014). Intraperitoneal curcumin decreased lung, renal and heart injury in abdominal aorta ischemia/reperfusion model in rat. *International Journal of Surgery*, *12*(6), 601–605. https://doi.org/10.1016/j.ijsu.2014.04.013

Behroozi-Lak, T., Ebrahimpour, M., Zarei, L., Pourjabali, M., Farhad, N., & Mohaddesi, H. (2018). Systemic administration of curcumin nanoparticles protects ischemia-reperfusion injury in ovaries: An animal model study. *Revista Da Associacao Medica Brasileira*, *64*(1), 22–31. https://doi.org/10.1590/1806-9282.64.01.22

Cipolla-Neto, J., & Amaral, F. G. (2018). Melatonin as a Hormone: New Physiological and Clinical Insights. In *Endocrine Reviews* (Vol. 39, Issue 6). https://doi.org/10.1210/er.2018-00084

Esatbeyoglu, T., Huebbe, P., Ernst, I. M. A., Chin, D., Wagner, A. E., & Rimbach, G. (2012). Curcumin-from molecule to biological function. *Angewandte Chemie - International Edition*, *51*(22), 5308–5332. https://doi.org/10.1002/anie.201107724

Eser, A., Hizli, D., Haltas, H., Namuslu, M., Kosus, A., Kosus, N., & Kafali, H. (2015). Effects of curcumin on ovarian ischemia-reperfusion injury in a rat model. *Biomedical Reports*, *3*(6), 807–813. https://doi.org/10.3892/br.2015.515

Esteban-Zubero, E., García-Gil, F. A., López-Pingarrón, L., Alatorre-Jiménez, M. A., Iñigo-Gil, P., Tan, D. X., García, J. J., & Reiter, R. J. (2016). Potential benefits of melatonin in organ transplantation: a review. *Journal of Endocrinology*, *229*(3), R129–R146. https://doi.org/10.1530/JOE-16-0117

Grams, M. E., & Rabb, H. (2012). The distant organ effects of acute kidney injury. *Kidney International*, *81*(10), 942–948. https://doi.org/10.1038/ki.2011.241

Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. *American Association of Pharmaceutical Scientists Journal*, *15*(1), 195–218. https://doi.org/10.1208/s12248-012-9432-8

Han, Y. S., Lee, J. H., & Lee, S. H. (2020). Melatonin suppresses ischemia-induced fibrosis by regulating miR-149. *Biochemical and Biophysical Research Communications*, *525*(2), 354–359. https://doi.org/10.1016/j.bbrc.2020.02.090

Hemadi, M., Saki, G., Shokri, S., & Ghasemi, F. M. (2011). Follicular dynamics in neonate vitrified ovarian grafts after host treatment with melatonin. *Folia Morphologica*, *70*(1), 18–23.

Hemadi, Masoud, Shokri, S., Pourmatroud, E., Moramezi, F., & Khodadai, A. (2012). Follicular Dynamic and Immunoreactions of the Vitrified Ovarian Graft After Host Treatment with Variable Regimens of Melatonin. *American Journal of Reproductive Immunology*, *67*(5), 401–412. https://doi.org/10.1111/j.1600-0897.2011.01087.x

Hongtao, C., Youling, F., Fang, H., Huihua, P., Jiying, Z., & Jun, Z. (2018). Curcumin alleviates ischemia reperfusion-induced late kidney fibrosis through the APPL1/Akt signaling pathway. *Journal of Cellular Physiology*, *233*(11), 8588–8596. https://doi.org/10.1002/jcp.26536

Kalyoncu, Ş., Yılmaz, B., Demir, M., Tuncer, M., Bozdağ, Z., İnce, O., Bozdayı, M. A., Ulusal, H., & Taysi, S. (2020). Melatonin attenuates ovarian ischemia reperfusion injury in rats by decreasing oxidative stress index and peroxynitrite. *Turkish Journal of Medical Sciences*, *50*(6), 1513–1522. https://doi.org/10.3906/sag-2004-135

Karaçor, T., Dogan, Z., Elibol, E., Bulbul, M., & Nacar, M. C. (2020). Effects of iloprost on experimental ischemia and reperfusion injury in rat ovary. *Biotechnic & Histochemistry*, *95*(5), 373–380. https://doi.org/10.1080/10520295.2019.1703219

Kirmizi, D. A., Baser, E., Okan, A., Kara, M., Yalvac, E. S., & Doganyigit, Z. (2021). 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

Koc, K., Erol, H. S., 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*, 631–637. https://doi.org/10.1016/j.biopha.2018.12.113

Kotha, R. R., & Luthria, D. L. (2019). Curcumin: Biological, Pharmaceutical, Nutraceutical, and Analytical Aspects. *Molecules*, *24*(16), 1–27. https://doi.org/10.3390/molecules24162930

Kunnumakkara, A. B., Bordoloi, D., Padmavathi, G., Monisha, J., Roy, N. K., Prasad, S., & Aggarwal, B. B. (2017). Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *British Journal of Pharmacology*, *174*(11), 1325–1348. https://doi.org/10.1111/bph.13621

Liu, Z., Ran, Y., Huang, S., Wen, S., Zhang, W., Liu, X., Ji, Z., Geng, X., Ji, X., Du, H, Leak, R. K., & Hu, X. (2017). Curcumin Protects Against Ischemic Stroke by Titrating Microglia/Macrophage Polarization. *Frontiers in Aging Neuroscience*, *9*, 233. https://doi.org/10.3389/fnagi.2017.00233

Ma, Z., Xin, Z., Di, W., Yan, X., Li, X., Reiter, R. J., & Yang, Y. (2017). Melatonin and mitochondrial function during ischemia/reperfusion injury. *Cellular and Molecular Life Sciences*, *74*(21), 3989–3998. https://doi.org/10.1007/s00018-017-2618-6

Moghaddam Aghili, S. N., Oskouie, M. N., Butler, A. E., Petit, P. X., & Barreto, G. E. (2018). Hormetic effects of curcumin: What is the evidence? *Journal of Cellular Physiology*, *234*(7), 10060–10071. https://doi.org/10.1002/jcp.27880

Mokhtari-Zaer, A., Marefati, N., Atkin, S. L., Butler, A. E., & Sahekbar, A. (2018). The protective role of curcumin in myocardial ischemia–reperfusion injury. *Journal of Cellular Physiology*, *234*(1), 214–222. https://doi.org/10.1002/jcp.26848

Noori Hassanvand, M., Soleimani Mehranjani, M., & Shojafar, E. (2019). Melatonin improves the structure and function of autografted mice ovaries through reducing inflammation: A stereological and biochemical analysis. *International Immunopharmacology*, *74*, 105679. https://doi.org/10.1016/j.intimp.2019.105679

Palareti, G., Legnani, C., Cosmi, B., Antonucci, E., Erba, N., Poli, D., Testa, S., & Tosetto, A. (2016). Comparison between different D-Dimer cutoff values to assess the individual risk of recurrent venous thromboembolism: Analysis of results obtained in the DULCIS study. *International Journal of Laboratory Hematology*, *38*(1), 42–49. https://doi.org/10.1111/ijlh.12426

Sak, M. E., Soydinc, H. E., Sak, S., Evsen, M. S., Alabalik, U., Akdemir, F., & Gul, T. (2013). The protective effect of curcumin on ischemia-reperfusion in rat ovary. *International Journal of Surgery*, *11*(9), 967–970. https://doi.org/10.1016/j.ijsu.2013.06.007

Shi, S., Lei, S., Tang, C., Wang, K., & Xia, Z. (2019). Melatonin attenuates acute kidney ischemia/reperfusion injury in diabetic rats by activation of the SIRT1/Nrf2/HO-1 signaling pathway. *Bioscience Reports*, *39*(1), BSR20181614. https://doi.org/10.1042/BSR20181614

Tang, J., & Zhuang, S. (2019). Histone acetylation and DNA methylation in ischemia/reperfusion injury. *Clinical Science*, *133*(4), 597–609. https://doi.org/10.1042/CS20180465

Tokgoz, V. Y., Sipahi, M., Keskin, O., Guvendi, G. F., & Takir, S. (2018). Protective effects of vitamin D on ischemia-reperfusion injury of the ovary in a rat model. *Iranian Journal of Basic Medical Sciences*, *21*(6), 593–599. https://doi.org/10.22038/ijbms.2018.26914.6581

Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., Fougerou, C., del Valle Bessone, C., Fajreldines, H. D., de Barboza, G. E. D., Tolosa de Talamoni, N. G., Allemandi, D. A., Carpentieri, A. R., Quinteros, D. A., Ghareghani, M., Scavo, L., Jand, Y., Farhadi, N., Sadeghi, H., … Perdana. (2017). Melatonin: Pharmacology, Functions and Therapeutic Benefits. *Current Neuropharmacology*, *15*(3), 434–443. https://doi.org/10.2174/1570159X14666161228122

Turkoz, Y., Celik, O., Hascalik, S., Cigremis, Y., Hascalik, M., Mizrak, B., & Yologlu, S. (2004). Melatonin reduces torsion-detorsion injury in rat ovary: Biochemical and histopathologic evaluation. *Journal of Pineal Research*, *37*(2), 137–141. https://doi.org/10.1111/j.1600-079X.2004.00146.x

Zang, X., Zhou, J., Zhang, X., Han, Y., & Chen, X. (2020). Ischemia Reperfusion Injury: Opportunities for Nanoparticles. *ACS Biomaterials Science & Engineering*, *6*(12), 6528–6539. https://doi.org/10.1021/acsbiomaterials.0c01197

Zhang, J., Tang, L., Li, G. Sen, & Wang, J. (2018). The anti-inflammatory effects of curcumin on renal ischemia-reperfusion injury in rats. *Renal Failure*, *40*(1), 681–687. https://doi.org/10.1080/0886022X.2018.1544565