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Protective effect of resveratrol on the kidney in rats under immunosuppression with tacrolimus

Hüseyin Özden [1], Muhammed Gömeç [2], Yaşar Şahin [3], Gökhan Karaca [4], Huri Bulut [5], Asuman Kilitçi [6]

[1] Ahi Evran University, Faculty of Medicine Department of General Surgery, Kırşehir, Turkey
[2] Cumhuriyet University, Faculty of Medicine Department of General Surgery, Sivas, Turkey
[3] Kırıkkale University, Faculty of Veterinary Medicine Department of Pharmacology And Toxicology, Kırıkkale, Turkey
[4] Yüksek İhtisas Hospital, Department of General Surgery, Kırıkkale, Turkey
[5] Bezmialem Vakif University Faculty of Medicine Department of Biochemistry, Istanbul, Turkey
[6] Ahi Evran University Faculty of Medicine Department of Pathology, Kırşehir, Turkey

ORCID ID of the author(s)

HÖ: 0000-0002-2786-3805
MG: 0000-0002-9127-3201
YŞ: 0000-0001-5936-4210
GK: 0000-0002-5107-5999
HB: 0000-0003-2706-9625
AK: 0000-0002-5489-2222

Corresponding Author
Hüseyin Özden
Ahi Evran University, Faculty of Medicine, Department of General Surgery, Kirsehir, Turkey
E-mail: huseyinozden@ahievran.edu.tr

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Abstract

Background/Aim: Tacrolimus is a commonly used agent for immunosuppression in organ transplantation with known nephrotoxic effects. We think that kidney-sparing therapy should be added to current treatment protocols. We aimed to observe the protective effect of resveratrol (RSV) against the adverse effects of tacrolimus (TAC) on rat kidneys.

Methods: Twenty-four rats were randomly divided into the following three groups of eight rats each: Control, TAC, and RSV. The control group was not treated in any way. Tacrolimus was administered to the TAC group. In addition to tacrolimus use, resveratrol was administered to the RSV group. At the end of the experiment, one kidney was used for histopathological examination and the other, for biochemical examination. Results were analyzed statistically.

Results: IL-1β, IL-6, TNF-Alpha levels in the control group were significantly lower than those in TAC and RSV groups (IL-1; $P<0.001$, $P<0.001$, IL-6; $P<0.001$ $P=0.002$, TNF-Alpha; $P<0.001$, $P<0.001$), and those in the RSV group were significantly lower than those in the TAC group (IL-1: $P=0.032$, IL-6: $P=0.001$ TNF-Alpha: $P=0.026$). TAS levels of the control group were significantly higher than those of the TAC group ($P=0.030$). TOS and OSI levels of the control group were significantly lower than those of the TAC and RSV groups (TOS: $P=0.002$, $P=0.012$, OSI: $P=0.001$, $P=0.004$). In histopathological evaluation, the TAC group showed the highest levels of fibrosis. The differences between the control and TAC groups and the TAC and RSV groups were statistically significant ($P=0.003$, $P=0.003$).

Conclusion: Resveratrol has a protective effect against the adverse effects of tacrolimus on the kidney, which may be because of its anti-inflammatory and antioxidant properties.

Keywords: Anti-inflammatory, Antioxidant, Immunosuppression, Resveratrol, Tacrolimus

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Introduction

It has been more than 60 years since the first human kidney transplantation. Over the years, it had a multidisciplinary nature [1]. Organ transplantation is one of the most complex and challenging areas of modern medicine today. It is a medical procedure in which an organ is removed from the donor and placed in the body of a recipient. After the organ is placed in the recipient's body, a complex process begins. The organ will survive healthily if the body accepts the organ. However, transplant rejection occurs when the immune system suppresses and rejects it. In this case, the transplanted organ may need to be removed. This bears high morbidity and mortality for the recipient.

For the organ transplantation to result in a healthy way, the immune system of the recipient body is suppressed. Suppression of the immune system prevents an excessive immune reaction and gives the new organ a chance to survive.

Transplant rejection is believed to develop through the activation of alloimmune responses mediated by effector CD4 + T cells [2]. Available studies show that the Th1 and Th17 cells, subsets of T cells, exhibit the highest activation [3].

Experiences have taught us that we need to use the right immunosuppressive drugs if we want to perform successful organ transplantation. Today, one of the most effective drugs used for this purpose is tacrolimus (TAC or FK506), which is a calcineurin inhibitor. It is a macrolide lactone isolated from streptomyces tsukubaensis [4].

TAC was first approved in the United States for use in liver transplantation. It was used in other organ transplants with the recognition of its effectiveness over time [4]. While the success of TAC in immunosuppression cannot be ignored, the need for individual dose adjustment due to the narrow therapeutic range has brought difficulties. Some studies report the nephrotoxic effect of TAC, however, it is a controversial topic in the literature [5]. Also, various studies that TAC was ineffective on Th17 [6].

In one study, combining TAC with a medical therapy that could have a modulatory effect on Th17 was suggested, and successful results were obtained with RSV [7]. Another study reported positive results about the effect of resveratrol in diabetic nephropathy. Resveratrol is a natural polyphenol compound [8].

In the present study, we aimed to examine the protective effect of resveratrol therapy on the kidney by combining it with tacrolimus.

Materials and methods

Written approval was obtained from the Local Ethics Committee of the Kırıkkale University on September 2, 2020 with the meeting number 2020/04, decision number 21.

A total of 24 Wistar albino male rats weighing 220-260 g were randomly divided into the following three groups with eight rats in each group: Control, TAC, and RSV. All animals were kept in collective cages at a controlled temperature (24°C) in daylight and dark conditions and had ad libitum access to water and food. The control group was not subject to any procedures. The TAC group was administered 0.5mg/kg tacrolimus (Prograf; Astellas Pharma Inc., Tokyo, Japan) perorally through oral gavage from day 7 to day 28. In addition to the procedure in the TAC group, 10 mg/kg of resveratrol (Interpharma Praha, Tokyo, Japan) was administered from day 1 to day 28 through oral gavage in the RSV group. On day 28, all animals were anesthetized with 8 mg/kg of ketamine intramuscularly and operated. Blood samples were obtained before the procedure. Laparotomy was performed with a 2 cm midline incision. Both kidneys were removed and submerged in isotonic NaCl solution. One kidney was used for histopathological examination and the other kidney, for biochemical examination. At the end of the experiment, all animals were sacrificed by decapitation (cervical dislocation).

Tissue homogenization and total protein assay

For this study, kidney samples were collected from twenty-four rats. Tissues samples were stored at -80°C until the experiments, and homogenized with PBS (Phosphate Buffer Saline, pH: 7.4) with a homogenizer (Fast prep-24, MP Biomedical, USA). The total amount of protein was measured by the Bradford method (Thermo scientific Pierce BCA) in all tissue samples with the spectrophotometer (Thermo Scientific Multiskan FC, 2011-06, USA).

Commercial kits

Rat IL-1β (Interleukin 1 Beta) ELISA Kit, Elabscience, Catalog No: E-EL-R0012, USA)

Rat IL-6 (Interleukin 6) ELISA Kit, Elabscience, Catalog No: E-EL-R0015, USA)

Rat TNF-Alpha ELISA Kit, Elabscience, Catalog No: E-EL-R0019, USA)

TAS (Total antioxidant status) Kit, Rel Assay Diagnostics, Turkey)

TOS (Total oxidant status) Kit, Rel Assay Diagnostics, Turkey)

ELISA analysis

Samples were thawed and Tumor Necrosis Factor α (TNF- α) ELISA kit (Elabscience, Catalog No: E-EL-R0019), Interleukin 1-β (IL-1 β) ELISA kit (Elabscience, Catalog No: E-EL-R0012) and Interleukin- 6 (IL-6) ELISA Kit (Elabscience, Catalog No: E-EL-R0015) were used for the quantitative measurement of TNF-α, IL-1 β and IL-6 in tissue homogenates. Samples and standards were added to appropriate wells which were pre-coated with Anti-Human monoclonal antibody before incubation. Biotin was added to all wells and combined with Streptavidin-HRP to form immune complex. Then, they were incubated and washed to remove the uncombined enzyme. Chromogen Solution A, B were added for the color of the liquid to change to blue, which later changed to yellow because of the acid. Optical density was read on a standard automated plate reader at 450 nm (Thermo Scientific Microplate Reader). The detection range of kits were between 78.13-5000 pg/mL for TNF-α, 31.25-2000 pg/ml for IL-1 β and 12.5-800 pg/mL for IL-6.

TAS and TOS measurement

Total Antioxidant Status (TAS) levels were measured spectrophotometrically using commercial kits (Rel Assay, Turkey). Assaying was performed at Thermo Scientific Microplate Reader, USA. Antioxidants in the sample reduce the dark blue-green colored ABTS radical to colorless ABTS form. The change of absorbance at 660 nm is related with the total

antioxidant level of the sample. Total antioxidant activities were expressed in mmol Trolox Equiv/L.

Total Oxidant Status (TOS) levels were measured by the spectrophotometric method using commercial kits (Rel Assay, Turkey). Assaying was performed at Thermo Scientific Microplate Reader, USA. Oxidants present in the sample oxidize the ferrous ion chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a color complex with chromogen in an acidic medium. The color intensity is related to the total amount of oxidant molecules present in the sample. Results are expressed in terms of μm H202 Equiv/L.

Macroscopic assessment

The excised kidney specimens were fixed in 10% neutral buffered formalin. All specimens were excised parallel to the longitudinal body axis and followed up for 1 night for histopathological examination.

Histopathologic assessment

After the tissues were embedded in paraffin blocks, four-micrometer sections were obtained and stained with hematoxylin and eosin (H&E) after deparaffinization and rehydration. Masson's trichrome staining and PAS (Periodic Acid Schiff's) histochemical staining were performed to better assess renal fibrosis, protein material accumulation, and glomerulosclerosis. Histopathological specimens were assessed using a light microscope by an experienced pathologist who was unaware of the experimental groups (Olympus CX41 microscope) (Olympus, Tokyo, Japan). A minimum of ten fields were examined for each slide and evaluated in terms of the severity of the changes.

Histopathologic scoring was made according to the highest field. Categories were determined by semi-quantitative analysis (0: None, 1: Minimal, 2: Mild, 3: Moderate, 4: Severe) and parameters were scored accordingly. The following parameters were used to decide the degree of tubular damage, glomerular damage, and interstitial damage: Tubular dilation (TD), proteinaceous material accumulation (PMA) in tubules, tubular epithelial cell change (ECC), glomerular damage (fibrosis, atrophy, thrombosis), interstitial fibrosis (IF), interstitial congestion/hemorrhage (IC/H), interstitial mononuclear inflammatory cell infiltration (ICI).

Statistical analysis

Statistical Analysis Statistical Package for Social Sciences version 21.0 software for Windows (IBM SPSS Statistics for Windows, Version 21.0 Armonk, NY: IBM Corp., USA) was used for the statistical analysis of the study. Assumption of normality was tested by Shapiro-Wilk tests. The normally distributed data were compared with one-way ANOVA, followed by a Tukey correction test (post hoc). Non-normally distributed data were analyzed with the Kruskal-Wallis test, followed by Games-Havell correction test. *P*-value <0.05 was considered significant.

Results

IL-1β, IL-6, TNF-Alpha levels in the control group were significantly lower than those in TAC and RSV groups (IL-1: $P<0.001$, $P<0.001$, IL-6: $P<0.001$, $P=0.002$, TNF-Alpha: $P<0.001$, $P<0.001$), and those in the RSV group were significantly lower than those in the TAC group (IL-1: $P=0.032$, IL-6: $P=0.001$ TNF-Alpha: $P=0.026$) (Table 1).

TAS levels of the control group were significantly higher than those of the TAC group ($P=0.030$, while TAS values were similar between the control-RSV group and TAC-RSV group ($P=0.063$, $P=0.359$). TOS and OSI levels of the control group were significantly lower than those of the TAC and RSV groups (TOS: $P=0.002$, $P=0.012$, OSI: $P=0.001$, $P=0.004$), while those of the TAC and RSV groups were similar ($P=0.757$). OSI was significantly lower in the control group compared to the TAC and RSV groups ($P=0.001$, $P=0.004$), but similar between the TAC and RSV groups ($P=0.884$) (Table 2).

Table 1: The results of IL-1, IL6 and TNF alfa levels

Group	IL1 Mean (SD)	IL6 Mean (SD)	TNF Alfa Mean (SD)
1	35.8 (9.6)	50.3 (9.4)	117.9 (29.2)
2	447.9 (128.6)	285.7 (59.0)	2009.7 (565.5)
3	291.0 (78.3)	142.9 (48.4)	1258.6 (428.9)
		P-value	
Group 1 vs. Group 2	<0.001	<0.001	<0.001
Group 1 vs. Group 3	<0.001	0.002	<0.001
Group 2 vs. Group 3	0.032	0.001	0.026

Table 2: The results of TAS, TOS and OSI levels

Group	TAS Mean (SD)	TOS Mean (SD)	OSI Mean (SD)
1	1.0 (0.1)	8.2 (0.4)	7.8 (0.5)
2	0.8 (0.1)	9.3 (0.4)	10.3 (1.5)
3	0.9 (0.1)	9.1 (0.6)	10.8 (1.4)
		P-value	
Group 1 vs. Group 2	0.030	0.002	0.001
Group 1 vs. Group 3	0.065	0.012	0.004
Group 2 vs. Group 3	0.359	0.757	0.884

Histopathological findings are presented in Table 3. Dilation, proteinaceous material accumulation (PMA), epithelial cell abnormalities (ECA) were analyzed to assess tubular damage, fibrosis/atrophy/thrombosis (FAT) was used to assess the degree of glomerular damage, and lymphoplasmacytic cellular inflammatory infiltration (CII), vascular congestion/hemorrhage (VCH), and fibrosis were used to determine the degree of interstitial damage. There was no difference between the groups, except interstitial fibrosis. The TAC group showed the highest levels in interstitial fibrosis. There was a significant difference between the control and TAC groups ($P=0.003$) and the TAC and RSV groups ($P=0.003$) (Figure 1, 2). There was no difference between the control and RSV groups ($P=1.00$).

Table 3: The results of the histopathological analysis

Group	TD Mean (SD)	pmA Mean (SD)	ECC Mean (SD)	ICI Mean (SD)	IC/H Mean (SD)	IF Mean (SD)
1	0.12	0.25	0.75	0.50	1.87	0.62
2	0.12	0.00	0.62	0.87	1.87	1.37
3	0.37	0.12	0.25	0.87	1.00	0.62
				P-value		
Group 1 vs. Group 2	1.000	0.442	0.798	0.234	0.959	0.030
Group 1 vs. Group 3	0.442	0.721	0.195	0.234	0.050	1.000
Group 2 vs. Group 3	0.442	0.721	0.234	1.000	0.083	0.030

Figure 1: Interstitial fibrosis in a large area in the kidney section of a rat in the tacrolimus group (Score: 2) (H&E, x100).

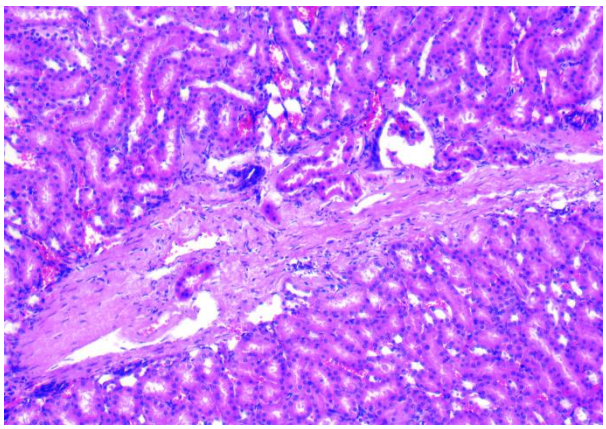
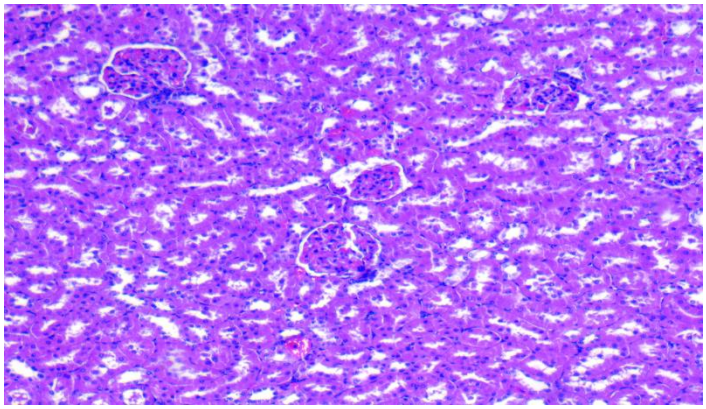


Figure 2: Normal histological appearance without fibrosis in the kidney section of a rat belonging to the resveratrol group (Score: 0) (H&E, x100).



Discussion

Organ transplantation involves obtaining an organ from a living human or cadaver and transferring it to a recipient's body. The donor and recipient may be at the same location, or organs may be transported from a donor's site to another location. The operation is quite risky, complex, and exhausting. Financial loss and disappointment are common. Modern medicine continues to develop to get better results from such an extensive process.

Organ transplantation is a multifaceted medical procedure. Various conditions must be met, and many obstacles must be overcome to execute successful organ transplantation. A multidisciplinary hospital with private and intensive care services is required, enabling the participation of all relevant branch physicians in the treatment of the patient when necessary. An immunological balance is vital for the patient. The higher the tissue compatibility, the more successful results can be obtained. For this reason, patients wait for a suitable donor for years. In some countries, it is a religiously controversial issue, on which restrictions have been imposed. It is regulated through legal processes by ethical committee decisions and regulations. Even if one overcomes these obstacles and manages to undergo organ transplantation, the immune system may reject the transplanted tissue. Therefore, any kind of study and research on the performance of organ transplantation in the safest way bears great value. In particular, suppression of immunity and protection of tissue are priorities.

Molecules on the transplanted organ, perceived as foreign by the host, are called allo-antigens. Antibodies and T cells formed in the host are alloreactive. Major Histocompatibility Complex (MHC) is responsible for the introduction of allo-antigens to host T cells. The compatibility of MHC molecules between the donor and recipient is essential for successful transplantation. Each individual's CD4 and CD8 T cells are selected to recognize the peptides presented by their MHC molecules during the transformation into mature cells in the thymus. CD4 and CD8 are transformed separately through thymic antigen-presenting cells. CD4 T-cells are called T helper (Th) cell and CD8 T-cells are called cytotoxic T lymphocytes (CTL). This way, T cells recognize their own specific MHC.

Transplant rejection can be classified as hyperacute, acute, or chronic. Hyperacute rejection is usually mediated by memory-infused antibodies during pregnancy or blood transfusions. Rejection may occur within minutes. Acute rejection is mainly evoked by T cells, and antibodies are less effective. It manifests with vascular and parenchymal damage. It is either caused by a direct cytotoxic T-cell attack on graft cells or damage through secreted inflammatory cytokines. The CD4+T cell (Th) play the most active role in this process. Immunosuppressive therapies used today aim to prevent or repress T cell-mediated rejection [9]. Chronic rejection is associated with progressive proliferation.

Popular immunosuppressive drugs developed to prevent acute rejection include tacrolimus, cyclosporine, and mycophenolate mofetil. They can be used alone or in combination to prevent graft rejection [10]. TAC is a highly effective immunosuppressive but also safer and better tolerated than others. However, in their study, Millis et al. [11] found that TAC has side effects such as neurotoxicity, nephrotoxicity, hepatotoxicity, glucose intolerance, gastrointestinal toxicity, post-transplant lymphoproliferative disorder, and infections. Another study emphasized that the use of TAC triggered the production of reactive oxygen species (ROS) and created oxidative stress [12].

While tacrolimus use has become popular in organ transplantation, its nephrotoxic effects were figured out over time, and medical treatment procedures are needed for prevention. Metabolites of tacrolimus are thought to be responsible for renal pathologies. It is difficult to determine accurate dosage since the minimum effective dose within the therapeutic range cannot be measured at the cell or tissue level. In our experimental study, we applied an equal dose of TAC in rats with equal weight and the same gene lineage. The dose we administered was determined on the basis of previous studies [13]. We obtained significant differences in laboratory tests and microscopic examination between the TAC group and the control group. Inflammation markers such as IL-1, IL-6, and TNF-alpha were significantly higher in the TAC group. Among the oxidative stress markers of TAS, TOS, and OSI, TAS was significantly lower, while TOS and OSI were significantly higher. In addition, the microscopic examination revealed that the TAC group exhibited the highest levels of interstitial fibrosis, which showed tacrolimus nephrotoxicity.

The use of tacrolimus may have nephrotoxic effects. Probable causes should be first ruled out in case of renal impairment. If no cause is found, a TAC dose adjustment may be required, or the immunosuppressive procedure may be changed. Hydration provides an effective treatment for kidney protection. Many drug modalities may be preferred in a normal transplant

follow-up. In the present study, we aimed to investigate the effect of resveratrol, which had antioxidant and anti-inflammatory properties.

Resveratrol (trans-3,4 ', 5- trihydroxystilbene) is a type of polyphenol called phytoalexin, and it is a plant-derived compound as a defense mechanism against diseases. It is found in peanuts and red wine. It can now be easily supplied and used as a food supplement [14].

A study reported that a drug called kojokon in China and Japan is the same drug that we call resveratrol [15]. We believe that this historical drug should be included in studies. In the literature, many studies have been conducted with resveratrol with successful outcomes. Available studies have mostly investigated its anti-inflammatory and antioxidant properties against malignancy, cardiovascular diseases, ischemia, toxicity, inflammation, and tissue injury [16-21].

Dolezelova et al. [22] performed a preliminary study and reported the minimum effective dose of resveratrol in rats as 10 mg/kg, which we administered. In the present study, no rat died due to resveratrol use. Inflammatory markers such as IL-1, IL-6, and TNF alpha were significantly lower in the RSV group compared to the TAC group. Oxidative markers such as TAL, TOS, and OSI revealed no significant difference although TAS was higher, and TOS was lower. In addition, histopathological examination revealed that interstitial fibrosis in the RSV group was lower than that in the TAC group. These results showed that the nephrotoxic effect of TAC regressed with the use of resveratrol.

Limitations

The small number of rats in our study constitutes a limit. Further studies with more sample size are needed.

Conclusion

We determined the nephrotoxic effects of TAC metabolites in the experimental animal model under immunosuppression with tacrolimus. Nephrotoxicity decreased with the use of RSV combined with TAC. We think that these positive effects are mostly due to the anti-inflammatory and antioxidant properties of RSV.

References

1. Daniel A, Rainer O, Uwe H, Ondrej V, Licia P, Christophe M, et al. Recent advances in kidney transplantation: a viewpoint from the Descartes advisory board. Nephrol Dial Transplant. 2018 Oct 1;33(10):1699-707. doi: 10.1093/ndt/gfx365.
2. Calvo-Turrubiartes M, Romano-Moreno S, García-Hernández M, Chevaile-Ramos JA, Layseca-Espinosa E, González-Amaro R, et al. Quantitative analysis of regulatory T cells in kidney graft recipients: a relationship with calcineurin inhibitor level. Transpl Immunol. 2009 May;21(1):43-9. doi: 10.1016/j.trim.2009.02.002.
3. Loong CC, Lin CY, Lui WY. Expression of interleukin-17 as a predictive parameter in acute renal allograft rejection. Transplant Proc. 2000 Nov;32(7):1773. doi: 10.1016/s0041-1345(00)01382-8.
4. David IP. Sourcing a chemical succession for cyclosporin from parasites and human pathogens. Drug Discov Today. 2005 May 15;10(10):688-91. doi: 10.1016/S1359-6446(05)03395-7.
5. Meng Y, Mouze L, Wei Z, Yingzi M. Pharmacokinetics, pharmacodynamics and pharmacogenetics of tacrolimus in kidney transplantation. Curr Drug Metab. 2018;19(6):513-522. doi: 10.2174/1389200219666180129151948.
6. Byung HC, Kyoung WK, Bo MK, Shang GP, Sun WL, Bum SC, et al. Dysregulation of Th17 cells during the early post-transplant period in patients under calcineurin inhibitor based immunosuppression. PLoS One. 2012;7(7):e42011. doi: 10.1371/journal.pone.0042011.
7. Anne LW, Joseph AB, Ole V. mTOR: more targets of resveratrol?. Expert Rev Mol Med. 2013 Sep 23;15:e10. doi: 10.1017/erm.2013.11.
8. Tomasz S, Katarzyna S. Resveratrol and diabetes: from animal to human studies. Biochim Biophys Acta. 2015 Jun;1852(6):1145-54. doi: 10.1016/j.bbadis.2014.10.013.
9. Lynn DC, R Neal S, Robert BC. Kidney transplantation: mechanisms of rejection and acceptance. Annu Rev Pathol. 2008;3:189-220. doi: 10.1146/annurev. pathmechdis.3.121806.151508.
10. Manuel RP, Marta GM, Douglas T, Brian RD, Emmanuel T, Kurinchi SG. Maintenance immunosuppression for adults undergoing liver transplantation: a network meta-analysis. Cochrane Database Syst Rev. 2017 Mar 31;3(3):CD011639. doi: 10.1002/14651858.CD011639.pub2.
11. Millis JM, Woodle ES, Piper JB, Bruce DS, Newell KA, Seaman DS, et al. Tacrolimus for primary treatment of steroid-resistant hepatic allograft rejection. Transplantation. 1996 May 15;61(9):1365-9. doi: 10.1097/00007890-199605150-00014.
12. Shang GP, Sun WL, Kyoung CD, Long J, Seong BH, Yu FZ. Combined treatment of tacrolimus and everolimus increases oxidative stress by pharmacological interactions. Transplantation. 2014 Jul 15;98(1):22-8. doi: 10.1097/TP.0000000000000146.
13. Akbulut S, Dogan Z, Baskiran A, Elbe H, Turkoz Y. Effect of a honey and arginine-glutamine-hydroxymethylbutyrate mixture on the healing of colon anastomosis in rats immunosuppressed with tacrolimus. Biotech Histochem. 2019 Oct;94(7):514-21. doi: 10.1080/10520295.2019.1601257
14. Belgaied J-E. Resveratrol content of Tunisian wines. Journal of Wine Research. 2001; 12:47-52
15. Savouret JF, Quesne M. Resveratrol and cancer: a review. Biomed Pharmacother. 2002 Mar;56(2):84-7. doi: 10.1016/s0753-3322(01)00158-5.
16. Amiot MJ, Riva C, Vinet A. Effects of dietary polyphenols on metabolic syndrome features in humans: a systematic review. Obes Rev. 2016 Jul;17(7):573-86. doi: 10.1111/obr.12409.
17. Rodica PB, Andrew Y. Resveratrol and malignancies. Curr Pharmacol Rep. 2015; Aug;1(4):266-71. doi: 10.1007/s40495-015-0030-1
18. Chibuike CU, Vanu RR, Rotimi EA, Peter JHJ. Potential of resveratrol in anticancer and anti-inflammatory therapy. Nutr Rev. 2008 Aug;66(8):445-54. doi: 10.1111/j.1753-4887.2008.00076.x.
19. Sara H, Karen LT, Steen BP, Hendrik V, Jacob G, Henning G. Effects of resveratrol in experimental and clinical non-alcoholic fatty liver disease. World J Hepatol. 2014 Apr 27;6(4):188-98. doi: 10.4254/wjh.v6.i4.188.
20. Alessandra F, Mariangela DG, Vincenzo Z. Antioxidant dietary approach in treatment of fatty liver: new insights and updates. World J Gastroenterol. 2017 Jun 21;23(23):4146-57. doi: 10.3748/wjg.v23.i23.4146.
21. Zakaria MMH, Hajipour B, Estakhri R, Saleh BM. Anti-oxidative effect of resveratrol on aluminum induced toxicity in rat cerebral tissue. Bratisl Lek Listy. 2017;118(5):269-72. doi: 10.4149/BLL_2017_053.
22. Eva D, Alena P, Jolana C, Alejandro C, Lucie H, Milos H, et al. Resveratrol modifies biliary secretion of cholephilic compounds in sham-operated and cholestatic rats. World J Gastroenterol. 2017 Nov 21;23(43):7678-92. doi: 10.3748/wjg.v23.i43.7678.

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