GÜSBD 2022; 11 (1): 112-117 | **Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi** | **Araştırma Makalesi**
GUJHS 2022; 11 (1): 112-117 | Gümüşhane University Journal of Health Sciences | Original Article

Ether Extract of Olive (*Olea europaea* L.) Leaf: Potential Effect on Streptozotocin-Induced Oxidative Stress in Rats

Zeytin (*Olea europaea* L.) Yaprağının Eter Ekstresi: Sıçanlarda Streptozotosin Kaynaklı Oksidatif Stres Üzerine Potansiyel Etkisi

Ahmet Gokhan AGGUL[1], Mine GULABOGLU[2]

ABSTRACT

The study's aim is to investigate the protective effects of olive (*Olea europaea* L.) leaf ether extract, due to its rich phenolic content and beneficial health effects, on streptozotocin (STZ)-induced oxidative stress in rats.

Diabetes in rats was intraperitoneally induced by STZ (40 mg/kg). Following the induction, significant increases in lipid peroxidation, blood glucose, and the levels of the hepatic enzymes (AST, ALP, and ALT) were observed. Moreover, there were significant decreases in GST, GPx, and SOD activities of the diabetic rats. The animals were treated with the ether extracts at two different doses by oral gavage for 14 days. At the end of the treatment, a decrease in lipid peroxidation and the hepatic enzyme levels of the diabetic rats and an increase in the antioxidant enzyme activities were observed. However, these values were not close to normal levels of the healthy rats. Interestingly, there were no significant differences between the blood glucose levels in all the groups throughout the treatment.

As a result, our findings have shown that the ether extract of olive leaf partially has a protective role on the STZ-induced oxidative damage in the rats due to inadequate treatment period. The antidiabetic effect of olive leaf might be due to some natural phenolic compounds it contains. In addition, we believe that the leaves could be used as potential therapeutic drugs or dietary supplements in diabetes management.

Keywords: Antioxidant, Ether Extract, Olive Leaf, Oxidative Stress, Streptozotocin

ÖZ

Bu çalışmanın amacı, zengin fenolik içeriği ve sağlığa yararlı etkileri nedeniyle zeytin (*Olea europaea* L.) yaprağının eter ekstresinin sıçanlarda streptozotosin (STZ) kaynaklı oksidatif stres üzerine koruyucu etkilerini incelemektir.

Sıçanlarda diyabet STZ (40 mg/kg) ile intraperitoneal olarak indüklendi. İndüksiyonu takiben lipit peroksidasyonu, kan glukozu ve karaciğer enzimleri (AST, ALP ve ALT) düzeylerinde belirgin artışlar gözlendi. Ayrıca diyabetik sıçanların GST, GPx ve SOD aktivitelerinde önemli azalmalar oldu. Sıçanlar, 14 gün boyunca oral gavaj ile iki farklı dozdaki eter ekstresi ile tedavi edildi. Tedavinin sonunda, diyabetik sıçanların lipit peroksidasyonunda ve karaciğer enzim seviyelerinde azalma ve antioksidan enzim aktivitelerinde artış gözlendi. Ancak bu değerler sağlıklı sıçanların normal seviyelerine yakın değildi. İlginç bir şekilde, tedavi boyunca tüm gruplarda kan glukoz seviyeleri arasında anlamlı bir fark yoktu.

Sonuç olarak bulgularımız, tedavi süresinin yeterli olmaması nedeniyle sıçanlarda STZ kaynaklı oksidatif hasara karşı zeytin yaprağının eter ekstresinin kısmen koruyucu bir role sahip olduğunu göstermektedir. Zeytin yaprağının anti-diyabetik etkisi, içerdiği bazı doğal fenolik bileşiklerin varlığından dolayı olabilir. Ayrıca yaprakların diyabet yönetiminde potansiyel terapötik ilaçlar veya diyet takviyeleri olarak kullanılabileceği kanaatindeyiz.

Anahtar Kelimeler: Antioksidan, Eter Ekstresi, Zeytin Yaprağı, Oksidatif Stres, Streptozotosin

Necessary permission from the Dean's Office of the Faculty of regarding the research and Ataturk University Scientific Research and Publication Ethics Committee permission were obtained (Permit number: B.30.2.ATA.0.22.02.00-208).

[1] Assistant Professor Dr., Ahmet Gokhan AGGUL, Agri Ibrahim Cecen University, Faculty of Pharmacy, Department of Biochemistry, agaggul@agri.edu.tr, ORCID: 0000-0003-0377-0388

[2] Professor Dr., Mine GULABOGLU, Ataturk University, Faculty of Pharmacy, Department of Biochemistry, minegulaboglu@atauni.edu.tr, ORCID: 0000-0002-3248-1502

İletişim / Corresponding Author: Ahmet Gokhan AGGUL
e-posta/e-mail: agaggul@agri.edu.tr

Geliş Tarihi / Received: 29.09.2021
Kabul Tarihi/Accepted: 09.03.2022

INTRODUCTION

Diabetes Mellitus is a metabolic disease, which is manifested by high blood glucose concentration because of insulin deficiency or ineffectiveness.[1, 2] DM is one of the public health emergencies of recent years and has the most aggressive course among chronic diseases. This disease is still one of the common causes of global death due to the lack of a cure for diabetes.[3] In diabetes, uncontrolled hyperglycemia can lead to the production of free radicals, which can accumulate in the body. When elimination of overwhelming free radicals is gradually impossible, oxidative stress generates.[4, 5] Consequently, it leads to complications affecting the eyes, kidneys, nerves, and blood vessels in the long term.[6] Many studies have reported its critical role in diabetes.[7, 8] Oxidative stress in diabetes results from the increased lipid peroxidation and the elevated liver enzyme levels,[7, 9] the changed glutathione redox status, and the decrease antioxidant enzyme activities.[10, 11] New developments in the diabetes treatment may only help to manage symptoms, and there is currently no cure. That has raised the demand for natural products by diabetic patients in recent years. In addition, many researchers have focused on the beneficial effects of natural antioxidants against oxidative damage related to diabetes.[12]

Olive (*Olea europaea* L.) leaf has been traditionally used as a herbal medicinal product throughout history, mainly due to its excellent biological activities.[13] Some studies have reported that olive leaves have antioxidant, hypoglycemic, hypolipidemic, antidiabetic, antimicrobial, antihypertensive, and antiatherosclerotic properties.[14-16] These properties are probably due to various bioactive phenolic compounds they contain. Recently, phenolic compounds obtained from plant extract have been popular due to their antioxidant properties. Their antioxidant potential is mainly related to their abilities to improve radical stability.[17, 18] Olive leaf contains a wide range of bioactive compounds, especially verbascoside, hydroxytyrosol, and oleuropein. Table 1 shows the main phenolic compositions and their amounts in the leaf extract.[18]

Table 1. Some Phenolic Compounds from Olive Leaf Extract

Compound	Dry weight (%)	Compound	Dry weight (%)
Luteolin	0.21	Verbascoside	1.11
Caffeic acid	0.34	Apigenin 7-glycoside	1.37
Diosmetin 7 glycoside	0.54	Luteolin 7-glycoside	1.38
Vannilic acid	0.63	Hydroxytyrosol	1.46
Tyrosol	0.71	Oleuropein	24.54

Among these natural compounds, oleuropein with the highest antioxidant activity is the main bioactive compound of olive leaf.[8, 11, 18] In our previous study, oleuropein have been tested that it has a strong antioxidant activity in the experimental animal model.[19] In our other studies, we have reported that the ethanol and water extracts of olive leaves has antioxidant effects in STZ-induced diabetic rats.[20, 21] In some studies, olive leaf extracts were prepared with different solvents such as ethanol and water, and analyzed to evaluate the antioxidant properties as *in vivo*. They have shown that the extracts have therapeutic effects on diabetes.[7, 22] However, no data that correlates antioxidant properties of the ether extract of olive leaf on STZ-induced oxidative stress in rats have been published.

In this study, we aimed to assess the protective effects of the ether extract of olive leaf against the STZ-induced oxidative stress in the rats.

MATERIAL AND METHOD

Animals

Male rats (Sprague-Dawley) in the experiment were supplied from the Experimental Animal Procurement Center at Ataturk University. All the animals (170-220 g b.wt) were housed in standard cages under standard conditions for the duration of the experiment. The rats were fed *ad libitum.*

Diabetes Induction

Diabetes was induced in the animals, which were fasted for 12-h overnight, by a single injection (i.p.) of STZ (40 mg/kg b.wt.) in its own buffer (0.01 mM citrate buffer, pH = 4.5). The healthy rats received an intraperitoneal injection of citrate buffer only. Following the STZ injection, the animals with the symptoms of polyuria and polydipsia, and 72-h glucose levels in the blood above 300 mg/dL were considered to be diabetic.[23]

Extract Preparation

The procedure for the extract preparation was provided in detail from some studies in literature.[21, 24] Briefly, the leaves were cleaned and dried at 22 ± 2 ºC. The dried leaves were powdered. The powder was then extracted by the Soxhlet apparatus at room temperature using diethyl ether.[24] At the end of 3 days, the extract was filtered through filter paper. After that, the solvent was removed, and the highly concentrated crude extracts were obtained.[7, 21]

Experimental Design

The experiment was carried out after three days of the STZ injection. The experimental animals (n = 25) were divided into five groups. Groups CD and C consisted of diabetic and healthy rats, respectively. Group CE (healthy rats) fed with 0.5 g/kg the extract alone. Groups E1 and E2 (diabetic rats) received 0.25 and 0.5 g/kg extracts, respectively. The rats were daily dosed by oral gavage for 14 days. The volume of all the administrations was kept constant at 2 ml. The group CE received the extract (0.5 g/kg) alone. The groups C and CD only received tap water throughout the experiment. The groups E1 and E2 were administered the extracts at the calculated doses.

Blood Glucose Measurement

The glucose levels in the blood, which were taken from the rat-tail vein, were measured by using a glucometer (Accu-Chek Active) at weekly intervals.

Sample Preparation

The procedures for the preparations of the tissue and blood samples were provided in detail in our previous study.[21]

Biochemical Assays

At the end of 14 days, the rats were euthanized with an administration of thiopental sodium at a high dose of 50 mg/kg. By the way, the liver tissues and intra-cardiac blood samples were taken from the rats. The liver tissues were used to determine lipid peroxidation and antioxidant enzyme activities. GPx, GST, and SOD activities were analyzed according to the previous methods.[25-27] MDA levels were assayed using the previous procedure.[28] The serum samples were used to determine the hepatic enzyme levels. ALP, ALT, and AST were analyzed by the Cobas c501 (Roche Ltd, Switzerland) analyzer. Protein concentrations were determined according to Bradford's method.[29]

Statistical Analyses

For statistical analysis, the SPSS Ver. 20.0 was used. The data were evaluated using One-Way Analysis of Variance (ANOVA) and the Duncan's test. The statistical case was significant if $p<0.05$.

Aspect of Research Ethics

The research permission of the current study (No: B.30.2.ATA.0.22.02.00-208) was obtained from the Ataturk University Scientific Research and Publication Ethics Committee in line with the procedures recommended by Authorized Institutes.

RESULTS AND DISCUSSION

Diabetes is one of the major public health emergencies of recent years. Despite its huge impact, there is still no cure for diabetes, and people with diabetes are unable to cope with this disease that can negatively affect life.[30] Therefore, every year thousands of people have begun to focus on the treatment of diabetes with natural products and the new treatment options to see whether they are safe. Among these natural products, olive plant has been a source of healing for the treatment of diseases throughout history.[13] Its leaf has been recommended for individuals with diabetes due to its health-beneficial properties.[7, 19] The leaves contain bioactive phenolic compounds such as hydroxytyrosol and oleuropein, which have antioxidant properties.[19, 31] The current study was conducted to evaluate the protective role of the ether extract of olive leaf against STZ-induced oxidative stress in the diabetic rats.

STZ, a unique agent used to treat metastatic pancreatic islet cell carcinoma, is generally used as a tool to create experimental diabetes model.[23, 32] In our current study, we have also employed such a model for experimental diabetes in rats. It is well-known that STZ injection increases gradually the blood glucose levels of living organisms. Three days after the STZ exposure, the blood glucose of the rats is remarkably higher than their initial concentrations ($p<0.05$).[19] The results were compatible with that of other studies.[7, 22] At the end of the experiment, the blood glucose levels of the groups were close to each other (data not shown). In contrary to many studies, our findings have shown that the olive leaf has no anti-hyperglycemic activity in short-term period.[7]

Diabetes induction increases some enzyme levels such as ALP in the blood. However, deterioration in only ALP value is not enough to show cellular function loss in the liver tissue during oxidative stress. Thus, in addition to ALP, ALT and AST are often examined.[20, 21] A significant increase in these enzyme levels often reflects liver damage. Eidi et al. (2009) have reported that STZ injection increased AST, ALP, and ALT values in the treated rats when compared to that in healthy rats.[7] In addition, our previous study has shown that STZ injection drastically increases the liver enzyme levels of the animals.[21] In the current study, three days after STZ injection, the diabetic rats significantly had the increased ALP, ALT, and AST levels when compared to that of healthy rats ($p<0.05$). This increase is probably due to the STZ injection. The findings of the current study were compatible with those of other studies.[7, 20, 21] The increases in these enzyme values were decreased in the presence of the extract, but not close to the normal enzyme levels of the healthy rats. The effects of the ether extract on the hepatic enzyme levels are shown in Table 2.

Table 2. The Changes of Hepatic Enzyme Levels among the Groups

Groups	*n*	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Group C	5	244.45 ± 32.50^{a}	55.31 ± 6.54^{a}	154.27 ± 17.58^{a}
Group CE	5	249.03 ± 30.41^{a}	59.07 ± 8.25^{a}	153.94 ± 18.58^{a}
Group CD	5	532.76 ± 53.20^{b}	111.16 ± 13.71^{b}	311.76 ± 30.17^{b}
Group E1	5	530.69 ± 50.28^{b}	108.81 ± 11.82^{b}	309.20 ± 32.32^{b}
Group E2	5	521.49 ± 52.88^{b}	104.11 ± 12.96^{b}	299.32 ± 30.65^{b}

The different letters within the same column are statistically significant. Groups CD and C consisted of diabetic and healthy rats, respectively. Group CE (healthy rats) received 0.5 g/kg the extract alone. Groups E1 and E2 (diabetic rats) received 0.25 and 0.5 g/kg extracts, respectively.

Experimental evidence supports the potential effect of oxidative stress on diabetic patients due to drastically changes in antioxidant enzyme activities.[33, 34] These enzymes (SOD, GPx, and GST) play a vital role in the antioxidant defense mechanism.[35] In our previous study, the STZ injection decreased significantly the above antioxidant enzyme activities in the rats.[21] We obtained similar results as in our previous study. MDA is a standard marker of lipid peroxidation, which formed in cellular injury prosess.[36, 37] It for The findings from our study have indicated that the diabetes induction can cause tissue injury in the rats. Because the STZ injection caused a significant increase in MDA levels of the diabetic rats ($p<0.05$). The presence of the ether extract caused a decrease in MDA levels of the treated diabetic rats, as well as an increase in GST, GPx, and SOD activities, but not close to the normal values of the healthy animals. In current study, the antioxidant effect of the ether extract was dose-dependent. However, the extract might be more effective at higher doses. Table 3 shows the effect of the ether extract of olive leaf on SOD, GSH, and MDA values of the rats at the end of 14 days.

Table 3. The Changes of the Lipid Peroxidation and the Antioxidant Parameters among the Groups

Groups	*n*	SOD (IU/mg protein)	GPx (IU/mg protein)	GST (IU/mg protein)	MDA (nmol/mg protein)
Group C	5	26.31 ± 1.17^{b}	9.16 ± 0.68^{a}	11.35 ± 0.73^{b}	363.77 ± 38.58^{a}
Group CE	5	25.66 ± 1.15^{b}	9.08 ± 0.74^{a}	11.02 ± 0.69^{b}	394.85 ± 35.84^{a}
Group CD	5	22.73 ± 1.08^{a}	6.05 ± 0.45^{b}	8.45 ± 0.64^{a}	480.91 ± 45.33^{b}
Group E1	5	23.01 ± 1.12^{a}	6.08 ± 0.45^{b}	8.48 ± 0.59^{a}	479.08 ± 44.18^{b}
Group E2	5	23.06 ± 0.92^{a}	6.55 ± 0.36^{b}	8.50 ± 1.49^{a}	456.99 ± 38.12^{b}

The different letters within the same column are statistically significant. Groups CD and C consisted of diabetic and healthy rats, respectively. Group CE (healthy rats) received 0.5 g/kg the extract alone. Groups E1 and E2 (diabetic rats) received 0.25 and 0.5 g/kg extracts, respectively.

CONCLUSION AND RECOMMENDATIONS

Solvents used in medicinal plant extraction are commonly water, ethanol, and ether. Although water and ethanol are polar solvents, ether is a nonpolar solvent. Ether is useful in the extraction of compounds such as coumarins, terpenoids, alkaloids, and fatty acids. The ethanol extract of the olive leaf has the richest total phenol and flavonoid contents as compared to the other extracts, which are prepared with solvents at different polarity such as water and ether. In our previous studies, the ethanol and water extracts of olive leaf exhibited antioxidant effects in the diabetic rats. In addition, the findings obtained from this study have shown that the ether extract partially had antioxidant activity in an experimental diabetic rat model. In conclusion, olive leaf extracts have a potent antioxidant effect due to the presence of phenolic compounds they contain. This has indicated that olive leaf is a beneficial source of antioxidant able to reduce the frequency of oxidative stress-related diabetes.

REFERENCES

1. Himsworth, H.P. (1939). "The Mechanism of Diabetes Mellitus". The Lancet, 234 (6047), 171-176.
2. Wood, M.E. (1998). "New Criteria for the Detection, Diagnosis, and Classification of Diabetes Mellitus". Nurse Practitioner Forum, 9 (2), 38-41.
3. International Diabetes Federation. (2015). "IDF Diabetes Atlas". Retrieved: https://diabetesatlas.org/atlas/seventh-edition/. (Accessed: March 26, 2021).
4. Pham-Huy, L.A, He, H. and Pham-Huy, C. (2008). "Free Radicals, Antioxidants in Disease and Health". International Journal of Biomedical Science, 4 (2), 89-96.

5. Alipour, G, Dashti, S. and Hosseinzadeh, H. (2014). "Review of Pharmacological Effects of Myrtus Communis L. and Its Active Constituents". Phytotherapy Research, 28 (8), 1125-36.

6. Wu, H, Cai, L, de Haan, J.B. and Giacconi, R. (2018). "Targeting Oxidative Stress in Diabetic Complications: New Insights". Journal of Diabetes Research, 2018 1909675.

7. Eidi, A, Eidi, M. and Darzi, R. (2009). "Antidiabetic Effect of Olea Europaea L. in Normal and Diabetic Rats". Phytotherapy Research, 23 (3), 347-50.

8. Al-Azzawie, H.F. and Alhamdani, M.S. (2006). "Hypoglycemic and Antioxidant Effect of Oleuropein in Alloxan-Diabetic Rabbits". Life Sciences, 78 (12), 1371-7.

9. Davi, G, Falco, A. and Patrono, C. (2005). "Lipid Peroxidation in Diabetes Mellitus". Antioxidant and Redox Signaling, 7 (1-2), 256-68.

10. Lutchmansingh, F.K, Hsu, J.W, Bennett, F.I. and Badaloo, A.V. (2018). "Glutathione Metabolism in Type 2 Diabetes and Its Relationship with Microvascular Complications and Glycemia". Plos One, 13 (6), e0198626.

11. Jemai, H, El Feki, A. and Sayadi, S. (2009). "Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats". Journal of Agricultural and Food Chemistry, 57 (19), 8798-804.

12. Raskin, I, Ribnicky, D.M, Komarnytsky, S. and Ilic, N. (2002). "Plants and Human Health in the Twenty-First Century". Trends in Biotechnology, 20 (12), 522-31.

13. El, S.N. and Karakaya, S. (2009). "Olive Tree (Olea Europaea) Leaves: Potential Beneficial Effects on Human Health". Nutrition Reviews, 67 (11), 632-8.

14. Soni, M, Burdock, G, Christian, M. and Bitler, C. (2006). "Safety Assessment of Aqueous Olive Pulp Extract as an Antioxidant or Antimicrobial Agent in Foods". Food and Chemical Toxicology, 44 (7), 903-15.

15. Somova, L.I, Shode, F.O, Ramnanan, P. and Nadar, A. (2003). "Antihypertensive, Antiatherosclerotic and Antioxidant Activity of Triterpenoids Isolated from Olea Europaea, Subspecies Africana Leaves". Journal of Ethnopharmacology, 84 (2-3), 299-305.

16. Lee, O.H. and Lee, B.Y. (2010). "Antioxidant and Antimicrobial Activities of Individual and Combined Phenolics in Olea Europaea Leaf Extract". Bioresource Technology, 101 (10), 3751-4.

17. Visioli, F, Bellomo, G. and Galli, C. (1998). "Free Radical-Scavenging Properties of Olive Oil Polyphenols". Biochemical and Biophysical Research Communications, 247 (1), 60-4.

18. Benavente-Garcıa, O, Castillo, J, Lorente, J. and Ortuño, A. (2000). "Antioxidant Activity of Phenolics Extracted from Olea Europaea L. Leaves". Food Chemistry, 68 (4), 457-62.

19. Aggul, A.G, Gulaboglu, M, Cetin, M, Ozakar, E, Ozakar Sevinc, R. and Aydin, T. (2020). "Effects of Emulsion Formulations of Oleuropein Isolated from Ethanol Extract of Olive Leaf in Diabetic Rats". Anais Da Academia Brasileira De Ciencias, 92 (4), e20190810. https://doi.org/10.1590/0001-3765202020190 810

20. Gur, F, Aggul, A.G. and Gulaboglu, M. (2020). "Su Ile Hazırlanan Zeytin Yaprağı Özütünün Ratlarda Streptozotosin Kaynaklı Oksidatif Stres ve Lipit Peroksidasyonu Üzerine Etkileri". Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 10 (4), 2406-15. https://doi.org/10.21597/jist.784425

21. Aggul, A.G, Gur, F. and Gulaboglu, M. (2020). "Streptozotocin-Induced Oxidative Stress in Rats: The Protective Role of Olive Leaf Extract". Bulletin of the Korean Chemical Society, 42 (2), 180-87. https://doi.org/10.1002/bkcs. 12157

22. El-Amin, M, Virk, P, Elobeid, M. and Almarhoon, Z.M. (2013). "Anti-Diabetic Effect of Murraya Koenigii (L) and Olea Europaea (L) Leaf Extracts on Streptozotocin Induced Diabetic Rats". Pakistan Journal of Pharmaceutical Sciences, 26 (2), 359-65.

23. Szkudelski, T. (2001). "The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas". Physiological Research, 50 (6), 537-46.

24. Abubakar, A.R. and Haque, M. (2020). "Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes". Journal of Pharmacy and Bioallied Sciences, 12 (1), 1-10. https://doi.org/10.4103 /jpbs.JPBS_175_19

25. Sun, Y, Oberley, L.W. and Li, Y. (1988). "A Simple Method for Clinical Assay of Superoxide Dismutase". Clinical Chemistry, 34 (3), 497-500.

26. Habig, W.H, Pabst, M.J. and Jakoby, W.B. (1974). "Glutathione S-Transferases: The First Enzymatic Step in Mercapturic Acid Formation". Journal of Biological Chemistry, 249 (22), 7130-39.

27. Paglia, D.E. and Valentine, W.N. (1967). "Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase". Journal of Laboratory and Clinical Medicine, 70 (1), 158-69.

28. Jain, S.K, McVie, R, Duett, J. and Herbst, J.J. (1989). "Erythrocyte Membrane Lipid Peroxidation and Glycosylated Hemoglobin in Diabetes". Diabetes, 38 (12), 1539-43.

29. Bradford, M.M. (1976). "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding". Analytical Biochemistry, 72 248-54.

30. Lobo, V, Patil, A, Phatak, A. and Chandra, N. (2010). "Free Radicals, Antioxidants and Functional Foods: Impact on Human Health". Pharmacognosy Reviews, 4 (8), 118-26.

31. Hamden, K, Allouche, N, Damak, M. and Elfeki, A. (2009). "Hypoglycemic and Antioxidant Effects of Phenolic Extracts and Purified Hydroxytyrosol from Olive Mill Waste in Vitro and in Rats". Chemico-Biological Interactions, 180 (3), 421-32.

32. Panjeshahin, M.R, Azadbakht, M. and Akbari, N. (2016). "Antidiabetic Activity of Different Extracts of Myrtus Communis in Streptozotocin Induced Diabetic Rats". Romanian Journal of Diabetes Nutrition and Metabolic Diseases, 23 (2), 183-90.

33. Ceriello, A. (2000). "Oxidative Stress and Glycemic Regulation". Metabolism, 49 (2 Suppl 1), 27-9.

34. Asmat, U, Abad, K. and Ismail, K. (2016). "Diabetes Mellitus and Oxidative Stress-A Concise Review". Saudi Pharmaceutical Journal, 24 (5), 547-53.

35. Ghosh, S, Bhattacharyya, S, Rashid, K. and Sil, P.C. (2015). "Curcumin Protects Rat Liver from Streptozotocin-Induced Diabetic Pathophysiology by Counteracting Reactive Oxygen Species and Inhibiting the Activation of P53 and Mapks Mediated Stress Response Pathways". Toxicology Reports, 2 365-76.

36. Ito, F, Sono, Y. and Ito, T. (2019). "Measurement and Clinical Significance of Lipid Peroxidation as a Biomarker of Oxidative Stress: Oxidative Stress in Diabetes, Atherosclerosis, and Chronic Inflammation". Antioxidants, 8 (3), 72.

37. Ayala, A, Munoz, M.F. and Arguelles, S. (2014). "Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal". Oxidative Medicine and Cellular Longevity, 2014 360438.