Effects Of Combination Sunkist and Lime Peel Nanoparticle Granules on Oxidative Stress and Heart Histopathology in Diabetes Rats

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Abstrak

Diabetes melitus dan konsekuensinya secara signifikan dipengaruhi oleh stres oksidatif. Jeruk sunkist dan jeruk nipis merupakan sumber nutrisi yang kaya akan nutrisi, menawarkan berbagai manfaat kesehatan karena kandungan antioksidan, vitamin, mineral, dan seratnya yang tinggi. Senyawa utama seperti antosianin, flavanon, dan asam hidroksisinamat berkontribusi pada potensi dampak positifnya untuk mengurangi stres oksidatif. Oleh karena itu, efek dari kombinasi butiran nanopartikel kulit jeruk sunkist dan kulit jeruk nipis diteliti pada tikus diabetes. Dua puluh ekor tikus Wistar jantan dibagi secara acak: Kelompok 1 sebagai kelompok kontrol yang diberi akuades, Kelompok 2 sebagai kelompok DM dengan aloksan, Kelompok 3 dengan DM + NGSLP 100mg/kgBB, Kelompok 4 dengan DM + NGSLP 200mg/kgBB. Tikus dengan diabetes diobati dengan injeksi intraperitoneal dengan aloksan 100 mg/kgBB. Perlakuan NGSLP diberikan selama 14 hari pada setiap kelompok perlakuan. Pada akhir penelitian, darah dan jaringan jantung dikumpulkan dan digunakan untuk menentukan enzim GPx, SOD, MDA, CAT dan evaluasi histologis. Induksi diabetes meningkatkan kadar MDA dan menurun setelah diobati dengan NGSLP. Pada tikus diabetes yang diobati dengan NGSLP menunjukkan adanya peningkatan SOD, GPX dan CAT dalam darah. Temuan histopatologi jaringan jantung menunjukkan efek protektif NGSLP pada tikus dengan diabetes. Penelitian ini mengungkapkan bahwa NGSLP memiliki efek antioksidan dalam darah dan jantung tikus diabetes yang diinduksi aloksan.

Kata kunci: diabetes, histopatologi, NGSLP, stres oksidatif

Abstract

Diabetes mellitus and its consequences are significantly influenced by oxidative stress. Sunkist and lime oranges are a nutritional powerhouse, offering a range of health benefits due to their high content of antioxidants, vitamins, minerals, and fiber. Key compounds like anthocyanins, flavanones, and hydroxycinnamic acid contribute to their potential positive impact to reduce oxidative stress. Therefore, the effect of nanoparticle granules combination of sunkist orange peel and lime peel was investigated on diabetic rats. Twenty male Wistar rats were randomly distributed: Group 1 as control group given distilled water, Group 2 as DM group with alloxan, Group 3 with DM + NGSLP 100mg / kgBW, Group 4 with DM + NGSLP 200mg / kgBW. Rats with diabetes were treated by intraperitoneal injection with 100 mg/kg alloxan. NGSLP treatment was given for 14 days to each treatment group. At the end, the blood and heart tissue were collected and used to determine the GPx enzyme, SOD, MDA, CAT and histological evaluation. Diabetes induction increased the level of MDA and decreased after treated with NGSLP. In diabetic rats treated with NGSLP showed an increase in SOD, GPX and CAT in blood. Histopathological findings of heart tissue showed the protective effect of NGSLP in rats with diabetic. This study revealed that NGSLP has antioxidant effects in the blood and heart of alloxan-induced diabetic rats.

Keywords: diabetes, NGSLP, oxidative stress, histopathology

Introduction

Diabetes mellitus is defined as impaired glucose metabolism associated with impaired protein, carbohydrate, and leptin metabolism as a result of insulin resistance, also known as insulin sensitivity (1). Hyperglycemia aggravates diabetic complications-related ocular symptoms (2). Enzymatic and non-enzymatic scavenger mechanisms protect cells from free radicals. However, cells are exposed to oxidative damage, which results in cell injury, if the scavenger system and free radical production somehow fall out of balance (3).

Hyperglycemia is a major contributor to increased reactive oxygen species (ROS) production, which promotes tissue damage and diabetic complications. Hyperglycemia promotes the formation of advanced glycation end products (AGEs), the polyol and hexosamine pathways, and the poly-ADP ribose polymerase (PARP) and protein kinase C (PKC) pathways. Excessive mitochondrial superoxide production activates or enhances these pathways. Excess mitochondrial superoxide generation activates or amplifies these pathways (4,5). This mechanism, which is a source of oxygen free radicals, is taken into consideration when trying to explain the pathogenesis of many complications in diabetes, especially those related to the vascular endothelium, retina, peripheral nervous system, and cardiovascular system. (6,7).

Traditional medicine (TM) is gaining popularity around the world. In many regions, it's part of the healthcare system. It’s offers potential benefits that have contributed to the discovery of modern medicines (8). Food plants, which have natural antioxidant and protective biochemical properties, are ideal for preventing or protecting against oxidative damage induced by free radical species (3). Orange peel, a recognized biological antioxidant, has been used in herbal medicine to cure or prevent a number of ailments such as cancer, cardiovascular disease, and diabetes (9). Orange peel is also reported to reduce hyperglycemia and hyperlipidemia while improving antioxidant status by reducing oxidative stress levels and boosting antioxidant enzyme synthesis (10,11). Flavonoids, natural compounds found in citrus fruits, have several health benefits including antioxidant, anti-inflammatory, and potential to prevent heart disease. Several studies show the flavonoids from Sunkist and lemon peel can help control blood glucose and fat, and improve heart health (9,12,13). However, the combination of orange peel has never been studied for its effectiveness. The purpose of this study is to evaluate the effect of NGSLP in the modulation of stres and antioksidan in diabetic rats' blood and tissues utilizing biochemical and histological markers.

Methods

Animal studies

This experimental study used a post-test-only control group design and conducted from February to July 2024 at the Laboratory of Universitas Prima Indonesia. Fan scales, ovens, analyzers, glucometer autocheck, glucose strips, mixers, petri dishes, sample bottles, microscopes, incubators, Pyrex beakers and glass jars, filter paper, gloves, masks, cages, and rat feeding and drinking stations were among the tools utilized. The materials utilized were sunkist and lime orange peel extract, distilled water, 96% ethanol, alloxan, simvastatin, phosphate-buffered saline (PBS) solution, 70% alcohol, and rat feeding pellets. Tweenty male Wistar rats (200-250 g) were placed in cage and every cage has five rats. Drinks and food were provided ad libitum. The study adhered to Universitas Prima Indonesia's standard of ethics (035/KEPK/UNPRI/II/2024). All rats fasted the day before the induction, and their blood glucose levels were assessed. Then, alloxan was administered intraperitoneally leading to diabetes in Wistar rats. The rats' levels of blood glucose were re-evaluated the next day after receiving an alloxan injection. Fasting blood sugar levels of more than 125 mg/dL were considered requirements for successful induction. Randomly, rats were distributed among four groups (n = 5):

1. Control (distilled water)
2. Negative control (alloxan at 100 mg/kg BW)
3. Combination of sunkist orange peel nanoparticle granules and lime peel at 100 mg/KgBW + alloxan,
4. Combination of sunkist orange peel nanoparticle granules at 200 mg/KgBW + alloxan.

The treatment with granule nanoparticles from the combination of sunkist orange peel and lime peel were given for 14 days. All rats were anesthetized with sodium pentobarbital at 35 mg/kg, ip, and the blood and heart tissue were collected from starved animals before they were euthanized.

Serum to analyze antioxidant enzymes

GPX, SOD, MDA and CAT levels were determined using blood samples. A spectrophotometer assessed SOD activity at 505 nm. Xanthine and xanthine oxidase are employed to create superoxide radicals, which combine with 2-(4-iodophenyl)-3 (4-nitrophenol)-5-phenyl tetrazolium chloride to give a red formazan dye. The xanthine was 0.05 mmol/L. The SOD activity values were obtained by comparing to the standard curve

and represented as µl/g hemoglobin (Hb) in blood (14,15). The percentage of inhibition was computed using the related calculation. A commercial kit was used to assess glutathione peroxidase (GPX) activity. GPX catalyzes cumene hydroperoxide's oxidation of glutathione (at 4 mmol/L). In the presence of glutathione reductase (at a concentration of ≥ 0.5 units/L) and NADPH 0.28 mmol/L, oxidized glutathione is quickly transformed to the reduced form, while NADPH is oxidized to NADP+. A spectrophotometer was used to quantify the change in absorbance at 340 nm (37 °C), and the GPx concentration was estimated using the following method (14,15).

Histophatology

After the rats were killed, a necropsy was done to extract the heart organ, which was then put in a labeled container and immersed in a solution (Neutralized Buffered Formaldehyde) NBF 10% to prepare histological sections. Following the insertion of the heart into the 10% NBF solution, many preparations were made, including tissue cutting, tissue processing, tissue block cutting, mounting, and Haematoxylin-Eosin staining (16). Reading and inspecting the preparations with 10x ocular magnification and 40x objective lens magnification.

Data Analysis

All data are presented as mean ± SD. Collected data were analyzed using SPSS; the results of Shapiro-Wilk test showed normality of data. So, in this study, we conducted One Way Anova test for the analysis.

Research Results

Antioxidant Enzymes

Based on the results of the study, MDA levels in the diabetes group increased significantly and after administration of NGSLP, MDA levels decreased almost the same as MDA levels in the control group. Likewise, the results of the SOD, GPX and CAT enzyme examinations increased after administration of NGSLP in mice with diabetes. There were statistically significant differences in MDA, SOD, GPx, and CAT activities in the blood between the diabetic group and control group. NGSLP administration at 200 mg/kg BW significantly increased MDA ($p<0.001$), SOD ($p < 0.001$), GPX ($p < 0.001$), and CAT ($p < 0.001$) levels compared to the control group (Figure 1).

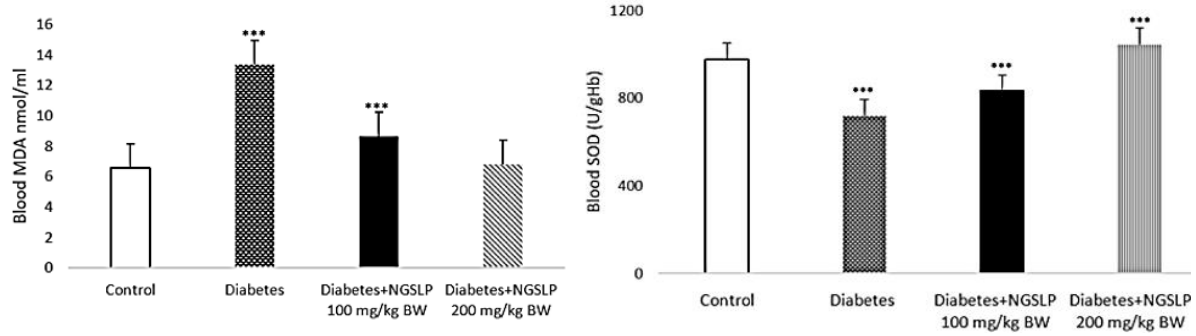

Figure 1. Effects of NGSLP on diabetic-induced changes MDA and SOD. Data were expressed as mean ± SE and analyzed using one-way ANOVA followed by post hoc test. P-values are considered significant when ∗ $p < 0.05$, ∗∗ $p < 0.01$, and ∗∗∗ $p < 0.001$ versus control group.

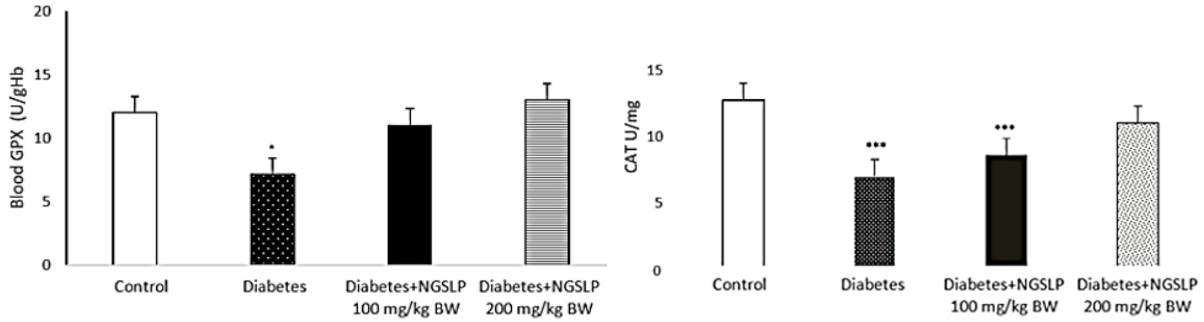

Figure 2. Effects of NGSLP on diabetic-induced changes GPX and CAT. Data were expressed as mean ± SE and analyzed using one-way ANOVA followed by post hoc test. P-values are considered significant when ∗ $p < 0.05$, ∗∗ $p < 0.01$, and ∗∗∗ $p < 0.001$ versus control group.

The administration of NGSLP 200 mg/kg BW for 14 days significantly increased the activities of antioxidant enzymes (SOD, GPx, and CAT) and decreased the level of malondialdehyde (MDA) in the blood of diabetic rats compared to the control group. These findings suggest that NGSLP has antioxidant properties and may be beneficial in reducing oxidative stress in diabetes.

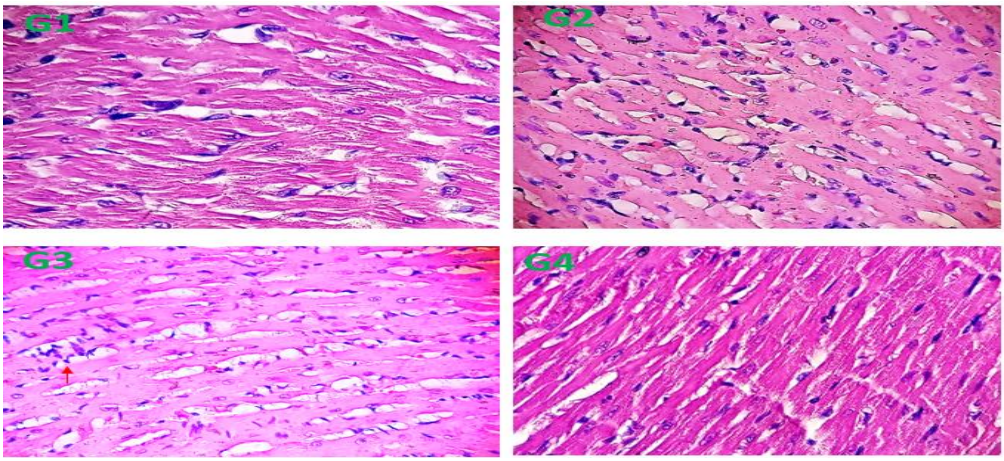

Figure 5. Histology of Heart. G1: control group, G2: Diabetes group, G3: Diabetes+ NGSLP 100mg/kg BW, G4: Diabetes+ NGSLP 200mg/kg BW

The findings suggest that the control group has normal cardiac tissue. Myocyte cell necrosis was seen in the diabetic group, indicating that alloxan induction might induce heart muscle cell death. Heart muscle fibers were destroyed, and the cell nucleus degenerated. In group G3, it was discovered that the myocyte cells seen were injured in just one fascicle, with this rise occurring when nanoparticle

Discussion

In this study, the treatment with NGSLP in diabetic rats showed a significant increase in antioxidant enzymes (SOD, GPX and CAT) indicating that NGSLP has a strong antioxidant effect in rats. In addition, histopathological findings showed that NGSLP treatment for 14 days reduced pathological changes in the heart of diabetic animals. Diabetes mellitus is associated with increased oxidative stress, characterized by elevated production of reactive oxygen species and decreased activity of antioxidant enzymes (17). This redox imbalance leads to damage of vital biomolecules and contributes to diabetic complications. SOD, GPx, and CAT are key antioxidant enzymes that protect against oxidative damage (18–20). Studies have shown reduced activity of these enzymes in diabetic patients, particularly those with long-standing diabetes or obesity. The presence of infections, such as Epstein-Barr virus, may further decrease antioxidant enzyme activity in diabetics (19). Oxidative stress affects pancreatic β-cell function and contributes to micro and macrovascular complications (20).

Since most antidiabetic drugs can have side effects, many studies have been conducted to identify natural substances that exhibit antidiabetic activity with fewer side effects(21). Our previous study showed a potential effect of sunkist peel to decrease the total cholesterol, LDL,

triglycerides, and HDL levels of rats with diabetes (22). Orange peel may be a suitable candidate for antidiabetic agents through its antioxidant effects. The peel contains flavonoids that exhibit antioxidant, α-glucosidase inhibitory, and antiglycation effects, which are crucial in managing diabetes and its complications (13,23). Orange peel extract, especially when combined with exercise, can ameliorate diabetes-induced dysglycemia, dyslipidemia, and upregulate GLUT4 expression (24). The flavonoids in citrus peel have been found to improve glucose metabolism, enhance insulin signaling, repair pancreatic islet cells, stimulate insulin secretion, and protect against diabetic complications (25). These effects are primarily attributed to enzyme inhibition, gene expression regulation, and antioxidant properties. While these findings are promising, further research on safety and efficacy is needed to develop orange peel flavonoids as an alternative therapy for diabetes mellitus. In the current study, administration of NGSLP also significantly enhanced the oxidative defense system in control rats, indicating that administration of NGSLP homogenate to controls activates almost the same mechanisms and pathways that enhance the antioxidant defense system. The underlying mechanisms responsible for the increased injury in diabetic myocardium are not fully understood. However, excessive free radical formation and/or depletion of endogenous antioxidant defense systems have been implicated (17,26). The main histopathological findings were that the myocardium of diabetic animals showed myonecrosis with edema, inflammatory cells, and separation of myocardial fibers. Treatment of rats with NGSLP homogenate ameliorated the cardiac tissue damage manifested as mild edema and inflammatory cells.

Conclusions and Suggestions

NGSLP inhibits lipid peroxidation and activates free radical scavengers in the blood and heart of alloxan-treated Wistar rats. Therefore, NGSLP has strong potential in helping to protect against free radical production in diabetes. However, further research is needed to fully understand the mechanisms of action and to optimize the dosage and treatment regimen.

References

1. Dilworth L, Facey A, Omoruyi F. Diabetes Mellitus and Its Metabolic Complications: The Role of Adipose Tissues. Int J Mol Sci [Internet]. 2021 Jul 16;22(14):7644. Available from: https://www.mdpi.com/1422-0067/22/14/7644
2. González P, Lozano P, Ros G, Solano F. Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections. Int J Mol

Sci [Internet]. 2023 May 27;24(11):9352. Available from: https://www.mdpi.com/1422-0067/24/11/9352

3. Akbari B, Baghaei-Yazdi N, Bahmaie M, Mahdavi Abhari F. The role of plant-derived natural antioxidants in reduction of oxidative stress. BioFactors [Internet]. 2022 May;48(3):611–33. Available from: https://iubmb.onlinelibrary.wiley.com/doi/10.1002/biof.1831
4. Babel RA, Dandekar MP. A Review on Cellular and Molecular Mechanisms Linked to the Development of Diabetes Complications. Curr Diabetes Rev [Internet]. 2021 May;17(4):457–73. Available from: https://www.eurekaselect.com/187544/article
5. Pardina E, Tondo M, Escolà-Gil JC, Rossell J, Julve J. Molecular mechanisms involved in hyperglycemia-induced cellular damage. In: Chronic Complications of Diabetes Mellitus [Internet]. Elsevier; 2024. p. 25–38. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323884266000129
6. Nellaiappan K, Preeti K, Khatri DK, Singh SB. Diabetic Complications: An Update on Pathobiology and Therapeutic Strategies. Curr Diabetes Rev [Internet]. 2022 Jan;18(1). Available from: https://www.eurekaselect.com/192146/article
7. Singh A, Kukreti R, Saso L, Kukreti S. Mechanistic Insight into Oxidative Stress-Triggered Signaling Pathways and Type 2 Diabetes. Molecules [Internet]. 2022 Jan 30;27(3):950. Available from: https://www.mdpi.com/1420-3049/27/3/950
8. Sarinastiti DD, Sudarsono TA, Dhanti KR, Wijayanti L, Muniroh NAN, Cahyaningrum WR, et al. The effect of a combination of kepok banana peel (musa acuminata balbisiana colla) and bay leaf (syzygium polyanthum) extracts on bleeding time in wistar rats (rattus norvegicus). MEDIA ILMU Kesehat [Internet]. 2024 Apr 28;13(1):58–68. Available from: https://ejournal.unjaya.ac.id/index.php/mik/article/view/1342
9. Sari M, Ginting CN, Yulizal O. Sunkist Peel Ethanol Extract Ameliorates Diabetic Nephropathy in Streptozotocin-Induced Diabetic Wistar Rats. Open Access Maced J Med Sci [Internet]. 2021 Oct 25;9(A):1086–91. Available from: https://oamjms.eu/index.php/mjms/article/view/7362
10. Mutia MS, Chiuman L. Hepatoprotective Effect of Citrus sinensis (L.) Osbeck Ethanol Extract in Paracetamol-Induced Rats. Maj Kedokt Bandung. 2019;51(4):189–93.
11. Liew SS, Ho WY, Yeap SK, Bin Sharifudin SA. Phytochemical composition and in vitro antioxidant activities of Citrus sinensis peel extracts. PeerJ. 2018;2018(8):1–16.
12. Pallavi M, Ramesh C K, Siddesha J M, Krishna V, Kavitha G C, Nethravathi A M, et

al. Anti-hyperlipidemic effects of citrus fruit peel extracts against high fat diet-induced hyperlipidemia in rats. Int J Res Pharm Sci [Internet]. 2021 Jul 5;12(3):2226–32. Available from: https://pharmascope.org/index.php/ijrps/article/view/4837

13. Sihotang WY, Putri IJD, Henny H, Suandy S, Mutia MS. Effect of sunkist orange peel nanoparticle granules on cardiac and aorta histopathology in diabetic rats. J Prima Med Sains [Internet]. 2023 Dec 10;5(2):94–9. Available from: http://jurnal.unprimdn.ac.id/index.php/JPMS/article/view/4426
14. Gusti AMT, Qusti SY, Alshammari EM, Toraih EA, Fawzy MS. Antioxidants-Related Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST), and Nitric Oxide Synthase (NOS) Gene Variants Analysis in an Obese Population: A Preliminary Case-Control Study. Antioxidants [Internet]. 2021 Apr 13;10(4):595. Available from: https://www.mdpi.com/2076-3921/10/4/595
15. Meca AD, Turcu-Stiolica A, Stanciulescu EC, Andrei AM, Nitu FM, Banita IM, et al. Variations of Serum Oxidative Stress Biomarkers under First-Line Antituberculosis Treatment: A Pilot Study. J Pers Med [Internet]. 2021 Feb 9;11(2):112. Available from: https://www.mdpi.com/2075-4426/11/2/112
16. Mutia MS, Suhartina. Role of Sunkist Peels Extract Against the Level of Estradiol and Maturity of Follicle in the Female Wistar Rat. Asian J Pharm Clin Res. 2021;14(4):120–3.
17. Ghasemi-Dehnoo M, Amini-Khoei H, Lorigooini Z, Rafieian-Kopaei M. Oxidative stress and antioxidants in diabetes mellitus. Asian Pac J Trop Med [Internet]. 2020;13(10):431. Available from: https://journals.lww.com/10.4103/1995-7645.291036
18. Omoruyi F, Sparks J, Stennett D, Dilworth L. Superoxide dismutase as a measure of antioxidant status and its application to diabetes. In: Diabetes [Internet]. Elsevier; 2020. p. 409–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128157763000413
19. Dworzański J, Strycharz-Dudziak M, Kliszczewska E, Kiełczykowska M, Dworzańska A, Drop B, et al. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. Pagano JS, editor. PLoS One [Internet]. 2020 Mar 25;15(3):e0230374. Available from: https://dx.plos.org/10.1371/journal.pone.0230374
20. Mehar Bisht A, Masood T, Shakeel T, Tiwari V, Pushkar R, Thapliyal N. “Correlation

Between Oxidative Stress Markers And Diabetic Cataract." INDIAN J Appl Res [Internet]. 2022 Feb 1;46–8. Available from: https://www.worldwidejournals.com/indian-journal-of-applied-research-(IJAR)/fileview/correlation-between-oxidative-stress-markers-and-diabetic-cataract_February_2022_4424890610_4604261.pdf

21. Bushnak R, El Hajj M, Jaber A. A Review on the Antidiabetic Potential of Medicinal Plants. Sumerianz J Med Healthc [Internet]. 2021 Nov 18;(44):172–89. Available from: https://www.sumerianz.com/?ic=journal-home&journal=31&info=archive-detail&month=12-2021&issue=4&volume=4
22. Mutia MS, Harahap S, Nurhady FZ, Gunawan R, Dewani Y, Chairul M, et al. Effect of sunkist orange peel extract nanoparticle granules on the lipid profile levels in diabetic wistar rat. J Prima Med Sains [Internet]. 2023 Dec 15;5(2):135–8. Available from: http://jurnal.unprimdn.ac.id/index.php/JPMS/article/view/4439
23. Zhang J, Zhang L, Lai C, Liang Y, Gao L, Kaliaperumal K, et al. Nutraceutical potential of navel orange peel in diabetes management: The chemical profile, antioxidant, α-glucosidase inhibitory and antiglycation effects of its flavonoids. Food Biosci [Internet]. 2022 Oct;49:101943. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2212429222004035
24. Olayaki LA, Okesina KB, Jesubowale JD, Ajibare AJ, Odetayo AF. Orange Peel Extract and Physical Exercise Synergistically Ameliorate Type 2 Diabetes Mellitus-Induced Dysmetabolism by Upregulating GLUT4 Concentration in Male Wistar Rats. J Med Food [Internet]. 2023 Jul 1;26(7):470–9. Available from: https://www.liebertpub.com/doi/10.1089/jmf.2023.0061
25. Herdien DR, Rahmadhani CA, Nurmala S, Yanuar A. Anti-Diabetes Mellitus Potential Compounds Of Flavonoid Citrus Peel: A Review On Mechanism. Berk Ilm Mhs Farm Indones [Internet]. 2020 Dec 30;7(2):58–74. Available from: https://bimfi.e-journal.id/bimfi/article/view/48
26. Matsumoto N, Omagari D, Ushikoshi-Nakayama R, Yamazaki T, Inoue H, Saito I. Hyperglycemia Induces Generation of Reactive Oxygen Species and Accelerates Apoptotic Cell Death in Salivary Gland Cells. Pathobiology [Internet]. 2021;88(3):234–41. Available from: https://karger.com/PAT/article/doi/10.1159/000512639