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Ethanolic Extract of Orange Leaves Ameliorates Dyslipidemia in Streptozotocin-induced Diabetic Rats

Eman Youssef[a,*], Abdelghafar M Abuelsaoud[b,c], Mona M Moghazee[d], Ohoud M Marie [a]

[a]*Chemistry Department, Faculty of Science, Suez Canal University, 41522, Ismailia, Egypt*

[b]*Botany Department, Faculty of Science, Suez Canal University, 41522, Ismailia, Egypt*

[c]*Department of Biology, College of Science, Imam Muhammad bin Saud Islamic University (IMSIU), 11623, Riyadh, Saudi Arabia*

[d]*Molecular and Biotechnology Department, Faculty of Agriculture, Ain Shams University, 11241, Cairo, Egypt*

Abstract

Dyslipidemia is among the main complications of diabetes mellitus. The aim of the current research is to assess the possible protection provided by orange leaves extract (OLE) against dyslipidemia experimentally generated by STZ-diabetes in rats. STZ was given as a single dose (45 mg/kg) to cause type 1 diabetes. 25 male adult albino Wistar rats were divided into five groups: the non-diabetic untreated group (control), the diabetic control group, non-diabetic group administered OLE (100 mg/kg/day), diabetic groups administered with OLE (100 mg/kg/day), and the diabetic group administered with metformin. The following parameters were assessed: FBG, TG, TC, LDL-c, HDL-c and VLDL-c. Our findings demonstrated that STZ dramatically increased FBS, TG, LDL-c, VLDL-c, while significantly decreased HDL-c level compared to the normal rats. OLE treatment counteracts these effects, lowering harmful lipids and raising beneficial HDL-c, but with less potency than metformin. In conclusion, OLE could have a therapeutic role against dyslipidemia in streptozotocin-induced diabetic rats with less bio-efficacy than metformin.

Keywords: Diabetes, Orange leaves extract, Polyphenols, Dyslipidemia, Streptozotocin, Lipid profile

1. Introduction:

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistently elevated blood sugar levels (hyperglycemia) due to impaired glucose metabolism and the body's reduced ability to utilize alternative energy sources efficiently. Insufficient insulin secretion, resistance to effect of insulin, or both are the causes of this metabolic disease **[1, 33].** Diabetes mellitus is linked to end-organ injury, malfunction, and collapse, including that of the kidney, retina, neurological system, circulatory system and the heart **[2].**

According to the International Diabetes Federation (IDF), 366 million people worldwide was recorded to have diabetes mellitus in 2011 and that number will continue to increase to 552 million by 2030 **[3].** Driven by its immense frequency, morbidity, and mortality, diabetes mellitus is eclipsing established public health threats like cancer and cardiovascular diseases as the third major non-communicable disease burden **[4].**

Dyslipidemia, characterized by abnormal serum lipid profiles, is a frequent companion to diabetes, regardless of underlying insulin deficiency or resistance. High plasma level of Low-density lipoprotein cholesterol (LDL-c), high plasma triglycerides and low amounts of HDL-c are the defining characteristics of diabetic dyslipidemia **[5] .** Among these lipid abnormalities, elevated low-density lipoprotein cholesterol (LDL-c) stands as a critical risk factor for atherosclerotic cardiovascular disease (CVD), including coronary artery disease **[6].**

* Corresponding author.
Email address: eman_yussuf@science.suez.edu.eg (Eman Youssef)

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Despite significant improvements in drug discovery, managing diabetes is difficult and poses a significant challenge for the medical profession. Also, there are several major unfavorable side effects linked with the use of synthesized oral hypoglycemic medications such sulfonylureas, thiazolidinediones and α-glucosidase inhibitors. As a result, researchers have concentrated on using herbal sources to create novel anti-diabetic drugs that maintain their therapeutic efficiency and have no negative implications [7]. Using herbal remedies in medical treatment is becoming increasingly well-known and popular on a global scale. Despite the widespread belief that natural remedies made from plants are harmless, scientists advise conducting adequate toxicological research before using any natural remedies **[8].**

Orange fruits are characterized by a rich abundance of phytochemicals and secondary metabolites serving crucial protective functions in their ecological interactions. Many flavonoids derived from orange fruits have been reported to reduce oxidative stress, improve glucose tolerance and insulin sensitivity, modulate lipid metabolism and adipocyte differentiation, suppress inflammation and apoptosis, and improve endothelial dysfunction **[9].**

Up to our knowledge, this study is the first study that investigate the potential of orange leaves extract (OLE) in ameliorating the lipid profile alterations characteristic of diabetes. Analysis of the chemical profile of OLE revealed the presence of many phenolic compounds such as rutin, quercetin, gallic acid, protocatechuic acid, caffeic acid, catechin and epicatechin [10]. These phenolic compounds were known with their hypoglycemic, hypolipidemic, and antioxidant effects **[11].**

Also, flavonoids as diosmin and hesperidin were found in OLE at reasonable concentrations. These flavonoids have some important biological activities, such as antioxidant, anti-inflammatory, and anti-apoptotic effects [12]. Recognizing the limited research on alternative therapeutic approaches for diabetic dyslipidemia, the presence of these potentially beneficial bioactive compounds in OLE motivated us to investigate its efficacy in managing lipid abnormalities associated with this metabolic condition.

The purpose of this research is to examine the effects of orange leaf extract (OLE) against dyslipidemia caused by diabetes mellitus experimentally generated by STZ in rats, through measuring of the following parameters: Fasting blood glucose (FBG), triglyceride (TG), low density lipoprotein (LDL-c), total cholesterol (TC), high density lipoprotein (HDL-c) and very low-density lipoprotein (VLDL-c).

2. MATERIALS AND METHODS:

2.1. Chemicals

Streptozotocin (STZ) was purchased from (MP Biomedicals, LLC, USA), ethanol and acetone were got from (LOBA CHEMIE PVT.LTD, India).

2.2. Plant Material:

Orange leaf samples were obtained from a local farm in Ismailia, Egypt, during the flowering season. The leaves were cleaned, rinsed, and dried naturally before being powdered with a blinder then weighted and extracted.

2.3. Extraction of Orange Leaves:

A solvent mixture was created by combining 96% ethanol and acetone in a 4:1 ratio. This type of solvent mixture is frequently employed for extracting polar and non-polar phytochemicals from plant materials. 2.5 kg of orange leaf powder was immersed in 12.5 L of the prepared solvent mixture. The mixture was then stirred and protected away from direct sunlight for 3 days. This prolonged maceration period allows for efficient diffusion of bioactive compounds from the leaves into the solvent. After 3 days, the extract was filtered to remove plant debris. The extraction process was repeated three times using fresh solvent each time to maximize the yield of extracted compounds. Finally, the combined extracts were filtered through a Whatman No. 1 filter paper to further remove any impurities. The extract was then concentrated using a rotary evaporator under reduced pressure and controlled temperature **[13].**

2.4. Animals:

25 male Wistar rats were obtained from the animal house, Faculty of Pharmacy, Suez Canal University. Wistar rats were 2-3 months old and weighed 175-225 g. This research was authorized by the Health Search Ethics Committee of the Faculty of Science, Suez Canal University (REC58/2020).

2.5. Experimental design:

Induction of diabetes mellitus:

Streptozotocin was used at dose of 45 mg/kg body weight dissolved in 0.1 M citrate buffer (pH 4.5) in a volume of 1mL/kg body weight that was freshly prepared and injected within 5 minutes by intraperitoneal (IP) route. Fasting blood sugar (FBS) was measured by reagent strips (Accu-Chek®, Roche, Germany) after three days to ensure the existence of diabetes. Rats with blood glucose range 300 mg/dL were considered diabetic **[14].**

Following a two-week acclimatization period, twenty-five male rats were randomly assigned to five equally-sized groups (n=5) for a 28-day experiment Group I: non-diabetic untreated rats that were gavaged daily with distilled water for 28 days. Group II: STZ-induced diabetic rats that were gavaged daily with distilled water for 28 days. Group III: non-diabetic rats that orally administered 0.1g/kg OLE over a period of 28 days [15]. Group IV: diabetic rats that orally administered 0.1g/kg OLE daily over a period of 28 days [15]. Group V: diabetic rats that orally administered 0.1g/kg metformin daily over a period of 28 days **[16].**

2.6. Sampling:

At the end of the experiment, all rats were fasted for 12 h then blood was drained in tubes and gathered under effect of tetrahydrofuran inhalation anesthesia. The tubes were centrifuged for 10 min using cooling centrifuge at 3000 rpm then plasma and sera were harvested. Sera were kept in deep freeze at -80 ∘C or determination of lipid profile.

2.6.1. Blood glucose:

The blood sugar level was measured using Strips and a commercial glucometer (Accu-Chek®, Roche, Germany).

2.6.2. Lipid profile:

Using CUSABIO enzymatic colorimetric kits, the levels of serum high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) total cholesterol (TC) and triglycerides (TG) were assessed **[17].**

2.6.3. Statistical analysis:

Each value was represented by its mean and standard deviation. The one-way ANOVA and post-Fisher's test were used to statistically analyze the data after Bartlett's test to validate equal variances. Statistical significance was defined as a P value ≥ 0.05. All statistical analyses were performed using GraphPad® software.

3. Results:

3.1. Effect of treatment by OLE on Fasting Blood Glucose Level (FBG :

A single IP injection of STZ in experimental rats successfully induced a diabetic state characterized by significantly elevated fasting blood glucose (FBG) levels compared to the control group ($p < 0.05$; Figure 1, table 1). This confirmed the effectiveness of the established STZ model in mimicking diabetic hyperglycemia. Oral administration of OLE for 1 month to diabetic rats demonstrated a potent anti-diabetic effect. Compared to the untreated diabetic group, OLE treatment significantly reduced FBG levels ($p < 0.05$; Figure 1, table 1). This finding suggests that OLE possesses promising therapeutic potential for managing hyperglycemia in diabetes.

3.2. Effect of treatment by OLE on Lipid Profile:

STZ-induced diabetes altered lipid metabolism, leading to significantly elevated levels of LDL-c, VLDL-c, TG, and TC, while simultaneously decreasing HDL-c compared to non-diabetic control rats. However, oral administration of OLE for one month to diabetic rats significantly increased their HDL-c levels ($P < 0.05$) in comparison to the diabetic groups. Also, OLE administration to diabetic rats for one month significantly decreased their TG and LDL-c levels ($P < 0.05$) compared to untreated diabetic rats, (figure1, Table 1).

Table 1: **Effect of OLE on fasting blood glucose level and lipid profile of diabetic rats**

Groups of experiment	Normal control	Diabetic control	OLE control	Diab+OLE	Metformin
FBG mg/dL	123.8 ± 10.59^{d}	560.6 ± 7.02^{a}	97.60 ± 16.06^{e}	360.2 ± 124.0^{b}	297.0 ± 181.0^{c}
TC mg/dL	55.30 ± 1.11^{d}	117.8 ± 3.48^{a}	70.09 ± 1.17^{c}	80.67 ± 0.69^{b}	69.19 ± 1.77^{c}
TG mg/dL	63.24 ± 1.63^{e}	143.3 ± 2.42^{a}	85.17 ± 1.59^{c}	101.2 ± 1.10^{b}	81.17 ± 0.85^{d}
HDL-C mg/dL	23.97 ± 0.64^{a}	14.73 ± 0.87^{d}	19.84 ± 0.34^{b}	20.57 ± 0.09^{b}	20.23 ± 0.31^{c}
LDL-C mg/dL	27.94 ± 1.68^{e}	64.77 ± 2.55^{a}	33.14 ± 1.09^{c}	39.90 ± 0.83^{b}	32.64 ± 2.04^{d}
VLDL-C mg/dL	12.65 ± 0.33^{e}	28.54 ± 0.51^{a}	17.03 ± 0.32^{c}	20.24 ± 0.21^{b}	16.27 ± 0.16^{d}

Every number denotes the mean ± SD (n = 5)Values that have distinct superscript letters in the same row changeconsiderably at (P<0.05). FBS: FastingBlood Glucose, TC: totalcholesterol, TG: triglycerides HDL-c: The high-density lipoproteincholesterol; LDL-c: low-densitylipoprotein cholesterol; and VLDL-c:very low-density lipoprotein cholesterol.

Every number denotes the mean ± SD (n = 5) Values that have distinct superscript letters in the same row change considerably at (P<0.05). FBS: Fasting Blood Glucose, TC: total cholesterol, TG: triglycerides HDL-c: The high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; and VLDL-c: very low-density lipoprotein cholesterol.

4. Discussion:

Diabetes is a chronic disease caused by inadequate pancreatic insulin synthesis or incorrect insulin utilization by the body [18]. Limitations of the cost and safety profiles of existing diabetes treatments motivate the pursuit of alternative therapeutic strategies [19]. Many plants serve as anti-diabetic agents based on the presence of bioactive compounds that have antioxidant and anti-diabetic activity (e.g., flavonoids, terpenoids, saponins, carotenoids, alkaloids, glycosides) with less harmful side effects **[20] .**

This research contributes to the ongoing exploration of natural therapeutic interventions for diabetic complications, potentially improving clinical management. This research aims to investigate the therapeutic potential of orange leaf extract (OLE) in mitigating dyslipidemia associated with streptozotocin (STZ)-induced diabetes in rats.

Diabetes pathogenesis is complicated and multifaceted. Diabetes is distinguished by hyperglycemia and is usually linked with a lipid metabolism disorder [21]. The present results showed that STZ significantly increased FBG level compared to normal rats. STZ preferentially damages pancreatic islets of Langerhans, leading to a deficiency in insulin production and action, which leads to reduced glucose utilization by tissues and, eventually, diabetes [6]. The administration of OLE to diabetic rats significantly decreased FBG compared to the diabetic rat. OLE is rich in polyphenols and flavonoids that target several molecules involved in the control of many pathways, such as alleviating hyperglycemia through regulating glucose metabolism in the liver [22]. These results agreed with [23] who suggested that orange leaf flavanones like naringin and naringenin hold promise for diabetes management due to their potential to enhance insulin sensitivity, boost glucose tolerance and reduce plasma lipids.

Dysregulation of lipid metabolism, characterized by altered levels and functions of various fats, plays a critical role in the pathogenesis of diabetes mellitus. Elevated triglycerides, low HDL-c, and accumulation of specific fatty acid metabolites contribute to insulin resistance and impaired glucose utilization [24]. In the present study, STZ significantly increased levels of LDL-c, VLDL-c, TG, and TC, while simultaneously decreasing HDL-c compared to control rats. This dyslipidemia was in line with [25] who discovered that development of diabetes caused a large increase in blood TG, TC, LDL-c, and a considerable drop in HDL-c. This marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of

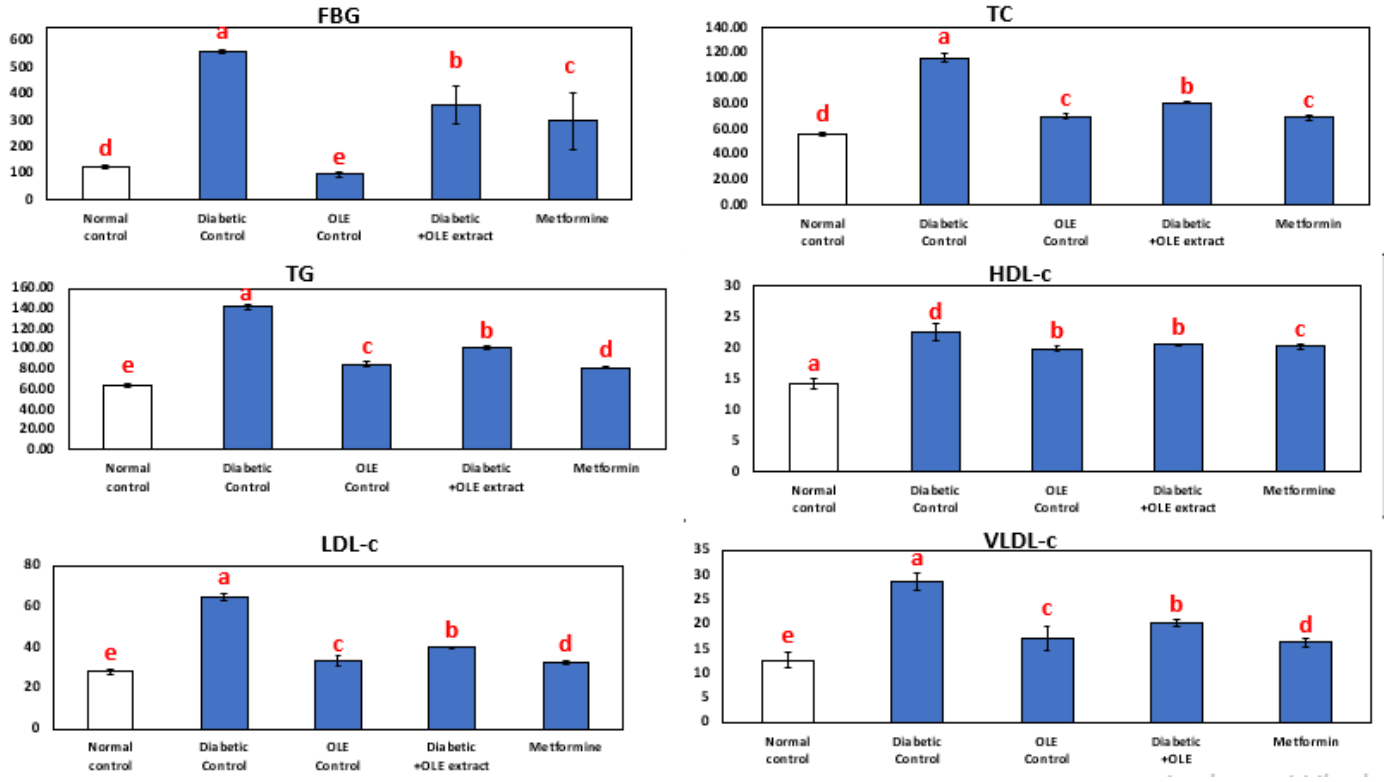

Figure 1: FBG mg/dL, TC, TG mg/dL, HDL-C mg/dL, LDL-C mg/dL and VLDL-C mg/dL at different treatment groups. Data presented as means and standard deviation. According to GraphPad® software, bars followed by various letters differ significantly.

the uninhibited release of fat depots from adipose tissue and the liver in response to energy demands of cells already deprived of glucose due to insulin deficiency or resistance, which identifies diabetes. Insulin deficiency results in activation of hormone-sensitive lipase (HSL) and consequently enhanced release of free fatty acids from adipose tissue. Thus, excess fatty acids in the plasma produced by the STZ-induced diabetes promote the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess triacylglycerol formed in the liver, may be discharged into the blood in the form of lipoproteins. resulting in hyperlipidemia. **[26].**

Also, in diabetic rats, hyperglycemia causes the production of hydrogen peroxide, which then results in the production of oxygen-free radicals like $O_2^{\cdot}$ and $OH^{\cdot}$ These reactive substances have the ability to peroxide lipids, producing endoperoxides and hydroperoxy fatty acids [27]. That may damage membranes by causing protein glycation and membrane lipid peroxidation **[28].**

OLE significantly improves STZ-induced dyslipidemia by improving TG breakdown in glycerol and fatty acids, furthermore, decreasing the action of acyl-CoA: cholesterol acyltransferase (ACAT) that provides a diminution in cholesterol ester levels. These results agreed with [29] who studied neohesperidin effects, which was extracted from orange leaves, on hypolipidemia and hypoglycemia in vivo. [29] found that neohesperidin prevented the buildup of lipids in diabetic rats' hepatocytes by suppressing the gene expression of acyl-CoA oxidase (ACOX), stearoyl-CoA desaturase (SCD-1), and fatty acid synthase (FAS).

In the present diabetic rat model, metformin demonstrated superior efficacy in alleviating hyperglycemia compared to OLE. While both of them displayed antihyperglycemic effects, metformin produced a greater reduction in elevated blood glucose levels. Metformin improves hyperglycemia mainly through the suppression of hepatic gluconeogenesis along with the improvement of insulin signaling [30]. This study revealed the differential effects of metformin and OLE on hyperglycemia in diabetic rats. Metformin exerts its antihyper-

glycemic action primarily through enhanced insulin sensitivity and increased glucose uptake in peripheral tissues, while OLE may act through alternative mechanisms, potentially involving antioxidant or anti-inflammatory properties **[31].**

Both OLE and metformin exerted favorable effects on HDL-c in diabetic rats, aligning with previous reports of HDL-c elevation following OLE supplementation [32]. Metformin displayed significantly greater improvement in LDL-c and VLDL-c levels compared to OLE, suggesting a stronger effect on atherogenic cholesterol fractions. These results matched with the superior hypoglycemic effect of metformin than OLE. While both OLE and metformin improved HDL-c, their mechanisms of action and overall lipid impacts might differ. Metformin primarily acts through enhancing insulin sensitivity and glucose uptake, leading to reduced hepatic cholesterol and TG production [33]. OLE's mechanisms are less understood, though its antioxidant and anti-inflammatory properties might play a role.

While metformin exhibited superior antihyperglycemic activity in this study, further investigation is needed to elucidate the precise mechanisms underlying OLE's effects and its potential role in combination therapy or as a complementary approach for managing hyperglycemia in diabetes.

5. Conclusion:

These findings suggest that OLE could be a potential therapeutic option for managing dyslipidemia associated with diabetes. However, further research is needed to fully understand the mechanisms of action and long-term efficacy of OLE in humans.

References

[1] A. A. Elberry, Methanolic extract of Marrubium vulgare ameliorates hyperglycemia and dyslipidemia in streptozotocin-induced diabetic rats, International Journal of Diabetes Mellitus 3 (1) (2015) 37–44.

[2] J. C. Ozougwu, The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus, J Physiol Pathophysiol 4 (4) (2013) 46–57.

[3] U. Alam, Chapter 15 - General aspects of diabetes mellitus, Elsevier, 2014.

[4] T. Ohkuwa, Y. Sato, M. Naoi, Hydroxyl radical formation in diabetic rats induced by streptozotocin., Life Sciences 56 (1995) 1789–1798.

[5] R. A. Kowluru, Hyperlipidemia and the development of diabetic retinopathy: Comparison between type 1 and type 2 animal models, Metabolism 65 (10) (2016) 1570–1581.

[6] P. Y. Konda, Pathophysiology of STZ-induced pancreatic β cell injury and dysfunction: traditional role of Boswellia ovalifoliolata Bal. & Henry on diabetes and dyslipidemia, Comparative Clinical Pathology 29 (3) (2020) 609–619.

[7] K. Madhuri, P. R. Naik, Ameliorative effect of borneol, a natural bicyclic monoterpene against hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic Wistar rats, Biomedicine & Pharmacotherapy 96 (2017) 336–347.

[8] E. Frederick, A. Kayode, U. Slyvester, Evaluation of the methanolic extract of mistletoe (Tapinanthus bangwensis) leaves grown on orange trees for the phytochemical properties and its physiological effects on streptozotocin induced diabetes mellitus in laboratory animals, World Applied Sciences Journal 9 (9) (2010) 975–979.

[9] C. A. Sanmartin, .-O, Cold-Pressing Olive Oil in the Presence of Cryomacerated Leaves of Olea or Citrus, Nutraceutical and Sensorial Features.

[10] S. Filip, Chemical composition and antimicrobial activity of Osage orange (Maclura pomifera) leaf extracts, Archiv der Pharmazie 354 (2) (2021) 2000195–2000195.

[11] S. M. Jeong, Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. , Nutrition research and practice 6 (2012) 201–207.

[12] G. R. Gandhi, Citrus Flavonoids as Promising Phytochemicals Targeting Diabetes and Related Complications: A Systematic Review of In Vitro and In Vivo Studies, Nutrients 12 (10) (2020) 2907–2907.

[13] H. Muhtadi, Antidiabetic and antihypercholesterolemic activities of Citrus sinensis peel: in vivo study, National Journal of Physiology, Pharmacy and Pharmacology 5 (5) (2015) 382–385.

[14] A. Ghasemi, S. Jeddi, Streptozotocin as a tool for induction of rat models of diabetes: A practical guide, EXCLI journal 22 (2023) 274–274.

[15] M. Z. Rizk, Therapeutic activity of sour orange albedo extract and abundant flavanones loaded silica nanoparticles against acrylamide-induced hepatotoxicity, Toxicology Reports 5 (2018) 929–942.

[16] S. M. Attia, G. K. Helal, A. A. Alhaider, Assessment of genomic instability in normal and diabetic rats treated with metformin, Chemico-Biological Interactions 180 (2) (2009) 296–304.

[17] P. Roeschlau, E. Bernt, W. Gruber, Enzymatic determination of total cholesterol in serum, Z Klin Chem Klin Biochem 12 (5) (1974) 226–226.

[18] G. A. Geberemeskel, Y. G. Debebe, N. A. Nguse, An-

tidiabetic Effect of Fenugreek Seed Powder Solution (Trigonella foenum-graecum L.) on Hyperlipidemia in Diabetic Patients, Journal of Diabetes Research (2019) 8507453–8507453.

[19] M. Przeor, Some Common Medicinal Plants with Antidiabetic Activity, Known and Available in Europe (A Mini-Review), Pharmaceuticals 15 (1) (2022) 65–65.

[20] B. Salehi, Antidiabetic Potential of Medicinal Plants and Their Active Components. , Biomolecules 9 (2019) 551–551.

[21] J. Zhai, Coptisine ameliorates renal injury in diabetic rats through the activation of Nrf2 signaling pathway., Naunyn-Schmiedeberg's Archives of Pharmacology 393 (2020) 57–65.

[22] R. K. Al-Ishaq, Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels, Biomolecules 9 (9) (2019) 430–430.

[23] J. M. Assini, E. E. Mulvihill, M. W. Huff, Citrus flavonoids and lipid metabolism, Current Opinion in Lipidology 24 (1) (2013) 34–40.

[24] U. Galicia-Garcia, Pathophysiology of type 2 diabetes mellitus., International journal of molecular sciences 21 (2020) 6275–6275.

[25] H. Barakat, Biological Assessment of Stevioside and Sucralose as Sucrose Substitutes for Diabetics on STZ-Induced Diabetes in Rats, Molecules 28 (3) (2023) 940–940.

[26] R. Ugbaja, Ameliorative effects of aloe vera gel extract on circulatory and tissue lipid profile status in streptozotocin-induced diabetic rats, Bangladesh Journal of Medical Biochemistry 9 (1) (2016) 11–26.

[27] A. A. S. Saleh, Effects of taurine and/or ginseng and their mixture on lipid profile and some parameters indicative of myocardial status in streptozotocin-diabetic rats, The Journal of Basic & Applied Zoology 65 (5) (2012) 267–273.

[28] L. Pari, M. Latha, Effect on lipid peroxidation in streptozotocin diabetes, Gen Physiol Biophys 24 (1) (2005) 13–26.

[29] S. Maksoud, Citrus aurantium L. Active Constituents, Biological Effects and Extraction Methods. An Updated Review, Molecules 26 (2019) 5832–5832.

[30] H. An, L. He, Current understanding of metformin effect on the control of hyperglycemia in diabetes, The Journal of endocrinology 228 (3) (2016) 97–97.

[31] Y. Mullugeta, Dyslipidemia associated with poor glycemic control in type 2 diabetes mellitus and the protective effect of metformin supplementation, Indian Journal of Clinical Biochemistry 27 (2012) 363–369.

[32] A. Tsolakou, Evaluation of the efficacy of the combination of Citrus aurantium, Cistus creticus and Olea europaea leaf extract on the lipid profiles of individuals with marginally elevated lipid levels, International Journal of Functional Nutrition 4 (1) (2023) 1–7.

[33] N. Apostolova, Mechanisms of action of metformin in type 2 diabetes: Effects on mitochondria and leukocyte-endothelium interactions., Redox biology 34 (2020) 101517–101517.