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Bioactivity effects of phlorizin extract on diabetic mice compared to Metformin

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Abstract Diabetes mellitus (DM) is a metabolic disorder that is characterized by hyperglycemia and glucose intolerance. Type 2 diabetes is the most common type of diabetes and accounts for approximately 90% of all diabetes cases with insulin resistance and impaired insulin secretion. Insulin resistance (IR) is the main pathological feature, as well as hypertension and dyslipidemia. This study aims to evaluate the effect of phlorizin extract in improving the symptoms associated with diabetes compared to metformin. In the present study, T2DM was induced by streptozotocin (STZ) (40 mg/kg, intraperitoneally) in male rats on a high-fat diet. Hence, the needs to develop new methods of treatment, the diabetic rats were assigned to receive two types of treatment: phlorizin (100 mg/kg/day) and metformin (250 mg/kg/day). Random levels of blood glucose, serum insulin, free fatty acids, total cholesterol, triglycerides and low-density lipoprotein, were detected in T2DM rats. The main goal of anti-diabetic therapy is to reduce the amount of glucose in the blood and increase the development of immunity. Phlorizin showed promising efficacy comparable to metformin in the management of diabetes and helped improve blood glucose levels, glucose tolerance and insulin sensitivity in mice with T2DM in the treated groups, in addition to reducing fat accumulation.

Keywords: Anti-diabetic therapy, Diabetes, Glucose, Metformin, Phlorizin

Introduction Diabetes is an endocrine disease characterized by chronic hyperglycemia with disturbances in the metabolism of carbohydrates, proteins, and fats resulting from a defect in the secretion or action of insulin, or both (1). It affects global health (2) and has three main types: insulin-dependent diabetes mellitus (T1DM), insulin-independent diabetes mellitus (T2DM) and gestational diabetes (GDM) which is a condition in which a woman develops high blood sugar levels during pregnancy (3, 4 and 5). Diabetes causes serious long-term complications resulting in cardiovascular problems (6), eyes and nerves (7). Nephropathy is one of its serious complications, which is characterized by a decrease in the glomerular filtration rate (GFR) and increased albumin excretion in the urine (proteinuria) (8, 9). In addition to liver damage caused by the effects of oxidative stress resulting from diabetes (10). Streptozotocin is one of the best substances used to create diabetes in animal models (11), which works to destroy and necrosis beta cells through DNA alkylation and prevents their development (12). Many medicines used to treat diabetes such as metformin, one of the main drugs for treating diabetes which reduce liver glucose production and increasing insulin sensitivity in body tissues (13, 14). However, it is not without side effects for the digestive system, as it causes indigestion, nausea and diarrhea (15). As a result, there is a need to find a treatment from natural medicines with lower side effects due to the interest in human health systems and diabetes prevention. Phlorizin is a member of the dihydrochalcone bicyclic flavonoid family found in apples (16). It is a high-essential polyphenol that can reduce glucose absorption in the gut and kidney through a mechanism of action via sodium-dependent glucose transporters (SGLTs) and thus lowering glucose levels. It has shown therapeutic and preventive effects on hyperlipidemia in diabetic rats (17).

Material and Methods

Ethical Approval

The current study protocol was approved by the Research Ethics Committee in the College of Veterinary Medicine, University of Al-Qadisiyah.

Animal preparation and Habitation

In this study, 40 adult male mice with weights of (25-38 g) were used, the mice were housed in the animal house of the College of Science / University of Al-Qadisiyah and a 12-hour light / 12-hour dark cycle was used to house the animals and the temperature was controlled at (24 ± 2 °C). The mice received a normal meal and distilled water during the experiment. After adapting week to the conditions, the study began (18).

Preparation of diet

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This study relied on feeding diabetic groups a high-fat diet (HFD) to induce insulin resistance followed by a dose of STZ to target pancreatic beta cells, which are phenotypically similar to and cause type 2 diabetes in humans (19). The diet included specific ratios of nutrients (Wang et al., 2020). The main components of this diet were (yellow corn, soybeans, wheat, barley, and dried milk in addition to sunflower oil) (20, 21).

Experimental designing

Forty male mice were divided into two groups as following:

1. Two healthy diabetic groups (16 mice, 8 mice in each group)

a. The Control Negative Group (C-): The group received a standard diet and purified water throughout the study.

b. Phlorizin group (Ph1): The group received phlorizin extract (100 mg/kg) for four weeks.

2. Induced diabetes groups (24 mice, 8 mice each group)

a. The Control positive group (C+): - mice were received no treatment

b. Metformin group (Met): - mice were received metformin (250 mg/kg/day orally) (22) for four weeks.

c. Phlorizin group (Ph2): - mice were received phlorizin extract (100mg/kg/day) for four weeks.

Induction of diabetes

The animals that were fasted for (12) hours in the three induction groups were injected with STZ at a dose of (40 mg/kg, by intraperitoneal injection) for each animal to obtain a diabetic animal. Then, the high blood sugar levels in the animals were confirmed 48 hours after the injection by taking a drop of blood from the tail of each animal and measuring the sugar level using a special measuring device where the blood glucose level was > 150 mg/dL (23)

Body weight

All animals were weighed at different times. The first weight was taken during the acclimatization period, and then a second weight was taken after feeding on the special high-fat diet. Then they were weighed in the second and fourth weeks of the study.

Sample preparation

At the end of the research period, blood samples were drawn for laboratory tests and analyses, then the animals were sacrificed, and the required organs were preserved for histological analysis (24).

Blood parameters

Blood glucose, lipid profile, IRI, C-reactive protein, and oxidative stress testing (MDA) and Total antioxidant capacity (T-AOC) were performed by spectrophotometry.

Percentage of weight of animal organs

After removing the organs (kidneys and liver) from each animal by making a median abdominal incision, the weight of the organs of each animal was taken and the ratio of the weight of the organ to the body weight was calculated according to the following equation:

Organ weight percentage %=(Organ weight)/(Total body weight)*%

Results and Discussion

Body weight

Diabetes is one of the most common diseases with serious complications leading to disability and death worldwide (25). STZ is considered the best model for inducing diabetes through its consumption by beta cells and production of reactive oxygen species (26. (

The results of the current research, as shown in table (1) and figure (1) after 14 days of relying on HFD (without diabetes), showed an increase in body weight for (C+, Met and Ph2) groups (32.08 ± 1.03, 34.72± 0.83 and 31.92 ± 0.99) respectively due to excess calorie consumption (27). In compared with both (C- and Ph1) groups (31.53± 1.01and 31.72 ± 1.18) which maintained normal weight changes.

In the second week after STZ injection, a clear decrease was observed in the weight of the (C+,Ph1 and Ph2) groups (29.67 ± 1.05, 30.82 ± 1.36 and 29.06 ± 0.66) respectively compared to the (C-) group (31.86± 1.01) especially the (C+) group, which showed a higher decrease in weight than the other groups due to not receiving any treatment and the appearance of a group of symptoms such as increased water intake, signs of fatigue and general weakness, which confirms the stimulation and development of T2DM symptoms (28). This is due to the accumulation of sugar levels outside the cells, so the body breaks down fats and proteins to obtain sufficient energy, which causes a decrease in body weight (29). Moreover, the result of the (Met) group showed a slight decrease in the weight (31.53± 0.75) due to the effect of metformin in alleviating the effects of diabetes and maintaining weight.

In the fourth week, a sharp decrease in the weight of the (C+) group was observed (25.62 ± 0.76) ($p < 0.05$) due to the increase in complications of diabetes. While the two groups (Met and Ph2) were able to maintain more stable body weights (29.91± 0.61 and

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29.95 ± 0.55) and a slight decrease compared to the (C-) group (32.47± 0.95). This confirms the effectiveness of metformin treatment and phlorizin extract in controlling weight loss associated with diabetes. The weight maintenance with some minor normal changes recorded in the results of the two (C- and Ph1) groups (32.47± 0.95 and 29.95± 0.55) due to a stable nutritional pattern supports the conclusion that the weight changes in the other groups are due to the induction of diabetes (30).

Table 1: Body weight of Animal

Groups	Zero time (without diabetes)	After 14 days (without diabetes)	2 weeks	4 weeks
C-	31± 1.08Aa	31.53± 1.01Aa	31.86± 1.01Aa	32.47± 0.95Aa
C+	1 ±31.5 ABa	32.08 ± 1.03Aba	29.67 ± 1.05Aa	25.62 ± 0.76Bb
Met	34.12± 0.76 Bab	34.72± 0.83Ba	31.53± 0.75Abc	29.91± 0.61Ac
Ph1	31.37± 1.19ABa	31.72 ± 1.18Aba	30.82 ± 1.36Aa	29.87 ± 1.66Aa
Ph2	31.37 ± 0.98ABab	31.92 ± 0.99Aba	29.06 ± 0.66Ab	29.95 ± 0.55Aab
LSD (p < 0.05	2.81			

* C-: control negative group, C+: control positive group, Met: metformin group, Ph1: phlorizin group (without diabetes), Ph2: phlorizin group (diabetic)

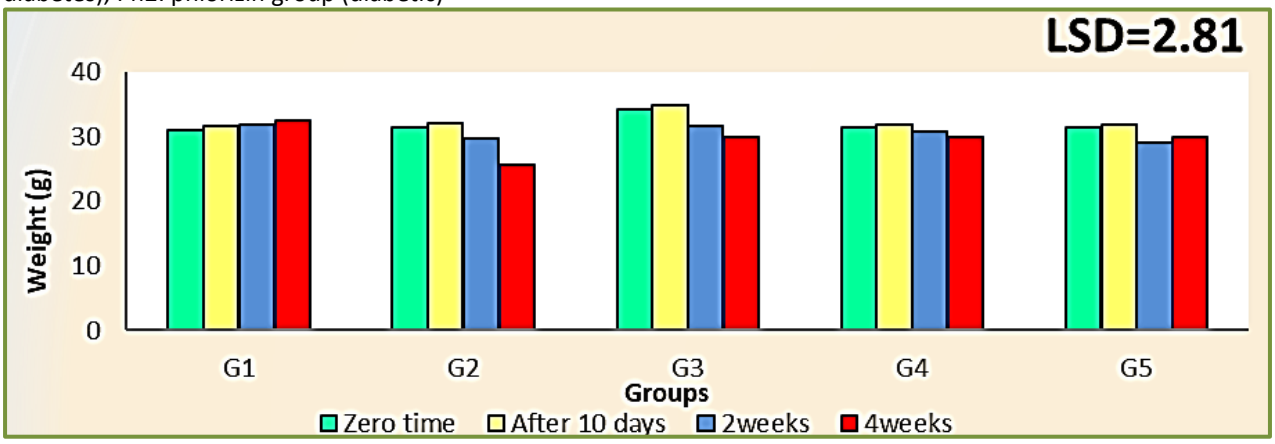

Figure 1: Body weights of experiment

* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

Blood parameters

1. Blood glucose, TC, and TG levels

The results of the study also showed the harmful effect of diabetes on glucose and lipid metabolism as shown in table (2) and figures (2, 3, 4), where (C-) group showed normal results for both blood sugar levels (101.25±3.27) and TC and TG values (124.37±1.22 and 96.62±2.20) respectively, and were within the normal range for lipid metabolism in the absence of diabetes, while the results of (C+) group showed a significant increase in blood sugar levels at (273±3.75 mg/dL), indicating severe hyperglycemia caused by diabetes, as well as a clear increase in TC and TG levels (216.12±3.32 and 115±6.07 mg/dL) compared to (C-) group due to the toxic and rapid effects of STZ on beta cells, which causes insulin resistance (31) and a disturbance in lipid metabolism (32)

In contrast, the (Met) group showed a significant improvement in blood sugar levels as it decrease to (126.62 ± 2.51 mg/dL) as well as a significant decrease in TC and TG levels (132.5 ± 3.69 and 101.12 ± 2.38 mg/dL) respectively compared to the (C+) group but higher than the (C-) group, which is due to the effective effect of metformin in increasing insulin sensitivity and managing diabetes (33). Similarly, the (Ph1 and Ph2) groups (121.51 ± 3.08 and 129.72 ± 2.07 mg/dL) respectively had significantly lower blood sugar levels than the (C+) group. TC levels (126.5 ± 1.97 and 132.87 ± 3.36 mg/dL) and TG levels (98.37 ± 2.12 and 106.5 ± 2.32 mg/dL) were also recorded to be lower than those in group (C+). This study

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ISSN P: 1818-5746
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demonstrated that phlorizin treats glucose and lipid metabolism disorders by lowering blood glucose levels, increasing its percentage in urine, improving lipid metabolism, and alleviating symptoms resulting from diabetes such as frequent thirst, frequent urination, laziness, and lethargy (34).

Table 2: Sugar levels and TC and TG

Groups	sugar	TC	TG
C-	101.25± 3.27C	124.37 ± 1.22C	96.62 ± 2.20C
C+	273± 3.75A	216.12 ± 3.32A	115 ± 6.07A
Met	126.62 ± 2.51B	132.5 ± 3.69BC	101.12± 2.38BC
Ph1	121.51 ± 3.08B	126.5 ± 1.97BC	98.37± 2.12BC
Ph2	129.72 ± 2.07B	132.87 ± 3.36B	106.5 ± 2.32AB
LSD (p< 0.05)	8.32	8.97	9.73

* C-: control negative group, C+: control positive group, Met: metformin group, Ph1: phlorizin group (without diabetes), Ph2: phlorizin group (diabetic)

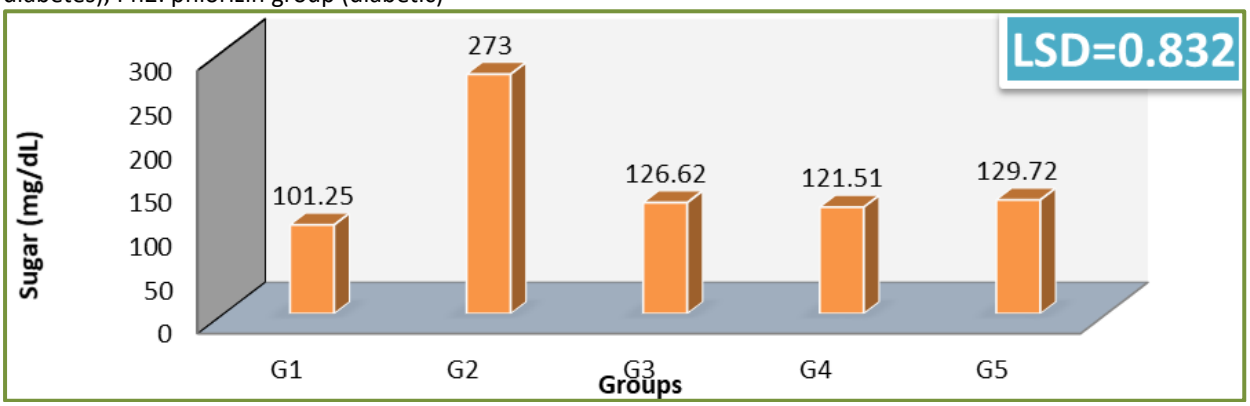

Figure 2**: Sugar levels**
*G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

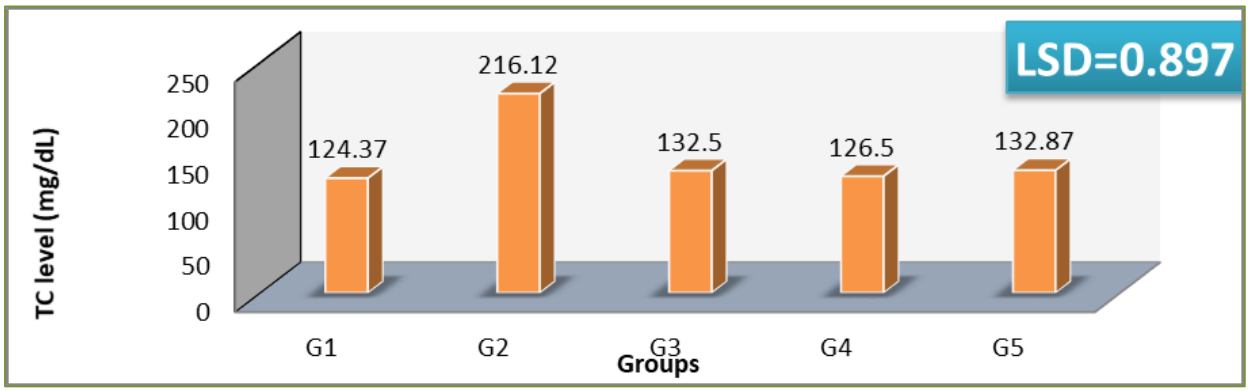

Figure 3: TC levels
* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

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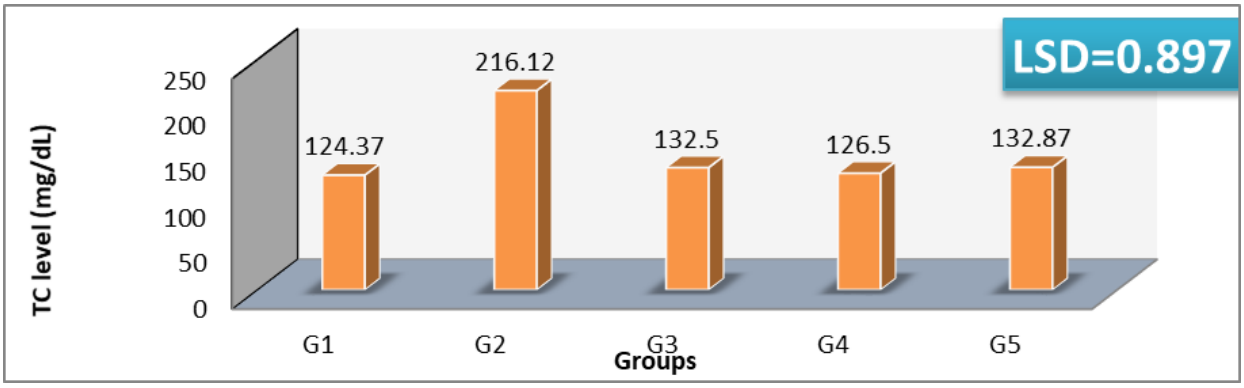

Figure 4: TG levels

* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

2. Lipoprotein Levels (LDL, HDL, VLDL)

The data in table (3) and figures (5, 6 and 7) presented the lipoprotein levels, including Very Low-Density Lipoprotein (VLDL), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) across various experimental groups.

The LDL, HDL and VLDL level in the (C-) group was (56.67 ± 2.23, 48.37± 1.89 and 19.32 ± 0.44 mg/dL) respectively was within normal limits, indicating a healthy lipid profile.

While the results recorded a significant increase in (C+) group for LDL and VLDL levels (156.1 ± 3.43 and 22.75 ± 1.32 mg/dl), at the same time, a lower level of HDL (36.5 ± 1.13 mg/dl) compared to (C-) group, which reflects the dyslipidemia associated with diabetes and insulin resistance this is consistent with study data provided by (35(.

In the same way the (Met) group showed a lower level of LDL and VLDL (54.65 ± 4.78 and 20.22 ± 0.47 mg/dL) respectively compare to (C+) group (156.1 ± 3.43 and 22.75 ± 1.32 mg/dl) but slightly higher than the (C-) group, while highest level of HDL (57.62 ± 1.61 mg/dL) compared to the other groups, this is due to the ability of metformin to enhance insulin sensitivity and reduce the severity of complications resulting from diabetes (36(.

The results of LDL level in the two groups (Ph1 and Ph2) (52.07 ± 2.78 and 61.82 ± 4.73 mg/dl) respectively was lower compared to the (C+) group, while the results showed no significant difference ($P<0.05$) in the VLDL levels which reached (19.55 ± 0.50 and 21.3 ± 0.46 mg/dl) respectively, compared to the (C-) group, and slightly higher compared to the group (C+). Similarly, the HDL levels in both (Ph1 and Ph2) groups (55 ± 1.22and 49.75 ± 1.83 mg/dl) respectively were higher compared to the (C-) group. This reason was that phlorizin inhibiting the reuptake of sodium glucose transporter (SGLT), increasing glucose entry into cells, and improving beta cell function and insulin sensitivity. The results obtained in the present study were consistent with previous reports, which supported the view that phlorizin effectively reduced high blood glucose and improved lipid metabolism in experimental diabetes model (37).

Table 3: Lipoprotein Levels (LDL, HDL, VLDL)

Groups	LDL	HDL	VLDL
C-	56.67 ± 2.23B	48.37± 1.89B	19.32 ± 0.44B
C+	156.1 ± 3.43A	36.5 ± 1.13C	22.75 ± 1.32A
Met	54.65 ± 4.78B	57.62 ± 1.61A	20.22 ± 0.47B
Ph1	52.07 ± 2.78B	55 ± 1.22A	19.55 ± 0.50B
Ph2	61.82± 4.73B	49.75 ± 1.83B	21.3 ± 0.46AB
LSD ($p < 0.05$)	10.73	4.51	2.08

* C-: control negative group, C+: control positive group, Met: metformin group, Ph1: phlorizin group (without diabetes), Ph2: phlorizin group (diabetic)

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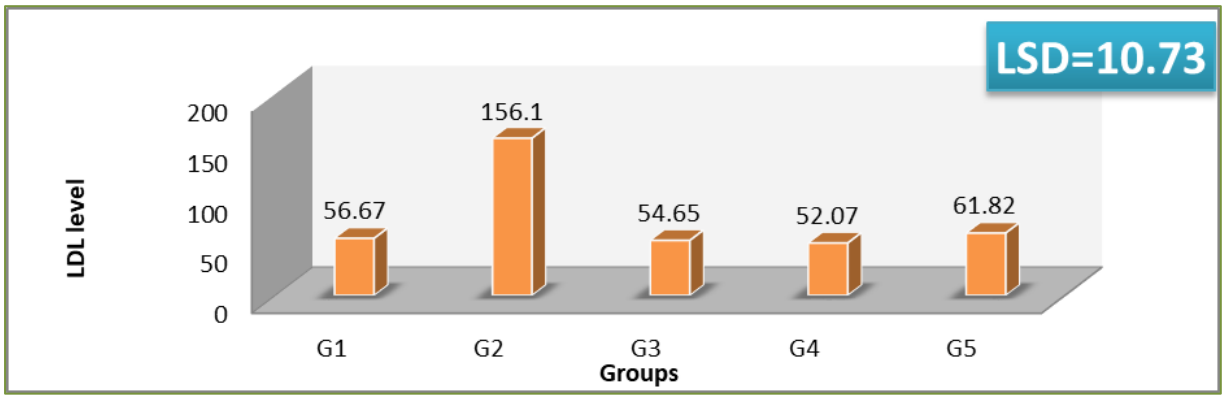

Figure 5: LDL level
* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

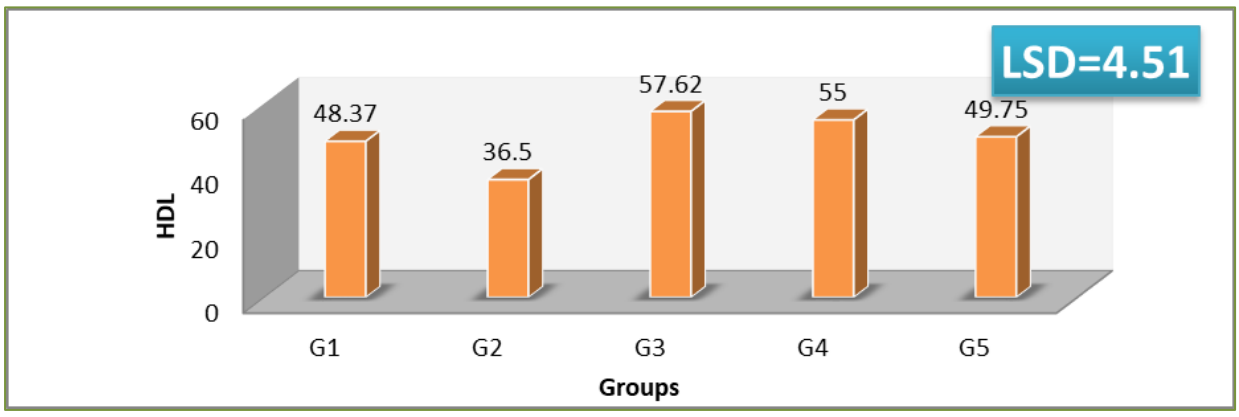

Figure 6: HDL levels
* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

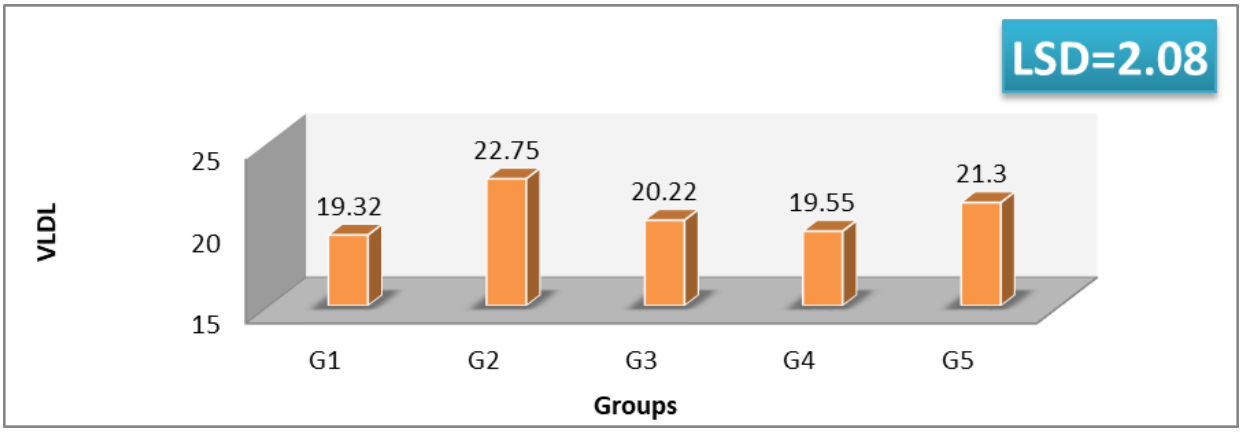

Figure 7: VLDL levels
* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

3. Increased Insulin Resistance (IRI) and C - reactive protein (CRP)

The data presented in table (4) and figures (8 and 9) illustrated the Homeostatic Model Assessment of

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increased insulin resistance (IRI) and C- reactive protein (CRP) levels across different experimental groups. These parameters are critical for assessing insulin resistance and inflammation, particularly in the context of diabetes and metabolic disorders.

Control negative group (C-) showed a normal insulin sensitivity (1.91 ± 0.17Uu/ml), and a normal level of inflammatory marker (CRP) (2.5 ± 0.03 mg/dl).

On the other hand, the data of (C+) group showed a significantly higher IRI level (3.98 ± 0.25 IU/ml) ($P<0.05$) as well as a significant increase ($P<0.05$) in CRP level in this group (3.25 ± 0.09 mg/dL) compared to (C-) group due to the toxic effect of STZ, as STZ-induced diabetic and has the ability to secrete large amounts of insulin, which is consistent with the study presented by (38). Group (Met) had a significant decrease in IRI and CRP levels (2.5 ± 0.26 IU/ml and 3.01 ± 0.07 mg/dL) respectively which were lower than (C+) group and a significant increase compared to (C-) group. This due to the high efficacy of metformin, which significantly improved blood glucose and insulin resistance in diabetic mice, which is agreed with the study content (39). Similarly, (Ph1 and Ph2) groups showed a significant reduction in IRI levels (1.83 ± 0.16 and 2.93 ± 0.17 IU/ml) respectively, as well as a reduction in CRP levels by (3.03 ± 0.06 mg/dL and 2.96 ± 0.07 mg/dL) respectively compared to (C+) group. Due to the efficacy of phlorizin, which is a biologically active plant molecule, and it acts as a natural regulator for the management of insulin resistance and T2DM (40)

Table 4: IRI and CRP Levels in Different Experimental Groups

Groups	IRI	CRP
C-	1.91 ± 0.17CD	2.5 ± 0.03C
C+	3.98 ± 0.25A	3.25 ± 0.09A
Met	2.5 ± 0.26BC	3.01 ± 0.07B
Ph1	1.83 ± 0.16 D	3.03 ± 0.06 B
Ph2	2.93 ± 0.17B	2.96 ± 0.07 B
LSD (P <0.05)	0.600	0.198

* C-: control negative group, C+: control positive group, Met: metformin group, Ph1: phlorizin group (without diabetes), Ph2: phlorizin group (diabetic)

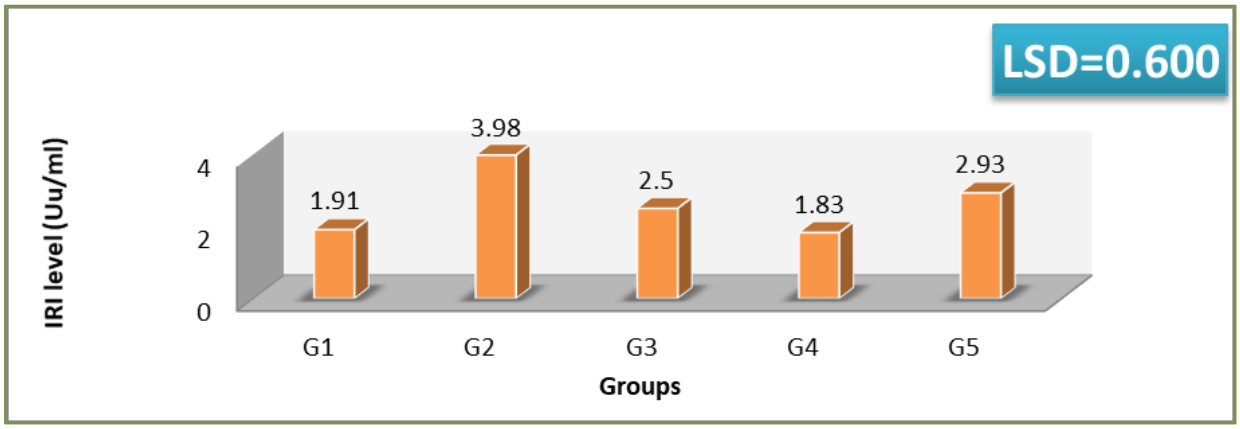

Figure 8: IRI levels

* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

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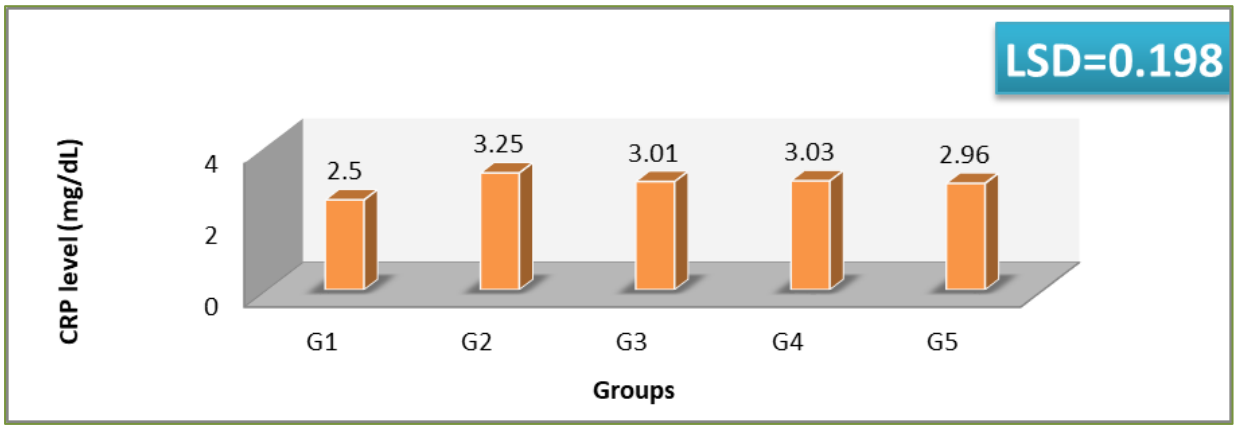

Figure 9: CRP levels
* G1: control negative group, G2: control positive group, G3: metformin group, G4: phlorizin group (without diabetes), G5: phlorizin group (diabetic)

Conclusion

The current study concluded that Phlorizin, a natural extract of apple tree, has shown promising therapeutic effects in reducing complications of diabetes, similar to the drug metformin. Phlorizin improved metabolic parameters by reducing blood sugar, cholesterol and triglyceride levels, in addition to alleviating liver and kidney injury in mice with hyperlipidemia due to it is anti-inflammatory properties, thus reducing inflammation associated with diabetes. Moreover, a comparative analysis between metformin and phlorizin extract treatments reveals subtle differences in their therapeutic effects both effectively improve metabolism and reduce harmful complications associated with diabetes, but there are similarities in their effectiveness.

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Conflict of Interest

The authors confirmed that they had no conflicts of interest.

References

1-Luchen CC, Chibuye M, Spijker R, Simuyandi M, Chisenga C, Bosomprah S, Chilengi R, Schultsz C, Mende DR, Harris VC. Impact of antibiotics on gut microbiome composition and resistome in the first years of life in low-to middle-income countries: A systematic review. PLoS medicine. 2023 Jun 27;20(6):e1004235. https://doi.org/10.1371/journal.pmed.1004235

2-Odularu AT, Ajibade PA. Challenge of diabetes mellitus and researchers' contributions to its control. Open Chemistry. 2021 May 26;19(1):614-34. https://doi.org/10.1515/chem-2020-0153

3- Genuth SM, Palmer JP, Nathan DM. Classification and Diagnosis of Diabetes. In: Cowie CC, Casagrande SS, Menke A, Cissell MA, Eberhardt MS, Meigs JB, Gregg EW, Knowler WC, Barrett-Connor E, Becker DJ, Brancati FL, Boyko EJ, Herman WH, Howard BV, Narayan KMV, Rewers M, Fradkin JE, editors. Diabetes in America. 3rd ed. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases (US); 2018 Aug. CHAPTER 1. PMID: 33651569.

4-Razia S, Sarker PR, Saha AK. Proportion of Gestational Diabetes Mellitus among the Pregnant Mothers and Their Socio-Demographic Characteristics Attending ANC Corner at ShaheedZiaur Rahman Medical College Hospital, Bogura. International Journal of Medical Science and Clinical Research Studies. 2021 Dec 15; 1(10):341-9.

5-Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences. 2020 Aug 30;21(17):6275. https://doi.org/10.3390/ijms21176275

6-Li Y, Liu Y, Liu S, Gao M, Wang W, Chen K, Huang L, Liu Y. Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. Signal transduction and targeted therapy. 2023 Apr 10;8(1):152. https://doi.org/10.1038/s41392-023-01400-z

7-Karim RA, Habib HA. Awareness regarding diabetes risk factors, prevention and management among community members in Diyala/Baqubah. Al-Kindy College Medical Journal. 2022 May 5;18(1):24-9. https://doi.org/10.47723/kcmj.v18i1.272

8-Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clinical journal of the American Society of Nephrology. 2017 Dec 1;12(12):2032-45. https://doi.org/10.2215/CJN.11491116

9.Sagoo MK, Gnudi L. Diabetic nephropathy: an overview. Diabetic nephropathy: methods and protocols. 2020:3-7. https://doi.org/10.1007/978-1-4939-9841-8_1

10- Mohamed J, Nafizah AN, Zariyantey AH, Budin S. Mechanisms of diabetes-induced liver damage: the role of oxidative stress and inflammation. Sultan qaboos university medical journal. 2016 May;16(2):e132. https://doi.org/10.18295/squmj.2016.16.02.002

11- Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. EXCLI journal. 2023;22:274.

12-Haghani F, Arabnezhad MR, Mohammadi S, Ghaffarian-Bahraman A. Aloe vera and streptozotocin-induced diabetes mellitus. Revista Brasileira de Farmacognosia. 2022 Apr;32(2):174-87. https://doi.org/10.1007/s43450-022-00231-3

13-Coll AP, Chen M, Taskar P, Rimmington D, Patel S, Tadross JA, Cimino I, Yang M, Welsh P, Virtue S, Goldspink DA. GDF15 mediates the effects of metformin on body weight and energy balance. Nature. 2020 Feb 20;578(7795):444-8. https://doi.org/10.1038/s41586-019-1911-y

14-Day EA, Ford RJ, Smith BK, Mohammadi-Shemirani P, Morrow MR, Gutgesell RM, Lu R, Raphenya AR, Kabiri M, McArthur AG, McInnes N. Metformin-induced increases in GDF15 are important for suppressing appetite and promoting weight loss. Nature Metabolism. 2019 Dec;1(12):1202-8. https://doi.org/10.1038/s42255-019-0146-4

15-Yuxin H, Cuiping J, Wen T, Jieyuzhen Q, Xiaoming T, Qin G, Haidong W, Jiao S, Zhijun B. Comparison of gastrointestinal adverse events with different doses of metformin in the treatment of elderly people with type 2 diabetes. Journal of Clinical Pharmacy and Therapeutics. 2020 Jun;45(3):470-6. https://doi.org/10.1111/jcpt.13087

16-Niederberger KE, Tennant DR, Bellion P. Dietary intake of phloridzin from natural occurrence in foods. British journal of nutrition. 2020 Apr;123(8):942-50. https://doi.org/10.1017/S0007114520000033

17-Raja M, Puntheeranurak T, Gruber HJ, Hinterdorfer P, Kinne RK. The role of transporter ectodomains in drug recognition and binding: phlorizin and the sodium-glucose cotransporter. MedChemComm. 2016;7(6):1056-68. https://doi.org/10.1039/C5MD00572H

18-Elamin NM, Fadlalla IM, Omer SA, Ibrahim HA. Histopathological alteration in STZ-nicotinamide diabetic rats, a complication of diabetes or a toxicity of STZ. Int J Diabetes Clin Res. 2018;5(3):1-8. https://doi.org/10.23937/2377-3634/1410091

19-Gheibi S, Kashfi K, Ghasemi A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomedicine & pharmacotherapy. 2017 Nov 1;95:605-13. https://doi.org/10.1016/j.biopha.2017.08.098

20-Wang B, Kong Q, Li X, Zhao J, Zhang H, Chen W, Wang G. A high-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. Nutrients. 2020 Oct 20;12(10):3197. https://doi.org/10.3390/nu12103197

21-Kapilevich LV, Zakharova AN, Dyakova EY, Kalinnikova JG, Chibalin AV. Mice experimental model of diabetes mellitus type ii based on high fat diet. Bulletin of Siberian Medicine. 2019 Oct 27;18(3):53-61. https://doi.org/10.20538/1682-0363-2019-3-53-61

22-Han X, Tao Y, Deng Y, Yu J, Sun Y, Jiang G. Metformin accelerates wound healing in type 2 diabetic db/db mice. Molecular medicine reports. 2017 Dec 1;16(6):8691-8. https://doi.org/10.3892/mmr.2017.7707

23-Furman BL. Streptozotocin-induced diabetic models in mice and rats. Current protocols. 2021 Apr;1(4):e78. https://doi.org/10.1002/cpz1.78

24-Couto M, Cates C. Laboratory guidelines for animal care. Vertebrate Embryogenesis: Embryological, Cellular, and Genetic Methods. 2019:407-30. https://doi.org/10.1007/978-1-4939-9009-2_25

25-Ghosh P, Azam S, Karim A, Hassan M, Roy K, Jonkman M. A comparative study of different machine learning tools in detecting diabetes. Procedia Computer Science. 2021 Jan 1;192:467-77. https://doi.org/10.1016/j.procs.2021.08.048

26-Jaishree V,Narsimha S.Swertiamarin and quercetin combination ameliorates hyperglycemia, hyperlipidemia and oxidative stress in streptozotocin-induced type 2 diabetes mellitus in wistar rats. Biomedicine & Pharmacotherapy. 2020 Oct 1;130:110561. https://doi.org/10.1016/j.biopha.2020.110561

27-Gallop MR, Wilson VC, Ferrante AW. Post-oral sensing of fat increases food intake and attenuates body weight defense. Cell reports. 2021 Oct 19;37(3). https://doi.org/10.1016/j.celrep.2021.109845

28.Care D. Care in diabetes-2022. Diabetes care. 2022 Jan 1;45:S17. https://doi.org/10.2337/dc22-S002

29-Rachdaoui N, Polo-Parada L, Ismail-Beigi F. Prolonged exposure to insulin inactivates Akt and Erk1/2 and increases pancreatic islet and INS1E β-cell apoptosis. Journal of the Endocrine Society. 2019 Jan;3(1):69-90. https://doi.org/10.1210/js.2018-00140

30-Drzewoski J, Hanefeld M. The current and potential therapeutic use of metformin-the good old drug. Pharmaceuticals. 2021 Feb 5;14(2):122. https://doi.org/10.3390/ph14020122

31-Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. EXCLI journal. 2023;22:274.

32-Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C. Pathophysiology of type 2 diabetes mellitus. International journal of molecular sciences. 2020 Aug 30;21(17):6275. https://doi.org/10.3390/ijms21176275

33-Tokhirovna EG. THE ROLE OF METFORMIN (GLIFORMIN) IN THE TREATMENT OF PATIENTS WITH TYPE 2 DIABETES MELLITUS. EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE. 2024 Apr 18;4(4):171-7.
34-Mudaliar S, Polidori D, Zambrowicz B, Henry RR. Sodium-glucose cotransporter inhibitors: effects on renal and intestinal glucose transport: from bench to bedside. Diabetes care. 2015 Dec 1;38(12):2344-53.
https://doi.org/10.2337/dc15-0642
35-Eid S, Sas KM, Abcouwer SF, Feldman EL, Gardner TW, Pennathur S, Fort PE. New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. Diabetologia. 2019 Sep 1;62:1539-49.
https://doi.org/10.1007/s00125-019-4959-1
36-Jin K, Ma Y, Manrique-Caballero CL, Li H, Emlet DR, Li S, Baty CJ, Wen X, Kim-Campbell N, Frank A, Menchikova EV. Activation of AMP-activated protein kinase during sepsis/inflammation improves survival by preserving cellular metabolic fitness. The FASEB Journal. 2020 May;34(5):7036-57.
https://doi.org/10.1096/fj.201901900R
37-Zhang W, Chen S, Fu H, Shu G, Tang H, Zhao X, Chen Y, Huang X, Zhao L, Yin L, Lv C. Hypoglycemic and hypolipidemic activities of phlorizin from Lithocarpus polystachyus Rehd in diabetes rats. Food Science & Nutrition. 2021 Apr;9(4):1989-96
https://doi.org/10.1002/fsn3.2165
38-Li M, Hu X, Xu Y, Hu X, Zhang C, Pang S. A possible mechanism of metformin in improving insulin resistance in diabetic rat models. International Journal of Endocrinology. 2019;2019(1):3248527.
https://doi.org/10.1155/2019/3248527
39-Sun J, Wang Y, Zhang X, He H. The effects of metformin on insulin resistance in overweight or obese children and adolescents: a PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. Medicine. 2019 Jan 1;98(4):e14249.
https://doi.org/10.1097/MD.0000000000014249
40-Kumar S, Sinha K, Sharma R, Purohit R, Padwad Y. Phloretin and phloridzin improve insulin sensitivity and enhance glucose uptake by subverting PPARγ/Cdk5 interaction in differentiated adipocytes. Experimental cell research. 2019 Oct 1;383(1):111480.
https://doi.org/10.1016/j.yexcr.2019.06.025