

Antidiabetic Activity of Eupatorin against Streptozotocin-Induced Diabetes in Rats: Biochemical and Histological Studies

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ABSTRACT

Background: Diabetes mellitus is a persistent metabolic condition that has emerged as a major public health issue globally. The prevalence of diabetes has been rapidly increasing, posing a major challenge to global healthcare systems. **Objectives:** The present work was aimed at investigating the anti-diabetic roles of the eupatorin against Streptozotocin (STZ)-induced rat model. **Materials and Methods:** The rats received 55 mg/kg of STZ to induce diabetes and were then treated with eupatorin for 15 days. At the end of treatments, the blood glucose concentration of experimental rats was evaluated. The biomarkers for renal impairment, including urea and creatinine, serum concentrations of liver marker enzymes, serum levels of lipid profile parameters and oxidative stress markers were assessed with commercial assay kits. The pancreas, liver and kidneys of the rats were subjected to histological studies to assess histological alterations. **Results:** The current findings demonstrated that eupatorin treatment significantly decreased the blood glucose, urea and creatinine concentrations in the serum of diabetic rats. The eupatorin treatment also reduced the serum concentrations of liver marker enzymes, regulated the lipid profile parameters and mitigated the oxidative stress via up-regulating antioxidants in the rats with STZ-induced diabetes. Moreover, the findings of histological analyses show that eupatorin treatment can protect vital organs from diabetes-induced histopathological alterations in diabetic rats. **Conclusion:** The current findings indicate that eupatorin may significantly mitigate diabetic conditions and its associated difficulties in STZ-induced rats. Consequently, the current data suggest that eupatorin may serve as an effective therapeutic alternative for diabetes management.

Keywords: Alkaline phosphatase, Eupatorin, Hyperglycemia, Oxidative stress, Triglycerides, Urea.

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INTRODUCTION

Diabetes mellitus is a collection of metabolic abnormalities marked by increased blood glucose levels, stemming from the body's incapacity to generate or efficiently utilize insulin, the hormone that regulates blood sugar. This condition is categorized into two primary types: type-1 diabetes, characterized by the immune system's destruction of pancreatic beta cells resulting in total insulin deficiency and type-2 diabetes, which develops when the body's cells exhibit resistance to insulin, referred to as insulin resistance.¹ The global prevalence of diabetes has increased, with around 451 million persons affected in 2017. The Figure is anticipated to rise to 693 million by 2045 if efficacious preventative strategies are not adopted. The disease prevalence is

higher in urban populations compared with rural populations, while the distribution between genders appears to be similar. The primary causes of diabetes mellitus are complex and multifactorial. Lifestyle variables, including inadequate nutrition, lack of physical activity and obesity, significantly contribute to the onset of type-2 diabetes. Additionally, genetic predisposition and autoimmune processes play an essential role in the onset of type-1 diabetes.²

Diabetes mellitus imposes a considerable burden on persons, healthcare systems and society. Individuals with diabetes face a high risk of developing various comorbidities, including cardiovascular disease, kidney disease, neuropathy and vision impairment, which can remarkably influence their quality of life and lifespan.³ The pathophysiology of type-2 diabetes is complicated and significantly influenced by both hereditary and environmental factors. In response to ongoing insulin resistance, pancreatic β -cells initially augment their mass and functionality to meet the heightened insulin requirements.⁴ Nevertheless,



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as nutrient surplus persists, hyperglycemia and heightened free fatty acids adversely affect β -cell functionality via multiple mechanisms, such as the production of ROS, modifications in metabolic pathways, escalations in intracellular calcium and the induction of endoplasmic reticulum stress. These activities ultimately hinder insulin production, reduce insulin gene expression and induce β -cell death, hence worsening the existing insulin resistance and facilitating the advancement of type-2 diabetes.⁵

The treatment for type-2 diabetes consists of lifestyle modifications, such as diet and exercise, followed by the introduction of oral anti-diabetic agents and exogenous insulin administration. Lifestyle interventions, including a healthy diet and regular exercise, are the first-line approach to managing hyperglycemia and reducing the risk of diabetic complications.⁶ When diet and exercise alone are insufficient to control blood glucose levels, patients are often prescribed oral anti-diabetic medications. These drugs include metformin, sulfonylureas, dipeptidyl peptidase-4 inhibitors and incretin mimetics, each with its mechanisms of action and potential side effects.⁷ Additionally, the long-term utilization of these medications can be accompanied by side effects, like hypoglycemia, gastrointestinal issues and even hepatorenal disorders.⁸ In light of the limitations of current treatment options, the use of plant-based bioactive compounds has appeared as a useful complementary method to manage type 2 diabetes. Medicinal plants and their derived phytochemicals have been broadly explored for their anti-diabetic effects. These natural compounds were shown to modulate various pathways that participated in the pathophysiology of type 2 diabetes, including insulin resistance, β -cell function and glucose homeostasis.⁹ Eupatorin, a 3',5'-dihydroxy-4',6,7-trimethoxyflavone is a major bioactive polymethoxyflavone compound present in the *Orthosiphon stamineus*. The various biological properties of the eupatorin were already well reported, including antitumor, antimicrobial and anti-inflammatory, neuroprotective, antioxidant and vasorelaxant activities.¹⁰⁻¹⁴ However, the anti-diabetic properties of the eupatorin was not reported yet. Therefore, this work was designed to examine the anti-diabetic roles of the eupatorin against Streptozotocin (STZ)-induced rat model.

MATERIALS AND METHODS

Experimental rats

The eight-week-aged (210-225 g) Wistar rats were employed in this research. The rats were maintained in sterile enclosures under laboratory settings, with a temperature range of $21 \pm 2^\circ\text{C}$, humidity levels of 60-70% and a 12 hr alternating light and dark series. Animals were granted free access to standard feed and water during the research.

Experimental groups

Following a one-week acclimatization period, the rats were distributed into four groups. Group I: non-diabetic control rats administered only standard feed; Group II: rats administered STZ at a dosage of 55 mg/kg to produce the diabetic condition. After a three-day administration of STZ, blood glucose concentration was evaluated and rats demonstrating glucose levels over 250 mg/dL were defined as diabetic and chosen for subsequent investigations. Group III: rats were stimulated with STZ and subsequently administered 10 mg/kg of glibenclamide, a standard anti-diabetic drug for 15 days. Group IV: rats were stimulated with STZ and administered with eupatorin (20 mg/kg) (Sigma-Aldrich USA) for 15 days. On the concluding day, rats were sedated with an overdose of isoflurane and sacrificed via cervical dislocation. The blood samples were collected and centrifuged at 5000 rpm for 15 min, with serum samples stored at 4°C for biochemical studies. The tissue samples were excised for histological and biochemical analyses.

Analysis of blood glucose levels

Blood samples were obtained in the fed condition via cardiac puncture and transferred to plain tubes for serum collection, remaining undisturbed for 15 min before centrifugation at 10,000 rpm for 10 min. Glucose concentrations in serum samples were assessed utilizing the GOD-PAP colorimetric method as per the manufacturer's protocol (Elabscience, USA).

Analysis of biochemical markers

The serum concentrations of urea and creatinine were assessed using commercial diagnostic kits. The tests were conducted with three replicates following the assay kit manufacturer's specifications (Abcam, USA). The serum concentrations of liver marker enzymes, including Alanine Transaminase (ALT), Alkaline Phosphatase (ALP) and Aspartate Transaminase (AST) were studied using commercial kits. Each assay was conducted in triplicates with the specifications of the kit's manufacturer (MyBioSource, USA).

Analysis of lipid profile markers

The serum concentrations of lipid parameters Triglycerides (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) were studied using commercially procured diagnostic kits (Elabscience, USA).

Analysis of oxidative stress markers

The harvested liver tissues were homogenized in iced saline and thereafter subjected to centrifugation for 15 min. The resulting supernatant was employed to assess the Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Malondialdehyde (MDA) concentrations were assessed using the kits. The tests

were conducted in triplicate using the manufacturer's prescribed methods (Elabscience, USA).

Histopathological analysis

Post-surgical excision, the pancreas, liver and kidney tissues were treated with formalin (10%) solution. Subsequently, the graded ethanol was utilized for tissue dehydration and then embedded in paraffin. The paraffinized tissues were cut to 5 μm size utilizing a rotary microtome. Eosin and hematoxylin were utilized for staining the tissues before microscopic examination.

Statistical analysis

The data from each assay was revealed as a mean \pm SD from three replicates, which is studied using GraphPad. A one-way ANOVA and Tukey's *post hoc* test were utilized to examine the significance, with $p < 0.05$ as significance.

RESULTS

Effect of eupatorin on blood glucose in experimental rats

The impact of eupatorin on the blood glucose concentration in the experimental rats was assessed on the 1st, 7th and 16th day of experiments, with results presented in Figure 1. The rats with STZ-induced diabetes revealed a significant increase in the glucose level. Interestingly, the treatment with 20 mg/kg of eupatorin considerably diminished the glucose concentrations in the diabetic rats. The effects of eupatorin were further supported by the findings of the glibenclamide (10 mg/kg) treatment, which similarly decreases the glucose concentration in diabetic rats.

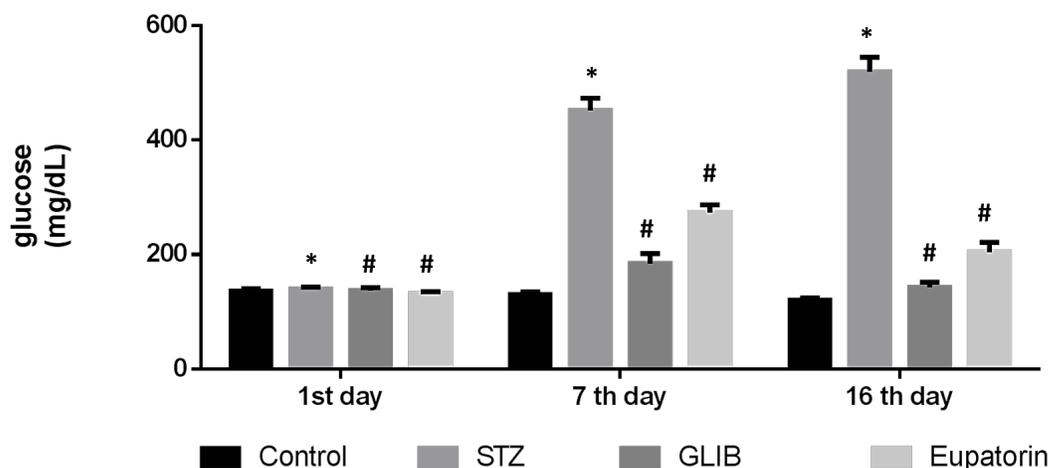


Figure 1: Effect of eupatorin on blood glucose level in the experimental rats. The findings are given as a mean \pm SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* tests were employed to assess the statistical significance. Note: "*" specifies statistical significance at $p < 0.01$ relative to the control (Group I); "#" specifies statistical significance at $p < 0.05$ relative to the STZ-induced diabetic group (Group II). STZ: Streptozotocin; GLIB: Glibenclamide.

Effect of eupatorin on urea and creatinine levels in experimental rats

Figure 2 illustrates the serum urea and creatinine concentrations of the experimental rats. The diabetic rats revealed a remarkable elevation in both creatinine and urea concentrations. Whereas, the eupatorin at 20 mg/kg concentration exhibited a considerable reduction in urea and creatinine concentrations in the diabetic rats. The standard drug glibenclamide (10 mg/kg) also considerably reduced urea and creatinine concentrations in diabetic rats, so further validating the efficacy of eupatorin.

Effect of eupatorin on hepatic marker enzymes in experimental rats

The serum concentrations of hepatic marker enzymes in the rats were studied and the findings are illustrated in Figure 3. The rats with diabetes demonstrated significant augmentations in ALT, ALP and AST concentrations in comparison to the control. Whereas, the treatment of 20 mg/kg of eupatorin remarkably diminished the ALT, ALP and AST concentrations in diabetic rats. The results of the eupatorin treatment were supported by the findings of glibenclamide (10 mg/kg) treatment, which similarly decreased the liver marker enzyme concentrations.

Effect of eupatorin on lipid parameters in experimental rats

The present findings demonstrated that the rats with STZ-induced diabetes displayed elevated TG, TC and LDL concentrations, along with a decreased HDL concentration in their serum (Figure 4). Interestingly, the 20 mg/kg of eupatorin treatment demonstrated a notable diminution in the TG, TC and LDL levels and an elevation in HDL levels in the serum of diabetic rats. Furthermore, glibenclamide (10 mg/kg) treatment also

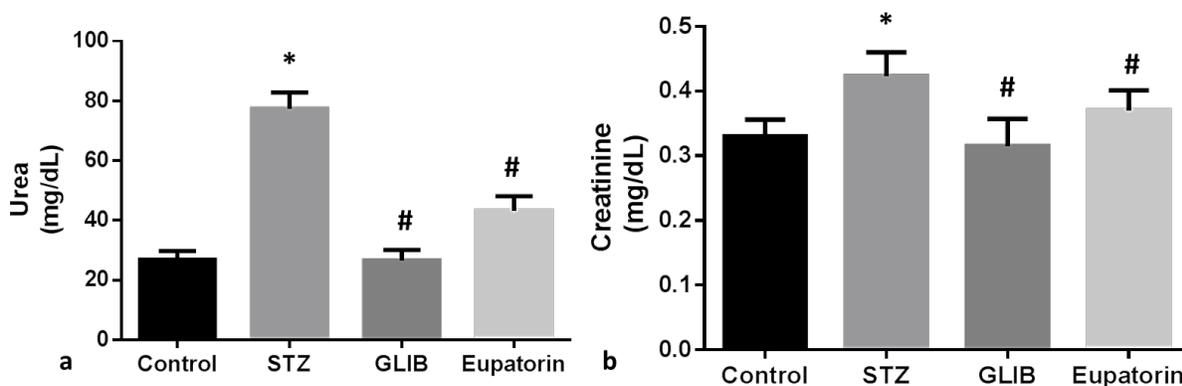


Figure 2: Effect of eupatorin on the urea and creatinine levels in the experimental rats. The findings are given as a mean \pm SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* tests were employed to assess the statistical significance. Note: '*' specifies statistical significance at $p < 0.01$ relative to the control (Group I); '#' specifies statistical significance at $p < 0.05$ relative to the STZ-induced diabetic group (Group II). (a): Urea (mg/dL); (b): Creatinine (mg/dL); STZ: Streptozotocin; GLIB: Glibenclamide.

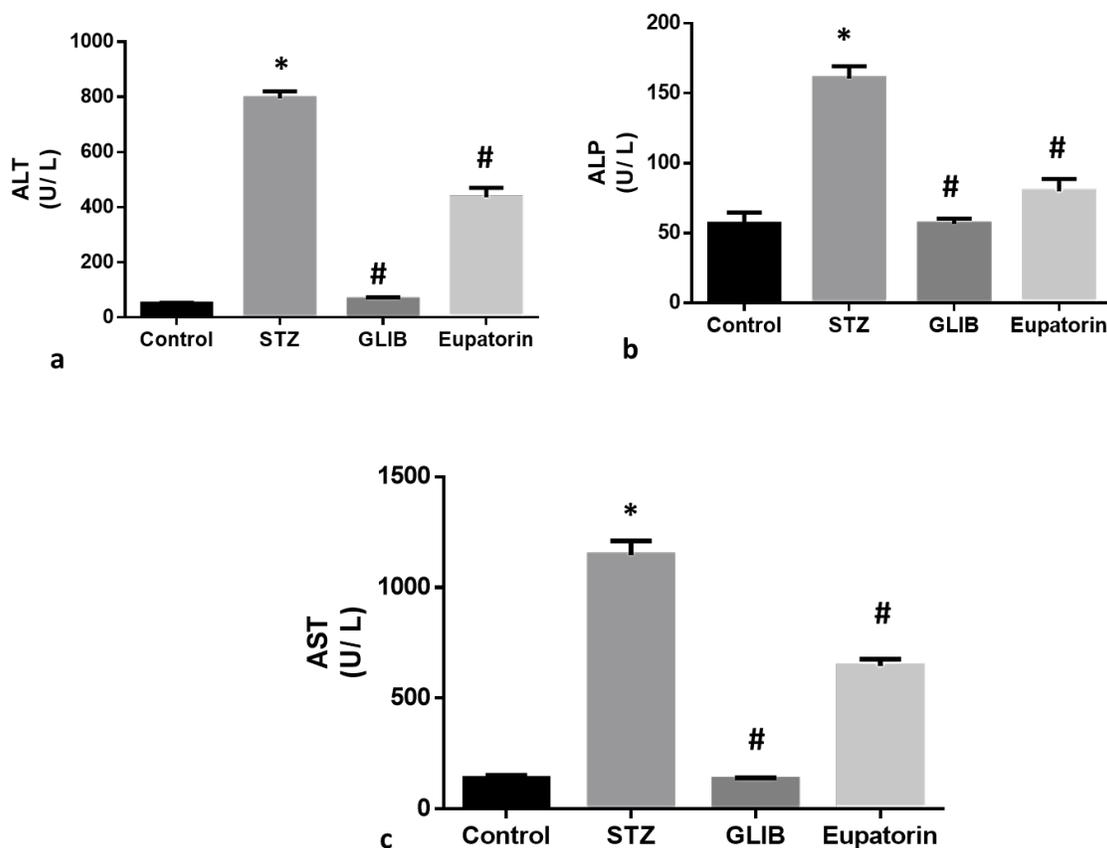


Figure 3: Effect of eupatorin on the liver marker enzymes in the experimental rats. The findings are given as a mean \pm SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* tests were employed to assess the statistical significance. Note: '*' specifies statistical significance at $p < 0.01$ relative to the control (Group I); '#' specifies statistical significance at $p < 0.05$ relative to the STZ-induced diabetic group (Group II). (a): ALT (U/L); (b): ALP (U/L); (c): AST (U/L); STZ: Streptozotocin; GLIB: Glibenclamide.

diminished the TG, TC and LDL concentrations and elevated the HDL level in the serum of diabetic rats, consequently supporting the activity of eupatorin.

Effect of eupatorin on oxidative stress markers in experimental rats

The current findings indicated that rats with STZ-induced diabetes exhibited reduced antioxidants SOD, CAT and GSH concentrations and a subsequent elevation in MDA in the liver tissues (Figure 5). Whereas, the eupatorin (20 mg/kg) treatment considerably augmented the antioxidants SOD, CAT and GSH levels while diminishing MDA levels in diabetic rats. Moreover, the glibenclamide (10 mg/kg) also increased the antioxidants and reduced the MDA in diabetic rats, thereby reinforcing the antioxidant properties of eupatorin.

Effect of eupatorin on pancreas, liver and kidney histology of the experimental rats

Figures 6-8 illustrate the results of a histological assessment of the pancreas, liver and renal tissues of the experimental rats, respectively. The pancreas, liver and kidney tissues from the non-diabetic control rats exhibited no changes and demonstrated usual cell structures. Conversely, the pancreatic tissue of STZ-induced diabetic rats exhibited increased inflammatory cell infiltrations, constriction of islet cells and enlarged adipose

tissue (Figure 6). The livers of the diabetic rats revealed marked inflammatory cell infiltrations and hepatocyte injury (Figure 7). Furthermore, the kidneys of the diabetic rats exhibited damaged glomerular architecture, vacuolar degeneration and increased inflammatory cell infiltrations (Figure 8). Captivatingly, these histological changes in the pancreas, liver and kidney tissues caused by STZ were significantly alleviated by the 20 mg/kg of eupatorin, as demonstrated by the reduction in pancreatic damages, adipose tissue size, inflammation, hepatocyte injury and glomerular damages. Similar results were also observed in the glibenclamide (10 mg/kg) treatment, which supports the therapeutic effects of eupatorin.

DISCUSSION

Diabetes mellitus is a common and debilitating metabolic condition marked by chronic hyperglycemia, which can result in numerous serious consequences if not managed promptly. One crucial aspect of diabetes management is the close monitoring and control of blood glucose levels. STZ-induced diabetes, a widely used animal model for studying the pathophysiology and treatment of the disease, provides a useful platform to investigate the importance and applications of blood glucose analysis.¹⁵ The analysis of blood glucose in the STZ-induced diabetes model is crucial for numerous applications. It offers a reliable model of the successful induction of the diabetic state, as STZ treatment

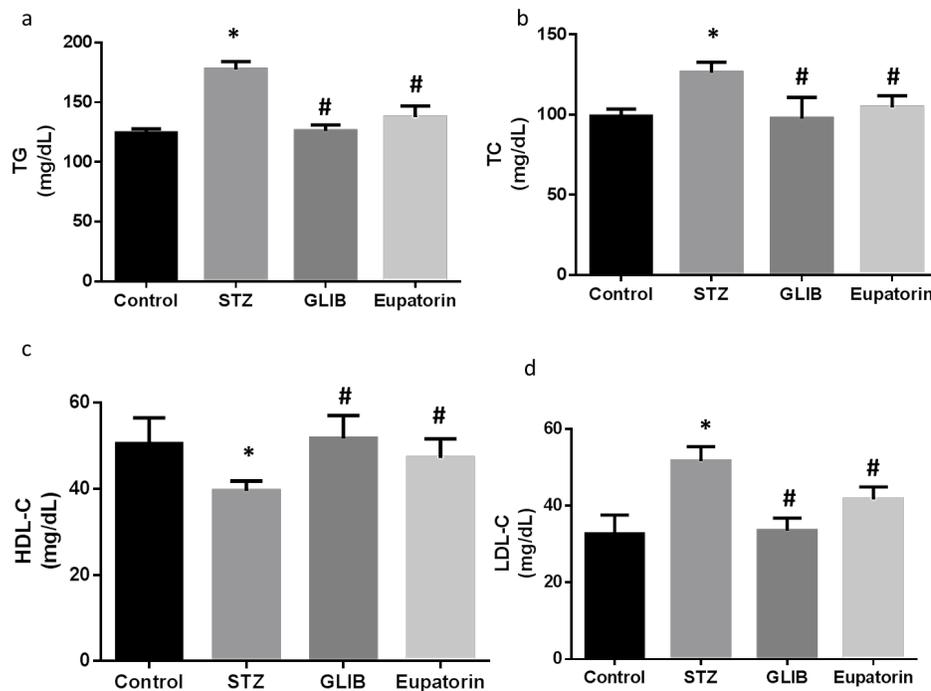


Figure 4: Effect of eupatorin on the lipid profiles in the experimental rats. The findings are given as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* tests were employed to assess the statistical significance.

Note: '*' specifies statistical significance at $p < 0.01$ relative to the control (Group I); '#' specifies statistical significance at $p < 0.05$ relative to the STZ-induced diabetic group (Group II). (a): TG (mg/dL); (b): TC (mg/dL); (c): HDL-C (mg/dL); (d): LDL-C (mg/dL); STZ: Streptozotocin; GLIB: Glibenclamide.

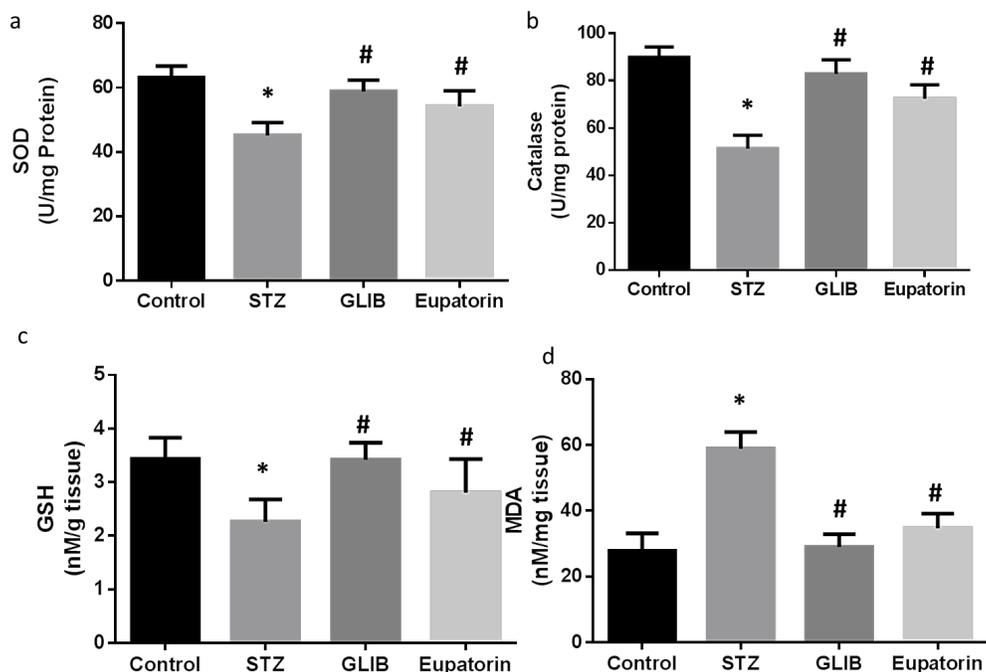


Figure 5: Effect of eupatorin on the oxidative stress markers in the experimental rats. The findings are given as a mean±SD of triplicate assays. A one-way ANOVA and Tukey's *post hoc* tests were employed to assess the statistical significance. Note: "*" specifies statistical significance at $p < 0.01$ relative to the control (Group I); "#" specifies statistical significance at $p < 0.05$ relative to the STZ-induced diabetic group (Group II). (a): SOD (U/mg protein); (b): CAT (U/mg protein); (c): GSH (nM/g tissue); (d): MDA (nM/mg tissue); STZ: Streptozotocin; GLIB: Glibenclamide.

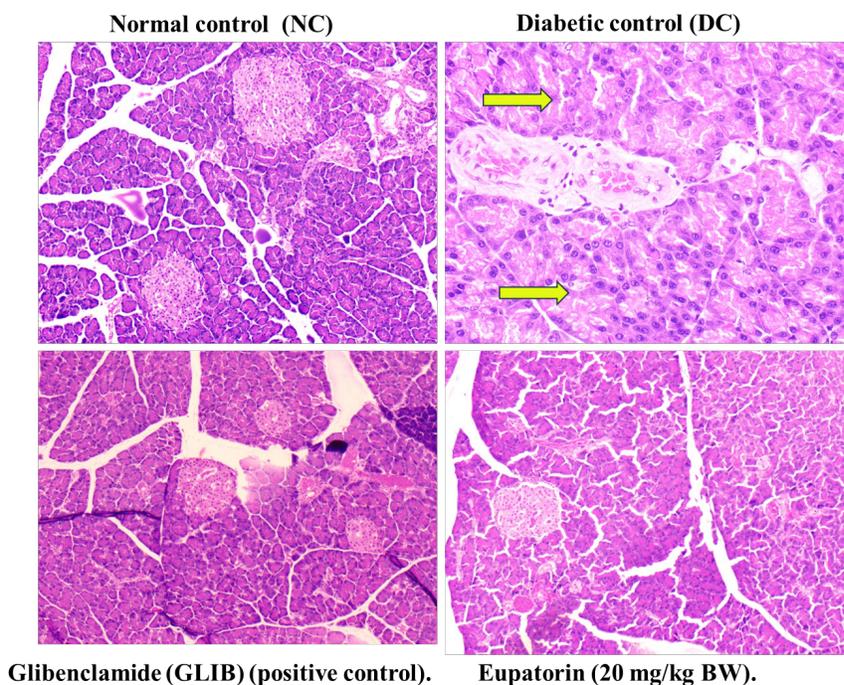


Figure 6: Effect of eupatorin on the pancreas tissue histopathology of the experimental rats. Control: The pancreas tissues from the healthy control rats demonstrated normal cellular architecture. STZ: The pancreas of STZ-induced diabetic rats exhibited increased infiltration of inflammatory cells, constriction of pancreatic islet cells and enlarged adipose tissue. GLIB and Eupatorin: The treatment with 10 mg/kg of glibenclamide and 20 mg/kg of eupatorin, respectively ameliorated the STZ-induced histological damage in the pancreas tissues of diabetic rats. STZ: Streptozotocin; GLIB: Glibenclamide.

is known to selectively damage pancreatic β -cells, resulting in a significant elevation in blood glucose levels.¹⁶ The continuous monitoring of blood glucose concentrations in STZ-induced diabetic models allows for the evaluation of the efficacy of various therapeutic interventions. By closely tracking the changes in blood glucose levels, researchers can assess the impact of treatments, such as insulin administration, dietary modifications, or novel pharmacological agents, on glycemic control. This data is crucial to developing and optimizing effective diabetes management techniques.¹⁷ It has been found that STZ-induced diabetic rats exhibited a range of characteristic symptoms, including decreased body weight, accompanied by significantly elevated glucose levels. These data suggest the significance of blood glucose analysis in verifying the successful induction of the diabetic state and monitoring the progression of the disease.¹⁸ In this work, we found that rats with STZ-induced diabetes demonstrated elevated glucose concentrations. However, the eupatorin treatment appreciably diminished the glucose concentrations in diabetic rats, which evidences its hypoglycemic activity.

Diabetes mellitus often leads to various microvascular and macrovascular difficulties. Diabetic kidney disease is a major consequence of diabetes that can advance to end-stage renal failure. Monitoring serum urea and creatinine levels in persons

with diabetes is essential for the early detection and treatment of diabetic kidney disease.¹⁹ Urea and creatinine are two important biomarkers that reflect the function of the kidneys. Urea is a by-product of protein metabolism and its concentration in the blood is affected by dietary protein consumption, hepatic activity and renal filtration rate. Creatinine, on the other hand, is a by-product of muscle metabolism and is primarily filtered out by the kidneys. In individuals with diabetes, the creatinine and urea in the serum can provide valuable insights into the onset of diabetic kidney disease.²⁰ The analysis of creatinine and urea levels in the serum of STZ-induced diabetic animal models can help researchers understand the pathophysiology of diabetic kidney disease and evaluate the effectiveness of potential therapeutic interventions.²¹ The urea and creatinine concentrations are typically augmented in diabetic animals than the non-diabetic counterparts, indicating the presence of impaired kidney function.²² Furthermore, the degree of creatinine and urea concentrations can be correlated with the severity of diabetic kidney disease, providing valuable information for disease monitoring and treatment. Similarly, the current findings also demonstrated the drastic elevation in the serum concentrations of urea and creatinine in diabetic rats. Fascinatingly, the eupatorin treatment exhibited a considerable reduction in both urea and creatinine concentrations in the diabetic rats. The findings evidence that eupatorin can protect kidney functions during diabetic conditions.

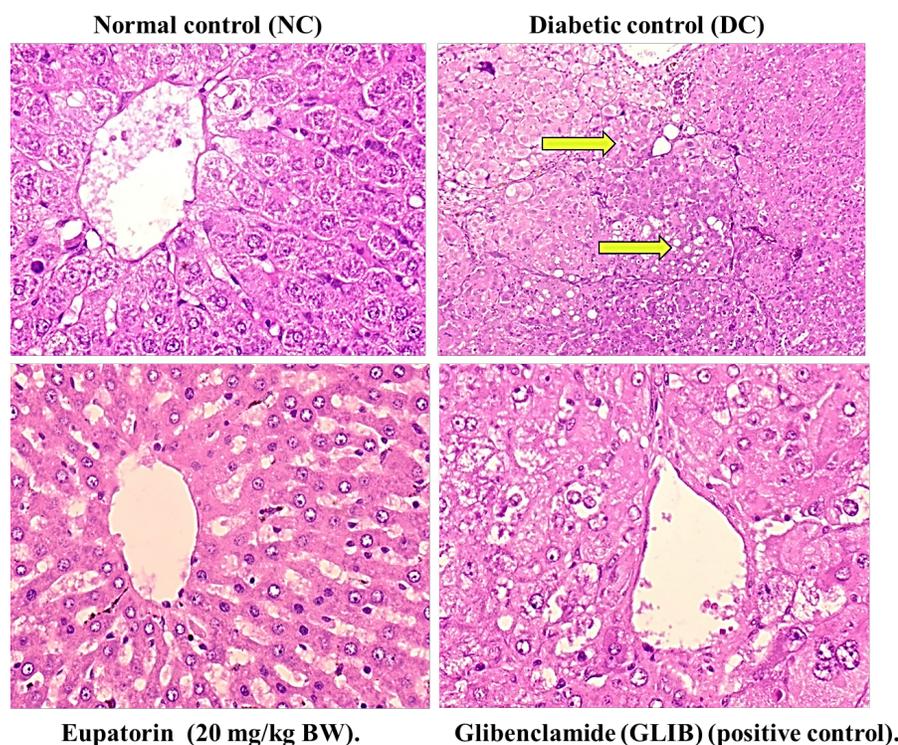


Figure 7: Effect of eupatorin on the liver tissue histopathology of the experimental rats. Control: The liver tissues from the healthy control rats demonstrated normal cellular arrangements. STZ: The livers of the STZ-induced diabetic rats displayed significant inflammatory cell infiltrations and hepatocyte injury. GLIB and Eupatorin: The glibenclamide (10 mg/kg) and eupatorin (20 mg/kg) treatments, respectively considerably reduced the STZ-induced histological changes in the liver tissues of diabetic rats. STZ: Streptozotocin; GLIB: Glibenclamide.

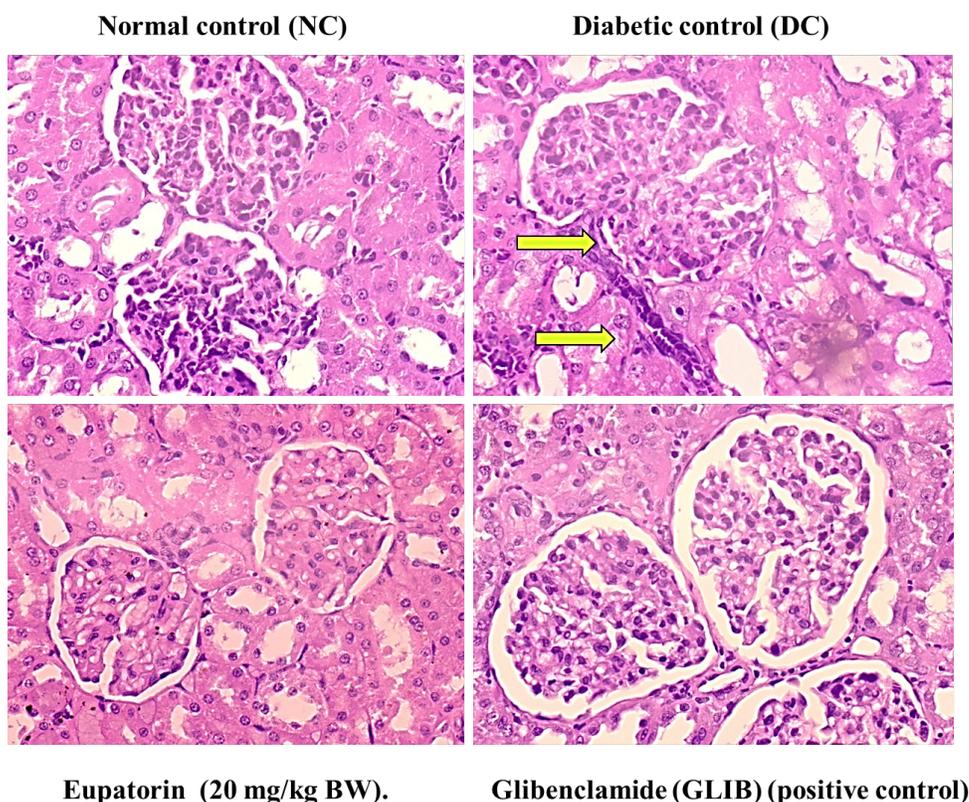


Figure 8: Effect of eupatorin on the kidney tissue histopathology of the experimental rats. Control: The kidney tissues from the healthy control rats displayed normal cell structures. STZ: The kidney tissues from the STZ-induced diabetic rats displayed damaged glomerular architecture, vacuolar degeneration and increased inflammatory cell infiltrations. GLIB and Eupatorin: The treatments with glibenclamide (10 mg/kg) and eupatorin (20 mg/kg), respectively demonstrated a remarkable reduction in the STZ-induced histological changes in the kidney tissues of diabetic rats. STZ: Streptozotocin; GLIB: Glibenclamide.

The analysis of specific liver marker enzymes is crucial for the understanding of metabolic and physiological changes associated with diabetic conditions. ALT is an enzyme primarily found in the liver and is considered a sensitive marker for hepatocellular injury. In STZ-induced diabetes, elevated levels of ALT have been observed, indicating the presence of liver damage or dysfunction. This elevation in ALT can be due to the disruption of normal liver function and the release of the enzyme into the bloodstream.²³ ALP, another liver marker enzyme, has also been shown to be elevated in STZ-induced diabetes. ALP levels reflect the integrity of bile ducts and the overall status of liver function. AST, a marker of both liver and muscle damage, is often elevated in diabetic conditions, including STZ-induced diabetes.²⁴ The analysis of these liver marker enzymes in diabetic models can provide valuable insights into the pathophysiology of the disease. For instance, the increased ALT, ALP and AST can indicate the presence of hepatic complications related to diabetes, such as liver inflammation and impaired liver function.²⁵ Furthermore, monitoring the changes in these liver enzymes over time can help researchers understand the onset of disease and the potential development of liver-associated problems in diabetic conditions. Analyzing liver marker enzymes in STZ-induced diabetes can also have important applications in the development

of new therapeutic interventions. Additionally, the use of liver enzyme markers can aid in the early detection and monitoring of diabetes-related liver complications, allowing for timely interventions and improved patient management.²⁶ The findings of this work indicated that the serum concentrations of liver marker enzymes, including ALT, AST and ALP were drastically increased in the serum of diabetic rats. Considerably, the eupatorin treatment reduced these enzyme concentrations in the diabetic rats. These findings evidenced that eupatorin treatment can maintain hepatic homeostasis and prevent hepatocellular injury during diabetes.

Dyslipidemia, defined by aberrant lipid and lipoprotein levels in the bloodstream, is a prevalent complication linked to diabetes and significantly contributes to the disease's pathogenesis. Dyslipidemia, marked by raised TG, reduced HDL and an elevation in LDL, is a prevalent characteristic of insulin resistance and diabetes. This dyslipidemic profile is thought to be driven by insulin resistance, which leads to increased hepatic LDL production and decreased clearance of TG-rich lipoproteins. In the context of diabetes, understanding the changes in these lipid profile parameters is essential to understanding the advancement of the disease and guiding clinical management.²⁷ TGs are lipids

that are stored in the body's adipose tissue and used as an energy source. In diabetic conditions, the increased TG levels can be attributed to the impaired regulation of lipid metabolism due to insulin deficiency or resistance. TC, on the other hand, is a measure of the overall cholesterol levels in the blood, including both LDL and HDL. In diabetic conditions, TC levels have been shown to increase, reflecting the dysregulation of cholesterol metabolism.²⁸ LDL is known as the "bad" cholesterol, as it develops the plaque in the arteries, resulting in the development of cardiovascular diseases. The LDL levels have been observed to increase in the diabetic condition, further exacerbating the risk of cardiovascular problems. HDL, commonly known as the "good" cholesterol, plays an essential role in the reverse transport of cholesterol from the tissues to the liver for excretion. HDL levels have been reported to decrease in diabetic conditions, indicating an impairment in the protective mechanisms against cardiovascular disease.²⁹ The alterations in lipid metabolism can participate in the onset of diabetic complications, like diabetic retinopathy, nephropathy and cardiovascular disease, highlighting the importance of monitoring and managing lipid profiles in diabetes management.³⁰ The results of this study also exhibited that the STZ-induced diabetic rats exhibited elevated TG, TC and LDL, along with a diminished HDL concentration in their serum. Captivatingly, the eupatorin treatment appreciably diminished the TG, TC and LDL and augmented the HDL concentration in diabetic rats. The current findings evidenced the antilipidemic properties of the eupatorin.

Oxidative stress is known as a disproportion between the production of free radicals and the neutralizing capacity of the body's antioxidant defense systems. Hyperglycemic circumstances result in the overproduction of ROS, which can cause oxidative injury to cellular proteins, lipids and DNA, so facilitating the onset of diabetic complications, including cardiovascular disease, neuropathy, nephropathy and retinopathy.³¹ The pathophysiology of insulin resistance, a hallmark of type-2 diabetes, has also been closely linked to oxidative stress. Oxidative stress can impair insulin signaling pathways, leading to decreased glucose uptake in peripheral tissues and facilitate insulin resistance. In addition to the direct cellular damage caused by free radicals, oxidative stress also plays a role in the activation of numerous metabolic signals that participate in the onset of diabetic complications.³² The primary antioxidants, such as SOD, CAT and GSH, are often altered during diabetic conditions, contributing to the onset of oxidative stress. SOD is responsible for the conversion of superoxide radicals to H_2O_2 , while CAT catalyzes the breakdown of H_2O_2 to water and oxygen. GSH plays an essential role in the neutralization of ROS and in maintaining the cellular redox balance. In addition to these primary antioxidants, MDA, a marker of lipid peroxidation, is commonly utilized to study the degree of oxidative stress in diabetic conditions.³³ The liver

is one of the primary organs that can be significantly impacted by the metabolic disturbances associated with diabetes. One of the hallmarks of diabetes-induced liver damage is the alteration of key antioxidant enzymes and metabolites.³⁴ The analysis of these parameters in diabetic animals can offer useful insights into the underlying mechanisms of diabetes-associated hepatic dysfunction.³⁵ Similarly, the present results demonstrated that STZ-induced diabetic rats exhibited diminished SOD, CAT and GSH concentrations and a subsequent elevation in MDA levels in the liver tissues. Fascinatingly, the eupatorin treatment markedly elevated the antioxidant concentrations while reducing the MDA level in diabetic rats. These data suggest that eupatorin treatment mitigates oxidative stress and maintains hepatic homeostasis in diabetic rats. Captivatingly, these histological changes in the tissues caused by STZ were significantly alleviated by the 20 mg/kg of eupatorin, as demonstrated by the reduction in pancreatic damage, adipose tissue size, inflammation, hepatocyte injury and glomerular damage.

CONCLUSION

The present results indicate that eupatorin may significantly mitigate diabetic conditions and their associated difficulties in STZ-induced diabetic rats. The treatment with eupatorin significantly reduced glucose, urea, creatinine and liver marker enzyme concentrations in the diabetic rats. Additionally, the eupatorin treatment regulated the lipid parameters and mitigated oxidative stress by subsequently elevating antioxidants in diabetic rats. Moreover, histological analyses of the pancreas, liver and kidney tissues show that eupatorin treatment can protect vital organs from diabetes-induced histological changes. Consequently, the present data highlight that eupatorin may serve as an effective therapeutic alternative for diabetes management.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

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ETHICAL STATEMENTS

Health Research Centre, Jazan University, Jazan, Saudi Arabia. (REC-46/03/1200).

ABBREVIATIONS

STZ: Streptozotocin; **ALT:** Alanine Transaminase; **ALP:** Alkaline Phosphatase; **AST:** Aspartate Transaminase; **TG:** Triglycerides; **TC:** Total Cholesterol; **HDL:** High-Density Lipoprotein; **LDL:** Low-Density Lipoprotein; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **GSH:** Glutathione; **MDA:** Malondialdehyde.

REFERENCES

- NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants. *Lancet*. 2024;404(10467):2077-93. doi: 10.1016/S0140-6736(24)02317-1, PMID 39549716 (NCD, RisC).
- Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 9th ed. 2019;157:107843. doi: 10.1016/j.diabres.2019.107843, PMID 31518657.
- Bodke H, Wagh V, Kakar G. Diabetes mellitus and prevalence of other comorbid conditions: A systematic review. *Cureus*. 2023;15(11):e49374. doi: 10.7759/cureus.49374, PMID 38146555.
- Lee SH, Park SY, Choi CS. Insulin resistance: from mechanisms to therapeutic strategies. *Diabetes Metab J*. 2022;46(1):15-37. doi: 10.4093/dmj.2021.0280, PMID 34965646.
- Galicia-García U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. *Int J Mol Sci*. 2020;21(17):6275. doi: 10.3390/ijms21176275, PMID 32872570.
- Davies MJ, Aroda VR, Collins BS, Gabbay RA, Green J, Maruthur NM, et al. Management of hyperglycaemia in type 2 diabetes, 2022. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*. 2022;65(12):1925-66. doi: 10.1007/s00125-022-05787-2, PMID 36151309.
- Galindo RJ, Trujillo JM, Low Wang CC, McCoy RG. Advances in the management of type 2 diabetes in adults. *BMJ Med*. 2023;2(1):e000372. doi: 10.1136/bmjmed-2022-000372, PMID 37680340.
- Holt RI, DeVries JH, Hess-Fischl A, Hirsch IB, Kirkman MS, Klupa T, et al. The management of type 1 diabetes in adults: a consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*. 2021;44(11):2589-625. doi: 10.2337/dci21-0043, PMID 34593612.
- Sukhikh S, Babich O, Prosekov A, Kalashnikova O, Noskova S, Bakhtiyarova A, et al. Antidiabetic properties of plant secondary metabolites. *Metabolites*. 2023;13(4):513. doi: 10.3390/metabo13040513, PMID 37110171.
- Abd Razak N, Yeap SK, Alitheen NB, Ho WY, Yong CY, Tan SW, et al. Eupatorin suppressed tumor progression and enhanced immunity in a 4T1 murine breast cancer model. *Integr Cancer Ther*. 2020;19:1534735420935625. doi: 10.1177/1534735420935625, PMID 32830560.
- González-Cortazar M, Salinas-Sánchez DO, Herrera-Ruiz M, Román-Ramos DC, Zamilpa A, Jiménez-Ferrer E, et al. Eupatorin and Salvianidin-A, with antimicrobial and anti-inflammatory effects from *Salvia lavanduloides* Kunth Leaves. *Plants (Basel)*. 2022;11(13):1739. doi: 10.3390/plants11131739, PMID 35807691.
- Rizou AE, Nasi GI, Paikopoulos Y, Bezantakou DS, Vraila KD, Spatharas PM, et al. A multilevel study of eupatorin and scutellarein as anti-amyloid agents in Alzheimer's disease. *Biomedicines*. 2023;11(5):1357. doi: 10.3390/biomedicines11051357, PMID 37239029.
- Akowiuh G, Zhari I, Norhayati I, Sadikun A, Khamsah S. Sinensetin, eupatorin, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. *Food Chem*. 2004;87:559-66.
- Yam MF, Tan CS, Ahmad M, Shiba R. Mechanism of vasorelaxation induced by eupatorin in the rats aortic ring. *Eur J Pharmacol*. 2016;789:27-36. doi: 10.1016/j.ejphar.2016.06.047, PMID 27370961.
- Singh R, Gholipourmalekabadi M, Shafikhani SH. Animal models for type 1 and type 2 diabetes: advantages and limitations. *Front Endocrinol*. 2024;15:1359685. doi: 10.3389/fendo.2024.1359685, PMID 38444587.
- Febriza A, Zahrah AA, Andini NS, Usman F, Idrus HH. Potential effect of curcumin in lowering blood glucose level in streptozotocin-induced diabetic rats. *Diabetes Metab Syndr Obes*. 2024;17:3305-13. doi: 10.2147/DMSO.S468059, PMID 39247432.
- Si Q, Guo J, Yang X, Guo Y, Wu L, Xie D, et al. Systematic assessment of streptozotocin-induced diabetic metabolic alterations in rats using metabolomics. *Front Endocrinol*. 2023;14:1107162. doi: 10.3389/fendo.2023.1107162, PMID 36761194.
- Rehman HU, Ullah K, Rasool A, Manzoor R, Yuan Y, Tareen AM, et al. Comparative impact of streptozotocin on altering normal glucose homeostasis in diabetic rats compared to normoglycemic rats. *Sci Rep*. 2023;13(1):7921. doi: 10.1038/s41598-023-29445-8, PMID 37193696.
- Chtourou Y, Morjen M, Ammar R, Mhiri R, Jemaà M, ELBini-Dhouib I, et al. Investigation of the renal protective effect of combined dietary polyphenols in streptozotocin-induced diabetic aged rats. *Nutrients*. 2022;14(14):2867. doi: 10.3390/nu14142867, PMID 35889824.
- Kokate D, Marathe P. Evaluation of effect of montelukast in the model of streptozotocin-induced diabetic nephropathy in rats. *Indian J Endocrinol Metab*. 2024;28(1):47-54. doi: 10.4103/ijem.ijem_414_22, PMID 38533280.
- Yan LJ. The nicotinamide/streptozotocin rodent model of type 2 diabetes: renal pathophysiology and redox imbalance features. *Biomolecules*. 2022;12(9):1225. doi: 10.3390/biom12091225, PMID 36139064.
- Rahmani AH, Alsahli MA, Khan AA, Almatroodi SA. Quercetin, a plant flavonol attenuates diabetic complications, renal tissue damage, renal oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Metabolites*. 2023;13(1):130. doi: 10.3390/metabo13010130, PMID 36677055.
- Ghanbari E, Nejadi V, Khazaei M. Improvement in Serum Biochemical Alterations and Oxidative Stress of Liver and Pancreas following Use of Royal Jelly in Streptozotocin-Induced Diabetic Rats. *Cell J*. 2016;18(3):362-70. doi: 10.22074/cellj.2016.4564, PMID 27602318.
- Amirkhosravi L, Kordestani Z, Nikooei R, Safi Z, Yeganeh-Hajhamedi M, Mirtajaddini-Goki M. Exercise-related alterations in MCT1 and GLUT4 expressions in the liver and pancreas of rats with STZ-induced diabetes. *J Diabetes Metab Disord*. 2023;22(2):1355-63. doi: 10.1007/s40200-023-01255-9, PMID 37975118.
- Azimi M, Mehrzad J, Ahmadi E, Orafi M, Aghaie F, Ahmadi A, et al. The effect of *Thymus vulgaris* on hepatic enzymes activity and apoptosis-related gene expression in streptozotocin-induced diabetic rats. *Evid Based Complement Alternat Med*. 2022;2022:2948966. doi: 10.1155/2022/2948966, PMID 35368767.
- Arabloei Sani M, Yaghmaei P, Hajebrami Z, Hayati Roodbari N. Therapeutic effect of P-cymene on lipid profile, liver enzyme and Akt/mTOR pathway in streptozotocin-induced diabetes mellitus in Wistar rats. *J Obes*. 2022;2022:1015669. doi: 10.1155/2022/1015669, PMID 35528246.
- Tiwari DD, Thorat VM, Pakale PV, Patil S, Chavan D. Evaluation of Lipid Profile Modulation by *Berberis asiatica*, *Withania somnifera* and Their Synergy in Type 2 Diabetic Wistar rats. *Cureus*. 2024;16(8):e67974. doi: 10.7759/cureus.67974, PMID 39347326.
- Andonova M, Dzhelebov P, Trifonova K, Yonkova P, Kostadinov N, Nancheva K, et al. Metabolic markers associated with progression of type 2 diabetes induced by high-fat diet and single low dose streptozotocin in rats. *Vet Sci*. 2023;10(7):431. doi: 10.3390/vetsci10070431, PMID 37505836.
- Raval KY, Tirgar PR. A preclinical study to investigate the antidiabetic potential of p-propoxybenzoic acid as a multi-target inhibitor in streptozotocin-nicotinamide-induced type-2 diabetic rats. *J Diabetes Metab Disord*. 2023;22(1):571-80. doi: 10.1007/s40200-022-01177-y, PMID 37255789.
- Bryl A, Mrugacz M, Falkowski M, Zorena K. The effect of hyperlipidemia on the course of diabetic retinopathy-literature review. *J Clin Med*. 2022;11(10):2761. doi: 10.3390/jcm11102761, PMID 35628887.
- Dos Santos JM, Zhong Q, Benite-Ribeiro SA, Heck TG. New insights into the role of oxidative stress in the development of diabetes mellitus and its complications. *J Diabetes Res*. 2023;2023:9824864. doi: 10.1155/2023/9824864, PMID 37546353.
- Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bull Exp Biol Med*. 2021;171(2):179-89. doi: 10.1007/s10517-021-05191-7, PMID 34173093.
- Shrivastav D, Dabla PK, Sharma J, Viswas A, Mir R. Insights on antioxidant therapeutic strategies in type 2 diabetes mellitus: A narrative review of randomized control trials. *World J Diabetes*. 2023;14(6):919-29. doi: 10.4239/wjcd.v14.i6.919, PMID 37383600.
- Najafi A, Pourfarzam M, Zadhoush F. Oxidant/antioxidant status in Type-2 diabetes mellitus patients with metabolic syndrome. *J Res Med Sci*. 2021;26:6. doi: 10.4103/jrms.JRMS_249_20, PMID 34084185.
- Mansouri RA, Ahmad A, Roushdy MM, Alshaihi HF, Ragab M. Pharmacological studies on the antidiabetic, antioxidant and antimicrobial efficacies of *Commiphora myrrha* resin in Streptozotocin-Induced Diabetes in Rats: A Preclinical Study. *J Diabetes Res*. 2023;2023:5478267. doi: 10.1155/2023/5478267, PMID 36825257.

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