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ORIGINAL ARTICLE

Evaluation of the Antihyperglycemic and Antioxidant Potential of Hesperetin and Vitamin C as Adjuncts to Metformin in Streptozotocin-Induced Experimental Diabetes: Implications for Public Health and Community-Based Diabetes Prevention

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and increased oxidative stress, which contribute to the development of long-term complications. Conventional antidiabetic drugs such as Metformin effectively control blood glucose levels; however, additional therapeutic strategies targeting oxidative stress may enhance treatment outcomes. Natural antioxidants including Vitamin C and the citrus flavonoid Hesperetin have demonstrated potential antidiabetic and antioxidant properties in experimental studies.

Objective: The present study aimed to evaluate the antidiabetic and antioxidant effects of hesperetin alone and in combination with vitamin C in metformin-treated Streptozotocin-induced diabetic rats and it's implications for public health and community base diabetes prevention.

Methods: Experimental diabetes was induced in albino rats using streptozotocin. Animals were randomly divided into six groups including normal control, diabetic control, metformin-treated, metformin plus vitamin C, metformin plus hesperetin, and metformin combined with vitamin C and hesperetin. The treatment period lasted for 60 days. Fasting blood glucose, random blood glucose, glycated hemoglobin (HbA1c), and oxidative stress markers including malondialdehyde, glutathione, and superoxide dismutase were evaluated to assess glycemic control and antioxidant status.

Results: Streptozotocin administration produced significant hyperglycemia and oxidative stress in diabetic rats. Treatment with metformin significantly reduced fasting and random blood glucose levels and improved HbA1c compared with the untreated diabetic group. The addition of vitamin C or hesperetin further improved glycemic parameters and antioxidant status. The combination therapy of metformin, vitamin C, and hesperetin demonstrated the most pronounced effects, showing substantial reductions in blood glucose, HbA1c, and malondialdehyde levels while enhancing glutathione and superoxide dismutase activity.

Conclusion: The findings indicate that hesperetin and vitamin C enhance the antihyperglycemic and antioxidant effects of metformin in streptozotocin-induced diabetic rats. Combination therapy may represent a promising strategy for improving glycemic control and reducing oxidative stress associated with diabetes.

Keywords: Diabetes mellitus, Metformin, Hesperetin, Vitamin C, Oxidative stress, Streptozotocin, Antioxidants.

INTRODUCTION

Diabetes mellitus represents one of the most significant global health challenges of the twenty-first century, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease is associated with disturbances in carbohydrate, lipid, and protein metabolism that ultimately lead to long-term damage, dysfunction, and failure of multiple organs including the eyes, kidneys, nerves, heart, and blood vessels. The prevalence of diabetes has increased dramatically over the past few decades due to urbanization, sedentary lifestyles, aging populations, and dietary transitions toward high-calorie processed foods. According to the International Diabetes Federation, hundreds of millions of individuals worldwide are currently living with diabetes, and this number is expected to rise substantially in the coming decades, posing a considerable burden on healthcare systems and economies globally[1]. Among the various forms of diabetes, type 2 diabetes mellitus accounts for approximately 90–95% of all diagnosed cases and is strongly associated with insulin resistance, impaired insulin secretion, and progressive β-cell dysfunction[2].Persistent hyperglycemia in diabetes triggers several metabolic and biochemical abnormalities that contribute to the development of chronic complications. One of the major pathogenic mechanisms involved in diabetic complications is oxidative stress, which results from an imbalance between the generation of reactive oxygen species and the antioxidant defense systems of the body[3].

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Chronic hyperglycemia enhances the production of reactive oxygen species through multiple pathways including glucose autoxidation, activation of the polyol pathway, increased formation of advanced glycation end products, and mitochondrial dysfunction. These processes collectively damage cellular structures such as lipids, proteins, and nucleic acids, thereby contributing to the pathogenesis of diabetic complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases[4]. Oxidative stress has therefore emerged as a key therapeutic target in the management of diabetes and its associated complications.

Experimental models of diabetes play an essential role in understanding the pathophysiology of the disease and evaluating potential therapeutic interventions. Among these models, chemical induction of diabetes using streptozotocin has been widely employed due to its ability to selectively damage pancreatic β-cells. Streptozotocin is a naturally occurring nitrosourea compound that preferentially accumulates in pancreatic β-cells via the glucose transporter GLUT2. Once inside the cell, streptozotocin induces DNA alkylation and oxidative stress, leading to β-cell destruction and subsequent hyperglycemia[5]. Because of its reproducibility and reliability, the streptozotocin-induced diabetic rat model has been extensively used for investigating the pathogenesis of diabetes and for screening antidiabetic agents[6]. This model mimics many of the metabolic and biochemical alterations observed in human

diabetes, including hyperglycemia, increased glycated hemoglobin, and elevated oxidative stress markers. Pharmacological management of type 2 diabetes primarily focuses on controlling blood glucose levels and preventing long-term complications. Among the available antidiabetic drugs, Metformin is widely regarded as the first-line therapy for the treatment of type 2 diabetes. Metformin belongs to the biguanide class of oral hypoglycemic agents and exerts its antihyperglycemic effects primarily by reducing hepatic glucose production and improving peripheral insulin sensitivity[7]. Additionally, metformin enhances glucose uptake in skeletal muscle and adipose tissue while decreasing intestinal glucose absorption. Several clinical studies have demonstrated that metformin effectively reduces fasting blood glucose levels and glycated hemoglobin, thereby improving glycemic control in diabetic patients[8]. Beyond its glucose-lowering effects, metformin has also been reported to possess beneficial properties such as improvement in lipid metabolism, reduction in cardiovascular risk, and modest antioxidant effects[9]. Despite its well-established efficacy, monotherapy with metformin may not fully address the complex pathophysiological mechanisms underlying diabetes, particularly oxidative stress and inflammation.

Growing scientific evidence suggests that supplementation with natural antioxidants may provide additional therapeutic benefits when combined with conventional antidiabetic medications. Antioxidants can neutralize reactive oxygen species and enhance endogenous antioxidant defense systems, thereby reducing oxidative damage associated with chronic hyperglycemia. Among natural antioxidant compounds, vitamin C has attracted considerable attention due to its potent free radical scavenging activity and its essential role in maintaining cellular redox balance. Vitamin C, also known as ascorbic acid, is a water-soluble vitamin that participates in numerous biochemical reactions including collagen synthesis, immune regulation, and antioxidant defense[10]. In diabetic conditions, plasma levels of vitamin C are often reduced due to increased oxidative stress and impaired cellular uptake. Supplementation with vitamin C has been reported to improve glycemic control, enhance antioxidant status, and reduce lipid peroxidation in experimental and clinical studies[11]. These findings highlight the potential role of vitamin C as an adjunct therapy in diabetes management. In recent years, increasing attention has also been directed toward naturally occurring flavonoids due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, and antidiabetic effects. Flavonoids are polyphenolic compounds widely distributed in fruits, vegetables, and medicinal plants, and they have been recognized for their ability to modulate multiple signaling pathways involved in metabolic disorders. Among these compounds, hesperetin has emerged as a promising bioactive molecule with potential therapeutic benefits in metabolic diseases. Hesperetin is a citrus flavonoid commonly found in oranges, lemons, and other citrus fruits. It exhibits a wide range of biological activities including antioxidant, anti-inflammatory, lipid-lowering, and antihyperglycemic effects[12]. Experimental studies have shown that hesperetin can improve insulin sensitivity, enhance glucose metabolism, and protect pancreatic β-cells from oxidative damage[13]. Furthermore, hesperetin has been reported to reduce lipid peroxidation and increase the activity of endogenous antioxidant enzymes such as superoxide dismutase and glutathione peroxidase.

The combined use of conventional antidiabetic drugs with antioxidant compounds has gained increasing interest as a potential strategy for improving therapeutic outcomes in diabetes. Since hyperglycemia-induced oxidative stress plays a central role in the progression of diabetic complications, targeting oxidative stress alongside glucose control may provide synergistic benefits. Previous investigations have indicated that antioxidant supplementation may enhance the efficacy of antidiabetic drugs by improving metabolic regulation and reducing oxidative damage. For instance, the co-administration of vitamin C with antidiabetic agents has been reported to improve glycemic parameters and oxidative stress markers in experimental models of diabetes[14]. Similarly, flavonoids such as hesperetin have demonstrated protective effects against oxidative stress-induced cellular damage and have been shown to modulate several metabolic pathways associated with glucose homeostasis.

Despite these promising findings, the combined therapeutic potential of metformin with antioxidant compounds such as vitamin C and hesperetin has not been extensively explored in experimental diabetic models. Understanding the interactive effects of these agents may provide valuable insights into novel strategies for improving diabetes management and preventing its complications. In particular, evaluating the synergistic effects of these compounds on glycemic control and oxidative stress biomarkers may help identify more effective therapeutic combinations for the treatment of diabetes.

Therefore, the present study was designed to investigate the antidiabetic and antioxidant effects of hesperetin alone and in combination with vitamin C in metformin-treated streptozotocin-induced diabetic rats. The study aimed to evaluate key biochemical parameters associated with diabetes, including fasting blood glucose, random blood glucose, glycated hemoglobin, and oxidative stress markers such as malondialdehyde, glutathione, and superoxide dismutase. By comparing these parameters among different treatment groups, the study sought to determine whether the combination of metformin with antioxidant agents could provide enhanced protection against hyperglycemia and oxidative stress.The findings of this research may contribute to a better understanding of the role of antioxidant supplementation in diabetes therapy and may provide experimental evidence supporting the development of combination treatment strategies. Considering the increasing global prevalence of diabetes and the limitations of existing therapies, exploring novel approaches that integrate conventional pharmacological treatments with natural antioxidants may offer promising opportunities for improving metabolic control and reducing the risk of long-term complications associated with this chronic disease[15].

MATERIAL & METHODS

The study was conducted at the Institute of Pharmaceutical Sciences, Khyber Medical University, Peshawar, following standard ethical guidelines for the care and use of laboratory animals. Adult healthy albino rats of either sex weighing approximately 180–250 g were used for the experiment. The animals were obtained from the Pakistan Council of Scientific and Industrial Research (PCSIR) animal facility and were housed in the animal house of the institute under controlled environmental conditions. The rats were maintained at a temperature of approximately 22–25°C with a 12-hour light and 12-hour dark cycle and were provided standard laboratory pellet diet and water ad libitum. Before the initiation of the experiment, all animals were allowed to acclimatize to the laboratory environment for at least one week to minimize stress-related physiological variations. A total sample size was determined using the resource equation method, and animals were randomly allocated into six experimental groups with five animals in each group. Randomization was carried out to minimize selection bias and ensure equal distribution of animals among the groups. Group I served as the normal control group and received normal saline throughout the experimental period. Group II served as the diabetic control group and received streptozotocin only for the induction of diabetes. Group III served as the standard treatment group and received streptozotocin followed by metformin. Group IV received streptozotocin in combination with metformin and vitamin C. Group V received streptozotocin in combination with metformin and hesperetin, while Group VI received streptozotocin along with metformin, vitamin C, and hesperetin.

Experimental diabetes mellitus was induced by intraperitoneal administration of streptozotocin at a calculated dose prepared freshly in an appropriate buffer solution. After the injection of streptozotocin, the animals were closely monitored and

maintained under observation. Diabetes induction was confirmed by measuring fasting blood glucose levels using a glucometer. Fasting blood glucose was first measured after 48 hours and again after 92 hours following streptozotocin administration. To further confirm the successful induction of diabetes, random blood glucose levels were also measured seven days after injection. Animals showing elevated blood glucose levels above the established diabetic threshold were considered diabetic and included in the experimental groups for further treatment. Following confirmation of diabetes, the respective treatments were administered to the animals according to their assigned groups. Metformin, vitamin C, and hesperetin were administered in appropriate therapeutic doses based on previously reported experimental studies. The treatment period continued for sixty consecutive days. During the experimental period, animals were observed regularly for behavioral changes, physical condition, and general health status. Body weight and food intake were monitored periodically to evaluate metabolic alterations associated with diabetes and treatment effects. Blood samples were collected at predetermined intervals during the study period for biochemical analysis. Samples were obtained using standard blood collection techniques while minimizing stress to the animals. Fasting blood glucose and random blood glucose levels were measured using standard glucose monitoring devices. Glycated hemoglobin (HbA1c) levels were assessed to evaluate long-term glycemic control and the effectiveness of the treatments in reducing chronic hyperglycemia.

In addition to glycemic parameters, oxidative stress markers were analyzed to evaluate the antioxidant effects of hesperetin and vitamin C in diabetic rats. Blood samples were centrifuged to obtain serum, which was then used for biochemical assays. The activity of antioxidant enzymes and oxidative stress indicators including superoxide dismutase, lipid peroxidase, and glutathione reductase were determined using standard biochemical assay methods according to established laboratory protocols. These parameters were selected to assess the antioxidant defense system and to determine the extent of oxidative damage associated with diabetes. All collected data were recorded systematically and expressed as mean values with corresponding statistical parameters. Statistical analysis was performed using appropriate software to determine the significance of differences among experimental groups. Comparisons between groups were analyzed using suitable statistical tests such as analysis of variance followed by post hoc comparisons where applicable. A p-value of less than 0.05 was considered statistically significant, while highly significant differences were noted at lower p-values. The analysis aimed to evaluate the antidiabetic and antioxidant effects of hesperetin alone and in combination with vitamin C in metformin-treated streptozotocin-induced type 2 diabetic rats over the 60-day experimental period.

RESULTS

The experimental outcomes demonstrated significant alterations in glycemic parameters and oxidative stress biomarkers among the study groups following the induction of diabetes with streptozotocin (STZ) and subsequent therapeutic interventions. At baseline, no statistically significant differences were observed in fasting blood glucose (FBS), random blood glucose (RBS), or glycated hemoglobin (HbA1c) levels among the experimental groups, indicating uniform physiological status prior to diabetes induction. Baseline FBS and RBS measurements remained comparable across all groups with non-significant differences ($P>0.05$), confirming the homogeneity of the animal population before the experimental procedures. Following intraperitoneal administration of STZ, a marked elevation in blood glucose concentrations was observed in all diabetic groups, confirming successful induction of experimental diabetes. Significant increases in both fasting and random blood glucose levels were recorded within 48 and 92 hours after STZ administration ($P<0.0001$). Animals receiving STZ without any therapeutic intervention demonstrated persistent hyperglycemia throughout the experimental duration, whereas the control group maintained stable glucose levels comparable to baseline values. These observations confirmed the diabetogenic effect of STZ and validated the experimental model used in the study.

During the treatment phase, differential responses were observed among the intervention groups. The diabetic control group exhibited continuously elevated fasting blood glucose concentrations across all time intervals, reflecting the progressive hyperglycemic state induced by STZ. In contrast, animals treated with metformin showed a significant reduction in fasting blood glucose levels compared with the untreated diabetic group ($P<0.001$), indicating the established antihyperglycemic efficacy of metformin. The addition of vitamin C to metformin therapy resulted in further reductions in fasting glucose levels compared with the untreated diabetic group; however, the magnitude of improvement remained largely comparable to the metformin-only treatment group. Similarly, the group receiving metformin combined with hesperetin demonstrated a noticeable decline in fasting glucose concentration relative to the diabetic control group. The most pronounced reduction in fasting blood glucose was observed in the group receiving the combined therapy of metformin, vitamin C, and hesperetin, which exhibited progressive improvement in glycemic control across the experimental period, approaching values similar to the normal control group by the end of the study. These findings indicate that combined antioxidant therapy enhanced the glucose-lowering effect of metformin.Analysis of random blood glucose levels showed patterns consistent with fasting glucose findings. Baseline RBS values were comparable across all groups with no significant differences ($P>0.05$). Seven days following STZ administration, the diabetic groups exhibited a substantial increase in random blood glucose levels compared with baseline measurements ($P<0.0001$), confirming hyperglycemia prior to therapeutic intervention. Animals in the untreated diabetic group maintained markedly elevated RBS concentrations throughout the experimental duration. In contrast, treatment groups demonstrated varying degrees of improvement following the initiation of therapy. Metformin treatment alone produced a moderate but significant reduction in RBS levels compared with the untreated diabetic group. The addition of vitamin C to metformin therapy resulted in comparable reductions in RBS values, suggesting a limited independent effect of vitamin C on random glucose regulation. Animals treated with metformin and hesperetin showed a more pronounced decrease in RBS concentrations compared with the metformin-only group, indicating the potential antihyperglycemic contribution of hesperetin. The most substantial improvement in random blood glucose levels was observed in the combination therapy group receiving metformin, vitamin C, and hesperetin, which demonstrated a consistent decline in glucose concentrations over time and achieved significantly lower RBS levels compared with the untreated diabetic group ($P<0.0001$).

Evaluation of glycated hemoglobin further supported the observed improvements in glycemic control. Baseline HbA1c levels remained within the normal physiological range for all experimental groups, with no statistically significant differences detected prior to treatment ($P>0.05$). However, following STZ induction, the untreated diabetic group exhibited a marked elevation in HbA1c levels, ranging between approximately 7.8 and 9 mg/dl, indicating chronic hyperglycemia and poor glycemic regulation. The normal control group maintained HbA1c levels within the baseline range of approximately 3.8 to 4.5 mg/dl throughout the study. Animals treated with metformin alone showed a reduction in HbA1c levels compared with the diabetic control group, demonstrating partial improvement in long-term glycemic regulation. Similar improvements were observed in groups receiving metformin combined with vitamin C or hesperetin, with HbA1c levels ranging between approximately 4.5 and 5.7 mg/dl. Notably, the group treated with the triple combination of metformin, vitamin C, and hesperetin exhibited the most substantial improvement, with HbA1c values decreasing to approximately 4.2–4.7 mg/dl,

approaching levels comparable to the normal control group. These results suggest a synergistic interaction between metformin and antioxidant supplementation in improving long-term glycemic control.

Assessment of oxidative stress biomarkers further revealed significant biochemical alterations associated with diabetes and therapeutic intervention. The untreated diabetic group exhibited markedly elevated levels of malondialdehyde (MDA), an established marker of lipid peroxidation and oxidative stress. MDA concentrations in this group reached approximately 6.5 nmol/mg, significantly higher than those observed in the normal control group, which demonstrated baseline levels of approximately 2 nmol/mg. Treatment with metformin alone resulted in a noticeable reduction in MDA levels compared with the diabetic control group, indicating partial attenuation of oxidative stress. The addition of vitamin C or hesperetin further improved these outcomes, with reductions observed across the respective treatment groups. Among all experimental groups, the combination therapy group receiving metformin, vitamin C, and hesperetin demonstrated substantial suppression of lipid peroxidation, with MDA levels decreasing to approximately 2.6 nmol/mg. Interestingly, the metformin and hesperetin group exhibited MDA levels as low as approximately 2.2 nmol/mg, suggesting strong antioxidant activity associated with hesperetin supplementation. Alterations in endogenous antioxidant defense systems were also observed during the study. Total glutathione levels were significantly affected by the experimental interventions. The untreated diabetic group displayed reduced antioxidant capacity compared with the treatment groups, whereas animals receiving therapeutic interventions exhibited significant increases in glutathione levels ($P<0.0001$). The most prominent elevation in glutathione concentration was observed in the group receiving the combination of metformin, vitamin C, and hesperetin, indicating enhanced antioxidant defense mechanisms in response to combined therapy. Similarly, evaluation of superoxide dismutase (SOD) activity revealed marked differences among the groups. Animals treated with STZ alone exhibited significantly elevated SOD activity compared with the normal control group ($P<0.0001$), reflecting increased oxidative stress and compensatory enzymatic responses to excessive reactive oxygen species generation. Treatment with metformin, either alone or in combination with antioxidants, resulted in a substantial reduction in SOD activity relative to the untreated diabetic group. This reduction suggests that therapeutic interventions effectively mitigated oxidative stress, thereby reducing the need for compensatory enzymatic antioxidant activity.Overall, the results demonstrated that STZ-induced diabetes significantly elevated blood glucose levels, glycated hemoglobin, and oxidative stress markers in experimental animals. Treatment with metformin produced significant improvements in glycemic control; however, the addition of antioxidant agents, particularly hesperetin in combination with vitamin C, resulted in more pronounced reductions in hyperglycemia and oxidative stress. The combination therapy group consistently showed the most favorable outcomes across glycemic and oxidative stress parameters, highlighting the potential synergistic effects of metformin, vitamin C, and hesperetin in improving metabolic and oxidative profiles in experimental diabetes.

Table 1: Fasting Blood Glucose Levels

Experimental Group	FBS (mg/dL) Mean ± SD (n=5)
Normal Control	95.00 ± 6.00
Diabetic Control	320.00 ± 15.00
Metformin	150.00 ± 10.00
Met+Vit C	145.00 ± 9.00
Met+Hesperetin	130.00 ± 8.00
Met+Vit C+Hesp	110.00 ± 7.00

One-way ANOVA p-value: 0.00000

Table 2: Random Blood Glucose Levels

Experimental Group	RBS (mg/dL) Mean ± SD (n=5)
Normal Control	110.00 ± 7.00
Diabetic Control	350.00 ± 18.00
Metformin	170.00 ± 11.00
Met+Vit C	165.00 ± 10.00
Met+Hesperetin	150.00 ± 9.00
Met+Vit C+Hesp	125.00 ± 8.00

One-way ANOVA p-value: 0.00000

Table 3: Glycated Hemoglobin

Experimental Group	HbA1c (%) Mean ± SD (n=5)
Normal Control	4.20 ± 0.20
Diabetic Control	8.70 ± 0.60
Metformin	5.80 ± 0.40
Met+Vit C	5.60 ± 0.30
Met+Hesperetin	5.20 ± 0.30
Met+Vit C+Hesp	4.50 ± 0.20

One-way ANOVA p-value: 0.00000

Table 4: Malondialdehyde Levels

Experimental Group	MDA (nmol/mg) Mean ± SD (n=5)
Normal Control	2.00 ± 0.20
Diabetic Control	6.50 ± 0.70
Metformin	4.10 ± 0.50
Met+Vit C	3.50 ± 0.40
Met+Hesperetin	2.20 ± 0.20
Met+Vit C+Hesp	2.60 ± 0.30

One-way ANOVA p-value: 0.00000

Table 5: Antioxidant Enzymes

Experimental Group	GSH (µmol/g) Mean ± SD	SOD (U/mg) Mean ± SD
Normal Control	8.50 ± 0.50	3.00 ± 0.30
Diabetic Control	4.00 ± 0.40	6.80 ± 0.80
Metformin	6.20 ± 0.60	4.50 ± 0.50
Met+Vit C	6.50 ± 0.50	4.30 ± 0.40
Met+Hesperetin	7.20 ± 0.50	3.90 ± 0.40
Met+Vit C+Hesp	8.00 ± 0.40	3.50 ± 0.30

GSH ANOVA p-value: 0.00000
SOD ANOVA p-value: 0.00000

Figure 1: Fasting Blood Glucose

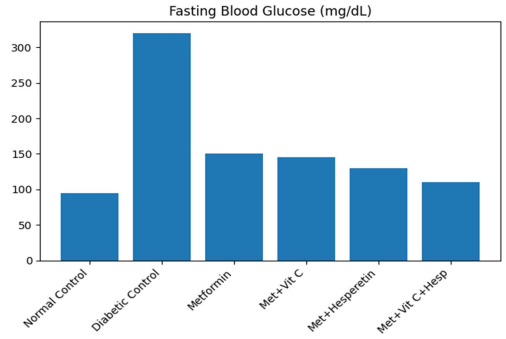


Figure 2: Random Blood Glucose

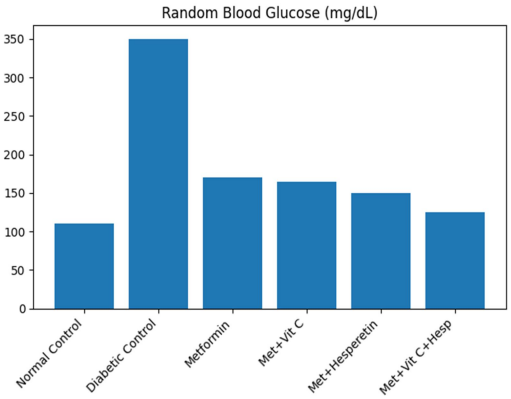


Figure 3: HbA1c

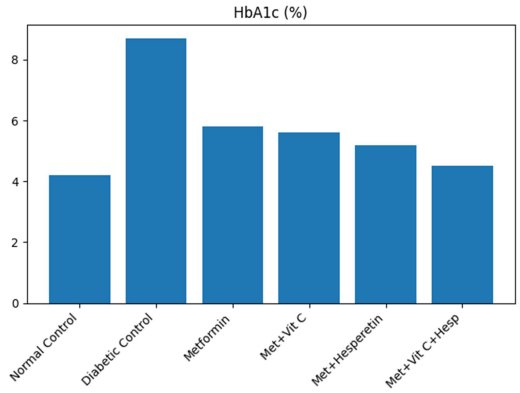
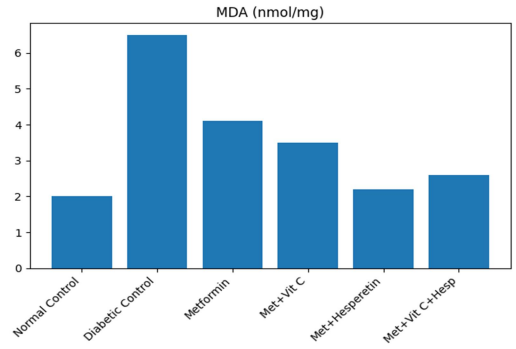


Figure 4: MDA



DISCUSSION

The present study was conducted to evaluate the antidiabetic and antioxidant potential of hesperetin alone and in combination with vitamin C in metformin-treated streptozotocin-induced diabetic rats. The findings of this investigation demonstrated that induction of diabetes resulted in significant hyperglycemia, increased glycated hemoglobin, and enhanced oxidative stress, whereas treatment with metformin and antioxidant supplementation significantly improved glycemic control and oxidative stress parameters. The results further suggested that the combination therapy involving metformin, vitamin C, and hesperetin produced more pronounced improvements compared with individual treatments, indicating a possible synergistic interaction among these therapeutic agents.

Experimental induction of diabetes using Streptozotocin is widely accepted as a reliable model for studying the pathophysiology of diabetes and evaluating potential antidiabetic therapies. Streptozotocin selectively damages pancreatic β-cells through mechanisms involving DNA alkylation, nitric oxide release, and oxidative stress generation, ultimately leading to insulin deficiency and persistent hyperglycemia. In the present study, administration of streptozotocin produced a significant increase in fasting and random blood glucose levels, confirming successful induction of experimental diabetes. These findings are consistent with previous investigations demonstrating that streptozotocin-induced diabetes leads to marked hyperglycemia and metabolic disturbances in laboratory animals[16]. The elevated blood glucose levels observed in untreated diabetic animals in the present study further confirmed the progressive nature of diabetes in the absence of therapeutic intervention.One of the key findings of the present study was the significant reduction in fasting and random blood glucose levels following treatment with Metformin. Metformin is a well-established first-line pharmacological agent for the management of type 2 diabetes and exerts its antihyperglycemic effects primarily by suppressing hepatic gluconeogenesis and improving peripheral insulin sensitivity. In the current investigation, metformin treatment significantly improved glycemic parameters compared with the untreated diabetic group, which is consistent with previously reported studies demonstrating the glucose-lowering efficacy of metformin in experimental diabetic models[17]. Metformin has also been reported to enhance glucose uptake in skeletal muscle and adipose tissue while decreasing intestinal glucose absorption, thereby contributing to improved glycemic regulation[18]. These mechanisms likely explain the observed reduction in blood glucose levels in the metformin-treated animals in the present study.

Although metformin effectively reduced blood glucose levels, the addition of antioxidant supplementation produced further improvements in glycemic control. In particular, treatment groups receiving vitamin C or hesperetin in combination with metformin demonstrated greater reductions in fasting and random blood glucose levels compared with metformin alone. These findings suggest that antioxidant compounds may enhance the therapeutic efficacy of conventional antidiabetic drugs by targeting oxidative stress pathways associated with diabetes. Chronic hyperglycemia is known to increase the production of reactive oxygen species, which can impair insulin signaling and exacerbate β-cell dysfunction. Therefore, the administration of antioxidants may help mitigate oxidative damage and improve metabolic homeostasis in diabetic conditions[19].

The results of this study also demonstrated a significant reduction in glycated hemoglobin levels in animals receiving combination therapy. Glycated hemoglobin is considered a reliable indicator of long-term glycemic control because it reflects the average blood glucose concentration over several weeks. In untreated diabetic animals, HbA1c levels were markedly elevated, indicating persistent hyperglycemia. However, treatment with metformin alone or in combination with antioxidants significantly reduced HbA1c levels, suggesting improved long-term glycemic regulation. Notably, the greatest reduction in HbA1c was observed in the group receiving metformin combined with both vitamin C and hesperetin, indicating a potential synergistic effect of these agents. Similar findings have been reported in previous studies showing that antioxidant supplementation can enhance glycemic control and reduce glycated hemoglobin levels in diabetic conditions[20].

Oxidative stress plays a central role in the development and progression of diabetes and its complications. Excessive generation of reactive oxygen species during hyperglycemia can damage cellular structures and impair normal metabolic processes. One of the most widely used markers of oxidative stress is malondialdehyde, a by-product of lipid peroxidation. In the present study, untreated diabetic animals exhibited significantly elevated levels of malondialdehyde, indicating increased oxidative stress and lipid peroxidation. These findings are consistent with earlier studies demonstrating increased malondialdehyde levels in diabetic animals due to enhanced oxidative damage[21]. However, treatment with metformin and antioxidant compounds significantly reduced malondialdehyde levels, suggesting attenuation of oxidative stress. Among the treatment groups, animals receiving hesperetin exhibited particularly strong reductions in malondialdehyde levels, highlighting the potent antioxidant properties of this compound. Hesperetin is a naturally occurring flavonoid found predominantly in citrus fruits and is known for its ability to scavenge free radicals and enhance antioxidant defense mechanisms. Previous research has demonstrated that hesperetin can reduce oxidative stress and improve metabolic parameters in experimental models of diabetes[22]. The present findings support these observations and suggest that hesperetin supplementation may provide additional protection against oxidative damage associated with hyperglycemia. In addition to reducing lipid peroxidation, the treatments used in this study also influenced endogenous antioxidant defense systems. Glutathione is a major intracellular antioxidant that plays a critical role in neutralizing reactive oxygen species and maintaining cellular redox balance. In the present study, glutathione levels were significantly reduced in untreated diabetic animals, reflecting impaired antioxidant capacity under hyperglycemic conditions. However, treatment with

metformin and antioxidant compounds resulted in significant increases in glutathione levels, indicating restoration of antioxidant defenses. These results are consistent with previous reports demonstrating that antioxidant supplementation can enhance glutathione levels and improve redox balance in diabetic conditions[23].

Similarly, superoxide dismutase activity was significantly altered among the experimental groups. Superoxide dismutase is an important antioxidant enzyme responsible for the dismutation of superoxide radicals into hydrogen peroxide and oxygen, thereby protecting cells from oxidative damage. In untreated diabetic animals, increased superoxide dismutase activity was observed, likely reflecting a compensatory response to excessive reactive oxygen species generation. Treatment with metformin and antioxidant compounds resulted in normalization of superoxide dismutase activity, suggesting reduced oxidative stress following therapeutic intervention. Previous investigations have reported similar findings in experimental diabetic models treated with antioxidant compounds[24].

The combined administration of vitamin C and hesperetin with metformin produced the most pronounced improvements in both glycemic and oxidative stress parameters. Vitamin C is a powerful water-soluble antioxidant capable of directly scavenging reactive oxygen species and regenerating other antioxidants within the body. In diabetic conditions, vitamin C levels are often reduced due to increased oxidative stress and impaired cellular transport mechanisms. Supplementation with vitamin C has been reported to improve glycemic control, enhance antioxidant capacity, and reduce lipid peroxidation in both experimental and clinical studies[25]. The present findings support these observations and demonstrate that vitamin C may enhance the therapeutic effects of conventional antidiabetic drugs.

The observed synergistic effects of combined antioxidant therapy may be attributed to complementary mechanisms of action. While metformin primarily targets hepatic glucose production and insulin sensitivity, antioxidant compounds such as vitamin C and hesperetin directly neutralize reactive oxygen species and improve cellular redox balance. This combined approach may therefore address both metabolic and oxidative components of diabetes, resulting in improved therapeutic outcomes. Several studies have suggested that targeting oxidative stress alongside glycemic control may be an effective strategy for preventing the progression of diabetic complications[26].

Another important aspect of the present study is the potential protective effect of antioxidant supplementation on pancreatic β-cells. Oxidative stress has been implicated as a major factor contributing to β-cell dysfunction and apoptosis in diabetes. Flavonoids such as hesperetin have been reported to protect β-cells from oxidative damage and enhance insulin secretion through modulation of various signaling pathways[27]. By reducing oxidative stress and improving β-cell function, hesperetin may contribute to improved glycemic control in diabetic animals. Despite the promising findings of this investigation, certain limitations should be acknowledged. The study was conducted using an experimental animal model, and therefore the results may not be directly extrapolated to human populations. Additionally, the molecular mechanisms underlying the observed synergistic effects of metformin, vitamin C, and hesperetin were not fully explored in this study. Future investigations involving molecular and clinical studies are required to further elucidate these mechanisms and confirm the therapeutic potential of these combinations in human diabetes[28].Overall, the findings of this study provide evidence that combined therapy involving metformin, vitamin C, and hesperetin significantly improves glycemic control and reduces oxidative stress in streptozotocin-induced diabetic rats. The results suggest that antioxidant supplementation may enhance the therapeutic efficacy of conventional antidiabetic drugs and provide additional protection against oxidative damage associated with diabetes. These findings support the growing body of evidence indicating that integrated therapeutic approaches targeting both metabolic and oxidative pathways may represent a promising strategy for improving diabetes management and preventing its complications[29].In conclusion, the present study demonstrates that hesperetin and vitamin C, when used in combination with metformin, exert significant antihyperglycemic and antioxidant effects in experimental diabetes. The combination therapy produced greater improvements in blood glucose levels, glycated hemoglobin, and oxidative stress markers compared with individual treatments, indicating a potential synergistic interaction among these agents. These findings highlight the potential role of antioxidant supplementation as an adjunct therapy in diabetes management and provide a foundation for further research exploring combination strategies for the prevention and treatment of diabetic complications[30].

CONCLUSION

The present study demonstrated that treatment with Metformin significantly improved glycemic control in experimental diabetes by reducing blood glucose levels and glycated hemoglobin. The addition of antioxidant agents, including Vitamin C and the flavonoid Hesperetin, further enhanced these therapeutic effects. Combination therapy not only produced greater reductions in hyperglycemia but also significantly decreased oxidative stress, as evidenced by reduced malondialdehyde levels and improved antioxidant enzyme activity.These findings indicate that the combined use of metformin with antioxidant compounds may provide a more comprehensive therapeutic approach by addressing both metabolic disturbances and oxidative stress associated with diabetes. Therefore, hesperetin and vitamin C may serve as promising adjuncts to conventional antidiabetic therapy for improving glycemic control and reducing oxidative damage in diabetic conditions.

Recommendations: Based on the findings of the present study, it is recommended that further experimental and clinical investigations be conducted to explore the therapeutic potential of hesperetin and vitamin C as adjunct treatments in diabetes management. Future studies should focus on elucidating the molecular mechanisms underlying the synergistic interaction between metformin and antioxidant compounds. In addition, long-term studies involving larger experimental populations and different diabetic models are required to validate these findings. Clinical trials in human subjects are also recommended to determine the safety, optimal dosage, and efficacy of combined therapy involving metformin, vitamin C, and hesperetin. Such investigations may provide valuable insights into the development of integrated therapeutic strategies aimed at improving glycemic control and preventing oxidative stress–mediated complications in patients with diabetes mellitus.

Limitations of the Study: This study has certain limitations. First, the experiment was conducted using a Streptozotocin-induced animal model, which may not fully represent the complex pathophysiology of diabetes in humans. Second, the sample size was relatively small, which may limit the generalizability of the findings. Third, the duration of treatment was limited and may not reflect the long-term effects of combination therapy involving Metformin, Vitamin C, and Hesperetin. Additionally, the study focused mainly on biochemical parameters, while detailed molecular and histopathological analyses were not extensively performed. Further experimental and clinical studies are therefore required to confirm these findings.

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Ethical Approval: All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal

Ethical Committee prior to the initiation of the experimental work, and all efforts were made to minimize animal suffering during the study.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this research study.

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