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The Role Of Metformin, Magnesium, And Vitamin D In Modulating Redox Enzymes In Streptozotocin-Induced Diabetes: An Albino Rat Model Study

Sukanya T[1], Chintha Madhusudhan[2], Kishore Salikineedy[3*]

[1,2,3*]*Department of Zoology, Sri Venkateswara University, Tirupati – 517502, INDIA.*

***Corresponding authors:** Kishore Salikineedy*
**Email: kishorecsb@gmail.com*

Abstract

This study investigates the role of metformin, magnesium, and vitamin D in modulating redox enzymes in streptozotocin-induced diabetes, employing an albino rat model. While numerous studies have explored the impact of metformin therapy in combination with various vitamins, there is a noticeable scarcity of research examining the effects of metformin therapy in conjunction with a combination of various vitamins along with manganese. The experimental design involved six groups of rats, each comprising six individuals. The control group (Group 1) served as the baseline, while Group 2 represented untreated diabetes induced by streptozotocin (DU). Group 3 received metformin for diabetes treatment (DTM), Group 4 received a combination of metformin and vitamin D (DTMV-D), Group 5 received a combination of metformin and magnesium (DTMM), and Group 6 received a combination of metformin, vitamin D, and magnesium (DTMMV-D).The organs, particularly the liver and kidney, were isolated after treatment for enzyme assays, including lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G-6-PDH). Results demonstrated that untreated diabetes led to significant increases in SDH, LDH, and G-6-PDH activities compared to the control group. While metformin treatments showed varying degrees of effectiveness in restoring these enzyme activities, the combination of metformin, vitamin D, and magnesium (DTMMV-D) consistently stood out, indicating potential benefits in mitigating diabetes-induced changes in redox enzyme levels. The study contributes valuable insights into the complex interplay of these treatments with redox enzymes, offering a foundation for future research on innovative therapeutic strategies in diabetes management.

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Keywords: Metformin; Diabetes; Redox enzymes; Magnesium; Vitamin D

Introduction

Diabetes mellitus, characterized by aberrant glucose metabolism, oxidative stress, and impaired antioxidant defenses, is a complex metabolic disorder affecting millions worldwide (Mule and Singh, 2018). Metformin, a cornerstone in the management of type 2 diabetes, plays a pivotal role in enhancing insulin sensitivity, reducing hepatic glucose production, and promoting glucose uptake (Su et al., 2023). Its weight-neutral or weight-loss effect and potential cardiovascular benefits underscore its significance (Terada. and Boulé, 2019). However,

combination therapy with metformin becomes crucial in cases of inadequate glycemic control, individual variability, or as diabetes progresses (Kuritzky, and Samraj, 2011; Padhi et al., 2020; Drzewoski,. and Hanefeld, 2021). Combining metformin with other medications, vitamins, nutritional essential elements etc., addresses these challenges by providing complementary mechanisms of action, offering a more tailored, comprehensive approach to diabetes management (Triggiani et al., 2006; Prabhakar et al., 2014; Owen et al., 2021). In this context, our research aims to elucidate the intricate interplay between metformin, magnesium, and vitamin D in modulating redox enzymes in a Streptozotocin (STZ)-induced diabetes albino rat model. STZ-induced diabetes in animal models mimics key aspects of human diabetes pathophysiology, providing insights into underlying mechanisms and potential therapeutic interventions (Yan, 2022).

This study emphasizes the role of redox enzymes as crucial contributors to maintaining cellular redox balance. It explores metformin's acknowledged properties in lowering glucose and acting as an antioxidant, magnesium's involvement in enzymatic reactions that regulate redox balance, and vitamin D's anti-inflammatory and antioxidant characteristics. The study systematically explores the individual and combined effects of these interventions on redox enzymes. The selected albino rat model, recognized for its genetic homogeneity and susceptibility to STZ-induced diabetes, provides a reliable platform to observe dynamic changes in redox enzymes and associated pathways. Through this research, our objective is to unravel the molecular mechanisms through which metformin, magnesium, and vitamin D collectively impact redox homeostasis in diabetic conditions. This exploration aims to provide valuable insights into potential therapeutic strategies for managing oxidative stress in diabetes, paving the way for novel approaches in diabetes management. Beyond glycemic control, our focus extends to addressing the intricate network of redox signaling.

Materials and Methods

Animals, Experimental Grouping and Ethical Considerations

Male albino Wistar rats, weighing 200 ± 20 g and aged 6-8 weeks, were employed in the experiment. The rats were housed in controlled conditions with a 12-hour light-dark cycle, providing continuous access to water and standard feed throughout the study. A total of six experimental groups were formed, each comprising six rats. The control group (Group 1, C) served as the baseline, while Group 2 represented untreated diabetes induced by STZ (DU). Group 3 received metformin for diabetes treatment (DTM), Group 4 received a combination of metformin and vitamin D (DTMV-D), Group 5 received a combination of metformin and magnesium (DTMM), and Group 6 received a combination of metformin, vitamin D, and magnesium (DTMMV-D). The experimental protocol strictly adhered to the guidelines established by the "Institutional Animal Ethics Committee of Sri Venkateswara University," ensuring the well-being of the experimental animals. Approval for the protocol and animal use was obtained from the Institutional Animal Ethics Committee (Resolution No. 10/(i)/a/CPCSEA/IAEC/SVU/ZOOL/SK/Dt. 08-07-2012), Sri Venkateswara University, Tirupati, INDIA.

Induction of diabetes and treatment administration

Diabetes was induced in Groups 2 to 6 through a single intraperitoneal (i.p.) injection of 40 mg/kg body weight of streptozotocin (STZ). Once diabetes was successfully induced, anti-diabetic treatment commenced in Groups 3 to 6. Metformin was administered at a dosage of 100 mg/kg body weight. Additional interventions were tailored to specific experimental groups: Groups 4 and 6 received oral supplementation of vitamin D at a dosage of 1000 IU/kg body weight three times a week, while Groups 5 and 6 received oral magnesium supplementation at a concentration of 10g/Litre. These interventions were strategically designed to assess their respective impacts on mitigating lipid metabolism in the streptozotocin-induced diabetic rat model. Following the treatment period, the rats were euthanized, and liver and kidney samples were isolated to estimate the lipid profile, enabling the assessment of lipid metabolism.

Estimation of redox enzyme activities

After treatment, organs from the treated rats, particularly the liver and kidney, were isolated by sacrificing the animals. The homogenate isolated organs were prepared in an ice-cold 0.25 M sucrose solution. Homogenates of the liver and kidney tissues (10% w/v) underwent centrifugation at 1000g for 15 minutes at 4°C. The resulting supernatant fraction was utilized for enzyme assays, including Lactate dehydrogenase (LDH), Succinate dehydrogenase (SDH), and Glucose-6-phosphate dehydrogenase (G-6-PDH).

The activity of LDH was determined at 495 nm in a spectrophotometer by measuring the formazone, following the protocol described by Nachlas et al. (1960) and recommended by Prameelamma and Swami (1975) with slight modifications. The results were expressed in moles of formazone formed per milligram of protein per

minute. Additionally, the specific activity of SDH was measured by assessing the absorbance at 495 nm in a spectrophotometer. Enzymes utilized FAD and INT to form formazone, following the method developed by Nachlas et al. (1960). The specific activity was expressed in micromoles of formazone formed per milligram of protein per minute. For Glucose-6-phosphate dehydrogenase activity, the calculation followed the method adapted from Lohr and Waller (1965), as modified by Mastanaiah et al. (1978). The results were expressed in micromoles of formazone formed per milligram of protein per minute.

Statistical analysis

All the experimental data given in the results were means of triplicates and followed Duncan's new Multiple range (DMR) test to find significant difference ($P<0.05$) between values of each sampling.

Results and Discussion

The experiment aimed to investigate the impact of different treatments on redox enzyme activities in the liver and kidney tissues of rats with induced diabetes. The activities of three key enzymes - SDH, LDH, and G-6-PDH were measured in the liver and kidney tissues after treatment. The results indicated that untreated diabetes led to significant increases in SDH, LDH, and G-6-PDH activities compared to the control group (Table 1 and 2). Treatment with metformin alone or in combinations with vitamin D and magnesium showed varying degrees of effectiveness in restoring these enzyme activities, suggesting potential benefits in mitigating diabetes-induced changes in redox enzyme levels.

In the liver, SDH, LDH, and G-6-PDH activities were elevated in untreated diabetes, and treatment groups generally exhibited reductions compared to the untreated diabetes group. The combination of metformin, vitamin D, and magnesium (DTMMV-D) showed a notable trend in restoring enzyme activities closer to control levels (Table 1). Similarly, in the kidney, enzyme activities were increased in untreated diabetes, and treatments, particularly the combined approach (DTMMV-D), demonstrated efficacy in mitigating these alterations (Table 2). These findings suggest that the combination of metformin, vitamin D, and magnesium may offer a more comprehensive approach to managing diabetes-induced changes in redox enzyme activities in both liver and kidney tissues. Overall, the study highlights the complex interplay of these treatments with redox enzymes and provides insights into potential therapeutic strategies for diabetes management.

Table 1. Influence of various treatments on liver redox enzymes: Means ± S.E., in each row, with values followed by the same letter signifying lack of significance ($P \leq 0.05$) as determined by the DMR Test.

Liver mg/dl	Group-I C	Group-II DU	Group-III DTM	Group-IV DTMV-D	Group-V DTMM	Group-VI DTMMV-D
SDH	12.74 ± 0.54^{a}	21.34 ± 1.64^{d}	16.14 ± 1.23^{b}	15.53 ± 1.43^{b}	17.21 ± 1.35^{c}	13.21 ± 1.21^{a}
LDH	7.14 ± 0.23^{a}	12.51 ± 1.32^{d}	9.14 ± 1.27^{c}	8.61 ± 1.43^{c}	7.42 ± 0.54^{b}	6.89 ± 0.52^{a}
G-6-PDH	3.64 ± 0.08^{a}	5.43 ± 0.32^{d}	4.54 ± 0.18^{c}	4.32 ± 0.05^{b}	4.21 ± 0.51^{b}	3.52 ± 0.15^{a}

The results of the experiment shed light on the intricate relationship between diabetes and the activities of redox enzymes, particularly SDH, LDH, and G-6-PDH, in the liver and kidney tissues of rats. Diabetes is known to induce oxidative stress, and redox enzymes play a crucial role in the cellular response to oxidative changes. SDH, involved in the citric acid cycle and the electron transport chain, is sensitive to alterations in the cellular redox state. The observed increase in SDH activity in untreated diabetes (DU) suggests heightened oxidative stress. Metformin, a commonly used diabetes medication, and its combinations with vitamin D and magnesium showed varying degrees of efficacy in mitigating this increase, indicating potential antioxidative effects. The combination of metformin, vitamin D, and magnesium (DTMMV-D) particularly demonstrated a noteworthy ability to restore SDH activity to levels closer to the control group, suggesting a synergistic effect of the combined treatment.

Table 2. Influence of various treatments on kidney redox enzymes: Means ± S.E., in each row, with values followed by the same letter signifying lack of significance (P ≤ 0.05) as determined by the DMR Test.

Liver mg/dl	Group-I C	Group-II DU	Group-III DTM	Group-IV DTMV-D	Group-V DTMM	Group-VI DTMMV-D
SDH	9.52±0.13[a]	17.52 ± 0.32[c]	12.73 ± 1.04[b]	12.36 ±1.21[b]	12.45 ± 1.14[b]	9.36 ± 0.54[a]
LDH	5.72±0.61[a]	9.63 ±1.41[d]	7.24 ± 0.36[c]	6.81 ± 0.14[b]	6.74 ± 0.21[b]	5.90 ± 0.12[a]
G-6-PDH	2.03±0.04[a]	3.86 ± 0.12[d]	3.04 ± 0.06[c]	2.87 ± 0.03[b]	2.75 ± 0.04[b]	1.83 ±0.02[a]

LDH, found in various tissues, is released from damaged cells, and its increased levels in the untreated diabetes group suggest tissue damage and oxidative stress. The treatments, especially metformin-based combinations, showed a trend toward reducing LDH activity, indicating a potential protective effect on tissues. The combination of metformin, vitamin D, and magnesium (DTMMV-D) again stood out in its efficacy. G-6-PDH, crucial for NADPH production in the pentose phosphate pathway, plays a role in antioxidant defense. The increased G-6-PDH activity in untreated diabetes aligns with the demand for NADPH under oxidative stress conditions. The treatments, once more, exhibited a trend toward restoring G-6-PDH activity, suggesting a potential role in enhancing antioxidant defense mechanisms. The results highlight the multifaceted nature of diabetes-induced changes in redox enzymes and the nuanced responses to different treatment modalities. The combination of metformin, vitamin D, and magnesium emerges as a promising approach, potentially offering a comprehensive strategy in managing diabetes-associated alterations in liver and kidney redox enzyme activities. However, further research is warranted to unravel the precise molecular mechanisms underlying these observed effects and to validate the translational relevance of these findings to human diabetes management.

Numerous studies have investigated the impact of metformin therapy combined with various vitamins (Kos et al., 2012; Infante et al., 2021; Haj-yahya et al., 2014). However, to our knowledge, there is a significant dearth of research exploring the effects of metformin therapy in conjunction with a combination of various vitamins along with manganese. The current study sheds light on a critical research gap by investigating the effects of metformin therapy in conjunction with a combination of various vitamins along with manganese, an area notably underexplored in existing literature. The combination treatment, specifically metformin, vitamin D, and magnesium, emerges as a promising therapeutic approach in mitigating diabetes-induced alterations in redox enzyme activities within the liver and kidney tissues. These results not only add to the collective knowledge regarding the impact of metformin and vitamins but also introduce manganese as a potential contributor to the observed effects. The findings suggest that the inclusion of manganese in the therapeutic combination may play a significant role in restoring redox enzyme activities to levels comparable to the control group. This introduces a novel dimension to the field, potentially offering a more comprehensive strategy for managing diabetes-associated oxidative stress. While these results are promising, the complexity of cellular processes warrants further investigation to decipher the underlying molecular mechanisms responsible for the observed effects. The identified therapeutic combination, DTMMV-D, presents a valuable avenue for future research, clinical trials, and potential applications in diabetes management. The insights gained from this study contribute not only to the field of diabetes research but also to the broader understanding of redox enzyme regulation and potential interventions for oxidative stress-related conditions. This work serves as a foundation for future explorations into the nuanced interplay of metformin, vitamins, and manganese, paving the way for innovative and targeted approaches in the treatment of diabetes and related metabolic disorders.

Summary and Conclusion:

The experiment investigated the effects of various diabetes treatments on redox enzyme activities in the liver and kidney tissues of rats. Six experimental groups were studied, including a control group, untreated diabetes, and groups treated with metformin alone, metformin with vitamin D, metformin with magnesium, and a combination of metformin, vitamin D, and magnesium. Key redox enzymes studied were SDH, LDH, and G-6-PDH. Results revealed significant alterations in enzyme activities in untreated diabetes, with treatments demonstrating varied effectiveness in mitigating these changes. The combination treatment DTMMV-D consistently stood out in restoring enzyme activities closer to control levels in both liver and kidney tissues. Untreated diabetes led to elevated SDH activity, indicative of increased oxidative stress. Treatments, especially

the combination of metformin, vitamin D, and magnesium, showed promising efficacy in restoring SDH activity to levels comparable to the control group. This suggests a potential antioxidative effect of the combined treatment. Increased LDH levels in untreated diabetes, indicating tissue damage and oxidative stress, were mitigated by various treatments. The DTMMV-D group consistently demonstrated effectiveness in reducing LDH activity, suggesting a protective role in tissues. Elevated G-6-PDH activity in untreated diabetes, reflecting increased demand for NADPH in oxidative stress conditions, was mitigated by treatments. The combination treatment DTMMV-D exhibited notable effectiveness in restoring G-6-PDH activity to levels comparable to the control group. In conclusion, the combination of metformin, vitamin D, and magnesium appears to be a promising therapeutic approach in mitigating diabetes-induced alterations in redox enzyme activities. These findings provide valuable insights into potential strategies for managing oxidative stress associated with diabetes, though further research is essential to validate these results and explore the underlying molecular mechanisms.

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Conflict of Interest

The authors do not have any conflict of interest.

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