ijlpr 2023; doi 10.22376/ijlpr.2023.13.1.P98-104

International Journal of Life science and Pharma Research

ISSN 2250-0480

Hexokinase Activator Attenuates Type 2 Diabetes Mellitus and Associated Endothelial Dysfunction in HFF-STZ-Induced Diabetic Rat

Ekta Patel[1*], Divyakant Patel[1], Kunjal Vegad[1], Yogesh Patel[1], Priya Shah[2], Dhwani Shah[1] and Nilesh Kanzariya[1]

[1] *Sharda school of pharmacy, Gandhinagar, Gujarat, India.*

[2] *L.J. institute of pharmacy, Ahmedabad, Gujarat, India.*

Abstract: Hexokinase is an enzyme involved in the glucose metabolism pathway. Magnesium is an inherent cofactor of the Hexokinase enzyme. The plethora of literature suggested that hexokinase level was decreased in diabetic rats. Sodium metavanadate shows insulin-mimetic action. We designed this work to determine the effect of magnesium on diabetes and associated vascular complications in rats. Type-2 diabetes was induced by a high-fat diet and a low dose of streptozotocin.. Diabetic rats were divided into groups, i.e. normal, diabetic, magnesium sulfate, sodium metavanadate, a combination of glibenclamide and metformin, a variety of sodium metavanadate with glibenclamide and metformin, and a combination of magnesium sulfate with glibenclamide and metformin. Magnesium sulfate was used as a hexokinase activator. Blood glucose levels were measured before initiation, between, and after 4 weeks of treatment. Biochemical and tissue parameters were estimated for additional confirmation. A vascular study took the contractile response of hydrogen peroxide in rat thoracic aortas of different groups. Statistical comparisons between groups were performed by two-tailed one-way ANOVA followed by the Dunnett test. P-values <0.05 were considered statistically significant. Treatment with magnesium sulfate and sodium metavanadate (S.M.V.) alone and in combination significantly ($p<0.05$) modified the elevated blood glucose and various altered biochemical parameters in diabetic rats. Treatment showed a significant decrease ($p < 0.001$) n elevated contractile responses of hydrogen peroxide in thoracic aortas of diabetic rats *Vs* Normal Control). Hexokinase activation by magnesium and sodium metavanadate significantly reduced (** $p < 0.01$) diabetes as well as vascular complications.

Keywords: Diabetes, Hexokinase, Magnesium Sulfate, Vascular Complication, and Hydrogen peroxide

***Corresponding Author**

Ekta Patel , Sharda school of pharmacy, Gandhinagar, Gujarat, India.

Received On 7 September, 2022

Revised On 8 November, 2022

Accepted On 15 November, 2022

Published On 2 January, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Ekta Patel, Divyakant Patel, Kunjal Vegad, Yogesh Patel, Priya Shah, Dhwani Shah and Nilesh Kanzariya , Hexokinase Activator Attenuates Type 2 Diabetes Mellitus and Associated Endothelial Dysfunction in HFF-STZ-Induced Diabetic Rat.(2023).Int. J. Life Sci. Pharma Res.13(1), P98-104 http://dx.doi.org/10.22376/ijlpr.2023.13.1.P98-104

1. INTRODUCTION

The Global prevalence of an estimated 537 million adults living with diabetes, according to the latest 2019 data from the International Diabetes Federation. Diabetes prevalence is increasing rapidly. The number is expected to rise to 643 million by 2030. [1]India leads the world with the largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". [2]Type 1 diabetes originates from a lack of insulin production by pancreatic b-cells. By contrast, type 2 diabetes (90% of cases) results from insulin resistance. [3]Glycolysis, the catabolism of glucose to pyruvate, is a central metabolic pathway. [4] Hexokinase catalyzes the conversion of glucose to glucose 6 phosphate, the main rate-limiting step in the path of glucose catabolism.[5] Hexokinase enzyme activity is decreased in diabetic rats. [6,5] One report on hexokinase showed that it has a preventive antioxidant defense activity. [7] Emerging evidence suggests that this mini 'oxidative burst' is important in signal transduction. [8] In mammalian cells, the physiological role for O_2 - H_2O_2 and nitric oxide (NO).is well characterized than that of another reactive oxygen species. H_2O_2 is a vasomotor agent, causing vessels to constrict [9] or relax.[10] Increased vascular activities of H_2O_2 may be an essential factor in the development of vascular disorders associated with chronic diabetes mellitus. [11] H_2O_2 was known as an insulin mimetic agent and produced tyrosine phosphorylation in Fao cells (Rat hepatoma cells) at 3 mM concentration. [12] H_2O_2 leading to enhanced intracellular production of $O^{\cdot H.-}$ Thus, OH^- is implicated in diabetes-induced endothelial dysfunction. [13] The phosphatase inhibitor Vanadate is a compound with insulin-mimetic properties both in vitro and in vivo, and it has been proposed in the treatment of diabetes mellitus. Vanadate lowers plasma glucose levels, increases peripheral glucose uptake, and improves insulin sensitivity. [14,15] The short-term effects, such as gastrointestinal discomfort and decreased body weight gain, have been reported. [16,17] Vanadate alters Mgi homeostasis[18]. Magnesium (Mg) is the second most abundant intracellular cation in living cells after potassium. Therefore, mg deficiency has been proposed as a factor implicated in the pathogenesis of diabetes complications. Mg supplementation in patients with Type II diabetes resulted in a significant decrease in total and LDL cholesterol and an increase in HDL cholesterol. [19] Metformin, for example, raises Mg levels in the liver. Pioglitazone, a thiazolidinedione antidiabetic agent that increases insulin sensitivity, has positive actions on Mg metabolism both in vitro and in vivo. [20] Mg deficiency is associated with increased free radical-dependent oxidative tissue damage. [21] Magnesium is an inherent cofactor for hexokinase activity.[22] Magnesium deficiency has been proposed as a novel factor implicated in the pathogenesis of late diabetic complications[23]. Hyperglycemia and Hyperinsulinemia are the hallmarks of type-II diabetes. This leads to the generation of oxidative stress, with O^{2-}, $H_2O_2$and OH^-. Overproduction of these R.O.S. may lead to vascular dysfunction. Hexokinase has a preventive antioxidant role. So, research was carried out to elucidate the role of hexokinase in diabetes and vascular complications. So, the present work was carried out to decipher the molecular targets which link diabetes to vascular dysfunctions and may become the drug target for treating diabetes and associated vascular dysfunctions. The main emphasis is whether magnesium as a hexokinase cofactor and vanadate has a role in vascular complication or not?

2. MATERIAL & METHODS

2.1 Animals

Male *Sprague Dawley (S.D.)* rats weighing 250-300g were procured from the central animal facility of the institute. The animals were maintained in controlled temperature and humidity and with a standard diet and water ad *libitium*. The experiment was carried out as per the instruction provided by the Institutional animal ethical committee of the institute (CPCSEA Reg. Number 197/CPCSEA/2000).

2.2 Chemicals

Streptozotocin in powder form was purchased from Enzo life sciences, U.S.A. Hexokinase, Glucose grade III, 2 mercaptoethanols, and cholesterol were purchased from S.D. Fine Chemical Ltd., India. 6-Phosphogluconate was purchased from Sigma chemicals company, U.S.A. while G6PD, NADP+ and ATP were from M.P. Biochemicals U.S.A. Sodium metavanadate was purchased from Central drug house (p) Ltd. New Delhi.

2.3 Induction of Diabetes in Rats

Healthy *SD* rats showing normal plasma glucose levels in the range of 80-120 mg/dl were used for the study. Animals were fed a high-fat diet for two weeks before Streptozotocin (S.T.Z.) injection. Then, a single dose of Streptozotocin (35mg/kg, i.p.) was administered to induce diabetes.[24] Plasma glucose level was measured after 72 hours of Streptozotocin treatment. After S.T.Z. administration animals were kept for 6 weeks for complete induction of diabetic complications. Those animals showing blood glucose more than or equal to 250 mg/dl were considered as diabetic and were used for further studies. Diabetic animals were also fed the high-fat diet till the experiment's termination. Plasma glucose was measured again at the end of every week to confirm consistent hyperglycemia.

2.4 Animal Grouping and Treatment Protocol

Male SD rats were grouped into 5 groups: normal control, diabetic control, Glibenclamide (500 mcg/Kg, p.o.) & metformin (50 mg/kg, p.o.), Sodium metavanadate (5 mg/Kg), Magnesium sulfate (Dose) and Sodium metavanadate(Dose) with metformin (50 mg/kg, p.o.) and glibenclamide (500 mcg/Kg, p.o.). The treatment was given for 4 weeks. The total duration of the study was 12 weeks. Blood glucose and other parameters were measured to confirm the complete development of vascular complications. At the end of treatment, liver hexokinase level and vascular reactivity studies were done.

2.5 Estimation of Biochemical Parameters

The blood sample (approximately 0.4 ml) was collected from the tail vein under light anesthesia in heparinized centrifuge tubes. The plasma was separated by centrifugation (5000 rpm, 10 min at 4°C). Estimating serum glucose, total cholesterol, and triglyceride was performed using commercially available spectrophotometric kits (Nicholas India Pvt. Ltd.). The samples were analyzed in a semi-auto analyzer (photometer 5010, Nicholas India Pvt. Ltd). A standard ELISA kit measured insulin level. Magnesium was estimated using the magnesium XL FS kit (DiaSys Diagnostic System GmbH, Germany).

2.6 *Hexokinase Enzyme Activity Measurement from Liver*

Hexokinase activity in liver tissue was determined spectrophotometrically: The assay mixture contained 3.7 mM glucose, 7.5 mM MgCl2, 11 mM thioglycerol, and 45 mM HEPES buffer. Tissue was homogenized in ice-cold 0.1M phosphate-buffered saline (pH 7.4) to a concentration of 50 mg/ml. A cuvette was added 0.9 mL of this assay mixture and 0.03 mL of 0.22M ATP and mixed well into a spectrophotometer. After that, 0.1 mL of the tissue supernatant was added to the cuvette, and absorbance was noted at 340 nm. One unit of hexokinase was expressed as µ g/mg of tissue. [25]

2.7 *In-Vitro and In-Vivo Vascular Reactivity Studies*

After 8 weeks of S.T.Z induction, the rats were sacrificed, and the thoracic aorta was isolated and cleaned of surrounding fat and connective tissues. Care was taken during the cleaning of the aorta. The strip of the aorta of 2-3 mm in width and 22 mm in length was cut with a sharp iris scissor and placed in a 10 ml organ bath containing Krebs henseleit buffer (NaCl 118 mM, KCl 4.7 mM, KH_2PO_4 1.2 mM, Magnesium sulfate. $7H_2O$ 1.2 mM, $CaCl_2.2H_2O$ 2.5 mM, $NaHCO_3$ 25mM and glucose 5.5 mM) of pH 7.4. The solution was continuously aerated with 5% carbogen at 37°C. A resting tension of 2 g was applied to the strips and allowed to equilibrate for 2 h. After 2h of equilibration, two equipotent responses of KCl (80 mM) were recorded. The increased vascular activity of H_2O_2may be an essential factor in the development of vascular disorders associated with chronic diabetes mellitus. [11] So, Concentration-response curves (C.R.C.s) (10-6 to 3×10-3M) of H_2O_2 were constructed in age matched control and in 4 weeks post S.T.Z. Induced diabetic animals in the presence of different concentrations of hexokinase were preincubated for 20 min. For in vivo study, in drug-treated groups, the C.R.C.s of $H_2O_2$are taken without preincubation. Concentration-response curves (C.R.C.) of H_2O_2 (10-6 to 10-3 M) were recorded in age-matched normal and diabetic rat thoracic aortas. Changes in the isotonic contraction were recorded. The maximum vasoconstrictor response to the H_2O_2in normal control aorta was considered as 100%. In drug-treated diabetic rats, contractile responses of H_2O_2 were taken and compared with responses obtained from untreated diabetic rats. All of the experiments were carried out in normal laboratory light, as the H_2O_2 is light-sensitive.

2.8 *Analysis of Dose-Response Curves*

The maximal contraction response of H_2O_2 was calculated as a percentage response (% Tension in mg) and expressed in mean ± S.E.M. in age-matched control and 4 weeks post-S.T.Z. Induced diabetes for each experiment was plotted against log [M] conc. of the H_2O_2. % Inhibition of contraction was calculated for control and Diabetic in the presence of hexokinase and compared.

3. STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (S.E.M.). The significance between two groups was determined using the unpaired student's t-test. Statistical comparisons between all groups were performed using two-tailed one-way ANOVA followed by the Dunnett test. P-values <0.05 were considered statistically significant.

4. RESULTS

4.1 *Effect of Various Treatments On Biochemical Parameters, Magnesium and Hexokinase Activity*

Table 1: Effect of various drug treatments on biochemical parameters

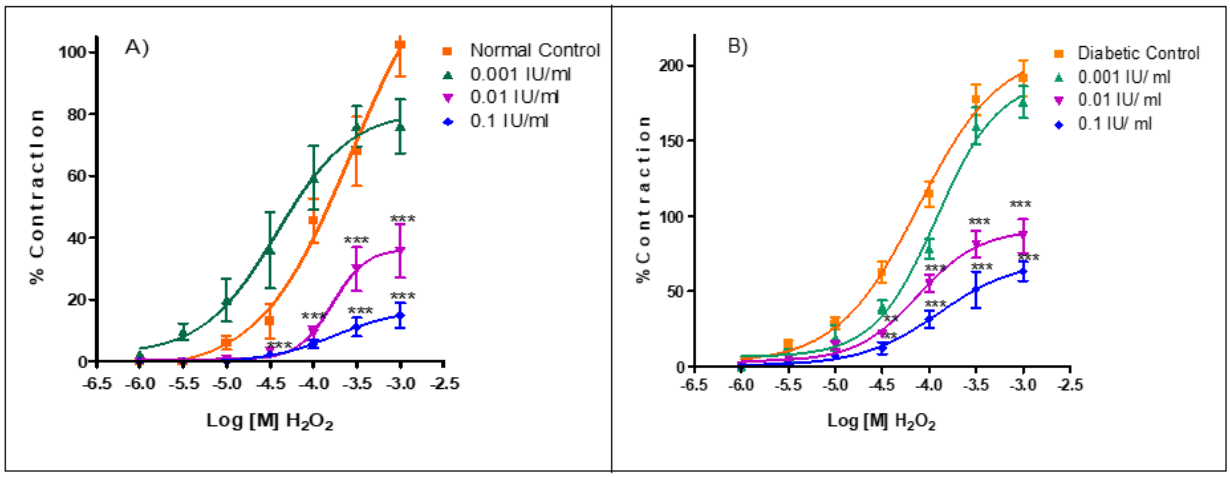
Biochemical parameters	Normal	Diabetic	Magnesium sulfate	Glibenclamide +Metformin	Glibenclamide + Metformin + Magnesium sulfate	S.M.V.	Glibenclamide + Metformin +SMV
Glucose (mg/dl)	89.05±10.95	484.5±50.01 ###	278±25**	222.45±22.45 ***	158.15±15.36 ***	327± 29 *	176.5±14.42 ***
Insulin (pmol/l)	176.31±10.6	256.2±13.23 ##	200.9 ±17.62 *	188.65±16.33 *	197.8±17.77 *	193.64±17.39 *	160.23±18.19 **
Triglyceride (mg/dl)	39.05±3.25	196.7±19.01 ###	130±10.15 *	98.63±12.09**	43±7.1***	110.01±13.01**	79±9 ***
Cholesterol (mg/dl)	57.62±6.33	190.76±9.98 ###	154.23± 8.41*	141.58±6.23**	78.32±4.92***	161.23±6.21*	150.51±5.93 **
Magnesium (mg/dl)	1.81±0.23	0.78±0.1##	3.63±0.37 ***	1.51±0.16**	4.11±0.41***	1.54±0.31*	2.43±0.42 **
Hexokinase (mg/gm of tissue)	1.096±0.16	0.29±0.045###	0.523±0.039**	0.401±0.029*	0.8±0.069***	0.69±0.051**	0.99±0.071 ***

Comparison of various biochemical parameters in normal, diabetic and various treatment groups. Each value is represented as Mean ± S.E.M. ## p < 0.01### p < 0.001 Vs. Normal Control. And *p < 0.05; ** p < 0.01; ***p < 0.001 Vs Diabetic Control. where SMV= sodium metaVanadate

Table 1 compares normal, diabetic, and treated animals with different parameters. Result suggests that the treatment of diabetic rat with Conventional drug in combination with Magnesium sulfate and vanadate reduce blood glucose level and Improve the lipid profile. Data reveals that Hexokinase activator might be helpful for Diabetic and other lipid complications. The conventional drug, combined with the Hexokinase activator, activates an enzyme's functioning, which might be one of the targets to treat late complications of diabetes.

4.2 *Effect of H_2O_2 On Normal and Diabetic Rat Thoracic Aorta*

4.2.1 *Effect of Hexokinase On H_2O_2 Induced Contraction in Normal Control and Diabetic Rat Thoracic Aorta*



*Effect of H_2O_2 on endothelium intact aortic spiral preparations obtained from untreated age-matched normal rats(A), high-fat diet, and a low dose of S.T.Z. induced diabetic rats(B) in the presence of a varying concentration of hexokinase 0.001 IU/ml (▲), 0.01 IU/ml (▼), 0.1 IU/ml (♦) Each point is represented as mean ± S.E.M. n = 4-6. For unpaired t-test *p < 0.05; ** p < 0.01; ***p < 0.001 Vs Diabetic Control.*

Fig 1: Cumulative concentration-response curves (C.R.C.s)

4.2.2 *Maximal Response (Emax) And Pd_2 Values of H_2O_2 On Spiral Aortic Preparation of Untreated Age Matched and HFF-STZ Diabetic Control Rat in the presence of Various Concentrations Hexokinase (In-Vitro).*

Table 2: Comparison of pD_2 values and %Emax

Groups	Non-Diabetic		Diabetic	
	pD_2 value	% Emax (mg)	pD_2 value	% Emax (mg)
Without incubation	3.825±0.19	101.30±6.42%	4.635±0.37	195.33±11.69%
0.001 IU/ ml	4.046±0.22	78.361±8.12%	4.304±0.15	180.13±10.23%
0.01 IU/ ml	3.781±0.137	35.88±4.95% ###	4.073±0.13	88.07±9.15% ***
0.1 IU/ ml	3.755±0.334	14.88±1.96% ###	3.35±0.33 *	63.41±5.78% ***

*Data shows the comparison of pD_2 values and %Emax of H_2O_2 in normal rat thoracic aorta and diabetic rat thoracic aorta with or without incubation of different concentrations of hexokinase, For unpaired t-test ### p < 0.001 Vs. Normal Control. And *p < 0.05; ** p < 0.01; ***p < 0.001 Vs Diabetic Control.*

In the present study, H_2O_2 induced contraction, % Emax and pD_2 value were higher in diabetic rat thoracic aorta. This indicates an increase in the release of H_2O_2 in diabetic rat aorta, which may be responsible for vascular complications [41]. Based on the pD_2 value and Emax result, it was concluded that an increase in the release of H_2O_2 in diabetic rats might be due to a decrease in Hexokinase activity [42].

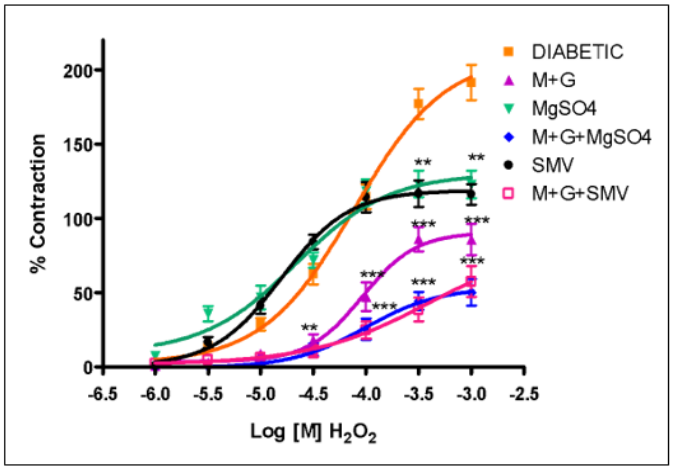
4.2.3 *Effect of Drug Treatment on H_2O_2-Induced Contraction (In-Vivo)*

Table 3: pD_2 values and % Emax of H_2O_2 induced contraction in different treatment groups

Animal Group	pD_2 value	% Emax
Normal	3.825±0.19	101.30±6.42%
Diabetic	4.635±0.204 #	195.33±11.69% ###
Magnesium sulfate	4.25±0.25 *	127.56±10.01% **
Glibenclamide and Metformin	3.98±0.165 *	88.380±7.09% ***
M + G + $MgSO_4$	3.08±0.086 ***	50.78±3.66% ***
Sodium metaVanadate	4.91±0.186	118.57±9.56% ***
M + G + SMV	3.20±0.11 ***	57.31±3.05% **

*Comparison of pD_2 values and % Emax of H_2O_2 in normal, diabetic rat thoracic aorta and various treatment groups. Each value is represented as Mean ± S.E.M. # p < 0.05, ### p < 0.001 Vs. Normal Control. *p < 0.05; ** p < 0.01; ***p < 0.001 Vs Diabetic Control.*

In-vivo administration of hexokinase activator magnesium sulfate and vanadate for four weeks showed a significant decrease in % Emax of H_2O_2. In addition, a combination of magnesium with Metformin and Glibenclamide significantly reduced the % Emax and pD_2 value of H_2O_2 compared to the diabetic control group. These results show that the hexokinase activator prevents free radical-induced vascular complications caused by diabetes [43].



*On endothelium intact, aortic spiral preparations were obtained from a high-fat diet and a low dose of S.T.Z. induced diabetic rats (■) and 4 weeks treated diabetic rats with M (50 mg/Kg) + G (500 mg/Kg) (▲), MgSO4 (50mg/Kg) (▼), M+G+MgSO4(♦), S.M.V. (5 mg/Kg) (●), M +G + S.M.V. (□) treated animals. Each point is represented as mean ± S.E.M. n = 4-6. For unpaired t-test *p < 0.05; ** p < 0.01; ***p < 0.001 Vs Diabetic Control.*

Fig 2: Cumulative concentration-response curves (C.R.C.s) to H_2O_2

5. DISCUSSION

According to the Diabetes Atlas 2014 published by the International Diabetes Federation, currently, the number of people with diabetes 387 million in the world and 75 million people in South-East Asia is expected to rise to 123 million by 2035 unless urgent preventive steps are taken.[2] The prime objective of this research project was to postulate the crucial molecular events linking hyperglycemia and vascular dysfunction associated with type II diabetes, with the main emphasis on hydrogen peroxide and hexokinase. The present study aimed to determine the hexokinase activity in type II diabetes and to check whether hexokinase impairs the H_2O_2-induced vascular dysfunction in diabetes.To prove a hypothesis, hexokinase activity in the liver of age-matched normal control and diabetic rats were measured. To determine the relation between hexokinase and H_2O_2, the C.R.C.s of H_2O_2 were constructed in aortic spiral preparations obtained from normal age-matched control and 10th weeks post high fat fed S.T.Z. diabetic rats. The present study expanded from in vitro vascular responses to *in vivo* study of a 4-week treatment of hexokinase activator MgSO4 & Sodium metavanadate in combination with currently available antidiabetic therapy. Vascular responses of H_2O_2 in treated rat aortas were taken to check in-vitro in-vivo correlation and calculated the pD2 values and % Emax. According to a previous study, magnesium improves glucose metabolism and improved insulin resistance.[26] Vanadate-like compounds have a potential role as adjunctive therapy in patients with IDDM and NIDDM diabetes mellitus.[27] In the present study, treatment of MgSO4 ($p<0.01$) and sodium metavanadate ($p<0.05$) alone showed a significantly decreased in blood glucose level, which reveals the hypoglycemic effect of the hexokinase activator. The hexokinase activator's hypoglycemic effect is less significant compared to metformin + glibenclamide treatment. Still, it showed a synergistic effect on decreased blood glucose levels when given in combination with metformin + glibenclamide. All these results proved the role of hexokinase in regulating blood glucose levels in diabetic rats (Table 1) [28, 29]. In the present study, treatment of MgSO4 ($p<0.05$) and sodium metavanadate ($p<0.001$) alone showed a significant decrease in triglyceride level, which reveals the antihyperlipidemic effect of the hexokinase activator. While total cholesterol level is significantly decreased in MgSO4 ($p<0.05$) and sodium metavanadate ($p<0.01$) treated animals. The antihyperlipidemic effect of hexokinase activator is less significant compared to Metformin + Glibenclamide treatment, but it showed a synergistic effect on decreased triglyceride and plasma cholesterol levels when given in combination with metformin + glibenclamide, which will comply with result proved by researcher G. Subrahmanyam (Table 1) [30]. From the glucose and lipid profile results, it can be stated that treatment of hexokinase activator with available hypoglycemic drugs may be beneficial in managing diabetic conditions. Magnesium sulfate orally is used as a laxative, while intravenous administration is helpful for hypomagnesemia, arrhythmia, and eclampsia. A concentration of 1.8 to 3.0 mmol/L has been suggested for treating eclamptic convulsions. [31] Diabetic rats showed a significant increase in blood glucose, Insulin, Triglyceride, and cholesterol. Treatment with magnesium sulfate and sodium metavanadate showed a significant reduction in all biochemical parameters, indicating it has the potential to be used as an antidiabetic agent.[26, 27] In combination with glibenclamide and metformin, it showed a higher effect, so we may conclude that it can be utilized in combination with currently utilized allopathy antidiabetic drugs. The relative synthesis rate of hexokinase II is approximately 1.9 times higher in the normal than in the diabetic rats. [32] The administration of insulin to the diabetic animal increases the rate of hexokinase synthesis to approximately normal levels. Hypomagnesaemia may have a negative impact on glucose homeostasis and insulin sensitivity in type II diabetes mellitus patients.[33] Further, Mg deficiency has been proposed as a novel factor implicated in the pathogenesis of late diabetic complications 4 having 25% to 39% prevalence. [34] Sodium metavanadate, a hexokinase activator, has an insulin-mimetic effect[18]. Present data show the efficacy of drug combination on hexokinase for restoration of the normal functioning of the liver. Treatment with S.M.V. significantly increased Mg ($p<0.01$) and hexokinase ($p<0.001$) as compared to Diabetic, but from these results, it can be observed that S.M.V. have a direct effect on hexokinase along with the effect on magnesium. A previous study had proven the role of vanadate and magnesium in hypoglycemia and hypolipidemic activity[35]. The results of this work confirmed that the hexokinase activator improves diabetic and microvascular complications. The hexokinase activity in the liver ($p < 0.001$) and magnesium level ($p < 0.01$) in blood were

significantly reduced in diabetic rats, while the treatment with magnesium sulfate significantly increased the hexokinase activity (** $p < 0.01$) and magnesium (***$p < 0.001$) level. And the treatment with sodium metavanadate significantly increased the hexokinase activity ** $p < 0.01$) and magnesium (*$p < 0.05$) levels, which indicates that magnesium is required for hexokinase activity which strengthens the proven report of hexokinase (Table 1)[36]. Evidence shows that excessive R.O.S. formation in diabetes leads to vascular complications. [37] As far as the H_2O_2 mediated contraction in pathological conditions was concerned, very few reports available, like the mediator of H_2O_2 induced contraction in S.H.R. was PLA2-COX-TXA2 pathway. [38,39] But information on the mediators of H_2O_2-induced contraction was still lacking in the aorta of diabetic rats. One report indicates higher contraction produced by H_2O_2 in the aorta of S.T.Z. induced diabetic rats. The increased vascular activity of H_2O_2 may be an important factor in developing vascular disorders associated with chronic diabetes mellitus. [25], [40] One relation between the hexokinase activity and H_2O_2 is that the Activation of mitochondrial-HK activity abolished the rate of H_2O_2 generation in Potato tuber mitochondria[41]. The role of hexokinase in R.O.S. stability in brain mitochondria was discussed in a previous study. [43] These results suggest that over-expression of hexokinase inhibits H_2O_2 release, which is involved in signaling pathways responsible for vascular dysfunction in HFF-STZ diabetic rats (Table 2 and Figure 1). However, this was the first study to prove the relation between free radical hydrogen peroxide and glycogen metabolizing enzyme hexokinase in isolated rat aorta. This study might prove that the hexokinase activator improves microvascular endothelial dysfunction, which might be one of the factors for late diabetic complications. So, data from the presented study suggests that increased hexokinase activity inhibits H_2O_2 release in High-fat diets and low doses of S.T.Z. diabetic rats. These all together reveal that hexokinase might be a novel therapeutic target for treating diabetes and its associated vascular complications (Table 3 and Figure 2).

6. CONCLUSION

Hexokinase activity is reduced in diabetic rats, which may be responsible for the increase in vascular responses of H_2O_2. Treatment with a Hexokinase activator reduces the diabetic state and lowers the effect of H_2O_2 on the vasculature of Diabetic rats. So, these agents might be useful as adjuvant therapy with conventional therapy because both are less productive at individual states compared to the combination.

7. ACKNOWLEDGEMENT

The authors are thankful to Troikaa pharma, Ahmedabad, for granting us the Raman Bhai V. Patel Master's best thesis award and a cash prize of 51000/- rupees for this research work.

8. AUTHORS CONTRIBUTION STATEMENT

Ekta Patel and Nilesh Kanzariya conceived the presented idea and made a hypothesis. Ekta Patel developed the method and performed the computations. Kunjal Vegad, Priya Shah, and Dhwani Shah verified the analytical methods. Yogesh Patel, Nilesh kanzariya, and Divyakant Patel encouraged Ekta Patel to investigate hexokinase-targeted drugs and supervised the findings of this work. All authors discussed the results and statistical aspects of the impact and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Federation I. I.D.F. Diabetes Atlas, tenth. Int Diabetes. 2021.
2. Patel E. Patel NJAriP, BioLogicals. Antidiabetic activity of leaves of Manilkara hexandra: role of carbohydrate metabolizing α-amylase enzyme. 2015;5(2):863-7.
3. Ekta D, Patel KLV, Shah DA, Patel YK, Kanzariya NR. A novel therapeutic target – glycogen synthase Kinase-3. Inventi Rapid. Mol Pharmacol. 2017;03:7.
4. Ronimus RS, Morgan HWJB. Communications br. Cloning Biochem Char Novel Mouse ADP-Depend Glucokinase. 2004;315(3):652-8.
5. Pda PE D, Patel YK, Shah DA, Vegad Kunjal L, Patel NJ. Enzyme Assay Guided Isolation of alpha-amylase Inhibitor from Manilkara hexandra Leaves. NeuroQuantology. 2022;20(11):9.
6. Frank SK, Fromm H.J.J. Aob, biophysics. Effect of streptozotocin-induced diabetes and insulin treatment on the synthesis of hexokinase II in the skeletal muscle of the rat. 1986;249(1):61-9.
7. da-Silva WS, Gómez-Puyou A, de Gómez-Puyou MT, Moreno-Sanchez R, De Felice FG, de Meis L, et al. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state A.D.P. formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. J Biol Chem. 2004;279(38):39846-55. doi: 10.1074/jbc.M403835200, PMID 15247300.
8. Finkel TJCoicb. Oxygen Radic Signal. 1998;10(2):248-53.
9. Hibino M, Okumura K, Iwama Y, Mokuno S, Osanai H, Matsui H, et al. Oxygen-derived free radical-induced vasoconstriction by thromboxane A2 in aorta of the spontaneously hypertensive rat. J Cardiovasc Pharmacol. 1999;33(4):605-10. doi: 10.1097/00005344-199904000-00013, PMID 10218731.
10. WYJTAjom F. The importance of insulin resistance in the pathogenesis of type 2 diabetes mellitus. 2000;108(6):9-14.
11. Karasu ÇJFrb, medicine. Increased activity of H2O2 in aorta isolated from chronically streptozotocin-diabetic rats: effects of antioxidant enzymes and enzyme inhibitors. 1999;27(1-2):16-27.
12. Heffetz D, Bushkin I, Dror R, Zick Y. The insulinomimetic agents H2O2 and vanadate stimulate protein tyrosine phosphorylation in intact cells. J Biol Chem. 1990;265(5):2896-902. PMID 2154464.
13. Pieper GM, Langenstroer P. Siebeneich WJCr. Diabet-Induced Endothelial Dysfunct Rat Aorta Role Hydroxyl Radic. 1997;34(1):145-56.
14. Goldfine AB, Simonson DC, Folli F, Patti M-E, Kahn CRM. Biochemistry c. In vivo andin vitro studies of vanadate in human and rodent diabetes mellitus. 1995;153(1):217-31.
15. Orvig C, Thompson K, Battell M. McNeill JJMiibs. Vanadium Compd Insulin Mimics. 1995;31:575-94.

16. Gupta BL, Preet A, Baquer NJ. Job. Protective effects of sodium orthovanadate in diabetic reticulocytes and aging red blood cells of Wistar rats. 2004;29(1):73-9.
17. Srivastava AKJM. Biochemistry C. Anti-Diabet Toxic Eff Vanadium Compd. 2000;206(1):177-82.
18. Barbagallo M, Dominguez LJ, Resnick LMJH. Insulin-mimetic action of vanadate: role of intracellular magnesium. Hypertension. 2001;38(3 Pt 2):701-4. doi: 10.1161/hy09t1.095392, PMID 11566960.
19. Barbagallo M, Dominguez LJJ. Aob, biophysics. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. 2007;458(1):40-7.
20. Barbagallo M, Dominguez LJ, Tagliamonte MR, Resnick LM, Paolisso GJH. Effects of glutathione on red blood cell intracellular magnesium: relation to glucose metabolism. Hypertension. 1999;34(1):76-82. DOI: 10.1161/01.hyp.34.1.76, PMID 10406827.
21. Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, et al. role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med. 2003;24(1-3):39-52. doi: 10.1016/s0098-2997(02)00090-0, PMID 12537988.
22. Weber C, Gauda E, Hecht E, Mizaikoff B, Kranz C, editors. Amperometric micro biosensors based on PQQ-dependent glucose dehydrogenase towards the development of an ATP biosensor for in vitro analysis. Munich, Germany: World Congress on Medical Physics and Biomedical Engineering. Springer; 2009.
23. Chaudhary DP, Sharma R, Bansal D.D.J. Beer. Implications of magnesium deficiency in type 2 diabetes: a review. 2010;134(2):119-29.
24. Srinivasan K, Patole PS, Kaul CL, Ramarao P. Reversal of glucose intolerance by by pioglitazone in high fat diet-fed rats. Methods Find Exp Clin Pharmacol. 2004;26(5):327-33. doi: 10.1358/mf.2004.26.5.831322. PMID 15319810.
25. Bera TK, De D, Chatterjee K, Ali KM, Ghosh DJI. JoAnn. Effect of Diashis, a polyherbal formulation, in streptozotocin-induced diabetic male albino rats. 2010;1(1):18.
26. Sohrabipour S, Sharifi MR, Sharifi M, Talebi A, Soltani NJF. Pharmacology c. Effect of magnesium sulfate administration to improve insulin resistance in type 2 diabetes animal model: using the hyperinsulinemic-euglycemic clamp technique. 2018;32(6):603-16.
27. Goldfine AB, Simonson DC, Folli F, Patti M-E, Kahn CRJT. JoCE, metabolism. Metabolic effects of sodium metavanadate in humans with insulin-dependent and noninsulin-dependent diabetes mellitus in vivo and in vitro studies. 1995;80(11):3311-20.
28. Garfinkel L, Garfinkel DJM. Magnesium regulation of the glycolytic pathway and the enzymes involved. Magnesium. 1985;4(2-3):60-72. PMID 2931560.
29. Xu MZ, Zhang AZ, Li XR, Xu W, Shen LW. Effects of vanadate on the activities of mice glucokinase and hexokinase. J Zhejiang Univ Sci. 2004;5(10):1245-8. doi: 10.1631/jzus.2004.1245, PMID 15362196.
30. Subrahmanyam G, Sankar KD, Ramalingam K, Bhanu P. Electrolytes. Hypolipidemic Anti-Atherogenic Eff Vanadium High-Fat Diet Rabbits. 2013;30(3).
31. Lu JF, Nightingale CHJCp. Magnesium sulfate in eclampsia and pre-eclampsia: pharmacokinetic principles. Clin Pharmacokinet. 2000;38(4):305-14. doi: 10.2165/00003088-200038040-00002, PMID 10803454.
32. Liang Q, Donthi RV, Kralik PM, Epstein PNJCr. Elevated hexokinase increases cardiac glycolysis in transgenic mice. Cardiovasc Res. 2002;53(2):423-30. doi: 10.1016/s0008-6363(01)00495-3, PMID 11827693.
33. Gommers LM, Hoenderop JG, Bindels RJ, de Baaij JHJD. Hypomagnesemia in type 2 diabetes: a vicious circle? Diabetes. 2016;65(1):3-13. doi: 10.2337/db15-1028, PMID 26696633.
34. Ankush R, Suryakar A. Ankush NJIjocb. Hypomagnesaemia in type-2 diabetes mellitus patients: a study on the status of oxidative and nitrosative stress. 2009;24(2):184-9.
35. Kornacki J, Gutowska I, Wiercioch M, Łukomska A, Tarnowski M, Drozd A, et al. Sodium orthovanadate changes fatty acid composition and increased expression of stearoyl-coenzyme A desaturase in THP-1 macrophages. Biol Trace Elem Res. 2020;193(1):152-61. doi: 10.1007/s12011-019-01699-2, PMID 30927246.
36. Voma C, Romani AJIL, UK. Role of magnesium in the regulation of hepatic glucose homeostasis. 2014:95-111.
37. Calver A, Collier J, Vallance PJT. Joci. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. 1992;90(6):2548-54.
38. Rodríguez-Martínez MA, García-Cohen EC, Baena AB, González R, Salaíces M. Marín JJBjop. Contractile responses elicited by hydrogen peroxide in aorta from normotensive and hypertensive rats. Endothelial modulation and mechanism involved. 1998;125(6):1329-35.
39. Gao Y. Lee RJBjop. Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A2 production. 2001;134(8):1639-46.
40. Hink U, Tsilimingas N, Wendt M. Münzel TJTie. Mech Underlying Endothelial Dysfunct Diabetes Mellitus. 2003;2(5):293-304.
41. Camacho-Pereira J, Meyer LE, Machado LB, Oliveira MF, Galina AJPP. Reactive oxygen species production by potato tuber mitochondria is modulated by mitochondrially bound hexokinase activity. Plant Physiol. 2009;149(2):1099-110. doi: 10.1104/pp.108.129247, PMID 19109413.
42. Zabala F, Besler T, Ari N, Karasu C, (Antioxidants in Diabetes-Induced Complications) The ADIC Study Group. Hydrogen peroxide-induced inhibition of vasomotor activity: evaluation of single and combined treatments with vitamin A and insulin in streptozotocin-diabetic rats. Int J Exp Diabetes Res. 2002;3(2):119-30. doi: 10.1080/15604280214484, PMID 11991198.
43. Cavalcanti-de-Albuquerque JP, de Souza Ferreira E, de Carvalho DP, Galina AJMN. Mitochondria-bound hexokinase (mt-HK) activity differs in cortical and hypothalamic synaptosomes: differential role of mt-HK in H2O2 depuration. Mol Neurobiol. 2018;55(7):5889-900. doi: 10.1007/s12035-017-0807-9, PMID 29119535.