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Original Article

Cardio- and Hepatoprotective Effects of Hydrogen Sulfide in a High-Fat Diet and Low-Dose Streptozotocin-Induced Type 2 Diabetic Rats

Hadis Abutalebian[1], Mohammad Esmaeil Shahaboddin[1], Maryam Akhavan Taheri [2], Azam Ghaseminezhad[1], Gholamreza Ghavipanjeh[3], Mojtaba Sehhat[4], Nejat Kheiripour[1*]

[1]Biochemistry and Nutrition Research Center in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[2]Germ Cell Production Research Center, Kashan University of Medical Sciences, Kashan, Iran
[3]Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran
[4]Trauma Research Center, Kashan University of Medical Sciences, Kashan, Iran

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***Corresponding author:**
Nejat Kheiripour
Email: nejatkh.bio@gmail.com

Abstract

Background: Hydrogen sulfide (H_2S) is the third most crucial gas that is produced inside the body, and at physiological levels, it increases a wide range of health properties, such as anti-inflammatory antioxidant effects.

Objectives: This study aimed at evaluating the impact of H_2S administration on oxidative stress (OS) in the heart and liver tissues of diabetic rats.

Methods: Twenty-eight Wistar rats were randomly divided into 4 groups, namely, the healthy control group, the diabetic group, and diabetic groups treated with 50 μM/kg and 100 μM/kg of H_2S. After 60 days of treatment, the animals were sacrificed, and biochemical and OS markers were determined using colorimeter methods. In addition, the liver tissue underwent histological assessment.

Results: The findings revealed that H_2S controlled the weight of rats and significantly decreased fasting blood sugar (FBS) in the treatment group with a dose of 50 μM/kg and 100 μM/kg ($P<0.05$) compared to the diabetic animals. Further, insulin concentration and insulin resistance in the H_2S 100 μM/kg group decreased in comparison to the diabetic group ($P<0.05$). The levels of triglyceride, cholesterol, atherogenic index, and low-density lipoprotein in H_2S-treated animals were markedly lower than in the diabetic group ($P<0.05$). The results of OS parameters demonstrated that H_2S 100 μM/kg reduced the malondialdehyde in the heart tissue compared to the diabetic group ($P<0.05$). The histological assessment also confirmed the effectiveness of H_2S in improving liver morphology and parenchymal structure.

Conclusion: According to the results of this study, H_2S can be considered a suitable therapeutic agent to prevent diabetes complications.

Keywords: Diabetes mellitus, Hydrogen sulfide, Oxidative stress, Heart, Liver

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Background

Currently, diabetes mellitus (DM) is one of the main chronic endocrine disorders in the world and occurs due to the production of insulin, or insulin resistance (1). DM can be considered one of the main causes of the incidence of heart and liver diseases (2). Hyperglycemia conditions induce oxidative stress (OS) through various mechanisms, such as the increased rate of reactive oxygen species production or the reduction of the antioxidant defense mechanism. The increased production of reactive oxygen species is associated with the pathogenesis and complications of DM (3).

Hydrogen sulfide (H_2S) is known as the third most crucial gas that is produced inside the body. H_2S is physiologically produced from cysteine and homocysteine (4,5). Numerous studies have revealed the role of endogenous H_2S in the pathogenesis and progression of type 2 DM (T2DM). H_2S has anti-inflammatory, anti-apoptotic, and antioxidant effects. In addition, available evidence indicates the role of H_2S in preventing diabetes complications such as nephropathy, cardiomyopathy, and hepatic disorders. Therefore, this endogenous gas shows a promising strategy for the management and prevention of diabetes and its complications (6). Although several

studies have confirmed the protective effects of H_2S gas on diabetes and its related disorders, its mechanism has not been fully elucidated yet. Accordingly, this study sought to investigate the effects of H_2S administration on biochemical and OS parameters in heart and liver tissues in T2DM animals induced by a high-fat diet (HFD) and single low-dose streptozotocin (STZ).

Materials and Methods

Twenty-eight male Wistar rats (200 ± 20 g) were used in this experimental study. The rats were maintained in standard conditions for one week (12/12 light/dark cycles). Then, they were randomly and equally divided into four groups (each containing 7 animals), including the control group, T2DM group, and T2DM groups treated with an intraperitoneal injection of 50 µM/kg and 100 µM/kg of H_2S. For the induction of T2DM, the rats received an HFD (35% fat, 24% protein, and 26% carbohydrate) for 60 days, and on the 61st day, while they were fasting for 12 hours, they received a single dose of STZ 40 mg/kg intraperitoneally. To confirm diabetes, 3 days later, fasting blood sugar (FBS) was determined by a glucometer. Rats with an FBS above 150 were considered diabetic (7). In the treatment groups, H_2S with doses of 50 µM/kg and 100 µM/kg was intraperitoneally administered for 60 days. To prepare H_2S in the selected daily doses, a certain amount of sodium hydrosulfide (NaHS) powder was taken based on its molecular weight (56.06 mol/g), dissolved in normal saline, and then injected into the diabetic-treated groups (8). The experimental timeline is shown in Figure 1. At the end of the study, the animals were anesthetized with a combination of low doses of ketamine and xylazine after an overnight fast, and the serum samples were collected and stored at −20 ˚C for biochemical tests. The activities of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), troponin I, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT) underwent measurement (9). In addition, heart and liver tissues were separated and stored for antioxidant and histological examinations.

Determination of Biochemical Parameters

The FBS was measured using a commercial glucometer through tail vein blood sampling. Insulin was measured using the enzyme-linked immunosorbent assay kit (RayBiotech). The insulin resistance index (homeostatic model assessment for insulin resistance: HOMA-IR) was calculated using the following formula (3):

$$HOMA\text{-}IR = Fasting\ insulin\ (\mu U/mL) \times FBS\ (mg/dL)/405$$

Further, heart and liver enzymes, troponin I, total and direct bilirubin, and lipid profiles of serums were measured by the BT3000 device.

Determination of Oxidative Stress Biomarkers

To measure the levels of OS, the heart and liver tissues were homogenized with liquid nitrogen, mixed in a lysing buffer containing anti-protease, and kept on ice for 20 minutes. Furthermore, total oxidant status (TOS), total antioxidant capacity (TAC), malondialdehyde (MDA), and total thiol groups (TTG) were estimated calorimetrically (ZellBio GmbH, Ulm, Germany), according to the manufacturer's instructions.

Liver Histology

For the histopathological analysis, the tissues were removed from all animals, and a small portion of liver tissue was fixed with 10% formalin and embedded in paraffin. Then, the serial sections (5-µm thick slices) were prepared and stained with hematoxylin and eosin (H&E), according to a previously published paper (10). The degree of fibrosis, hyperplasia of bile ducts, necrosis, and inflammation of the liver tissue were graded according to the METAVIR system (11).

Statistical Analysis

The data were analyzed by SPSS-16 software using analysis of variance and post-hoc analysis. Moreover, a repeated-measures test was used for parameters such as weight and FBS that were measured during the period, and $P < 0.05$

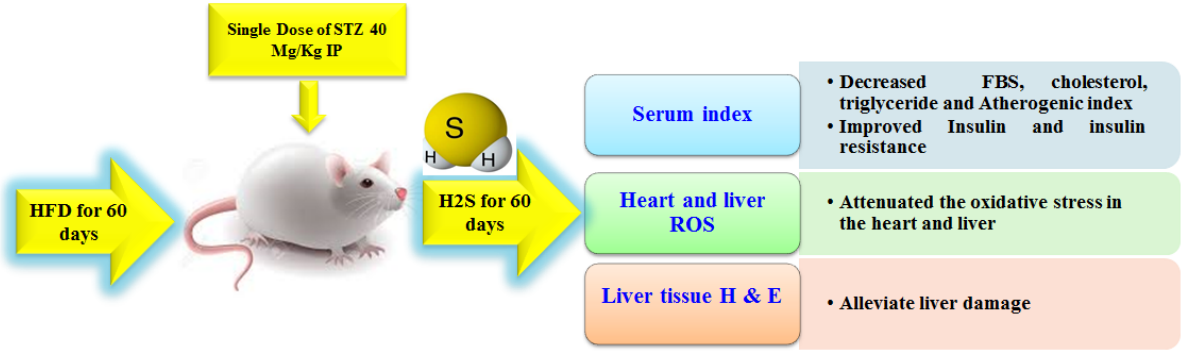

Figure 1. Experimental Time Line: Effects of H_2S on the Heart and Liver Tissues of T2DM Rats. *Note*. T2DM: Type 2 diabetes: H_2S: Hydrogen sulphide; STZ: Streptozotocin. The T2DM model was induced by receiving a high-fat diet (35% fat, 24% protein, and 26% carbohydrate) for 60 days, and on the 61st day, while they were fasting for 12 hours, they received a single dose of STZ 40 mg/kg intraperitoneally, which was confirmed by a fasting glucose level of ≥ 150 mg/dL 3 days later. One day after the confirmation of diabetes, treatment was started with doses of 50 µM/kg H_2S and 100 µM/kg H_2S (daily intraperitoneally) and continued for 60 days. At the end of the experiment, biochemical and oxidative stress markers were measured using spectrophotometric methods. In addition, the liver tissue was histologically assessed

was considered statistically significant. Finally, the Kolmogorov-Smirnov test was utilized to confirm that the study variables were normal.

Results

Effects of Hydrogen Sulfide on Rats' Body Weight

As shown in Figure 2, the body weight of groups treated with 50 µM/kg and 100 µM/kg H_2S was elevated compared to that of diabetic rats, but this weight gain was not significant.

Effects of Hydrogen Sulfide on Biochemical Parameters

The concentration of FBS in the treatment group with 50 µM/kg and 100 µM/kg H_2S doses reduced markedly ($P<0.05$) compared to the diabetic group. However, the FBS level was not reversed to the level of the control group (Figure 3). The results showed that insulin concentrations were significantly decreased in the diabetic group rats with 100 µM/kg H_2S in comparison to the diabetic group ($P<0.05$). HOMA-IR was significantly decreased in the diabetic groups treated with 50 µM/kg H_2S and 100 µM/kg H_2S when compared to diabetic control rats ($P<0.05$, Table 1).

The findings related to lipid parameters are provided in Table 1. In this study, triglyceride, low-density lipoprotein (LDL), and the atherogenic index were significantly increased in diabetic rats' cholesterol. On the other hand, treatment with 50 µM/kg and 100 µM/kg H_2S caused a significant decrease in cholesterol, LDL, and atherogenic index ($P<0.05$). Additionally, treatment with 100 µM/kg H_2S led to a significant decrease in triglyceride levels ($P<0.05$). There was no significant difference in HDL levels in the diabetic group compared to the H_2S-treated groups. In this study, no significant change was observed in total and direct bilirubin concentrations (Table 1).

According to the results, CK-MB, LDH, and troponin I levels were higher in the diabetic group than in the other 3 groups. In the H_2S-treated groups, this level was close to that of the control group, but there was no significant difference in the other groups (Figure 4). The levels of ALT and ALP enzymes in the diabetic group increased compared to the control group ($P<0.05$). The amount of AST, ALT, and ALP enzymes in the treatment groups decreased in comparison to the diabetic rats, but this

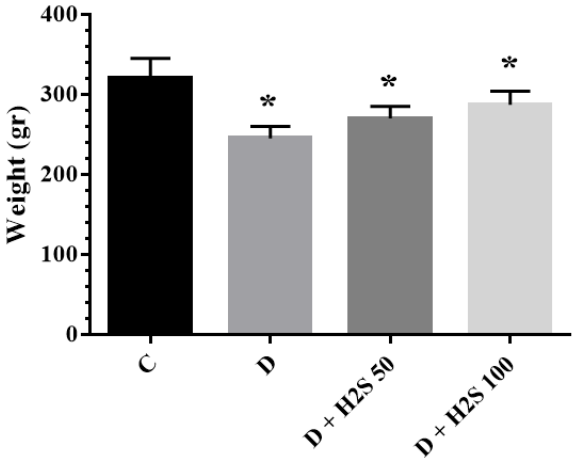

Figure 2. The Weight of the Studied Groups at the End of the Experiment. *Note*. The results are presented as means ± standard errors. C: Healthy control; D: Diabetic; H_2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); *: Significant comparison with group C

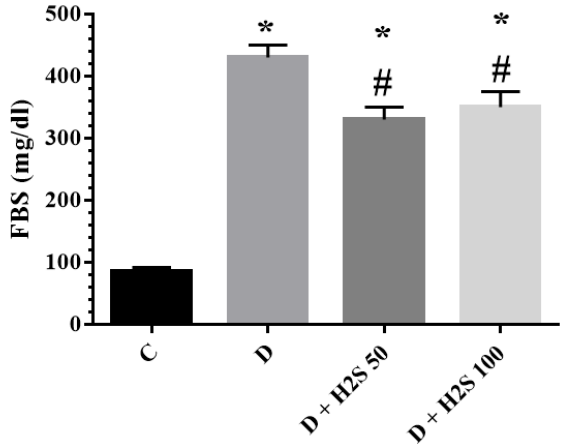

Figure 3. The Fasting Blood Sugar of the Studied Groups at the End of the Experiment. *Note*. The results are presented as means ± standard error. C: Healthy control; D: Diabetic; H_2S: Hydrogen sulfide (50 100 µM/kg or 100 µM/kg); *: Significant comparison with group C; #: Significant comparison with group D ($P<0.05$)

Table 1. Insulin, HOMA-IR, and Serum Biochemical Parameters of Studied Groups

Group parameters	C	D	D+H_2S 50µM/kg	D+ H_2S 100µM/kg
Insulin (pmol/L)	210.67 ± 12.63	109.31 ± 5.33[a]	103.60 ± 10.99[a]	81.65 ± 8.85[ab]
HOMA-IR	45.77 ± 4.04	98.30 ± 6.96[a]	90.20 ± 11.55[a]	74.60 ± 12.80[ab]
Cholesterol(mg/dL)	40.42 ± 7.81	56.01 ± 6.27	38.80 ± 5.77[b]	38.21 ± 1.92[b]
Triglyceride (mg/dL)	63.05 ± 21.15	61.35 ± 13.47	35.42 ± 10.71[a]	28.61 ± 13.57[ab]
HDL-C (mg/dL)	25.85 ± 3.85	19.21 ± 2.21[a]	20.00 ± 3.58[a]	19.21 ± 2.62[a]
LDL-C (mg/dL)	11.61 ± 2.65	17.65 ± 3.21[a]	11.43 ± 1.21[b]	11.41 ± 2.07[b]
Atherogenic index (TG/HDL-C)	2.49 ± 0.87	3.67± 0.55	1.67 ± 0.39[b]	1.53 ± 0.80[b]
Bili-total (mg/dL)	40.23±1.96	42.67± 2.00	42.45 ± 2.21	40.12±2.01
Bili-direct (mg/dL)	0.1± 0.01	0.12 ± 0.02	0.12 ± 0.00	0.1±0.00

Note. HOMA-IR: Homeostatic model assessment for insulin resistance. The results are presented as means ± standard errors. HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; C: healthy control, D: Diabetic; H_2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); HOMA-IR: Homeostasis assessment model of insulin resistance.
[a]significant comparison with group C;
[b]significant comparison with group D ($P< 0.05$).

decrease was not significant (Figure 5).

Effects of Hydrogen Sulfide on Oxidative Stress Biomarkers

In the present study, OS was evaluated by detecting the content of TAC, TOS, MDA, and TTG in the heart and liver by spectrophotometric methods. The amount of TAC in the heart and liver tissues decreased in the diabetic animals compared to the control group, and H_2S increased the TAC in comparison to diabetic group, but these changes were not significant. The results showed that the amount of TOS in the heart and liver tissue in the diabetic rats increased compared to the control group. Based on the results, the TOS concentration decreased in the groups treated with doses of 50 µM/kg and 100 µM/kg H_2S compared to both the control and diabetic groups. However, this change was not statistically significant. The amount of MDA in the heart and liver tissues in the diabetic group increased compared to the control group, but it was not significant. Conversely, the MDA level was reduced in the heart and liver tissues of groups treated with 50 µM/kg and 100 µM/kg H_2S doses compared to

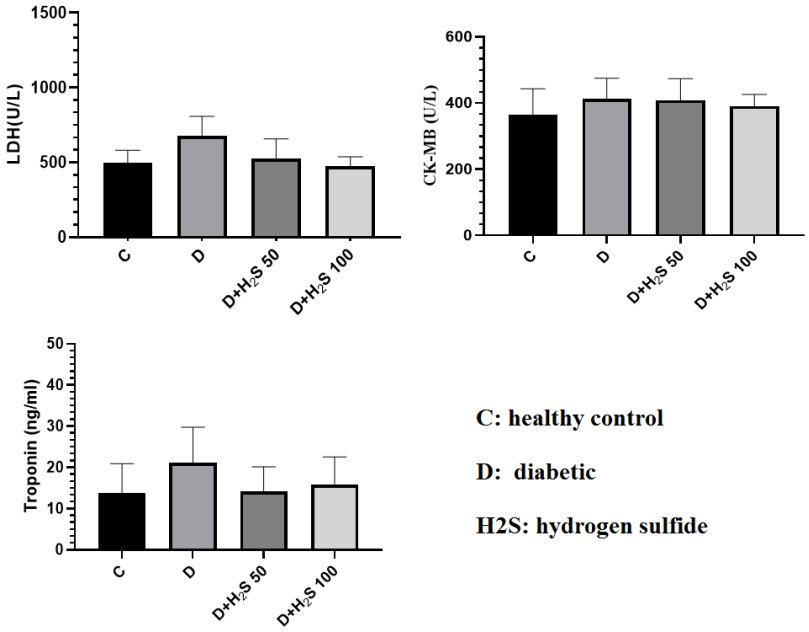

Figure 4. The Effect of H_2S on the Amount of Cardiac Enzymes and Troponin I in the Serum. *Note*. The results are presented as means ± standard errors. C: Healthy control; D: Diabetic; H2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); LDH: Lactate dehydroogenase; CK-MB: Creatine kinase MB

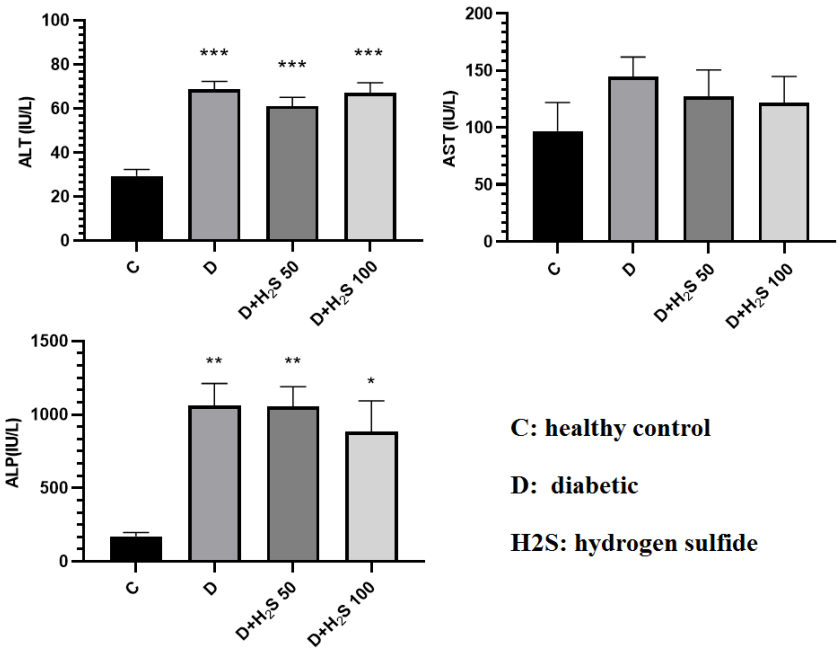

Figure 5. The Effect of H_2S on the Amount of Liver Enzymes (ALT, ALP, and AST) in the Serum. *Note*. ALT: Alanine transaminase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase. The results are expressed as means ± standard errors. C: Healthy control; D: Diabetic; H_2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); * Significant comparison with group C ($P < 0.05$)

the diabetes group, but only the treatment group with 100 µM/kg H2S was significant compared to the diabetic group ($P < 0.05$).

TTG levels in the heart and liver tissues of the diabetic group were reduced compared to the control rats. Moreover, TTG increased in the treatment groups compared to the diabetic group; however, this change was not statistically significant ($P > 0.05$, Figures 6 and 7).

Liver Histological Observations

In this study, after processing the liver tissue and staining it with H&E, the degree of liver tissue damage was graded according to the METAVIR system. A small amount of focal lymphocytes was observed in some parts of the liver tissue of the diabetic control group. The results of the liver tissue examination revealed inflammation, fat deposition, liver fibrosis, and other tissue changes in the sections of the liver of diabetic rats. The livers of the treated diabetic rats with low and high doses of H_2S demonstrated improvements in structure, inflammation, fat deposition, and liver fibrosis compared to those of the untreated diabetic rats (Figure 8).

Discussion

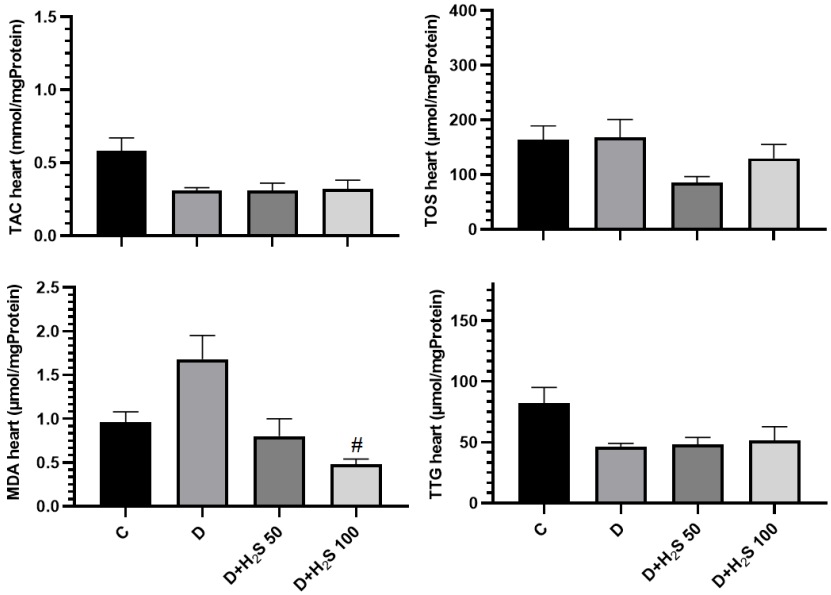

Figure 6. The Effect of H_2S on the Amount of Oxidative Stress Parameters in the Heart Tissue. *Note*. The results are presented as means ± standard errors. C: Healthy control; D: Diabetic; H_2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); TAC: Total antioxidant capacity; TOS: Total oxidative status; MDA: Malondialdehyde; TTG: Total thiol groups; # Significant comparison with group D ($P < 0.05$)

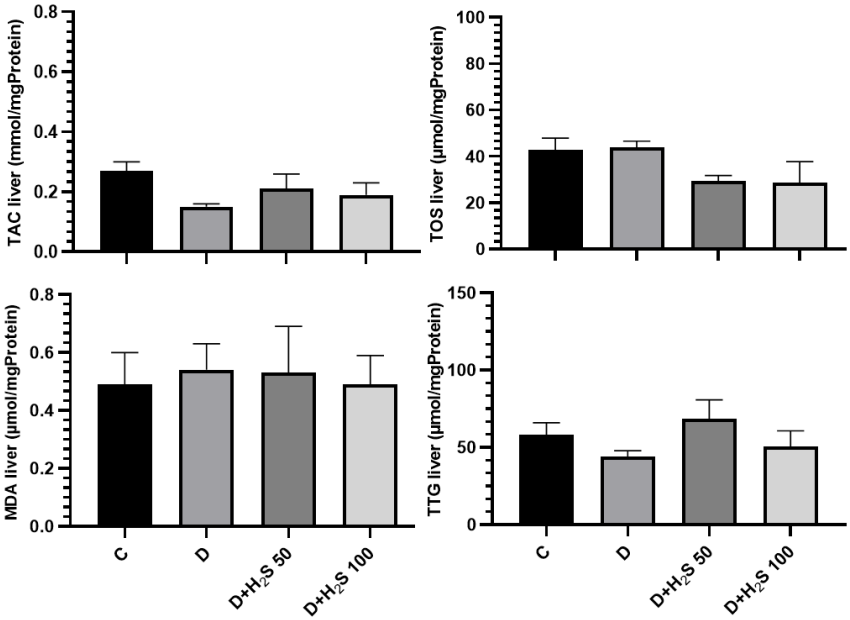

Figure 7. The Effect of H_2S on the Amount of Oxidative Stress Parameters in the Liver Tissue. *Note*. The results are provided as means ± standard errors. C: Healthy control; D: Diabetic; H_2S: Hydrogen sulfide (50 µM/kg or 100 µM/kg); TAC: Total antioxidant capacity; TOS: Total oxidative status; MDA: Malondialdehyde; TTG: Total thiol groups

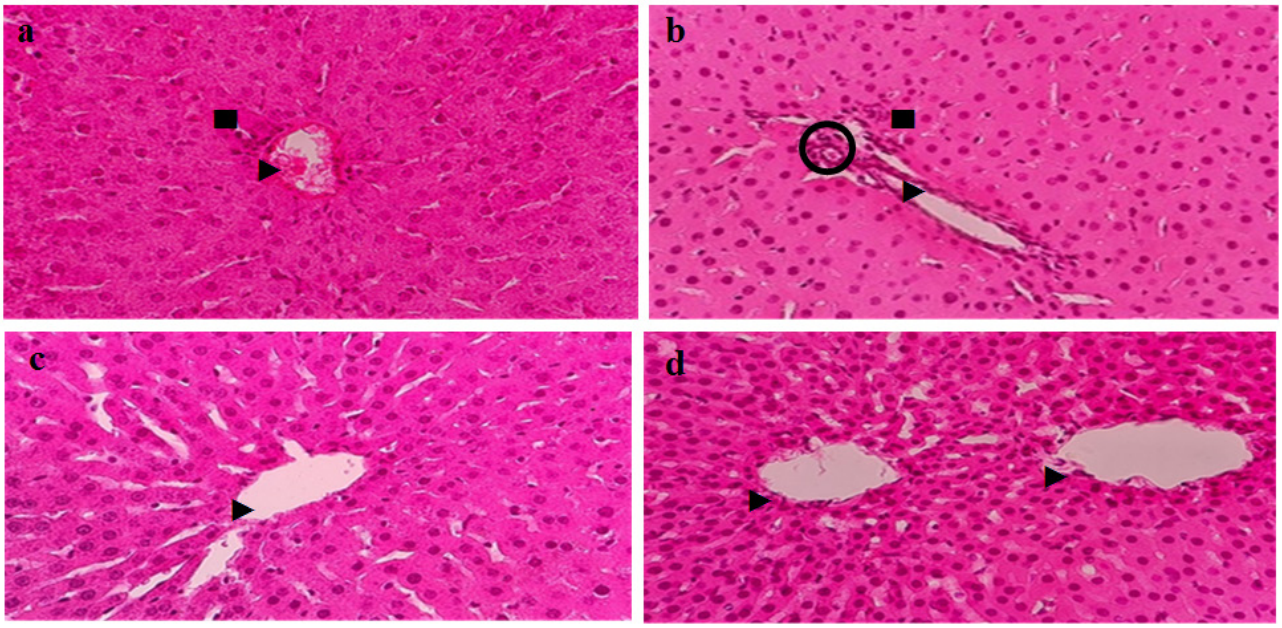

Figure 8. Images of Liver Tissue Sections Stained With Hematoxylin and Eosin. *Note*. 1 mm magnification × 400. ►: Bile ducts; ■: Focal accumulation of lymphocytes; **O**: Hepatic artery. a: Healthy control; b: Diabetic; c: Hydrogen sulfide (50 µM/kg) and d: Hydrogen sulfide (100 µM/kg)

The research findings indicated that plasma H_2S concentrations decreased in DM patients. A decrease was found in the levels of H_2S related to the pathogenesis of DM. Bahadoran et al reported that H_2S levels decreased in diabetic patients (12). Similarly, Suzuki et al concluded that the amount of H_2S was reduced in T2DM patients (13), and Dutta et al reported that the concentration of H_2S was reduced in type 2 diabetic rats by using STZ. On the other hand, the findings revealed that increases in H_2S levels are linked to a decrease in the FBS concentration (14). Therefore, considering the beneficial effects of H_2S in many studies and the reduction of this compound in diabetes, this research investigated the effect of H_2S in the heart and liver tissues of HFD/low-dose STZ T2DM rats.

Weight loss in T2DM-induced rats was due to muscle wasting and loss of muscle protein. The result of the present study showed the weight of diabetic rats decreased significantly in comparison with the control group, and treatment with 50 and 100 µM/kg H_2S, elevated body weight compared to the diabetic group. Consistent with our expectations, Cai et al and Yang et al indicated that treatment with H_2S or an increase in endogenous H_2S biosynthesis could increase fat mass accumulation in mice and fruit flies, whereas the decrease of endogenous H_2S biosynthesis prevented HFD-induced fat mass (15,16).

The results of this study revealed that the FBS level in diabetic animals was significantly increased compared to control rats. However, our findings confirmed that treatment with H_2S 100 µM/kg/day for 60 days significantly reduced FBS, insulin, and insulin resistance. Previous studies indicated that STZ enters pancreatic β cells through the glucose transporter (GLUT2) and leads to DNA damage, causing increased activity of the poly (ADP-ribose) polymerase 1 enzyme to repair DNA. Poly ADP-ribosylation leads to the depletion of cellular NAD + and ATP inside β cells. Decreased levels of ATP inhibit insulin synthesis and secretion by pancreatic beta cells (17,18). Several studies reported the protective role of H_2S in regulating pancreatic β-cell function. Interestingly, H_2S can regulate energy homeostasis and glucose metabolism through various mechanisms in adipose tissue, the pancreas, skeletal muscle, and the liver (19). Kaneko et al demonstrated that NaHS (100 µmol/L) reduced high glucose concentrations while increasing glutathione levels in the mouse islet β cell line (20). It has also been shown that H_2S inhibits the inflammatory or oxidative signaling pathways of pancreatic cells (21).

Based on the results of the current study, the lipid profile in the H_2S-treated groups was improved than that in the diabetic group. Studies have reported different molecular mechanisms for the effect of H_2S on lipid profile; in human adipocytes and Wistar rats, diallyl sulfide was an H_2S donor, which down-regulated the mRNA and protein expression of lipolytic genes, such as hormone-sensitive lipase and adipose triglyceride lipase, whereas it increased the expression of lipogenic genes, such as PPARγ, thereby reducing free fatty acid release (22).

The findings of the current research revealed that liver and heart enzymes improved in the treated groups compared to the diabetic group. In agreement with these findings, Gheibi et al and Jeddi et al observed that the amount of ALP, AST, and ALT increased significantly in diabetic groups. In addition, the amount of these enzymes in the diabetic group treated with NaHS was reduced compared to the diabetic group (23,24). Moreover, a study reported that H_2S alleviates liver function and decreases inflammation and OS. H_2S significantly decreased serum levels of ALP, AST, ALT, hepatic MDA, total bilirubin, and

expression of protein kinase B, lipocalin-2, transforming growth factor-beta, and alpha-smooth muscle actin (25).

Our findings showed that treatment with H_2S for 60 days reduced the heart enzyme, but this change was not statistically significant. Multiple studies have also demonstrated that H_2S has protective effects on the heart against hypertrophy, myocardial infarction, arrhythmia, heart failure, ischemia-reperfusion injury, and fibrosis. Some cardioprotective effects of H_2S are antioxidative action, preservation of mitochondrial function, reduction of angiogenic actions, apoptosis, inflammation, and increased production of nitric oxide (4). In their study on the effect of H_2S on ischemia in diabetic rats, Peake et al observed that the troponin I level after stroke was significantly lower in rats that received H_2S before stroke compared to the diabetic group (26). In the study of Liu et al, the level of CK-MB in diabetic rats that received NaHS was lower than in the diabetic control group (27). The amount of LDH and CK-MB in the study of Ansari et al was higher in the diabetic group receiving HFD than in the group treated with 20 μM NaHS after stroke (28).

The obtained data confirmed the antioxidant effects of H_2S regarding protecting the liver and heart from OS. T2DM causes an increase in lipid peroxidation and OS in the liver and heart tissue, which is associated with an increase in parameters such as MDA and TOS. On the other hand, it reduces the amount of TAC and GSH, which are antioxidants (29). In the research on the effect of H_2S on the progression of diabetic cardiomyopathy by Zhou et al, higher levels of MDA and lower thiol groups were found in the heart tissue of the diabetic groups compared to the diabetic group receiving H_2S (30), which is in line with the results of the study by Liu et al (31). Olas and Wachowicz concluded that H_2S could protect neurons by reducing the levels of MDA and reactive oxygen species. Furthermore, H_2S could inhibit the peroxynitrite ($ONOO^-$) formation (32). The results of the current study also represented that heart tissue MDA in H_2S 100 μM/kg/day was significantly lower than that in diabetic animals. The results of the present study also revealed that H_2S improved the TAC, TOS, and TTG in the liver and heart of the diabetic group, but these changes were not significant.

It has been shown in many studies that people with diabetes are more exposed to liver inflammation and fibrosis than others, and in humans, diabetes is a strong factor in the development of fibrosis, followed by the development of hepatocellular carcinoma (33). Rajapaksha et al, by examining the liver tissue, concluded that the METAVIR degree of inflammation and liver fibrosis in diabetic rats was related with HFD increases (34). After staining the liver tissue with H&E and examining it, Yusuf et al found that the amount of tissue changes, fat deposition, and death of liver cells in diabetic rats with HFD/STZ was higher than that in the control group, and this amount decreased in groups treated with H_2S (35). The findings also revealed that H_2S could reduce liver injury. Assessment of the livers of the treated diabetic rats with low and high doses of H_2S showed improvements in the structure, inflammation, fat deposition, and liver fibrosis.

Limitations

The present study had some limitations. Long-term H_2S intervention can have better effects on OS and biochemical parameters. In addition, there were no molecular investigations or heart histological observations in this research.

Conclusion

Our results confirmed that H_2S has potential hypoglycemic ability and has a significant effect on weight, FBS, insulin, insulin resistance, and lipid profile. It also improved the heart and liver enzymes and OS factors. After further investigation, H_2S can be used as a strategy to prevent the complications of T2DM.

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Authors' Contribution

Conceptualization: Nejat Kheiripour.
Data curation: Hadis Abutalebian, Mohammad Esmaeil Shahaboddin.
Formal analysis: Gholamreza Ghavipanjeh, Mojtaba Sehhat.
Funding acquisition: Nejat Kheiripour.
Investigation: Hadis Abutalebian, Azam Ghaseminezhad.
Methodology: Hadis Abutalebian, Mohammad Esmaeil Shahaboddin, Maryam Akhavan Taheri.
Project administration: Nejat Kheiripour.
Resources: Mohammad Esmaeil Shahaboddin, Nejat Kheiripour.
Software: Gholamreza Ghavipanjeh, Mojtaba Sehhat.
Supervision: Mohammad Esmaeil Shahaboddin, Nejat Kheiripour.
Validation: Nejat Kheiripour.
Visualization: Nejat Kheiripour.
Writing–original draft: Hadis Abutalebian, Mohammad Esmaeil Shahaboddin, Nejat Kheiripour.
Writing–review & editing: Nejat Kheiripour.

Competing Interests

The authors declared no conflict of interests.

Ethical Approval

The present study was approved by the Medical Ethics Committee of the Kashan University of Medical Sciences (IR.KAUMS.MEDNT.REC.1398.110).

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