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Hydrogen Sulfide Ameliorative Role in Induced Diabetes in Rat by Regulating Endoplasmic Reticulum Stress Signaling and miRNA-27a

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Abstract

The present study was carried out to investigate the effects of garlic (A*llium sativum* Linn) and leek (*Allium porrum* L.) on biochemical parameters, lipid profile and gene expression in high fructose diet (HDF)- induced diabetes in rat. In this study, we used 80 males Wistar rats for 18 weeks, HDF was administered daily in diet to induce diabetes. A high and low dose of garlic oil and leek powder were given orally daily to HDF-diabetic rats. Compared to rats in the diabetic groups, the garlic oil and leek powder reduced serum cholesterol, triacylglycerol, low-density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) levels. The garlic oil and leek powder also helped reduce hepatic destruction. A reduction was found in the gene expression in the hepatic homogenate of activating transcription factor 6 (ATF6) and X-Box Binding Protein1 (XBP1), Binding immunoglobulin protein (BIP), C/EBP homologous protein (CHOP), Jun N-terminal kinase (JNK), and peroxisome proliferator- activated receptor gamma (PPAR-γ). On the other hand, there was a significant upregulation in the mRNA expression of has been found in the promoter of glucose transporter 2 (Glut2), and miRNA 27a which is also a dose- and time-dependent manner. These results suggest that H_2S donor as garlic oil and leek powder exhibits therapeutic potential for diabetes, which is most likely related to its protective effects against ER stress and regulating miRNA 27a and its target gene.

KEYWORDS
Type 2 diabetes, Hydrogen sulphide, endoplasmic reticulum stress pathway, insulin release, miRNA

INTRODUCTION

Diabetes mellitus is becoming more common and is now recognized as a major health problem on a global scale. Hyperinsulinemia, caused by malfunctioning of pancreatic cells, acts as a compensatory mechanism, and contributes to the etiology of diabetes (Zhang *et al.*, 2021). Hyperglycemia due to insulin resistance or inadequate insulin production is a main of type 2 diabetes (affecting over 90% of DM patients). One of the primary sources of oxidative stress and widely acknowledged as a primary culprit in the development of type 2 DM problems is progressive hyperglycemia (Melino *et al.*, 2019).

The endoplasmic reticulum (ER) plays a crucial role in regulating insulin synthesis by beta cells. Beta cells boost insulin production in response to insulin resistance to meet the increased peripheral insulin demand, which in turn improves ER function. Excessive overload can lead to chronic ER stress activation and death, resulting in beta cell malfunction, despite the unfolded protein response (UPR)'s adaptive nature and its goal of improving the ER's capacity to fold proteins. Activating transcription factor 6 (ATF6) and X-Box Binding Protein (XBP), have recently been identified as being unique to the UPR in mammals. Binding immunoglobulin protein (BIP) separated from the luminal side of the ER stress transducers inositol-requiring transmembrane kinase endoribonuclease-1 (IRE1), and ATF6, activating them when misfolded proteins accumulate in the ER lumen. In addition to activating c-Jun N-terminal kinase (JNK), IRE1 degrades ER-targeted mRNAs to reduce protein synthesis in the ER. BIP and ATF6 also stimulates the transcription of XBP1 and C/EBP homologous protein (CHOP) (Kadowaki and Nishitoh, 2013; Grajales *et al.*, 2022).

The pancreatic islet expresses peroxisome proliferator- activated receptor gamma (PPAR-γ) has been found in the promoter of glucose transporter 2 (Glut2) that is responsible for glucose-stimulated insulin release and maintain function of n β-cell lines, rodent models of progressive type 2 diabetes, and humans at risk for type 2 diabetes. In diabetes and insulin resistance, PPAR-γ may have direct impacts on islet function through the mitigation of ER stress (Evans-Molina *et al.*, 2009).

An increasing body of research points to the role of microRNAs (miRNAs), a type of short noncoding RNA molecule, in the development of diabetes. MiRNAs are gaining recognition as critical regulators of fundamental biological processes due to their role as translational repressors. Glucose homeostasis is just one of many physiological processes that may be influenced by miRNAs. The etiology of diabetes and its associated complications may be heavily influenced by microRNAs, according to recent studies (He *et al.*, 2021). Many studies have shown that miRNA-27a is highly expressed in obese patients, and this miRNA has also been shown to be associated with diabetes (Zhuang and

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Li, 2022).

Hydrogen sulphide (H_2S) is considered as a gasotransmitter, although it is harmful gas, the human body synthesize it and uses it in a wide range of physiological processes. It produced by enzymatic and non-enzymatic mechanisms in the body. H_2S is an odourless, colourless gas. Several studies demonstrated that H2S inhibited ER stress, apoptosis, inflammation, and oxidation. H_2S is neuroprotective and may have pharmacological benefits in people with Parkinson's disease and Alzheimer's disease because it operates on the nervous system (Li *et al*., 2016; Gheibi *et al*., 2020).

Plants sulfur-containing components such as garlic, leek, and onion have recently been demonstrated to have therapeutic effects in attenuating outcomes associated with cardiovascular disease and inflammation, possibly through a mechanism connected to the H_2S signalling pathway (Rodrigues and Percival, 2019). However, it has not yet been established if H_2S can prevent the pathogenic process of diabetes by reducing ER stress and stimulating insulin release. Therefore, our present work examined whether H_2S suppresses the ER stress in high fructose diet (HDF)-exposed diabetic rats.

MATERIALS AND METHODS

Preparation of garlic oil and leek powder

After adding distilled water at 35°C for 4 hours, fresh garlic cloves were coarsely chopped and subjected to hydro distillation in essential oil testing equipment for 2 hours. Water was removed from the extracted essential oils using anhydrous sodium sulphate, and the oils were then stored in a cool, dark environment (Yang *et al*., 2018). The garlic oil was dissolved in 1% Tween 80.

The *Allium porrum* L. used here was sourced from an Egyptian market. Due to the excellent condition of the acquired leek, it was sliced into chips and dried in an oven set to 40 degrees Celsius for a full week. The dried chips were ground into a powder using industrial-grade blenders. For later examination, the powdered material was stored in a sealed container and frozen (El-Khabery *et al*., 2016).

Experimental animals

All experimental methods were carried out in the Faculty of Veterinary Medicine at Zagazig University, Egypt. Eighty male Wistar rats weighing between 150 and 170 g were housed in polycarbonate cages, with each mouse having its own space, and maintained at a constant temperature (22 2 °C) and humidity (55%). The rats were separated into 8 groups (n = 10 per group) after a week of acclimation to their new surroundings. The experimental protocol was approved by Suez Canal University _ IACUC Committee approval number (Suez Canal University _ REC 63/2022).

Study design

The HDF was prepared by mixing 60% wt/wt fructose with standard rodent diet (Maithilikarpagaselvi *et al*., 2016), Fructose-induced diabetes was evaluated by an increase in fasting blood glucose 10 weeks.

Control group: rats were fed with standard rodent chow throughout the experimental period of 18 weeks.

Diabetic group: rats were fed with 60% HDF through-out the experimental period of 18 weeks. THGO group (high garlic oil treated group): diabetic rats were given a high dose of garlic oil (92.6 mg/kg·bw/d); orally daily for 8 weeks (Yang *et al*., 2018). TLGO group (low garlic oil treated group): diabetic rats were given a low dose of garlic oil (11.6 mg/kg·bw/d) orally daily for 8 weeks (Yang *et al*., 2018). Tleek group (leek powder treated group): the diabetic rats were given diet containing 11% leek powder for 8 weeks (El-Khabery *et al*., 2016). PHGO group (high garlic oil prophylactic group): rats were fed on HDF and high dose of garlic oil (92.6 mg/kg·bw/d) for 10 weeks as prophylactic dose. PLGO group (low garlic oil prophylactic group): rats were fed on HDF and low dose of garlic oil (11.6 mg/kg·bw/d) for 10 weeks. PTleek group (leek powder prophylactic group): rats were fed on HDF and diet containing 11% leek powder for 10 weeks.

Table 1. Primers sequences for the real time PCR.

Gene	Sequence	Accession number
ATF-6	F 5′-AAGTGAAGAACCATTACTTTATATC-3' R 5'- TTTCTGCTGGCTATTTGT -3'	NM_001107196.1
BIP	F 5′-AACCAAGGATGCTGGCACTA-3' R 5'-ATGACCCGCTGATCAAAGTC-3'	NM_013083.2
CHOP	F 5′-CACAAGCACCTCCCAAAG-3' R 5'-CCTGCTCCTTCTCCTTCAT-3'	NM_001109986.1
JNK	F 5′- AGTGTAGAGTGGATGCATGA-3' R 5'-ATGTGCTTCCTGTGGTTTAC-3'	NM_053829.2
XBP-1	F 5′-TTACGAGAGAAAACTCATGGGC-3' R 5'- GGGTCCAACTTGTCCAGAATGC -3'	NM_001004210.2
PPAR-γ	F 5′- CCTGAAGCTCCAAGAATACC -3' R 5'- GATGCTTTATCCCCACAGAC -3'	NM_013124.3
GLUT-2	F 5′- CTCTGTGCTGCTTGTGGAGA -3' R 5'- CGGCACAGAAAAACATGCCA -3'	NM_012879.2
GADPH	F 5′-GCATCTTCTTGTGCAGTGCC-3' R 5'-TACGGCCAAATCCGTTCACA-3'	NM_017008.4
miRNA 27a	R 5'- AACGGCTTCACAGTGGCTA-3' R 5'- GTCGTATCCAGTGCAGGGT-3' Stem-loop primer; GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCGGAA	
U6	R 5'- GCTCGCTTCGGCAGCACA-3' R 5'- GAGGTATTCGCACCAGAGGA-3' Stem-loop primer; AACGCTTCACGAATTTGCGTG	

Measuring body weight and BMI

The BMI (Weight (g)/Length (cm^2)) was calculated at day zero, after 10 weeks, and at the end of the experiment (after 18 week).

Collection of samples and preparation of tissue homogenate

After collecting blood from the vena cava, the samples were allowed to clot before being centrifuged at 3,000 rpm for 15 minutes. The serum samples were stored at -20 degrees Celsius until additional analysis could be performed. Rats were treated for 18 weeks before being slaughtered and their liver dissected. Separated liver tissue was divided in half; the first half was used to test for gene expression. Hematoxylin and eosin (H&E) staining was used for histopathological examinations of liver tissue that had been preserved in 10% formalin solution at room temperature for 24 hours.

Biochemical analysis

Glucose levels were detected with the use of the Glucose assay kit MyBioSource, USA, and insulin with the use of insulin assay kit MyBioSource, USA, cholesterol (HDL and LDL/VLDL, USA), triacylglycerol (Quantification Abcam, USA), High-density lipoprotein-cholesterol (HDL-c) (Fluorometric Abcam, USA), LDL-c (Crystal Chem's Rat LDL, USA), in accordance with the manufacturer's instructions. HOMA-IR index, was calculated according to the following equation:

HOMA IR=(Blood glucose (mg/dl)x Insulin (ng/ml))/405

Hemoglobin A1C (HbA1c) Test

The monoclonal antibody used here only reacted with one particular amino acid sequence (the NH_2-terminus) in the beta chain of glycated Hb. Both glucose and this particular sequence of amino acids were necessary for the antibody binding. In the absence of HbAlc, the second reagent agglutinated monoclonal antibody attached to latex beads. Glycated haemoglobin competed with the agglutinating agent for antibody binding sites, preventing agglutination. The agglutination reaction was shown to increase the absorbance at 531 nm. The proportion of HbAlc in the total Hb was used to represent the HbAlc concentration. The concentration of HbA1c was calculated as a proportion of total haemoglobin (Hsieh *et al*., 2013).

Quantitative real-time PCR

Trizol (Invitrogen; Thermo Fisher) was used to extract total RNA from 30 mg of liver tissue. Making use of a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware; USA). The TaqManTM Small RNA Assays were used to generate miRNA from 10 ng, in accordance with the manufacturer's instructions. The universal reverse primer, the Stem-loop RT specific primers, and the miRNA specific primers (Table 1) were all designed using assay design software (http://genomics.dote.hu:8080/ mirnadesigntool) (Czimmerer *et al*., 2013). Real-time PCR was performed using the Maxima SYBR Green/Rox qPCR Master Mix (2X). After normalization to housekeeping GADPH (for mRNA) or U6 (for miRNA), fold change in gene expression was reported as $2^{-\Delta\Delta CT}$ relative to control.

Histopathology examination

Histological investigation of liver tissue samples involved fixing the tissue in 10% buffered formalin solution, paraffin embedding it, and cutting it into 5 µm sections. After being deparaffinized and stained with hematoxylin and eosin (H&E) for histopathological changes.

Statistical analysis

The results were presented as a mean ± standard error. The effects of the groups on the various biochemical indicators were compared using one-way analysis of variance (ANOVA) followed by the Duncan multiple-test post hoc analysis. $P < 0.05$ was used as the threshold for statistical significance. Statistical analyses and generate charts were created using SPSS 24.0 (IBM Corp., Armonk, NY) and Graph Pad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Effects on body weight and BMI in HFD-induced diabetic rat

Figure 1 revealed non-significant change in body weight and BMI in all groups at day zero. At week 10 the highest significant

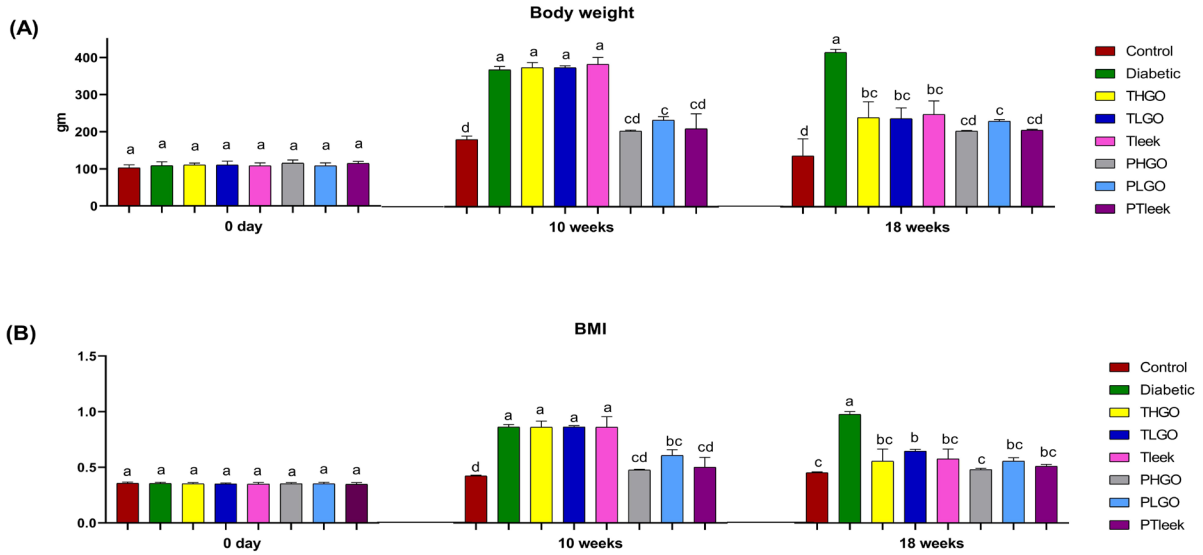

Fig. 1. Effects of garlic oil and leek powder on body weight (gm) and BMI in HFD-induced diabetic rats.

increase in body weight and BMI were in treatment groups and diabetic group. The body weight of diabetic rats and BMI in the treatment groups and diabetic group were significantly higher than control group and prophylactic groups at week 10. At week 18, the diabetic group exhibited the highest weight gain and BMI, and it was significantly changed than other groups. Prophylactic groups and treatment groups were non-significant to each other at week 18.

Effects on HbA1c, insulin, and HOMA-IR levels in HFD-induced diabetic rat

Figure 2 demonstrated that HbA1c, insulin, and HOMA-IR levels were the highest in diabetic groups in comparison with other groups. While their levels were decreased significantly in the prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek) in comparison with diabetic groups. HbA1c levels were non-significantly differ in prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek). Insulin levels were non-significantly differ in between prophylactic groups (PHGO, PLGO) and treatment groups (THGO, Tleek). HOMA-IR were non-significantly differ in between prophylactic groups (PLGO, PTleek) each other, and treatment groups (THGO, TLGO, Tleek) were non-significantly differ in between each other. Also control group were non-significant to PHGO prophylactic group.

Effects on lipid profile in HFD-induced diabetic rat

Results in Figure 3 revealed that the highest level of cholesterol, triacylglycerol, LDL-c and VLDL-c levels were in diabetic groups. While their levels were significantly decreased in the prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek) in comparison with diabetic groups. HDL-c levels were non-significantly differ in diabetic groups, prophylactic groups (PLGO, PTleek) and treatment groups (THGO). HDL-c levels were non-significantly differ in between prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek).

Effects on gene expression in HFD-induced diabetic rat

In Figure 4 the mRNA expression of ATF-6, BIP, XBP1 and CHOP levels were at the highest ($p < 0.05$) levels in diabet-

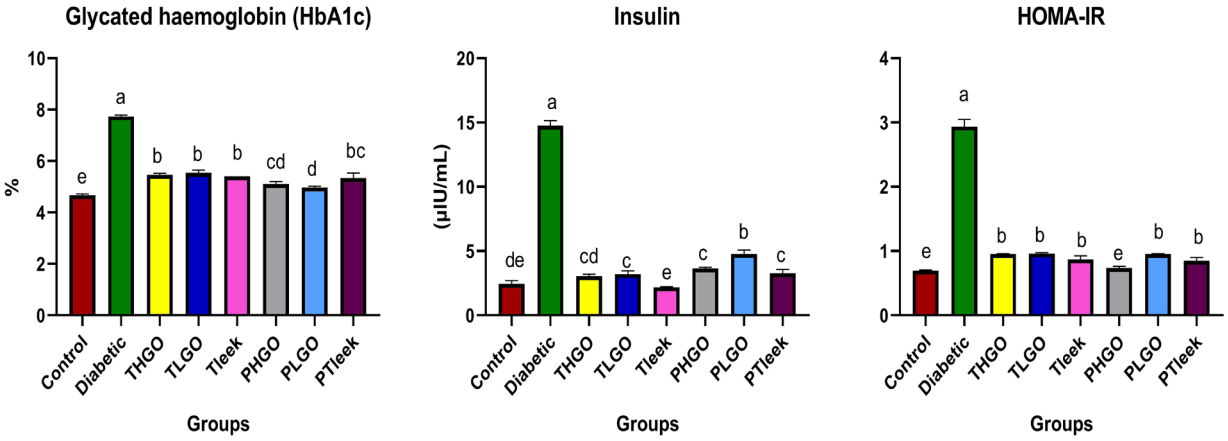

Fig. 2. Effects of garlic oil and leek powder in the levels of HbA1c (%), insulin (µIU/mL), and HOMA-IR in HFD-induced diabetic rats.

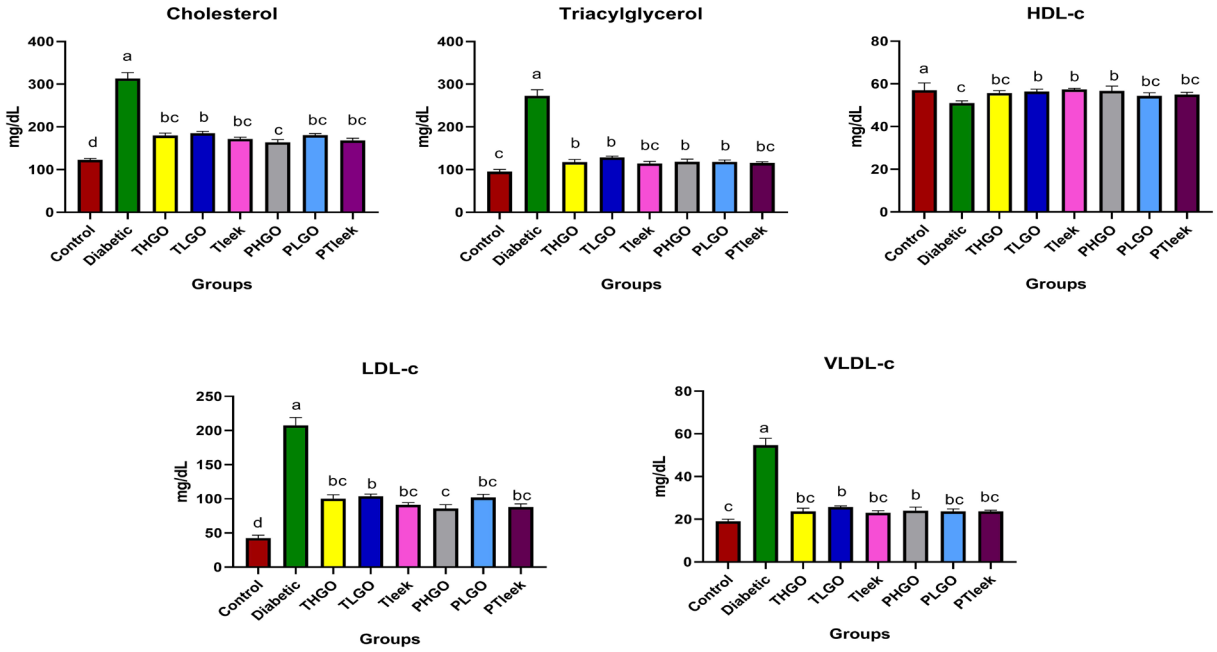

Fig. 3. Effects of garlic oil and leek powder in lipid profile in HFD-induced diabetic rats.

ic groups and significantly downregulated in the prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek). The lowest (P < 0.05) levels were revealed in control groups. ATF-6 levels were non-significantly differ in between prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek), but significantly differ from control group. BIP levels were non-significantly differ in between prophylactic groups (PHGO, PTleek) each other and treatment groups (TLGO, Tleek) each other, also TLGO and PHGO treated groups were non-significantly to each other, but all prophylactic and treatment groups were significantly differing from control group.

The expression of JNK, and PPAR-γ in Figure 5 were at the highest (p < 0.05) levels in diabetic groups and significantly downregulated in the prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek). The lowest (p < 0.05) levels were revealed in control groups. JNK levels were non-significantly differ in between THGO, Tleek treated groups. Also, TLGO, PHGO, PLGO treated groups were non-significant to each other, while THGO, and Tleek were also non-significant to each other. The prophylactic and treatment groups were significantly differing from control group. PPAR-γ levels were non-significantly differ in between THGO, Tleek treated groups. Also, TLGO, Tleek, and PTleek treated groups were non-significant to each other, while PHGO, and PLGO were also non-significant to each other. The prophylactic and treatment groups were significantly differing from control group except PHGO treated group.

On the other hand, GLUT-2, miRNA 27a activity were the lowest (p < 0.05) level in diabetic groups and significantly upregulated in the prophylactic groups (PHGO, PLGO, PTleek) and treatment groups (THGO, TLGO, Tleek). The highest (p < 0.05) level was revealed in control negative group. GLUT-2 levels were non-significantly differ in between THGO, Tleek treated groups. Also TLGO, and PLGO treated groups were non-significant to each other, while PHGO, and PTleek were also non-significant to each other. The prophylactic and treatment groups were significantly differing from control group. miRNA 27a levels were non-significantly differ in between THGO, Tleek treated groups. Also, TLGO, PHGO, and PTleek treated groups were non-significant to each other, while TLGO, and PLGO were also non-significant to each other. The prophylactic and treatment groups were significantly differing from control group.

Histopathological findings

In control negative group liver tissues were normal structure in Figure 6A. In diabetic group the liver tissue showed significant fatty degeneration of most of the hepatocytes (Figure 6B). The garlic oil and leek powder treated groups (prophylactic and treatments) showed restroration in the affected cells by the action of HDF (Figure 6C-H).

DISCUSSION

Although many studies have shown that H_2S production is diminished in diabetes, the function of H2S in diabetes metabolism is still up for debate. H_2S is critical for keeping insulin bioactivity and glucose absorption, according to studies. H_2S inhibited resident adipose macrophages from producing inflammatory cytokine, a known contributor to insulin resistance in adipose and other metabolic tissues. The role of ER stress in the development of insulin resistance and diabetes is widely speculated upon (Cheng and Kishore, 2020).

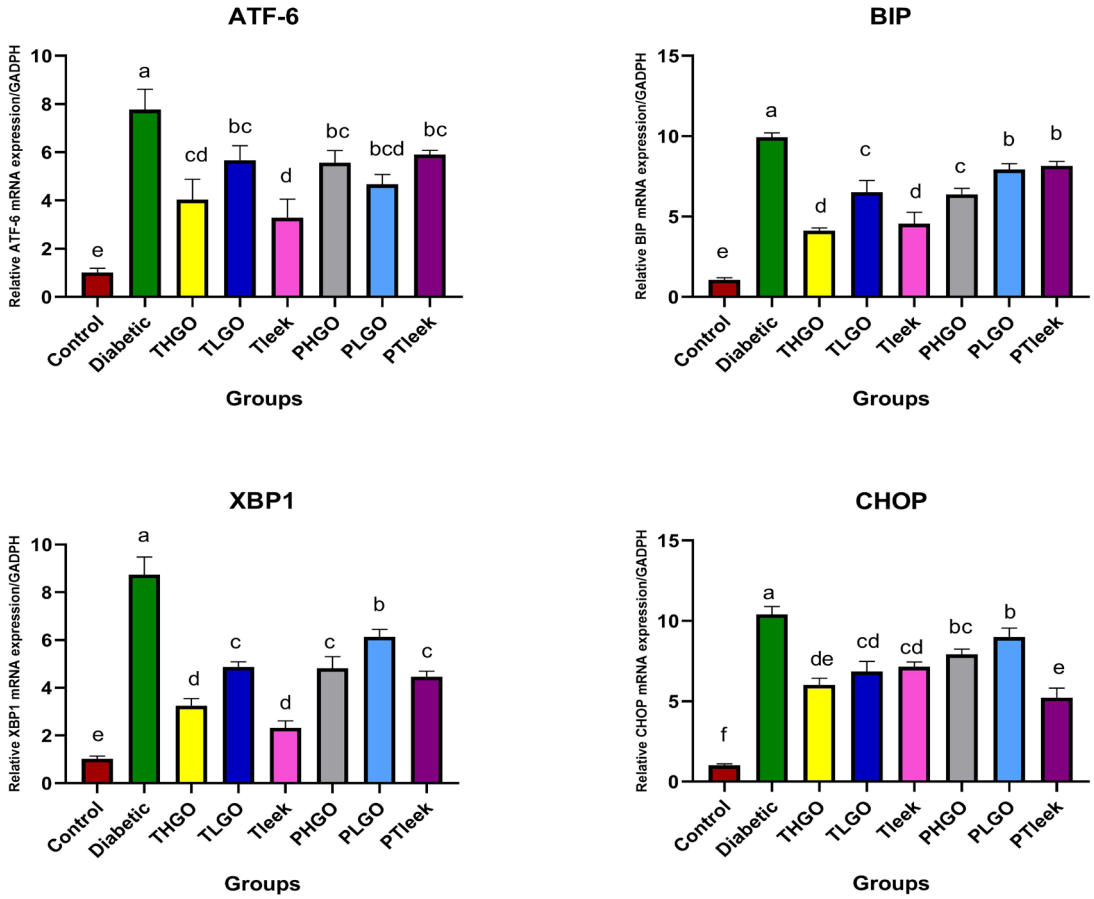

Fig. 4. Effects of administration of garlic oil and leek powder in the mRNA expression of some genes in HFD-induced diabetic rats.

In this study, the effects of garlic oil and leek powder on diabetes induced by a HDF were assessed in animals. Garlic oil and leek powder were tested for their ability to regulate diabetes indicators in rats. Rats fed the high dose of garlic oil and leek powder had significantly decreased lipid profile and insulin, HbA1C indices compared to diabetic group. These findings point to the anti-diabetic effects garlic oil and leek powder (Gheibi *et al.*, 2020).

Blood glucose levels have been reported to have worsened, improved, or not changed at all, hence the reports are essentially dichotomous. Possible causes for this discrepancy include variations in treatment length, species involved, H2S donor dose and type, and animal models tested. Using the same animal model of T2D, another study showed that high doses of NaSH (1.6-5.6 mg/kg) worsen carbohydrate metabolism, while moderate doses of NaSH (0.28 and 0.56 mg/kg) have no effect on these parameters. Other work demonstrates that H_2S reduces insulin production in a dose-dependent manner in pancreatic β-cells (Gheibi *et al.*,

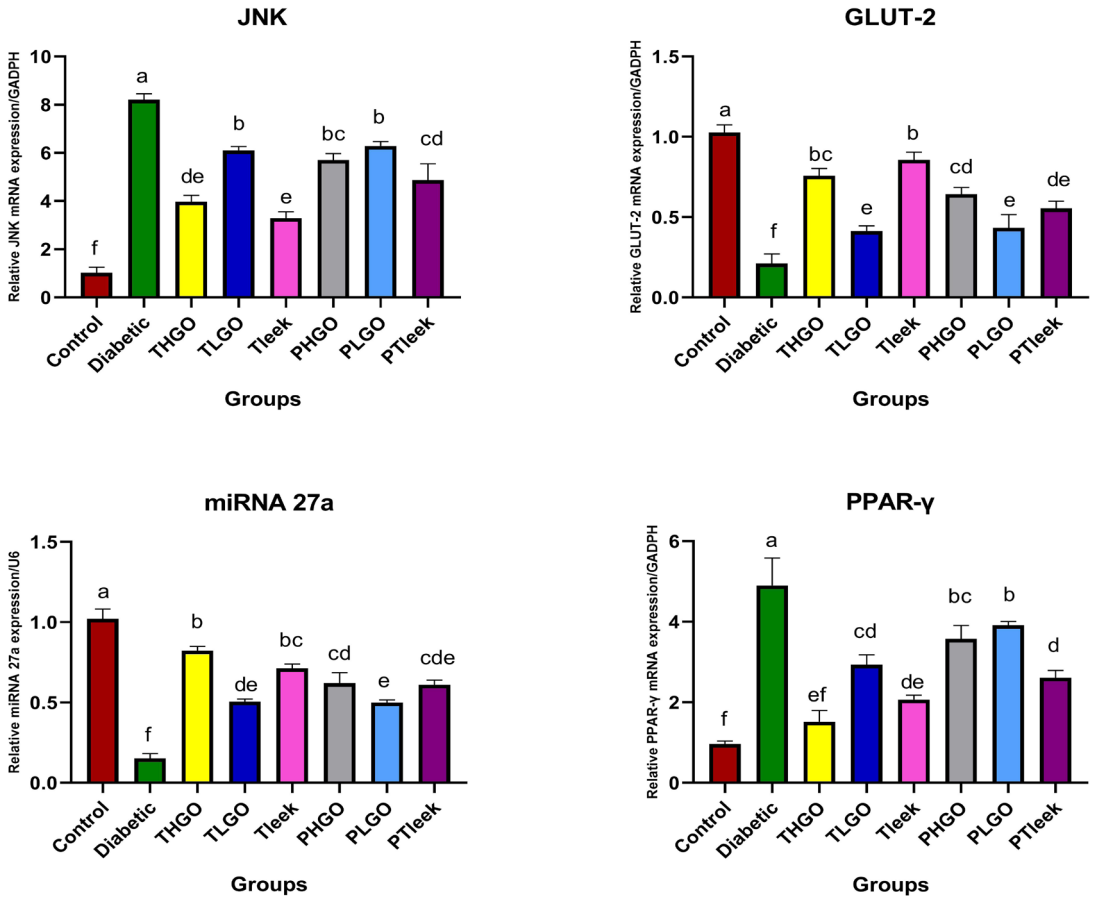

Fig. 5. Effects of administration of garlic oil and leek powder in the expression of some genes in HFD-induced diabetic rats.

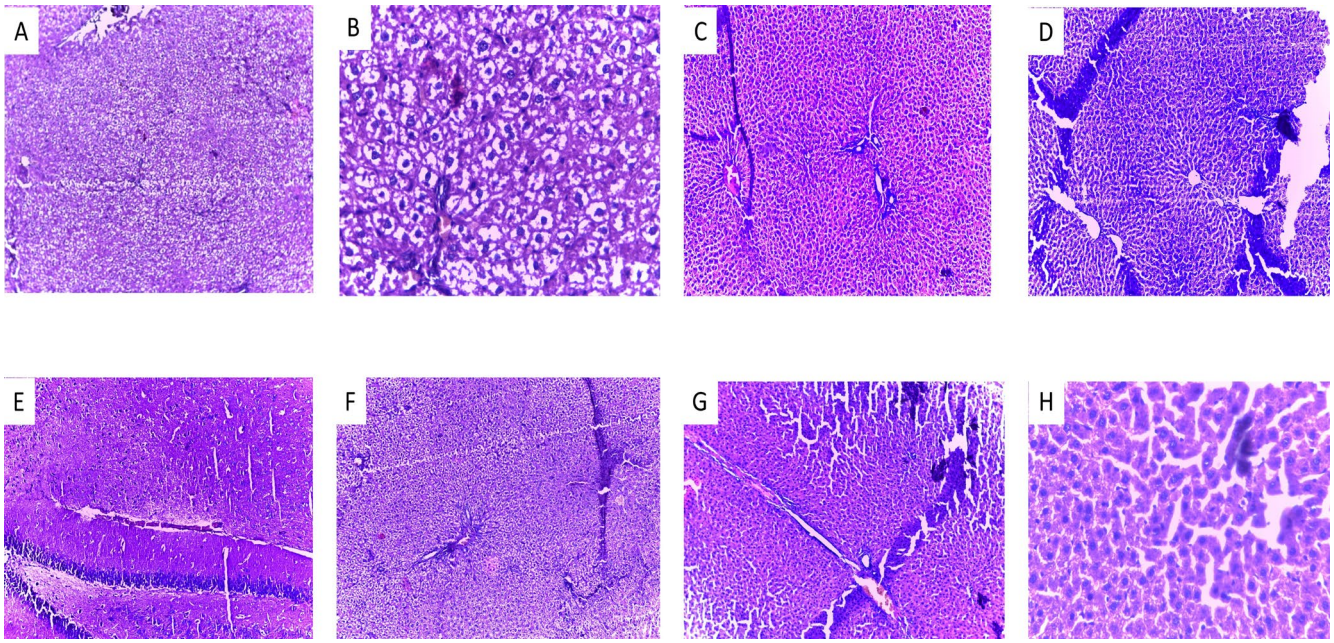

Fig. 6. Histopathological results in liver tissue in: (A) control rat, H&E, X 40. (B) diabetic rat, H&E, X 40. (C) THGO treated rat, H&E, X 40. (D) TLGO treated rat, H&E, X 40. (E) Tleek treated rat, H&E, X 40. (F) PHGO treated, H&E, X 40. (G) PLGO treated rat, H&E, X 40. (H) PTleek treated rat rats, H&E, X 40.

2019a; Gheibi *et al*., 2019b).

H_2S can control blood sugar levels and affect insulin secretion. Both mouse islets and the pancreatic beta cell line showed an inhibition of glucose (10 mM)-induced insulin secretion by sodium hydrosulfide and L-cysteine (Kaneko *et al*., 2006). This effect was not seen at a low glucose concentration (3 mM) in either cell type. In addition, insulin secretion from HIT-T15 cells was suppressed by roughly 70% after being treated with NaSH (100 M) (Suzuki *et al*., 2011). Basal insulin secretion was not affected, but high glucose (16 mM)-stimulated insulin secretion was nearly eliminated when CSE was overexpressed in INS-1E cells. Indeed, it has been shown that elevated levels of extracellular glucose reduce intracellular H_2S production, leading to an increase in insulin secretion. Evidence from this research demonstrates that H_2S inhibits the release of insulin in response to glucose (Yang *et al*., 2005).

The ER has been implicated in the pathophysiology of diabetes, where it contributes to the death of beta cells in the pancreas and insulin resistance. Research suggests that beta-cell vulnerability to persistent high glucose and fatty acid exposure, agents that contribute to beta-cell failure in type 2 diabetes, Insulin resistance and obesity may also be related to ER stress in people with type 2 diabetes. Insulin signalling is downregulated by activation of JNK in response to high-fat eating and obesity-induced ER stress in the liver (Eizirik *et al*., 2008).

Our study revealed a reduction in the gene expression in the hepatic homogenate of ATF6 and XBP1, BIP, CHOP, JNK, and PPAR-γ in prophylactic and treatment groups. On the other hand, there was a significant upregulation in the mRNA expression of has been found in the promoter of Glut2, and miRNA 27a. The liver histology was deteriorated with HDF, and garlic oil and leek powder restored liver structure. The following studies were in line with our results.

H_2S has been shown to reduce diabetes-related illnesses by controlling ER stress in a growing number of investigations in recent years. The CHOP, BIP, and sterol regulatory element binding protein-1c were all upregulated by H_2S, all of which are indications of endoplasmic reticulum stress. Furthermore, anti-CHOP siRNA suppressed apoptosis induced by H_2S (Yang *et al*., 2007).

Another study showed that compared to the control, ERK1/2 phosphorylation was significantly boosted by both garlic preparations, but p38MAPK and JNK activations were significantly reduced. Diabetes and obesity-related heart problems may be mitigated by garlic's ability to regulate the activities of Glut-4 and PPARs. The role of garlic in obesity has been the subject of very few studies to far. Although PPAR-α and PPAR-γ were both increased in both types of garlic preparations, PPAR was decreased in the processed garlic group and increased in the fresh garlic group after I/R (Mukherjee *et al*., 2009).

Wei Zou and coworkers, injected streptozotocin (STZ) intraperitoneally. Diabetic rats treated with NaHS (a H_2S-releasing substance) showed a marked improvement in their cognitive function. NaHS reversed the elevated levels of glucose-regulated protein 78 (GRP78), cleaved caspase-12, and CHOP in the hippocampus of STZ-induced diabetic rats, demonstrating that H2S inhibited diabetes-associated ER stress (Zou *et al*., 2017).

Several studies linked polymorphisms within miRs and the dysregulation of their expression to metabolic diseases like cancer, type 2 diabetes, cardiovascular disease, and gestational diabetes mellitus because of the roles miRs play in these processes. Adipocyte development from preadipocytes, insulin resistance, and type 2 diabetes are all processes adversely regulated by miR-27a because it targets the PPAR-γ gene (Choi and Hong, 2022). Overexpression of miR-27a in pre-adipocytes inhibited expression of PPAR-γ and the development of adipocytes, leading Ciccacci *et al.* (Chen *et al*., 2019) to conclude that miR-27a displayed a protective effect in T2DM. Furthermore, Gong *et al.* (Gong *et al*., 2014) demonstrated that miR-27a inhibited the transcriptional induction of PPAR-γ from T2DM patients. By increasing peripheral insulin sensitivity and decreasing hepatic glucose levels (Chen *et al*., 2019), activation of PPAR-γ, an anti-inflammatory factor, lowered hyperglycemia. Since insulin resistance contributes to tissues' decreased sensitivity to insulin-mediated biological function, it can exacerbate DM patients' obesity, hypertension, and dyslipidemia (Wang *et al*., 2020).

CONCLUSION

Our findings suggested that garlic oil and leek powder as H_2S donors are a possible candidate for the prevention and treatment of diabetes mellitus. Regulating endoplasmic reticulum stress signaling is how garlic oil and leek powder can lower blood HOMA-IR, insulin levels, lipid profile, and liver structural damage in diabetic rats. Further research is required to understand the mechanism by which H_2S regulates ER stress, and in particular the conditions under which H_2S promotes ER stress and the conditions under which it inhibits ER stress.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Chen, T., Zhang, Y., Liu, Y., Zhu, D., Yu, J., Li, G., Sun, Z., Wang, W., Jiang, H., Hong, Z., 2019. MiR-27a promotes insulin resistance and mediates glucose metabolism by targeting PPAR-γ-mediated PI3K/AKT signaling. Aging 11, 7510.

Cheng, Z., Kishore, R., 2020. Potential role of hydrogen sulfide in diabetes-impaired angiogenesis and ischemic tissue repair. Redox Biol. 37, 101704.

Choi, Y., Hong, S.H., 2022. Genetic association between miR-27a and miR-449b polymorphisms and susceptibility to diabetes mellitus. Biomed. Rep. 16, 37.

Czimmerer, Z., Hulvely, J., Simandi, Z., Varallyay, E., Havelda, Z., Szabo, E., Varga, A., Dezso, B., Balogh, M., Horvath, A., 2013. A versatile method to design stem-loop primer-based quantitative PCR assays for detecting small regulatory RNA molecules. PloS one 8, e55168.

Eizirik, D.L., Cardozo, A.K., Cnop, M., 2008. The role for endoplasmic reticulum stress in diabetes mellitus. Endocr. Rev. 29, 42-61.

El-Khabery, M.A., Sitohy, M., Hefnawy, H., Gomaa, A.M., 2016. Effect of using Egyptian leek leaves powder (*Allium porrum* l.) as unconventional feed on growth performance, carcass quality and some serum biochemical analysis in broiler chickens. ZJAR. 43, 555-567.

Evans-Molina, C., Robbins, R.D., Kono, T., Tersey, S.A., Vestermark, G.L., Nunemaker, C.S., Garmey, J.C., Deering, T.G., Keller, S.R., Maier, B., Mirmira, R.G., 2009. Peroxisome Proliferator-Activated Receptor γ Activation Restores Islet Function in Diabetic Mice through Reduction of Endoplasmic Reticulum Stress and Maintenance of Euchromatin Structure. Mol. Cell. Biol. 29, 2053-2067.

Gheibi, S., Jeddi, S., Carlström, M., Kashfi, K., Ghasemi, A., 2019a. Hydrogen sulfide potentiates the favorable metabolic effects of inorganic nitrite in type 2 diabetic rats. Nitric Oxide 92, 60-72.

Gheibi, S., Jeddi, S., Kashfi, K., Ghasemi, A., 2019b. Effects of hydrogen sulfide on carbohydrate metabolism in obese type 2 diabetic rats. Molecules 24, 190.

Gheibi, S., Samsonov, A.P., Gheibi, S., Vazquez, A.B., Kashfi, K., 2020. Regulation of carbohydrate metabolism by nitric oxide and hydrogen sulfide: Implications in diabetes. Biochem. Pharmacol. 176, 113819.

Gong, W., Xiao, D., Ming, G., Yin, J., Zhou, H., Liu, Z., 2014. Type 2 diabetes mellitus-related genetic polymorphisms in microRNAs and microRNA target sites. J. Diabetes 6, 279-289.

Grajales, D., Vázquez, P., Alén, R., Hitos, A.B., Valverde, Á.M., 2022. Attenuation of Olanzapine-Induced Endoplasmic Reticulum Stress Improves Insulin Secretion in Pancreatic Beta Cells. Metabolites 12, 443.

He, X., Kuang, G., Wu, Y., Ou, C., 2021. Emerging roles of exosomal miRNAs in diabetes mellitus. Clin Transl Med. 11, e468.

Hsieh, F.-C., Lee, C.-L., Chai, C.-Y., Chen, W.-T., Lu, Y.-C., Wu, C.-S., 2013. Oral administration of Lactobacillus reuteri GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutr. Metab. 10, 35.

Kadowaki, H., Nishitoh, H., 2013. Signaling Pathways from the Endoplasmic Reticulum and Their Roles in Disease. Genes 4, 306-333.

Kaneko, Y., Kimura, Y., Kimura, H., Niki, I., 2006. L-cysteine inhibits insulin

release from the pancreatic β-cell: possible involvement of metabolic production of hydrogen sulfide, a novel gasotransmitter. Diabetes 55, 1391-1397.

Li, F., Luo, J., Wu, Z., Xiao, T., Zeng, O., Li, L., Li, Y., Yang, J., 2016. Hydrogen sulfide exhibits cardioprotective effects by decreasing endoplasmic reticulum stress in a diabetic cardiomyopathy rat model. Mol. Med. Rep. 14, 865-873.

Maithilikarpagaselvi, N., Sridhar, M.G., Swaminathan, R.P., Zachariah, B., 2016. Curcumin prevents inflammatory response, oxidative stress and insulin resistance in high fructose fed male Wistar rats: Potential role of serine kinases. Chem. Biol. Interact. 244, 187-194.

Melino, S., Leo, S., Toska Papajani, V., 2019. Natural Hydrogen Sulfide Donors from Allium sp. as a Nutraceutical Approach in Type 2 Diabetes Prevention and Therapy. Nutrients 11, 1581.

Mukherjee, S., Lekli, I., Goswami, S., Das, D.K., 2009. Freshly crushed garlic is a superior cardioprotective agent than processed garlic. J. Agric. Food Chem. 57, 7137-7144.

Rodrigues, C., Percival, S.S., 2019. Immunomodulatory Effects of Glutathione, Garlic Derivatives, and Hydrogen Sulfide. Nutrients 11, 295.

Suzuki, K., Olah, G., Modis, K., Coletta, C., Kulp, G., Gerö, D., Szoleczky, P., Chang, T., Zhou, Z., Wu, L., 2011. Hydrogen sulfide replacement therapy protects the vascular endothelium in hyperglycemia by preserving mitochondrial function. PNAS 108, 13829-13834.

Wang, Y., Yang, L.-Z., Yang, D.-G., Zhang, Q.-Y., Deng, Z.-N., Wang, K., Mao, X.-J., 2020. MiR-21 antagomir improves insulin resistance and lipid metabolism disorder in streptozotocin-induced type 2 diabetes mellitus rats. Ann. Palliat. Med. 9, 394-404.

Yang, C., Li, L., Yang, L., Lǚ, H., Wang, S., Sun, G., 2018. Anti-obesity and Hypolipidemic effects of garlic oil and onion oil in rats fed a high-fat diet. Nutr. Metab. 15, 43.

Yang, G., Yang, W., Wu, L., Wang, R., 2007. H2S, endoplasmic reticulum stress, and apoptosis of insulin-secreting beta cells. J. Biol. Chem. 282, 16567-16576.

Yang, W., Yang, G., Jia, X., Wu, L., Wang, R., 2005. Activation of KATP channels by H_2S in rat insulin-secreting cells and the underlying mechanisms. J. Physiol. 569, 519-531.

Zhang, H., Huang, Y., Chen, S., Tang, C., Wang, G., Du, J., Jin, H., 2021. Hydrogen sulfide regulates insulin secretion and insulin resistance in diabetes mellitus, a new promising target for diabetes mellitus treatment? A review. J. Adv. Res. 27, 19-30.

Zhuang, Y., Li, M., 2022. MiRNA-27a mediates insulin resistance in 3T3-L1 cells through the PPARγ. Mol. Cell. Biochem. 477, 1107-1112.

Zou, W., Yuan, J., Tang, Z.-J., Wei, H.-J., Zhu, W.-W., Zhang, P., Gu, H.-F., Wang, C.-Y., Tang, X.-Q., 2017. Hydrogen sulfide ameliorates cognitive dysfunction in streptozotocin-induced diabetic rats: involving suppression in hippocampal endoplasmic reticulum stress. Oncotarget 8, 64203.