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RESEARCH ARTICLE

Protective effect of acetylcysteine, histidine, and their combination against diabetes vascular complications in type-2 diabetic rats via reducing NF-kβ pathway signaling

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Abstract

Purpose The nuclear factor-kappa B (NF-κB) signaling participates in diabetes complications. Therefore, the reduction of NF-κB signaling may be a goal to prevent or improve them. Thus, we investigated the effects of acetylcysteine (AC), histidine (His), and their combination on the NF-κB expression and its different activators in type 2 diabetic rats.

Methods The survey was performed on 50 rats that were allotted equally into five groups composed of control, diabetic, diabetic treated with (AC, 0.06%), (His, 0.1%), and (AC & His) groups. Treated groups have received the treatments daily in drinking water for two months. Metabolic profile (glucose, insulin resistance indices, lipid profile, and cardiovascular indices) and renal dysfunction parameters (creatinine and urinary protein excretion) were measured. Plus, diverse glycation (early, intermediate, and end), oxidative stress (Oxidized LDL, Reduced glutathione), and inflammatory markers (interleukine-1β, myeloperoxidase, and NF-kβ expression) were determined.

Results Glucose, insulin resistance indices, cardiovascular indices, renal dysfunction parameters, different markers of glycation, oxidative stress, and inflammation as well as NF-κB expression, were the lowest in the (AC & His) treated diabetic rats. Besides, the cited parameter was lower in the Ac treated one than His treated ($p > 0.001$).

Conclusion The combination of AC and His had the most protective effect against diabetes complications and advantageous effect on metabolism, β-cell activity, and insulin function due to the most reductive effect on the NF-κB pathway rather than More than any of the amino acids alone.

Keywords Acetylcysteine · Histidine · NF-kappa β · Diabetes complications

Introduction

In the twenty-first century, diabetic vascular complications as life-threatening diseases and medical emergencies have been considered [1]. Insulin resistance is the cardinal manifestation and reason of type 2 diabetes and related complications. Hyperglycemia or glyoxalase-I (Glo-I) deficiency increases gly-oxidation products. Gly-oxidation products interfere with β-cell and insulin functions by intensifying oxidative stress and inflammation [2]. The nuclear factor-NF-kβ (NF-kβ) pathway in the liver manipulates liver and insulin functions [3]. Early to end glycation products, oxidative stress, Free fatty acids (FFTs), oxidized LDL, myeloperoxidase (MPO), and interleukine-1β (IL-1β) elevate stress-induced NF-kβ signaling. Probably, the hepatic NF-kβ motivates glycemia, insulin resistance, dyslipidemia, and diabetes complications [4, 5]. The activation of the NF-κB signaling causes diabetic nephropathy by elevating transforming growth factor-β1(TGF-β1) expression [6]. Thus, the repression of glycation products and other activators of the expression of NF-κB may be a goal for ameliorating diabetic kidney disease (DKD). Likely, amino acids are functional safety biomolecules to prevent or improve diabetes and diabetes complications [7–9].

Acetylcysteine (AC) has an advantageous effect on cardiovascular diseases and nephropathy [10] via antioxidant, anti-glycation, and anti-inflammatory properties [8, 11].

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Histidine (His) low level leads to inflammation, oxidative stress, and insulin resistance [12]. Further, it improves insulin resistance and diabetes complications via antioxidant and anti-inflammatory properties [13]. Following previous studies, we designed a new combination therapy to find more functional therapies based on the synergistic effect of treatments to prevent and improve the complications of diabetes [8, 13]. Hence, we compared the effect of AC, His, and their combination on the NF-kβ expression and its activators in a rat model of type-2 diabetes.

Materials and methods

Materials

Perchlorate sodium, reduced and oxidized glutathione, Guaiacol, H_2O_2, ketamine and xylosine, phosphate buffer components, 2,4-dinitrophenylhydrazine, citric acid were bought from Sigma Chemical Company.

Type 2 diabetes induction in rats

The Faculty of Veterinary Medicine, Tehran University, Iran was selected for purchasing male Wistar rats with weights of 190 ± 10. Animals are maintained in regulated conditions with liberating approachability to food and water. In addition, the rats were nourished on a normal rat diet. This project was allowed by the Ethics Committee of our University (IR.ARUMS.REC.1397.176). Type 2 diabetes was motivated in rats via a mixture of nicotinamide and alloxan (110 + 50 mg/kg) (1). After one week, rats had fasting blood sugar levels between 8 and 16 mmol/l, and postprandial blood sugar levels > 11 mmol/l were acceded as type 2 diabetic rats. Besides, to confirm induction Type 2 diabetes insulin resistance indices were calculated at the last. Fifty rats were allotted into five equal groups. Control and diabetic groups that were introduced respectively as (C) and (D), as well as diabetic rats received 0.06% AC, 0.1% His, and a combination of AC and His that were named respectively as (DA), (DH), and (DAH). The treated groups received the treatment daily for two months a week after diabetes induction. The dose of the treatments was chosen according to our previous studies (2, 3). The weight of rats was detected a week before the end of the study. Then, 24 h urine samples of them were collected in the metabolic cages. After 16 h fasting and desensitizing with IP injection of ketamine & xylazine, blood samples were gathered from the rat heart and poured into the test tubes. Immediately, upon completion of blood collection, the rats were euthanized. The rat's liver and kidneys were taken away and weighted promptly.

Measuring of Biochemical items

Fasting blood glucose (FBG), lipid profile, creatinine (Cr). The diverse cardiovascular indices were computed with the determination of LDL/HDL, TC/HDL, and TG/HDL proportions. Glomerular filtration rate (GFR) was determined was determined via Eq. 1 [14].

$$\mathrm{GFR} = \frac{\text{Urine creatinine}}{\text{serum creatinine}} \times \frac{\text{Urine volume}}{\text{Body weight}} \quad (1)$$

A rat insulin enzyme-linked immunosorbent assay (ELISA) kit (ZellBio GmBH, Germany) was applied for measuring insulin levels. Further, the original homeostasis model assessment of insulin resistance (HOMA1) was computed.

Besides, the updated HOMA model (HOMA2), the percentage of beta-cell activity (%B), and the percentage of insulin subtlety (%S) were calculated with the HOMA2 calculator software [15].

Determination of glycated products and Glo-I activity

Glycated albumin (g-Alb) was measured based on the tetrazolium chloride reduction and read absorbance at 530 nm [16]. Glycated LDL (g-LDL) was quantified via measuring the hydroxymethylfurfuraldehyde absorbance at 443 nm [17]. Methylglyoxal (MG) was detected by HPLC through measuring the DNPH derivatives at a wavelength of 330 nm [18]. AGEs were determined with a fluorimeter via the determination fluorescence intensity at 370 nm [19]. The Glo-I activity was quantified by detecting the primary rate generation of S-D-lactoylglutathione at 240 nm [20].

Measurement of oxidative stress and inflammatory markers

Malondialdehyde (MDA) was determined by reading the absorbance at 532 nm. Advanced oxidation protein products (AOPP) in diluted serum with citrate solution (200 mmol/l) were detected via measuring absorbance in wavelength 340 nm [21]. Primary LDL oxidation products were quantified at 234 nm [22] and final LDL oxidation products were detected with a fluorimeter at 360 nm [23]. UV-HPLC at 210 nm was applied for reduced glutathione (GSH) measuring [24]. The paraoxonase-I (PON1) activity was detected by determining one minute the absorbance of p-nitrophenol at 412 nm [25]. Catalase (CAT) activity was assayed with a changed procedure [26]. Momentarily, 5 µl sample was put on one ml of phosphate- buffered saline (buffer 50 mmol/l, pH = 7 and containing 10 mm of H_2O_2) and read absorbance at 240 nm to twenty seconds.

The inflammatory marker as interleukin-1β (IL-1β) and transforming growth factor-β (TGF-β1) were measured with the ELISA kits (Immunotech, France). The sera myeloperoxidase (MPO) activity was determined by detecting absorbance at 470 nm.

NF-κB expression in the rat liver

The liver total RNA was separated with TRIzol reagent (Invitrogen, USA). Its quantity and quality were determined by a Nanodrop at 260 nm and 260/280 nm ratios, respectively. Reverse transcription (MBI Fermentas, Lithuania) was done for cDNA generation. qRT-PCR was fulfilled with a high quality SYBR-Green PCR kit (Toyobo, Japan). The ABI 7300 (Applied Biosystems, Germany) was applied for gene specific PCR amplification. B-actin (ACTB) normalized the gene expression data. RT-PCR primer sequences were as comes behind: NF-kβ: 5´-CCTGTCTGCACC TGTTCCAA-3´ (forward) and 3´ACTCCTGGGTCTGTG TTGTT-5´(reverse) as well as ACTB: 5´-GGAGAA GAT TTGGCACCACACT-3´ (forward) and 3´-CGGTTGGCC TTAGGGTTCAGA-5´ (reverse). After the normalization, $2^{-\Delta\Delta CT}$ method determined the correlative gene expression levels. The first ΔCT is the variation in threshold round between the NF-kβ and ACTB: $\Delta CT = CT$ (NF-kβ)-CT (ACTB) [2].

in vitro study

Investigation the impact of the treatments on the generation of glyco-oxidation parameters of albumin and LDL

Extracted albumin and LDL from rat serum maintained with and without glucose and AC (60 mg/l) and His (1000 mg/L) as well as the combination of AC and His. The complete procedure was resembling what we described in an earlier study [8].

Measurement of gly-oxidized products

Alb and LDL gly-oxidation products were quantified with the cited procedures in the in vivo section.

Statistical analysis

Diverse parameters in the rat groups were contrasted with the multiple analysis of variance (MANOVA-TUKEY) test of SPSS version 16. $p < 0.05$ was admitted as statistical significance.

Results

in vivo study

Table 1 shows metabolic profile quantities (FBS, insulin, HOMA1, HOMA2, %B, and %S as well as lipid profile, FFA, and atherogenic indices) and renal dysfunction parameters in the rat groups with or without receiving AC, His, and their combination. The levels of FBS, insulin, HOMA1, and HOMA2 were more in the diabetic group than in the treated ones. Furthermore, %B, and %S was lower. The most decrease in FBS and insulin resistance indices, as well as an increase in %B and %S, was seen respectively in DAH, DA, and DH *($P < 0.001$)*. The quantities of lipid profile, FFA, and diverse indices (LDL/HDL, TG/HDL, TC/HDL, MPO/HDL) in AC, His, and their combination treated diabetic groups were less than the no treated ones. The levels of TC, LDL, as well as the cited indices in DAH, were less than in other diabetic treated groups. GFR level reduction along with TGF-β, Cr, and PU elevation was observed in the type 2 diabetic rat group. DAH had the most level of GFR and the lowest levels of TGF-β, Cr, and PU. The levels of the cited parameters expect %B, %S, and GFR were lower in DA than DH *($P < 0.001$)*.

The comparison of oxidative stress, inflammatory, and glycation markers along with GLO-I activity in the rats, are represented in Table 2. The levels of proteins, lipids, and LDL oxidation products as well as all glycation and inflammatory markers increased in untreated diabetic rats rather than in other groups. Furthermore, GSH levels along with Glo-I, PON-1, and CAT activities were lower in untreated diabetic rats. Glycation, oxidative stress, and inflammatory markers or triad markers were less in the treated diabetic groups than in no treated diabetic group. The lowest triad markers were observed in DAH. Moreover, quantities of the cited markers were lower in DA than in DH *($p < 0.001$)*.

The ratio of hepatic NF-kβ to ACTB gene expression has been shown in Fig. 1. The ratio of it in D groups was more than in other groups. The most decrease in the ratio of hepatic NF-kβ to ACTB gene expression was observed in DAH. Further, the level of it in DA and DH groups were in the next ranks, respectively *($P < 0.001$)*.

in vitro study

The effect of AC, His, and their combination on the formation of different Alb (g-Alb, MGO, and AGEs) and LDL (g-LDL and LDL oxidation products) gly-oxidation products of albumin and LDL has been shown in Table 3. The

Table 1 The levels of metabolic profile and renal dysfunction parameters in the control (C), diabetic (D), and treated diabetic rats with acetylcysteine (DA), histidine (DH), their combination (DAH)

Parameter	Groups				
	C	D	DA	DH	DAH
FBS (mmol/L)	3.98 ± 0.27	18.46 ± 1.24*	8.94 ± 0.63*, #	12.03 ± 0.73*, #, @	7.38 ± 0.48 *, #, @, $
Insulin (μU/mL)	16.14 ± 0.91	22.58 ± 1.36*	17.73 ± 1.29 *, #	18.98 ± 1.56 *, #, @	17.01 ± 0.53 *, #, @, $
HOMA1	2.85 ± 0.18	18.70 ± 1.28*	7.04 ± 0.41*, #	10.14 ± 0.65*, #, @	5.57 ± 0.36*, #, @, $
HOMA2	1.94 ± 0.13	5.56 ± 0.39*	2.58 ± 0.23*, #	2.95 ± 0.28*, #, @	2.38 ± 0.19*, #, @, $
%β	246.30 ± 14.03	22.90 ± 1.16*	56.50 ± 3.42 *, #	36.10 ± 1.69 *, #, @	77. 6 ± 3.22 *, #, @, $
%S	51.60 ± 2.37	18.00 ± 0.94*	38.80 ± 2.06 *, #	33.90 ± 1.94 *, #	42.10 ± 2.15 *, #, @, $
TG (mmol/L)	0.99 ± 0.07	1.98 ± 0.14*	1.43 ± 0.12 *, #	1.39 ± 0.10 *, #	1.16 ± 0.09 *, #, @, $
TC (mmol/L)	1.60 ± 0.13	2.75 ± 0.18*	1.95 ± 0.14*, #	2.08 ± 0.16*, #	1.77 ± 0.11 *, #, @, $
HDL (mmol/L)	0.78 ± 0.06	0.52 ± 0.03*	0.74 ± 0.05 *, #	0.70 ± 0.06 *, #	0.68 ± 0.04 *, #
LDL (mmol/L)	0.41 ± 0.03	1.40 ± 0.10*	0.82 ± 0.06 *, #	0.92 ± 0.08 *, #, @	0.73 ± 0.07 *, #, @, $
LDL/HDL	0.52 ± 0.04	2.69 ± 0.24 *	1.05 ± 0.09*, #	1.31 ± 0.10 *, #, @	0.94 ± 0.08 *, #, @, $
TC/HDL	2.05 ± 0.13	5.28 ± 0.36 *	2.41 ± 0.17 *, #	2.97 ± 0.22 *, #, @	2.54 ± 0.19*, #, @, $
TG/HDL	1.26 ± 0.08	3.80 ± 0.23 *	1.93 ± 0.12 *, #	1.98 ± 0.14 *, #	1.61 ± 0.11*, #, @, $
MPO/HDL	0.91 ± 0.07	11.90 ± 0.86	3.22 ± 0.15*	4.92 ± 0.28 *, #, @	2.42 ± 0.19 *, #, @, $
FFAs (μmol/L)	385.07 ± 29.05	697.29 ± 38.05 *	436.05 ± 25.64 *, #	534.57 ± 36.71 *, #, @	366.43 ± 23.85 *, #, @, $
TGF-β1 (pg/ml)	11.02 ± 0.64	73.86 ± 5.29 *	29.13 ± 1.70 *, #	42.18 ± 3.23 *, #, @	20.48 ± 1.30 *, #, @, $
Cr (μmol/L)	50.26 ± 3.87	111.07 ± 6.74 *	68.32 ± 4.51 *, #	80.54 ± 5.27 *, #, @	60.21 ± 4.03 *, #, @, $
PU (mg/dl/24 h)	11.03 ± 2.32	296.62 ± 14.51 *	97.32 ± 6.28 *, #	126.03 ± 8.24 *, #, @	78.32 ± 8.24 *, #, @, $
GFR (ml/min)	3.09 ± 0.26	1.46 ± 0.14 *	2.10 ± 0.18*, #	1.82 ± 0.16 *, #, @	2.38 ± 0.21 *, #, @, $

* Compared control group with other groups ($p < 0.001$)

Compared diabetic group with other groups ($p < 0.001$)

@ Compared acetylcysteine treated diabetic group (DA) with other groups ($p < 0.001$)

$ Compared histidine treated diabetic group (DH) with other groups ($p < 0.001$)

FBG: fasting blood sugar; HOMA1: the original homeostasis model assessment of insulin resistance; HOMA2: the updated of HOMA model; %B: the percentage of β−cell function %S: the percentage of insulin sensitivity; TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; MPO; myeloperoxidase FFATs: free fatty acids; TGF−β: transforming growth factor; Cr: creatinine; PU: proteinuria in 24−h; GFR: glomerular filtration rate

treatments diminished various Alb and LDL gly-oxidation products. Further, the levels of the cited products were the lowest in the presence of AC & His and the highest in the presence of His.

Discussion

Acetylcysteine, histidine, and their combination corrected hyperglycemia, insulin resistance, and dyslipidemia through the hepatic-NF-kβ down-regulation (Fig. 1). The treatments decreased NF-kβ expression following their reductive effects on diverse NF-kβ activators such as different glycation (early to end), oxidative stress (LDL oxidation products), and inflammatory (IL-1β and MPO) markers (Table 2) as well as FFTs (Table 1). Moreover, the best one was their combination and AC was better than His. The elevation of IL-1β [27], MPO [28], and gly-oxidation products, consequent hyperglycemia and the reduction of Glo-I activity, leads to intensifying oxidative stress and inflammation, contributing to interfering with β-cell and insulin activities. The induction of glycolytic enzymes gene expression and the activities contribute to insulin dysfunction [29]. AC [30] has an advantageous effect on insulin action and glycemia with a decrease of glycolytic enzymes expression and their activity. Moreover, it increases glycogenosis [31]. In addition, Cys with a reversible cysteine sulfenic acid generation is a critical pathway for the activation and proliferation of β-cell [32]. His also by downregulation of the gluconeogenic enzymes gene expression improves glucose metabolism [33]. AC had a more inducing effect on Glo-I activity and a more lowering effect on the generation of different gly-oxidation products (g-Alb, MG, AGEs, g-LDL, and Ox-LDL) in the rats (Table 2) and test tube (Table 3). The higher level of glycoxidation products, IL-1β, and MPO activity (Table 2) in the diabetic group than other groups were correlated with the higher level of insulin resistance indices, β-cell dysfunction, and lower insulin sensitivity.

Table 2 Comparison the levels of glycation, oxidative stress and inflammatory markers in the control (C), diabetic (D), and treated diabetic rats with acetylcysteine (DA), histidine (DH), and their combination (DAH)

Parameter	Groups				
	C	D	DA	DH	DAH
g-Alb (μmol/L)	75.76 ± 4.72	377.65 ± 16.38 [*]	286.97 ± 14.61 [*, #]	324.50 ± 17.43 [*, #, @]	242.05 ± 13.62 [*, #, @, $]
g-LDL (μmol/L)	42.70 ± 2.26	229.52 ± 11.56 [*]	162.56 ± 9.78 [*, #]	188.05 ± 10.09 [*, #, @]	130.77 ± 7.86 [*, #, @, $]
MG (μmol/L)	13.66 ± 0.74	107.39 ± 6.42 [*]	50.44 ± 3.10 [*, #]	71.01 ± 3.58 [*, #, @]	43.92 ± 2.64 [*, #, @, $]
AGEs (FI, A.U)	35.94 ± 2.47	428.66 ± 26.12 [*]	287.10 ± 16.59 [*, #]	325.78 ± 18.24 [*, #, @]	217.21 ± 11.08 [*, #, @, $]
CD (μmol/L)	10.45 ± 0.77	107.45 ± 6.28 [*]	69.97 ± 4.06 [*, #]	86.40 ± 5.26 [*, #, @]	59.62 ± 3.73 [*, #, @, $]
FOPL (μmol/L)	188.96 ± 9.38	525.30 ± 28.07 [*]	411.35 ± 24.52 [*, #]	427.61 ± 26.34 [*, #, @]	363.94 ± 18.35 [*, #, @, $]
AOPP (μmol/L)	9.84 ± 0.62	118.06 ± 7.24 [*, #]	53.50 ± 3.08 [*, #]	66.61 ± 4.12 [*, #, @]	41.39 ± 2.06 [*, #, @, $]
MDA (μmol/L)	16.12 ± 0.86	75.28 ± 4.42 [*, #]	29.53 ± 2.51 [*, #]	41.04 ± 3.13 [*, #, @]	27.31 ± 1.60 [*, #, $]
GSH (μmol/L)	207.80 ± 12.39	97.64 ± 5.28 [*, #]	161.86 ± 7.01 [*, #]	121.88 ± 8.56 [*, #, @]	177.61 ± 9.38 [*, #, @, $]
IL-1β (pg/ml)	65.60 ± 2.58	318.33 ± 16.02	100.21 ± 6.05 [*, #]	127.66 ± 7.28 [*, #, @]	85.33 ± 5.28 [*, #, @, $]
Glo-I (U/ml)	39.63 ± 1.97	18.60 ± 1.05 [*]	29.67 ± 1.42 [*, #]	25.65 ± 1.51 [*, #, @]	31.23 ± 1.47 [*, #, @, $]
PON-I (U/ml)	141.53 ± 8.27	47.53 ± 3.05	106.31 ± 6.42 [*, #]	77.71 ± 4.90 [*, #, @]	114.01 ± 7.23 [*, #, @, $]
CAT (U/ml)	141.55 ± 9.61	62.34 ± 3.90 [*, #]	97.86 ± 5.23 [*, #]	83.64 ± 4.80 [*, #, @]	115.84 ± 6.02 [#, @, $]
MPO (U/ml)	0.71 ± 0.05	6.19 ± 0.43	2.39 ± 0.13 [*]	3.45 ± 0.22 [*, #, @]	1.65 ± 0.11 [*, #, @, $]
MPO/HDL	0.91 ± 0.07	11.90 ± 0.86	3.22 ± 0.15 [*]	4.92 ± 0.28 [*, #, @]	2.42 ± 0.19 [*, #, @, $]

[*] Compared control group with other groups ($p < 0.001$)

[#] Compared diabetic group with other groups ($p < 0.001$)

[@] Compared acetylcysteine treated diabetic group (DA) with other groups ($p < 0.001$)

[$] Compared histidine treated diabetic group (DH) with other groups ($p < 0.001$)

g-Alb: glycated albumin; g-LDL: glycated LDL; MG: methylglyoxal; AGEs: advanced glycation end products; CD: conjugated dines; FOPL: fluorescent oxidation products of LDL; AOPP: advanced oxidation end products; MDA: malondialdehyde; GSH: reduced glutathione; IL-1β: interleukine-1β; Glo-I: glyoxalase-I; PON-I: paraoxonase-I; CAT: catalase; MPO: myeloperoxidase

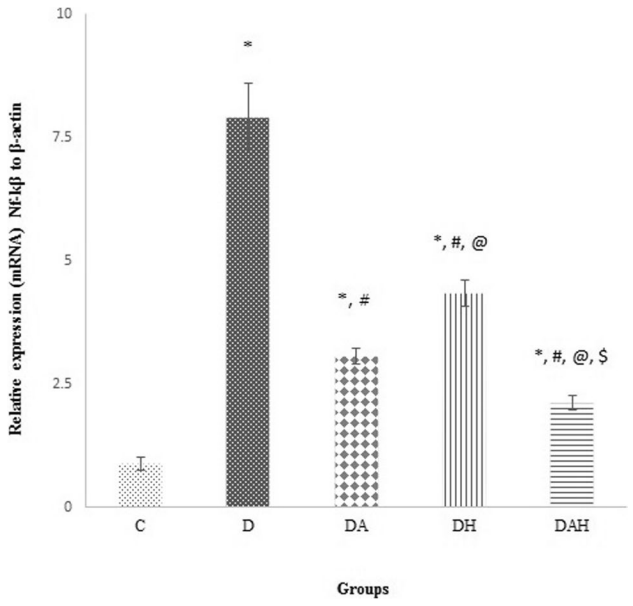

Fig. 1 The comparision effect of acetylcysteine (A), histidine (H), and their combination (AH) on the NF-kβ gene expression in type 2 diabetic rat model. * Indicates significance of data comparing control group with other groups ($p < 0.001$). # Indicates significance of data comparing diabetic group with other groups ($p < 0.001$). @ Indicates significance of data comparing acetylcysteine treated diabetic group (DA) with other groups ($p < 0.001$). $ Indicates significance of data comparing histidine treated diabetic group (DH) with other groups ($p < 0.001$)

An increase in IL-1β level via the elevation of the NF-kβ expression, insulin level, and insulin resistance as well as lipase activity cause dyslipidemia [2, 34]. There is a two-way and synergistic relationship between inflammatory processes and dyslipidemia [35]. In this study, induction type-2 diabetes in rats leads to dyslipidemia and is elevated prone to diabetes vascular complications by an increase of different atherogenic or cardiovascular indices (LDL/HDL, TC/HDL, TG/HDL, and MPO/HDL) (Table 1). Moreover, the elevated levels of FFAs [36], TG/HDL, and MPO/HDL result in an increased risk of diabetes vascular complications [37] with a decrease in levels of HDL and Apo-AI in HDL [35] The treatments represented anti-atherogenic impact by the betterment of lipid metabolism and reducing cardiovascular indices. AC with more lowering effect on TC, LDL, TC/HDL, LDL/HDL, and MPO/HDL than His in the diabetic rats had a more beneficial effect on lipid metabolism but both amino acids along with had the best advantageous effect on lipid metabolism. Cys [38] and His [39] decrease lipogenesis following the reduction of expressions and activities enzymes that have roles in fatty acid and cholesterol synthesis. Furthermore, Cys participates in the hepatic management of APO-A1 as the main component of HDL [40]. Probably, the more effectiveness of AC on glucose metabolism, β-cell

Table 3 The effect of glucose (Glc) and the treatments (Acetylcysteine, AC and histidine, His, and their combination) on the formation of various rat serum albumin (RSA) glycation products and LDL glycation and oxidation products in the *test tube* experiment

	Tube Content	Rat serum albumin glycation products		
		Glycated albumin (μmol/L)	Methylglyoxal (μmol/L)	AGEs (AU)
Albumin	RSA	103.05 ± 1.98	2.96 ± 0.14	43.92 ± 0. 86
	RSA + Glc	990.28 ± 8.73	37.51 ± 0.64	501.23 ± 5.63
	RSA + Glc + AC	349.11 ± 2.84	7.02 ± 0.25	259.82 ± 3.04
	RSA + Glc + His	493.21 ± 3.01	19.16 ± 0.76	376.03 ± 4.16
	RSA + Glc + (AC & His)	292.35 ± 1.87	5.78 ± 0.24	193.17 ± 2.01
	Tube Content	LDL glycation and oxidation products		
		Glycated LDL (μmol/L)	Early oxidation products (μmol/L)	End Oxidation products (AU)
LDL	LDL	14.26 ± 0.51	10.18 ± 0.32	19.74 ± 0.77
	LDL + Glc	298.75 ± 7.46	132.41 ± 2.89	520.63 ± 8.54
	LDL + Glc + AC	48.13 ± 2.08	34.48 ± 0.51	397.41 ± 4.52
	LDL + Glc + His	71.06 ± 3.29	69.32 ± 0.93	446.01 ± 5.98
	LDL + Glc + (AC & His)	27.54 ± 1.06	21.62 ± 0.45	302.14 ± 3.48

function, and insulin sensitivity, and lipid metabolism (Table 1) may be on higher anti-glycation, antioxidant, anti-inflammatory activities (Table 2) of it as well as the more lowering effect on FFA Level (Table 1). The combination of AC and His with more anti-glycating, antioxidant, anti-inflammatory activities than the alone amino acids improved glucose and lipid metabolism through a beneficial effect on β-cell function and insulin function. Lately, a beneficial effect of AC [8] and His [13] on glycemia and dyslipidemia respectively in diabetic-atherosclerotic and type-2 diabetic rats models have been represented. The effect of AC, His, and their combination on the hepatic-NF-kβ pathway, Glo-I, and MPO activities has not been investigated. Furthermore, the combinatorial treatment effect on hyperglycemia and dyslipidemia in the rats that induced type-2 diabetic has not been reported.

Glycated and oxidized LDL take part in vascular and renal cellular dysfunction (34). LDL glycated and oxidized are more in no treated the type 2 diabetic group model than in other groups. The beneficial effect of the treatments on lipid metabolism and PON-I activity along with the lower MPO activity in the treated groups lead to the reduction of glycated and oxidized LDL products in the cited groups rather than untreated diabetic ones. The combination of the amino acids had the most protective effect on vascular and renal cells via the lowest levels of glycated and oxidized LDL. Furthermore, AC was a more potent inhibitor of LDL modifications than His. The advantageous effect of AC on levels of modified LDL products in diabetic-atherosclerotic rats has been reported [8]. The effect of His, AC, and their combination on PON-I activity and the effect of His and the combination of it with AC on LDL gly-oxidation products in type 2 diabetic rats for the first time, was represented.

Proteinuria and a decrease in GFR level, as well as an increase in the sera TGF-β1 and Cr concentrations in the diabetic group (Table 1), corroborated renal dysfunction in rats following type-2 diabetes induction. The renoprotective effect of the treatments is due to their corrective effect on glycemia, insulin resistance (Table 1), glycation, oxidative stress, inflammation (Table 2), and dyslipidemia (Table 1). Glycation products are the most important stimulant in the development of diabetes related complications due to the initiation and intensification of oxidative stress, inflammation, hyperglycemia, and insulin resistance [41, 42]. The kidneys and Glo-I following the reduction of glycation products contribute to the defense against diabetes complications [42, 43]. A decrease in Glo-I activity and the elevation IL-1β level are the most important factors in the progression of DKD and atherosclerosis. lately, Glo-I activation and the decrease of IL-1β have been considered therapeutic targets for diabetic complications [44, 45]. IL-1β and diverse glycation products in the diabetic group were higher but the activity of Glo-I (Table 2) was lower than in other groups. Induction of Oxidative stress and the elevation of MPO activity participate in diabetic nephropathy progression [46]. The elevation of the MPO activity in diabetes not only induces the NF-kβ pathway but also by generating 3-chlorotyrosine may cause renal dysfunction and cardiovascular complications. The activation of the NF-κB signaling pathway causes an increased TGF-β1 expression as a pivotal pathogenic factor in diabetic nephropathy [6, 47]. Thus, the inhibition of the AGE-RAGE-NFκB axis may be a goal for ameliorating DKD [48]. In this study, oxidative stress markers levels (AOPP and MDA) and MPO activity increased in the diabetic group. In addition, the GSH level (Table 2) decreased. The treatments reduced oxidative stress markers and MPO

activity along with the elevation of GSH in diabetic rats. The treatments reduced free radical's generation and inflammatory process together with the increase of GSH in diabetic rats. In this study, AC had a more antioxidant property than His in the diabetic rats as well as their combination generated the most potent antioxidants. Presumably, the protective effect of the amino acids against nephropathy was due to a decrease of the AGE-RAGE-NFκB axis. Diminution glycation, oxidative stress, and inflammatory events cause the reduction of TGF-1β as a central player in DKD. The levels of TGF-β1, Cr, and PU in AC treated diabetic group were lower than His as well as their combination had the best renoprotective effect. We want to study the effect of the treatments on the AGE-RAGE-NFκB axis in diabetic patients in the future.

Conclusions

The combination of acetylcysteine and histidine had the most protective effect against diabetes complications in type 2 diabetic rats. It has the most beneficial effect on glycemia, dyslipidemia, β-cell function, and insulin sensitivity due to the most reductive effect on the NF-κB gene expression. Moreover, it had the highest anti-glycating, antioxidant, and anti-inflammatory activities. Besides, AC was a more potent protector against diabetes vascular complications than His.

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2) Manuscript writing and revising: Mahdavifard.

3) Ultimate sanction of the version to be submitted and any revised version: Mahdavifard & Nakhjavani.

The survey is a genuine study that it has not been lately send to or admitted by any other journal, that is has been ratified by the authors, that ethics assent and written informed consent have been attained, and describe if any author has a conflict of interest.

Declarations

Monetary sustain Ardabil University of medical sciences.

Conflict interest Authors proclaim that they have not any conflict of interest.

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