In vivo Assessment of Combined Effects of Glibenclamide and Losartan in Diabetic Rats

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Significance of the Study

- A combination of glibenclamide and losartan therapy diminished the consequences of diabetic hyperglycemia in this study. The combined therapy showed a benefit on hyperglycemia-induced oxidative and inflammatory reactions. However, the combined effects did not demonstrate a significant advantage over single drug therapy. These findings may be of relevance to the treatment of hypertensive diabetics.

Keywords

Diabetes mellitus · Glibenclamide · Losartan · Combined therapy · Oxidative stress · Inflammation

Abstract

Objective: Diabetic complications involve multiple pathological pathways, including hyperglycemia-induced oxidative stress and inflammation. Combination therapy is usually employed to improve treatment outcomes and to lower potential adverse effects. In this study, we evaluated the effects of antidiabetic and antihypertensive agents, glibenclamide (GLI) and losartan (LT), on diabetes mellitus (DM)-associated metabolic changes in rats. **Materials and Methods:** Streptozotocin-induced diabetic animals were orally treated with GLI 5 mg/kg and/or LT 25 mg/kg for 4 weeks.

Blood glucose, insulin, aspartate aminotransferase, alanine aminotransferase, urinary creatinine, and urea levels were measured. Serum, liver, and kidney values of inflammatory markers, such as interleukin-1 β , tumor necrosis factor alpha, and interleukin-6 were assessed, along with lipid peroxidation products (e.g., thiobarbituric acid reactive substances), endogenous antioxidants (e.g., glutathione), as well as antioxidant enzyme activities (e.g., catalase, superoxide dismutase, and glutathione peroxidase). Finally, histological changes in liver and kidney tissues were evaluated. **Results:** DM markedly induced systemic, hepatic, and renal inflammation and lowered antioxidant defense mechanisms. Treatment of diabetic rats with either GLI or LT significantly improved liver and kidney functions and histological structure. Moreover, both medications reduced signs of oxidative stress and inflammation in blood, liver, and kidney sam-

ples. Combining GLI and LT showed similar protective potential against systemic, hepatic, and renal oxidative stress and inflammation. **Conclusion:** Adding LT to GLI therapy revealed prospective antioxidant and anti-inflammatory action, while no synergistic or additive effects were observed.

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Introduction

Diabetes mellitus (DM) is an endocrine disorder characterized by multiple etiological factors, including chronic hyperglycemia and alterations in the metabolism of carbohydrates, fats, and proteins caused by defects in insulin secretion and/or action. The associated chronic hyperglycemia may lead to long-term oxidative cellular injury and dysfunction of various organs, particularly the kidneys, eyes, nerves, liver, heart, and blood vessels. The provoked oxidative stress and lipid peroxidation act as a potential pathophysiological mechanism underlying diabetic complications through the overproduction of reactive oxygen species and by triggering the production of proinflammatory cytokines, which together with reactive oxygen species are deemed to play vital roles in the pathogenesis of DM [1]. Furthermore, the use of antioxidants has been found to reduce the burden of diabetic complications in several experimental and clinical studies [1–3].

Glibenclamide (GLI) is a common oral hypoglycemic agent that works primarily by stimulating insulin secretion via blockage of the ATP-sensitive K⁺ channels and depolarization of pancreatic beta cells. GLI also functions by the same mechanism of action in extrapancreatic sites, including the liver, kidneys, heart, as well as skeletal and smooth muscles [4]. In addition, the ability of GLI to reduce the biomarkers of oxidative stress and lipid peroxidation during DM has been documented in several experimental studies where GLI was used as a reference antidiabetic agent [5].

The renin-angiotensin system (RAS) is a well-documented endocrine system that regulates the physiological electrolyte and fluid balance. The octapeptide angiotensin-II induces vasoconstriction and proinflammatory markers. Inhibition of the production or action of angiotensin-II is a target for different groups of antihypertensive agents, including angiotensin-converting enzyme inhibitors and angiotensin-II type 1 (AT₁) receptor blockers. Evidence supports the pathological role of RAS components in the development and progression of diabetic complications by exacerbating oxidative injury and inflammation [6]. Losartan (LT) is an AT₁ receptor block-

er that is characterized by its antioxidant defense properties during DM [7]. Moreover, LT reduced DM-induced inflammation and lipid peroxidation in diabetic rats [8].

In recent decades, there has been increasing interest in combination therapy or polytherapy, which refers to the concurrent consumption of multiple medications against one or several medical conditions. As hypertension is considered one of the macrovascular complications of DM, the concomitant use of GLI and LT in diabetic patients could be beneficial to treating DM. Therefore, this study was designed to evaluate the impact of combined GLI and LT therapies on oxidative and inflammatory changes in diabetic rats.

Materials and Methods

Animals

Male Wistar albino rats weighing between 260 and 300 g were obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University.

Experimental Procedures

DM was induced by a single intraperitoneal injection of 60 mg/kg streptozotocin (STZ). STZ was freshly dissolved in citrate buffer (0.1 M, pH = 4.5). The remaining control animals were injected with an equivalent volume of citrate buffer. After 72 h, blood glucose levels were measured with a glucometer (ACCU-CHEK Active, Mannheim, Germany), and values >250 mg/dL were considered diabetic. Animals were then randomly divided into five groups (*n* = 6 each) as follows: (A) control group; (B) DM (STZ) group; (C) DM + GLI group (5 mg/kg oral dose); (D) DM + LT group (25 mg/kg oral dose); and (E) DM + GLI + LT group (5 and 25 mg/kg oral dose, respectively). GLI (Sigma-Aldrich Chemie, Steinheim, Germany) and LT (TRC Inc., Toronto, ON, Canada) were suspended in 0.5% carboxymethyl cellulose and administered via gastric gavage in a volume of 0.5 mL/100 g body weight. The doses for GLI and LT were selected in line with previous experimental reports [9, 10]. The control and STZ groups received similar volumes of 0.5% carboxymethyl cellulose during the treatment period. Treatment started 1 week after the induction of DM and lasted for 4 consecutive weeks, which is sufficient time for diabetic metabolic changes to appear. Then 24-h urine samples were collected in metabolic cages, and after centrifugation at 3,000 rpm for 5 min, the supernatants were transferred to 1.5-mL tubes. Next, blood samples were collected via cardiac puncture and centrifuged at 3,000 rpm for 10 min to obtain the sera. Liver and kidney tissues were desiccated, weighed, and homogenized in a 10-fold volume of ice-cold phosphate-buffered saline (pH = 7.4). The homogenates were transferred to 1.5-mL tubes and stored at –80 °C until use.

Biochemical Analysis

Serum glucose and insulin levels were measured by a commercially available kit (RANDOX Laboratories Ltd., UK and SPI bio, France, respectively). Serum aspartate aminotransferase and alanine aminotransferase were determined by diagnostic kits (Hu-

Table 1. Effects of GLI and/or LT on serum and urine biochemistry in diabetic rats

	Control	STZ	GLI	LT	GLI + LT
Glucose, mmol/L	4.8±1.56	11.2±1.45 ^a	6.1±0.79 ^b	9.8±1.05 ^a	6.4±1.37 ^b
Insulin, mAU	476.5±41.38	138.9±21.77 ^a	290.3±20.16 ^b	143.3±13.73 ^a	196.3±28.42 ^b
IL-1 β , pg/mL	99.8±5.97	177.1±19.82 ^a	123.7±15.34 ^b	116.4±8.22 ^b	139.3±15.26 ^b
TNF- α , pg/mL	85.1±4.64	126.5±12.49 ^a	106.3±10.41 ^b	113.1±6.08 ^b	116.4±8.83 ^b
IL-6, pg/mL	197.5±12.28	353.6±129.12 ^a	247.3±11.92 ^b	221.8±18.31 ^b	228.6±15.65 ^b
TBARS, nmol/mL	299.7±35.41	546.5±33.91 ^a	423±20.57 ^b	350.5±59.27 ^b	370.7±42.41 ^b
GSH, mg/dL	35.9±2.24	27.1±0.82 ^a	29.4±0.35 ^b	29.6±0.24 ^b	28.9±0.67 ^b
CAT, nmol/min/mL	8.5±0.32	3.8±0.25 ^a	9.8±0.75 ^b	9±0.21 ^b	7.6±0.24 ^b
SOD, U	25.5±1.36	17.2±1.93 ^a	23.5±0.65 ^b	22.4±1.57 ^b	23.3±0.72 ^b
GPx, mU/L	7.7±0.81	5.3±0.26 ^a	6.8±0.34 ^b	8.7±0.42 ^b	8.4±0.42 ^b
AST, U/L	37.1±2.75	49.7±2.35 ^a	38.7±2.57 ^b	39.9±1.77 ^b	40.8±2.55 ^b
ALT, U/L	30.5±1.48	105.5±2.93 ^a	89.9±1.46 ^b	81.5±3.53 ^b	97.3±2.51 ^b
Urinary creatinine, mmol/L	8.3±2.25	3.5±0.54 ^a	5.7±0.77 ^b	5±0.72 ^b	5.9±0.52 ^b
Urinary urea, mmol/L	111.2±6.24	454.3±29.76 ^a	233.4±41.01 ^b	295.5±23.31 ^b	257.9±29.31 ^b

Numerical values are presented as mean $\pm$ standard deviation and were statistically analyzed using one-way ANOVA followed by Newman-Keuls multiple comparison test. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; GLI, glibenclamide; GPx, glutathione peroxidase; GSH, glutathione; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; LT, losartan; SOD, superoxide dismutase; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; TNF- α , tumor necrosis factor alpha. Statistically significant difference: ^a $p < 0.01$ versus control group, ^b $p < 0.01$ versus STZ group.

man Diagnostics Worldwide, Wiesbaden, Germany). Urine creatinine and urea were estimated by colorimetric methods (Linear Chemicals, Barcelona, Spain). Serum, liver, and kidney levels of interleukin-1 β (IL-1 β), tumor necrosis factor alpha (TNF- α), and interleukin-6 (IL-6) were determined by an ELISA technique (Thermo Scientific, Rockford, IL, USA).

Estimation of Lipid Peroxidation and Oxidative Stress Markers

Serum, liver, and kidney concentrations of thiobarbituric acid reactive substances (TBARS) were assayed by a biochemical kit provided by Zepto Metrix Inc., USA. Levels of reduced glutathione (GSH) were determined by the Sedlak and Lindsay method [11]. The enzymatic activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were evaluated by kits (Cyman Chemical Company, Ann Arbor, MI, USA).

Histology

Liver and kidney specimens were fixed in 10% formalin and subsequently sliced into segments and embedded in paraffin wax squares. Then the paraffin pieces were cut with a microtome and stained with hematoxylin and eosin. The recolored segments were mounted and examined for histopathological variations by a histopathologist in a blinded manner. Histological assessment of liver injury was conducted as follows. Grading of hepatocyte architecture: (0) no fibrosis, (1) $\leq 25\%$ fibrotic damage, (2) 26–50% fibrotic damage, (3) 51–75% fibrotic damage, and (4) $> 75\%$ fibrotic damage. Grading of inflammatory infiltrate: (0) no lymphocytes, (1) $\leq 25\%$ of lymphocytes and inflammatory exudate, (2) 26–50% of lymphocytes and inflammatory exudate, (3) 51–75% of lymphocytes and inflammatory exudate, and (4) $> 75\%$ of lymphocytes and inflammatory exudate. Histological grading of kidney injury:

Grading of glomerular damage: (0) no damage, (1) $\leq 25\%$ of glomeruli involved, (2) 26–50% of glomeruli involved, (3) 51–75% of glomeruli involved, and (4) $> 75\%$ of glomeruli involved. Grading of acute tubular necrosis: (0) no damage, (1) $\leq 25\%$ of renal tubules involved, (2) 26–50% of renal tubules involved, (3) 51–75% of renal tubules involved, and (4) $> 75\%$ of renal tubules involved. Grading of tubulointerstitial nephritis: (0) no damage, (1) leukocytes confined within the interstitium, and (2) leukocytes within the interstitium and tubular epithelial cells.

Statistical Analysis

The results of the current study are presented as the mean $\pm$ standard deviation. Statistical analyses were carried out using one-way ANOVA followed by a Newman-Keuls multiple comparison test applied on GraphPad Prism version 5. Differences were considered statistically significant when p values were < 0.05 .

Results

Glucose, aspartate aminotransferase, and alanine aminotransferase levels increased significantly ($p < 0.01$) in the STZ group compared to the control group, while these levels were significantly decreased in the GLI- and LT-treated groups compared to STZ ($p < 0.01$) (Table 1). The insulin concentration in the STZ group showed significantly lower levels relative to those of the control group ($p < 0.01$), while insulin levels significantly increased in

the GLI group ($p < 0.01$) compared to the STZ group. The diabetic groups that were treated showed a significant increase in urine creatinine in comparison with the STZ controls ($p < 0.01$). On other hand, there were significant decreases in urine urea levels in the treated groups compared to those in the STZ group ($p < 0.01$) (Table 1).

The serum, liver, and kidney levels of IL-1 β , TNF- α , and IL-6 showed a significant ($p < 0.01$) increase in the STZ group compared to the control group. All treated groups demonstrated significant ($p < 0.01$) corrected values of IL-1 β , TNF- α , and IL-6 (Tables 1, 2). In the STZ group serum, hepatic, and renal levels of TBARS were significantly elevated ($p < 0.01$), while GSH levels were significantly reduced ($p < 0.01$) compared to control animals. Treatment of the animals with GLI and/or LT significantly ($p < 0.01$) attenuated the altered TBARS and GSH concentrations (Tables 1, 2). The enzymatic activities of CAT, SOD, and GPx enzymes were significant ($p < 0.01$) in the diabetic untreated group compared to the control group. The altered antioxidant enzymatic activities were significantly ($p < 0.01$) corrected in the GLI, LT, and GLI + LT groups compared to the STZ group (Tables 1, 2).

Histopathological assessment of liver sections revealed normal- and benign-looking liver hepatocytes in the control animals (Fig. 1a). In the diabetic untreated rats the hepatocytes were degenerated, dilated, and surrounded by inflammatory cells infiltrating along with fatty degeneration (Fig. 1b). Treatment of diabetic animals with GLI reduced hepatocellular fibrosis, degeneration, and inflammation (Fig. 1c). Hepatocytes from the diabetic group treated with LT looked benign and well-arranged in structure, with limited inflammatory exudates (Fig. 1d). Similarly, liver sections from the diabetic group treated with GLI and LT in combination showed normal and well-structured hepatocytes without fibrosis or degenerative changes (Fig. 1e).

Histopathological evaluation of kidney sections revealed normal- and benign-looking renal tissues along with prominent cortex and medulla in the control animals (Fig. 2a). In the diabetic untreated group, there were significantly congested renal tubules with scattered intraluminal eosinophilic sections (Fig. 2b). Diabetic animals treated with GLI had preserved renal structure with a few scattered eosinophilic intratubular secretions (Fig. 2c). LT markedly preserved the renal cortex and medulla and reduced the cloudy degeneration of renal tubules in the kidneys of diabetic animals (Fig. 2d). Kidney sections from diabetic animals treated with GLI and LT in combination had perfect and normal appearance

Table 2. Effects of GLI and/or LT on hepatic and renal inflammatory and oxidative stress parameters in diabetic rats

	Control		STZ		GLI		LT		GLI + LT	
	hepatic	renal	hepatic	renal	hepatic	renal	hepatic	renal	hepatic	renal
Organ weight, g/100 g BW	3 $\pm$ 0.12	1.3 $\pm$ 0.17	4.7 $\pm$ 0.36 ^a	3.3 $\pm$ 0.21 ^a	4.6 $\pm$ 0.24	2.3 $\pm$ 0.16 ^b	4 $\pm$ 0.18 ^b	2.4 $\pm$ 0.17 ^b	4.5 $\pm$ 0.24	2.2 $\pm$ 0.25 ^b
IL-1 β , pg/mg protein	0.6 $\pm$ 0.07	0.8 $\pm$ 0.11	1.9 $\pm$ 0.04 ^a	1.1 $\pm$ 0.13 ^a	0.9 $\pm$ 0.14 ^b	0.9 $\pm$ 0.08	0.8 $\pm$ 0.11 ^b	0.9 $\pm$ 0.12	0.9 $\pm$ 0.11 ^b	0.9 $\pm$ 0.16
TNF- α , pg/mg protein	1.1 $\pm$ 0.09	1.4 $\pm$ 0.12	2.2 $\pm$ 0.17 ^a	1.9 $\pm$ 0.14 ^a	1.5 $\pm$ 0.13 ^b	1.4 $\pm$ 0.21 ^b	1.5 $\pm$ 0.11 ^b	1.4 $\pm$ 0.18 ^b	1.6 $\pm$ 0.11 ^b	1.3 $\pm$ 0.21 ^b
IL-6, pg/mg protein	1.6 $\pm$ 0.19	1.7 $\pm$ 0.32	3.1 $\pm$ 0.16 ^a	3.2 $\pm$ 0.36 ^a	2.2 $\pm$ 0.12 ^b	2.7 $\pm$ 0.32 ^b	2.4 $\pm$ 0.17 ^b	2.9 $\pm$ 0.32	2.3 $\pm$ 0.09 ^b	2.5 $\pm$ 0.36 ^b
TBARS, nmol/mg protein	7.2 $\pm$ 0.61	13.9 $\pm$ 0.37	16.8 $\pm$ 0.92 ^a	31.7 $\pm$ 0.42 ^a	13.4 $\pm$ 0.61 ^b	29.1 $\pm$ 0.76 ^b	11.2 $\pm$ 0.93 ^b	27.2 $\pm$ 0.33 ^b	9.5 $\pm$ 0.31 ^b	27.8 $\pm$ 0.74 ^b
GSH, mg/mg protein	34.9 $\pm$ 1.85	20.2 $\pm$ 0.08	22.1 $\pm$ 1.46 ^a	16.2 $\pm$ 0.08 ^a	29.3 $\pm$ 1.94 ^b	19.8 $\pm$ 0.18 ^b	23.2 $\pm$ 0.86 ^b	13.4 $\pm$ 0.08 ^b	22 $\pm$ 0.94 ^b	15.8 $\pm$ 0.13 ^b
CAT, pmol/min/mg protein	19.1 $\pm$ 0.63	2.8 $\pm$ 0.21	17.1 $\pm$ 0.88	2 $\pm$ 0.26 ^a	17.2 $\pm$ 0.86	3.3 $\pm$ 0.22 ^b	17.5 $\pm$ 0.69	2.1 $\pm$ 0.21	15.2 $\pm$ 0.55	2.3 $\pm$ 0.17 ^b
SOD, U/mg protein	3.8 $\pm$ 0.26	0.3 $\pm$ 0.01	1.5 $\pm$ 0.06 ^a	0.2 $\pm$ 0.01 ^a	1.4 $\pm$ 0.08	0.2 $\pm$ 0.01 ^b	2 $\pm$ 0.17 ^b	0.2 $\pm$ 0.01 ^b	2.1 $\pm$ 0.11 ^b	0.3 $\pm$ 0.01 ^b
GPx, mU/mg protein	11.1 $\pm$ 0.54	1.5 $\pm$ 0.14	6.7 $\pm$ 0.33 ^a	0.8 $\pm$ 0.08 ^a	6.8 $\pm$ 0.41	1.2 $\pm$ 0.09 ^b	7.5 $\pm$ 0.55	1.2 $\pm$ 0.11 ^b	7.4 $\pm$ 0.57	1.3 $\pm$ 0.12 ^b

Numerical values were presented as mean $\pm$ standard deviation and were statistically analyzed using one-way ANOVA followed by Newman-Keuls multiple comparison test. BW, body weight; CAT, catalase; GLI, glibenclamide; GPx, glutathione peroxidase; GSH, glutathione; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; LT, losartan; SOD, superoxide dismutase; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; TNF- α , tumor necrosis factor alpha. Statistically significant difference: ^a $p < 0.01$ versus control group, ^b $p < 0.01$ versus STZ group.

Color version available online

Fig. 1. Effects of GLI and/or LT on liver histopathological changes in diabetic rats ($\times 400$). **a** Liver of a control animal showing benign normal-looking hepatocytes. **b** Liver of a diabetic untreated animal. The arrows indicate inflammatory cell infiltrate with ballooning degeneration and few fatty degenerated cells. **c** Liver of a diabetic animal treated with GLI. The arrows show limited peripor-

tal inflammatory cells with no fibrosis and degenerative changes. **d** Liver of a diabetic animal treated with LT. The arrows demonstrate benign well-arranged hepatocytes with few inflammatory infiltrate. **e** Liver of a diabetic animal treated with GLI and LT. The arrows indicate typical-looking hepatocytes with no degenerated cells or inflammatory infiltrate. GLI, glibenclamide; LT, losartan.

Table 3. Histological assessment of liver and kidney injuries in diabetic rats

	Control	STZ	GLI	LT	GLI + LT
<i>Liver</i>					
Hepatocyte architecture	0	3	2	1	1
Inflammatory infiltrate	0	3	2	2	1
Total score	0	6	4	3	2
Liver injury grade	no	moderate	mild	mild	mild
<i>Kidneys</i>					
Glomerular damage	0	3	1	2	1
Acute tubular necrosis	0	2	2	1	2
Tubulointerstitial nephritis	0	2	1	1	1
Total score	0	7	4	4	4
Kidney injury grade	no	moderate	mild	mild	mild

GLI, glibenclamide; LT, losartan; STZ, streptozotocin. The total score of liver samples evaluation indicated the degree for liver injury, where 0–1 classified as no injury, 2–4 as mild injury, 5–6 as moderate injury, and 7–8 as severe injury. The degree of kidney injury was evaluated based on the renal total score, where 0–1 classified as no injury, 2–4 as mild injury, 5–7 as moderate injury, and 8–10 as severe injury.

Fig. 2. Effects of GLI and/or LT on kidney histopathological changes in diabetic rats ($\times 400$). **a** Kidney of a control animal showing normal benign-looking nephron. **b** Kidney of a diabetic untreated animal. The arrows indicate congested and inflamed renal tubules. **c** Kidney of a diabetic animal treated with GLI. The arrows show preserved renal cortex and medulla with partial inflammation.

d Kidney of a diabetic animal treated with LT. The arrow points to conserved renal cortex and medulla with no congestion. **e** Kidney of a diabetic animal treated with GLI and LT. The arrow points to lack of renal inflammatory exudate or degeneration. GLI, glibenclamide; LT, losartan.

of renal glomeruli and tubules (Fig. 2e). The total histological assessment of liver and kidney injuries is provided in Table 3.

Discussion

Chemically induced DM in rats by STZ is a widely utilized experimental model. STZ accumulates after administration in the beta cells, where glucose transporter type 2 selectively transports it, intracellularly causing DNA alkylation and loss of cellular function [12]. Oxidative stress has been reported in several studies to exacerbate DM complications through triggering lipid and nucleic acid damage along with harming antioxidant defense mechanisms [13, 14]. Studies have also reported that hyperglycemia provokes the production of proinflammatory cytokines, which contribute to considerable pathology during DM-associated inflammation and apoptosis [15]. Together, oxidative stress and inflammation form a

malicious series of biological events that eventually lead to tissue injury. Similarly, signs of augmented systemic and tissue oxidative injuries and inflammation were observed in the present study. Moreover, DM-associated hepatotoxicity was confirmed by elevated liver function enzymes and histological variations, while renal histological analysis and altered creatinine and urea levels verified the renal toxicity in diabetic untreated animals. This was associated with higher levels of proinflammatory cytokines and lipid peroxidation along with diminished endogenous antioxidant molecules and enzyme activities in the STZ group.

In the present study, GLI and LT therapies lowered signs of oxidative stress, lipid peroxidation, and inflammation. Moreover, both medications preserved hepatic and renal functions and structures in diabetic animals. Several clinical and experimental studies have reported comparable protective effects for AT₁ receptor antagonism by LT against DM-induced hepatic and renal injuries through its antioxidant and anti-inflammatory prop-

erties [16]. Interestingly, co-administration of LT with sitagliptin, an antidiabetic agent that belongs to the dipeptidyl peptidase-4 inhibitor group, suppressed the prognosis of nondiabetic nonalcoholic steatohepatitis in diabetic rats [17]. Blockage of the AT₁ receptor has been reported to improve liver function in hypertensive patients [18, 19]. In diabetic rats, LT also provided protection against vascular dysfunction seen in diabetic nephropathy via modulation of renal oxidative stress and inflammation [7]. On the other hand, GLI is a well-known oral antidiabetic agent. Numerous studies have reported its antioxidant and anti-inflammatory properties in different biological systems of diabetic animals [20–22]. Studies have also revealed a marked impact of GLI therapy on DM inflammation and oxidative stress in humans [23]. In contrast, GLI, as a K_{ATP} channel blocker, abolished sodium hydrosulfide neuroprotection against oxidative stress in one study [24]. Moreover, GLI monotherapy had unfavorable effects on lipid peroxidation in the liver and pancreatic tissues of diabetic animals, suggesting the need for combination therapy to restore the antioxidant potential of the drug [25]. Interestingly, Chaturphonprasert et al. [26] reported that GLI co-therapy with berberine did not provide additional value for either medication, which indicates that the therapeutic value may be restricted to single, but not combined, treatments.

Although both GLI and LT showed significant preventive effects against oxidative stress, inflammation, and histological impairments, we did not observe any synergistic or additive properties. Different sites of action, specifically related to blood glucose lowering abilities, may explain these findings. Furthermore, other studies have reported a conflicting action between GLI and anti-RAS medications [27]. Williams et al. [28] reported that chronic GLI therapy resulted in higher blood pressure following infusion of Ang-II in diabetic patients. In addition,

GLI treatment in dogs markedly inhibited the coronary vasodilatory effect of AT₁ receptor blockage, where modulation of K_{ATP} channels was deemed to play a vital role [29]. We did not notice a significant change in fasting elevated blood glucose levels in LT-treated diabetic rats either alone or in combination with GLI. Another AT₁ receptor antagonist, eprosartan, was also unable to significantly alter the 24-h plasma glucose profile in patients with type 2 DM who had previously been stabilized on glyburide [30].

In conclusion, the present study revealed the remarkable systemic, hepatic, and renal antioxidant and anti-inflammatory actions of both GLI and LT in diabetic rats. These preventive abilities were neither harmed nor improved by combining both therapies. Therefore, concurrent administration of both medications may have a therapeutic value in diabetic patients without conferring a synergistic benefit.

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Statement of Ethics

All experimental protocols were performed in agreement with the Laboratory Animals Usage Guidelines of the National Institutes of Health and ethically approved at the College of Pharmacy, King Saud University.

Disclosure Statement

The authors declare no conflicts of interest.

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