Evaluation of regression of diabetes-induced nephropathy and vascular dysfunction in rats by Montelukast via antioxidative and anti-inflammatory actions

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ABSTRACT

This research investigated the effects of montelukast (MONT), a leukotriene receptor antagonist, on diabetes-associated nephropathy and vascular dysfunction in streptozotocin (STZ) diabetic rats. STZ-diabetic Sprague-Dawley rats (a single STZ injection, 50 mg/kg, i.p.) were randomly allocated into three groups (n = 8 each): STZ (received the drug vehicle), STZ-LOS (received Losartan (LOS), 25 mg/kg/day, orally), and STZ-MONT (received MONT, 10 mg/kg/day, orally). Drug administration started 2 weeks after the induction of diabetes and continued till the end of the experiments (10 weeks). A group of age-matched normal rats was set as a control. After 70 days, urine and serum specimens were obtained for biochemical assessments. Moreover, in renal and/or aortic tissue homogenates, levels of reduced glutathione, superoxide dismutase, malondialdehyde, nitric oxide, tumor necrosis factorα (TNF-α), and transforming growth factor-β1 (TGF-β1) were assessed. Pathological alterations in diabetic kidneys and aorta were examined and the vascular reactivity of isolated aortic rings was investigated. MONT attenuated body weight loss, reduced diabetic renal hypertrophy, ameliorated glycated hemoglobin levels, improved renal functions, and lessened renal and aortic oxidative stress in STZ rats. Moreover, MONT reduced kidney levels of TNF-α and TGF-β1 compared to the untreated STZ group. MONT reduced histopathological alterations in renal tissues and diminished aortic medial thickness in diabetic animals. MONT also attenuated enhanced contractile reactivity of STZ aortas to phenylephrine. The effects of MONT were comparable to or surpassed those brought about by LOS treatment. In conclusion, MONT could offer comparable renoprotective and vasculoprotective effects to LOS in type 1 diabetic rats.

INTRODUCTION

Diabetes is a significant contributor to morbidity and mortality through its associated micro- and macrovascular complications (Dabelea et al., 2017; Graves and Donaghue, 2020). Type 1 diabetes, an autoimmune disease recognized by its early onset in childhood or adolescence, has become more prevalent in recent years (Dabelea, 2018; Hamman et al., 2014). Although diabetic patients currently have increased awareness of the importance of strict glycemic control, chronic vascular complications are still an ongoing burden for type 1 diabetic youths (Dabelea et al., 2017; Libby et al., 2005).

Streptozotocin (STZ) is an antibiotic that causes selective destruction of pancreatic islet β-cells and is widely used to induce type 1 diabetes in experimental animals (Furman, 2021). The STZ-diabetic model mimics the main features of human

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diabetes and its associated vascular complications (Kara et al., 2022; Lim et al., 2022; Wei et al., 2003).

A plethora of mechanisms may underlie diabetic vascular complications. These include, but are not limited to, sustained hyperglycemia, oxidative vascular damage, glycation end products (AGEs) accumulation, diminished bioavailability of vascular nitric oxide (NO), and increased systemic and vascular tissue inflammation (Ceriello, 2006; Devaraj et al., 2007; Graves and Donaghue, 2020; Satoh et al., 2005; Wu et al., 2006).

Patients with type 1 diabetes are extremely vulnerable to microvascular issues such as retinopathy and nephropathy (Libby et al., 2005). Nephropathy can result in end-stage renal disease (ESRD) (Kuhad and Chopra, 2009) and is characterized by elevation of protein and albumin in urine, renal hypertrophy, glomerulosclerosis, decrease in the GFR, and eventually tubulointerstitial fibrosis (Mogensen, 1995; Soldatos and Cooper, 2008).

One of the key causes in the onset and progression of nephropathy, which in turn sets off a number of inflammatory variables that cause renal fibrogenesis, is the activation of the renal renin-angiotensin system (Aggarwal et al., 2017; Giacchetti et al., 2005). Currently, the major goals of employing ACE inhibitors and angiotensin receptor blockers (ARBs) in diabetes are to delay the onset of diabetic nephropathy (Aggarwal et al., 2017; Giacchetti et al., 2005; Ruggenenti et al., 2010).

However, these drugs are not able to provide stable and full renoprotection for diabetic patients (Benigni et al., 2003; Bilous et al., 2009; Mauer et al., 2009). Therefore, more effective alternative therapies for patients with diabetic nephropathy are warranted.

Losartan (LOS) is a potent, orally active, and highly specific ARB drug. It showed protective influences against nephropathy (Manni et al., 2012; Murali and Goyal, 2001; Volpini et al., 2003; Yao et al., 2018) and ameliorated endothelial dysfunction (Ateyya et al., 2018; Sleem et al., 2014) in diabetic rats. LOS also prevented the progression of early diabetic nephropathy, reduced the incidence of ESRD, and improved endothelial function in type 2 diabetic patients (Brenner et al., 2001; Cheetham et al., 2001; Weil et al., 2013).

Montelukast (MONT) is an FDA-approved antiasthmatic drug for children and adolescents. MONT is a leukotriene receptor antagonist that selectively blocks the cysteinyl leukotriene 1 (CysLT1) receptor (Nayak, 2004). Several studies showed that MONT may mitigate experimental tissue injury by diminishing oxidative stress and inflammation (El-Boghday et al., 2017; Khodir et al., 2014; Saad et al., 2014; Said and Bosland, 2016). MONT protected renal tissues against ischemia/reperfusion injury by attenuating oxidative stress and reducing the generation of inflammatory mediators (Sener et al., 2006). Moreover, MONT diminished renal damage in rats with unilateral ureteral obstruction (Otuncemur et al., 2015) and lipopolysaccharide-challenged rats (Khodir et al., 2014) via its antioxidant and anti-inflammatory potential.

Interestingly, MONT prevented early diabetic retinopathy in mice by inhibiting proinflammatory leukotriene generation and superoxide accumulation (Bapputy et al., 2019). However, the renoprotective and vasculoprotective effects of MONT in diabetic rats were not investigated.

In this research, the beneficial effects of MONT on renal and aortic tissues of STZ-diabetic rats were compared to those brought about by the ARB drug Losartan.

**MATERIALS AND METHODS**

**Drugs and chemicals**

MONT was purchased from Sigma Chemicals (St. Louis, MO). LOS was obtained from Amriya Pharmaceutical Industries (Cairo, Egypt). Before giving either medication to rats, it was suspended in 0.5% carboxymethyl cellulose (CMC). STZ and all other chemicals were from Sigma Chemicals.

**Animals**

Male Sprague-Dawley rats (weighing 200–250 g) were placed in a temperature-controlled environment (23°C–2°C) with a 12-hour light–dark cycle. Animals had unrestricted access to food pellets and water. The Mansoura University Faculty of Pharmacy’s Research Ethics Committee approved experimental protocols that complied with national and international NIH standards for using animals in research.

**Experimental protocol**

To induce diabetes, rats were given a single intraperitoneal injection of STZ at a dose of 50 mg/kg in ice-cold saline (Amin et al., 2020). Rats with hyperglycemia levels more than 250 mg/dl 48 hours after STZ treatment were deemed diabetic and enrolled in the study. To lower mortality, insulin (4 IU/kg, subcutaneous, twice weekly) was administered to all diabetic rats. Insulin administration limits excessive hyperglycemia and diminishes extreme body weight loss (Alderson et al., 2004).

Diabetic rats were randomly distributed into three groups (n = 8 each), as follows: STZ, received 0.5% CMC (3 ml/kg/day, orally); STZ-LOS, received LOS (25 mg/kg/day, orally); and STZ-MONT, received MONT (10 mg/kg/day, orally). Drug administration started 2 weeks after the induction of diabetes and continued till the end of the experiments (10 weeks). Doses of LOS and MONT were selected based on former rat studies (Abdel-Reaheim and Khedr, 2014; Gad et al., 2017; Khodir et al., 2014; Manni et al., 2012; Sleem et al., 2014). Age-matched normal rats (n = 8) received the vehicle of drug administration (0.5% CMC, 3 ml/kg/day, orally) and served as the Control group. On day 70, whole blood, serum specimens, and 24-hour urine outputs were obtained from rats. Rats were also euthanized, and the kidney and aorta were taken out, cleaned with ice-cold saline, dried off, and weighed. They were then used for tissue homogenate preparation (1:10 w/v in 0.9% NaCl, pH 7.4) and histopathological examinations. Moreover, vascular contractile responsiveness of isolated aortic rings to PE was assessed. The experimental protocol is summarized in Figure 1.

**Body weight change and kidney mass index**

By deducting each rat’s ultimate body weight (at day 70) from its starting body weight (on day 1), the change in rat weight was computed. To determine kidney mass index, kidney weight was standardized to body weight.

**Glycemia and renal function**

Glycated hemoglobin A\text{\textsubscript{1c}} (HbA\text{\textsubscript{1c}}) levels were assessed in whole blood samples using a kit from Biosystems (Spain). Rat
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urine and/or serum were examined to find the levels of creatinine, urea, albumin, and total albumin using commercial kits from Biodiagnostic (Giza, Egypt). Creatinine and urea clearance, indices of GFR, were determined using serum and urine concentrations of creatinine and urea, respectively, as previously mentioned (Bazzano et al., 2015).

**Oxidative stress parameters**

Superoxide dismutase (SOD) activity levels in tissues were measured spectrophotometrically (Marklund, 1985). Moreover, renal and aortic tissue levels of malondialdehyde (MDA), measured as thiobarbituric acid reactive species (Ohkawa et al., 1979), and reduced glutathione (GSH), assessed as acid-soluble sulfhydryl compounds (Ellman, 1959), were quantified.

**Total nitrite/nitrate (NOx)**

Using a colorimetric kit from R&D Systems (catalog number KGE001, Minneapolis, USA), a commercially available product, tissue NOx concentrations were evaluated. The assay is based on using reductase enzyme to reduce the nitrate content to nitrite. Using the Griess reaction, the total nitrite was measured as a colored azo-dye product that absorbs light at 540 nm (Bories and Bories, 1995).

**Tumor necrosis factor-α (TNF-α) and TGF-β1 levels**

Following the manufacturer’s instructions, the levels of TGF-1 and TNF- in tissue homogenates were assessed using rat ELISA kits (catalog numbers BMS623-3 and KRC3011, respectively, from Thermo Fisher Scientific, MA, USA).

**Aortic ring responsiveness to PE**

Vascular reactivity of isolated aortic rings to PE [10^{-7}–10^{-5} M] was assessed, as previously described (Shawky et al., 2019).

**Histopathological analyses**

Specimens of the kidney and aorta were embedded in paraffin, fixed in 10% buffered formalin, and cut into 4-μm slices. Periodic acid-Schiff (PAS) and/or Masson’s trichrome were then used to stain them. On PAS-stained renal tissues, renal glomerulosclerosis was semiquantitatively graded on a scale from 0 to 4. (Benigni et al., 2003). Furthermore, using ImageJ software, percentage areas of collagen deposition within the glomeruli and interstitium were evaluated on Masson’s trichrome-stained renal sections (National Institutes of Health, USA) (Shang et al., 2013). ImageJ software was also used to determine aortic media thickness.

**Statistics**

The data are shown as means ± SEM. One-way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc tests was used to compare the group means. The histopathological scores were analyzed using Kruskal-Wallis followed by Dunn’s post hoc test. Cumulative concentration-response curves for aortic ring reactivity to PE were fitted using nonlinear regression analysis. GraphPad Prism was used to create all graphs and statistical analyses (San Diego, CA). Statistical significance was set at $p < 0.05$ (Amin et al., 2022; Meyerholz et al., 2019).

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**Figure 1.** An illustration of the experimental protocol. CMC, carboxymethyl cellulose; LOS, Losartan; MONT, montelukast; STZ, streptozotocin.
RESULTS

Body weight, kidney relative weight, and glycemic levels

STZ rats had significantly lower body weights ($p < 0.0001$ vs. control rats). Both LOS and MONT prevented diabetes-induced body weight loss ($p < 0.0001$ vs. STZ group). However, STZ-LOS and STZ-MONT rats still showed significantly lower weight gains than the control group ($p < 0.0001$).

Moreover, STZ rats exhibited significantly higher kidney relative weights than the control group (by 108%, $p < 0.0001$). MONT, but not LOS, significantly diminished diabetic renal hypertrophy (by 23%, $p < 0.01$ vs. untreated STZ group).

Expectedly, there were significant increases in diabetic levels of serum glucose (by 362%, $p < 0.0001$) and glycated HbA$_\text{l,c}$ (by 43%, $p < 0.0001$) when compared to those of control rats. LOS and MONT treatments significantly reduced serum glucose levels by 23.02% ($p < 0.001$) and 19.67% ($p < 0.01$), respectively, relative to untreated STZ glycemic levels. However, MONT achieved better long-term glycemic control than LOS, as indicated by near-normal levels of HbA$_\text{l,c}$ in STZ-MONT rats ($p < 0.0001$ vs. STZ and STZ-LOS groups). These results are shown in Table 1.

Renal function

STZ rats exhibited significantly lower levels of serum albumin (by 24.15%, $p < 0.001$) and significantly higher levels of serum creatinine (by 193%, $p < 0.001$), blood urea (by 133%, $p < 0.0001$), and urinary protein (by 206%, $p < 0.0001$) when compared to control levels. These findings indicate an impaired renal function in STZ-diabetic rats. In line with this, they also showed diminished clearance rates of creatinine (by 64.6%, $p < 0.01$) and urea (by 75.8%, $p < 0.05$) compared to those of control rats.

Both MONT and LOS comparably restored renal function parameters in STZ rats to near-normal levels. These findings are presented in Table 2.

Renal histological changes

To determine the amount of collagen deposition in the study groups’ renal tissues, Masson’s trichrome was used as a stain (Fig. 2A). When compared to control rats, the kidneys of STZ-diabetic animals exhibited interstitial fibrosis, segmental glomerulosclerosis, and vascular tuft atrophy. STZ-LOS kidneys exhibited partially resolved glomerular lesions with residual mild segmental glomerular mesangial expansion and no interstitial fibrosis. On the other hand, STZ-MONT rats demonstrated perfect renal histology with no evidence of glomerular lesions or interstitial fibrosis.

PAS-stained renal tissues are shown in Figure 2B. Focal segmental glomerulosclerosis and shrinkage of the vascular glomerular tuft were also observed in diabetic kidneys. STZ-LOS rats showed normal glomerular histology with mild shrinkage tuft and mild focal tubular atrophy. Renal specimens from the STZ-MONT group showed normal histology with no evidence of glomerular lesions or tubular atrophy.

Quantification of renal fibrosis areas in kidneys and renal glomerulosclerosis scores are presented in Figure 2C and D, respectively.

Renal oxidative status

Chronic STZ diabetes elicited a significant increase in renal MDA (by 59%, $p < 0.001$, Fig. 3A) and significant reductions in renal levels of GSH (by 53.27%, $p < 0.0001$, Fig. 3B). SOD activity (by 68.00%, $p < 0.0001$, Fig. 3C), and NO$_\text{x}$ (by 19.16%, $p < 0.0001$, Fig. 3D) relative to corresponding levels in the control group. Treatment with MONT and LOS attenuated diabetes-induced renal oxidative stress, suggesting the antioxidant properties of both drugs in diabetic rats (Fig. 3A–D).

Renal cytokine levels

Type 1 diabetic rats showed significantly higher concentrations of TNF-α (by 347%, $p < 0.0001$, Fig. 4A) and TGF-β1 (by 128%, $p < 0.0001$, Fig. 4B) than corresponding levels in the control group.

MONT treatment significantly reduced both renal TNF-α (by 38.5%, $p < 0.05$) and TGF-β1 (by 49.3%, $p < 0.0001$) relative to untreated STZ levels. While LOS failed to lessen renal TNF-α ($p > 0.05$ vs. STZ group), it was able to diminish renal TGF-β1 concentrations (by 33.9%, $p < 0.01$) compared to the model STZ group.

Aortic reactivity to PE and NOx levels

STZ aortas exhibited significantly higher responses (Emax values) and enhanced sensitivity (pEC50 values) to PE-induced contractility in comparison to control aortic rings (Fig. 5A). In contrast to the STZ-LOS group, aortic rings from the STZ-MONT group showed significantly diminished Emax of contractile responsiveness to PE compared to those of untreated STZ aortas.

Diabetic aortas showed a significant reduction in NO$_\text{x}$ concentrations (by 53.1%, $p < 0.01$, Fig. 5B) compared to control

Table 1. Effect of MONT treatment (10 mg/kg/day) on body weight, kidney relative weight, and glycemic levels in STZ-diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ</th>
<th>STZ-LOS</th>
<th>STZ-MONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight change (g)</td>
<td>45.13 ± 2.40</td>
<td>−24.38 ± 2.36</td>
<td>8.38 ± 1.36*</td>
<td>5.88 ± 3.28*</td>
</tr>
<tr>
<td>Kidney relative weight</td>
<td>2.98 ± 0.13</td>
<td>6.21 ± 0.31*</td>
<td>5.35 ± 0.21*</td>
<td>4.78 ± 0.39*</td>
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<tr>
<td>× 10^3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood glucose level (mg/dl)</td>
<td>155.50 ± 6.48</td>
<td>718.40 ± 28.25*</td>
<td>553.00 ± 30.09*</td>
<td>577.10 ± 24.67*</td>
</tr>
<tr>
<td>Glycated HbA$_\text{l,c}$</td>
<td>5.05 ± 0.04</td>
<td>7.22 ± 0.06*</td>
<td>6.92 ± 0.12*</td>
<td>5.30 ± 0.33*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM, n = 8 rats/group.

* $p < 0.05$ versus control, STZ, and STZ-LOS groups, respectively.

LOS, Losartan; MONT, STZ, streptozotocin; HbA$_\text{l,c}$, glycated hemoglobin. STZ group refers to the untreated diabetic group.
MONT treatment, but not LOS, was able to restore altered aortic NOx in STZ rats to almost control levels. Aortic oxidative stress

Both MONT and LOS treatments significantly reduced aortic MDA levels in STZ rats (by 72.9% and 69.8%, respectively, \( p < 0.0001 \), Fig. 6A) relative to model STZ levels. Moreover, STZ-MONT and STZ-LOS aortas showed near-normal levels of SOD activity as compared to significantly diminished aortic SOD levels in STZ rats (Fig. 6C). However, MONT, but not LOS, was able to restore aortic GSH to almost control levels (\( p < 0.0001 \) vs. STZ group and \( p < 0.01 \) vs. STZ-LOS group, Fig. 6B).

Aortic medial thickness and TGF-β1 levels

Figure 7A demonstrates PAS-stained aortic sections from the study groups. In contrast to control aortas, STZ aortic walls exhibited structural alterations, including increased thickness of the medial layer (quantified in Fig. 7B) and disorganized appearance of elastic fibers. STZ-MONT rats significantly showed reduced thickness of the medial layer compared to model STZ and STZ-LOS groups (Fig. 7A and B). There were no significant differences in terms of aortic TGF-β1 levels in the experimental groups (Fig. 7C).

DISCUSSION

This work showed that chronic administration of the CysLT1 receptor antagonist MONT to STZ-diabetic rats elicited comparable reno- and vasculoprotective influences to the ARB blocker LOS. Primarily, MONT ameliorated diabetic hyperglycemia and renal functions, diminished renal interstitial fibrosis and glomerulosclerosis, reduced aortic medial thickness, and attenuated aortic hypercontractility to PE in diabetic animals.
These effects are possibly mediated via diminishing renal and aortic tissue oxidative stress and inflammation.

In this research, a rat STZ-diabetic model was used to investigate the effect of MONT on vascular complications. This model is robustly used to investigate the pathogenesis of diabetic vascular complications (Amin et al., 2022; Kara et al., 2022; Lim et al., 2022; Sleem et al., 2014), as it simulates many aspects of human diabetes (Wei et al., 2003). The duration of experimental protocol was selected based on several studies which reported that diabetic rats developed nephropathy and renal fibrosis within 8 weeks following STZ administration (Jia et al., 2019; Mestry et al., 2017). LOS was used as a standard renoprotective agent in this research. LOS showed protective influences against nephropathy (Manni et al., 2012; Murali and Goyal, 2001; Volpini et al., 2003; Yao et al., 2018) and ameliorated endothelial dysfunction (Ateyya et al., 2018; Sleem et al., 2014) in diabetic rats. ARB drugs have been shown to offer significant renal benefits in ameliorating nephropathy and slowing the progression of renal failure in diabetic patients (Wang et al., 2018). LOS also reduced the incidence of ESRD in type 2 diabetic patients (Brenner et al., 2001). MONT and LOS treatments were started 2 weeks following the induction of diabetes to give diabetic kidneys sufficient time...
to recuperate from the modest nephrotoxic effects of STZ, as previously observed (Kraynak et al., 1995).

Both LOS and MONT reduced random blood glucose levels in diabetic animals. However, only MONT treatment was able to significantly reduce glycated HbA\textsubscript{1c} values, an index of chronic hyperglycemia, compared to levels in untreated STZ rats. This may explain, at least in part, why MONT could significantly lessen diabetic relative kidney weight as high blood glucose levels in diabetic rats were associated with increased kidney weight (Rasch and Dorup, 1997). Supporting these findings, LOS administration reduced diabetic hyperglycemia by only ~17% and failed to alter hypoinsulinemia in STZ-diabetic rats (Murad and Goyal, 2001). MONT diminished fasting blood glucose and normalized insulin levels in rats with metabolic syndrome (Ibrahim et al., 2014). A 6-week MONT therapy in type 2 diabetic patients (n = 6) elicited reductions, albeit insignificant, in HbA\textsubscript{1c} compared with baseline levels (Faul et al., 2009). It was reported that MONT promoted glucose-stimulated insulin secretion (GSIS) in a dose-dependent manner in pancreatic MIN6 β-cells (Guo et al., 2018), an effect that might explain the lowering effect of MONT on fasting glycemia and HbA\textsubscript{1c} levels in the present work. Impaired GSIS in β-cells have been associated with STZ-induced diabetes (Delaney et al., 1995).

Renal injury was evident in STZ rats, which showed increased kidney mass index, proteinuria, elevated blood urea, and diminished serum albumin levels compared to control animals. Increased proteinuria is a marker of glomerular damage, which indicates GFR decline (Marques et al., 2022). Reduced urea and creatinine clearances in the STZ group supported altered GFR in diabetic animals (Matboli et al., 2017; Zhang et al., 2017). Both MONT and LOS improved assessed parameters of renal function to comparable levels. In line with these findings, MONT showed renoprotective effects in models of acute kidney injury (Abdel-Raheem and Khedr, 2014; Khodir et al., 2014; Sener et al., 2006).

Oxidative stress is a central contributor to the pathogenesis of diabetic nephropathy. Renal tissues in STZ rats exhibited significantly elevated levels of the lipid peroxidation marker MDA and diminished contents of the antioxidant components, reduced glutathione, and SOD activity. These findings agree with other studies (Qi et al., 2020; Tang et al., 2020). Sustained diabetic hyperglycemia was shown to enhance ROS generation and attenuate antioxidant mechanisms via the glycation of scavenging enzymes (Ha and Kim, 1999). Both MONT and LOS treatments mitigated renal oxidative stress in STZ rats. Previously, MONT enhanced the antioxidant capacity of renal tissues in rats with experimental sepsis (Coskun et al., 2011; Khodir et al., 2014). Moreover, MONT diminished the nephrotoxic effects of methotrexate in rats via the mitigation of renal oxidative stress (Abdel-Raheem and Khedr, 2014). Furthermore, MONT lessened MDA and boosted reduced glutathione levels in renal tissues of rats with renal ischemia/reperfusion injury (Sener et al., 2006). MONT attenuated kidney damage in rats with unilateral ureteral obstruction via its antioxidant effects (Otuncemur et al., 2015). MONT also reduced interleukin II-1β-induced oxidative stress in chondrocytes (Li et al., 2021).

NOx levels were significantly lower in diabetic renal tissues compared to control tissues, which may be attributed to glomerular eNOS uncoupling (Alaofi, 2020; Satoh et al., 2005). NO regulates several vital processes in the kidney, including glomerular and medullary hemodynamics, renal blood flow, GFR, and mesangial matrix accumulation. NO is also involved in the tubuloglomerular feedback response and regulation of the extracellular fluid volume (Kone, 1997). Both LOS and MONT returned renal NOx levels to near the normal range.

Diabetes-associated oxidative damage results in the enhancement of the formation of inflammatory mediators and proinflammatory cytokines (Alaofi, 2020). Renal tissue contents of TNF-α and TGF-β1 were significantly elevated in STZ animals compared with those in control rats. Similar findings were revealed in other studies (Alaofi, 2020; Ko et al., 2008). Inflammatory markers were significantly elevated in type 1 diabetic patients who harbored macrovascular complications (Schram et al., 2003).

TNF-α is cytotoxic to mesangial cells, which directly causes kidney failure (Bertani et al., 1989; Ortiz et al., 1995). TNF-α also boosts the production of ROS which disrupts the glomerular protein permeability barrier (McCarthy et al., 1998). Numerous studies demonstrated that MONT decreased renal TNF-α (Abdel-Raheem and Khedr, 2014; Khodir et al., 2014) and plasma proinflammatory cytokine levels (Sener et al., 2006). TGF-β1, a profibrotic cytokine, stimulates glomerular hypertrophy, proteinuria, and extracellular matrix buildup. Consequently, it is crucial to the emergence and development of diabetic nephropathy (Zhao et al., 2020). MONT was more effective than LOS in lowering increased kidney levels of TNF-α and TGF-β1 in diabetic rats.

Aortas from STZ rats exhibited enhanced responsiveness to PE-induced contractility. Vascular irregularities and endothelial dysfunction are greatly attributed to enhanced oxidative stress and reduced NO bioavailability in diabetic vessels (Ateyaa et al., 2018; Ceriello, 2006; Liu et al., 2014; Satoh et al., 2005). In line with this, the diabetic aorta showed diminished NO bioavailability, reduced antioxidant levels of SOD and GSH, and increased concentrations of MDA compared to those of control animals. Structurally, the medial layer of diabetic aortas showed increased medial thickening and atypical elastic fiber organization, which indicated vascular hypertrophic alterations. Several studies reported similar findings (Baluchnejadmoharadj and Roghani, 2008; Elbe et al., 2014; Fukuda et al., 2005; Jandeleit-Dahm et al., 2000; Sleem et al., 2014; Xavier et al., 2003).

Aortic medial layer thickening is a characteristic aspect of arterial wall remodeling in diabetic patients (Astrand et al., 2007; Frost and Beischer, 1998). It is regarded as an independent risk factor for vascular complications in patients with diabetes (Harrington et al., 2010). Increased vascular smooth muscle cell proliferation is the cause of these structural anomalies in the vessels (Ruiz et al., 2006) as well as a lower medial elastin content (Salum et al., 2014). The indifferent aortic TGF-β1 contents between the groups in this study may be a sign that extracellular matrix deposition in the diabetic aorta has not changed at this stage of the disease’s development as previously reported (Akhtar et al., 2014; Salum et al., 2012).

The abnormal functional alteration in the aortic reactivity to PE and the structural changes in STZ rats was attenuated to a greater extent by MONT than by LOS. MONT was also able to restore aortic NOx levels and showed greater antioxidant activity than LOS on diabetic aortic tissues. Therefore, MONT ability to
ameliorate vascular dysfunction may be related to its effects on NO levels and vascular oxidative stress. Moreover, CysLT1 receptors were reported to mediate vasoconstrictor effects on diabetic aortas (Hardy et al., 2001).

In conclusion, according to the current research, MONT treatment for experimental STZ diabetes resulted in vasculoprotective and renoprotective effects that were comparable to or even greater than those caused by LOS treatment. MONT administration might provide a prospective treatment option for diabetic individuals who do not receive the full benefit of ARB medication for kidney and vascular protection. Multiple mechanisms may mediate MONT effects in the current investigation, including attenuation of inflammation and oxidative stress. Glycemic management is improved, and diabetic rats’ kidney and blood arteries directly benefit. It should be investigated in the future whether MONT-mediated effects also occur in people and other disease models.

AUTHOR CONTRIBUTIONS
All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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The protocol was approved by the Research Ethics Committee at Dubai Pharmacy College for Girls, Dubai, United Arab Emirates (Reference # FEC/FD/2020/02).

CONFLICTS OF INTEREST
The authors declared they have no conflicts of interest.

DATA AVAILABILITY
All data generated and analyzed are included in this research article.

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