Original Article

Evaluation of Effect of Montelukast in the Model of Streptozotocin Induced Diabetic Nephropathy in Rats

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Abstract

Background: Diabetic nephropathy is a progressive condition and a leading cause of end-stage renal disease. Oxidative stress and inflammation play an important role in its pathogenesis. In pre-clinical studies, Montelukast had shown renoprotective and anti-oxidant properties, hence the study was planned to evaluate the effect of Montelukast in a Streptozotocin (STZ) induced model of diabetic nephropathy. **Methods:** 40 Wistar rats of either sex were randomly divided into four groups *viz*. 1. Vehicle control group, 2. Enalapril (5 mg/kg), 3. Montelukast low-dose (10 mg/kg) and 4. High-dose (20 mg/kg) group. On day 1, diabetes was induced using a single dose of STZ (60 mg/kg) intraperitoneally. Diabetes induction was verified based on fasting blood glucose (FBG) levels on day 7 and from day 8 to day 42, rats were given study drugs. FBG, serum creatinine, blood urea nitrogen (BUN) and urine microalbumin levels were assessed pre-study and post-study. Assessments of kidney malondialdehyde (MDA), reduced glutathione (GSH) and renal histopathology were carried out at the end of the study. **Results:** Montelukast 10 mg/kg group showed significantly lower urine microalbumin levels compared to the vehicle control group ($p < 0.05$). Montelukast 20 mg/kg group showed significantly lower levels of FBG, serum creatinine, BUN and urine microalbumin compared to the vehicle control group ($p < 0.05$). In addition, Montelukast 20 mg/kg group also showed better effects on kidney MDA and GSH levels ($p < 0.05$) and histopathological scores compared to the vehicle control group. **Conclusion:** Montelukast showed a protective effect in the model of diabetic nephropathy because of its antioxidant effect.

Keywords: Diabetic mellitus, microalbuminuria, montelukast, oxidative stress, renoprotection

Introduction

Diabetic nephropathy (DN) is an important microvascular complication of type 1 as well as type 2 diabetes mellitus. It is estimated that more than 40% of people with diabetes mellitus develop chronic kidney disease and end-stage renal disease (ESRD).[1,2] A population-based study conducted in India (2002-2005) showed that DN accounted for approximately 44% of cases of ESRD.[3] Risk factors associated with DN include genetic susceptibility, polyol pathway activation, renin-angiotensin system activation, reactive oxygen species (ROS), activation of the protein kinase C pathway, increased advanced glycation end-products (AGE) and glomerular hyperfiltration.[4,5] There may be no signs or symptoms in the early stages of DN. However, in the late stages, we may observe alterations in blood pressure, fluid imbalance, elevated urine albumin excretion and reduced glomerular filtration rate as a result of persistently elevated blood glucose levels.[6] Current treatment options for patients with DN having microalbuminuria are angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) which are used alongside antidiabetic drugs for glycemic control. Management of ESRD includes dialysis and renal transplant.[7] Even with these treatments, the risk of DN progression remains high. There is currently no approved drug that can prevent or slow the progression of DN.[2] There are several drugs which have shown beneficial effects in animal studies but have failed in clinical trials either due to lack of efficacy or due to safety concerns. Because of the unmet need, it was of interest to explore newer agents in this therapy area.[8]

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Persistent hyperglycemia and the formation of AGEs in diabetes mellitus lead to the release of monocyte chemo-attractant protein (MCP-1) from renal parenchyma which attracts monocytes into kidneys and causes monocyte activation and differentiation. Activated monocytes then release ROS, proinflammatory cytokines like interleukins (IL-1, IL-6, IL-18) and tumour necrosis factor (TNF-α). These inflammatory cytokines alongside ROS cause renal parenchymal cellular apoptosis and necrosis. This pathophysiological pathway driven by oxidative damage and inflammation are new targets for exploring the effects of novel agents.[9-11]

Montelukast is an FDA-approved drug used for the prevention and treatment of asthma, exercise-induced bronchoconstriction and allergic rhinitis.[12] It blocks the action of cysteinyl leukotrienes—LTC4, LTD4 and LTE4 on cysteinyl leukotriene receptor cysLT1.[13] Montelukast has been shown to reduce MCP-1 expression and to possess anti-oxidant properties in previous experimental studies.[14,15] It has also been shown to have promising renoprotective effects in various animal models of nephrotoxicity.[16,17]

Thus, we decided to investigate the effect of Montelukast in an experimental model of diabetic nephropathy with the objectives to evaluate its renoprotective effect and explore the mechanism of action using oxidative stress markers.

Material and Methods

The permission of the institutional animal ethics committee was obtained before the initiation of the study (Reference approval number- AEC/15/2017). The animals bred in the Central Animal House of the institute [registered under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) which is a statutory Committee under the Government Ministry] were used and the study was conducted according to CPCSEA guidelines.

Experimental animals: The study was carried out in Wistar rats of either sex, each weighing between 150 and 250 grams. The rats were housed in polypropylene cages with stainless steel top grills having facilities for providing food and water. The rats had free access to UV-filtered water and food which was administered in the form of pellets. Paddy husk was used as the bedding in the cages. Regulated conditions were maintained with temperature 23 ± 4°C, humidity 30%–70% and 12-hour light-dark cycles.

Study drugs and chemicals: Disease-inducing chemical agent Streptozotocin (STZ) (Product number-572201), study drugs Montelukast (Product number-PHR-1603-1G) and Enalapril (Product number-PHR-1603-1G) were purchased from Sigma-Aldrich. STZ was administered intraperitoneally at a dose of 60 mg/kg in 0.1 M sodium citrate buffer (pH-4.5). Montelukast and Enalapril were given orally mixed in normal saline (0.9%) and 0.5% carboxy methyl cellulose, respectively. Two doses of Montelukast that is 10 mg/kg and 20 mg/kg[16-18] were used as a test drug and Enalapril 5 mg/kg was used as a positive control.[19,20]

Study procedure:[21] Forty Wistar rats of the required weight range were randomly allocated to four groups each group containing ten rats. The experimental study groups were: Vehicle control (STZ + Normal saline), Enalapril group (STZ + Enalapril 5 mg/kg), low-dose group (STZ + Montelukast 10 mg/kg) and high-dose group (STZ + Montelukast 20 mg/kg). The STZ-induced diabetic nephropathy model employed in this study had previously been standardised in identical lab settings.[19] Rats were kept fasting overnight in metabolic cages, the day before initiation of the study. Blood was collected from all rats from the retro-orbital plexus using non-heparinized micro-hematocrit capillary tubes and the samples were used for baseline estimation of fasting blood glucose (FBG), serum creatinine and blood urea nitrogen (BUN). Also, 24-hours urine samples were collected from metabolic cages to measure urine microalbumin levels. Then rats were housed according to the groups and the cages were labeled. On day 1, all rats were administered STZ 60 mg/kg single dose intraperitoneally and on day 7, FBG levels were assessed to confirm the induction of diabetes (levels above 150 mg/dL). The study drugs that is Montelukast and Enalapril were started as once-daily oral gavage using a rat-feeding needle from day 8 to day 42. At the end of the study, blood was collected from all the rats from the retro-orbital plexus to evaluate FBG, creatinine and BUN levels, and urine collection over 24-hours was done for urine microalbumin levels. After the collection of blood and urine samples, the rats were sacrificed. Exploratory laparotomy was performed and both the kidneys of the rats were dissected The kidneys were washed in cold saline and dried with the help of filter paper and immediately weighed on a digital weighing balance and the volume of the kidney was measured using the displacement method. One gram of kidney tissue was transferred to a glass bulb filled with 9 ml of cold phosphate buffer solution (PBS). The kidney tissue samples in the PBS glass bulbs were homogenised using homogenizer apparatus under constant motor speed. The entire process for homogenisation was carried out under strict cold chain control of the samples. Following the homogenisation, samples were processed for estimation of the kidney tissue malondialdehyde (MDA) and reduced glutathione (GSH) levels. The remaining second kidney was placed in a glass bulb filled with 10% neutral buffered formalin which was further processed for histopathological analysis.

Biochemical analysis: FBG, serum creatinine and BUN were assessed using an automated analyser. Urine microalbumin levels and MDA-GSH levels in kidney tissue were measured using double antibody sandwich enzyme-linked immunosorbent assay (ELISA) method. MDA and GSH levels were measured to quantify the amount of oxidative stress induced by STZ. ELISA kits were purchased from Kinesis Dx, Los Angeles, USA.

Histopathological examination of kidneys: Kidneys in the formalin glass bulbs were sent to the laboratory for preparation of the slides and the slides were stained using

haematoxylin and eosin. The slides were examined by a trained and experienced pathologist. Based on the current knowledge regarding histopathological changes that occur in STZ-induced diabetic nephropathy in rats, we devised a scoring system in consultation with the pathologist as shown in Table 1. This scoring was indirectly based on a study by Ozdemir O *et al.*[22]

Statistical analysis: Data from each study group were compiled and were expressed as Mean ± standard deviation (SD). The data were analysed using GraphPad InStat version 3.0. The level of significance was set at $P < 0.05$. Normality was checked by Shapiro-Wilk test. The study variables (except histopathological examination) were analysed using one-way ANOVA followed by post hoc Tukey's test. The scoring of histopathological findings was expressed as median and range.

Results

Out of 40 Wistar rats at the beginning of the experiments, 29 rats survived through the six weeks of the study period. Four rats died in the vehicle group, three in the low-dose group and two in each of the Enalapril and high-dose groups.

The baseline mean body weights of the rats were comparable among the groups. At the end of six weeks, though all groups showed weight gain only Enalapril and high-dose Montelukast group illustrated statistical significance compared to baseline as shown in Table 2.

Table 2 also shows that the kidney weight across the groups was similar. Although it was slightly higher in the Enalapril group compared to the vehicle as well as the Montelukast groups, the difference was not statistically significant. The volume of the kidney was significantly higher in the Enalapril group when compared to the vehicle control group ($p < 0.05$). The mean volume of the kidneys in both the Montelukast groups was observed to be slightly higher than the vehicle control group but comparable to the Enalapril group. Baseline FBG levels amongst all four groups were comparable and within normal limits (50–125 mg/dL).[23] At the end of six weeks, FBG levels were raised in all the groups, however, both the Enalapril and high-dose Montelukast group showed significantly lower levels compared to the vehicle control group as seen in Table 3.

Table 4 shows that baseline serum creatinine levels across the four groups were comparable and within the normal range (≤ 0.50 ± 0.07 mg/dL)[24] and at day 42, serum creatinine was significantly elevated in all the groups. However, the rise in creatinine levels was significantly less in the Enalapril group ($p < 0.001$) and high-dose Montelukast group ($p < 0.01$) when compared statistically with the vehicle control group. Similarly, mean BUN levels at day 0 were comparable across all the groups and at the end of the study, there was a rise in mean BUN levels in all the groups. The rise in the BUN levels was significantly less in the Enalapril group and high-dose Montelukast group when compared to the vehicle control group. Results between the Enalapril group and the high-dose Montelukast group were statistically comparable in terms of end of study FBG, creatinine and BUN levels.

Figure 1 depicts baseline mean urine microalbumin levels were comparable amongst all groups and at the end of the study, there was a significant rise in urine microalbumin levels in vehicle control, Enalapril and low-dose Montelukast group from the baseline. In high dose Montelukast group, the rise in urine microalbumin levels between baseline (1.29 ± 0.39) and day 42 (1.54 ± 0.56) is not statistically significant. At the end of the study, Enalapril (0.95 ± 0.39), low dose (3.46 ± 0.78) and high dose Montelukast (1.54 ± 0.56) groups showed significantly lesser urine microalbumin levels compared to the vehicle control group (4.46 ± 0.84). Also, mean urine microalbumin levels observed in the high-dose Montelukast group were comparable to the Enalapril group.

Biochemical analysis of rat kidney tissue samples for oxidative markers showed lesser MDA levels and higher GSH levels in Enalapril (MDA 4.49 ± 1.69, GSH 137.24 ± 31.76)

Table 1: Scoring system for histopathological examination of kidneys

Score	Description
0	No light microscopy changes
1	Minimal changes, >5 and <10 tubules
2	Mild changes, >10 and <15 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules
3	Moderate changes, >15 and <20 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules
4	Severe changes, >20 tubules in 5 LPF with vacuolar degeneration and cystic dilatation of tubules OR mesangial expansion

Table 2: Baseline (day 0) and end of study (day 42) rat body weights (grams) mean kidney weights (grams/100 gm body wt.) and kidney volumes (ml)

Groups	Rat body weight		Kidney weight	Kidney volume
	Day 0	Day 42		
Vehicle control	177.33±23.21	186±12.16	0.61±0.11	2.30±0.26
Enalapril	175.75±20.44	211.12±22.52*	0.72±0.12	2.79±0.28#
Low-dose Montelukast (10 mg/kg)	177.43±22.80	189.29±11.63	0.67±0.15	2.47±0.42
High-dose Montelukast (20 mg/kg)	185.63±18.37	209±14.28*	0.67±0.07	2.74±0.32

Values expressed as Mean±SD. *P<0.05 compared to mean body weight of day 0 using paired t-test. #P<0.05 compared to kidney volume in vehicle group using one-way ANOVA with post hoc Tukey's test

and high-dose Montelukast group (MDA 6.12 ± 2.69, GSH 126.64 ± 16.19) compared to vehicle control (MDA 14.03 ± 4.12, GSH 89.01 ± 15.07) and the difference was statistically significant (as shown in Figure 2). Low-dose Montelukast failed to offer significant protection against oxidative damage as suggested by the marker levels (MDA 11 ± 3, GSH 101.82 ± 15.36).

Histopathological examination of rat kidneys

Histopathology grades given to kidneys are expressed as median and range as shown in Table 5. The median score that was observed in the vehicle control (range 1-2) and low-dose Montelukast (range 0-2) group was 2 indicating vacuolar degeneration of epithelial cells and cystic dilatation of tubules (as shown by arrows in Figure 3) which was observed in both the groups. While Enalapril and the high-dose Montelukast group showed a median score of 0 (range 0-1) indicating near normal kidney histology with minimal changes.

Discussion

As per the current understanding of the pathophysiology of DN, oxidative stress and release of inflammatory cytokines caused due to persistently high blood glucose levels and the formation of AGEs lead to renal parenchymal damage. Montelukast in previous experimental studies had shown to reduce cytokines expression and to possess anti-oxidant properties.[14,15] Further, it has been demonstrated to have a protective effect in gentamicin and amikacin-induced nephrotoxicity models.[16,17] Recent studies published by Bapputty R *et al.*[25] and Pham B *et al.*[26] showed the potential role of Montelukast in preventing diabetic retinopathy which is also an important microvascular complication seen in long-standing diabetes by reducing proinflammatory leukotriene generation and superoxide accumulation. The available data on the antioxidant and renoprotective potential of Montelukast from the previous studies were compelling and hence, it was decided to test its effect in the DN model in the present study. Two doses (10 mg/kg and 20 mg/kg) of Montelukast were chosen for this study based on the previous preclinical studies.[16-18] Since ACE inhibitors are a class of drugs currently used clinically in the management of DN, Enalapril was selected as the positive control.[13,19,20]

The STZ-induced model of diabetes is known to be associated with 30%–40% mortality, especially in the case of a single high-dose STZ model.[27,28] In the present study, out of 40 Wistar rats, 11 (27.5%) died due to STZ toxicity. Higher survival rates (80%) were observed in the Enalapril and the high-dose Montelukast groups, respectively.

The baseline mean body weights of the rats were comparable across the groups. The weight gain over a six weeks period was the least in the vehicle control group, reflecting the weight loss due to the induction of diabetes. Previous preclinical studies evaluating renoprotective drugs have also illustrated a marked reduction in mean body weight in STZ-treated diabetic rats.[29,30] Possible mechanism for weight loss in diabetic rats is excessive loss of muscle mass because of tissue protein catabolism in a state of hyperglycemia. At the end of six weeks, though all the groups showed weight gain, a statistically significant increase in weight was illustrated in the Enalapril and high-dose Montelukast groups only and it

Table 3: Fasting blood glucose (mg/dL) at day 0 and day 42

Groups	Baseline values (day 0)	End of study values (day 42)
Vehicle control	81±7.62	271.17±19.47
Enalapril	82.37±8.38	210.38±22.49*
Low-dose Montelukast (10 mg/kg)	85.14±9.32	244.86±36.03
High-dose Montelukast (20 mg/kg)	80.13±8.35	216.88±15.96*

Values expressed as Mean±SD. *P<0.05 compared to vehicle group one-way ANOVA with Tukey's post-test

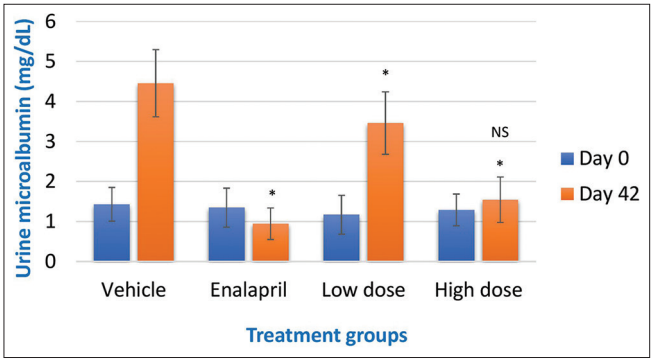

Figure 1: Urine Microalbumin (mg/dL) at Day 0 and Day 42. Values expressed as Mean ± SD. *P<0.05 compared to vehicle group using one-way ANOVA with Tukey's post test. NS: Not significant compared to urine microalbumin levels at day 0 using paired t-test

Table 4: Comparison of serum creatinine levels (mg/dL) and blood urea nitrogen (mg/dL) at day 0 and day 42

Groups	Serum creatinine		Blood urea nitrogen	
	Day 0	Day 42	Day 0	Day 42
Vehicle control	0.33±0.08	0.92±0.19	18.16±3.10	28.19±2.77
Enalapril	0.36±0.07	0.55±0.13@	18.19±2.55	19.72±2.38*
Low-dose Montelukast (10 mg/kg)	0.36±0.07	0.83±0.22	19.60±3.52	24.40±4.66
High-dose Montelukast (20 mg/kg)	0.35±0.09	0.57±0.15@	17.92±3.51	20.02±3.09*

Values expressed as Mean±SD. @P<0.01 compared to vehicle group, using one-way ANOVA with Tukey's post-test. *P<0.001 compared to vehicle group, using one-way ANOVA with Tukey's post-test

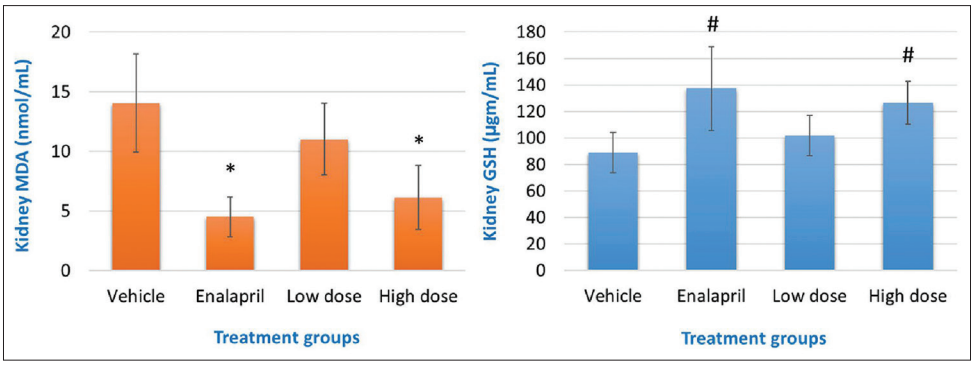

Figure 2: Kidney MDA (nmol/mL) and GSH (μgm/mL). Values expressed as Mean ± SD. *$P<0.001$ compared to mean kidney MDA level in vehicle group and #$P<0.05$ compared to mean kidney GSH level in vehicle group using one-way ANOVA with Tukey's post-test

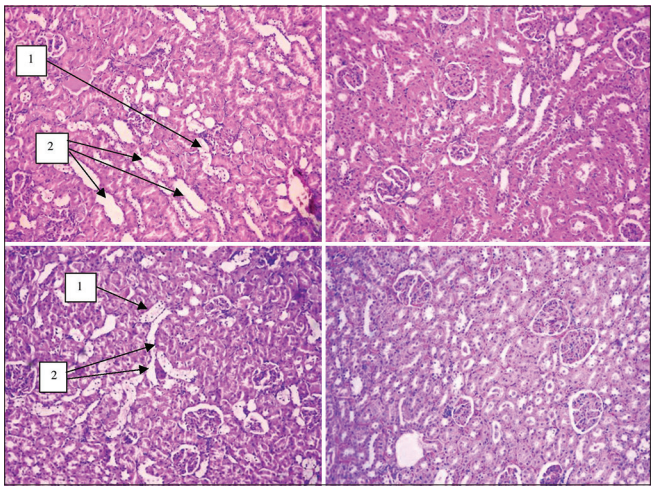

Figure 3: Histopathological Section of Kidney (Image: H&E stain under 100x magnification). Study groups: Vehicle control (top left), Enalapril (top right), Low dose Montelukast (10 mg/kg) (bottom left), High dose Montelukast (20 mg/kg) (bottom right), Histopathological changes - 1: Vacuolar degeneration, 2: Cystic dilatation of tubules

was comparable. Also, there was a significantly higher mean body weight in the Enalapril treatment group compared to the vehicle control group. It is observed by Guneli E *et al.*[31] and Andallu B and Varadacharyulu NC[32] that drugs with free radical-scavenging properties protect against weight loss in diabetic rats.

There have been mixed reports in previous preclinical studies regarding the effect of diabetes on organs like kidneys in terms of weight and volume. Some researchers reported a decrease in weight, while others have reported an increase or no significant change in kidney weight.[33-35] In our study, kidney volume and weight were higher in the Enalapril group compared to the vehicle control group. However, there was no statistically significant difference in mean kidney weights among the different groups.

Various preclinical studies using the STZ model which evaluated the renoprotective effect of experimental agents have used the standard biochemical tests *viz.* FBG, BUN, serum creatinine and urine microalbumin.[21,35-43] In our study, baseline

Table 5: Histopathological examination of kidney

Groups	Histopathological grades: Median (range)
Vehicle control	2 (1-2)
Enalapril	0 (0-1)
Low-dose Montelukast (10 mg/kg)	2 (0-2)
High-dose Montelukast (20 mg/kg)	0 (0-1)

FBG levels of the rats were found to be comparable across all the groups and were within the normal range. There was a dramatic rise in FBG after one week of STZ administration indicating successful induction of diabetes. The cut-off level of FBG was kept at 150 mg/dL based on the previous preclinical studies.[21,44] At the end of six weeks, FBG levels increased in all the groups. There was a significant difference in blood glucose levels in the Enalapril group and high-dose Montelukast (20 mg/kg) group compared to the vehicle control indicating their protective action against diabetes. Also, FBG levels in the Enalapril group were comparable to both doses of Montelukast. Enalapril in previous studies also has been reported to lower blood glucose levels by improving insulin signalling and insulin sensitivity caused by RAAS disruption.[45-47]

Similarly, at baseline, serum creatinine and BUN levels in all four groups were comparable and within the normal range.[24] At the end of the study, there was a significant increase in the levels of these variables suggesting the induction of nephropathy due to sustained high blood glucose levels. In the advanced stages of nephropathy, there is a fall in GFR caused by constriction of glomerular arterioles and mesangial expansion. These changes lead to a rise in serum creatinine and BUN levels.[48] In our study, serum creatinine and BUN levels had increased at the end of the study compared to baseline. A study by Kundu A *et al.*[38] also showed a marked rise in creatinine and BUN levels in their three weeks model of STZ-induced DN. Enalapril and high-dose group showed the least rise in serum creatinine and BUN levels among all the groups and it was statistically significant when compared to the vehicle control group. Both the groups of Montelukast showed dose-dependent effects on biochemical variables with

high-dose Montelukast showing a comparable effect to the Enalapril group.

Development of microalbuminuria is one of the important clinical feature and earliest signs of DN.[49] Microalbuminuria in STZ induced diabetes model is caused by glomerular basement membrane thickening, destruction of podocytes and increased intracellular spaces.[33] Earlier preclinical studies screening renoprotective agents in STZ-induced DN have also used urine microalbumin levels as a variable for detecting nephropathy.[37-40,43,44] Baseline albuminuria in Wistar rats according to previous studies lies between 0 and 1.5 mg/24 hr.[50,51] No formal grading or classification is available correlating the level of albuminuria and the severity of renal injury. In a preclinical study conducted by Bahaa Al-Trad *et al.*[52] reported that a reduction in podocytes and reduced expression of mRNA and proteins related to nephrin and podocin led to albuminuria after a single dose of STZ treatment. A study conducted by Dubey VK *et al.*[35] showed significant albuminuria after four weeks of administration of STZ. In our study too, the vehicle control group showed higher microalbuminuria and Enalapril as well as both the Montelukast groups offered protection against it. The dose-dependent renoprotective effect was seen with Montelukast and the results of the high-dose group matched with those of the Enalapril group. Also, the rise in urine microalbumin levels from baseline till six weeks was significant in all the groups except in the high-dose montelukast group, indicating its renoprotective role in the early stages of DN.

Various studies, evaluating the anti-oxidant property of novel agents in STZ-induced DN have previously used antioxidants like superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO) alongside MDA and GSH for estimation of oxidative stress.[37,38,42,53] In above-mentioned studies, alleviation of oxidative stress was interpreted in terms of the ability to raise anti-oxidant levels and decrease MDA levels. In our study, mean kidney MDA levels were significantly lesser in the Enalapril and the high-dose Montelukast group compared to the vehicle control. The mean kidney GSH levels also showed higher values in the Enalapril and high-dose Montelukast groups demonstrating anti-oxidant potential.

The gold standard for diagnosis of DN is renal biopsy. Zhao Y *et al.*[36] have reported that diabetic rats showed increased matrix in the mesangium and vacuolar degeneration of glomerular epithelial cells after 28 days of STZ administration. Other preclinical studies by Zhou X *et al.*,[42] Han H *et al.*,[41] Elbe H *et al.*[29] and Yuan H *et al.*[43] reported similar histopathological results showing glomerular basement membrane thickening, vacuolar degeneration and renal tubular damage. In the present study, a median score of 2 was observed in the vehicle control group (range 1-2) and low-dose Montelukast (range 0-2) group indicating vacuolar degeneration of epithelial cells and cystic dilatation of tubules in the kidney. On the other hand, the Enalapril and high-dose Montelukast group showed median score of 0 (range 0-1) indicating near-normal kidney histology with minimal changes. Thus, Enalapril and high-dose Montelukast preserved the renal architecture in the face of high glucose levels. The finding is interesting and future research should be directed to confirm whether Montelukast would be useful as a prophylactic agent to prevent DN.

In vitro studies have proved that at high concentrations of glucose, proteoglycans, fibronectin, type-4 collagen, and laminin levels as well as TGF-β expression are increased. TGF-β inhibits collagenase production and leads to the accumulation of extracellular matrix (ECM) proteins in glomerular and tubular basal membranes by preventing its degradation.[41,54] In light of these studies, it will be worthwhile to evaluate the effect of Montelukast on TGF-β expression in future studies.

The findings of this study are promising since the renoprotective effects of the higher dose Montelukast group were not inferior to Enalapril which was considered the treatment of choice for patients with diabetes and elevated urinary albumin excretion (30–299 mg/day) according to clinical practice guidelines.[55] However, the interpretations of the results and inferences drawn from the present study should be considered given certain limitations as only the anti-oxidant action of montelukast was probed in the present study. The possible effect of Montelukast on inflammatory markers like TGF-β and MCP-1 (which may have contributed to its protective action) was not addressed in this study. The STZ model was selected in this study as a screening test. It is more useful as a model of early changes in diabetic nephropathy as it fails to induce hypertension and advanced renal pathological features like severe mesangial matrix accumulation, nodular lesions in glomeruli and severe tubular cell damage.[56] Also, the limitation of our study is the non-inclusion of a nondiabetic normal control group which would have been better for the overall interpretation of results.

Conclusion

In the present study, Montelukast showed a potential renoprotective effect as evident from the assessment of renal biochemical and histopathological variables in STZ-induced diabetic nephropathy. The protective effect was mediated by its anti-oxidant property and it needs to be explored further.

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Conflicts of interest

There are no conflicts of interest.

References

1. American Diabetes Association. Standards of medical care in diabetes-2014. Diabetes Care 2014;37(Suppl 1):S14-80.
2. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. Clin J Am Soc Nephrol 2017;12:2032-45.
3. Modi GK, Jha V. The incidence of end stage renal disease in India: A population-based study. Kidney Int 2006;70:2131-3.

4. Raile K, Galler A, Hofer S, Herbst A, Dunstheimer D, Busch P, *et al*. Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes: Effect of diabetes duration, A1C, hypertension, dyslipidemia, diabetes onset and sex. Diabetes Care 2007;30:2523–8.
5. Eberhard R. Diabetic nephropathy. Saudi J Kidney Dis Transplant 2006;17:481–90.
6. Nazar CM. Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease. J Nephropharmacol 2014;3:15-20.
7. Longo DL, Fauci AS, Kasper DL, Houser SL, Jamson J, Loscalzo J. Harrison's principle of internal medicine. 2015;19:2422-6.
8. Perez-Gomez MV, Sanchez-Niño MD, Sanz AB, Martín-Cleary C, Ruiz-Ortega M, Egido J, *et al*. Horizon 2020 in diabetic kidney disease: The clinical trial pipeline for add-on therapies on top of renin angiotensin system blockade. J Clin Med 2015;4:1325-47.
9. Ritz E. Limitations and future treatment options in type 2 diabetes with renal impairment. Diabetes Care 2011;34(Supplement 2):S330-4.
10. Banba N, Nakamura T, Matsumura M, Kuroda H, Hattori Y, Kasai K. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney Int 2000;58:684-90.
11. Tesch GH. MCP-1/CCL2: A new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. Am J Physiol Renal Physiol 2008;294:F697-701.
12. Paggiaro P, Bacci E. Montelukast in asthma: A review of its efficacy and place in therapy. Ther Adv Chronic Dis 2011;2:47-58.
13. Brunton L, Knollman B, Hilal-Dandan R. Goodman & Gillman's The Pharmacological Basis of Therapeutics, 13th Edition. New York, United States: Mc Graw- Hill Education- Europe; 2018.
14. Ge S, Zhou G, Cheng S, Liu D, Xu J, Xu G, *et al*. Anti-atherogenic effects of montelukast associated with reduced MCP-1 expression in a rabbit carotid balloon injury model. Atherosclerosis 2009;205:74-9.
15. Mohamadin AM, Elberry AA, Elkablawy MA, Gawad HS, Al-Abbasi FA. Montelukast, a leukotriene receptor antagonist abrogates lipopolysaccharide-induced toxicity and oxidative stress in rat liver. Pathophysiology 2011;18:235-42.
16. Otunctemur A, Ozbek E, Cekmen M, Cakir SS, Dursun M, Polat EC, *et al*. Protective effect of montelukast which is cysteinyl-leukotriene receptor antagonist on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney. Ren Fail 2013;35:403-10.
17. Kose E, Beytur A, Dogan Z, Ekincioglu Z, Vardi N, Cinar K, *et al*. The effects of montelukast against amikacin-induced acute renal damage. Eur Rev Med Pharmacol Sci 2012;16:503-11.
18. Gad AM, El-Raouf OM, El-Sayeh BM, Fawzy HM, Abdallah DM. Renoprotective effects of montelukast in an experimental model of cisplatin nephrotoxicity in rats. J Biochem Mol Toxicol 2017;31:e21979.
19. Bhide SS, Maurya MR, Gajbhiye SV, Tadavi FM. Evaluation of nephroprotective effect of Bryonia lacinosa on streptozotocin induced diabetic nephropathy in rats. Int J Basic Clin Pharmacol 2017;6:1193.
20. Karadeniz T, Cavusoğlu T, Turkmen E, Uyanıkgil Y, Karadeniz M, Akdemir O, *et al*. Experimental comparison of protective characteristics of enalapril and trimetazidine in diabetic nephropathy. Ren Fail 2014;36:1283-90.
21. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol 2015;70:5.47.1-20. doi: 10.1002/0471141755.ph 0547s70.
22. Ozdemir O, Akalin PP, Baspinar N, Hatipoglu F. Pathological changes in the acute phase of streptozotocin-induced diabetic rats. Bull Vet Inst Pulawy 2009;53:783-90.
23. Brăslaşu ED, Brădălan CĂ, Cornilă M, Săvulescu I, Cojmăleală RO, Brăslaşu MC. Normal blood glucose in white wistar rat and its changes following anesthesia. Lucr Ştiinłifice Med Vet 2007;XL: 120-3.
24. Castro BB, Colugnati FA, Cenedeze MA, Suassuna PG, Pinheiro HS. Standardization of renal function evaluation in Wistar rats (Rattus norvegicus)from the Federal University of Juiz de Fora's colony. J Bras Nefrol 2014;36:139-49.
25. Bapputty R, Talahalli R, Zarini S, Samuels I, Murphy R, Gubitosi-Klug R. Montelukast prevents early diabetic retinopathy in mice. Diabetes 2019;68:2004-15.
26. Pham B, Matsumiya W, Akhavanrezayat A, Uludag G, Yasar C, Ghoraba H, *et al*. Oral montelukast is associated with decreased odds of diabetic retinopathy. Invest Ophthalmol Vis Sci 2021;62:1152.
27. Deeds MC, Anderson JM, Armstrong AS, Gastineau DA, Hiddinga HJ, Jahangir A, *et al*. Single dose streptozotocin-induced diabetes: Considerations for study design in islet transplantation models. Lab Anim 2011;45:131-40.
28. Wang YJ, Xie XS, Feng SG, Long QX, Ai N, Wang BF. Causes of death in STZ-induced rat models of diabetes mellitus. Sichuan Da Xue Xue Bao Yi Xue Ban 2014;45:691-5.
29. Elbe H, Vardi N, Esrefoglu M, Ates B, Yologlu S, Taskapan C. Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats. Hum Exp Toxicol 2015;34:100-13.
30. Hasanvand A, Amini-Khoei H, Jahanabadi S, Mehr SE, Dehpour AR. Metformin attenuates streptozotocin-induced diabetic nephropathy in rats through activation of AMPK signaling pathway. J Nephropathol 2018;7:37-42.
31. Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S, Uysal N. Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur Surg Res 2008;40:354-60.
32. Andallu B, Varadacharyulu NC. Antioxidant role of mulberry (Morus indica L. cv. Anantha) leaves in streptozotocin-diabetic rats. Clin Chim Acta 2003;338:3-10.
33. Coldiron AD Jr, Sanders RA, Watkins JB III. Effects of combined quercetin and coenzyme Q10 treatment on oxidative stress in normal and diabetic rats. J Biochem Mol Toxicol 2002;16:197-202.
34. Garman JH, Mulroney S, Manigrasso M, Flynn E, Maric C. Omega-3 fatty acid rich diet prevents diabetic renal disease. Am J Physiol-Renal Physiol 2009;296:F306-16.
35. Dubey VK, Patil CR, Kamble SM, Tidke PS, Patil KR, Maniya PJ, *et al*. Oleanolic acid prevents progression of streptozotocin induced diabetic nephropathy and protects renal microstructures in Sprague Dawley rats. J Pharmacol Pharmacother 2013;4:47-52.
36. Zhao Y, Huang W, Wang J, Chen Y, Huang W, Zhu Y. Taxifolin attenuates diabetic nephropathy in streptozotocin-induced diabetic rats. Am J Transl Res 2018;10:1205-10.
37. Malik S, Suchal K, Khan SI, Bhatia J, Kishore K, Dinda AK, *et al*. Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF-κB-TNF-α and TGF-β1-MAPK-fibronectin pathways. Am J Physiol-Renal Physiol 2017;313:F414-22.
38. Kundu A, Dey P, Sarkar P, Karmakar S, Tae IH, Kim KS, *et al*. Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy. Food Chem Toxicol 2020;135:110873.
39. Matboli M, Eissa S, Ibrahim D, Hegazy MG, Imam SS, Habib EK. Caffeic acid attenuates diabetic kidney disease via modulation of autophagy in a high-fat diet/streptozotocin-induced diabetic rat. Sci Rep 2017;7:2263.
40. Wu Z, Xie Z, Liu J, Wu Q, Wang X. Renoprotective effect of berberine on streptozotocin-induced diabetic nephropathy rats. Int J Pharmacol 2017;13:247-56.
41. Han H, Cao A, Wang L, Guo H, Zang Y, Li Z, *et al*. Huangqi decoction ameliorates streptozotocin-induced rat diabetic nephropathy through antioxidant and regulation of the TGF-β/MAPK/PPAR-γ signaling. Cell Physiol Biochem 2017;42:1934-44.
42. Zhou X, Feng Y, Zhan Z, Chen J. Hydrogen sulfide alleviates diabetic nephropathy in a streptozotocin-induced diabetic rat model. J Biol Chem 2014;289:28827-34.
43. Yuan H, Zhang X, Zheng W, Zhou H, Zhang BY, Zhao D. Minocycline attenuates kidney injury in a rat model of streptozotocin-induced diabetic nephropathy. Biol Pharm Bull 2016;39:1231-7.
44. Zheng H, Piao S, Sun L, Zhao H, Jin J, Jin J, *et al*. Renoprotective effects of L-Carnitine in streptozotocin-induced diabetic nephropathy. Int J Clin Exp Med 2018;11:4459-69.
45. Agrawal NK, Gupta U, Singh SP. Effects of enalapril on blood glucose level and interaction with the oral anti-diabetic drugs in alloxan-induced diabetic rats. Asian J Pharm Clin Res 2013;6:66-9.
46. Rosenthal T, Erlich Y, Rosenmann E, Grossman E, Cohen A. Enalapril improves glucose tolerance in two rat models: A new hypertensive diabetic strain and a fructose-induced hyperinsulinaemic rat. Clin Exp Pharmacol Physiol 1995;22:S353-4.
47. Ahmed OM, Ali TM, Abdel Gaid MA, Elberry AA. Effects of enalapril and paricalcitol treatment on diabetic nephropathy and renal expressions

of TNF-α, p53, caspase-3 and Bcl-2 in STZ-induced diabetic rats. PloS One 2019;14:e0214349.
48. Pourghasem M, Shafi H, Babazadeh Z. Histological changes of kidney in diabetic nephropathy. Caspian J Intern Med 2015;6:120-7.
49. Roshan B, Stanton RC. A story of microalbuminuria and diabetic nephropathy. J Nephropathol 2013;2:234-40.
50. Figueira MF, Castiglione RC, de Lemos Barbosa CM, Ornellas FM, da Silva Feltran G, Morales MM, *et al*. Diabetic rats present higher urinary loss of proteins and lower renal expression of megalin, cubilin, ClC-5, and CFTR. Physiol Rep 2017;5:e13335. doi: 10.14814/phy2.13335.
51. Cadaval RA, Kohlman O, Michelacci YM. Urinary excretion of glycosaminoglycans and albumin in experimental diabetes mellitus. Glycobiology 2000;10:185-92.
52. Al-Trad B, Ashankyty IM, Alaraj M. Progesterone ameliorates diabetic nephropathy in streptozotocin-induced diabetic Rats. Diabetol Metab Syndr 2015;7:97.
53. Pierini D, Bryan NS. Nitric oxide availability as a marker of oxidative stress. In: Advanced Protocols in Oxidative Stress III. New York, NY: Humana Press; 2015. p. 63-71.
54. Shankland SJ, Scholey JW. Expression of transforming growth factor-β1 during diabetic renal hypertrophy. Kidney Int 1994;46:430–42.
55. ADA Standards of Medical Care in Diabetes 2016 | World diabetes foundation. Available from: https://www.worlddiabetesfoundation.org/files/ada-standards-medical-care-diabetes-2016-0. [Last accessed on 2022 Mar 23].
56. Kitada M, Ogura Y, Koya D. Rodent models of diabetic nephropathy: Their utility and limitations. Int J Nephrol Renovasc Dis 2016;9:279-90.