EFFECT OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA-AGONISTS ON DIABETES-INDUCED ACUTE KIDNEY INJURY: ROLE OF OXIDATIVE STRESS AND HYPERLIPIDEMIA

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ABSTRACT

Objective: The present study investigated the possible effect of fenofibrate and gemfibrozil peroxisome proliferator-activated receptor-alpha agonist in diabetes-induced acute kidney injury (AKI) in rats.

Methods: Rats were administered streptozotocin (STZ) (50 mg/kg, i.p., single dose) to induce experimental diabetes mellitus. The development of diabetic AKI was assessed biochemically and histologically. In addition, the diabetes-induced lipid profile and renal oxidative stress were assessed. The single dose of STZ produced diabetes, which induced renal oxidative stress, altered the lipid profile and subsequently produced kidney injury. AKI in 7 weeks by increasing serum creatinine, blood urea nitrogen (BUN), proteinuria, and glomerular damage. Treatment with fenofibrate and gemfibrozil (30 mg/kg p.o, 7 weeks) normalized the altered lipid profile by decreasing serum cholesterol, triglycerides, and increasing serum high-density lipoprotein in diabetic rats. Lisinopril {1 mg/kg, p.o., 7 weeks, reference compound} prevents lipid alteration and development of diabetic AKI.

Result: Fenofibrate and gemfibrozil, besides hyperglycemia, significantly prevented the development of diabetic AKI by reducing (serum and tissue) oxidative stress, hyperlipidemia, serum BUN, creatinine, and urinary protein. Further, fenofibrate, but not gemfibrozil, considerably reduced renal structural and functional abnormalities in diabetic rats. The fenofibrate was more effective in attenuating the diabetes-induced AKI and renal oxidative stress as compared to treatment with and gemfibrozil.

Conclusion: The fenofibrate and gemfibrozil treatment markedly prevented the diabetes-induced AKI. In comparison, the fenofibrate is found to be a superior approach to attenuate the diabetic AKI than gemfibrozil.

Keywords: Diabetes, Fenofibrate, Gemfibrozil, Nephropathy.

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia followed by micro- and macro-vascular complications [1]. Diabetic nephropathy is an important microvascular complication of diabetes and is widely recognized as the most common cause of the end-stage renal failure. Diabetes mediated acute kidney injury (AKI) is one of the leading causes of morbidity and mortality. Hyperglycemia, vascular endothelial dysfunction, and hyperlipidemia are considered as possible reasons behind the pathogenesis and progression of diabetic nephropathy [2]. Hyperglycemia mainly targets the almost all the kidney cellular elements such as glomerular endothelial cells, mesangial cells, podocytes, and tubular epithelial injury.

Further, the growing glomerulosclerosis, thickening of the glomerular basement membrane, glomerular hypertrophy, mesangial cell expansion, podocyte loss, renal-cell hypertrophy, and tubulointerstitial fibrosis are major pathological changes during the course of diabetic nephropathy, which ultimately results in functional consequences, including progressive albuminuria, reduction in glomerular filtration rate, elevation of arterial blood pressure, and fluid retention [3]. It is interesting that recent studies demonstrated numerous pleiotropic effects of peroxisome proliferator-activated receptor-alpha (PPAR-α) agonist such as fenofibrate and gemfibrozil. Gemfibrozil is well known to produce antihyperlipidemic action by activation of PPAR-α. In addition to this, upregulation of PPAR-α had an important role in affording the protection against experimental acute kidney failure. A recent study revealed the mechanism of cardioprotection by gemfibrozil by attenuating oxidative stress. Importantly, research exhibiting the most pronounced benefits of gemfibrozil in preventing vascular events by direct activation and protection of guanylyl cyclase (sGC) the key mediator of NO signaling [4]. The gemfibrozil mediated activation of guanylyl cyclase may be a rational concept to attenuate the oxidative stress and hyperlipidemia. Further, fenofibrate is well documented to attenuate diabetes-induced endothelial function by upregulating eNOS and by decreasing oxidative stress and hyperlipidemia [5]. Further, the nephroprotective role of fenofibrate is reported in diabetes by ameliorating oxidative stress and hyperlipidemia. Furthermore, fenofibrate has been shown to reduce glomerular hypertrophy, mesangial matrix expansion and suppress the expression of plasminogen activator inhibitor-1 and transforming growth factor-b [5,6]. Therefore, the renovascular protective role of PPAR-α agonist may be a rational therapeutic strategy to ameliorate diabetes mediated AKI.

METHODS

Experimental animals
The Institutional Animal Ethical Committee approved the experimental protocol used in the present study. Age-matched young Wistar rats weighing about 200–250 g were employed in the present study. The animals were housed in the room maintained at approximately 24±1°C temperature and humidity of 55±5% with 12-h light/dark cycle. Free access to food (standard chow from Ashirwad Industries, Ropar, India) and water was allowed. The animals were acclimatized for at least 4–5 days before the initiation of the experiment and were observed for any sign of disease. The animals were maintained under proper conditions until the termination of the experiment. The animals were sacrificed after a predetermined period of the treatment as per the study design to evaluate various parameters.
Experimental protocol
Seven groups were employed in the present study and each group comprised 8 animals each. The fenofibrate and gemfibrozil were dissolved in 1% w/v of carboxymethylcellulose (CMC) and water, respectively.

Drug-treated diabetic group
Group I (normal control)
Rats were maintained on standard food and water, and no treatment was given.

Group II (diabetic control)
Rats were administered streptozotocin (STZ) (50 mg/kg, i.p., once) dissolved in citrate buffer (pH 4.5).

Group III (fenofibrate per se)
Rats were administered fenofibrate (30 mg/kg p.o.) dissolved in 1% w/v CMC for 7 weeks.

Group IV (gemfibrozil per se)
Rats were administered gemfibrozil (30 mg/kg p.o.) dissolved in water for 7 weeks.

Group V (fenofibrate treated diabetic group)
The diabetic rats after 1 week of STZ administration were treated with fenofibrate (30 mg/kg p.o.) for 7 weeks.

Group VI (gemfibrozil treated diabetic group)
The diabetic rats after 1 week of STZ administration were treated with gemfibrozil (30 mg/kg p.o.) for 7 weeks.

Group VII (lisinopril treated diabetic group)
The diabetic rats after 1 week of STZ administration were treated with lisinopril (1 mg/kg p.o.) for 7 weeks (Fig. 1).

Drugs and chemicals
STZ and acetylcholine hydrochloride were obtained from Sigma-Aldrich Ltd, St. Louis, USA. 1,1,3,3 tetramethoxypropane and carboxymethyl cellulose were purchased from V. K. Chemicals and Instruments, Ambala, India. The fenofibrate and gemfibrozil were obtained from Ranbaxy Laboratory Ltd., Gurgaon, India. Lisinopril was obtained from Dr. Reddy’s Laboratory Ltd., Hyderabad, India. All other chemicals used in the present study were of analytical grade.

Assessment of serum glucose
At the end of the experimental protocol, the blood samples were collected, and serum was separated after allowing to clot followed by centrifugation. The glucose concentration was estimated by glucose oxidase-peroxidase GOD-POD method using the commercially available kit (Bio Lab Diagnostics India Private Limited) [5].

Assessment of lipid profile
Estimation of serum total cholesterol
The total cholesterol was estimated by cholesterol oxidase peroxidase CHOD-POD method using the commercially available kit (Bio Lab Diagnostics India Private Limited) [5,6].

Estimation of serum triglycerides
The serum triglyceride was estimated by GOD-POD method using the commercially available kit (Bio Lab Diagnostics India Private Limited) [5,6].

Estimation of high-density lipoprotein (HDL)
The HDL was estimated by CHOD-POD method using the commercially available kit (Bio Lab Diagnostics India Private Limited) [5,6].

Assessment of oxidative stress
The oxidative stress in serum samples was assessed by estimating serum thiobarbituric acid reactive substance (TBARS) and superoxide anion generation.

Estimation of TBARS
Serum
1 ml of 20% trichloroacetic acid was added to 100 µL of serum and 1% TBA reagent (1.0 mL) which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1000 g for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard curve using 1, 1.3, 3, tertramethoxypropane (1–10 µM) was plotted to calculate the concentration of TBARS [5,6].

Tissue
The kidney was excised and washed with ice-cold isotonic saline and weighed. The kidney weight to body weight ratio was calculated. The kidney was then minced, and a homogenate (10% w/v) was prepared in chilled 1.15% KI. The homogenate was used for the estimation of renal TBARS and glutathione (GSH).

The renal TBARS, an index of lipid peroxidation, were estimated according to the method described earlier. The reaction mixture was prepared by mixing 0.2 mL of tissue homogenate, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution (adjusted to pH 3.5 with NaOH), and 1.5 mL of 0.8% aqueous solution of TBA. The reaction mixture was made up to 4.0 mL with distilled water and then incubated at 95°C for 60 min. After cooling in tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1 v/v) were added to reaction mixture and shaken vigorously using vortex shaker. The test tubes were centrifuged at 12,520 g (radius of the rotor was 7 cm) for 10 min (REMI Cooling Centrifuge, India). The absorbance of the developed pink color was measured spectrophotometrically at 532 nm. The standard curve using 1, 1.3, 3, tertramethoxypropane (1–10 µM) was plotted to calculate the concentration of TBARS and the results were expressed as nM/g wet weight of renal tissue [7].

Estimation of reduced GSH
The renal GSH level was estimated using the methods described by Ellman. Ellman’s reagent (5,5’-dithiobis-[2-nitrobenzoic acid] [DTNB]) is a chemical used for measuring the amount of thiol group. Thiol react with this compound, cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (NTB-), which ionizes to the NTB2- dianion in water at neutral and alkaline pH. This NTB2- ion has a yellow color. The NTB2- is quantified in a spectrophotometer by measuring the absorbance at 412 nm.

The renal homogenate of the rat was mixed with 10% w/v trichloroacetic acid in 1:1 ratio and centrifuged at 4°C for 10 min at 1957 g. The supernatant (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 mL of distilled 1,1,3,3 tetramethoxypropane and 0.002 M TBA reagent (1.0 mL) which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1000 g for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard curve using 1, 1.3, 3, tertramethoxypropane (1–10 µM) was plotted to calculate the concentration of TBARS [5,6].

The oxidative stress in serum samples was assessed by estimating serum thiobarbituric acid reactive substance (TBARS) and superoxide anion generation.
water. Then, 0.25 mL of 0.001 M freshly prepared DTNB (dissolved in 1% w/v sodium citrate) was added to the reaction mixture, and then incubated for 10 min. The absorbance of the yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using the reduced form of GSH (0.1–1 mM), and the results were expressed as mM/g wet weight of renal tissue [7].

Assessment of AKI
The serum creatinine concentration was estimated by the alkaline picrate kinetic method using the commercially available kit (Span Diagnostics Ltd., India).

The blood urea was estimated by a method using the commercially available kit (Span Diagnostics Ltd., India).

The proteinuria was estimated by pyrogallol red method using the commercially available kit (Span Diagnostics Ltd., India) [5-7].

Histopathological study
The early diabetic changes in glomeruli were assessed histopathologically. The kidneys were excised and immediately immersed in 10% formalin. The kidney was dehydrated in graded concentrations of alcohol, immersed in xylene and then embedded in paraffin. From the paraffin blocks, sections of 5-µm thickness were made and stained with hematoxylin and eosin to assess pathological changes in glomeruli using light microscopy [×400] [5,6].

Statistical analysis
All values were expressed as mean ± S.E.M. The data for serum/tissue levels of TBARS, serum creatinine, blood urea and proteinuria, serum glucose, and lipid profile were statistically analyzed using one-way analysis of variance followed by Tukey’s multiple comparison tests. p<0.05 was considered to be statistically significant.

RESULT
Pharmacological account of drug in diabetic model
The administration of fenofibrate (30 mg/kg, p.o., 7 weeks) or gemfibrozil (30 mg/kg, p.o., 7 weeks) or lisinopril (1 mg/kg, p.o.,...
Admistration of STZ (50 mg/kg, i.p., single dose) produced hyperglycemia after 72 h (serum glucose >180 mg/dL) of injection. After 7 days of STZ administration, the rats which showed blood glucose level >250 mg/dL were selected and were assigned in subsequent groups. Fenofibrate, gemfibrozil, and lisinopril were administered to diabetic rats after 7 days of injection of STZ, and their treatment was continued for 7 weeks. All the parameters were assessed at the end of 7 weeks of treatment, i.e., 8 weeks of diabetes induction in all groups. Overall, mortality among diabetic animals with or without drug treatments for 8 weeks was <10%.

Effect of pharmacological interventions on serum glucose
The marked increase in serum concentration of glucose was noted in diabetic rats when compared with normal rats. Treatment with fenofibrate (30 mg/kg p.o., 7 weeks) or gemfibrozil (30 mg/kg p.o., 7 weeks) did not affect the serum glucose concentration in diabetic rats. However, treatment with lisinopril (1 mg/kg p.o., 7 weeks) slightly reduced the glucose level in diabetic rats; the result was not statistically significant (Fig. 2).

Effect of pharmacological interventions on serum TBARS
The increase in serum concentration of total cholesterol and triglycerides and decrease in HDL were noted in diabetic rats when compared with normal rats. The treatment with fenofibrate (30 mg/kg p.o., 7 weeks) or gemfibrozil (30 mg/kg p.o., 7 weeks) or lisinopril (1 mg/kg p.o., 7 weeks) alone significantly attenuated diabetes-induced alterations in serum lipids. In addition, treatment with lisinopril partially prevented a diabetes-induced increase in total cholesterol and triglycerides and decrease in HDL level (Fig. 3-5).

Effect of pharmacological interventions on renal TBARS and GSH
A marked increase in renal TBARS was noted in diabetic rats as compared to normal rats. The treatment with fenofibrate (30 mg/kg p.o., 7 weeks) or gemfibrozil (30 mg/kg p.o., 7 weeks) or lisinopril (1 mg/kg p.o., 7 weeks) significantly attenuated the diabetes-induced increase in renal TBARS and decrease in renal GSH. In addition, the marked reduction in serum TBARS was noted in diabetic rats treated with fenofibrate as compared to treatment with gemfibrozil and but not superior than lisinopril (Fig. 6).

Effect of pharmacological interventions on histopathological study on kidney
The diabetes was noted to develop pathological changes in the glomeruli such as reduced capillary size and extracellular mesangial expansion. The diabetes was noted to develop pathological changes in the glomeruli such as reduced capillary size and extracellular mesangial expansion in diabetic rats after 7 weeks when compared with normal rats. Treatment with fenofibrate (30 mg/kg p.o., 7 weeks) or gemfibrozil (30 mg/kg p.o., 7 weeks) or lisinopril (1 mg/kg p.o., 7 weeks) attenuated diabetes-induced pathological changes in glomeruli. In addition, the marked pathological changes in glomeruli were noted in diabetic rats treated with fenofibrate as compared to treatment with gemfibrozil and but not superior than lisinopril (Fig. 12a-c).

DISCUSSION
One of the most important functions of the kidney is the filtration and excretion of nitrogenous waste products from the blood. The measurements of elevated blood urea nitrogen (BUN) and creatinine serve as indicators of decreased renal function indicative of the decreased clearance of these waste products. AKI is currently defined as a rapid decline in the glomerular filtration rate resulting in retention of nitrogenous wastes, primarily creatinine and BUN [8]. Concurrently, major structural hallmarks of AKI include renal enlargement, mesangial cell expansion, tubular injury, and glomerular basement membrane thickening [9,10]. There are several reasons for kidney damages, the
primary focus on the potential causes of accounting for the majority of nephrology disease as diabetes. There are several mechanisms studied, but still, the management of kidney dysfunctions associated with diabetes remains obscure.

The animal models for nephropathy share many features which are common to human nephropathy and have been delineated by targeting proteinuria, glomerulosclerosis, glomerulonephritis, glomerular hyper trophy, tubulointerstitial nephritis, tubular necrosis, and reduced glomerular filtration rate. The number of patients with end-stage renal failure requiring dialysis is increasing. Therefore, suitable animal models that exhibit progressive renal lesions are necessary to identify the mechanisms involved in human nephropathy and to develop potential drugs/drug targets for the treatment of renal complications. We have used the well-established animal model of diabetes using STZ.

At present, angiotensin-converting enzyme (ACE) inhibitors such as captopril, lisinopril, and fosinopril are employed to treat diabetic nephropathy [11]. However, clinical evidence suggests that ACE inhibitors are not sufficient to control the symptoms of nephropathy [3,12,13]. The present study has been aimed to explore the potential use of PPAR-α to prevent diabetic-induced AKI.

The STZ, a single dose of 50 mg/kg ip, was employed in the present study to induce experimental diabetes in rats. STZ developed hyperglycemia within 72 h (serum glucose >180 mg/dL). After 7 days of STZ administration, rats which showed the blood glucose level >240 mg/dL were selected for the study and are named as diabetic rats. The strong association between diabetes and vascular endothelial dysfunction and mediated nephropathy is demonstrated in various studies. Frequently, the strong correlations between oxidative stress and hyperlipidemia in the induction of nephropathy have been reported [5,13]. In diabetes, the reactive oxygen species (ROS) one of the main culprits plays a key role in the progression of pathophysiologic processes of renal diseases. Moreover, high glucose-induced ROS generation through activation of

Fig. 8: Effect of fenofibrate, gemfibrozil, and lisinopril on plasma glutathione. All results are expressed in mean±SEM. *p<0.001 versus normal control, **p<0.01, *p<0.05 versus diabetic control, #p<0.05 versus gemfibrozil + diabetic animals (n=6)

Fig. 9: Effect of fenofibrate, gemfibrozil, and lisinopril on plasma blood urea nitrogen. All results are expressed in mean±SEM. ***p<0.001 versus normal control, **p<0.01, *p<0.05 versus diabetic control, #p<0.05 versus gemfibrozil + diabetic animals (n=6)

Fig. 10: Effect of fenofibrate, gemfibrozil, and lisinopril on serum creatinine. All results are expressed in mean±SEM. **p<0.001 versus normal control, *p<0.01, *p<0.05 versus diabetic control, #p<0.05 versus gemfibrozil + diabetic animals (n=6)

Fig. 11: Effect of fenofibrate, gemfibrozil, and lisinopril on urinary protein. All results are expressed in mean±SEM. **p<0.001 versus normal control, **p<0.01; *p<0.05 versus diabetic control, #p<0.05 versus gemfibrozil + diabetic animals (n=6)
properties on the diabetic kidney. The overall observed beneficial effect of fenofibrate in preventing diabetic AKI may be due to their antioxidant activity. Thus, it may be suggested that the direct renoprotective potential of fenofibrate or gemfibrozil may play a role in the pathogenesis of diabetic AKI.

In the present study, diabetes has been noted to increase the renal TBARS and decrease the GSH levels in the kidney. Also, it has been demonstrated that diabetes increases the production of ROS, lipid, and consequently reduces the synthesis and bioavailability of no, which result in AKI.

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This contention is supported by the results obtained in the present study that increases in serum/tissue TBARS level and the decrease in tissue GSH are regarded as an index of development of oxidative stress. Further, the oxidative stress has been documented to play a major role in the progression of VED and nephropathy. It has been demonstrated that diabetes increases the production of ROS, lipid, and consequently reduces the synthesis and bioavailability of no, which result in AKI.

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gemfibrozil the other PPAR-α agonist noted to be useful to control diabetic dyslipidemia and control diabetic nephropathy. Importantly, the fenofibrate was noted to be superior in preventing diabetic AKI than gemfibrozil. The present work emphasized the rationale for employing PPAR-α agonists in the management of diabetic nephropathy.

CONCLUSION
On the basis of above discussion, diabetes-mediated lipid alteration and oxidative stress could play a role in the development of AKI. Fenofibrate and gemfibrozil show antihyperlipidemic, antioxidant property, and contributed in renoprotection in diabetic rats with AKI. Apart from antihyperlipidemic action fenofibrate showed, the significant renoprotection action of than gemfibrozil by drastically restructures the kidney architect.

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AUTHOR’S CONTRIBUTION
VA Chakkarwar, research scholar, constructing an idea or hypothesis for research and/or manuscript. Planning methodology to reach a conclusion. Reviewing the article before submission not only for spelling and grammar but also for its intellectual content. PS. Kawtikwar, supervising, organizing, and supervising the course of the project or the article along with constructing a hypothesis and taking the responsibility to complete research work.

CONFLICTS OF INTEREST
None.

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