

Research Article**A protective effect of DPP4 inhibitor and dual PPAR agonist combination on diabetes and its complications****Chitrang Patel¹, Raju Dangar², Yagnik Bhalodia¹, Jayesh V. Beladiya², Vandana R. Thakur², Anita A. Mehta^{2*}**¹Department of Pharmacology, C. U. Shah University, Wadhawan, Gujarat, India²Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad, Gujarat, India

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Abstract

Objective: The prevalence of type-2 diabetes mellitus and their complications are rapidly rising all over the globe at an alarming rate. Numerous drugs are available for treatment of diabetes. However, the prominent drug combination is yet not identified for the management of diabetes and its complications. Aim of present study was investigate the effects of PPAR agonist (saroglitazar) alone and in combination with DPP4 inhibitors (Teneligliptin and linagliptin). **Materials and Methods:** Rats were divided into seven groups (n=8). All the groups except Normal Control (NC) were fed with high fat diet for four weeks, followed by single bolus dose of streptozotocin (STZ, 40mg/kg, i.p.) while NC received vehicle (citrate buffer, 1ml/kg, i.p.). The treatment of saroglitazar (4mg/kg/d, p.o.), teneligliptin (4mg/kg/d, p.o.) and linagliptin (4mg/kg/d, p.o.) were given alone and in combination (saroglitazar (2mg/kg/d) + teneligliptin (2mg/kg/d), and saroglitazar (2mg/kg/d) + teneligliptin (2mg/kg/d)) for 21 days in diabetic rats. At the end of the experiment, various biochemical parameters such as glucose, cholesterol, triglyceride, urea, creatinine, LDH and CK-MB levels were determined in the serum of all rats. **Results:** The treatment of saroglitazar, teneligliptin, and linagliptin alone; and in combination significantly attenuated serum glucose, cholesterol, triglyceride, creatinine, urea, CK-MB, LDH level in diabetic rats as compared to control rats. However, the protective effects of saroglitazar in combination with DPP4 inhibitors did not show higher significance as compared to the individual therapy. **Conclusion:** The current study indicated that saroglitazar has additive protective effects with DPP4 inhibitors against diabetes and diabetic complications. However, it did not show the supraadditive effects.

Keywords: Diabetes, saroglitazar, teneligliptin, linagliptin

Introduction

Diabetic complications are detrimental problem and the most prevalent cause of morbidity and mortality among the people with Type 2 diabetes mellitus (Morrish et al., 2001). Type 2 diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from declining beta cell function and insulin resistance (American Diabetes Association, 2011). India has the highest population suffering from diabetes across the world (Kaveeshwar and Cornwall J, 2014). The prevalence of diabetes was 415 million in 2015 and expected to rise to 642

million by 2040 with a maximum increase in India (Whiting et al., 2011). Epidemiological studies demonstrated, 85% of diabetics in the world are suffering from diabetic dyslipidemia. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL-C) levels and a high proportion of small dense low-density lipoprotein cholesterol (Mooradian, 2009). Despite numerous anti-diabetic drugs are identified, the prominent combination therapy for management of diabetes is still unavailable. The combination therapy based on the rationale with the multi-targeted approach has a vital role in the management of diabetes and related complications. Therefore, there is need to identify the combination that improves glycemic and lipid control and reduce the risk of life threatening complications.

DPP4 inhibitors (teneligliptin, linagliptin), improves glucose

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control by inhibiting the DPP4 enzyme in T2DM, critically reducing glycosylated hemoglobin, with low risk for hypoglycemia and without weight gain (Sharma et al., 2016). Further, Peroxisome proliferator-activated receptors (PPARs) are potential therapeutic target in treatment of hyperglycemia and lipid metabolism. Numerous studies suggested beneficial effect of PPAR- γ agonist in insulin resistance and lowering blood glucose levels in diabetic patients (Buchanan et al., 2002; DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) Trial Investigators, 2006). Moreover, PPAR- α agonists have crucial role in the lipid metabolism and decreases TG levels by amplifying the expression of lipoprotein lipase (LPL) (Monsalve et al., 2013). Additionally, previously reported study demonstrated role of PPAR- α agonists in the treatment of dyslipidemia. Saroglitazar is a dual peroxisome proliferator-activated receptor (PPAR- α/γ) agonist having the beneficial effect on both hyperglycemia and lipid profile (Jain et al., 2015). Further combining Saroglitazar to DPP4 inhibitors (Linagliptin or Tenueligliptin) may show the additive effect by showing improved glycemic and lipids control among the diabetic patients. Therefore we have aimed to study the efficacy of linagliptin, tenueligliptin, and Saroglitazar alone and in combination on diabetes and its complications in the rats.

Material and Methods

Animals

The protocol (ARL/PT/027/2015) of the experiment was approved by the institutional animal ethical committee (IAEC). Male Wistar rats (200-250 g) were procured (Cadila Pharmaceutical Ltd, Dholka, Dist. Ahmedabad, India) for the experiment. All Rats were housed at ambient temperature ($22 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 5\%$) and 12/12 h light/dark cycle. Animals had access to standard pellet diet and water given ad libitum. All experiments were carried out as per the guidance of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, New Delhi, India.

Chemicals and reagents

Streptozotocin (STZ) was purchased from Sigma chemicals (St Louis, USA). Citric acid, sodium citrate, and other solvents (like diethyl ether, ethanol etc.) were obtained from Merck (Mumbai, India). Kits for estimation of serum glucose, triglyceride and total cholesterol, LDH, CK-MB, urea and creatinine were purchased from Span Diagnostics Ltd. (Surat, Gujarat, India).

Experiment design and Diabetes Mellitus induction

Rats were divided into seven groups ($n=8/\text{group}$).

Group-1 (NC): Normal Control rats

Group-2 (DC): Diseased Control rats (HFD/STZ (40mg/kg, i.p.) induced diabetic rats)

Group-3 (Saro): Saroglitazar (4mg/kg, p.o.) treated diabetic rats

Group-4 (Lina): Linagliptin (4mg/kg, p.o.) treated diabetic rats

Group-5 (Tenedi): Tenueligliptin (4mg/kg, p.o.) treated diabetic rats

Group-6 (Saro+Lina): Saroglitazar (2mg/kg, p.o.) + Linagliptin (2mg/kg, p.o.) treated diabetic rats

Group-7 (Saro+Tenedi): Saroglitazar (2mg/kg, p.o.) + Tenueligliptin (2mg/kg, p.o.) treated diabetic rats

Diabetes was induced by High Fat Diet (HFD), followed by single bolus injection of streptozotocin (40mg/kg, i.p.) Rats were fed with High-fat diet (HFD) for 4 weeks, except normal rats, followed by a single bolus injection of STZ (40mg/kg) in citrate buffer (pH 4.5) intraperitoneal. Drinking water was replaced with 5% glucose solution for 5 days before and after STZ administration. The serum glucose level was measured after a one week of STZ administration. Those rats showed the blood glucose level of higher than 200 mg/dl considered as diabetic rats and were involved in the study. The treatment of Saroglitazar (4mg/kg, p.o.), Linagliptin (4mg/kg, p.o.), Tenueligliptin (4mg/kg, p.o.), and combinations, (Saroglitazar (2mg/kg, p.o.) + Linagliptin (2mg/kg, p.o.); Saroglitazar (2mg/kg, p.o.) + Tenueligliptin (2mg/kg, p.o.)) were administered daily for next 21 days. At the end of experiment, metabolic parameters (glucose, cholesterol, triglyceride), renal damage markers (urea and creatinine), cardiac damage markers (serum lactate dehydrogenase (LDH) and creatinine kinase (CK-MB)) levels were measured in the serum of all rats.

Blood sample collection

Blood were withdrawn from retro-orbital plexus of anesthetized rats (under light diethyl ether anesthesia). They kept at room temperature for 30 min, centrifuged at 1000 g for 15 min, and serum was isolated by aspiration (Thakur et al., 2018).

Measurement of metabolic, cardiac damage and renal damage markers

The metabolic parameters, serum (glucose, cholesterol, triglyceride) levels, cardiac damage markers (serum LDH and CK-MB) levels and renal damage markers serum (urea and creatinine) levels were estimated using spectrophotometry based kits (Span Diagnostic Ltd., Surat, Gujarat, India) (Beladiya et al., 2018).

Statistical analysis

All the data were expressed as mean \pm SEM. Group

comparison was performed by one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test using Graphpad prism 5.0 Software. $P < 0.05$ is considered statistically significant.

Results

The administration of STZ in rats significantly increased the serum glucose ($P < 0.001$), serum cholesterol ($P < 0.001$) and serum triglyceride ($P < 0.01$) level as compared to the normal control rats. Apart from this, the cardiac damage markers, serum creatinine kinase (CK-MB) ($P < 0.001$) and serum lactate dehydrogenase (LDH) ($P < 0.001$) levels; the renal damage markers, serum urea ($P < 0.01$) and creatinine ($P < 0.001$) levels were significantly increased in the diabetic rats as compared to normal control rats.

Treatment of Saroglitazar significantly reduced the serum glucose ($P < 0.001$, Figure 1), serum cholesterol ($p < 0.05$, Figure 1B), serum triglyceride ($P < 0.001$, Figure 1C), serum creatinine kinase (CK-MB) ($P < 0.001$, Figure 1D), serum lactate dehydrogenase (LDH) ($P < 0.001$, Figure 2A), serum urea ($P < 0.001$, Figure 2B) and creatinine ($P < 0.001$, Figure 2C) levels in STZ induced diabetic rats.

Similarly, Linagliptin and Teneligliptin treated STZ induced

diabetic rats showed significant decrease in serum glucose ($P < 0.05$, $P < 0.01$ respectively, Figure 1A), serum cholesterol ($P < 0.05$, $P < 0.05$ respectively, Figure 1B), serum triglyceride ($P < 0.05$, $P < 0.01$ respectively, Figure 1C), serum creatinine kinase (CK-MB) ($P < 0.05$, $P < 0.01$ respectively, Figure 1D), serum lactate dehydrogenase (LDH) ($P < 0.05$, $P < 0.01$ respectively, Figure 2A), serum urea ($P < 0.01$, $P < 0.001$ respectively, Figure 2B) and creatinine ($P < 0.01$, $P < 0.001$ respectively, Figure 2C) levels as compared to untreated STZ induced diabetic rats.

Moreover, the half dose of saroglitazar and DPP4 inhibitors in combination treatment, Saroglitazar + Linagliptin and Saroglitazar + Teneligliptin resulted in more significant decrease in serum glucose ($P < 0.001$; $P < 0.001$ respectively, Figure 1A), serum cholesterol ($p < 0.01$; $P < 0.001$ respectively, Figure 1B), serum triglyceride ($P < 0.001$; $P < 0.001$ respectively, Figure 1C), serum creatinine kinase (CK-MB) ($P < 0.001$; $P < 0.001$ respectively, Figure 1D), serum lactate dehydrogenase (LDH) ($P < 0.001$; $P < 0.001$ respectively, Figure 2A), serum urea ($P < 0.001$; $P < 0.001$ respectively, Figure 2B) and creatinine ($P < 0.001$; $P < 0.001$ respectively, Figure 2C) levels in STZ induced diabetic rats compared to untreated groups.

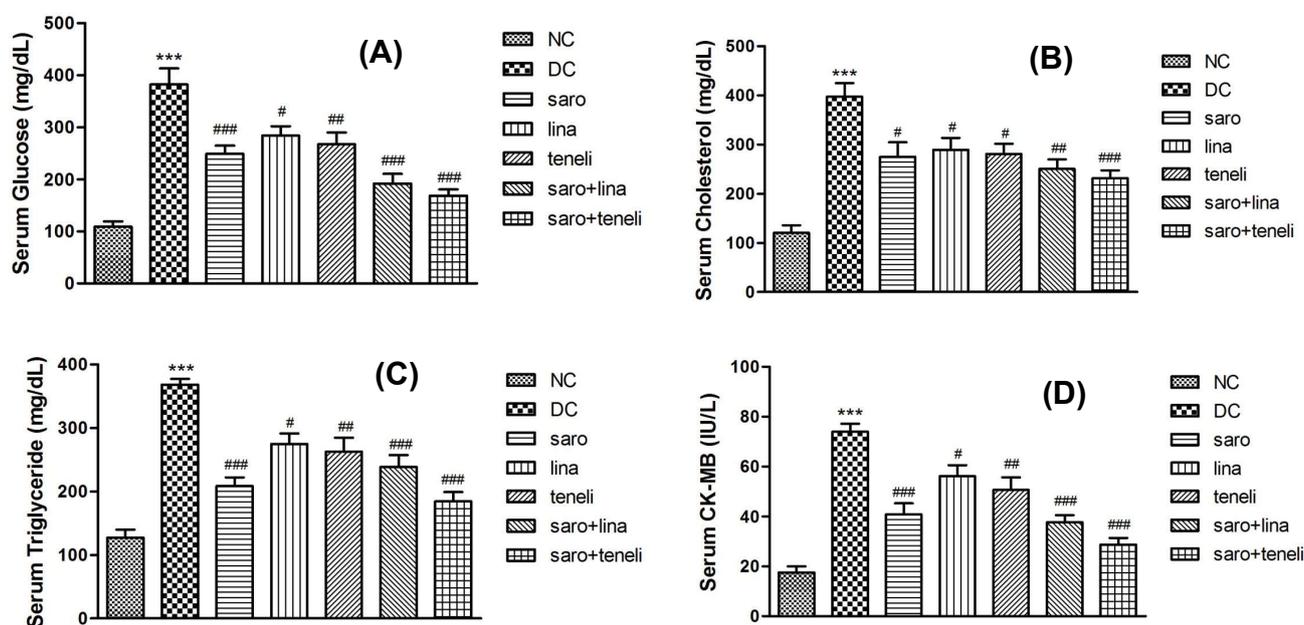


Figure 1. Effect of PPAR agonist, DPP4 inhibitors and their combination in normal and STZ induced diabetic rats on: (A) Serum glucose level, (B) Serum cholesterol, (C) Serum triglyceride, (D) Serum CK-MB. NC= Normal Control, DC= Diseased Control, STZ (40mg/kg) induced diabetic rats, Saro= Diabetic Saroglitazar (4mg/kg) treated, Lina= Diabetic Linagliptin (4mg/kg) treated, Teneli= Diabetic Teneligliptin (4mg/kg) treated, Saro+Lina= Diabetic Saroglitazar (2mg/kg) + Linagliptin (2mg/kg) treated, Saro+Teneli= Diabetic Saroglitazar (2mg/kg) + Teneligliptin (2mg/kg) rats. *** $p < 0.001$ vs. NC. ### $p < 0.001$, ## $p < 0.01$ and # $p < 0.05$ vs. DC rats.

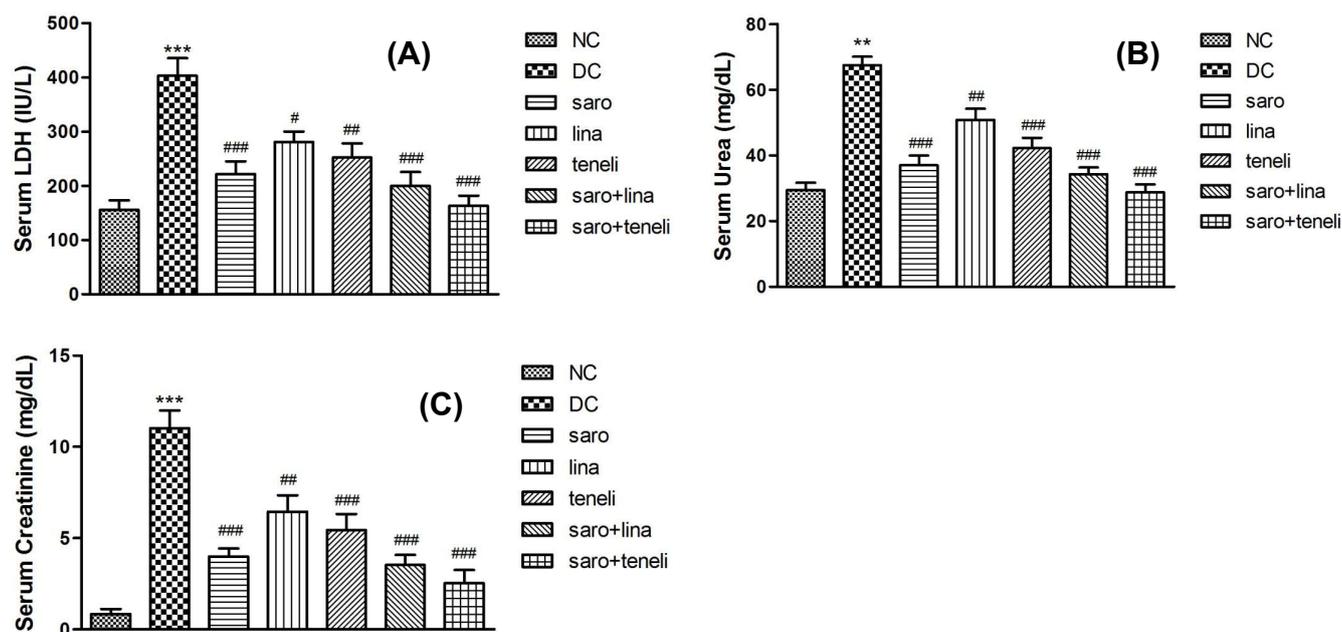


Figure 2. Effect of PPAR agonist, DPP4 inhibitors and their combination in normal and STZ induced diabetic rats on: (A) serum LDH, (B) serum urea, (C) serum creatinine. NC= Normal Control, DC= Diseased Control, STZ (40mg/kg) induced diabetic rats, Saro= Diabetic Saroglitazar (4mg/kg) treated, Lina= Diabetic Linagliptin (4mg/kg) treated, Teneli= Diabetic Teneligliptin (4mg/kg) treated, Saro+Lina= Diabetic Saroglitazar (2mg/kg) + Linagliptin (2mg/kg) treated, Saro+Teneli= Diabetic Saroglitazar (2mg/kg) + Teneligliptin (2mg/kg) rats. ***p<0.001 vs. NC. ###p<0.001, ##p<0.01 and #p<0.05 vs. DC rats.

Discussion

The current study showed the protective action of dual agonist of PPAR (saroglitazar) and DPP4 inhibitors (teneligliptin, linagliptin) in the combination on diabetic complications. In STZ-induced diabetic rats, insulin deficiency or hypoinsulinemia develops as a consequence of the irreversible destruction of the β -cells of the pancreas resulting in hyperglycemia (Rakieten et al., 1963). Tight glycemic control is recognized as a standard therapeutic goal in the prevention of diabetic complications (Nathan et al., 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998). In this study, we measured the serum glucose level which is highly implicated in the diabetes manifestations. We observed a significant increase in the serum glucose level in the STZ induced diabetic rats as compared to normal rats. Further, insulin deficiency results in increased lipolysis in adipose tissues and augmentation of free fatty acids (FFA) entry to the liver, which is additionally reported in the triglyceride synthesis (Coppack et al., 1994; Ohno et al., 2000). The reports have also been suggesting cardio-renal damage in STZ induced diabetic rats (Dobrzynski et al., 2002). Consistent with these findings, results from our study also showed increased levels of triglycerides, cardiac (CK-MB, LDH) and renal (urea and creatinine) damage markers in the diabetic rats as compared to normal rats.

The present study showed treatment with linagliptin, Teneligliptin and saroglitazar significantly improved glycemic

control as compared to the untreated diabetic animals. Further, reduction in serum triglyceride and cholesterol levels in Linagliptin, Teneligliptin, and Saroglitazar treated groups seems to relate with the previous findings in amelioration of dyslipidemia (Fukuda-Tsuru et al., 2012; Chatterjee et al., 2015; Tanaka et al., 2016). Additional, improvement in the cardiac and renal damage marker suggested the treatment have cardio-reno protective actions.

Although all the three drugs alone improved the metabolic parameters (serum glucose and serum triglycerides), cardiac damage markers (CK-MB, LDH) and renal damage markers (Urea, creatinine). However, the beneficial effect of teneligliptin was found to be more compared to linagliptin but lower than saroglitazar. Serum cholesterol level was found to be comparable in all the treatment groups. While, combination (Linagliptin + Saroglitazar) and (teneligliptin + Saroglitazar), further reduced both serum glucose and serum triglycerides in an additive manner; however, no significant difference was observed among the saroglitazar alone treated group and the combinations treated groups. Conversely, a significant synergistic effect was observed in the cholesterol level among saroglitazar alone and in combination with linagliptin and teneligliptin. Further, when saroglitazar was added to linagliptin or teneligliptin, there was an

insignificant decrease in cardiac damage markers (CK-MB, LDH) and renal damage markers (Urea, creatinine) in the serum as compared to saroglitazar alone.

In summary, the DPP4 inhibitors, (linagliptin & teneligliptin) and PPAR α/γ agonist (saroglitazar) having a different mechanism of action, in combination showed insignificant but additive benefits compared to treated alone. However significant reduction was determined merely in the cholesterol level of combined treatment. Moreover, among both the combinations, saroglitazar added to teneligliptin shows more benefit as compared to saroglitazar with linagliptin.

Conclusion

The combination of saroglitazar (dual PPAR agonist) with DPP4 inhibitor (Teneligliptin and linagliptin) showed the additive effect in the protective action of alone therapy against diabetes and diabetic complications. However, it did not show the supraadditive effect in the protective action of alone therapy against diabetes and diabetic complications.

Conflicts of interest

The authors declare no conflicts of interest.

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