TRIGONELLINE AND SITAGLIPTIN ATTENUATES NICOTINAMIDE-STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY IN WISTAR RATS

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ABSTRACT

Objective: The objective of present study was to evaluate the effect of trigonelline (TRIG), sitagliptin (SITA) alone and concomitant administration in nicotinamide-streptozotocin induced diabetic nephropathy in Wistar rats.

Methods: Diabetes was induced by streptozotocin (65 mg/kg *i.p.*) injected 15 min after nicotinamide (110 mg/kg, *i.p.*). Four week later rats were randomly selected and divided into five groups (n=6). The rats were divided into following groups. Group I: non-diabetic. Group II: diabetic control, Group III: diabetic + TRIG (50 mg/kg), Group IV: diabetic + SITA (5 mg/kg), Group V: diabetic + [TRIG (50 mg/kg) + SITA (5 mg/kg)].The drugs were administered for next 4 weeks starting from 5th week post nicotinamide-STZ injection. Serum glucose (SG) and body weight were measured weekly. Serum creatinine, blood urea nitrogen, serum uric acid, urine volume and creatinine were measured at 0, 4th and 8th week of the study. While superoxide dismutase, reduced glutathione, malondialdehyde and kidney weight measurement and histopathological were carried out at the end of study period.

Results: Concomitant administration of TRIG + SITA showed significant decrease in SG, kidney weight, serum creatinine, blood urea nitrogen, serum uric acid, urine volume and the level of malondialdehyde. While significant increase in urine creatinine, activity of superoxide dismutase and glutathione were observed compared to effect of either drug alone. Histopathological study confirmed that TRIG + SITA prevented structural kidney damage.

Conclusion: Concomitant administration of trigonelline with sitagliptin prevented development of diabetic nephropathy in rats.

Keywords: Diabetic nephropathy, Trigonelline, Sitagliptin, Renal function test, Reactive oxygen species.

INTRODUCTION

The chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality [1]. It can be divided as vascular and nonvascular complications [2].Vascular complications are sub-divided into microvascular retinopathy, neuropathy, nephropathy and macrovascular complications are coronary artery disease, peripheral vascular disease and cerebrovascular disease [3]. Nonvascular complications include problems such as gastroporesis, sexual dysfunction and skin changes [2]. Diabetic nephropathy (DN) is the leading cause of chronic renal failure and end-stage renal disease worldwide [4]. Previous reports suggest that 43% of the chronic renal failure (CRF) patients on dialysis have diabetic nephropathy, 60% death cases of diabetes mellitus patients are due to diabetic nephropathy, death cases of diabetes mellitus patients due to renal failure are 17 times more as compared to non-diabetes mellitus patients [5-6]. Metabolic and hemodynamic factors interactions are mainly causative to diabetic nephropathy which activates common pathways for renal damage [7]. Metabolic derangement, glomerular hypertension, oxidative stress and advanced glycation end products are responsible for progression of diabetic nephropathy [8]. It includes clinical irregularities of kidney which consists of increased creatinine level and also elevation in urea, albuminuria, arterial blood pressure and retention of fluid [9].

Pathologically diabetic kidney shows thickening of glomerular basement membrane and mesangial expansion which leads to microalbuminurea, hyperfiltration, intertubular fibrosis along with increase in extracellular matrix. In post stage of the disease, due to glomerulosclerosis; urine albumin increases to a level which can be detected by normal urinalysis [10]. Therefore, prevention of the occurrence and the development of DN has become a very urgent issue.

Food and Drug Administration (U.S.) in October 2006 has approved sitagliptin (SITA) as first dipeptidyl peptidase-IV (DPP-IV) inhibitor for the treatment of type 2 diabetes [11]. After ingestion of meal it enhances levels of incretin hormones like glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) [12]. Both GLP-1 and GIP hormones enhance glucose-dependent insulin biosynthesis and release of glucagon-like peptide-1, additionally inhibit glucagon secretion, delays gastric emptying [13]. Moreover in animal models DDP-IV inhibitors are shown to enhance glycemic control, insulin secretion and proliferation and differentiation of pancreatic beta cells [14]. These effects may be beneficial in preserving the pancreatic beta cell mass and function [15]. Various clinical studies showed sitagliptin decreased postprandial glucose excursion, fasting plasma glucose and hemoglobin levels with neutral weight effect and a low chance of hypoglycemia and gastrointestinal adverse effect [16-18]. DPP-IV inhibitors are beneficial for acute renal failure and chronic renal disease [19]. DPP-IV inhibitor (linagliptin) combined with telmisartan was associated with marked reduction in albumin urea, an early marker for DN and also reduction in tumor necrosis factor alpha (TNF-α) an early indicator for systemic inflammation, which is found during hyperfiltration stage in diabetic nephropathy [20]. SITA protects renal ischemia reperfusion induced renal damage in diabetes [21]. Chronic administration of SITA treatment ameliorated all lesions glomerular, tubulointerstitial, and vascular of kidney [22].

Trigonelline, the major isolated component of seeds of Trigonella *foenum graceum L.*, is commonly used to treat diabetes in China [23]. TRIG is found in various plants and in some animal species including sea urchins and jellyfish [24]. Trigonelline (TRIG) is a plant alkaloid which is claimed to have hypoglycemic, antiseptic, hypocholesterolemic, anticarcinogenic and antimigraine activities [25]. TRIG is reported to have a hypoglycemic effect in normal and in alloxan induced diabetes in mice [26]. Fenugreek has been reported to restore the kidney function of diabetic rats via its antioxidant and anti-inflammatory activities [27]. TRIG showed renoprotective effect in alloxan induced early diabetic nephropathy in rats [28]. Trigonelline ameliorated diabetic hypertensive nephropathy by suppression of oxidative stress in kidney in streptozotocin induced neonatal diabetic rats [29].

The effect of trigonelline and sitagliptin together in diabetic nephropathy has not been reported. The objective of present study was to investigate the effect of concomitant administration of trigonelline (TRIG) and sitagliptin (SITA) on renal marker and oxidative stress in nicotinamide-STZ induced diabetic nephropathy in Wistar rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (200-250 g) were purchased from National Toxicology Center, Pune. During the experiment, rats were housed in standard housing conditions like temperature of 25± 1ºC, relative humidity of 45%-55% and 12 h light: 12 h dark cycle. Rats had free access to food pellets (Navmaharashtra Chakan Oil Mills Ltd., Sangli, India) and tap water *ad ibitum* during the experiment. The experiments were performed after approval of the protocol (CPCSEA/72/12) by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune. Constituted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Chemicals and Reagents

Trigonelline hydrochloride (Batch No - BCBH2678V, Sigma Aldrich, Mo.USA) and Sitagliptin (Batch No- 20120223, Hangzhou longshine Bio-Tech Co.,LTD,China), Streptozotocin (Batch No - 082K0827,Sigma Aldrich, Mo.USA), nicotinamide (Batch No - 0001448241,Sigma Aldrich, Mo.USA), Biphasic isophane insulin injection (Batch No - B-50431, Human Mixtard, Torrent Pharmaceuticals LTD, India) were procured. Other chemicals such as disodium ethylenediamine tetra acetic acid, potassium dihydrogen phosphate, sodium chloride, hydrochloric acid, sucrose, tris buffer, thiobarbituric acid, trichlocroacetic acid, tetraethoxy propene, sodium bicarbonate, sodium carbonate, ethylenediamine tetra acetic acid, epinephrine bitartarate, sodium phosphate, 2-nitro benzoic acid, sodium carbonate, copper sulphate, anesthetic ether other chemicals were purchased from local vendors. All chemicals used were of analytical grade. Glucose kit (GOD/POD) (Batch No -16312), BUN kit (Batch No - 23710), Uric acid kit (Batch No - L39809), Creatinine kit (Batch No - 06212) were procured from Acuurex Biomedical Pvt. Ltd., Mumbai, India.

Preparation of solutions

Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. The solutions were prepared fresh each time.

Induction of diabetes and experimental design

For experimental induction of diabetic nephropathy in Wistar rats, we followed the protocol reported by [30-31]. In brief overnight fasted rats were injected with nicotinamide (110 mg/kg, *i.p*) and streptozotocin (65 mg/kg, *i.p.*) was injected 15 min after nicotinamide injection in all the groups except group (I) which was non-diabetic group. Rats were fed with glucose solution (4%) for 12 h to avoid hypoglycemia. Hyperglycemia confirmed after 72 h. Steady state of hyperglycemia reached after 7 days. Serum glucose was determined by the glucose oxidase peroxidase method. Rats having serum glucose more than 250 mg/dl were labelled 'diabetic' and selected for the study. In order to prevent subsequent development of ketonuria, biphasic isophane insulin (2 to 4U/rat) was injected subcutaneously on alternate days to maintain serum glucose levels during first four weeks. The body weight and serum glucose levels were measured every week.

Control and diabetic rats were randomly selected and divided into five groups, six animals in each group.

Group I: non-diabetic group.

Group II: diabetic group (nicotinamide 110 mg/kg + STZ 65 mg/kg *i.p.*),

Group III: diabetic treated with trigonelline (TRIG, 50 mg/kg *p.o.*),

Group IV: diabetic treated with sitagliptin (SITA, 5 mg/kg *p.o.*),

Group V: diabetic treated with trigonelline (TRIG, 50 mg/kg *p.o.*) + sitagliptin (SITA 5 mg/kg *p.o.*).

The rats were allowed to develop diabetic nephropathy for next four weeks. TRIG, SITA and concomitant TRIG + SITA were administered for next 4 weeks starting from 5th week of nicotinamide-STZ

injection. After 8 weeks, rats were killed by deep anesthesia and both kidneys were immediately isolated.

Collection of blood and determination of serum glucose

Blood from the experimental rats was withdrawn by retro orbital plexus technique using heparinized capillary glass tubes. The collected blood was placed in Eppendorff tubes (1.5 ml). The serum was separated by centrifugation using Eppendorff Cryocentrifuge (Model no 5810, Germany) maintained at 4 ⁰C and run at speed of 7000 r.p.m. for 15 min. 10 μl of serum and 1 ml of working reagent (GOD/POD) were mixed and incubated for 15 min at 37 ⁰C. The UV/ Visible spectrophotometer (Jasco V-530, Japan) reading was adjusted to 0 by measuring the absorbance of blank (distilled water). The absorbance of sample (A_s) and standard (A_{std} provided by manufacturer) were measured against blank at 505 nm. Glucose was estimated using formula: Glucose (mg/dl) = A_s / A_{std}*100, Where, A_s = Sample reading; A_{std} = Standard reading.

Body weight measurement

During the study period of 8 weeks, the body weight of rat was recorded daily using electronic balance. From this data, mean change in body weight and S.E.M were calculated.

Kidney weight determination

At the end day of treatment (8th week), kidney weight was measured using electronic weighing balance. From this data, mean change in kidney weight and S.E.M were calculated.

Renal function tests

Levels of serum creatinine, urine creatinine, blood urea nitrogen (BUN) and uric acid were measured using commercial diagnostic kits; mean change and S.E.M were calculated.

Evaluation of oxidative stress

At the end of the treatment, right kidney of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris –HCl buffer (pH7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD), reduced glutathione (GSH), lipid per-oxidation (MDA).SOD activity was determined by the method of Misera and Fridovich, 1972 [32]. GSH assay was carried out by method of Moron et al., 1979 [33]. Malondialdehyde assay was performed by method of Slater and Sawyer, 1971 [34]. From obtained data mean change in GSH, MDA, SOD and S.E.M were calculated.

Histological examination of kidneys

Isolated left kidney was cut into small pieces and preserved in 10% formalin for 24 h. Specimens were cut in section of 3-5μm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain and renal fiber staining by Masson's trichome (MT) stain. The stained samples were observed under microscope and analysed by cell imaging software for life sciences microscopy (Olympus soft imaging solution, Munster, Germany).

Statistical analysis

All of the data are expressed as mean ± S.E.M. Data obtained for serum glucose, body weight and biochemical estimation were analyzed separately by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 5 software. Separate analysis was performed for data on kidney weight, SOD, GSH, MDA levels on last day of study period (on week 8th) by one-way ANOVA followed by Dunnet's test for significance.

RESULTS

Effect of TRIG, SITA and their concomitant administration on serum glucose level

There was no significant difference in SG level in diabetic control rats compared to non diabetic rats before induction of diabetes. After four weeks nicotinamide-STZ injection resulted in significant (*P*<0.001) increase in SG level in diabetic rats (Group II), as compared to non diabetic rats (Group I). In the rats with confirmed

diabetes, TRIG (50 mg/kg) treatment showed significant decrease ($P<0.01$; $P<0.001$ and $P<0.001$) in SG levels after 2nd, 3rd and 4th week of treatment i.e. at 6th, 7th and 8th week post nicotinamide-STZ injection respectively as compared to diabetic rats. SITA (5 mg/kg) showed significantly decrease ($P<0.01$; $P<0.001$; $P<0.001$ and $P<0.001$) in SG levels at 1st, 2nd, 3rd and 4th week of treatment i.e. 5th, 6th, 7th and 8th week post nicotinamide-STZ injection, respectively as compared to diabetic rats. Moreover, treatment with concomitant administration of TRIG (50 mg/kg) + SITA (5 mg/kg) showed maximum reduction in SG ($P<0.01$; $P<0.001$; $P<0.001$ and $P<0.001$) levels at 1st, 2nd, 3rd and 4th week of treatment than that of drug alone (**Fig. 1**).

Effect of TRIG, SITA and their concomitant administration on body weight and kidney weight

There was no significant difference in body weight in diabetic control rats as compared to non diabetic rats before induction of diabetes. Four week post nicotinamide-STZ injection resulted in significant decrease in body weight in diabetic rats as compared to non diabetic rats. TRIG (50 mg/kg) treated rats showed significant increase in body weight ($P<0.001$) at 3rd and 4th week of treatment i.e. at 7th and 8th week post nicotinamide-STZ injection as compared to diabetic rats. SITA (5 mg/kg) showed significantly increase ($P<0.05$; $P<0.001$ and $P<0.001$) in body weight at 2nd, 3rd and 4th week of treatment as compared to diabetic control rats. Moreover, treatment with concomitant administration of TRIG (50 mg/kg) + SITA (5 mg/kg) showed more significant ($P<0.01$; $P<0.001$ and $P<0.001$) increase in body weight at 2nd, 3rd and 4th week of treatment than that of drug alone (**Fig. 2**).

Kidney weight after 8th week in diabetic control rats was significantly ($P<0.001$) increased as compared to non diabetic rats. TRIG and SITA showed marked ($P<0.01$) decrease in kidney weight as compared to diabetic control rats. Concomitant administration of (TRIG + SITA) showed more significant ($P<0.001$) reduction in kidney weight as compared to other groups (**Table 2**).

Effect of TRIG, SITA and their concomitant administration on serum creatinine, uric acid and BUN levels

Serum creatinine, serum uric acid and BUN did not significantly different in nicotinamide-STZ control rats as compared to non diabetic rats before induction of diabetes. Four week post nicotinamide-STZ *(i.p.)* injection showed significant increase in serum creatinine, uric acid and BUN levels as compared to non diabetic rats. Treatment with TRIG (50 mg/kg), SITA (5 mg/kg) and (TRIG+SITA) resulted in significant ($P<0.05$; $P<0.01$ and $P<0.001$) reduction in serum creatinine level at 4th week of treatment i.e. 8th week post nicotinamide-STZ injection compared to diabetic rats.

Treatment of TRIG, SITA and (TRIG+SITA) resulted in significant ($P<0.001$) reduction in serum uric acid level at 4th week of treatment compared to diabetic rats.

Treatment of TRIG, SITA and (TRIG+SITA) resulted in significant ($P<0.001$) reduction in BUN level at 4th week of treatment compared to diabetic rats (**Table 1**).

Effect of TRIG, SITA and their concomitant administration on urine creatinine and urine volume

Urine creatinine and urine volume did not differ significant in nicotinamide-STZ control rats as compared to non diabetic rats before induction of diabetes. Four week post nicotinamide-STZ showed significant reduction in urine creatinine and increase in urine volume levels of diabetic rats as compared to non diabetic rats. Chronic treatment with TRIG (50 mg/kg), SITA (5 mg/kg) and TRIG+SITA resulted in significant ($P<0.05$; $P<0.01$ and $P<0.001$) increase in urine creatinine levels after 4th week of treatment to diabetic control group.

Chronic treatment of TRIG, SITA and (TRIG+SITA) resulted in significant ($P<0.001$) reduction in urine volume at 4th week of treatment compared to diabetic control group. TRIG+SITA showed maximum increase in urine creatinine and decrease in urine volume than that of drug alone (**Table 1**).

Effect of TRIG, SITA and their concomitant administration on parameters of oxidative stress in renal tissue

The levels of MDA were significantly ($P<0.001$) increased in renal tissue of diabetic control rats as compared to non diabetic rats after eight weeks of study. The treatment of diabetic control rats with TRIG, SITA resulted in a significant ($P<0.05$) decrease in levels of MDA as compared to diabetic control rats. The level of MDA in diabetic rats treated with TRIG+SITA showed significant reduction ($P<0.01$) as compared to other groups.

In diabetic control rats significant ($P<0.001$) reduction in GSH and SOD levels were observed compared to non diabetic rats. The treatment with TRIG, SITA resulted in a significantly increase in GSH ($P<0.05$) and SOD ($P<0.05$) levels as compared diabetic control rats. Further, concomitant administration of (TRIG+SITA) showed significant increase in level of GSH ($P<0.01$) and SOD ($P<0.01$) compared with diabetic rats (**Table 2**).

Effect of TRIG, SITA and their concomitant administration on histological examination of renal tissue

After eight weeks of study examination of renal sections stained with hematoxylin & eosin (H&E) and Masson's trichome (MT) did not show changes in the architecture of the renal tissue of non diabetic rats. While renal tissue sections of diabetic animals showed severe glomerular injury, tubular swelling. MT stained sections of kidney diabetic animals showed glomerular fibrosis and peritubular fibrosis. In contrast kidney sections of TRIG treated animals showed moderate injury, tubular swelling with H&E stained. MT stained renal tissue of TRIG treated animals showed mild glomerular fibrosis and moderate peritubular fibrosis. H&E and MT stained renal tissue of SITA treated animals showed mild glomerular necrosis, moderate tubular swelling and moderate glomerular fibrosis and mild peritubular fibrosis. Treatment with (TRIG + SITA) showed mild glomerular fibrosis, absence of tubular swelling with peritubular fibrosis (**Fig. 3 and 4**).

DISCUSSION

Diabetic nephropathy in type two diabetes has become the single most important cause of end stage of renal disease worldwide [35]. Preventing the progression of diabetic nephropathy has been a tough goal in biomedical research [22]. Increased levels of serum glucose, creatinine, BUN, uric acid are the markers for diabetic nephropathy that can be commonly seen in this complication [36]. Administration of nicotinamide-STZ in Wistar rats produced marked and sustained increase in glucose level [37]. STZ cause diabetes by the rapid depletion of β-cells of pancreas [38-39]. Moreover, when administered along with nicotinamide, it causes minor damage to pancreatic β-cells [40].

High glucose level is the main factor responsible for structural alteration at the renal level [8]. Mesangial cells produce endogenous TGF-β1 under high glucose conditions which increase glucose uptake and glucose transport by inducing over expression of mRNA and protein GLUT-1. Ultimately, it initiates glucose induced metabolic abnormalities in mesangial cells [41]. Diabetic Control and Complication Trial Research Group in 1993 elucidated that hyperglycemia is directly linked to diabetic microvascular complications, particularly in kidney [42].

In the present study, concomitant administration of TRIG with SITA showed significant reduction in SG levels than treatment with either TRIG or SITA after four weeks of treatment. Previous report showed that trigonelline improved pancreatic regeneration in alloxan induced diabetes in mice [26]. Earlier findings suggest that hypoglycemic effect of trigonelline may be mediated by stimulating insulin synthesis and/or secretion from the beta pancreatic cells of Langerhans [43]. Sitagliptin enhance release of incretin hormone GLP-1 from small intestine that stimulates insulin secretion from the pancreas [19]. Literature has also revealed that ratio of pancreatic β-cells is maintained by sitagliptin in diabetic mice [44]. Nicotinamide-STZ induced type two diabetes caused a severe weight loss due to degradation or loss of proteins which contribute to the body weight [45-47]. In present study kidney weight in diabetic rats significantly increased as compared to normal rats. Concomitant administration

of TRIG and SITA significantly increased body weight and decreased kidney weight. These findings suggest that concomitant administration of TRIG and SITA may prevent kidney hypertrophy.

The abnormalities in kidney function progress by alteration in renal haemodynamics which leads to proteinuria, glomerulosclerosis and renal dysfunction [48]. Management of renal hemodynamic abnormality and reduction of proteinuria are important to prevent the decline of kidney function. Degradation of protein and nucleic acid results in the formation of non-protein nitrogenous compounds such as urea and creatinine [49]. A significant elevation in serum creatinine, uric acid and BUN levels is indicative of impaired renal function in diabetic animals. These are independent predictor for DN [50]. The present study demonstrated that concomitant administration of TRIG and SITA improved renal function, which was evident from the lowered serum uric acid, creatinine and blood urea nitrogen levels.

Glomerular filtration rate is calculated by creatinine levels in blood and urine which is indication of renal function. High creatinine level in urine is ideal while high blood creatinine level indicate alteration in the kidney function. GFR is ultimate indication for assessment of excretory functions of kidney [51-53]. Significant decrease in urine volume observed in the study; probably appear as a result of the normalization of SG level. The present study demonstrated that concomitant administration of TRIG and SITA significantly increased urine creatinine level and decreased urine volume than monotherapy.

Previous studies suggest that oxidative stress is provoked in DN [54]. Metabolic activity within the nephron generates a large amount of reactive oxygen species (ROS) that are balanced by antioxidant enzymes and free radical scavenging systems [55]. Various biological effects such as peroxidation of cell membrane lipids, oxidation of proteins, renal vasoconstriction and damage to DNA are aggravated by ROS [56]. High glucose level is associated with oxidative stress through generation of ROS from the mitochondrial electron transport chain [57]. Alterations in glucose metabolism disturb several pathways such as polyol pathway, advanced glycation and uncoupling of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) [58-59].

Table 1: Effect of TRIG, SITA and their concomitant administration on serum creatinine, uric acid, BUN and urine creatinine, urine volume levels

Parameters /Groups	W	ND	DC	TRIG	SITA	TRIG+SITA
Serum creatinine (mg/dl)	0	0.61 ± 0.12	0.58 ± 0.14^{ns}	0.54 ± 0.11	0.55 ± 0.09	0.57 ± 0.06
	4th	0.59 ± 0.08	$1.43\pm0.13^{\#\#\#}$	1.38 ± 0.12	1.41 ± 0.16	1.40 ± 0.28
	8th	0.57 ± 0.09	$1.84\pm0.19^{\#\#\#}$	$1.17\pm0.17^{*}$	$1.13\pm0.19^{**}$	$0.92\pm0.21^{***}$
BUN (mg/dl)	0	13.86 ± 0.47	14.70 ± 1.09^{ns}	12.11 ± 0.37	11.70 ± 0.40	12.27 ± 0.93
	4th	16.21 ± 0.81	$35.30\pm3.81^{\#\#\#}$	33.03 ± 2.52	34.39 ± 1.92	34.54 ± 3.25
	8th	18.71 ± 1.06	$39.11\pm3.14^{\#\#\#}$	$22.27\pm2.35^{***}$	$21.61\pm1.98^{***}$	$18.68\pm1.87^{***}$
Serum	0	1.82 ± 0.11	1.90 ± 0.05^{ns}	1.88 ± 0.06	1.90 ± 0.07	1.87 ± 0.07
Uric acid (mg/dl)	4th	2.03 ± 0.31	$4.20\pm0.23^{\#\#\#}$	4.15 ± 0.26	4.12 ± 0.10	4.16 ± 0.10
	8th	2.01 ± 0.27	$4.62\pm0.17^{\#\#\#}$	$3.26\pm0.14^{***}$	$2.99\pm0.10^{***}$	$2.26\pm0.08^{***}$
Urine	0	56.56 ± 3.29	57.40 ± 1.86^{ns}	55.88 ± 1.00	56.66 ± 1.11	57.25 ± 0.80
creatinine (mg/dl)	4th	57.25 ± 3.88	$24.84\pm2.08^{\#\#\#}$	24.45 ± 2.28	23.76 ± 2.10	24.61 ± 2.29
	8th	56.63 ± 6.40	$13.81\pm1.69^{\#\#\#}$	$26.08\pm4.60^{*}$	$29.47\pm5.15^{**}$	$35.03\pm4.04^{***}$
Urine volume (ml)	0	9.83 ± 0.30	10.66 ± 0.42^{ns}	10.50 ± 0.76	9.58 ± 0.73	10.16 ± 0.60
	4th	11.16 ± 0.60	$51.48\pm2.51^{\#\#\#}$	49.50 ± 1.43	49.75 ± 1.23	50.11 ± 1.27
	8th	12.75 ± 0.35	$55.91\pm2.47^{\#\#\#}$	$35.58\pm1.25^{***}$	$32.41\pm1.57^{***}$	$27.08\pm1.64^{***}$

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; ns= non significant, $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$ as compared with diabetic control, $^{\#\#\#}$ $P< 0.001$ as compared with non diabetic. W: Week, ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.

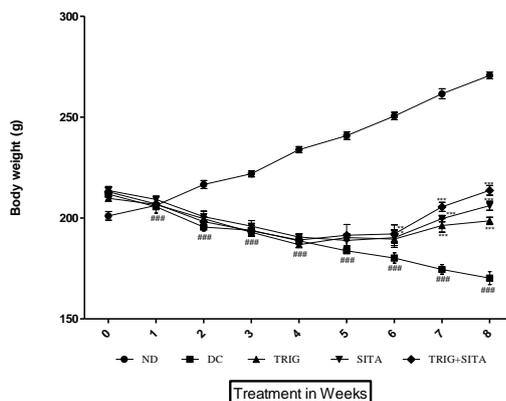**Table 2: Effect of TRIG, SITA and their concomitant administration on Oxidative stress**

Parameters /Groups	ND	DC	TRIG	SITA	TRIG+SITA
Kidney weight (g)	0.64 ± 0.03	$1.26\pm0.03^{\#\#\#}$	$1.01\pm0.06^{**}$	$0.99\pm0.03^{**}$	$0.87\pm0.06^{***}$
MDA (nmol of MDA/mg protein)	4.17 ± 0.39	$8.57\pm0.88^{\#\#\#}$	$6.05\pm0.89^{*}$	$5.51\pm0.33^{*}$	$4.46\pm0.36^{**}$
GSH (g of GSH/mg protein)	41.09 ± 1.39	$22.45\pm0.76^{\#\#\#}$	$29.80\pm1.13^{*}$	$31.69\pm3.24^{*}$	$33.74\pm1.88^{**}$
SOD (Unit /mg protein)	17.73 ± 2.12	$07.58\pm0.67^{\#\#\#}$	$13.14\pm1.10^{*}$	$13.43\pm1.22^{*}$	$15.04\pm0.98^{**}$

Number of rats per group = 6; data on each parameter were analyzed by one-way ANOVA followed by Dunnet's test; $^{*}P< 0.05$, $^{**}P< 0.01$, *** $P< 0.001$ as compared with diabetic control, $^{\#\#\#}P< 0.001$ as compared with non diabetic. ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.
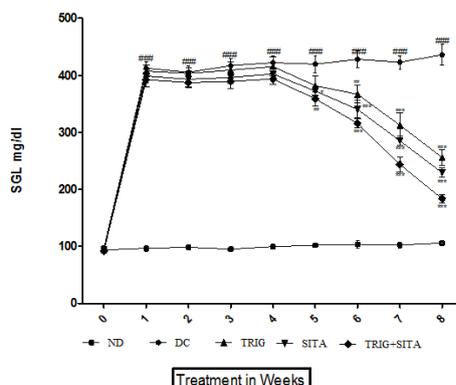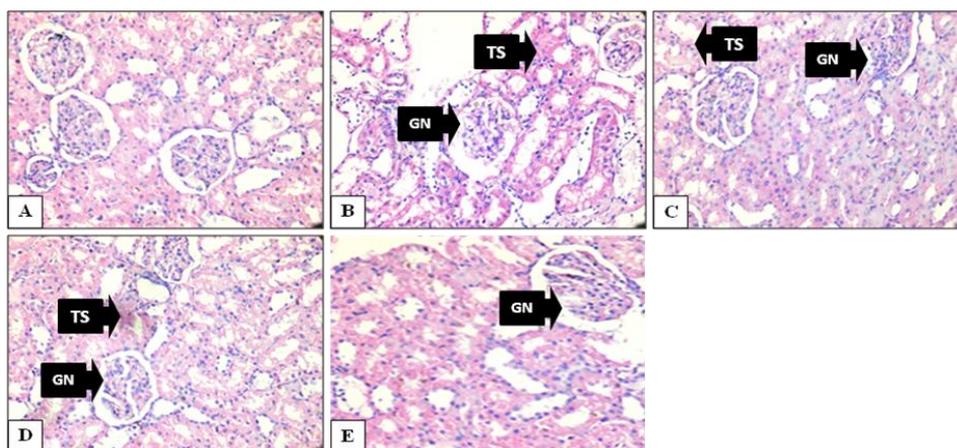

Fig. 1: Effect of TRIG, SITA and their concomitant administration serum glucose level

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; *P< 0.05, **P< 0.01, ***P< 0.001 as compared with diabetic control, ### P< 0.001 as compared with non diabetic. W: Week, ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.
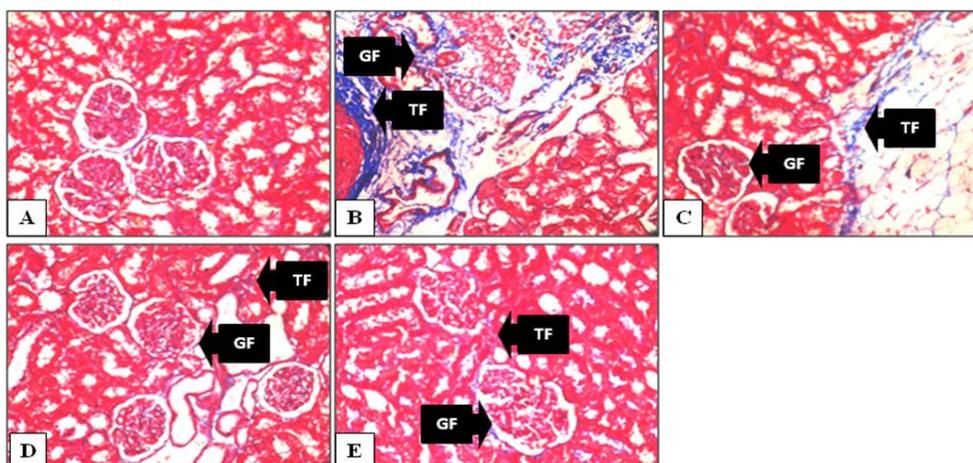
Fig. 2: Effect of TRIG, SITA and their concomitant administration on body weight

Number of rats per group = 6; data were analyzed by two-way ANOVA followed by Bonferroni post tests; *$P< 0.05$, **$P< 0.01$, ***$P< 0.001$ as compared with diabetic control, ### $P< 0.001$ as compared with non diabetic. W: Week, ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.

Fig. 3: Effect of TRIG, SITA and their concomitant administration on histological examination of renal tissue with H&E stain

A)Non diabetic group: Normal (Grade -); **B)**Diabetic control group: glomerular necrosis (Grade +++), tubular swelling (Grade +++); **C)**Trigonelline (TRIG) 50mg/kg: glomerular necrosis (Grade ++), tubular swelling (Grade ++); **D)**Sitagliptin (SITA) 5 mg/kg: glomerular necrosis (Grade +),tubular swelling (Grade ++); **E)**Trigonelline (TRIG) 50 mg/kg+ Sitagliptin (SITA) 5 mg/kg: glomerular necrosis (Grade +), tubular swelling (Grade -). Stain with hematoxyline and eosin. Grade (-) Normal, Grade (+++) severe injury, Grade (++) moderate injury, Grade (+) mild injury. (Magnification: 40x)

Fig. 4: Effect of TRIG, SITA and their concomitant administration on histological examination of renal tissue with MT stain

A)Non diabetic group: Normal (Grade -); **B)**Diabetic control group: glomerular fibrosis (Grade +++), peritubular fibrosis (Grade +++); **C)**Trigonelline (TRIG) 50 mg/kg: glomerular fibrosis (Grade +), peritubular fibrosis (Grade ++); **D)**Sitagliptin (SITA) 5 mg/kg: glomerular fibrosis (Grade ++), peritubular fibrosis (Grade +); **E)**Trigonelline (TRIG) 50 mg/kg+ Sitagliptin (SITA) 5 mg/kg: glomerular fibrosis (Grade +), peritubular fibrosis (Grade +).Stain with Masson's trichome. Grade (-) Normal, Grade (+++) severe injury, Grade (++) moderate injury, Grade (+) mild injury. (Magnification: 100x)

Oxygen free radicals exert their cytotoxic effects on membrane phospholipids resulting in the formation of lipid peroxidation product malondialdehyde (MDA) [60]. MDA is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation [61]. The present study showed that the renal concentration of MDA was elevated and that of GSH, SOD decreased in diabetic animals. The obtained data are in line with previous work [8, 29]. Chronic treatment with concomitant administration of TRIG+SITA significantly decreased MDA content and increased SOD, GSH concentration than alone treatment. Elevated concentration of MDA is due to reactive oxygen species (ROS). Moreover decreased SOD activity and GSH concentration may be due to defense mechanism against oxygen free radicals.

In previous study, non diabetic rat kidneys showed normal morphology of glomerular and tubular architecture [62]. Kidneys of diabetic rat showed glomerular damage and severe destruction of tubules occurs due to factors such as glomerular hyperplasia, tubular hypertrophy and interstitial expansion [63]. In the present study, non diabetic kidney showed normal morphology; while diabetic kidney showed glomerular necrosis, tubular swellings, glomerular and renal fibrosis. Concomitant administrations of TRIG + SITA showed renal prevention the damage was mild in nature.

CONCLUSION

The result of the present study showed that combination of trigonelline and sitagliptin compared to monotherapy have better renoprotective effects in diabetic rats. However, the concomitant administration of trigonelline along with sitagliptin not only attenuated the glucose homeostasis but also showed significant decrease in kidney weight, serum creatinine, BUN, serum uric acid, urine volume, the level of lipid peroxidation product, MDA and significant increase in the urine creatinine, activity of endogenous antioxidants such as SOD and GSH. Results obtained from histopathological study confirmed that concomitant administration prevented kidney damage, which provided structural support for the renal shielding effect.

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