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Research Article

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Concomitant administration of trigonelline and sitagliptin attenuates nicotinamide-streptozotocin induced diabetic neuropathy in wistar rats

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ABSTRACT

The objective of the present study was to investigate the effect of concomitant administration of trigonelline (TRIG) along with sitagliptin (SITA) on glucose level, behavior, oxidative stress and histological studies in nicotinamide-streptozotocin induced diabetic neuropathy in Wistar rats. Diabetes was induced by administration of streptozotocin (65 mg/kg i.p), 15 min after nicotinamide (110mg/kg) injection. After developing hyperglycemia, control and diabetic rats were randomly selected and divided into five groups (n=6). These includes, Group 1 was: non-diabetic, Group 2: diabetic control, Group 3: diabetic + TRIG (50 mg/kg), Group 4: diabetic + SITA (5 mg/kg), Group 5: diabetic + TRIG (50mg/kg) + SITA (5 mg/kg). TRIG, SITA and TRIG+SITA administered for 4 weeks. Serum glucose (SG) levels and body weights of animals were measured at every week of study period. Motor nerve conduction velocity and behavioral parameters were also screened at 0, 4th and at 8th weeks of study. Levels of SOD, MDA and histopathological study were carried out on the last day of study. Administration of these drugs resulting significant reduction in SG levels, decreasing lipid peroxidation and increase in the SOD levels. Histopathology examination of the sciatic nerve sections from TRIG+SITA treated rats showed absence of necrosis, mild congestion and swellings as compared to monotherapy. Study undertaken demonstrated that concomitant treatment of TRIG + SITA showed promising therapeutic effect against experimental diabetic neuropathy in rats than monotherapy.

Keywords: Trigonelline, sitagliptin, diabetic neuropathy.

INTRODUCTION

Diabetic neuropathy is most frequent microvascular complication of diabetes mellitus [1]. Predominance of diabetic neuropathy is rising with the global burden of type 2 diabetes [2,3]. Diabetes affects approximately 246 million people worldwide [4]. Recently, it is estimated that 20-30 million people are exaggerated by diabetic neuropathy [5,6]. Diabetic neuropathy commonly classified as peripheral, autonomic, proximal, focal and multifocal or mixed [7]. Peripheral neuropathy is the most common type of diabetic neuropathy, clinically apparent that it causes either negative (lack of sensation) and positive (painful) symptoms, or both [8]. Symptoms characteristically associated with damage to motor nerve are muscle weakness, cramps, spasms, loss of balance and coordination [9]. Damage to sensory nerve may produce tingling, numbness and burning pain, which is typically characterized by two common features such as mechanical and thermal hyperalgesia or allodynia [8,10,11]. Diabetic peripheral neuropathy is present in up to 50% of all diabetic patients with long duration of disorder and is a major cause of morbidity which is also associated with increased mortality [12].

Clinical and preclinical literature has also revealed that long term hyperglycemia plays a major role in the diabetic neuropathy through much metabolic and structural rearrangement [13,14]. It includes various pathogenic mechanisms such as increased production of advanced glycation end products, polyol pathway, activation of protein kinase C isoforms and increased oxidative stress, and abnormalities of nitric oxide production [15]. It affects all

peripheral nerves including pain fibers, motor neurons and the autonomic nervous system which ultimately affect all organs and systems [16].

Neuronal pharmacotherapy's from several class includes selective serotonin reuptake inhibitors, tricyclic antidepressant, opoids and antioxidant, anticonvulsants, protein kinase C (PKC) inhibitors, growth factors, COX-2 inhibitors and non-steroidal anti-inflammatory drugs as mild analgesics [17,18]. Monotherapy treatment is often inadequate due to associated side effects and partial effectiveness and therefore in clinical practice drugs with different mechanisms of actions are generally combined for their synergistic or additive effects and to reduce the unwanted side effects of existing drug therapy [19]. Single drug only inhibits one or two pathways only [20]. Hence, combination of drugs may be used to prevent various side effects which occur due to single drug therapy [21]. Furthermore, diseases can be cured possibly by acting through different mechanism [22].

Treatment of diabetic neuropathy is therefore a major goal [23, 24]. Now a day, diabetes health care professionals such as health care providers, researchers, educators and people acknowledge their interest in alternative medicines [25]. Trigonelline is a plant alkaloid [26]. Preclinical and clinically marked that has antihyperglycemic activity [27, 28]. It has also shown antioxidant and hypolipidemic effects on diabetes [29, 30]. Sitagliptin is currently approved by the U.S. Food and Drug Administration (FDA) on October 17, 2006 and available in many countries as an antihyperglycemic agent in type 2 diabetic patients [31]. In response to food intake it synthesize and secrete incretin hormones like Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) from the L-cells of the gastrointestinal tract [32]. GLP-1 and related peptides improves glucostasis by stimulating endogenous insulin secretion [33]. GLP-1 possesses neurotrophic and neuroprotective effects [34].

Concomitant administration of trigonelline (TRIG 50mg/kg, *p.o*) +sitagliptin (SITA 5mg/kg, *p.o*) significantly reduced serum glucose, glycated hemoglobin, insulin levels and modifies the disturbed architecture of pancreas in nicotinamide-streptozotocin induced diabetes in Wistar rats [35]. Through literature survey revealed that; no reports are available on the concomitant administration of trigonelline and sitagliptin on diabetic neuropathy.

Hence, objective of the study undertaken was to investigate the effect of concomitant administration of trigonelline (TRIG) and sitagliptin (SITA) in nicotinamide (NICO) - streptozotocin (STZ) induced neuropathy in Wistar rats.

EXPERIMENTAL SECTION

Animals

Male Wistar rats (200-250 g) were purchased from National Toxicology Center, Pune. The animals were housed in polypropylene cages at an ambient temperature of 25±1ºC and relative humidity (45-55%), with a 12:12 h light/dark cycle. Commercial food pellets provided by Navmaharashtra Chakan Oil Mills Ltd., Sangli, India and water *ad libitum*. The research protocol was approved by Institutional Animal Ethical Committee (IAEC), Poona College of Pharmacy, Pune. The Animals were acclimatized to laboratory conditions for at least one week before using them for experiments and were subjected only once to the experimental conditions. Principles of laboratory animal care CPCSEA guidelines were followed in the research study.

Chemicals and Reagents

Trigonelline (Sigma Aldrich, Mo. USA), Sitagliptin (Hangzhou longshine Bio-Tech Co. Ltd, China).Streptozotocin (Sigma Aldrich, Mo. USA) and nicotinamide(Sigma Aldrich, Mo.USA), Glucose kit (GOD/POD) provide by (Acuurex Biomedical Pvt. Ltd., Mumbai, India). 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 tetracetic acid and other chemicals were purchased from respective vendors. All chemicals used were of analytical grade. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline.

Preparation of the drug solutions

Trigonelline and sitagliptin solutions were prepared by dissolving in distilled water. The volumes of drug solutions were calculated based upon the body weight of the animal. The drug solution was prepared fresh daily and stored at cool and dry place in dark before use.

Induction of diabetes and experimental design

Induction of diabetic neuropathy was performed as per reported methods [36, 37]. Overnight fasted rats of all groups except group1 non-diabetic group were injected with NICO (110 mg/kg, *i.p*) then after 15 min rats injected with STZ (65 mg/kg, *i.p*). Rats were fed with glucose solution (4%) for 12 h to avoid hypoglycemia. Hyperglycemia was confirmed after 72 h. Steady state hyperglycemia was reached after 7 days. Serum glucose was determined by the

GOD/POD method. Rats having serum glucose more than 250 mg /dl were labelled 'diabetic' and selected for the study. To prevent subsequent development of ketonuria, diabetic rats were given alternate day subcutaneous injections of insulin (2-4 U/rat) to maintain serum glucose levels in the range of 250-300 mg/dl throughout the experimental period. The serum glucose levels and body weight were measured weekly.

After establishing hyperglycemia, control and diabetic rats were randomly selected and divided into five groups (n=6). Group 1: non-diabetic control. Group 2: diabetic control group (NICO 110 mg/kg + STZ 65 mg/kg *i.p*), Group 3: diabetic + trigonelline (TRIG, 50 mg/kg *p.o*), Group 4: diabetic + sitagliptin (SITA, 5 mg/kg *p.o*), Group 5: diabetic + trigonelline (TRIG, 50mg/kg *p.o*) + sitagliptin (SITA 5 mg/kg *p.o*).The rats were allowed to develop diabetic neuropathy for four weeks. TRIG, SITA and their concomitant administration of TRIG+ SITA for next 4 weeks starting from 5^{th} week to 8^{th} week. Radiant heat test, Randall–Selitto paw pressure test, Von-Frey hair test, motor nerve conduction velocity (MNCV) were assessed from week 0 to week 8 for all groups on regular basis. After 8 weeks, rats were killed under deep anesthesia and sciatic nerve were immediately isolated and fixed in 10% formalin for morphological examination and nerve tissue homogenate was prepared in 0.1 M tris –HCl buffer (pH 7.4) for the biochemical assay.

Blood sample collection and determination of serum glucose

Blood from the experimental rats was withdrawn by retro orbital plexus technique using 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 ^{0}C 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 ^{0}C. The UV Visible spectrophotometer (Jasco V-530, Japan) the analysis. The absorbance of sample and standard provided by manufacturer (Acuurex Biomedical Pvt Ltd., Mumbai, India) were measured against blank at 505 nm wavelength.

Effect on body weight

Body weight of rats was recorded daily using electronic balance. From this data, mean change in body weight and S.E.M were calculated.

Assessment of behavioral tests

Thermal hyperalgesia (Radiant heat test)

Radiant heat hyperalgesia of the left hind paw was assessed using the radiant heat lamp source as per reported method [38], for assessing the reactivity to noxious thermal stimuli. The intensity of the radiant heat stimulus was maintained at 55 ± 0.1°C. Response of left hind paw withdrawal threshold was noted. Cut-off time of 10 s was maintained.

Mechanical hyperalgesia (Randall–Selitto paw pressure test)

The nociceptive flexion reflex was quantified using the Randall-Sellito paw pressure device (UGO Basile SRL Biological Research Apparatus, Italy) as per the method available in the literature [39] which applies a linearly increasing mechanical force in (g) to the dorsum of the rat hind paw. Nociceptive threshold, expressed in (g), were applied by increasing pressure to the hind paw until squeak (vocalization threshold) was entitled. The paw of the rat was placed under the tip and the progressive pressure applied until the rat vocalized. The nociceptive threshold was measured three or four times in order to obtain two consecutive values that differed more than 10% and respecting an interval of at least 10 min between two measures.

Mechano-tactile allodynia (Von-Frey hair test)

Mechanical allodynia was assessed using Von-Frey hair apparatus as per published method described by [40]. Rats were placed individually on an elevated mesh in a clear plastic cage and adapted to the testing environment for at least 15 min. Von-Frey hairs (IITC, Woodland Hills, USA) with calibrated bending forces in (g) of different intensities were used to deliver mechanical stimuli of varying intensity. Starting with the lowest filament force, Von-Frey hairs were applied from below the mesh floor to the planter surface of the hind paw, with sufficient force to cause slight bending against the paw and held for 1sec. Each stimulation was applied five times with and simultaneous interval of 4-5 sec. Care was taken to stimulate random locations on the planter surface. A positive response was noted if the paw was robustly and immediately withdrawn.

Electrophysiological study (MNCV)

Electrophysiological study [41] rats were anesthetized using ketamine (50 mg/kg *i.p.*). To minimize effects of differences in body temperature on motor nerve conduction velocity (MNCV), subjects were allowed to acclimatize under a 40 W light bulb for 15 min before procedure. The left leg of rat was shaved and cleaned. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch, respectively by a 200 µs square wave

pulse delivered through a pair of mono-polar needle electrodes through stimulator. Recordings were obtained by using Student Power Lab 8 channel data acquisition system (AD Instrument Pvt. Ltd., Lab Chart 7.3, Australia).

Biochemical estimation

On the last day (8^{th} week) of study period, sciatic nerve of individual rat were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris –HCl buffer (pH 7.4). The supernatant obtained was used to estimate superoxide dismutase (SOD) and lipid per-oxidation malondialdehyde (MDA). SOD activity was determined by the method of [42]. MDA assay was performed by method of [43].

Histopathological examination of the sciatic nerve

At the end of 8^{th} week, rats were killing under deep anesthesia and sciatic nerves were carefully removed. Isolated nerves were kept in fixative solution (10%) formalin. It was then cut in section of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain as [44]. H&E staining was performed to analyze nerve section quantitatively under light microscope (40X) for histopathological alterations such as necrosis, swelling and congestion.

Statistical analysis

Data was expressed as mean ± standard error mean (SEM). Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, USA).Stastical analysis was performed by ONE WAY ANNOVA followed by *Dunnets test* for oxidative stress and TWO WAY ANNOVA followed by *Borfforoni test* for SG, body weight, thermal hyperalgesia andmechanical hyperalgesia, mechano tactile allodynia. A value of $P < 0.05$ was considered to be statistically significant.

Pharmacological Evaluation

Effect of TRIG, SITA and concomitant administration of TRIG + SITA on serum glucose level in NICO-STZ induced diabetic neuropathy in Wistar rats

Before induction of diabetes there was no significant change in SG level in diabetic control rats as compared to nondiabetic rats. At the end of after 4^{th} week, after NICO-STZ injection there was significant (P< 0.001) increase in SG levels as compared to normal rats. In this respect, treatment with TRIG (50mg/kg) for 4 week showed significant (*P*<0.01, *P*<0.001 and *P*<0.001) reduction in SG at 6^{th}, 7^{th} and 8^{th} week of treatment. SITA (5 mg/kg) treated diabetic rats showed significant (*P*<0.05, *P*<0.01, *P*<0.001 and *P*<0.001) reduction in SG at 5^{th}, 6^{th}, 7^{th} and 8^{th} week of treatment. Moreover, concomitant administration of TRIG+SITA showed maximum reduction in SG (*P*<0.01, *P*<0.001, *P*<0.001 and *P*<0.001) on 5^{th}, 6^{th}, 7^{th} and 8^{th} than its monotherapy [Fig- ure 1(A)].

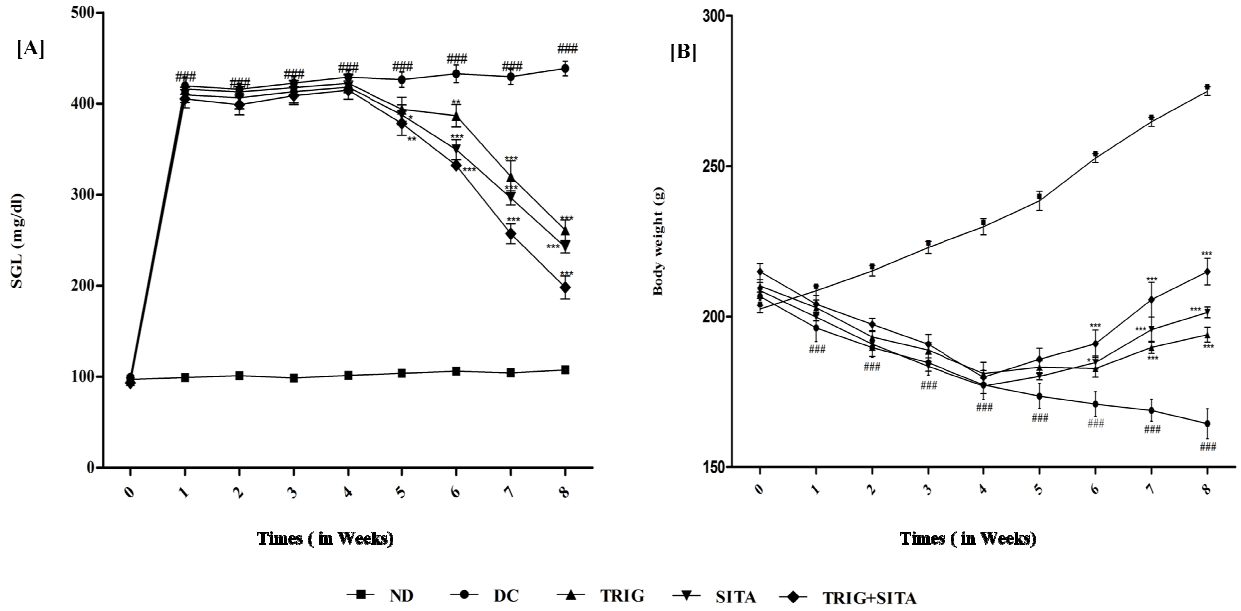

Figure 1: Effect of TRIG, SITA and Concomitant administration of TRIG + SITA on SG levels and body weight in NICO-STZ induced diabetic neuropathy in Wistar rats

*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< 0.05, ## P< 0.01 ### P< 0.001 as compared with non diabetic. W: Week, ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.*

Effect of TRIG, SITA and concomitant administration of TRIG + SITA on body weight in NICO-STZ induced diabetic neuropathy in Wistar rats

Before induction of diabetes there was no significant change in body weight in diabetic rats as compared to nondiabetic rats. Post four weeks of NICO-STZ injection, there was significant reduction (P< 0.001) in body weight of diabetic rats (Group 2) as compared to non diabetic rats (Group 1). Treatment with TRIG (50mg/kg) from 4th week of diabetic neuropathy induction up to 8th week, there was significant increase (*P*<0.001 and *P*<0.001) in SG at 7th and 8th week of treatment. Treatment with SITA (5 mg/kg) showed significant reduction (*P*<0.05, *P*<0.001 and *P*<0.001) in SG at 6th, 7th and 8th week of treatment. However, concomitant administration of TRIG+SITA showed significant (*P*<0.001, *P*<0.001 and *P*<0.001) increase in body weight at 6th, 7th and 8th week of treatment, indicating effectiveness of concomitant therapy in preventing weight loss than its monotherapy [Figure 1(B)].

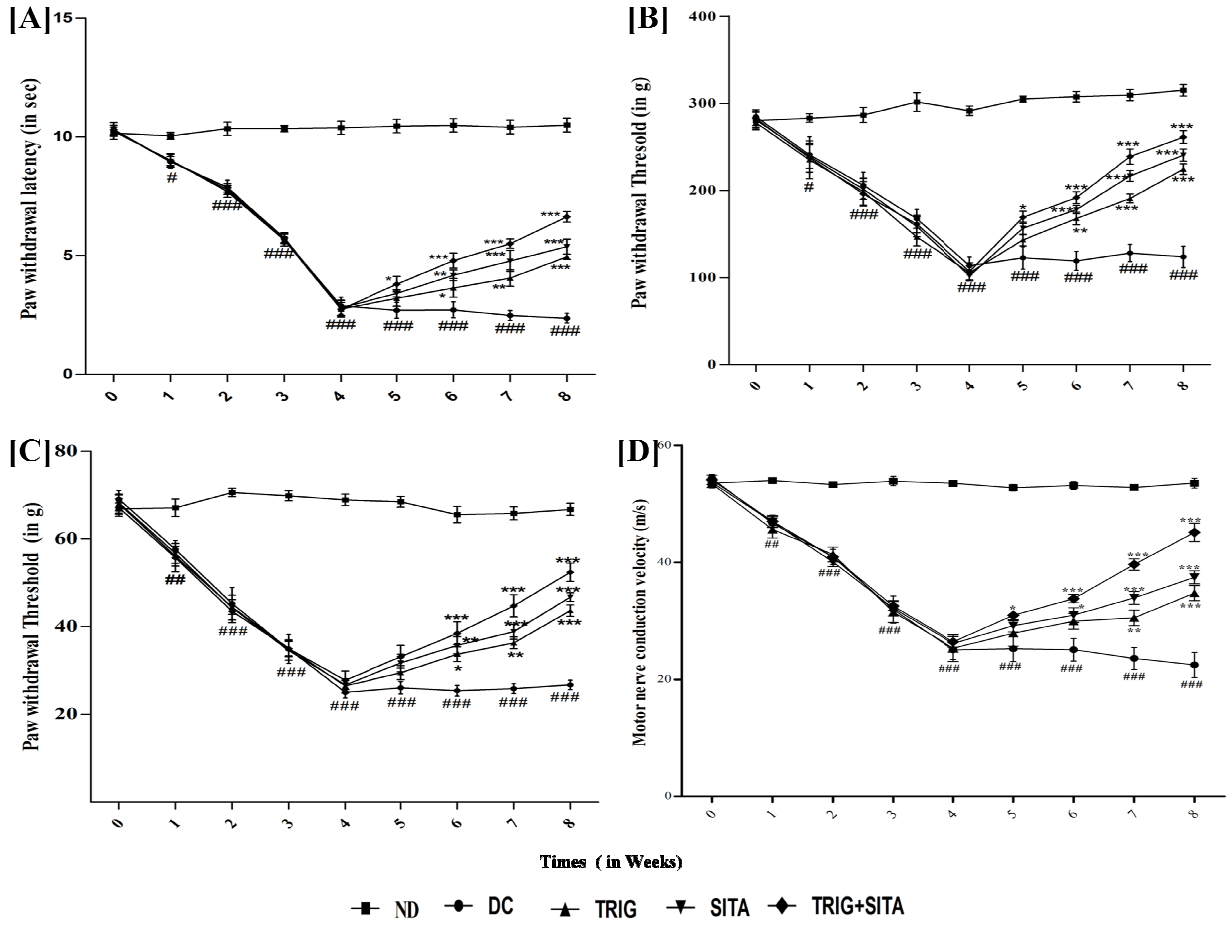

Figure 2: Effect of TRIG, SITA and their Concomitant administration of TRIG + SITA on [A] Thermal hyperalgesia, [B] Mechanical hyperalgesia, [C] Mechano-tectile allodynia and [D] Electrophysiological study (MNCV) in NICO-STZ induced diabetic neuropathy in Wistar rats

Number of rats per group (n= 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< 0.05, $^{\#\#}$ P< 0.01 $^{\#\#\#}$ P< 0.001 as compared with non diabetic. W: Week, ND: Nondiabetic, DC: Diabetic control, TRIG: Trigonelline, SITA: Sitagliptin, TRIG+SITA: Trigonelline + Sitagliptin.

Effect of TRIG, SITA and concomitant administration of TRIG+SITA on thermal hyperalgesia in NICO-STZ induced diabetic neuropathy in Wistar rats

Paw withdrawal latency in diabetic rats before induction of diabetic neuropathy was not significantly different than that in nondiabetic rats on day 0. Significant decrease (*P*<0.001) in paw withdrawal latency was observed in diabetic rats after four weeks of intraperitoneal injection of NICO-STZ as compared to diabetic rats. Diabetic rats treated with TRIG (50mg/kg) for four weeks showed significant (*P*<0.05, *P*<0.01, *P*<0.001) increase in paw withdrawal latency at 6th, 7th and 8th week of treatment. Whereas SITA (5mg/kg) showed significant increase (*P*<0.01, *P*<0.001, *P*<0.001) in paw withdrawal latency at 6th, 7th and 8th week of treatment. However, concomitant administration of TRIG+SITA showed more significant (*P*<0.05, *P*<0.001, *P*<0.001 and *P*<0.001) increased in paw withdrawal at 5th, 6th, 7th and 8th latency as compared to TRIG or SITA alone treated groups [Figure 2(A)].

Effect of TRIG, SITA and TRIG+SITA on mechanical hyperalgesia in NICO-STZ induced diabetic neuropathy in Wistar rats

There was no significant difference in mechanical hyperalgesia i.e. paw withdrawal threshold in diabetic rats before induction of neuropathy on day 0. Four week post NICO-STZ injection showed significant reduction in paw withdrawal threshold in diabetic rats as compared to nondiabetic rats. Chronic treatment with TRIG (50mg/kg) for four weeks showed significant (P<0.01, P<0.001 and P<0.001) increase in paw withdrawal threshold at 6th, 7th and 8th week of treatment as compared to control rats. SITA (5mg/kg) also significant increase (P<0.01, P<0.001 and P<0.001) in paw withdrawal threshold at 6th, 7th and 8th during four week of treatment when compared with diabetic control. However, increase in paw withdrawal threshold by concomitant administration of TRIG+SITA was more significant (P<0.001, P<0.001 and P<0.001) at 5th , 6th, 7th and 8th as compared to TRIG or SITA alone treated groups [Figure 2(B)].

Effect of TRIG, SITA and concomitant administration of TRIG+SITA on mechano- tactile allodynia in NICO-STZ induced diabetic neuropathy in Wistar rats

Paw withdrawal threshold in diabetic rats before induction of diabetic neuropathy was not significantly different than that of non diabetic rats on day 0. Four week of post intraperitonelly NICO-STZ injection, the significant decrease in paw withdrawal threshold was recorded in diabetic rats as compared to non diabetic rats. TRIG treated rats showed significant increase (P<0.05, P<0.01 and P<0.001) in paw withdrawal threshold at 6th, 7th and 8th week of treatment when compared with diabetic control rats. SITA (5mg/kg) showed significant (P<0.01, P<0.001 and P<0.001) increase in paw withdrawal threshold at 6th, 7th and 8th week of treatment. However, TRIG+SITA showed more significant increase (P<0.001, P<0.001 and P<0.001) in paw withdrawal threshold at 6th, 7th and 8th week of treatment compared to TRIG or SITA alone treated groups [Figure 2(C)].

Effect of TRIG, SITA and TRIG + SITA on electrophysiological study

There was no significant change in motor nerve conduction velocity (MNCV) in diabetic rats compared to nondiabetic rats on day 0.A significant reduction in MNCV in diabetic rats after four weeks of intraperitoneal injection of NICO-STZ injection as compared to nondiabetic rats. Chronic treatment with TRIG (50 mg/kg *p.o.*) showed significant increase (P<0.01, P<0.001) in MNCV on 7th and 8th week of treatment as compared to diabetic rats. SITA (5mg/kg *p.o.*) showed significant increase (P<0.01, P<0.001, P<0.001) in MNCV on 6th, 7th and 8th week of treatment when compared with diabetic rats. However, treatment with TRIG+SITA showed more significant (P<0.01, P<0.001, P<0.001) increase in MNCV at 6th, 7th and 8th week of treatment as compared to TRIG or SITA alone treated groups [Figure 2(D)].

Effect of TRIG, SITA and TRIG+SITA on SOD and MDA levels in NICO-STZ induced diabetic neuropathy in Wistar rats

The level of SOD was significantly decreased (P<0.001) in sciatic nerve tissue of diabetic rats as compared to non diabetic rats. Treatment with TRIG and SITA showed significant increase (P<0.01) in level of SOD as compared to diabetic rats. However TRIG+SITA treated rats showed more significant increase (P<0.001) in level of SOD as compared to TRIG or SITA alone treated groups. In diabetic rats there was significant increase (P<0.001) in MDA level as compared to non diabetic rats. Treatment with TRIG and SITA resulted in significant (P<0.01, P<0.001) decrease in the level of MDA as compared to diabetic rats. Further, treatment with TRIG+SITA showed more significant (P<0.001) decrease in the MDA level as compared to TRIG or SITA alone treated groups [Table 1].

Table 1. Effect of TRIG, SITA and their concomitant administration of TRIG+SITA oxidative stress

Parameters /Groups	ND	DC	TRIG	SITA	TRIG+SITA
SOD (Unit /mg protein)	22.12±4.93	5.30±2.24$^{###}$	14.98±2.02**	16.21±3.95**	18.40±3.40***
MDA (nmol of MDA/mg protein)	3.83±0.31	9.74±0.87$^{###}$	7.39±0.51**	6.34±1.34***	4.36±0.53****

Number of rats per group (n= 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.

Histological examination of sciatic nerve in NICO-STZ induced diabetic neuropathy in Wistar rats

Sections of Sciatic nerve of diabetic rats showed various histological alterations as necrosis, swelling, congestion compared to nondiabetic rats. With TRIG(50 mg/kg) treatment for four weeks showed decrease in necrosis (++),swelling (+),congestion (+).The histology of sciatic nerve of tats treated with SITA(5mg/kg) showed; necrosis (++), swelling (+), congestion (+).The histology of sciatic nerve of tats treated with concomitant administration of TRIG+SITA showed higher attenuation in necrosis (--), swelling (+), congestion (+) as compared to TRIG or SITA alone treated groups [Figure 3].

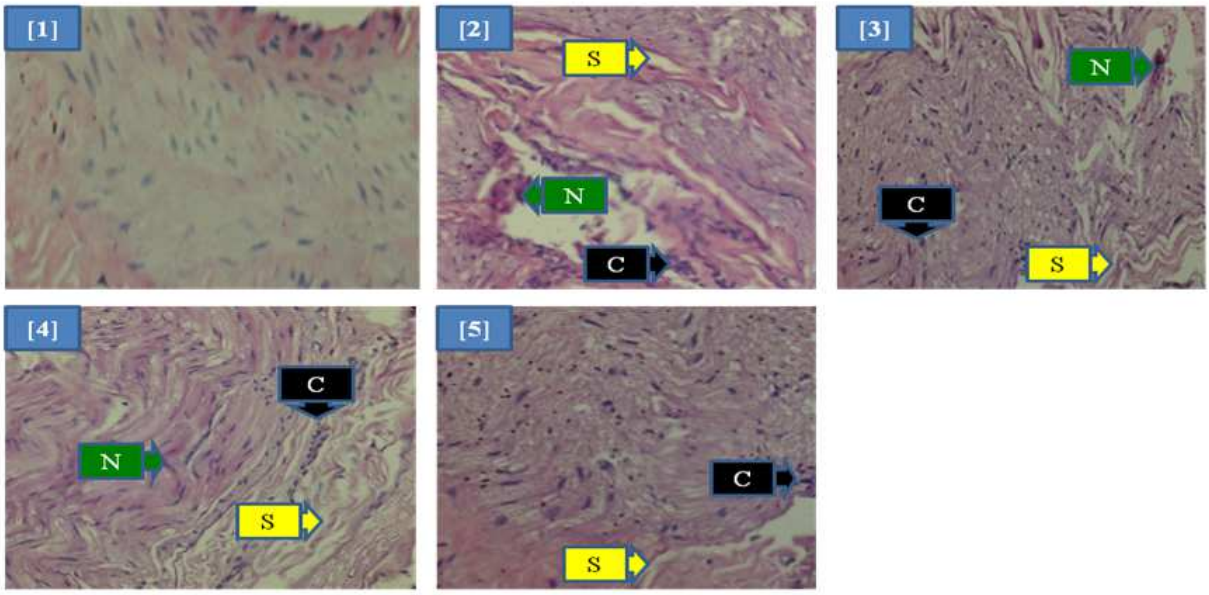

Figure 3: Effect of treatment of TRIG (50mg/kg) and SITA (5mg/kg) on histopathological examination of sciatic nerve in NICO-STZ induced diabetic neuropathy with H & E stain

Where [1] normal rats, [2] diabetic rats, [3] TRIG (50mg/kg), [4] SITA (5mg/kg) and [5] TRIG (50mg/kg) +SITA (5mg/kg) concomitant treated rats (microscopic examination under 40X light microscopy). Where, N- necrosis; S- swelling; C-congestion and +++ = more than 75%; ++ = more than %; + = more than 25% and -- = absence.

DISCUSSION

Diabetic neuropathy threatens quality of life affecting function, mood and sleep pattern of the majority of affected patients [45]. About 33.5% of patients with diabetic neuropathy are strongly related to serious mortality and mobility rates [46]. It is characterized by clinical features like allodynia, hyperalgesia due to elevated nociceptive response, reduced motor nerve conduction velocity and reduced threshold to painful stimuli [40]. STZ induced neuropathy is well known model for diabetic neuropathy especially in rats [47]. Intraperitonelly administered STZ causes damage to islets of langerhans of pancreatic beta cells that leads to decrease in insulin secretion [48,49]. When administered along with suitable dose of nicotinamide it is characterized by stable hyperglycemia and glucose intolerance [50, 51].

In previous study, we have reported that, administration of TRIG (50 mg/kg) and sitagliptin (5 mg/kg) alone increased body and reduced SG level in NICO-STZ induced type 2 diabetes mellitus in Wistar rats after four week treatment period [37]. In the present study, concomitant administration of TRIG with SITA for four weeks, it showed significant reduction in SG level. Trigonelline reduced SG level it might be due to regeneration in pancreatic beta cells [52]. It has been reported that sitagliptin enhance release of GLP-1 that stimulates insulin secretion from pancreatic beta cells [31].

In the study undertaken, behavioral techniques to distinguish nociceptor functions in diabetic rats was used. For behavioral studies Von Frey hairs, Randall Selitto and tail flick are reported methods to measure mechanical hyperalgesia, thermal hyperalgesia in preclinical studies [53]. STZ causes damage in sensory and motor fibers resulting in reduction in pain threshold [54]. Significant increase in pain threshold was observed in the TRIG+ SITA treated group. Accordance with previous study of enzyme dipeptidyl peptidase IV (DPP- 4) inhibitors showed amelioration in pain threshold due to reverse alteration in damaged motor and sensory fibers in rats [55, 56].

Reduced MNCV in diabetic rats is due to high glucose level which leads to neuronal dysfunction and nerve reperfusion to cause the endoneurial hypoxia [57]. Present study demonstrated that MNCV decreased in diabetic rats was improved by concomitant administration of TRIG + SITA in treated rats. It is apparent that TRIG and SITA protect neuronal dysfunction (sciatic nerve) by alteration in morphological and neuronal architecture.

High glucose level is responsible for generation of reactive oxygenase species (ROS) that leads to imbalance between radical production and radical scavenging system resulted in generation of oxidative stress [58,59]. MDA is endogenous biomarker mainly responsible for oxidative damage causing vascular endothelial dysfunction [60]. SOD provides protection to all aerobic cells by scavenging superoxide radicals and hydrogen peroxide (H_20_2). Present

study showed increased level of MDA and reductions in the activity of SOD in sciatic nerve of diabetic rats as compared to nondiabetic. Increased MDA level in stress condition, is responsible for lipid membrane destruction resulted tissue injury, which resembles with the reported literature [61,62].Concomitant administration of TRIG+ SITA showed decreased level of MDA and amelioration in the level of SOD as compared to alone. Previous findings suggest that trigonelline partially normalizing glucose homeostasis and restored altered enzyme activities [63, 64]. Sitagliptin showed antioxidant activity might be due to reduced lipid peroxidation and reduced overproduction of ROS.TRIG+ SITA promoted antioxidant activity in NICO-STZ induced diabetic peripheral neuropathy.

Sciatic nerve of non diabetic rats showed normal morphology and architecture. Diabetic rats showed severe necrosis, swelling and congestion. Trigonelline (50mg/kg) group showed moderate necrosis, mild swelling and congestion when compared with the diabetic control. The sitagliptin group at 5mg/kg showed moderate alteration in sciatic nerve. Concomitant administration (TRIG 50 mg/kg and SITA 5mg/kg) showed significant changes in the sciatic nerve architecture with mild swelling and congestion.

Trigonelline effect in diabetic neuropathy may be partly attributed to it regulation of GLP-1R/p 38 MAPK signaling pathway. GLP- 1R has regenerative effect on peripheral nervous system via reduced ROS production [65, 66, 67]. DPP-4 inhibitors might be act through inhibition of polyol pathway which leads to improved nerve blood flow and nerve fiber damage in patient with diabetic neuropathy [68].

CONCLUSION

The additive effect of concomitant administration of TRIG+SITA might be able to improve multiple mechanisms involved in the pathophysiologic events of diabetic neuropathy.

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