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Effect of Teneligliptin, and Teneligliptin Combined with Gabapentin on Alloxan-Induced Diabetic Neuropathy in Albino Mice

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Abstract

Diabetic neuropathy is a nerve disorder manifested by different faceted condition affecting up to half of individuals with persistent diabetes. Symptoms associated with this disease such as nerve palsy, painful polyneuropathy, thoracoabdominal neuropathy, autonomic neuropathy, diabetic amyotrophy, mononeuropathy multiplex, caused by motor, sensory, and autonomic nerve dysfunction which affects peripheral nervous system, gastrointestinal, pain receptors, urogenital, and cardiovascular system. In this study, type 2 diabetes was induced with alloxan in albino mice. After induction, drug treatment was initiated on the day 15, with different regimen on different group of mice that is teneligliptin, sitagliptin, combination of teneligliptin, and gabapentin. After investigation on day 21, 28, 35, and 42 found significantly improved glycemic control, paw jumping response, and grip strength ($P < 0.001$). Mice treated with different regimen on day 21, 28, 35, and 42 were observed significant increase in blood protein ($P < 0.001$). Alloxan caused marked nerve cell degeneration, teneligliptin and sitagliptin showed tissue regeneration and neutral effect on body weight. In the conclusion, treatment with teneligliptin and teneligliptin combined with gabapentin results an increase in pain sensitivity, grip strength, neural protection, and reverses the alteration of biochemical parameters but neutral effect on body weight in alloxan-induced type 2 diabetic mice.

Keywords: Alloxan, Diabetic neuropathy, Gabapentin, Sitagliptin, Teneligliptin

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Introduction

Diabetic neuropathy caused by persistent hyperglycemia, leading to damages nervous system, resulting unintended effect in many parts of the body such as feet, legs, heart, bladder, and reproductive and gastrointestinal system.[1] The symptoms caused by motor, sensory, and autonomic nerve dysfunction show pain, tingling, and numbness that start in the feet. Hand can be affected at the late stage and of diabetic neuropathy. In rare case of diabetic neuropathy, the unusual sensation can reach out to arms, trunk, and legs. Diabetic neuropathy includes third nerve palsy, painful polyneuropathy, thoracoabdominal neuropathy, autonomic neuropathy, diabetic amyotrophy, mononeuropathy multiplex results from diabetic microvascular, as well macrovascular injury.[2] Prescriber generally prefers combination of oral medication to treat sever painful diabetic neuropathy.[3] Medication used for diabetic neuropathic pain include: (1) anticonvulsants, such as pregabalin, gabapentin, valproate, carbamazepine, and lamotrigine; (2) antidepressants, such as amitriptyline, imipramine, desipramine, duloxetine, venlafaxine, bupropion, paroxetine, and citalopram; (3) opioids, such as tramadol, tapentadol ER, dextromethorphan, morphine sustained release, oxycodone; and (4) others treatment that are applied to the skin, specially to the feet, such as capsaicin cream, topical nitrate sprays, and lidocaine patches.[4] US Food and Drug Administration (FDA) and Health Canada approved drug pregabalin and duloxetine are specially used for the treatment of diabetic neuropathy pain.[5] Opioids should not advise as first-line therapy or use with caution for painful neuropathy due to risk of tolerance, dependency, abuse, and addiction.[6] Many studies suggest nitrate spray and lidocaine patch can relive neuropathic pain of feet. Studies of an antioxidant alpha-lipoic acid or ALA and evening primrose oil shown relieve symptoms and may build on nerve function.[7] Dipeptidyl peptidase 4 (DDP-4) inhibitors act for novel anti-diabetic treatment method by decreasing glucose level, decreasing glucagon levels and sustaining insulin levels, in type 2 diabetes mellitus.[8] The mechanism of actions of DPP-4 inhibitor is to block DPP-4 enzyme to prevent metabolism of Glucagon Like Peptide-1 (GLP-1) incretins. GLP-1 is secreted in the distal small intestine and is also produced in the brain stem of central nervous system to stimulate insulin secretion from β-cells of pancreas, inhibits glucagon secretion, decreases gastric emptying, improves insulin signaling pathway, decreases blood glucose levels, and protects β-cells from apoptosis showing neuroprotective functions.[9] DDP-4 inhibitors and GLP-1 Receptor Agonists are a breakthrough in the field of neural regeneration research.[10] At present, there is absence of available large randomized clinical trials that focusing on the effects of DPP-4 inhibitors on diabetic neuropathy. Animal studies showed promising beneficial effects of DPP-4 inhibitors on diabetic neuropathy. It is one of the most concern regarding the fastest growing health issues in the world. Current drug is insufficient to control the hyperglycemia and associated complication that may cause unintended side effect such as episodes of hypoglycemia and weight gain.[11] Therefore, newer DPP-4 inhibitors have been developed that showing more effective drugs and playing a significant role. The first drug of DPP-4 inhibitors class is sitagliptin, with β-amino amide

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derivatives, approved by the US, FDA in 2006 with trade name Januvia. Sitagliptin works to competitively inhibit around 97% of DPP-4 enzyme and reduces blood glucose levels, either in the postprandial or the fasting state. It presents a bioavailability of 87% and can be taken with or without food. The hepatic metabolism of sitagliptin is minimal (mainly by cytochrome P450 3A4) and it is largely (70–80%) excreted by the urine in its unchanged form, with a half-life of around 12 h. Those with severe renal impairment require dose adjustment due to its metabolism and elimination; however, patients with mild or moderate renal or hepatic impairment do not. Drug interactions involving pharmacokinetics are rare with sitagliptin. Sitagliptin shown effectiveness in lowering HbA1c, fasting plasma glucose, and 2-h postprandial glucose levels, as well as in raising the percentage of patients meeting target HbA1c values. Body weight is unaffected by sitagliptin. Sitagliptin is well tolerated and the risk of adverse events, including hypoglycemia, is very low.[12] Teneligliptin is a third-generation gliptin, which offers a pharmacodynamic and pharmacokinetic advantage. Teneligliptin was originally synthesized by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan) and was the first drug of its kind to be synthesized in Japan in 2012 with trade name Tenelia. Teneligliptin exhibits a unique structure that is characterized by five consecutive rings and is peptidomimetic.[13] The main interaction between the phenyl ring on the pyrazole and the S2 extensive subsite of DPP-4, which is shown by the X-ray co-crystal structure of teneligliptin, which not only enhances the potency of the drug but also increases its selectivity.[14] Teneligliptin provides 1/4th–1/5th low-cost treatment in comparison to another agent of the same class. This study was designed with an aim to evaluate the effectiveness and safety of teneligliptin when switched from other gliptins in patients not controlled on oral anti-diabetic drugs in T2DM.[15] Clinical evidence in a different demographic is needed and lacking, because the majority of studies focused on Japanese and Korean (Asian) T2DM patients. Teneligliptin has to be the subject of an extensive, carefully planned, and long-term safety research.[16] Diabetic neuropathy can be effectively treated with gabapentin. Mechanism of action associated with gabapentin is to decreases release of glutamate, norepinephrine, and substance P, with ligands on α2-δ subunit of voltage-gated calcium channel. No clinically significant drug interactions found with this drug.[17] This study was conducted to assess the effect of teneligliptin, and teneligliptin combined with gabapentin on alloxan-induced diabetic neuropathy in albino mice.

Materials and Methods

Animals and Drugs

Adult Swiss Albino Mice weighing 25–30 g bred in the Animal House, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, were used. All animal experiments were approved by the Institutional Animal Ethical Committee (IAEC, NIMS Institute of Pharmacy), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA No. 1302/PO/RE/S/09/CPCSEA). The animals were house in polycarbonate cages in a room with a 12-h day and 12-h night cycle, temperature of 24 ± 2°C, and humidity of 45–64%. During the whole experimental period, animals were feed with a balanced commercial diet and water *ad libitum*.

Alloxan was purchased from Central Drug House (P) Ltd, Daryaganj, New Delhi, India, Batch No.- 090217. Teneligliptin was purchased from Ami Lifesciences Pvt. Ltd, Vadodara, Gujrat, India, Batch No.- TNG/RD/20980618, Gabapentin from Alkem

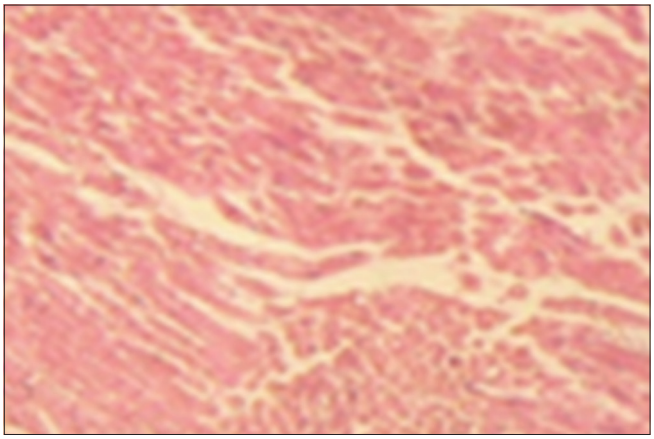

Figure 1: Normal, untreated rats. Cross-sectional view of mice sciatic nerve showing normal structure

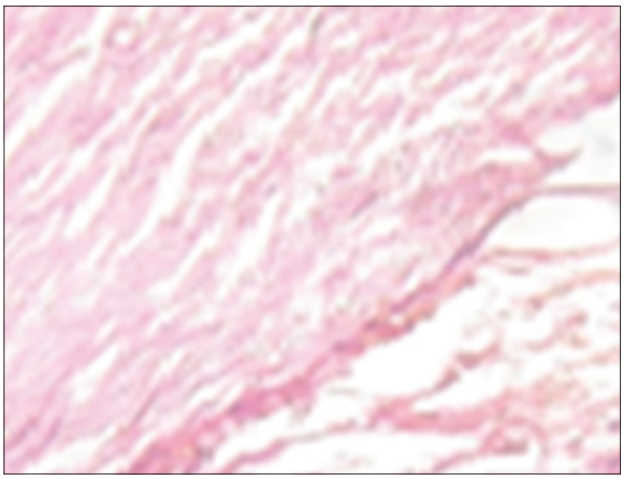

Figure 2: Diabetic control mice. Cross-sectional view mice sciatic nerve treated with Alloxan (150 mg/kg).* showing significant degeneration of nerve cells. * = single dose

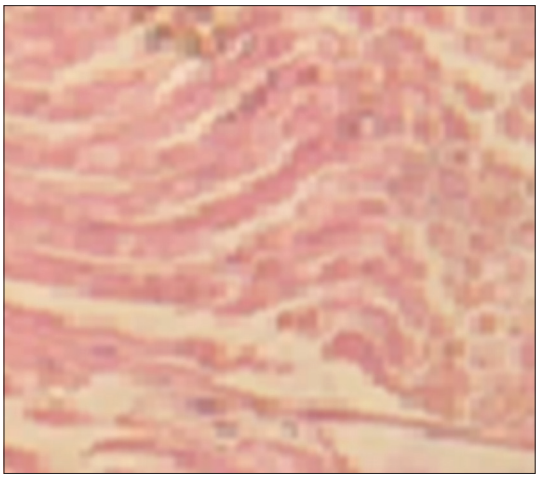

Figure 3: Diabetic mice given standard drug sitagliptin (100 mg/kg of body weight). Cross-sectional view mice sciatic nerve

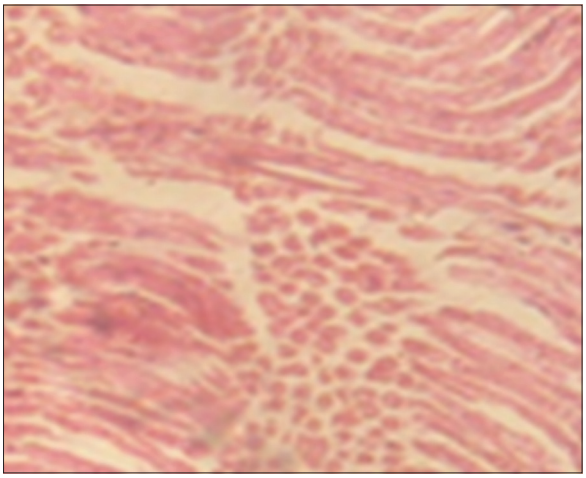

Figure 4: Diabetic mice given teneligliptin (20 mg/kg of body weight). Cross-sectional view mice sciatic nerve

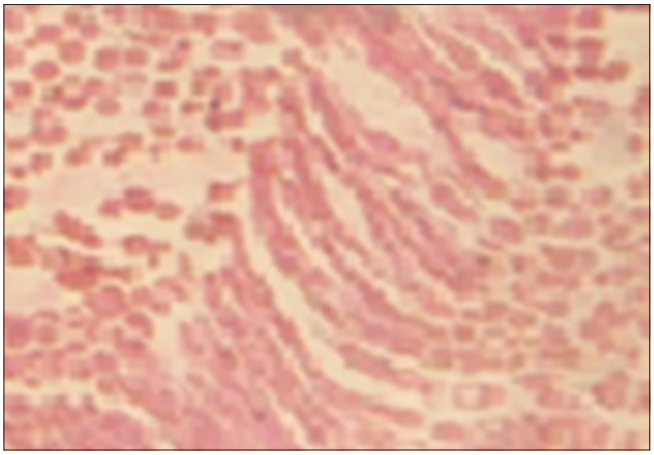

Figure 5: Diabetic mice given teneligliptin and gabapentin (600 mg/kg of body weight). Cross-sectional view rat sciatic nerve

Laboratories Limited, Navi Mumbai, India, Batch No.-813R201388 in this study.

Induction of Diabetic Neuropathy

Induction of type 2 diabetes in the mice with alloxan (180 mg/kg). Non-insulin-dependent diabetes mellitus was induced by a single intraperitoneal injection of 180 mg/kg body weight alloxan in overnight fasting mice. The injection site was dorsal midpoint between the pelvis and ribs close to the right side of the spine. Elevated blood glucose concentration of animal, more than 200 mg/dl represent hyperglycemia that included in study.[18]

Treatment Protocol

Animals used included insulin-resistant (IR) type 2 diabetic mice (induced by alloxan). IR mice of either sex was randomly allotted into different experimental groups (n = 5), each group containing six animals: Group I – Normal mice group (without any drug); Group II – Alloxan-induced diabetic mice (Alloxan only); Group III – Standard drug sitagliptin of alloxan treated type 2 diabetes mellitus (Mono therapy Sitagliptin 0.4 mg); Group IV – Test group alloxan treated type 2 diabetes mellitus (Mono therapy Teneligliptin 0.08 mg); and Group V – Test group alloxan treated type 2 diabetes mellitus + neuropathy (Dual therapy: Teneligliptin 0.08 mg + Gabapentin 2.3 mg).[8] These drug treatment will carry out for 42 days with the help of an oral catheter in every morning. At day 14, 21, 28, 35, and 42, blood glucose level, blood protein level, body weight, and different activity report monitored through different apparatus such as – Rota rod for muscular grip strength, Eddy's Hot plate for induced thermal pain, were evaluated in mice with alloxan induced diabetic neuropathy. At the end of the experiment proceeded for histopathology of mice sciatic nerve.

Effects of Drug Treatment on Grip Strength, Body Weight, Pain Sensitivity, and CNS Activity in Mice

Grip strength of diabetic animal was evaluated using the Rota rod apparatus. Grip strength test was used to find muscle strength or neuromuscular activity in rodents which could be altered by muscle relaxants, sedatives, and toxic chemical substances. The Rota rod is made up of experimental compartments and a common rotating rod with variable speeds of roughly 5, 10, 15, 20, and 25 revolutions per minute and a diameter of about 25 mm. The interval counters are provided in each compartment. The device runs on single phase, 50 Hz, 220/30 V AC power. On the floor of each compartment, there is a cantilever platform that is hinged at the rear end. The Rota rod apparatus consists of horizontal metal rod of 3 cm diameter and 23 cm length, divided into three sections of disks, coated with rubber and their speed was adjusted to 25 rpm.[19] The animals are prevented from leaving the roller by cages beneath the segment. Only those animals were employed in the test that showed the ability to hang onto the rotating rod for at least 1 min. Every week, the mice were placed on the rotating rod. The time to fall was measured in all five-group using a single dose of teneligliptin and its combinations with gabapentin. Throughout the research period, all diabetic animals had their body weights checked every week. By measuring pain threshold, the sensory function was evaluated. The hot plate test was conducted using the Eddy's *et al*. method. Animals were put on hot plates that were kept at a constant temperature of 55°C, and the reaction time was measured as response latency. Before and following treatment, the response latencies were measured. Hot-plate delay has a 10 s cutoff time. It was timed to see how long the animals took to lick their paws or jump. To determine the mouse's response to electrical heat-induced pain, each mouse was separately placed on the hot plate (licking of the fore paws and eventually jumping). The time it took mice to exhibit their initial signs of pain (raising of the hind paws, hind paw licking, or jumping) was recorded.[20]

Determination of Biochemical Indicators

Following the delivery of the testing chemicals, blood glucose levels were monitored weekly using a glucometer. The biuret end-point assay (Central laboratory Biochemistry department of NIMS University, Jaipur, Rajasthan, India) was used to detect total blood protein. Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet colored complex. The intensity of the color formed is directly proportional to the amount of proteins present in the sample. Serum and Heparinized/EDTA Plasma were used as sample in Biuret Method. In this method, two reagent were used- Reagent 1 : Biuret Reagent

and Reagent 2 : Protein Standard 6 g/dl. Micropipettes and Tips with Colorimeter or Bio-Chemistry Analyzer were used to measure the absorbance of the standard Abs. S and sample Abs. T against the reagent blank.[21] The total protein concentrations in blood plasma were determined using the following formula: Total protein concentration (g/dL) = Absorbance of test/Absorbance of standard × 6.5.

Histopathological Examination of the Sciatic Nerve

Before and after therapy, the sciatic nerve underwent a histopathological analysis. The sciatic nerve was examined histopathologically using albino mice as a mammalian model. In preparation for surgery, mice were anesthetized by intraperitoneal ketamine (80 mg/kg)[22] and maintained under sedation with additional boluses of ketamine for the duration of each individual experiment. The right and left sciatic nerves were exposed throughout the length of the femur after the animals had been given general anesthesia. From the gluteus muscles to the popliteal area, a posterior-lateral incision was performed. This made it possible to see the precise motor branches of the sciatic nerve that go to the biceps femoris, gastrocnemius, and distal muscles (number of fibularis and number of tibialis). The sciatic nerve exits the pelvic cavity at the level of the knee. With the help of this surgical operation, a large enough area was made accessible for compound muscular action potentials (CNAPs) together with (CMAPs) from the biceps femoris and gastrocnemius muscles. To expose the nerve surface and preserve the epineurial coating, the muscle fascia covering the nerve was carefully removed. The energy needed for stimulation is significantly reduced when this fascia is removed. Throughout the acute research, normal saline was administered continuously to the nerves to prevent desiccation. In this investigation, the typical sciatic nerve stimulation of mice had a diameter of about 2 mm. Even though the average number of fascicles per nerve varied widely among mammalian species, the usual fascicle thickness was always between 200 and 400 m.[22]

Statistical Analysis

Using Sigma Stat 3.5 and Sigma Plot 10.0 (Sigma), all statistical analyses were carried out, and all values were reported as mean ± SEM. Using paired Student's t-tests to determine, whether differences between variables before and after treatment were statistically significant, and one-way analyses of variance to evaluate differences between different groups followed by Tukey-Kramer multiple comparison test. $P < 0.05$ were considered statistically significant.

Results

Teneligliptin, Sitagliptin, and Teneligliptin in Combination with Gabapentin has No Effect on the Body Weight of Diabetic Mice

The body weight decreased rapidly in mice with alloxan induced diabetic neuropathy. Measurement of the body weight of mice in all experimental groups is shown in Table 1. Significant decreases in body weight were observed in alloxan-induced diabetic mice on week 1 post-injection (28.05 ± 0.36 g in non-diabetic mice vs. 27.45 ± 0.36 g in diabetic control mice at 1 week after alloxan treatment, $P < 0.01$). A progressive loss of body weight was noted from week 0 to week 8 in the diabetic control mice (28.05 ± 0.36 g at week 0 vs. 23.73 ± 0.34 at week 8, $P < 0.001$). When compared to the negative control group, the animals in the other groups similarly had considerable weight loss till day 21. On day 15, animals treated with standard protocol sitagliptin (100 mg human adult dose [HAD], 0.38 mg for adult mice dose), teneligliptin in combination with gabapentin (600 mg HAD, 2.314 mg for adult mice dose), and teneligliptin (20 mg HAD, 0.078 mg for adult mice dose) did not induce any apparent body weight gain.

Teneligliptin, Sitagliptin, and Teneligliptin in Combination with Gabapentin Significantly Reduces Blood Glucose Level in Diabetic Mice

The blood glucose levels of mice in all experimental groups, except the normal control group, increased significantly after the alloxan injection until day 14 [Table 2]. On day 21, 28, 35, 42, 49, and 56 post-induction, significantly increased blood glucose levels were detected in the negative control group, compared with the normal control group ($P < 0.001$). In the diabetic control group (negative control), blood glucose increased to the peak level of 355.5 ± 3.50 mg/dL on day 56 and was found to be significantly increased ($P < 0.001$) compared with the value on day 1 (88.01 ± 0.71 mg/dL). Control animals remained normoglycemic during the entire testing period of 56 day. The treatment with standard protocol sitagliptin (100 mg HAD, 0.38 mg for adult mice dose), teneligliptin in combination with gabapentin (600 mg HAD, 2.314 mg for adult mice dose), and teneligliptin (20 mg HAD, 0.078 mg for adult mice dose) on day 15 exhibited significantly decreased blood glucose levels compared with those in the normal control group on d 21, 28, 35, 42, 49, and 56 ($P < 0.001$) [Table 2].

Teneligliptin, Sitagliptin, and Teneligliptin in Combination with Gabapentin Improves Muscular Grip Strength of Diabetic Mice

Measurement of muscular grip strength was used to evaluate diabetic neuropathy after 14 day of alloxan injection. Muscular grip strength was reduced significantly in all mice with alloxan-induced diabetes. In the normal control group, muscular grip strength was normal (92.33 ± 2.52 vs. 94.66 ± 1.45 N), and no statistically significant difference was found ($P > 0.05$), while significant difference of muscular grip strength was found in the diabetic control group (88.16 ± 2.19 vs. 9.50 ± 0.82 N, $P < 0.001$). The grip strength of all treated animals increased significantly compared with the positive control group on day 49 and 56 ($P < 0.001$). The grip strength of mice treated with standard protocol and combination of teneligliptin and gabapentin was significantly greater on 49 and 56, when compared with the negative control group [Table 3].

Effects of Teneligliptin, Sitagliptin, and Teneligliptin in Combination with Gabapentin on Pain Sensation (thermal pain)

In mice, a single systemic injection of alloxan induced a hyperalgesic reaction observed on day 14 after the onset of diabetic neuropathy. In this study, hyperalgesic reaction was evaluated for a period of 14 day post-treatment with alloxan. The Eddy's hot plate was used to gauge the paw jumping reaction.

There was significant difference in paw jumping response 14 day post-induction of diabetic neuropathy in mice, but there was no significant difference found in the control group, in which diabetes was not induced (6.23 ± 0.60 vs. 6.68 ± 0.93). In diabetic mice (negative control), there was significant increase in paw jumping response (6.33 ± 0.49 vs. 15.50 ± 0.88). The paw jumping responses of all treated mice on day 21, 28, 35, 42, 49, and 56 were reduced significantly compared with the negative control group [Table 4]. The paw jumping response of mice treated with sitagliptin (100 mg HAD 0.38 mg for adult mice dose), teneligliptin in combination with gabapentin (600 mg HAD, 2.314 mg for adult mice dose), and teneligliptin (20 mg HAD, 0.078 mg for adult mice dose) was found to be significantly different from that in the normal control group. Combined treatment of teneligliptin and gabapentin on day 15–56 caused significant effect in pain threshold compared the negative control group.

Effects of Teneligliptin, Sitagliptin, and Teneligliptin in Combination with Gabapentin on Blood Protein Levels

The blood protein levels in all experimental groups, except the normal control group, were significantly decreased after alloxan injection [Table 5]. On day 21, 28, 35, 42, 49, and 56 post-induction, statistically significant decrease in blood protein level in the negative control group was observed in comparison with the normal control group ($P < 0.001$) [Table 5]. In the diabetic group (negative control), the blood protein level decreased from the maximum value of 6.13 ± 0.033 versus 3.21 ± 0.019 mg/dL, and statistically significant differences were observed ($P < 0.001$). However, normal blood protein level was observed in the control group (6.11 ± 0.032 vs. 6.10 ± 0.033 mg/dL) during the period of the study [Table 5]. Significantly increased blood protein level was detected in the animals treated with different treatment protocols, compared with the negative control group on day 21, 28, 35, 42, 49, and 56 ($P < 0.001$).

Histopathology of Sciatic Nerve

Histopathological examination of the sciatic nerve revealed that the nerve cells of the alloxan treated group showed marked degenerations. Increases in tissue regeneration capacity were observed in mice treated with standard protocol, while normal cell growth in tissues was found in the normal control group. In addition, combined treatment of teneligliptin and gabapentin

Table 1: Effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin on body weight (g) in mice (mean±SEM, *n*=6)

Group	*Day-1*	*Day-7*	*Day-14*	*Day-21*	*Day-28*	*Day-35*	*Day-42*
Control	22.55±0.19	23.1±0.27	23.53±0.29	24.08±0.28	24.63±0.26	25.25±0.25	25.75±0.28
Neg. Control	28.05±0.36	26.45±0.36*	25.83±0.33*	24.2±0.31*	23.76±0.29*	22.03±0.29*	21.61±0.29*
Sitagliptin	27.66±0.80	25.38±0.85*	23.01±0.85*	22.81±0.84#	22.93±0.82#	22.86±0.85#	22.71±0.82#
Teneligliptin	27.83±0.87	25.78±0.86*	24.68±0.89*	23.55±0.87*	23.58±0.82*	23.61±0.88#	23.55±0.89#
Teneligliptin+Gabapentin	28.75±0.54	25.40±0.49*	23.36±0.53*	21.30±0.54*	21.31±0.54*	21.28±0.57#	21.40±0.52#

Values are given as mean±SEM for groups of six animals each, compare to the normal control group, *P<0.01; Compare to the negative control group, #P<0.01; Neg.: Negative

Table 2: Effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin on blood glucose (mg/dl) in mice (mean±SEM, *n*=6)

Group	*Day-1*	*Day-7*	*Day-14*	*Day-21*	*Day-28*	*Day-35*	*Day-42*
Control	86.41±0.75	86.43±0.58	86.48±0.69	86.26±0.65	86.2±0.61	86.13±0.62	86.35±0.62
Neg. Control	88.01±0.71	105.16±1.88*	126.5±1.47*	135.8±1.30*	168.5±2.65*	254.3±2.65*	291±2.33*
Sitagliptin	84.38±1.24	192.7±2.41*	288.3±2.64*	248.2±2.58*	195.3±3.32*#	147.8±3.57*#	121.3±2.88*#
Teneligliptin	90.16±1.42*	199.6±2.66*	292.2±1.01*	252.6±1.85*	197.5±3.31*	145.3±1.94*#	118±2.63*#
Teneligliptin+Gabapentin	92.66±1.92*	202±2.93*	291.5±3.39*	245.2±1.22*	192.6±3.75*#	140.6±1.22#	116.5±2.32#

Values are given as mean±SEM for groups of six animals each, compare to the normal control group, *P<0.01; Compare to the negative control group, #P<0.01; Neg.: Negative

Table 3: Effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin on muscle grip strength (s) in mice (mean±SEM, *n*=6)

Group	*Day-1*	*Day-7*	*Day-14*	*Day-21*	*Day-28*	*Day-35*	*Day-42*
Control	92.33±2.52	88.83±2.84	89.66±1.94	86.26±0.65	93.33±1.76	90.0±1.98	91.50±1.83
Neg. Control	88.16±2.19	52.50±1.92*	46.50±1.76*	36.33±1.66*	28.66±1.88*	22.83±1.65*	19.66±1.55*
Sitagliptin	87.17±3.67	51.25±2.51*	43.67±0.96*	48.83±1.89*	51.16±1.21*#	58.32±1.03#	61.21±0.94#
Teneligliptin	88.34±3.25	50.83±1.84*	40.21±1.23*	49.50±0.87*	53.32±1.49*#	59.21±1.45*#	66.12±0.95*#
Teneligliptin+Gabapentin	86.28±2.95	51.36±1.86*	44.36±1.42*	52.56±1.25*	58.46±0.98*#	64.25±1.86#	69.84±1.10#

Values are given as mean±SEM for groups of six animals each, compare to the normal control group, *P<0.01; Compare to the negative control group, #P<0.01; Neg.: Negative

Table 4: Effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin on thermal pain (s) in mice (mean±SEM, *n*=6)

Group	*Day-1*	*Day-7*	*Day-14*	*Day-21*	*Day-28*	*Day-35*	*Day-42*
Control	6.23±0.60	6.45±0.47	6.40±0.36	6.50±0.42	6.66±0.76	6.81±0.94	6.33±0.55
Neg. Control	6.33±0.49	9.83±1.24*	11.00±1.39*	11.5±1.02*	12.83±1.24*	13.00±1.15*	13.83±1.22*
Sitagliptin	6.83±0.60	9.66±1.22*	12.16±1.07*	11.23±0.90#	11.15±0.66#	10.83±0.47#	10.33±0.21#
Teneligliptin	6.50±0.42	9.16±0.83*	11.66±0.80*	10.83±0.40#	10.66±0.66#	10.16±0.30#	9.66±0.21#
Teneliglipti+Gabapentin	6.66±0.33	9.16±0.47*	10.66±0.21*	11.16±0.30*#	12.00±0.44#	12.33±0.42*#	12.83±0.90*#

Values are given as mean±SEM for groups of six animals each, compare to the normal control group, *P<0.01; Compare to the negative control group, #P<0.01; Neg.: Negative

Table 5: Effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin on blood protein level (g/dl) in mice (mean±SEM, *n*=6)

Group	*Day-1*	*Day-7*	*Day-14*	*Day-21*	*Day-28*	*Day-35*	*Day-42*
Control	6.11±0.032	6.15±0.045	6.14±0.040	6.21±0.044	6.25±0.037	6.19±0.032	6.11±0.035
Neg. Control	6.13±0.033	5.11±0.038*	4.85±0.042*	4.32±0.023*	4.11±0.020*	3.91±0.033*	3.62±0.019*
Sitagliptin	6.21±0.015	5.15±0.013*	4.88±0.018*	4.96±0.021*	4.98±0.017*#	5.15±0.012#	5.21±0.016#
Teneligliptin	6.21±0.015	4.98±0.023*	4.78±0.010*	4.94±0.016*	4.99±0.032*#	5.18±0.021*#	5.24±0.012*#
Teneligliptin+Gabapentin	6.23±0.021	5.12±0.023*	4.88±0.025*	4.91±0.027*	4.96±0.046*#	5.11±0.040#	5.23±0.035#

Values are given as mean±SEM for groups of six animals each, compare to the normal control group, *P<0.01; Compare to the negative control group, #P<0.01; Neg.: Negative

showed significant tissue regeneration capacity when compared with the control group as well as the sitagliptin treatment group in the figure.

Discussion

The present study investigated the effects of teneligliptin, sitagliptin, and teneligliptin in combination with gabapentin drugs on alloxan-induced diabetic neuropathy in mice. Alloxan is most commonly used to induce diabetes in experimental animals and administration of alloxan caused diabetic neuropathy,[23] most likely as a result of the pancreatic islets of Langerhans-cells being destroyed, excessive blood glucose production, and impaired tissue glucose uptake, all of which contribute to the primary cause of hyperglycemia in diabetes mellitus. Hyperglycemia accompanied by weight loss is seen in adult rats treated with alloxan and is stable for weeks, indicating irreversible destruction of β-cells of the islets of Lengerhans of the pancreas. Teneligliptin has been shown, in several clinical studies to improve metabolic control in type 2 diabetes. There is a very little risk of hypoglycemia, and teneligliptin has an excellent safety profile and high tolerability. Teneligliptin also has no effect on body weight or only slightly reduces it. In more advanced stages of the condition, teneligliptin may also be taken alone or in conjunction with insulin.[24] Teneligliptin, a DPP-4 inhibitor, is thought to work in type 2 diabetes patients by delaying the inactivation of incretin hormones. Teneligliptin raises the concentrations of the intact active hormones, which increases and prolongs the action of these hormones. The intestine continuously releases incretin hormones, such as GLP-1 and GIP, and their levels rise in response to meals. The enzyme DPP-4 quickly deactivates these hormones. The incretins are a component of an endogenous system that controls glucose homeostasis physiologically. Using intracellular signaling pathways including cyclic adenosine monophosphate, GLP-1 and GIP boost insulin synthesis and release from pancreatic beta-cells whether blood glucose levels are normal or increased. In addition, GLP-1 reduces pancreatic-cells' production of glucagon, which, in turn, results in less hepatic glucose generation. Teneligliptin enhances insulin release and reduces glucagon levels in the blood in a glucose-dependent manner by raising and extending active incretin levels. At concentrations that are similar to those from therapeutic dosages, teneligliptin exhibits selectivity for DPP-4 and does not block DPP-8 or DPP-9 activity *in vitro*.[25] In response to nutritional intake, the small intestine secretes the enteric hormone GLP-1. GLP-1 is of interest, because it can increase insulin production that is induced by glucose. The dependence of GLP-1 on glucose concentration is seen as a distinct safety advantage due to a lesser risk of hypoglycemia compared to medicines that increase insulin secretion through glucose-independent pathways. The proteolytic enzyme DPP-4, which has two biologically active processes and has a short half-life of only 2 min, quickly degrades GLP-1, which limits its usefulness. The membrane-spanning form of DPP-4 and its circulating soluble form are the first to demonstrate its enzyme activity. GLP-1 is one of the substrates that DPP-4 preferentially cleaves, because it has an amino-terminal proline or alanine. Second, DPP-4 binds to adenosine deaminase and transmits intracellular signals by dimerization and the activation of intracellular pathways, independent of its enzymatic characteristics. Emerging as a therapeutic approach to improve GLP-1 activity is the prevention of GLP-1 breakdown through inhibition of the DPP-4 enzyme. Teneligliptin therapy improves GLP-1's capacity to create insulin in response to high blood glucose levels, prevents glucagon release after meals, delays the pace of nutrient absorption into the bloodstream, slows the rate of stomach emptying, and reduces food intake.[26] An essential physiological role in controlling blood glucose levels is played by GLP-1 and GIP. Incretins are released after eating, and they promote the synthesis and release of insulin (GLP-1 and GIP), while suppressing the release of glucagon (GLP-1), GLP-1 increases satiety and delays stomach emptying.[27] The effects to increase insulin and decrease glucagon levels are dependent on blood glucose levels; they are not present when blood glucose levels are low. These methods help to reduce fasting glucose concentrations by reducing the post-meal surge in glucose. Due to DPP-4's quick deactivation, these glucoregulatory effects of GLP-1 and GIP are transient.[28] By preventing the breakdown and subsequent deactivation of incretins, DPP-4 inhibitors improve their activity. The half-life and concentrations of circulating intact (active) GLP-1 and GIP are increased by DPP-4 inhibition. Increased levels of incretins that are active both before and after meals as a result of DPP-4 inhibition boost insulin release, decrease glucagon levels, and enhance fasting and post-meal glucose concentrations. With normal GLP-1 function, post-meal GLP-1 concentrations are lower in patients with type 2 diabetes. Therefore, DPP-4 inhibition corrects a flaw that may be responsible for hyperglycemia in this condition. After eating, intact (active) GLP-1 and GIP are produced from gut endocrine cells, which lower blood sugar levels by promoting glucose-dependent insulin release from pancreatic beta-cells (GLP-1 and GIP) and inhibiting glucose-dependent glucagon release from pancreatic beta-cells (GLP-1). Sitagliptin, vildagliptin, and saxagliptin are three orally active DPP-4 inhibitors that have been developed to treat type 2 diabetes. Sitagliptin was authorized for use in the United States in October 2006 to treat people with type 2 diabetes. Due in part to the limits of current anti-hyperglycemic medicines that are linked to side effects like hypoglycemia or weight gain, many people with type 2 diabetes continue to get insufficient care. DPP-4 inhibitors are an important new addition to the treatment toolbox for managing type 2 diabetes, giving doctors a well-tolerated option for enhancing 24-h glucose control by including glucose-dependent physiological processes. Whether chronic treatment will be an significant, unresolved question that has to be addressed in

long-term clinical research is whether increases in-cell function are related with changes in the natural history of diabetes. Diabetes-related kidney damage from renal ischemia and reperfusion is protected by teneligliptin.[23] In people with normal or depressed moods, gabapentine reduces the discomfort of diabetic neuropathy. When administered alone or in conjunction with other anti-diabetic medications, DDP-4 inhibitors help individuals with type 2 diabetes mellitus maintain better glycemic control (metformin, sulfonylurea, or thiazolidinedione). Teneligliptin shields normal mice from the metabolic and hormonal abnormalities, increased death of beta cells, and hepatic steatosis brought on by a fructose-rich diet. By preventing the incretin hormones' breakdown, DDP-4 inhibitors help type 2 diabetics maintain healthy glucose levels. The first evidence for an interaction hemodynamic effect of DDP-4 and ACE inhibition in humans was provided by DDP-4 inhibition, which also inhibits the breakdown of the vasoconstrictive neuropeptide Y.[28] In between 60% and 70% of diabetic patients, diabetic neuropathy, a long-term consequence of diabetes, is seen. It appears early in the course of the disease. It is well recognized that diabetic neuropathy is a nerve degenerative condition marked by axonal degeneration, demyelination of nerve fibers, and a decrease in the quantity of medium- to large-diameter nerve fiber, particularly in peripheral nerve. Hyperglycemia, which causes a constant, rapid flux of glucose, causes diabetic neuropathy. Aldose reductase is the enzyme that restricts the pace of this process. Increased protein kinase C metabolism, oxidative stress, rapid glycation, and decreased endoneural capillary perfusion are all consequences of the increased flux through the polyol pathway, which eventually results in nerve degeneration. In this investigation, teneligliptin administration and its combination with other medications resulted in a hypoglycemic effect in mice with alloxan-induced hyperglycemia. Alloxan's ability to induce diabetic neuropathy is also accompanied by the typical decrease of body weight that results from increased muscle wasting and protein loss in tissue. Teneligliptin treatment prevented muscular atrophy in diabetic mice, which may explain why they did not gain weight compared to the diabetic control group. It has been demonstrated that diminished muscle strength in diabetes mellitus is correlated with the existence and severity of diabetic neuropathy. In the current investigation, diabetic mice treated with teneligliptin and its combination with other medications showed noticeably improved motor behavior, particularly grip strength. When compared to the diabetic control group, the treatment with gabapentin and teneligliptin combinations significantly increased grip strength, but the negative control group significantly decreased grip strength. In both spontaneous and experimental types of diabetic neuropathy, hyperalgesia is a recurring aspect of sensory impairment. According to our research, diabetic rats treated with gabapentin and teneligliptin combinations had higher pain thresholds, which contributed to the improvement in hot-plate response. The response in the group receiving gabapentin plus teneligliptin was shown to be more favorable than in groups receiving other medications. The combination of gabapentin and teneligliptin medication resulted in a significant increase in pain threshold. The outcome supports the value of combining gabapentin with teneligliptin for the symptomatic management of excruciating diabetic neuropathy. Blood protein levels drop in diabetic neuropathy, according to numerous research. This is in line with what our study's diabetic control group of mice found. The negative control group's reduced blood protein level may have been caused by its metabolism. According to studies using mice treated with alloxan, osmotic shrinkage or a delay in normal axonal development causes the peripheral nerve to become ischemic and hypoxic. The cross-sectional profiles of the mice's sciatic nerves in the diabetic group revealed a considerable loss of nerve fibers, which are consistent with earlier research. Teneligliptin and gabapentin medication together resulted in normal sciatic nerve development when compared to the healthy control group.

Conclusion

The significant effect of teneligliptin and its combination with other drugs on diabetic neuropathy in mice was observed. This may be explained by the combined therapy's synergistic and potentiating effects, because they include a wide range of active ingredients that can target various conditions and mechanisms involved in the pathophysiology of diabetic neuropathy. The treatment of teneligliptin only and in combination with gabapentin causes no weight gain and an increase in grip strength and pain sensitivity, which indicates neural protection. Administration of teneligliptin alone and combined treatment of teneligliptin and gabapentin reverses the alteration in biochemical parameters, causes morphological changes in sciatic nerve, and improves the general behavioral parameters in diabetic mice induced by alloxan.

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