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Evaluation of Protective Effect of Gemigliptin and Rizatriptan in Streptozotocin induced Diabetic Neuropathy in Rats

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A typical micro-vascular consequence of diabetes mellitus is diabetic neuropathy. The prevalence of diabetic neuropathy patients is rising in spite of strict glycemic control, blood pressure, and lipid lowering medication. New prevention and treatment methods are required because of the drawbacks and side effects of current medicines. Serotonin, a neurotransmitter implicated in the transmission of pain, is being investigated for its potential to process pain and to reduce inflammatory responses. Gemigliptin and rizatriptan are being studied for the treatment of hyperglycemic mortality. In this investigation, STZ (60 mg/kg) was injected intraperitoneally once to cause diabetic neuropathy. Tests were conducted on the neuroprotective potential of Gemigliptin (5 mg/kg p,o) alone and in combination with variable dosages of Rizatriptan (0.5, 1 mg/kg, i.p.) administered at intervals of 72 hours and one month. According to the study, a medicine combination of gemigliptin and rizatriptan successfully reduces the symptoms of diabetic neuropathy by lowering cholesterol, triglycerides, and serum glucose levels—all of which contribute to diabetes complications. Additionally, the combination reduces nerve damage-related hyperalgesia and significantly increases locomotor activity. Oxidative stress is decreased, which helps prevent additional difficulties, and the combination raises levels of antioxidants like SOD and CAT. Overall, Gemigliptin and Rizatriptan work well together to reduce diabetic neuropathic pain.

Keywords: Antioxidant; Blood glucose; Diabetic neuropathy; Hyperalgesia; Neuroprotection; Oxidative stress.

Neuropathic pain (NP) is a type of persistent pain brought on by diseases or injuries that have harmed the nerve system. Dysesthesia (an unpleasant aberrant sensation), hyperalgesia (an heightened reaction to painful stimuli), and allodynia (pain to stimuli that typically do not trigger pain) are the sensory abnormalities that set NP apart from other conditions. Both forms of diabetes can result in NP. In patients with chronic conditions, it happens more than 50% of the time and occurs in roughly 8% of new patients.[1]

Inflammatory stress[2] Diabetes-related problems including neuropathy are raised as a result of prolonged hyperglycemia. This oxidative stress also leads to the development of apoptosis in supporting glial cells, which may be the

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mechanism behind diabetic nerve injury Certain medications that lower hyperglycemia-mediated mitochondrial ROS restrict the development of diabetic sequelae by reducing the synthesis of advanced glycation end products, glucose-induced activation of protein kinase C, accumulation of sorbitol, and activation of NF-B (nuclear factor B).[3] The majority of pain-producing stimuli cause neuronal damage, making human testing to assess NP challenging. Therefore, animal research is necessary to comprehend the numerous NP-related mechanisms. The STZ (Streptozotocin) induced neuropathy model, which resembles diabetic neuropathy, is commonly used. STZ is an antibiotic that fights cancer and is chemically related to nitrosoureas. STZ preferentially destroys pancreatic beta cells when injected 45 to 60 mg/kg intravenously or intraperitoneally, and after three to four days, develops diabetes in mice.[4] Peripheral neuropathies can be identified by symptoms such hyperalgesia, hyperesthesia, cold or hot allodynia, hyperglycemia-induced nitrosative stress, and oxidative stress. These symptoms are a key component of diabetic neuropathy. Both afferent and efferent nerve conduction abnormalities are brought on by ROS.[5] It can be difficult to maintain consistent hyperglycemia control, and even patients with strong glucose management might develop neuropathy. The long-term quality of life of diabetic patients must be preserved by drugs that also target several pathways causing hyperglycemia-mediated issues.[6] Additionally, microvascular and neuronal impairments, which are significant risk factors for diabetes complications, can be mediated by hyperglycemia-induced oxidative stress. In order to treat diabetes and its complications, antioxidants may be useful until blood glucose levels can be completely under control.[7] Natural remedies are a safer therapeutic alternative to current medications for treating neuropathy because modern medications have a number of side effects. Rats with neuropathy have been treated with a variety of plant phytoconstituents. Numerous polyphenols and flavonoids have been investigated for their potential to act as antioxidants and reduce inflammation in the treatment of neuropathic pain. Animal neuropathy must be treated, and isolated plant bioactive compounds hold considerable promise as free radical scavengers.[8]

In addition to their capacity to neutralize free radicals and act as antioxidants, phenolic acids have also been shown to have neuroprotective properties since they also protect glial cells in addition to neurons. Numerous studies have demonstrated how different phenolic acids, such as chlorogenic acid, CAPE (caffeic acid phenethyl ester), ferulic acid, and protocatechuic acid, can be used to treat neurological problems such as neuropathic pain.

In light of the aforementioned information, the current study sought to determine how Rizatriptan and Gemigliptin fared against STZ-induced diabetic neuropathy.

MATERIAL AND METHODS

Animals and drugs

For the investigation, Wistar strain rats (230–250g, either female) were employed. 64 animals were used during the trial. Under standard laboratory environmental conditions, including a temperature of 25 °C, a 12-hour day and night cycle, and a relative humidity of 55 %, animals were housed in polypropylene cages with free access to food and water. Animals were isolated before the test to let them get acclimated to the lab setting. All of the tests were conducted during the light time. The research was conducted in compliance with the recommendations made by the committee New Delhi, India's Centre for the Control and Supervision of Animal Experiments (CPCSEA). The study's protocol was authorised by the M.V.P.S. College of Pharmacy's institutional animal ethical committee (IAEC), located in Nashik-02. IAEC/Jan2019/03 is the approval number. The companies Sigma Aldrich, Aarti Distributors, Sanofi, and Sun Pharma provided the drugs streptozotocin, rizatriptan, gemigliptin, and pregabalin, respectively.

Induction of diabetes in rats by streptozotocin

By administering STZ intraperitoneal in doses of 60 mg/kg, i.p rats were rendered diabetic. For each animal, STZ was first weighed based on body weight before being dissolved in 0.05 M citrate buffer at pH 4.5. After that, it was intraperitoneal infused. For 24 hours, 5% glucose was given to prevent abrupt hypoglycemia. When rats receive a single dose of STZ, hyperglycemia develops within 72 hours.

Experimental design

Eight groups were created for the animals. Each batch has six 230–250 gm animals.

Group I: Control, Rats administered with DW p.o. for 15 days straight.

Group II: STZ (60mg/kg, i.p.) on day 1

Group III: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o) for 15 days straight.

Group IV: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o)+ Rizatriptan (0.5mg/kg, i.p.) Treatment after 72 hrs for 15 days straight

Group V: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o)+ Rizatriptan (0.5mg/kg) Treatment after 1 month for 15 days straight

Group VI: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o)+ Rizatriptan (1mg/kg i.p.) Treatment after 72 hrs for 15 days straight

Group VII: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o)+ Rizatriptan (1mg/kg i.p.) Treatment after 1 month for 15 days straight

Group VIII: STZ (60mg/kg, i.p.) on day 1 + Gemigliptin (5mg/kg p,o) + Pregabalin (10 mg/kg i.p.) for 15 days straight

Behavior Parameters

Tail Flick method

A variation of the tail flick model that uses radiant heat is the use of immersion of the tail. In this technique, the animal's tail was submerged in hot water that was 55°C heated for 5 cm from the tip of the tail and response that was tail flicking and escape of tail from water was observed. Tail flick and escape/ withdrawal of tail with respect to time was noted[8].

Hot Plate Method

The hot plate approach produces hyperalgesia responses through a mix of cerebral and peripheral mechanisms. In this procedure animals were placed one by one on the floor (metallic plate) in the blank space. The plate heated to a constant temperature at 55!. The animal's basal reaction time is determined by watching to see if its rear paw licks the hot plate or jumps in response (depending on which occurred first). Animals often respond within 5-8 seconds. To protect the paws, a 15-second cutoff time is maintained[8].

Photo Actometer

The locomotor activity (horizontal activity) can be readily evaluated by Actophotometer which operate photoelectric cells. In this procedure the machinery is turned on (check all the photocells for precise recording) and position each rat in the activity cage for 5 minutes separately. Note all the animal's basal activity[8].

Biochemical Parameter

Serum Analysis

Serum separated by centrifugation was used to determine the biochemical parameters like Serum Glucose, Serum Triglyceride and Serum Cholesterol using commercially available kits.

Nerve collection and preparation of homogenate

At the end of the treatment, a rat from each group was sacrificed by cervical dislocation, and sciatic nerve was removed. The sciatic nerve was rinsed with isotonic saline. A 10 % (w/v) tissue homogenate was prepared in 0.5 M Tris Buffer. Centrifuging the homogenate at 1000 rpm for 20 minutes at 4 °C to acquire the post nuclear fraction for the catalase assay and at 12,000 rpm for 60 minutes at 4 °C to obtain the post nuclear fraction for other enzyme assays. The following test was conducted using an Ultra-Violet Spectrophotometer (UV-160A, Shimadzu)[8].

Catalase Activity

The Luck's approach was used to measure the catalase activity. Supernatant of the tissue homogenate (10%) is combined with H2O2 phosphate buffer (3 ml) in the following situation. After 1 minute, At 240 nm, an alteration in absorbance is seen. The millimolar extinction coefficient of H2O2 (0.07/mmol/cm) was used to calculate the enzyme activity. Results were given in terms of micromoles of H2O2 decomposed each minute per milligrams of protein[8,9].

Superoxide Dismutase Activity

Using Kono's method, the activity of superoxide dismutase was evaluated. 0.1 mM EDTA, 24 M NBT (Nitrobluetetrazolium), and 0.03% Triton X100 were combined with tissue homogenate's (10%) after nuclear fraction. After that, To the reaction mixture, 1 mM of hydroxylamine hydrochloride was added. After 20 mins at 37 °C, the mixture's absorbance at 560 nm was measured. The amount of SOD required to reduce the rate of reaction by 50% was used to define one unit of enzyme, which was then expressed as one unit per milligramme of protein[10].

Nitric oxide (NO) Activity

Sodium Nitroprusside in phosphate buffer is mixed with Post nuclear fraction of homogenate (10%). It was then incubated at room temperature

for 2.5 hours. The incubated sample is then mixed with Griess reagent and the absorbance was taken after 10 min at 546 nm[11].

Histopathological Examination

Hematoxylin and eosin were used to stain sections of formalin-fixed sciatic nerve. Samples were evaluated for signs of demyelination, inflammation, vascular damage, and damage to nerve fibers under a light microscope[12,13].

Statistical Analysis

The data from the investigations were subjected to one way analysis of variance (ANOVA) in order to determine the significance of the difference. In order to determine intergroup significance, Dunnett's t-test was utilized. P values under 0.05 were considered significant. The data was all presented as mean± SEM. ns – non-significant, * pÂ 0.05, ** pÂ 0.01 and *** pÂ 0.001.

RESULTS

Biochemical Estimation

Serum Glucose

Group I showed the normal level of serum glucose. Group II showed significantly (pÂ 0.001) increased in serum glucose level compared to Group I. Group III, IV, V, VI.VII, VIII (pÂ 0.001) showed significantly decreased in serum glucose level as compared to Group II.

Serum Triglyceride

The serum triglyceride level in Group I was normal. In comparison to Group I, Group II's serum triglyceride level was considerably ($p < 0.001$) higher. When compared to group II, group III's serum triglyceride level decreased, but not significantly. Serum triglycerides significantly decreased in groups IV, VI, VII, and VIII ($p<0.001$) and group V ($p<0.01$) as compared to group II.

Serum Total Cholesterol

Group I showed the normal level of serum cholesterol. Group II showed significantly (pÂ 0.001) increased in serum cholesterol level compared to Group I. Group III showed non-significant decrease in serum cholesterol as compared to group II. Group IV ($p<0.05$), V ($p< 0.01$), VI, VII, VIII ($p<0.001$) showed significant decrease in serum triglyceride as compare to group II.

Behavioral Parameters

Tail Flick Method

Group I showed normal reaction time in tail flick latency. Group II showed significant (pÂ 0.001) decrease in tail flick latency compared to Group I. Group III showed non-significant change in tail flick latency as compared to group II. Group IV ($p<0.01$), group V, VIII ($p<0.001$), group VI, VII ($p<0.001$) showed significant increase in tail flick latency as compared to Group II.

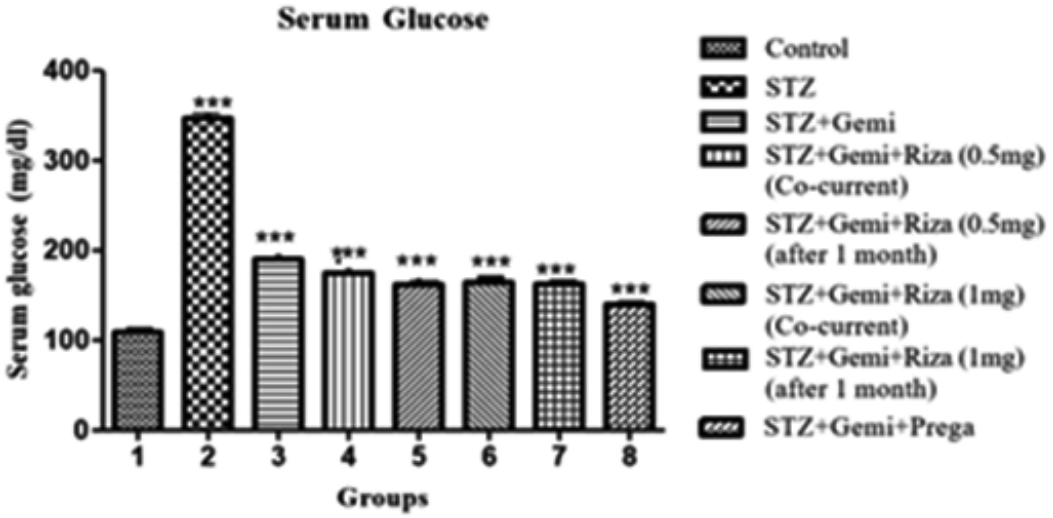

Fig. 1. Effect of Gemigliptin and Rizatriptan on serum glucose level in Streptozotocin induced diabetes

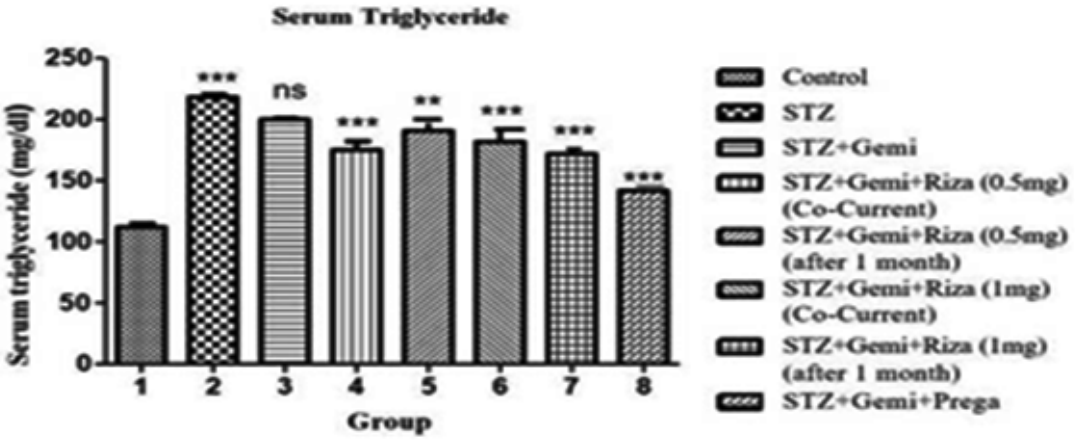

Fig. 2. Effect of Gemigliptin and Rizatriptan on Serum Triglyceride level in Streptozotocin induced diabetes

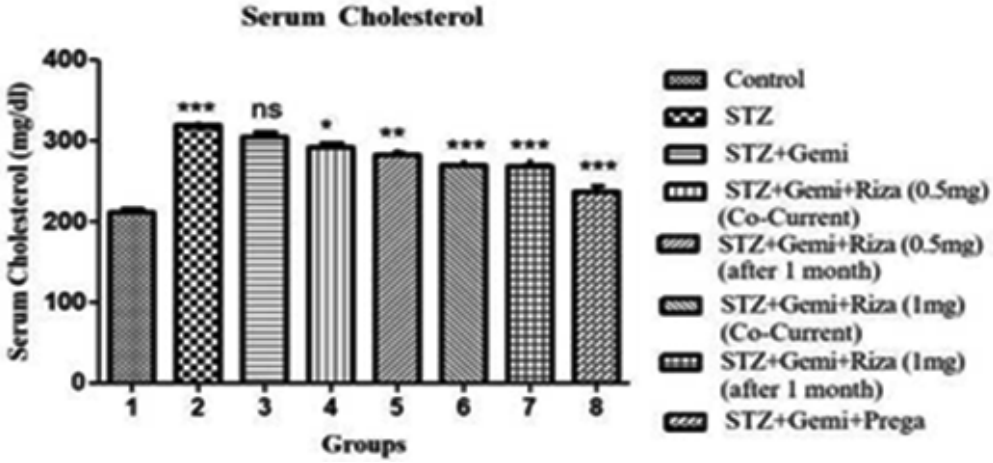

Fig. 3. Effect of Gemigliptin and Rizatriptan on Serum Cholesterol level in Streptozotocin induced diabetes

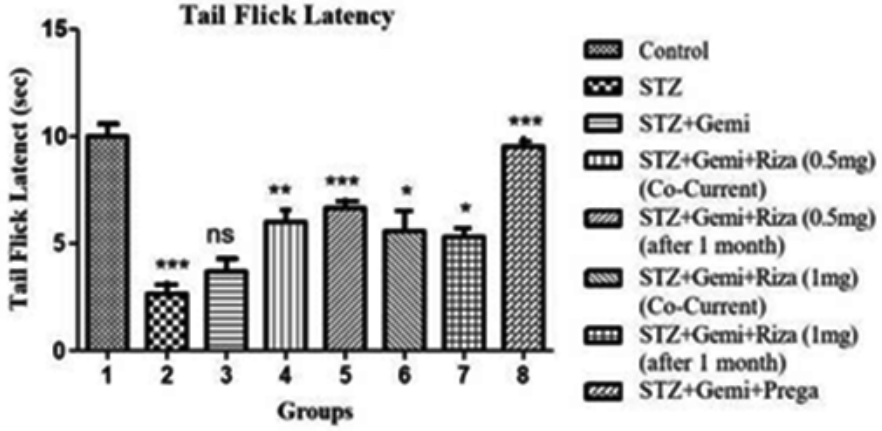

Fig. 4. Effect of Gemigliptin and Rizatriptan on Tail Flick Latency in Streptozotocin induced diabetes

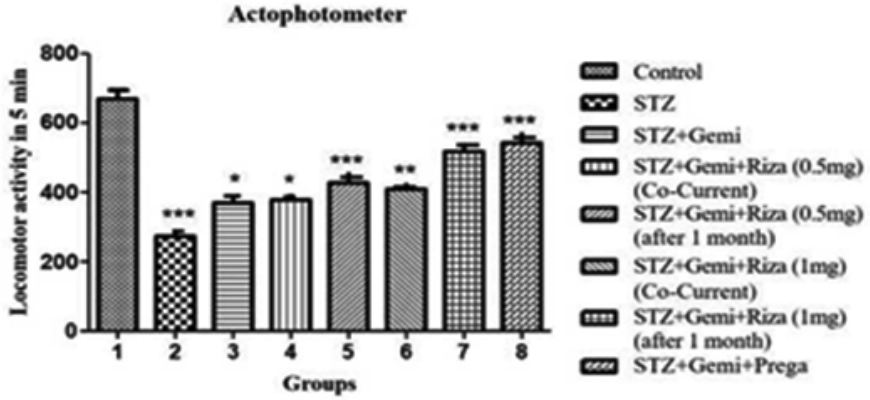

Fig. 5. Effect of Gemigliptin and Rizatriptan on Locomotor Activity in Streptozotocin induced diabetes

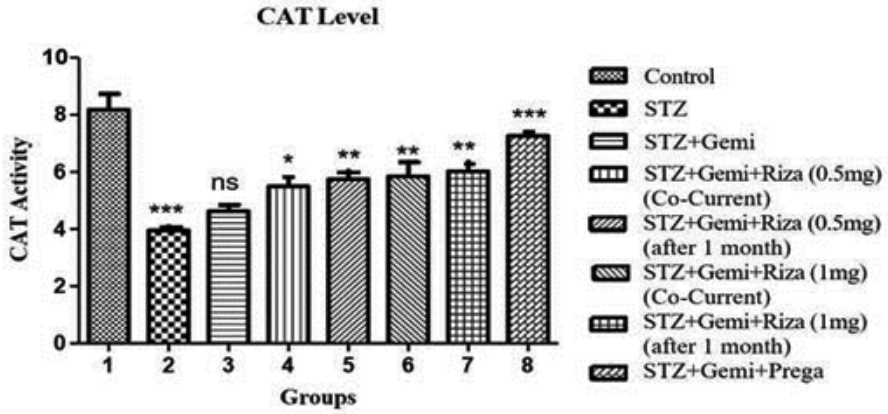

Fig. 6. Effect of Gemigliptin and Rizatriptan on CAT level in streptozotocin induced diabetic neuropathy

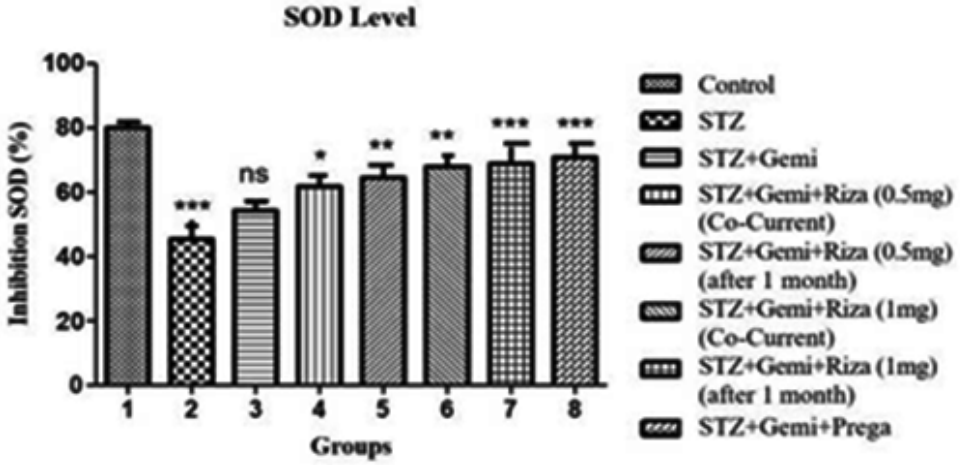

Fig. 7. Effect of Gemigliptin and Rizatriptan on Superoxide Dismutase level in streptozotocin induced diabetic neuropathy

Hot Plate Method

Control group showed the normal reaction time on hot plate. The streptozotocin treated group showed significantly (pÂ 0.001) decreased reaction time compared to control group. Treated groups III, IV, VI, showed (pÂ 0.01) and groups V, VII, VIII showed ($p<0.001$) that is significant increase in reaction time compared to Group II.

Antioxidant Activity

Effect of Catalase (CAT) level

Group I had a normal amount of catalase. When compared to Group I, Group II had a considerably ($p<0.001$) lower CAT level. CAT levels in Group III were not substantially higher than in Group II. While CAT levels in Groups V, VI, and VII were considerably ($p<0.01$) higher than those in Group II. When compared to Group II, Group IV's CAT level exhibited a marginally significant increase, whereas Group VIII's increase was extremely significant ($p<0.001$).

Effect of Superoxide Dismutase (SOD) level

Group I displayed a normal level of superoxide dismutase. Superoxide dismutase levels in Group II were considerably ($p<0.001$) lower than in Group I. Results for Group III are not significant. The SOD level has increased significantly ($p<0.05$) in Group IV. When compared to the STZ provided group, groups V and VI demonstrate ($p<0.01$) significance and VII and VIII demonstrate ($p<0.001$) extremely significant growth.

Effect of Nitric Oxide (NO) level

Group I displayed a normal quantity of nitric oxide. When compared to Group I, Group II's NO level decreased significantly ($p<0.001$) from that of Group I. Comparing Group III to the diabetic group, the amount of NO did not alter significantly. The recovery in NO levels was highly significant ($p<0.001$) in groups IV, VI, VII, and VIII. While compared to the other groups, Group V demonstrated significance in a lesser degree ($p<0.05$).

DISCUSSION

The patients with diabetes in the world have increased Four Times since 1980. In 2012 according to WHO's Global health report nearly 1.5 million death were due to diabetes. Although many treatment regimens are there not a single treatment is sufficient to cure the diabetic condition. Rather than diabetes the further complications due to diabetes are responsible for diminishing the quality of life in greater extent. On of such complication is diabetes induced neuropathy which if progresses further can cause severe conditions which ultimately leads to amputation of foot. In this study Streptozotocin induced diabetic neuropathy model is used as it closely resembles to the symptoms seen in humans. As STZ is responsible for the destruction of the â cells in pancreas we

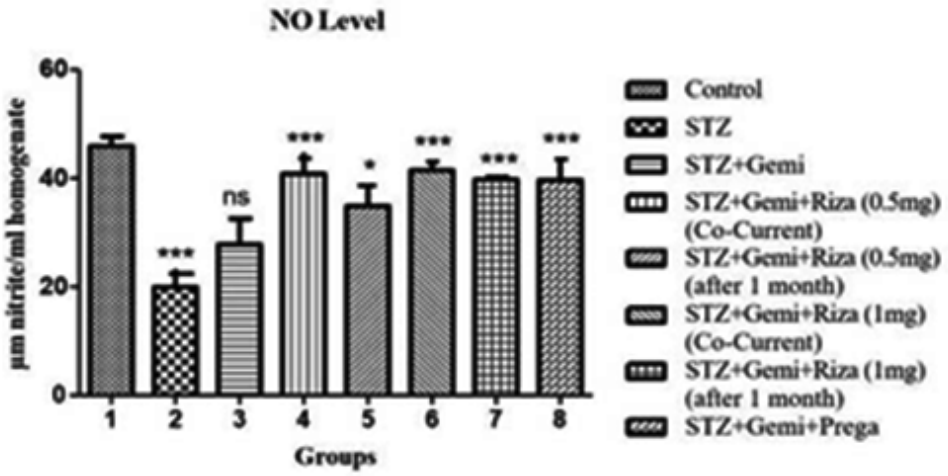

Fig. 8. Effect of Gemigliptin and Rizatriptan on Nitric Oxide level in streptozotocin induced diabetic neuropathy

were able to produce hyperglycaemia within 72 hours after single intraperitoneal injection of STZ. To check the effect of drugs under study two treatment regimen were followed, in which first regimen consist the start of treatment after neuropathy is confirmed to check the curative effect of drugs. While the second regimen started after 72 hours of inducer administration that is after the hyperglycaemic condition is confirmed to check the protective effect of the drug. Prior to inducer administration Pain threshold and locomotor activities of animals were checked by using Hot plate methods and Actophotometer respectively which showed normal observations which indicates normal pain threshold and locomotor activity. Single dose of STZ (60 mg/kg) was given to animals by intraperitoneal route, 5% solution of glucose was effective as no sudden death occurred due to sudden rise in serum glucose. After 72 hours urine analysis was done using Mission reagent strips, the change in colour of strip from bluish green to brown indicates presence of glucose in urine which primarily indicates hyperglycaemic condition. The actual glucose

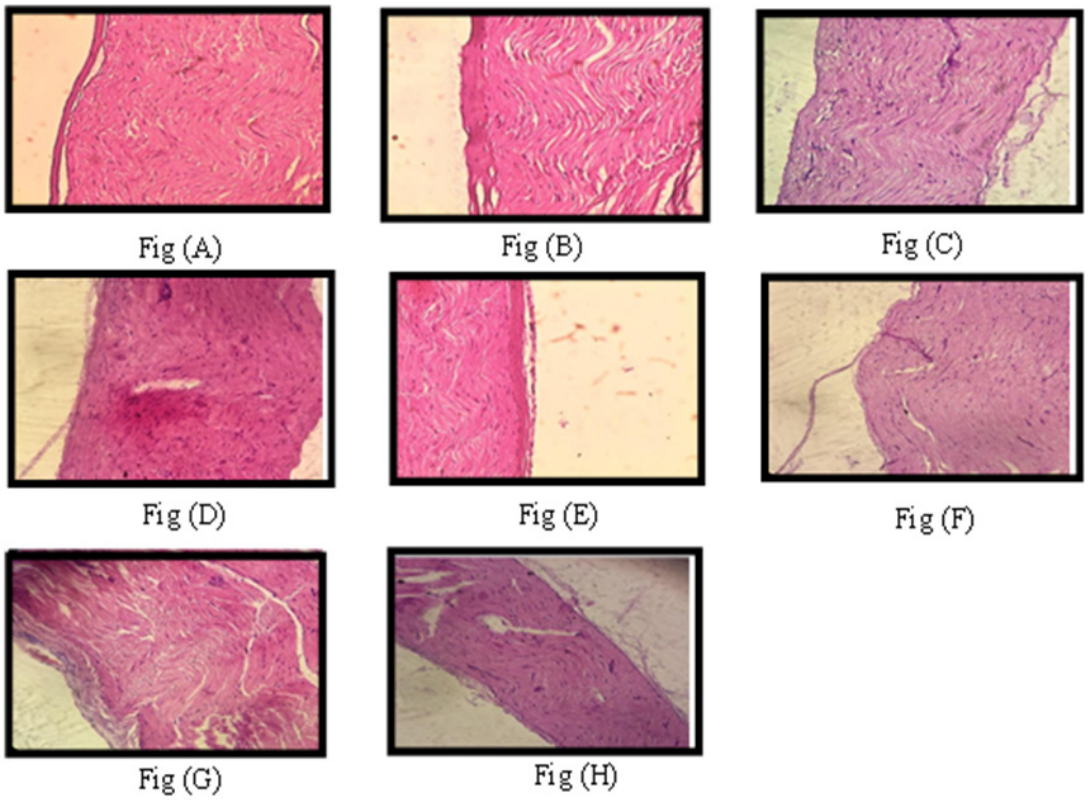

Fig (A): Histopath of sciatic nerve of control rat which showed normal morphology.
Fig (B): Histopath of sciatic nerve of diabetic rat showed beginning of demyelination and nerve starts to lose its normal morphology.
Fig (C): Histopath of sciatic nerve treated with only 5mg Gemigliptin showed non- significant changes as compared to diabetic nerve.
Fig (D): Histopath of sciatic nerve of animal which undergone co-current treatment of 0.5mg Rizatriptan showed slight recovery as compared to diseased nerve.
Fig (E): Histopath of sciatic nerve of animal which is treated with 0.5 mg of Rizatriptan after the neuropathy exhibited a considerable recovery compared to the preceding group.
Fig (F): Histopath of sciatic nerve of animal which is treated with 1mg of Rizatriptan in co- current manner showed less extent of nerve damage as compared to other nerves.
Fig (G): Histopath of sciatic nerve of animal treated after 1 month with 1mg Rizatriptan showed less extent of demyelination as compared to diseased nerve.
Fig (H): Histopath of sciatic nerve of animal which is treated with 10 mg of pregabalin showed lesser damage as compared to fig B.

Fig. 9. Histopathology of sciatic nerve

levels were determined after 15 days of inducer administration and level of glucose in serum were more than 300 mg/dl which is reported can cause diabetic neuropathy. Before treatment diabetic neuropathy was confirmed by checking behavioural parameters which shows hypersensitivity towards pain stimuli and reduced locomotor activity which are classic symptoms of neuropathy. Once neuropathy was confirmed treatment started which lasted for two weeks. Throughout the course of study body weight of animals were monitored. Control group showed non-significant change in body weight throughout the study. Diseased group showed drastic decrease in weight due to diabetic condition. Treatment groups showed satisfactory recovery in body weight especially group with 0.5mg rizatriptan given co-currently which showed highly significant ($p< 0.001$) recovery. In present study as the disease prolongs the level of serum glucose increased as the pancreatic cells are partially destroyed. This showed development of Hyperglycemia as reported. Apart from group II all other groups showed significant recovery ($p<0.001$) in blood sugar because they were treated with Gemigliptin which acts as DPP-4 inhibitor there by nearly normalising the serum glucose level. Along with serum glucose level serum cholesterol, triglyceride was also elevated in STZ given animals.When compared to the sick group, the treatment with Gemigliptin and Rizatriptan demonstrated a significant decrease in these values. Since pain is the most common sign of diabetic neuropathy, we used both the tail flick method and the hot plate method in this study to assess how the animals responded to heat stimulation. As neuropathy is the result of nerve damage, this nerve damage leads to hypersensitivity to stimuli in early stages of neuropathy and hyposensitivity in the late stage of neuropathy.In the current investigation, diabetic rats exhibited a reduction in tail flick latency and early withdrawal or paw licking. Significant increase in the latency was seen in the drug treated groups. Especially group V and VII showed significant recovery ($p<0.001$) in the hypersensitivity. Diabetic neuropathy in early stages not only affect the sensory nerves but also affects the motor nerves. To check the effects on this both nerves locomotor activities of animals were observed using actophotometer. In present study locomotor activity significantly improved ($p<0.001$) in groups where treatment was given after a month as compared to diabetic control group. In this study the antioxidant effect of Gemigliptin and Rizatriptan is studied with the help of assays of enzymes like superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO). In case of levels of nitric oxide, levels of NO were decreased in diabetic animals which is probably due to high oxidative stress.in present study combination of our drugs was able to increase the level of NO as compared to diabetic group. The levels of SOD and CAT were decreased significantly in the diabetic group. Rizatriptan in the dose of 1mg/kg was able to significantly normalise the levels of this enzymes. In histopathological study, animals with diabetes shows abnormal morphology in sciatic nerve with demyelination of several nerves also perineural fibrosis was observed in diabetic animals. Rizatriptan belongs to a group of drugs known as selective agonists of serotonin receptors. It functions by constricting blood arteries in the brain, preventing the brain from receiving pain signals, and preventing the production of several naturally occurring compounds that cause pain. Additionally, gemigliptin is GLT2 inhibitors modulate stroke risk variables, including lipids, insulin resistance, glucose levels, and body fat mass, by reducing antioxidant, anti-inflammatory, and antiapoptotic pathways. This results in a neuroprotective effect.Treatment with Gemigliptin and Rizatriptan shows significant decrease in nerve damage.

CONCLUSION

Results of the present study showed that drug combination of Gemigliptin and Rizatriptan is successful to improve symptoms of diabetic neuropathy. Combination of this drugs shows promising results by improving the levels of serum glucose, triglycerides and cholesterol levels whose imbalance is responsible for the diabetic related complications. Also, the drug combination improves the hyperalgesia, which is caused due to nerve damage, significant recovery in locomotor activity is also seen. Oxidative stress is reported to be underlying cause of the further complications of diabetes and the drug combination is seen to improve the level of antioxidants like SOD and CAT is seen. Thus, going through all the parameters

combination of Gemigliptin and Rizatriptan surely has positive effect in ameliorating diabetic neuropathic pain.

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Conflict of Interest

Authors declare no conflict of interest

Funding Source

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