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INVESTIGATION OF NEUROPROTECTIVE EFFECT OF RASAGILINE IN DIABETIC NEUROPATHY IN STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Background One of the most common complications of diabetes is the involvement of the peripheral and autonomic nervous systems . An important risk factor for the development of diabetic neuropathy in patients with type 1 or type 2 diabetes is the duration and severity of hyperglycaemia. Diabetic neuropathy is "the presence of symptoms and/or signs of peripheral nerve dysfunction in persons with diabetes after the exclusion of other causes." Till date there is no effective treatment available for diabetic neuropathy, However, it can be managed using certain pharmacological and, non-pharmacological treatments. The aim of this study is to investigate the neuroprotective effect of rasagiline in diabetic neuropathy. **Methods:** Diabetes was induced by a single dose of STZ (60 mg/kg i.p). After the administration of Streptozotocin the animals showed marked hyperglycaemia, reduction in body weight and other signs of neuropathy like thermal allodynia (hot and cold), thermal hyperalgesia and motor co-ordination was compared to the animals of the control group. **Results :** After 28 days of treatment with Rasagiline (0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg) in combination with Glimepiride (10 mg/kg), rats showed significant reduction in serum glucose level, and improvement in body weight. Rasagiline combined with Glimepiride showed good results in different parameters such as thermal allodynia (hot and cold), thermal hyperalgesia and motor co-ordination in comparison with diabetic control group. The most significant effect was seen with 1.0 mg/kg of rasagiline in combination with Glimepiride (10 mg/kg) after 28th day of treatment. **Conclusion:** Rasagiline can, therefore, be concluded to be effective treatment option for diabetic complications like neuropathy. This is due to its inhibitory effect on MAO-B.

KEYWORDS: thermal hyperalgesia, thermal allodynia, cold allodynia and motor inco-ordination.

INTRODUCTION

Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality . Diabetic nephropathy has been classically defined by the presence of proteinuria 0.5 g/24 h. This stage has been referred to as overt nephropathy, clinical nephropathy, proteinuria, or macroalbuminuria.[1] One of the most common complications of diabetes is the involvement of the peripheral and autonomic nervous systems. An important risk factor for the development of diabetic neuropathy in patients with type 1 or type 2 diabetes is duration and severity of hyperglycemia.[2] A simple definition of diabetic neuropathy is "the presence of symptoms and/or signs of peripheral nerve dysfunction in person with diabetes after the exclusion of other causes.".[3] In the Western world, Diabetes is the leading cause of neuropathy and happens to be most common complication and greatest source of morbidity and mortality in diabetes patients.[4] All peripheral nerves including pain-fibres, motor neurons and the autonomic nervous system are affected by Diabetic neuropathy. The pathogenesis of diabetic neuropathy is yet to be understood completely. It is suggested that hyperglycemia leads to changes in the nerve tissue.[5] In some patients, the manifestations of diabetic neuropathy may precede glucose intolerance and the diabetic neuropathy may be thought to be of a different origin then diabetes[6]. Patients with diabetic neuropathy commonly complain of symptoms of neuropathic pain.[7] Focal mononeuropathies can result in third and sixth cranial nerve palsies, painful intercostal neuropathy, and isolated muscle weakness involving the hip girdle.[5] Patients with diabetic peripheral neuropathy manifest painful symptoms, which are commonly characterized as burning, aching, tingling, cold, lancinating, allodynia, and/or numbness.[8]

The understanding of the pathogenesis of diabetic neuropathy in recent years has increased to a great extent, and new drugs that target the pathophysiological mechanisms are currently being studied.[9] The pathological mechanisms implicated in diabetic neuropathy, include microvascular damage, metabolic

disorders, and changes in the interactions between neuronal and immunological systems in parallel with glial cells activation.[10]

Various factors such as Metabolic and vascular/hypoxic appear to be involved in diabetic polyneuropathy. Advanced glycosylation end products may damage capillaries, inhibit axonal transport, Na + /K + -ATPase activity and cause axonal degeneration. Hyperglycemia and increased intracellular Glucose may saturate normal glycolysis. Extra glucose may enter the polyol pathway and activates aldose, which converts it to fructose and sorbitol. Their Accumulation results in reduced nerven myoinositol and membrane Na + /K+-ATPase activity, impaired axonal transport leading to structural damage. Nerve ischemia may result from increased endoneural vascular resistance to hyperglycemic blood. In experimental animals, the metabolism of nerve growth factor (NGF) is impaired, which is the basis for clinical studies.[11] Recent evidences have suggested that hyperglycemia contributes to a state of heightened oxidative stress and the generation of reactive oxygen species that are important ‘in the development of neuropathy and other microvascular diabetes complications. various metabolic pathways such as polyol pathway probably contribute to hyperglycemia-induced oxidative stress, including the, protein kinase C (PKC) activation, and accumulation of the end products of autoglycation (ie, advanced glycation endproducts).[12]

There is no specific treatment available for DPN but this decease could be managed using different therapeutic strategies. Drugs used to treat DPN include antidepressants (primarily tricyclic antidepressants [TCAs], antiepileptics, NSAIDS, Opioids, Inhibitors of Protein Kinase C pathway and others. Carbamazepine was the first medication studied for use in the treatment of DPN. Amitriptyline was first studied in an open-label study in 1977.[13] The other agents used for treatment of DPN include opiates, capsaicin, lidocain, l-carnitine & alpha-lipoic acid.[14]

MATERIALS AND METHODS

Chemicals

Rasagiline was obtained from Sun Pharma., India. STZ and nicotinamide where obtained from Sigma-Aldrich. Sodium citrate and citric acid were obtained from Central Drug House, New Delhi. All the other chemicals and biochemical reagents, of highest analytical grade, were used.

Animals

Wistar rats of either sex, weighing 100-150 gms were procured from the departmental animal house of Division of Pharmaceutical Sciences, SGRRITS, Dehradun. Animals were acclimatized to the environment of the animal house facility of department and were group housed (n=8 per cage) in 12hr light/dark cycle. During the whole study animals were fed with a standard chow diet and water ad libitum. All experiments were carried out between 9:00 and 17:00 o’clock and using age-matched animals in an attempt to avoid variability between experimental groups. Animal body weight was measured at the beginning and at the end of the experiment. All the experiments were carried out as per CPCSEA guidelines and were performed after approval of project by IAEC of Division of Pharmaceutical Sciences, SGRRITS. (Regd No. 264/CPCSEA).

***Induction and assessment of diabetes (type2) in rats.*[15]**

A single dose of 60mg/kg Streptozotocin prepared in citrate buffer (pH 4.4.0.1M) was administered intraperitoneally to overnight fasted animals to induce diabetes. NAD+ 235mg/kg was admistered prior (15 minutes) to STZ administration. The control rats received an equal volume of citrate buffer and were used along with diabetic animals. Serum glucose level was estimated on 0th, 7th, 14thand 28th and 75th day respectively, after STZ administration by enzymatic GOD-POD (glucose oxidase peroxidase) diagnostic kit. The rats having fasting plasma glucose levels more than 250mg/dL were selective for the study. STZ treated rats were provided with 10% glucose solution after 6 hrs to prevented fatal hypoglycemia, since STZ is potent enough to cause fatal hypoglycemia as a result of massive pancreatic insulin release.

Study protocol

The animals were divided into six groups of 8 animals each.

Group I: Sham control: Citrate buffer administered as a vehicle.

Group II: Diabetic control: STZ (60mg/kg, i.p) + NAD^{+}(235mg/kg), administered [15 minutes] prior to STZ administration.

Group III: Active control: Glimepiride (10mg/kg) + Amitriptyline (10mg/kg), administered to diabetic animals for 4 weeks.

Group IV: Glimepiride (10mg/kg) + Rasagiline (0.25mg/kg, i.p) were administered to diabetic animals for 4 weeks.

Group V: Glimepiride (10mg/kg) + Rasagiline (0.5mg/kg, i.p) were administered to diabetic animals for 4 weeks.

Group VI: Glimepiride (10mg/kg) + Rasagiline (1.0 mg/kg, i.p) were administered to diabetic animals for 4 weeks.

Behavioral test paradigm

The behavioral parameters were assessed 4 weeks after treatment with STZ.

Thermal hyperalgesia (hot plat method)[16] Rats were transported to the testing room in their home cages. Rats were allowed to acclimatize for 15 mins. Hot plate apparatus was switched on to heat up the surface of the hot plate to a constant temperature of 55±0.2˚C. Rats were place on the hot plate surrounded by a clear acrylic protection casing. The paw withdrawal time, displayed on the dial of the apparatus, was noted down. If the rat

did not respond within 30 secs, the test was terminated and the rat was removed from the hot plate.After testing the last rat, the apparatus was wiped and cleaned with detergent then with 70% ethanol.

***Thermal Allodynia (hot plate method)*[17]**
Animals were placed into a glass cylinder on a hot plate adjusted at 38˚C. The time at which animal withdrew its paw was recorded as paw withdrawal latency with a cut-off time of 30 secs. Heating was terminated to prevent tissue damage if an animal failed to withdraw its paw prior to the cutoff.

***Cold Allodynia*[18]**
Rats were transported to the testing room from their home cages. Rats were allowed to acclimatize for 15 mins. Animals were placed on an ice platform submerged approximately 1cm below the surface of cold water (4˚C), such that hairy and glabrous skin of the animal's feet was in contact with water. Paw withdrawal latency was recorded was recorded with a cutoff time of 30 secs.

***Motor Coordination Test*[19]**
Motor coordination (grip muscle strength) was evaluated by a Rotarod apparatus. Rats were transported to the testing room in their home cages. Allowed to acclimatize for 15 mins. Rotarod apparatus was turned on. The apparatus was set at an acceleration of 4rpm, to constantly increase the rotation to 25rpm. Rats were placed on the rotating rod for one minute

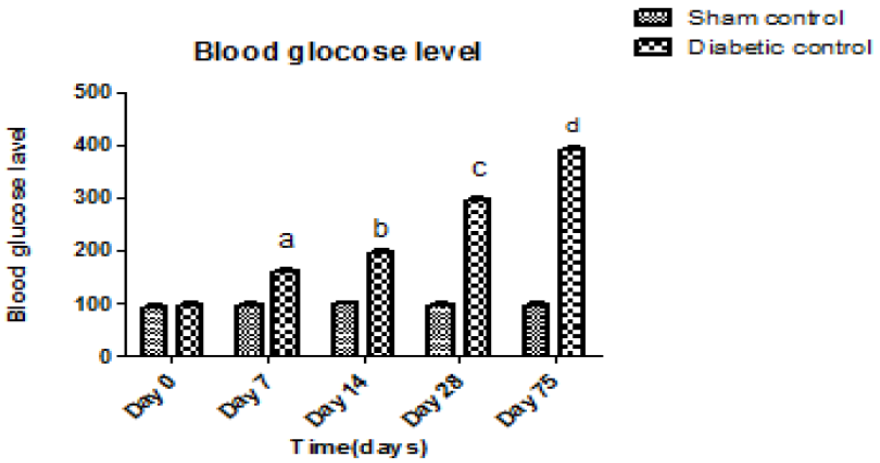
The time taken for falling off the rod (within one minute) was recorded. After testing the last rat, the apparatus was cleaned and wiped with detergent and 70% ethanol.

Statistical Analysis
The data obtained from the results was analyzed using two-way ANOVA followed by Bonferroni's post-test with Graph Pad Prism 5.4 software. All the data was expressed as the mean ± SEM of their means.

RESULTS
Effect of Streptozotocin on Serum Glucose Level in STZ induced diabetic rats
In the control group the Serum glucose level was observed to be 95.4 mg/dl. Serum glucose level was estimated in 0^{th} 7^{th}, 14^{th}, 28^{th} and 75^{th} days in all STZ treated groups. The diabetic animals were selected whose serum glucose level was found more than 250 mg/dl after administration of STZ**. (Figure 1).**

Group	Day 0	Day 7	Day 14	Day 28	Day 75
Sham control	94.850±2.682	97.213±2.488	98.887±2.329	96.988±2.771	95.400±3.776
Diabetic control	98.188±2.181	161.038±3.272[a]	196.025±3.195[b]	298.138±2.520[c]	390.462±3.903[d]

All data are presented as mean±SEM . Sham control represents the group treated with citrate buffer. Diabetic control represents the group treated with STZ. aP<0.01. bP<0.5.



a= p≤0.01, b= p≤0.01, c= p≤0.01, d= p≤0.01
Figure 1: Effect of STZ on blood glucose level in rats.

Effect of Streptozotocin on Body Weight in STZ induced diabetic rats
Over a period of 75 days after the administration of STZ, there was a significant reduction in body weight observed in diabetic rats (116.0 g) as compared to control group .**(Figure 2).**

Group	Day 0	Day 7	Day 14	Day 28	Day 75
Sham control	150.625±1.125	151.000±1.669	148.825±1.481	147.125±2.601	140.500±6.897
Diabetic control	152.750±0.701	149.000±1.225[a]	144.125±1.043[b]	135.000±1.254[c]	116.000±1.547[d]

All data are presented as mean±SEM . Sham control represents the group treated with citrate buffer. Diabetic control represents the group treated with STZ. aP<0.01. bP<0.5

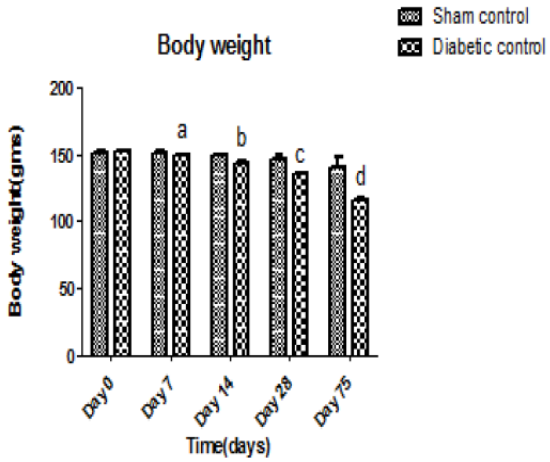
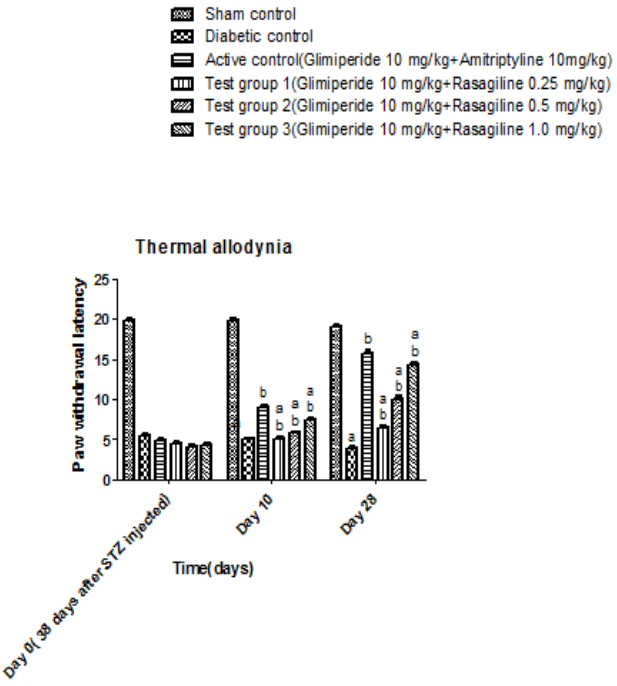
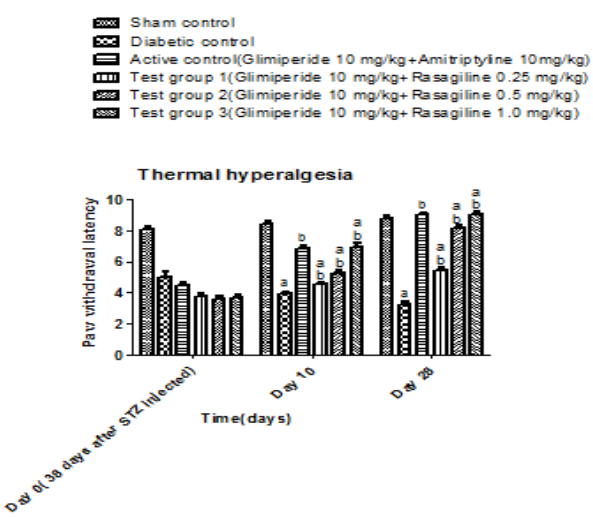
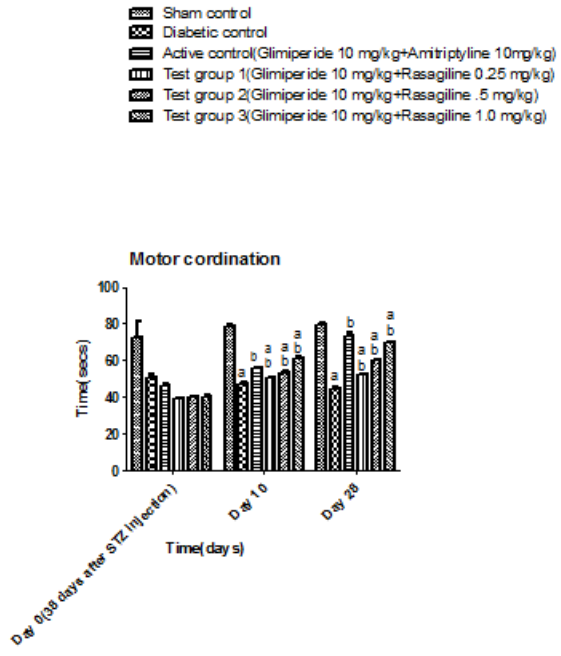


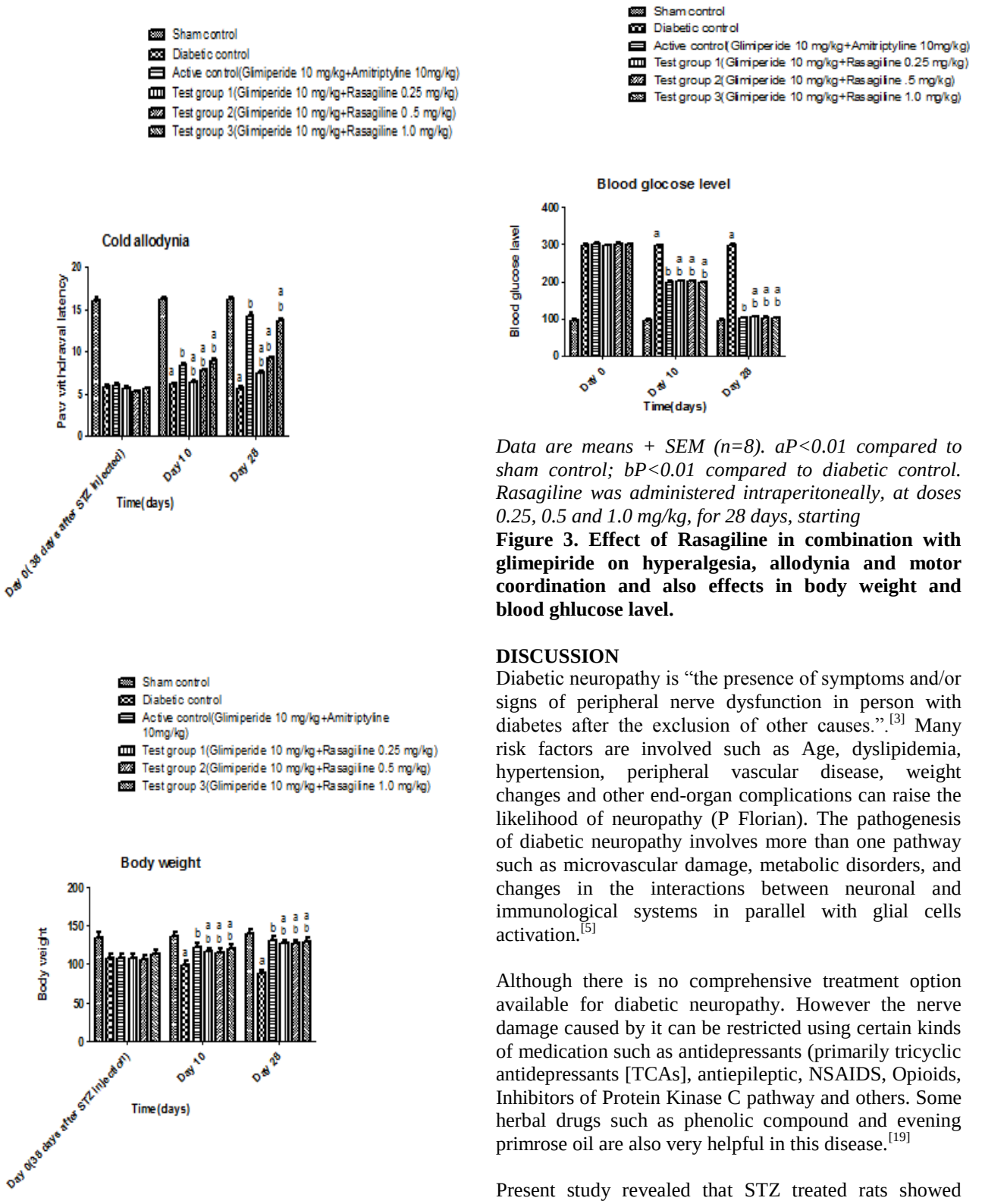
Figure 2 a= p≤0.01, b= p≤0.01, c= p≤0.01, d= p≤0.01

Effect of rasagiline in combination with glimipride on thermal hyperalgesia, allodynia and motor coordination The threshold of thermal hyperalgesia, thermal allodynia and cold allodynia was markedly reduced by day 28 after STZ administration. rasagiline, administred in combination with glimepiride, started on day 28 after STZ administration, was able to bring about an improvement in the pain threshold, in a dose dependent manner, over a period of 28 days. The motor coordination was similar seen to improve, with an increase in the fall-off time, over the same period. The body weight were improved and the blood glucose lavel was decrease in the given treatment.









Data are means + SEM (n=8). aP<0.01 compared to sham control; bP<0.01 compared to diabetic control. Rasagiline was administered intraperitoneally, at doses 0.25, 0.5 and 1.0 mg/kg, for 28 days, starting

Figure 3. Effect of Rasagiline in combination with glimepiride on hyperalgesia, allodynia and motor coordination and also effects in body weight and blood ghlucose lavel.

DISCUSSION

Diabetic neuropathy is "the presence of symptoms and/or signs of peripheral nerve dysfunction in person with diabetes after the exclusion of other causes.".[3] Many risk factors are involved such as Age, dyslipidemia, hypertension, peripheral vascular disease, weight changes and other end-organ complications can raise the likelihood of neuropathy (P Florian). The pathogenesis of diabetic neuropathy involves more than one pathway such as microvascular damage, metabolic disorders, and changes in the interactions between neuronal and immunological systems in parallel with glial cells activation.[5]

Although there is no comprehensive treatment option available for diabetic neuropathy. However the nerve damage caused by it can be restricted using certain kinds of medication such as antidepressants (primarily tricyclic antidepressants [TCAs], antiepileptic, NSAIDS, Opioids, Inhibitors of Protein Kinase C pathway and others. Some herbal drugs such as phenolic compound and evening primrose oil are also very helpful in this disease.[19]

Present study revealed that STZ treated rats showed marked hyperglycemia, thermal allodynia (hot and cold), thermal hyperalgesia and motor co-ordination as compared to control group which continued to increase for 8-9 weeks after treatment. These pathological changes might serve as a simulated of human diabetic neuropathy. It was also seen that diabetic rats lose their

body weight compared to non- diabetic rats after the treatment of STZ which continued to decrease for 8-9 weeks and onwards.

Our study shows that rasagiline a MAO-B inhibiter is an effected treatment option in diabetic neuropathy.

The motor effectiveness produced by rasagiline has been demonstrated in many studies, with durable response, with additional studies showing rapid onset of action. Considering also the simplicity of administration, good compliance, and the low side effects, the importance of rasagiline in the treatment of patients with Parkinson disease at all stages of the disease is well established.[20] Acute rasagiline treatment enhanced object recognition and spatial learning in healthy rats.[21]

Rasagiline prevents dopamine metabolism irreversibly, thus increasing levels of dopamine, the result being a symptomatic benefit in patients with PD. As adjunctive therapy, rasagiline has proved efficacious and well-tolerated in reducing "off"-time, and therefore provides an additional option, with a simpler dosing schedule, to dopamine agonists and COMT inhibitors for those with motor fluctuations.[22] So in following studies rasagiline inhibits the MAO-B which inturn supresses dopamine metabolism in the brain. As result of which free radicals are not produced that intern to leads to prevention of nerve cell damage. So in all this studies proves that rasagiline may show greater therapeutic potential for treating diabetic neuropathy. Therefore we were interested in studying its effect in model of type 2 diabetes.

Our result proves that the rasagiline helps to treat the following condition of diabetic neuropathy in a dose dependent manner. Rasagiline combine with Glimeperide (10 mg/kg) showed significant reduction in hyperglycemia, body weight loss, thermal allodinia (hot and cold), thermal hyperalgesia and moter co-ordination, when compared to rats of diabetic control group. The most significant effect was seen with the dose of Rasagiline (1.0 mg/kg) in combination with Glimepiride (10mg/kg) after 28 days of treatment.

Our study shows that Rasagiline could be used as a treatment option in diabetic neuropathy, which could be attributed to its MAO-B inhibitory action. Further studies are required to establish the above mentioned fact.

CONCLUSION

Rasagline combine with Glimiperide (10 mg/kg) significant reduce the symptoms of diabetic neuropathy in a dose dependent manner. The most significant effect of Rasagiline in combination with Glimiperide (10 mg/kg) was noted at a dose of (1.0 mg/kg) on day 28th of treatment. Rasagiline, after 28 days, at 1.0 mg/kg, showed effects that were close to those produced by the standard drug (Amitryptiline10 mg / kg).

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