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ROLE OF RASAGILINE, A MAO-B INHIBITOR IN STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY.

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ABSTRACT

Introduction: Diabetic nephropathy is a chronic micro vascular complication of diabetes mellitus which affects the kidneys and is one of the common reasons for End Stage Renal Disease leading to mortality and morbidity. Hyperglycemia induces oxidative stress and leads to activation of multiple biochemical pathways which are a major source of kidney damage. Thus, prevention and treatment of diabetic nephropathy can reduce the incidence of end stage renal disease, death and ultimately the economic burden. Currently treatments available for diabetic nephropathy are limited so more investigations are needed to improve the condition of the patients. The present study involves investigation of the effect of MAO-B inhibitor in diabetic nephropathic animals. **Methods:** Male wistar rats, weighing 150-250 g were randomized into nine groups (n=7). Diabetes was induced by administering Streptozotocin (65mg/kg, i.p.) 15 min after nicotinamide (110 mg/kg, i.p.) injection. Starting from 5th week, post nicotinamide (NAD)-Streptozotocin (STZ) injection, Rasagiline (RG) and Glimepiride (GP) were administered for 4 weeks. Serum glucose, body weight, serum creatinine, and serum urea were measured weekly. Malondialdehyde measurement and histopathology of kidney was carried out at the end of the study. **Results:** After 28 days of treatment with RG (0.5, 1.0 mg/kg, i.p), significant reduction in serum urea, serum creatinine and lipid peroxidation was observed. No significant effect was observed on serum glucose and body weight as compared to diabetic control. RG (0.5, 1.0 mg/kg, i.p) in combination with GP (10mg/kg, i.p) showed significant reduction in serum glucose, serum urea, serum creatinine and lipid peroxidation. Improvement in body weight was also observed in STZ induced diabetic rats as compared to diabetic control. **Conclusion**: The present study concludes that Combination of RG and GP not only attenuates the diabetes but also reverses the nephropathic signs through its nephroprotective actions and thus RG may serve as a new therapeutic alternative for management in nephropathy associated with type-2 diabetes.

KEYWORDS: Diabetic nephropathy, Rasagiline, oxidative stress, renal function test.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder characterized by the existence of hyperglycemia due to deficient insulin secretion or deficient insulin action or both, affecting metabolism of carbohydrate, protein, and fat. It also affects β cells of the pancreas, kidneys, and liver. It is classified into type-1 and type-2 DM. Type-2 DM is a heterogeneous disorder characterized by insulin resistance, followed by the pancreatic β cell dysfunction wherein they are unable to compensate for insulin resistance.[1,2,3]

The secondary complications of diabetes mellitus influences many organ systems and are the major source of elevated mortality and morbidity. It can be divided as micro vascular and macro vascular complications. Micro vascular complications include retinopathy, neuropathy, nephropathy and macro vascular complications comprise of coronary artery disease, peripheral vascular disease and cerebrovascular disease. It also includes problems like gastroparesis, sexual dysfunction and dermatological complications.[4,5,6]

Diabetic Nephropathy is also known as Kimmelstiel–Wilson syndrome. It is the condition in which kidneys lose their capability to function properly due to diabetes and is the most common cause of chronic renal failure in both developing and developed countries.[7,8] Interactions between metabolic and hemodynamic factors contribute to evolution and consequences of diabetic nephropathy. Activation of common pathways like metabolic derangement, glomerular hypertension, oxidative stress, and advanced glycation end products are responsible for progression of diabetic nephropathy and ultimately renal damage. It also includes clinical irregularities of kidney which consists of increased creatinine level and also elevation in urea, and retention of fluid.[9,10,11]

Currently oxidative stress is suggested as mechanism underlying diabetes and its complications, which results from an imbalance between radical generating and

radical scavenging systems. Oxidative stress plays an important role in the advancement of diabetic complications. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction which causes the activation of 5 major pathways involved in the pathogenesis of complications: polyol pathway flux, increased formation of AGEs (advanced glycation end products), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway. Through these pathways, increased intracellular reactive oxygen species (ROS) activate a number of proinflammatory pathways leading to diabetic nephropathy.[12,13,14]

Monoamine Oxidases and its substrates are present in both exocrine and endocrine parts of the pancreas and are involved in the oxidative deamination of catecholamine, serotonin, and dopamine. MAO is a hydrogen peroxide generating enzyme; this property makes it an important substance in redox status of cells.[15] In individuals with Type-2 DM, a drop in dopamine levels is thought to lead to an inadequate hypothalamus response, resulting in an elevated levels of blood glucose, free fatty acids (FFA), and triglycerides (TG), which contribute to insulin resistance, visceral adiposity, and beta-cell dysfunction. MAO-B inhibitors, such as rasagiline or selegiline, bind to and inhibit MAO-B, preventing dopamine degradation. This results in greater stores of dopamine available for release and can reduce hepatic and renal gluconeogenesis due to reduced cortisol level in response to dopamine.[16]

These nephroprotective effects of rasagiline or selegiline are not only due to MAO-B inhibition, but also due to many other effects, such as suppression of free radical formation, up-regulation of the antioxidant enzymes, superoxide dismutase and catalase and attenuation of apoptosis.[17,18]

Therefore in this study we have investigated the effect of rasagiline in STZ induced diabetic nephropathy as Rasagiline may be useful in the treatment of diabetic nephropathy because of its reduced hepatic and renal gluconeogensis and nephroprotective actions which are independent of its inhibitory action on MAO-B.

MATERIALS AND METHODS

Animals

Male Wistar rats (150-250 g) were procured from Shri Guru Ram Rai Institute of Technology, Dehradun. During the experiment, rats were housed in standard housing conditions like temperature of 25± 1ºC, relative humidity of 45%-55% and 12 h light: 12 h dark cycle. Rats had free access to food pellets and tap water *ad libitum* during the experiment. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and was carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India.

Chemicals and Reagents

Glimepride was gifted from PIL, Rasagiline, Streptozotocin and nicotinamide was purchased from Sigma Aldrich. Other chemicals such as potassium dihydrogen phosphate, sodium chloride, hydrochloric acid, sucrose, thiobarbituric acid, trichlocroacetic acid, heparin, anesthetic ether other chemicals were supplied by departmental laboratory at SGRRITS Dehradun. All chemicals used were of analytical grade. Serum glucose estimation kit (GOD/POD), Serum urea estimation kit, serum creatinine estimation kit was procured from Himgiri traders Dehradun.

Preparation of solutions

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

Induction of diabetes and experimental design

NAD (110 mg/kg, i.p) was injected in overnight fasted rats followed by STZ (65 mg/kg, i.p.) injection after 15 min of NAD administration, in all the groups except non-diabetic group. STZ was freshly prepared by dissolving in 0.1 M citrate buffer (pH 4.8) while NAD was prepared in physiological saline. The STZ treated rats were fed with glucose solution (4%) for 12 h to avoid hypoglycemia. Rats having serum glucose more than 250 mg/dl after 72 hrs of induction were considered 'diabetic' and selected for the further study. All the animals were allowed free access to water, pellet diet and kept in polyethylene cages.

Control and diabetic rats were randomly selected and divided into nine groups, each group consisting of seven animals.

Group I: Non-Diabetic group. [Normal animals without any treatment]

Group II: Diabetic control: Rats treated with NAD (110mg/kg, i.p.) followed by Streptozotocin (65mg/kg, i.p.) to develop type II DM. Vehicle (citrate buffer) treated group

Group III: Diabetic rats treated with Glimepiride (10 mg/kg, i.p.)

Group IV: Diabetic rats treated with test drug Rasagiline (0.25mg/kg, i.p)
Group V: Diabetic rats treated with test drug Rasagiline (0.5mg/kg, i.p)

Group VI:. Diabetic rats treated with test drug Rasagiline (1.0mg/kg, i.p)

Group VII: Diabetic rats treated with test drug Rasagiline (0.25mg/kg, i.p) + Glimepiride (10 mg/kg, i.p.)

Group VIII: Diabetic rats treated with test drug Rasagiline (0.5mg/kg, i.p) + Glimepiride (10 mg/kg, i.p.)

Group IX: Diabetic rats treated with test drug Rasagiline (1.0mg/kg, i.p) + Glimepiride (10 mg/kg, i.p.)
The rats were allowed to develop diabetic nephropathy for next four weeks. RG (test drug) was administered for next 4 weeks starting from 5th week of NAD-STZ injection.

The blood sample was collected from tail vein technique and the serum was separated by centrifugation at 4500 rpm for 10 min. The serum glucose, serum urea, and serum creatinine of all the animals was estimated at weekly interval basal, 1st week, 2nd week, 3rd week, 4th week, 5th, 6th, 7th and 8th week during the experimental period. At the end of the experiment rats were sacrificed by cervical dislocation under light anesthesia and both kidneys were immediately isolated for the evaluation of lipid peroxidation levels and histopathological studies.

BIOCHEMICAL PARAMETERS

- **Estimation of serum glucose[19]**

Serum glucose levels were estimated by glucose oxidase-peroxidise (GOD-POD) method.

Principle: Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of Peroxidase (POD) oxidizes the chromogen 4-Aminoantipyrine/ phenolic compound to a red coloured compound. The red colour so developed was measured spectrophotometrically at 505nm. The intensity is proportional to the concentration of the glucose present in the sample.

$Glucose + O_2 + H_2 \rightarrow$ gluconic acid + H_2O_2

H_2O_2 + Phenolic compound + 4- Amino-antipyrine → Red compound + 2H2O

Procedure: In this method, 1000 µl working glucose reagent was added to 10 µl of serum (obtained from blood samples), 10 µl of standard glucose (100 mg/dl) and 10 µl of purified water to prepare test, standard and blank sample respectively. All the test tubes were incubated at room temperature for 30 min. To each test tube, 1000 µl of purified water was added. The absorbance of test and standard samples were measured against blank.

- **Body weight measurement**

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

Estimation of serum urea[20]

Principle: Urease splits urea into ammonia and carbon dioxide. Ammonia released in this reaction reacts with hypochlorite and Phenolic chromogen to produce green colored compound (indophenols). The intensity of color produced at 578nm is directly proportional to the concentration of urea in the sample.

Procedure: 1000 µl of working reagent-I containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10µl of serum, 10µl of standard urea (40mg/ml) and 10µl of purified water to prepare test, standard and blank, respectively. The absorbance of test and standard samples were measured against blank.

Estimation of Serum Creatinine[21]

Principle: Creatinine in alkaline medium reacts with picrate to produce orange colored complex and this colour absorbs light at 520 nm. The rate of increase in absorbance is directly proportional to the concentration of creatinine in specimen.

Procedure: 1000 µL working reagent was added to 100 µL of serum, 100 µL of standard creatinine, 100 µL of purified water to prepare test, standard and blank samples respectively. To each test tube, 1000 µL of purified water was added. The absorbance of test and standard samples were measured against blank.

Estimation of MDA[22]

The renal thiobarbituric acid reactive substances, an index of lipid peroxidation, were estimated.

15 ml centrifuge tubes for the standards and samples was labeled accordingly. 100 ml of 8.1% sodium lauryl sulphate solution to tube was added and swirled to mix. 1.5ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of thio barbituric acid was added to each tube. The final volume was made up to 4 ml with biological grade water. The tubes were heated at 95-100˚C in a boiling water bath for one hour. After one hour the tubes were cooled immediately under running tap water for 10 min and centrifuged at 4000 rpm for 10 mins. The supernatant was collected and the optical density was read at 532 nm using UV-Visible spectrophotometer.

Histological examination of kidneys

Left kidney was isolated and sample was sent for analysis to Dr. Lal pathology Lab. Dehradun, India for further histopathological study.

Statistical analysis

All of the data were expressed as mean ± S.E.M. Data was analyzed by two-way ANOVA followed by Bonferroni test using the Graph Pad Prism Version 6 software. Separate analysis was performed for data on MDA levels on last day of study period (on week 8th) by one-way ANOVA followed by Tukey's test for significance.

RESULTS

- **Effect of STZ on markers of Diabetic Nephropathy**

Significant increase ($p<0.001$) in serum glucose level, serum urea, serum creatinine, lipid peroxidation levels was observed in all groups in comparison with non-diabetic group till the start of treatment and significant ($p<0.001$) reduction in Body weight was also observed.

- **Effect of Rasagiline (RG) on body weight**

Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) did not have any significant effect on body weight Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) with glimepiride (10 mg/kg, i.p) showed significant ($p<0.05$) improvement in body weight from 3rd week onwards in comparison with diabetic control. **FIG 1**

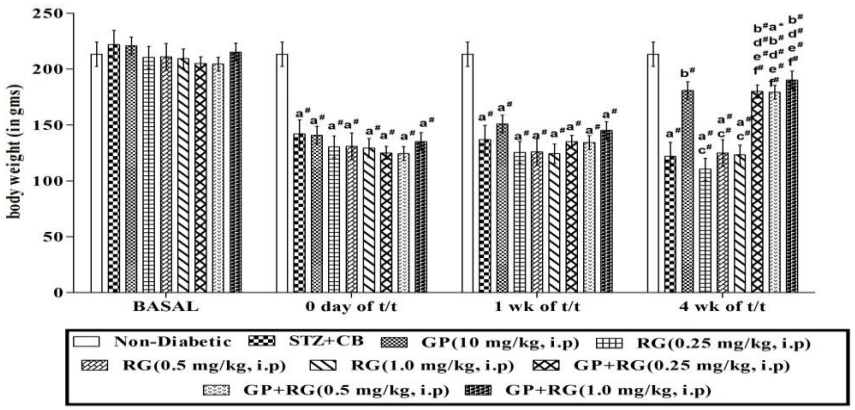


Figure1. Effect of Rasagiline on body weight activity of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose.

***represents $p<0.05$, # represents $p<0.001$. Values are expressed as Mean±SEM. n=7 in each group.**

GP- glimepiride, RG-rasagiline,

STZ- streptozotocin, CB- citrate buffer.

- **Effect of Rasagiline on body weight Level**

Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) did not have any significant effect on fasting glucose level till 3rd week of treatment. In the 4th week Rasagiline (0.5, 1mg/kg, i.p) showed significant ($p<0.05$, $p<0.01$) lowering of fasting Serum glucose in comparison with diabetic control. Low dose of Rasagiline did not have any significant effects on glucose levels. Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) with glimepiride (10 mg/kg, i.p) showed significant ($p<0.001$) reduction in glucose levels in comparison with diabetic control**.** **Fig 2**

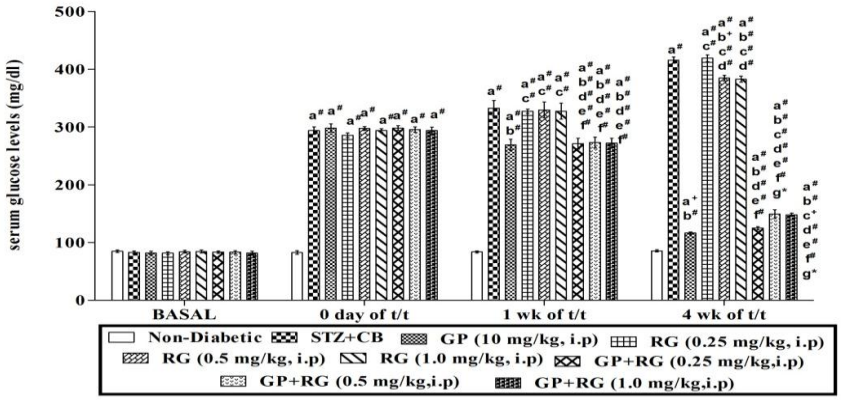


Figure 2 Effect of Rasagiline on fasting glucose levels of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose, g' represents versus glimepiride + Rasagiline low dose.

***represents $p<0.05$, + represents $p<0.01$, # represents $p<0.001$.Values are expressed as Mean±SEM. n=7 in each group.**

GP- glimepiride, RG-rasagiline,

STZ- streptozotocin, CB- citrate buffer.

• Effect of Rasagiline on Post Prandial Serum Glucose Level

Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) did not have any significant effect on post prandial glucose level till 3rd week of treatment. In the 4th week Rasagiline (0.5, 1.0 mg/kg, i.p) showed significant ($p<0.01$, $p<0.01$) lowering of fasting Serum glucose in comparison with diabetic control. Low dose of Rasagiline did not have any significant effects on glucose levels. Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) with glimepiride (10 mg/kg, i.p) showed significant ($p<0.001$) reduction in glucose levels in comparison with diabetic control**. Fig 3**

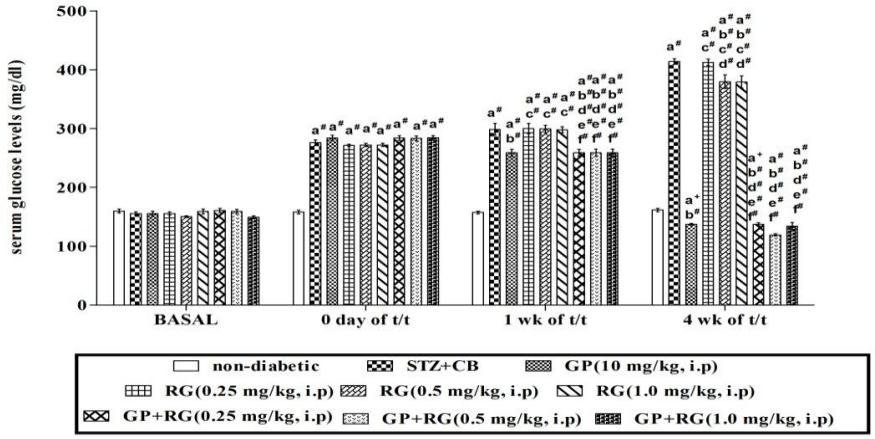


Figure 3 Effect of Rasagiline on Post prandial glucose levels of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose.

***represents p<0.05, + represents p<0.01, # represents p<0.001.Values are expressed as Mean±SEM. n=7 in each group.**

GP- glimepiride, RG-rasagiline,

STZ- streptozotocin, CB- citrate buffer.

• Effect of Rasagiline on Serum Creatinine Levels (mg/dl): Administration of Rasagiline (0.5, 1.0 mg/kg, i.p) showed significant ($p<0.001$) reduction in serum creatinine levels from the first week itself in comparison with diabetic control. Low dose of Rasagiline did not have any significant effects in serum creatinine levels. Administration of Rasagiline (0.5, 1.0 mg/kg, i.p) with glimepiride (10 mg/kg, i.p) showed significant ($p<0.001$) reduction in serum creatinine levels from 1st week on wards in comparison with Diabetic control whereas low dose of Rasagilne (0.25mg/kg, i.p) with glimepiride showed similar effects from 2nd week onwards. **Fig 4**

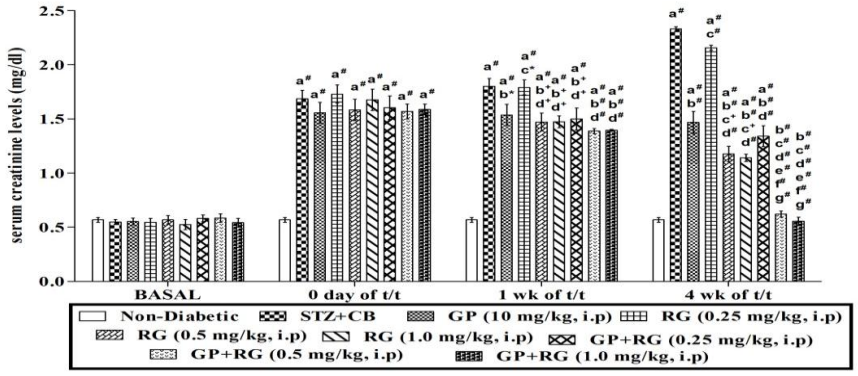


Figure 4: Effect of Rasagiline on creatinine levels of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose, g' represents versus Glimepiride + Rasagiline low dose.

***represents p<0.05, + represents p<0.01, # represents p<0.001.Values are expressed as Mean ± SEM. n=7 in each group.**

GP- glimepiride, RG-rasagiline,

STZ- streptozotocin, CB- citrate buffer.

• **Effect of Rasagiline on Serum Urea Level (mg/dl)**
Administration of Rasagiline (0.5, 1.0 mg/kg, i.p) showed significant ($p<0.001$) reduction in serum urea levels from the first week itself in comparison with diabetic control. Low dose of Rasagiline did not have any significant effects in serum urea levels. Administration of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) with glimepiride (10 mg/kg, i.p) showed significant ($p<0.001$) reduction in serum urea levels from 1st week on wards in comparison with diabetic control. **Fig 5.**

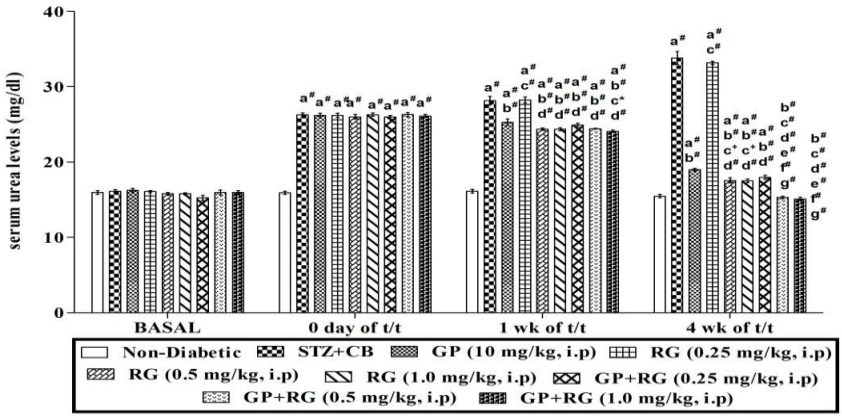


Figure 5 Effect of Rasagiline on urea levels of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose, g' represents versus Glimepiride + Rasagiline low dose.
***represents $p<0.05$, + represents $p<0.01$, # represents $p<0.001$.Values are expressed as Mean ± SEM. n=7 in each group.**
GP- glimepiride, RG-rasagiline,
STZ- streptozotocin, CB- citrate buffer.

• **Effect of Rasagiline on Lipid Peroxidation activity**
Treatment with Rasagiline (0.5, 1.0 mg/kg, i.p) significantly ($p<0.001$) inhibited the hyperglycemia induced rise in kidney oxidative stress levels whereas low dose did not showed any beneficial effect in comparison with diabetic control. However, the combination of Rasagiline (0.25, 0.5, 1.0 mg/kg, i.p) with Glimepiride was seen to be significantly ($p<0.001$) effective in decreasing MDA levels when compared with diabetic control group. **Fig 6.**

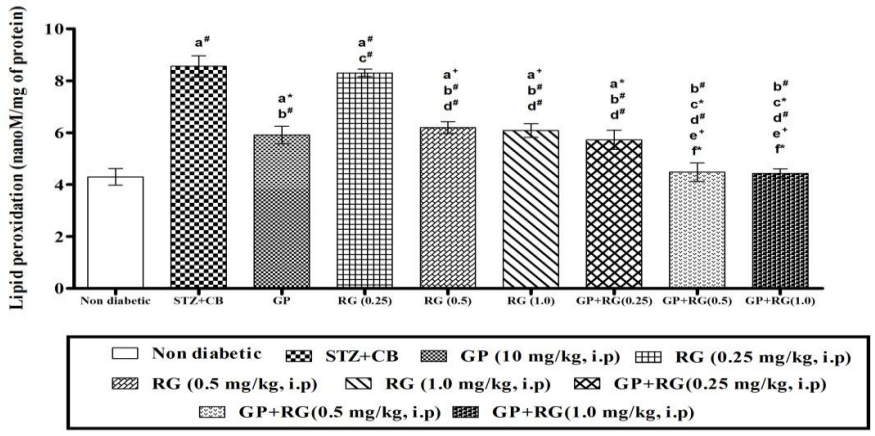
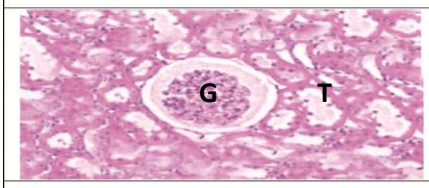
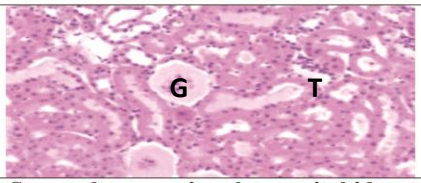
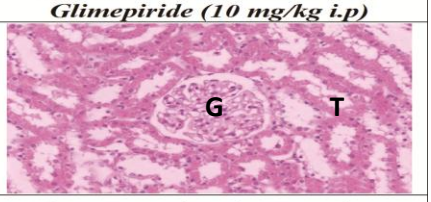
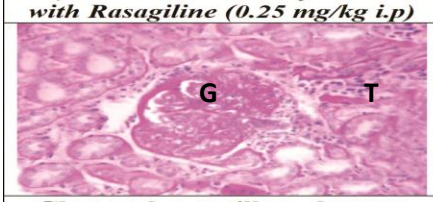
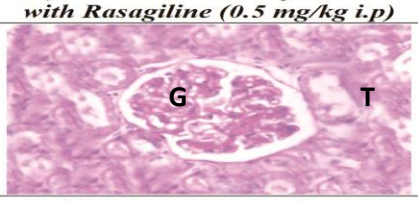
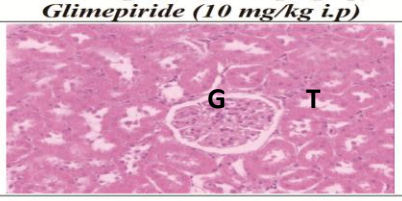


Figure 6 Effect of Rasagiline on Lipid Peroxidation activity of STZ treated rats.

'a' indicates significance versus naïve control, 'b' indicates significance versus diabetic control, 'c' indicates significance versus std, d' indicates significance versus Rasagiline low dose, e' indicates significance versus Rasagiline medium dose, f' indicates significance versus Rasagiline high dose.
***represents $p<0.05$, + represents $p<0.01$, # represents $p<0.001$.Values are expressed as Mean±SEM. n=7 in each group.**
GP- glimepiride, RG-rasagiline,
STZ- streptozotocin, CB- citrate buffer.

Histopathology of kidneys

A) Non-Diabetic Group	*B) Diabetic Control*	*C) Diabetic rats treated with Glimepiride (10 mg/kg i.p)*
		
Normal structure of the glomerulus and the tubules	Severe degenerative changes in kidney tubules and damaged glomeruli with glomerular mesangial cell expansion.	Mild regeneration of glomeruli and tubules.
D) Diabetic rat kidney treated with Rasagiline (0.25 mg/kg i.p)	*E) Diabetic rat kidney treated with Rasagiline (0.5 mg/kg i.p)*	*F) Diabetic rat kidney treated with Rasagiline (1 mg/kg i.p*
		
Glomerulus capillary damage with degeneration of tubules	Mild changes in glomerulus with degeneration of tubules.	Moderate change in glomerulus with degeneration of some tubules.
G) Diabetic rat kidney treated with Rasagiline (0.25mg/kg i.p) + Glimepiride (10 mg/kg i.p)	*H) Diabetic rat kidney treated with Rasagiline (0.5 mg/kg i.p) + Glimepiride (10 mg/kg i.p)*	*I) Diabetic rat kidney treated with Rasagiline (1mg/kg i.p) + Glimepiride(10 mg/kg i.p*
		
Regeneration of glomeruli and tubules.	Regeneration of tubules and glomeruli appears to be restored	Glomerulus shows reversal to nearly normal structure in its tuft. Tubules are also nearly restored.

DISCUSSION

Diabetic nephropathy in type-2 diabetes has become the single most important cause of end stage of renal disease worldwide**.** Hyperglycemia induces oxidative stress and leads to activation of multiple biochemical pathways which are a major source of kidney damage. Increased levels of serum glucose, serum creatinine, and serum urea are the markers for diabetic nephropathy that can be commonly seen in this complication Thus, prevention and treatment of diabetic nephropathy can reduce the incidence of kidney damage. However, diabetic nephropathy, still, remains a huge clinical problem despite implementation of overall intensified glycemic control in these patients. Accordingly, there is an ongoing need for development of new therapeutic strategies to prevent the development or retard the progression of diabetic nephropathy.[23, 24]

In present study we investigated the effect of Rasagiline alone and effect of rasagiline in combination with glimepiride on various markers of diabetic nephropathy. As per previous reports the inhibition of MAO-A or MAO-B reduces the enhanced level of oxidative stress and rasagiline also reduces dopamine oxidative metabolism. Rasagiline which is a selective MAO-B inhibitor increases antioxidant enzyme activity and in addition it also attenuates apoptosis by scavenging free radicals.[25, 26]

In animals MAO-B inhibitors have been shown to prevent dopamine degradation by MAO inhibition activity which results in greater stores of dopamine available for release and thus can reduce hepatic gluconeogenesis, renal gluconeogenesis due to reduced cortisol level in response to dopamine. Thus, Rasagiline may show greater therapeutic potential for treating diabetic nephropathy which was studied in present study in model of type 2 diabetes.

Diabetes mellitus was chemically induced in rats by administrating single dose of streptozotocin (65 mg/kg i.p.) which produces cytotoxicity to β-cells of islets of langerhans by increasing the activity of xanthine oxidase and poly (ADP-ribose) polymerase (PARP), which consequently causes apoptotic and necrotic cell death in pancreatic β-cells. STZ was used in the present study because of its properties such as selective β-cell cytotoxicity and minimal toxicity to other organs as compared to alloxan. Moreover, the halflife (t1/2) of single dose of STZ is about 15 min which is higher than the t1/2 of alloxan.[27, 28]

In this study there was a significant increase in the markers of diabetic nephropathy i.e, serum glucose, serum urea, serum creatinine, and lipid peroxidation after the administration of STZ as compared to non-diabetic group which continued to increase for 7-8 weeks post STZ treatment and reduction in body weight was also observed in STZ treated animals.

Rasagiline (0.5mg/kg, i.p and1.0 mg/kg, i.p) markedly showed reduction in the levels of serum urea, serum creatinine and lipid peroxidation as compared to diabetic control. Low dose (0.25mg/kg, i.p) showed less effect as compared to medium (0.5mg/kg, i.p) and high dose (1.0mg/kg, i.p). Glimepiride alone did not show significant effects in reduction of serum urea, serum creatinine and lipid peroxidation levels as compared to diabetic control. Effects of Rasagiline (0.5 mg/kg, i.p and 1.0 mg/kg, i.p) in combination with glimepiride (10mg/kg, i.p), were significantly better. This may be attributed to reduction in ROS and glucose levels as the hyperglycemia induced activation of polyol, PKC, AGE and hexosamine pathway have been shown to play a key role in the pathogenesis of diabetic nephropathy.

Rasagiline (0.25mg/kg, i.p, 0.5mg/kg, i.p and 1.0mg/kg, i.p) did not have significant effect on glucose levels (fasting and post prandial). There was no significant difference between Rasagiline in combination with glimepiride (10mg/kg, i.p) and glimepiride alone (10mg/kg, i.p) as suggesting that the reduction in glucose levels was due to glimepiride which is a proven anti diabetic agent.

Histopathological study in kidney of untreated diabetic rats showed degeneration of glomeruli and thickening of the basement membrane as compared to control group, Glomerular capillaries were irregular, widened. Furthermore, mesangial cell number was slightly higher in diabetic group. However, the above changes were reduced significantly in the group where Rasagiline (0.25, 0.5 mg/kg, 1 mg/kg) was given with Glimepiride treated group and moderately in glimepiride perse treated group.

The findings of the present study strongly suggest that oxidative stress plays a key role in the pathogenesis of diabetic nephropathy and Rasagiline suppresses free radical formation as suggested by decrease in lipid peroxidation. Further, the histopathology of kidney indicates that Rasagiline is a good nephroprotective and could be used as a therapeutic treatment in diabetic nephropathy when given in combination with glimepiride. These nephroprotective effects of Rasagiline may be due to induction of proteins interfering with the apoptotic pathway, and reduction renal gluconeogenesis by prevention of dopamine degradation.

Rasagiline was found to be more effective when given in combination with glimepiride as the combination not only had beneficial effect on the kidney but also controlled glucose, serum urea, serum creatinine levels. Thus, Rasagiline in combination with glimepiride not only attenuated the diabetic condition but also reversed the nephropathic signs through its nephroprotective actions.

CONCLUSION

From the above discussion and results it can be concluded that

- Rasagiline shows promise as a nephroprotective agent in STZ induced experimental diabetic nephropathy in rats.

- Treatment with Rasagiline (0.5 and 1mg/kg, i.p) in combination with Glimepiride (10mg/kg, i.p) had better ameliorative effects on diabetic nephropathy as compared to monotherapy of either drug.

- The concomitant administration of Rasagiline along with Glimepiride not only attenuated the glucose levels but also showed significant decrease in serum creatinine, serum urea, level of lipid peroxidation and improvement in body weight as compared to monotherapy of either drug.

- Thus, Rasagiline in combination with glimeripide can be an effective treatment for not only diabetic nephropathy but also for other associated complication like diabetic neuropathy due to its dopaminergic activity and potent nephroprotective actions through multiple mechanisms which are independent of its MAO inhibition activity but more research needs to be done to further evaluate the effect.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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