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MOFEGILINE HELPS ALLEVIATE SYMPTOMS OF NEUROPATHY IN DIABETIC RATS

Muneeb Ahmad Bhat*

Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun, India.

***Author for Correspondence: Muneeb Ahmad Bhat,**
Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun, India

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ABSTRACT

Background: Diabetic neuropathy (DN) is defined as the signs and symptoms of neuropathy in a patient with diabetes in whom no other causes of neuropathy are known. DN is one of the commonest causes of peripheral neuropathy. It accounts for more frequent hospitalisations than by other complications of diabetes and also is the most frequent cause of non-traumatic amputation. A simple and internationally agreed definition of DPN is ―the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes. There are approximately 246 million people worldwide, out of which an estimated 20–30 million people are affected by symptomatic diabetic neuropathy. The prevalence of diabetic neuropathy increases with time and poor glycemic control, and poorly controlled diabetes can lead to severe diabetic polyneuropathy in young adults within a few months after the onset. The conditions predisposing an individual to diabetic neuropathy include hypertension, albuminuria (either microalbuminuria or macroalbuminuria), any retinopathy, history of cardiovascular disease, and history of smoking. The risk of developing a disabling peripheral neuropathy could be decreased by optimum glycemic control, but the risk of hypoglycemia increases. Besides, glycemic control, various other treatment options are available to treat patients with NP, such as: opioids, anticonvulsants, tricyclic antidepressants, serotonin reuptake inhibitors, NMDA receptor antagonists, lipoic acid, protein kinase C inhibitors, NSAIDs, ARIs, carnitine and capsaicin. The aim of this study is to explore the neuroprotective effects of Mofegiline in diabetic neuropathy. **Methods and Results:** Diabetes was induced with a single dose of Streptozotocin(60 mg/kg). The diabetic animals exhibited marked hyperglycemia, reduction in body weight and symptoms of neuropathy like thermal hyperalgesia, thermal allodynia, cold allodynia and motor inco-ordination, in comparison with the control animals. After 28 days of Mofegiline administration (10 mg/kg, 20 mg/kg, 30 mg/kg) in combination with Glimepiride (10 mg/kg), a significant reduction was seen in the serum glucose levels, along with an improvement in body weight. Mofegiline caused the symptoms of diabetic neuropathy to improve in a dose dependent manner. The most profound effects of Mofegiline were observed at the dose 30 mg/kg, combined with Glimepiride (10 mg/kg), compared to diabetic control. **Conclusion:** Mofegiline improves hyperalgesia and allodynia in STZ model of DN, by possible MAO-B inhibitory mechanism, resulting in suppression of oxidative stress.

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

INTRODUCTION

Diabetic neuropathy is the most common complication of diabetes mellitus characterized by hyperalgesia, allodynia, parasthesias, dysthesias, foot ulcers and amputations.[1] An internationally agreed simple definition of DPN for clinical practice is ―the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes‖.[2] Over 170 million people suffer from diabetes worldwide, out of whom 20-30 million develop symptoms of diabetic neuropathy. The prevalence of diabetic neuropathy increases with time and poor glycemic control, and poorly controlled diabetes can lead to severe diabetic polyneuropathy in young adults within a few months after the onset.[3] Age, dyslipidemia, hypertension, peripheral vascular disease, weight changes, and other end-organ complications raise the likelihood of neuropathy.[4] The factors that are implicated in the pathogenesis of DN include involvement oxidative-nitrosative stress due to generation of advanced glycation end-products[5], mitochondrial dysfuntion[6], and activation of NF-κB.[7] Oxidative stress is considered the final common pathway of cellular injuries in hyperglycemia. Several mechanisms of hyperglycemia-induced cellular injury were described in the vascular endothelium. An overproduction of ROS by the mitochondrial electron transport chain is induced by the hyperglycemia. Binding of superoxides to nitric oxide (NO) produces peroxynitrite that injures endothelial cells.[8] Reactive

oxygen species (ROS) sensitize nociceptors, which respond more vigorously to the noxious stimuli and start to respond to normally sub-threshold stimuli. This peripheral sensitization results in direct induction of pain as well as induction of central sensitization in the spinal cord, which also indirectly contributes to pain. Superoxide and peroxynitrite mediate the pain accompanying inflammation and are implicated in diabetes-induced motor and sensory nerve conduction deficits and peripheral nerve energy deficiency.[9]

Beside glycemic control, several treatment options, such as TCAs, SSRIs, opioids, NMDA receptors antagonists, anticonvulsants, alpha-lipoic acid, protein kinase C inhibitors, NSAIDs, aldolase reductase, AGE inhibitors, capsaicin and acetyl-l-carnitine, are employed for the treatment of DN. The most commonly used drugs are duloxetine, gabapentine and pregabaline.[10] Drug therapy of DN is often limited and unsatisfactory due to partial effectiveness and associated side effects and further their development is hindered due to incomplete knowledge about induction and maintenance of DN. Mofegiline is an irreversible inhibitor of monoamine oxidase B enzyme.[11] The MAO-B is responsible for the oxidative deamination of dopamine, that results in formation of reactive oxygen species, which in turn can cause damage to neuronal cells. Mofegiline, by inhibiting MAO-B, causes suppression of ROS production, and thereby limits the neuronal damage. Therefore, the present work was aimed to estimate the effect of mofegiline on experimental DN in rats.

MATERIALS AND METHODS

Chemicals

Mofegiline was obtained from Santa Cruz Biotech., USA. 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[12]

A single dose of 60 mg/kg Streptozotocin prepared in citrate buffer (pH 4.4.0.1M) was administered intraperitoneally to overnight fasted animals to induce diabetes. NAD+ 235 mg/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, 14th, 28th day respectively, after STZ administration by enzymatic GOD-POD (glucose oxidase peroxidase) diagnostic kit. The rats having fasting plasma glucose levels more than 250 mg/dl were selective for the study. STZ treated rats were provided with 10% glucose solution after 6 hrs to prevent 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 (60 mg/kg, i.p) + NAD+(235 mg/kg), administered [15 minutes] prior to STZ administration.

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

Group IV: *Test group I*: Glimepiride (10 mg/kg) + Mofegiline (10 mg/kg, i.p)[13] were administered to diabetic animals for 4 weeks.

Group V: *Test group II*: Glimepiride (10 mg/kg) + Mofegiline (20 mg/kg, i.p)[13] were administered to diabetic animals for 4 weeks.

Group VI: *Test group III*: Glimepiride (10 mg/kg) + Mofegiline (30 mg/kg, i.p)[13] were administered to diabetic animals for 4 weeks.

Behavioral test paradigm

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

Thermal hyperalgesia: The hyperalgesic response on the hot plate is considered to result from a combination of central and peripheral mechanisms.[14,15] Hyperalgesia may include both a decrease in threshold and an increase in suprathreshold response.[16] Rats were allowed to acclimatize to laboratory conditions 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 placed 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.a cut-off time of 30 secs was used in the test, to avoid tissue damage.

Thermal Allodynia: Allodynia is the pain in response to a non-nociceptive stimulus. This term is used when the test stimulus is not capable of activating nociceptors.[16] 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 cutoff time of 30 secs.

Cold Allodynia: Pain is elicited by cold, and a major feature of many neuropathic pain states is that normally innocuous cool stimuli begin to produce pain.[17] 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 (rotarod): Motor coordination test was performed on rotarod apparatus. Rats underwent training for three days on the apparatus before the test was conducted.[17] The apparatus was set at an acceleration of 4rpm, to constantly increase the rotation to 25 rpm. Rats were placed on the rotating rod for one minute. The time taken for falling off the rod (within one minute) was recorded.

Statistical analysis

Data are expressed as mean ± SEM and statistically analyzed using one-way ANOVA followed by Bonferroni's test. $P \leq 0.05$ was considered as statistically significant.

RESULTS

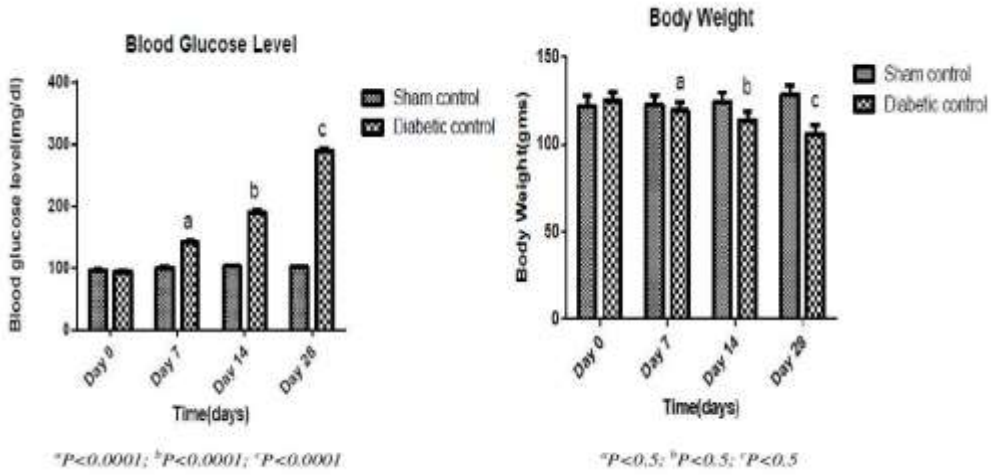
Effect of STZ on blood glucose level and body weight

Rats threated with STZ were examined for their blood glucose levels on 0th, 7th, 14th, and 28th day after STZ administration. The STZ treated rats showed a signifincant increase in the blood glucose levels and body weights as compared to the rats of the sham control group. (figure 1)

Table 1: Effect of STZ on blood glucose level and body weight

Group	Initial blood glucose level	Final blood glucose level		Initial body weight	Final body weight
Sham Control	95.875±1.817	101.313±1.066		121.625±5.852	128.250±5.612
Diabetic control	93.550±2.527	289.350±3.283[a]		124.750±5.230	105.625±5.281[b]

Data are the means + SEM. Sham control represents the group treated with citrate buffer. Diabetic control represents the group treated with STZ. aP<0.0001. bP<0.5

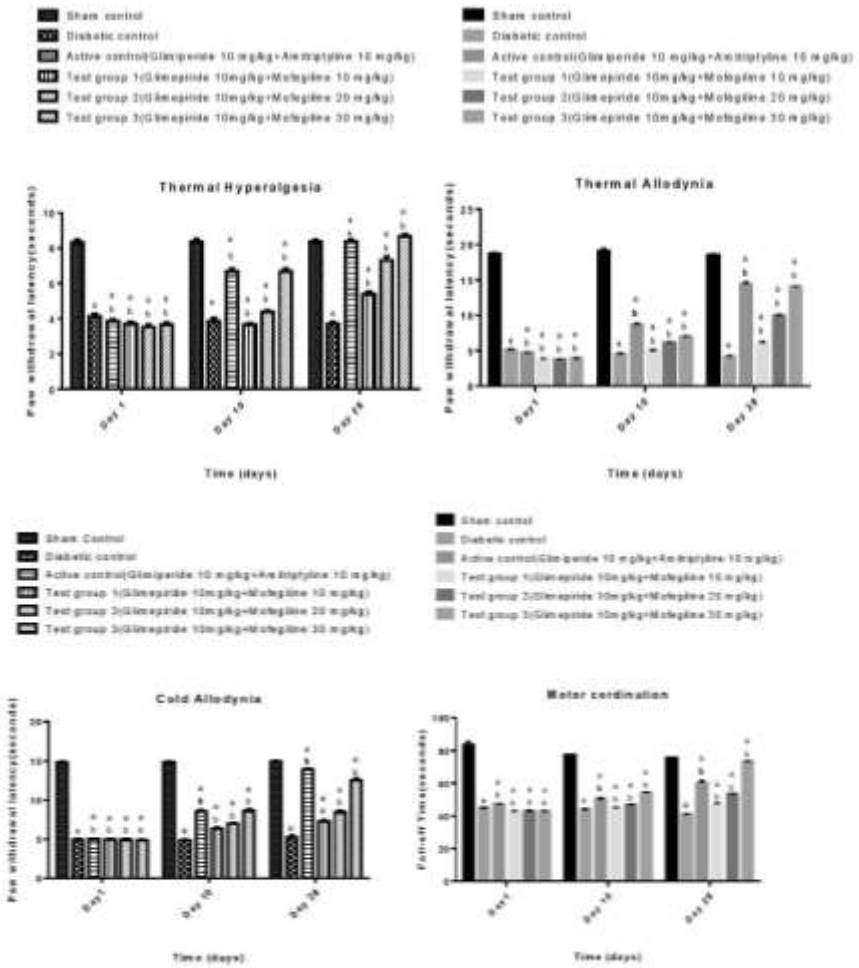


[a]P<0.0001; [b]P<0.0001; [c]P<0.0001 *[a]P<0.5; [b]P<0.5; [c]P<0.5*

Figure 1. Effect of STZ on blood glucose level and body weight

Effect of mofegiline 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. Mofegiline, 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.



Data are means + SEM (n=8). aP<0.0001 compared to sham control; bP<0.0001 compared to diabetic control. Mofegiline was administered intraperitoneally, at doses 10, 20 and 30 mg/kg, for 28 days, starting

Figure 2. Effect of mofegiline in combination with glimepiride on hyperalgesia, allodynia and motor coordination

DISCUSSION

The mechanisms leading to diabetic neuropathy, include polyol pathway, glycation, ROS, pro-IC and altered protein kinase C activity, which affect cellular proteins, gene expression and receptor expression and result in diabetic complications.[18]

In the present study, diabetic rats had significantly lower nociceptive threshold and body weight gain, and higher blood glucose. Treatment of diabetic rats with mofegiline significantly increased nociceptive threshold. Thus, it is clear from the behavioral studies that mofegiline attenuated the development of DN and suggested the involvement of MAO-B enzyme and production of ROS in nociceptor hypersensitization in diabetes.

Hyperglycemia induces oxidative stress through various pathways such as increased aldose reductase activity, glycation, protein kinase C activity, prostanoids production and superoxide generation.[19,18,20] All these pathways unify in producing oxidative stress and thus, results in NF-κB[21] and TNF-α activation[22] and cycloxygenase-2 (COX- 2) gene expression.[23] ROS, nitrosative and oxidative stress have been implicated in the central sensitization, development and maintenance of DN[6,24], which are attenuated by antioxidants GSH, α-lipoic acid, taurine.[6] Oxidative stress can also be prevented by inhibition of the enzyme MAO-B. studies indicate that mofegiline is a potent irreversible inhibitor of MAO-B.[11,25,26,27] The present study confirms that mofegiline causes attenuation of symptoms of diabetic neuropathy, bringing about an improvement in hyperalgesia, allodynia and motor coordination. These findings suggest that mofegiline could be a promising candidate for use in the management of DN.

In conclusion, mofegiline improves hyperalgesia and allodynia in STZ model of DN, by possible MAO-B inhibitory mechanism, resulting in suppression of oxidative stress.

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