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Repositioning of Olmesartan by Exploring Its Protective Potential Against STZ Induced Diabetic Neuropathy in Rodents

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ABSTRACT:
Olmesartan (OLM), an angiotensin II receptor blocker (ARB) initially recognized for its blood pressure-lowering properties, exhibits significant potential in addressing disorders associated with inflammation. This suggests promising prospects for its use in conditions such as neuropathy, and nephropathy. This study aimed to evaluate the impact of OLM (5 mg/kg/day) on streptozotocin (STZ) induced diabetic neuropathy in Sprague Dawley (SD) rats. The animals were divided into four groups, each consisting of six rats (n = 6). Group I, the nondiabetic/normal control (NCG), received normal saline. Group II served as the diabetic control (DCG). Group III, the treatment group (TG), received OLM at a dosage of 5 mg/kg/day and STZ. Group IV, the *perse* group (PSG), received OLM at the same dosage. Parameters measured included blood glucose levels, and various behavioural assessments such as motor coordination, thermal hyperalgesia and heat allodynia. Functional biomarkers, including Slow nerve conduction velocity (SNCV), Motor nerve conduction velocity (MNCV) & Na+K+ATPase activity was also assessed. OLM results in neuroprotective efficacy, characterized by enhanced myelination, reduced axonal swelling of nerve fibers, improvements in SNCV and MNCV. This study substantiates the beneficial impact of OLM and underscores its significance in diabetic neuropathy treatment. Further clinical trials hold the potential to unveil innovative pharmacological treatments for diabetes and its associated complications.

Keywords: Olmesartan, Diabetic Neuropathy, Myelination, Slow nerve conduction velocity, Sciatic nerve.

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1. Introduction

Diabetic neuropathy (DN) is a notable consequence of diabetes which is the primary reason of excruciating nerve damage. It is divided into four different forms based on the specific location of the body where neurons are predominantly impacted: proximal, peripheral, autonomic, and focal [1]. With a frequency of around 50% among diabetics and a potential 20% presence at the time of diabetes diagnosis, it is the most common microvascular consequence of the disease (Saraiva et al., 2023). The common indications include a lack of sensation, a prickling sensation, discomfort, and a decrease in strength that originates in the

farthest parts of the lower limbs. However, treatment of hyperglycaemia is not enough to prevent neuropathy in those with type 2 diabetes [3].

DN possesses a multifactorial pathophysiology such as hyperglycaemia causes the activation of polyol pathway, non-enzymatic glycation and generation of oxidative stress [4]. The development of DN includes several molecular processes, including oxidative stress, inflammation, and pathways related to advanced glycation end products (AGE), polyol, hexosamine, and protein kinase C (PKC) [5]. Oxidative stress is produced when AGE binds to the receptor for advanced glycation end products (RAGE), activating NADPH oxidase in the process [6]. The onset of diabetic neuropathy in rats is mostly linked to the production of pro-inflammatory cytokines, such as TNF (tumour necrosis factor), IL-6, and IL-1 (interleukin). The presence of both redox imbalance and inflammatory cascades strongly indicates the cause of hyperglycaemia [6]. The pro-inflammatory cytokines, which are generated by glia and immune cells, have been identified in many studies as a potential cause for neuropathic pain of diverse origins. [7]. Apart from this, modification of PKC [8] and enhancement in mitogen-activated protein kinase (MAPK) and upregulation of nuclear factor-NF-kB causes functional and structural alterations in peripheral neuropathy [9].

Clinically effective treatments for diabetic neuropathy include substances that limit the action of the renin-angiotensin system (RAS), such as ARBs or ACE inhibitors. In both animal models and human clinical research, ACE inhibitors and ARBs have been shown to alleviate the nerve conduction deficit in DN [10]. The inhibition of RAS has demonstrated efficacy in ameliorating diabetic nephropathy. However, there is limited evidence on the preventive effects of ACE inhibitors or angiotensin II receptor antagonists on diabetic neuropathy. Coppey et al. reported that ACE inhibitors and/or ARBs can effectively combat diabetes, as well as vascular and neurological dysfunction. This is achieved by inhibiting or reversing the production of superoxide in the epineural arterioles of the sciatic nerve [12]. As diabetes is known to hinder the ability of blood vessels in the sciatic nerve to relax in response to acetylcholine and calcitonin gene-related peptide (CGRP). This results in a decrease in MNCV and contributes to decreased nerve function. So, ARBs and ACE inhibitors are also capable of preventing this pathogenesis in the progression of DN [11].

OLM is an AT1R antagonist, has demonstrated neurotrophic effects on spinal motor neurons both in laboratory settings and in living organisms [13]. It enhances the development of nerve fibers and boosts the activity of choline acetyltransferase in primary cultures of ventral spinal cord. In vivo tests also indicate that OLM effectively inhibits the apoptosis of spinal motor neurons induced by sciatic nerve transection (Iwasaki et al., 2002). Additionally, it has been observed that the AT1R blocker increases insulin receptor via inhibiting the MAPK pathway in peripheral nerves, improving DN [13]. However, there has been limited investigation into the impact of AT1R blockers on STZ induced DN. This study examines the impact of OLM on neuronal activities, blood glucose levels, behavioral tests, biochemical tests, and antioxidant assessments in animals with DN.

2. Material and Methods

2.1 Animals

In this experimental design, twenty-four SD rats (150-200 gm), procured from CDRI, Lucknow. All the animals were acclimatized separately one week prior to the experiment in controlled conditions (12/12 h L/D cycle, 25 ± 2° C), with food and water *ad libitum.* Ethical approval was taken from Institutional Animal Ethical Committee (IU/IAEC/21/10) Integral University, Lucknow.

2.2 Induction of DN

Diabetes was induced in rats with an intraperitoneal injection of STZ (60 mg/kg) that was dissolved in ice-cold 0.1 M citrate buffer (pH 4.5). Following a period of 72 hours, blood samples were obtained from the tail vein and subjected to analysis utilizing the ACCUCHEK-ACTIVE kit made by Roche, Germany. Animals exhibiting plasma glucose levels over 200 mg/dL were classified as diabetic and utilized for investigations on DN (Shoaib et al., 2019). At weeks 4^{th} & 10^{th} SNCV and MNCV were measured. The diabetic animals (n = 24) were divided in four groups: Group 1 (n = 6) served as normal control (NCG) in which animals were given normal saline(1ml/day, orally), Group 2 (n = 6) served as Diabetic control (DCG), Group 3 (n = 6) served as treatment group (TG) in which animals were treated with OLM (5

mg/kg/day) orally from week 4th till week 10th and Group 4 (n = 6) served as *perse* group (PSG) (positive control group). At week 4th a neuropathy had already developed, as shown by a significant slowing of SNCV and MNCV i.e. 23.20±1.7 ms^{-1}& 34.69±1.8 ms^{-1} respectively [15]. Under the anesthesia, the rats were sacrificed, and blood was drawn by retro-orbital sinus. The sciatic nerves were promptly extracted and homogenized in a solution of 10% phosphate-buffered saline with a pH of 7.4.

2.3 Validation of DN Rats

After a period of four weeks following the introduction of diabetes, the animals were evaluated to see if they had developed diabetic neuropathy. The physical validation of neuropathic pain involved the examination of the following criteria. Motor incoordination by Rota rod apparatus, Neuropathic Pain (Thermal hyperalgesia), Hot Allodynia test. After successful development of DN, animals were administered with OLM 5 mg/kg/day for 6 weeks. After six weeks, physical parameters viz., motor coordination, hot allodynia, hyperalgesia were studied, and animals were sacrificed for further studies.

2.4 Measurement of blood glucose

The body weight was measured on week 0, 4 & 10, blood glucose levels of animals were measured at different time intervals, i.e. 0, 2, 6, 8 and 10th week from tail's vein blood (by ACCUCHEK-ACTIVE kit made by Roche, Germany).

2.5 Behavioural Tests

2.5.1 Motor Coordination

Rotarod activity was assessed to analyse the effects of OLM in all the groups on motor coordination [16]. Prior to commencing the experiment, the rats had a three-day training period when they were used to a consistent pace of 20 to 25 rpm for five minutes each day. On the test day, rats were positioned on the spinning rod, which started at a speed of 4 rpm (revolutions per minute) and subsequently increased to 20-25 rpm. A decrease in muscular grip signifies muscle relaxation. A muscular relaxation index is calculated by measuring the difference in drop-off time from the spinning rotarod between different groups. [17].

2.5.2 Hot Allodynia

This approach is used to estimate the nociceptive response to OLM. The animals were subjected to a continuous temperature of 55°C on a hot plate for the length of the experiment. Digital stopwatches were used to measure jumping and paw licking in order to evaluate thermal hyperalgesia. These acts were categorized as favourable responses to heat. After the allotted amount of time, animals were removed from the hot plate until their original values were restored.[18].

2.5.3 Hyperalgesia

This technique considers the removal of the tail from the heat as the last point. To avoid tail damage, it was discovered that a duration of 10-12 seconds should be removed while using a hot plate at a temperature of 55°C. Animals who fail to retract their tails within a time frame of 3-5 seconds are excluded from the research. The response time was recorded at 5, 15, 30, and 60 minutes following the administration of OLM [18].

2.6 Functional Biomarkers

2.6.1 Assessment of SNCV & MNCV

MNCV measurements were taken non-invasively from the left sciatic tibial nerve in four groups between the 4th and 10th week of the trial. The measurements were obtained in a temperature-controlled setting while the animals were under ether anaesthesia. The rectal temperature was regulated at 37°C using a heating light and pad. The sciatic nerve on the left side was stimulated using bipolar electrodes. The stimulation was done at two points: proximally at the sciatic notch and distally at the ankle. The stimuli were supramaximal, with a strength of 6 mA, and were delivered using rectangular pulses that lasted for 0.3 ms. The stimulator was set at a frequency of 10 Hz and the procedure was performed using the Neuropack 2 EMG device manufactured by Nihon Kohden. The electrical activity of the first interosseous muscle in the left hind limb was measured using unipolar pin electrodes. The latencies were quantified from the occurrence of the sensory artifact to the initiation of the negative M wave deflection [19]. The SNCV was measured by recording at the dorsum of the foot and stimulating antidromically with supramaximal stimulation at the ankle [20].

2.6.2 Assessment of Na^+K^+ ATPase activity

The activity of the Na^+K^+ATPase enzyme in the sciatic nerve was quantified in samples obtained from all of the experimental groups. It was measured by removing the perineurium from sciatic nerves and determining the activity using the spectrophotometric enzymatic technique reported by Greene & Lattimer in 1983 [21]. The ouabain-sensitive activity of Na^+K^+ATPase was determined by subtracting the ATPase activity measured in the presence of 1 mmol/l ouabain from the activity measured in its absence. [22].

2.7 Assessment of Oxidative Stress

2.7.1 Catalase

Johansson and Borg's method was adopted to measure the activity of Catalase using its peroxidase function [23]. The reaction started with 50 µl of 250 mM potassium phosphate buffer at pH 7.0 was incubated with 50 µl of methanol and 10 µl of 0.27% hydrogen peroxide. 100 µl of enzyme sample were then added at room temperature 25±1˚C, and the reaction was allowed to proceed with continuous agitation. After 20 min, the reaction was stopped using 50 µl of 7.8 M KOH, and 100 µl of purpald was added, and the reaction was stopped again at 25±1˚C for 10 more minutes with continuous agitation. The colour compound was achieved by adding 50 µl 65.2 mM KON at the end, and absorbance was read at 550 nm using a spectrophotometer.

2.7.2 SOD (Superoxide dismutase)

SOD catalyses the neutralization of superoxide radicals with the formation of H_2O_2 and O_2. The reaction mixture includes nitro blue tetrazolium (300 µM, 0.3 ml), sodium pyrophosphate buffer (0.052 mM, pH 7.0, 1.2 ml) and phenazine methosulphate (186 µM, 0.1 ml), which was supplemented with tissue homogenate (0.2 ml). One minute of the enzyme reaction was halted by adding 1ml of glacial acetic acid, after 0.2 ml of NADH (780 µM) had been added. The colour intensity at 560 nm was used to calculate the amount of chromogen that had generated. The expressed results are U/protein (mg) [24].

2.8 Statistical Analysis

The data was reported as mean ± SEM, and the statistics were performed by one-way & two-way ANOVA, followed by Dunett post hoc test using Graph pad prism 8.0.1 software. It was deemed statistically significant when $p < 0.05$.

3. Results

3.1 Effect of OLM on blood glucose

Blood glucose level and body weight were estimated in OLM treated rats as shown in Table 01. There was significant ($p < 0.01$) increase in blood glucose level in all the STZ induced groups than control group of rats on 4th week of protocol. Treatment with OLM ameliorates the altered level of glucose in STZ induced DN rats on 4th-10th week of protocol (Table 01).

Table 01: Effect of OLM on blood glucose level

Blood Glucose levels (mg/dl)				
Weeks	NC	DC	TG (5mg/kg/day)	Perse Group
Week 0	82.200 ± 10.354	218.200 ± 39.789###	246.600 ± 14.117	93.200 ± 9.884
Week 2	84.800 ± 17.499	235.400 ± 30.892###	241.800 ± 33.848	91.000 ± 10.124
Week 4	84.600 ± 11.610	248.400 ± 22.84###	252.200 ± 24.633	79.00 ± 07.649
Week 6	84.600 ± 11.610	248.400 ± 22.854###	198.600 ± 28.130**	76.800 ± 5.541
Week 8	93.600 ± 13.520	286.800 ± 11.735###	131.400 ± 9.529**	84.400 ± 6.229
Week 10	90.300 ± 13.547	287.900 ± 12.453###	127.800 ± 8.948***	68.900 ± 5.454

3.2 OLM attenuates motor coordination

Motor coordination was measured using rotarod apparatus in STZ induced DN rats on 4th and 10th week (Fig 1). There was significant ($p < 0.001$) decrease in motor coordination in DCG group than NCG rats on 4th week i.e. 53.80 ± 3.0 s & 28.20 ± 4.6 s respectively to confirm diabetic neuropathy. OLM treatment significantly ($p < 0.01$) enhances the motor coordination in TG rats i.e. 35.40 ± 3.0 s on 10th week.

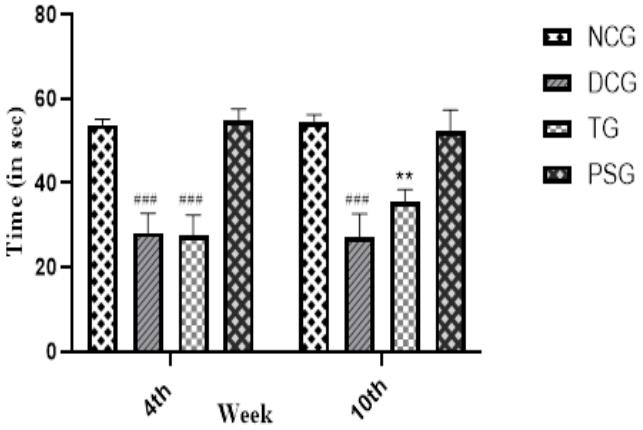

Fig. 01 Effect of OLM on motor incoordination at 4th and 10th week. ###$p < 0.001$ v/s NCG & **$p < 0.01$ v/s DCG.

3.3 OLM attenuates neuropathic pain

The effect of OLM on neuropathic pain was measured using tail flick and hot plate test. There was a significant decrease in withdrawal latency (i.e. hyperalgesia) in DCG & TG group which is 9.40 ± 0.83 s & 9.60 ±0.54 s respectively on 4th week. OLM treated group significantly ($p < 0.01$) improves withdrawal latency i.e. 7.80 ± 0.83 s than DCG group at 10th week i.e. 9.6 ± 1.5 s (Fig 2 A). The DCG & TG group (7.80 ± 0.84 s & 8.01 ± 1.24 s) also decrease paw withdrawal latency to thermal stimuli as compared with NCG group (3.80 ± 0.83 s) on week 4th which confirmed DN. OLM treatment significantly ($p < 0.01$) increased (6.40 ± 0.54 s) the withdrawal latency than DCG rats on 10th week (Fig. 2B).

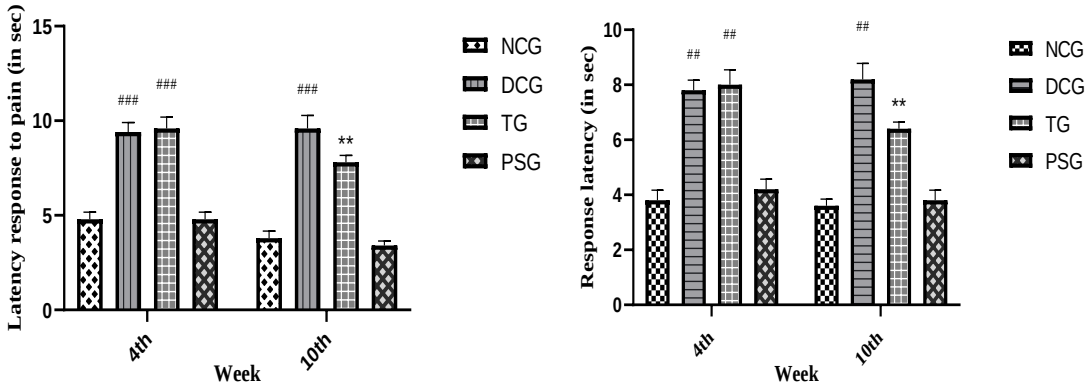

Fig.2 Effect of OLM on neuropathic pain in STZ induced DN rats on 4th & 10th week. A: Hyperalgesia by tail flick test; B: Hot allodynia by Eddy's hot plate test ###$p < 0.001$ v/s NCG and **$p < 0.01$ v/s DCG.

3.4 OLM attenuates Motor & Sensory NCV

There was significant decrease in MNCV & SNCV viz., 34.60 ± 1.8 m sec^{-1} & 23.20 ± 1.4 m sec^{-1} respectively in DCG group when compared to NCG i.e. 50.80 ± 1.6 m sec^{-1} & 34.20 ± 1.6 m sec^{-1}respectively at week 4th which confirmed the presence of DN. There was a significant increase in NCV of motor & sensory

nerve in the TG group (40.75 ± 1.2 m sec^{-1} & 27.74 ± 0.9 m sec^{-1}) when compared with the DCG at week 10th which showed the positive effect of OLM at week 10th (Fig 3 A & B).

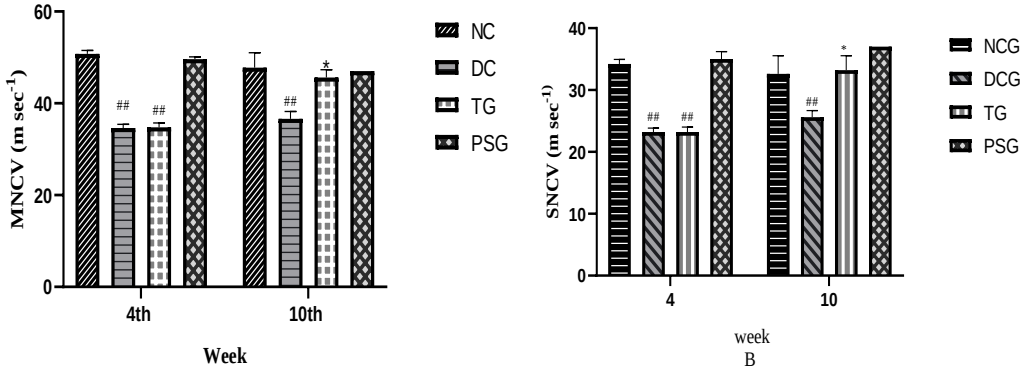

Fig. 3 Effect of OLM on Motor & Sensory NCV in rats. A: MNCV at 4th & 10th week; B: MNCV at 4th & 10th week; ##p < 0.01 v/s NCG, *p < 0.05 v/s DCG

3.5 OLM attenuates Na^+ K^+ ATPase activity

In diabetic control rats, composite ATPase activity was substantially lower but treatment group activity tended to be somewhat greater than diabetic rats' (p < 0.05). The untreated diabetic rats' ouabain-sensitive Na+ K+ ATPase activity was only around 43% of the levels seen in the non-diabetic control animals. OLM treatment decreased this loss by 54% (p < 0.01 compared to diabetic rats not receiving treatment). There was no variation in the Na+ K+ ATPase activity's ouabain-resistant percentage across the experimental groups.

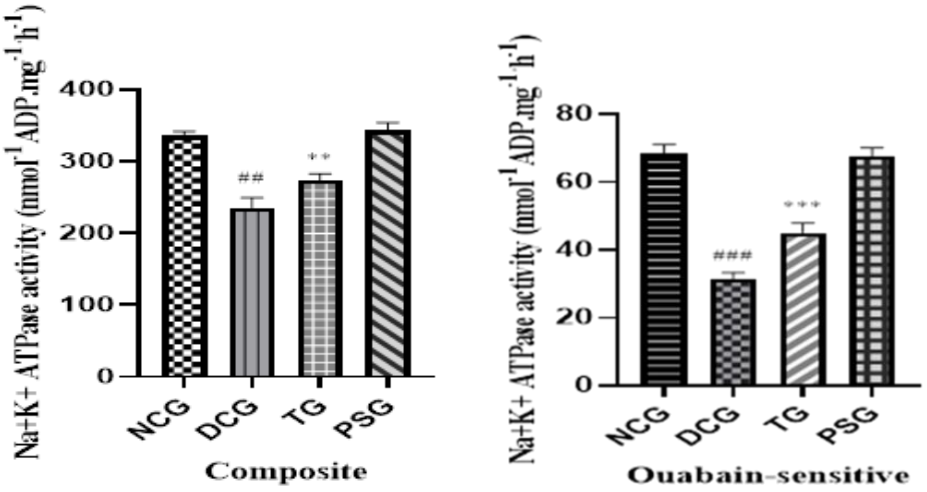

Fig. 4 Effect of OLM on Na+ K+ ATPase activity in rats: ##p < 0.01 v/s NCG & ***p < 0.001 v/s DCG.

3.6 OLM on oxidative stress

The DCG rats had a substantial decrease (p < 0.001) in SOD activity, measuring 13.753 ± 2.19 U/mg protein, in comparison to normal rats. Regarding the discrepancy, the group of rats who were given OLM at a dosage of 5mg/kg had elevated levels of SOD (22.354 ± 3.78U/mg protein) (Fig 5 A).

Catalase levels were significantly (p < 0.001) reduced in DCG rats (5.368 ± 0.582 U/mg of protein) than NCG group (10.842 ± 1.470 U/mg of protein). A significant increase (p<0.01) in CAT levels has been seen in rats treated with OLM (8.530±0.553 U/mg protein) (Fig 5 B).

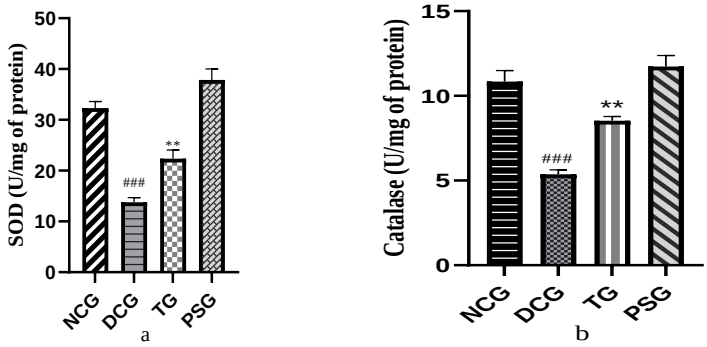

Fig.5 Effect of OLM on Oxidative stress. A: SOD level; ###$p < 0.001$ v/s NCG & **$p < 0.01$ v/s DCG. B. Catalase level; ###p < 0.001 v/s NCG & **p < 0.01 v/s DCG.

4. Discussion

DN is a progressive and degenerative condition of peripheral nerves that affects sensory, motor, and autonomic neurons. Studies suggest that entire neuron is targeted in diabetes i.e. from perikaryon to the terminal [25] It is often observed in around 50% of individuals with diabetes who have advanced and chronic illness. It can be attributed to a combination of causes, including oxidative stress and other unknown factors. Nevertheless, the advancement of DN can be averted by the regulation of blood glucose levels in the body [26].

The current investigation showcased the neuroprotective properties of OLM by exhibiting substantial enhancements in several behavioral, functional and biochemical biomarkers. Chronic hyperglycemia along-with altered insulin signaling causes the alterations in neurons, glia and vascular cells, ultimately leads to nerve dysfunction. In a diabetic rat model, produced by STZ, a characteristic feature is the reduced insulin response to glucose, resulting in changes in blood glucose concentration and body weight due to impaired insulin-secreting cells in the pancreatic islets [27]. Amoghimath et al., reported that OLM reduces glucose levels from 355.5 mg/dl to 200.8 mg/ml, an indication that it corrects insulin resistance and increases glucose transport [28]. In our study, the decreased glycemic levels from 287.9 mg/dl to 127.8 mg/dl shows the protective effect of OLM in hyperglycemia and eventually in nerve dysfunction. It is also reported that Chronic hyperglycemia leads to metabolic abnormalities and contribute to nerve damage by activating dysfunctional biochemical processes, such as the production of advanced glycation end products, increased activity of the polyol pathway, and PKC activity [29].

Prolonged presence of diabetes in animals leads to neuronal sensitization caused by motor and sensory fiber loss, resulting in a decrease in paw withdrawal latency [30]. Neuronal dysfunction in diabetes is mainly via DNA damage, increased endoplasmic reticulum stress, mitochondrial dysfunction, neurodegeneration and loss of neurotrophic signaling, and can trigger macrophage activation [31]. In our study, we discovered that TG group (OLM 5 mg/kg) exhibited a noteworthy elevation (P < 0.01) in hyperalgesia, hot allodynia, and motor coordination at the 10th week, in comparison to the DCG group. Thermal hyperalgesia, allodynia, and motor activity have been identified as behavioral characteristics used to assess diabetic neuropathy in rats produced by STZ.

The present study results, a notable decrease in nerve conduction in the experimental group of rats compared to the control group. This indicates that neuropathy was successfully induced in these rats by the fourth week. At the 10th week, the TG group exhibited a noteworthy rise in NCV of both the sciatic motor and sensory nerves, in comparison to the DCG group, this indicates a beneficial impact of OLM (5 mg/kg). Previous research has also corroborated our discoveries, demonstrating that the hindered transmission of nerve signals in neuropathy arises from the enduring elevation of blood sugar levels, resulting in oxidative stress and diminished blood circulation to the nerves, ultimately leading to nerve degeneration [20].

Inflammation in peripheral tissues causes upregulation of voltage-gated sodium channel which is associated with dorsal root ganglia. The increase in sodium influx during the

inflammation lead to upregulation of Na+K+ATPase pump which causes swelling of neurons and ultimately neuronal death [32]. Many reports indicate that a reduction in Na^+K^+ATPase activity is a key factor in the development of electrophysiological and morphological abnormalities observed in many animal models of diabetes mellitus, which are associated with neuropathic consequences [33]. In our study, the Na^+ K^+ ATPase activity was significantly reduced in DCG rats, although composite ATPase activity tended to be slightly higher in TG group than in DCG group ($P < 0.01$) (Table 5). In untreated diabetic rats, Ouabain sensitive Na + K + ATPase activity was found to be approximately 57% less than that of non-diabetic control animals. Administration of OLM significantly decreased this loss by 54%. The Ouabain resistant fraction of Na + K + ATPase activity did not differ between the groups studied.

Biochemical changes that occur in DM leads to excessive generation of ROS and reduced antioxidant levels in tissues & cells. In present study, SOD enzyme activity is reported to be increased in TG group as compared with DCG group. While PSG group had no significant changes after the treatment as well when compared with the NCG group. Besides that, Catalase activity is also significantly increased in TG group as compared with control group which explain that oxidant-antioxidant imbalance might also be the major cause in progression of diabetic neuropathy. According to Vincet et al., antioxidant imbalance is mainly associated with hyperglycemic effect as both of TG & DCG throughout the study period. An increase in blood glucose levels promotes production of high level of oxidative stress via glucose metabolism, [34] predominating direct damage to nerve parenchyma, reduced blood flow, vascular damage, formation of AGEs and others in development of DN [35]. In conclusion, Olmesartan is a viable candidate for repurposing in a variety of disorders due to its adaptable influence on pathways connected to inflammation: The drug's good attributes and ongoing pre- and clinical studies make it more appealing for future research and possible use in a variety of medical settings.

Limitations

In summary, while the repositioning of OLM against STZ-induced diabetic neuropathy in rodents is very promising, several limitations must be taken into consideration while translating these findings into the clinic. Side effects and safety profiles after long-term use should be carefully assessed since the adverse reactions derived from its use in rodents may or may not occur in humans. Inter-species variations in pharmacokinetics and pharmacodynamics can also alter drug efficacy and safety. Thus, despite the encouraging results reported in this article, thorough clinical trials and scrutiny of these limitations are needed for the successful translation of OLM repositioning into a feasible therapeutic strategy for diabetic neuropathy.

5. Conclusion

The outcomes of this research substantiate the beneficial impact of OLM in the context of DN and pave the way for further exploration in clinical trials. The potential for innovative pharmacological treatments for diabetes and its associated complications is encouraging, opening new avenues for therapeutic interventions and improved patient outcomes.

Declaration

Competing interest: The authors declare no competing interest.
Funding: No funding received
Ethics approval: The Institutional Animal Ethics Committee (IAEC) of the faculty of Pharmacy, Integral University, Lucknow (U.P.), India, approved the study process with approval number (IU/IAEC/21/10), (Reg no. 1213/PO/Re/S/08/CPCSEA, 5 June 2008).
Data Availability: All data generated and analyzed are included in this research article.
Consent to participate: Not required
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