Behavioral and Biochemical Evaluation of the Neuroprotective Role of Naringin in Streptozotocin-Induced Diabetic Peripheral Neuropathy in Rats

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Abstract: Background: Diabetic neuropathy is the most prevalent diabetic complication, impacting up to 60% of people with diabetes. It is characterized by distal symmetrical loss of sensory function in the lower extremities, presenting with spontaneous excruciating neuropathic pain, hyperalgesia, and allodynia that impairs quality of life. The current work aims to evaluate the neuroprotective potential of naringin (a citrus flavonoid) and examine its ability to improve Streptozotocin (STZ)-induced diabetic neuropathic pain by investigating its hypoglycemic, lipid-lowering, anti-inflammatory, and antioxidant effects. Methods: out of 40 adult male Sprague-Dawley rats, diabetes was induced in 30 rats through a single injection of STZ (45 mg/kg) dissolved in citrate buffer. Ten days after induction of diabetes, rats were divided into four groups (10/group): normal control, diabetic control, naringin-treated diabetic rats, and glimepiride-treated diabetic rats as a positive control group. At the end of the experiment, serum and brain tissue samples will be collected then the hypoglycemic, lipid-lowering, anti-inflammatory, and antioxidant effects of naringin will be assessed and compared with that of standard drug glimepiride. Hyperalgesia and the effect of the studied drugs on it will also be evaluated by observing pain related behaviors in diabetic rats using hot plate, tail immersion, and mechanical sensitivity (von Frey) tests. Results: Significant increase in the serum levels of glucose, triglyceride, total cholesterol, low-density lipoprotein-cholesterol, and nitric oxide, with a concomitant decrease in body weight, plasma insulin and high-density lipoprotein-cholesterol were observed in diabetic rats. Also, the brain level of malondialdehyde was increased, while that of reduced glutathione, glutathione peroxidase, catalase, and superoxide dismutase were markedly decreased. Furthermore, diabetic rats showed a marked increase in plasma levels of inflammatory cytokines including interleukin-6 and tumor necrosis factor-α. Moreover, hot plate, tail immersion, and von Frey tests revealed hyperalgesia in diabetic rats. Treatment with naringin and glimepiride reduced pain hypersensitivity, restored body weight and nearly normalized the altered biochemical parameters, more significantly with naringin than with glimepiride. Conclusion: These results may highlight the potential effects of naringin as a therapeutic strategy for diabetes and its complications including peripheral neuropathy.

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Introduction

Diabetes mellitus (DM) is a chronic condition marked by high blood sugar levels due to inability of the body to produce or use insulin effectively. This condition disrupts the metabolism of carbohydrates, fats, and proteins, leading to various health complications. The main types of diabetes include type 1 diabetes (an autoimmune condition in which the body attacks insulin-producing pancreatic β-cells), type 2 diabetes (where the body becomes resistant to insulin or doesn't produce enough), and gestational diabetes diagnosed or develops during early or mid-pregnancy [1].

Diabetes mellitus can lead to a range of serious long-term complications affecting various parts of the body. Long-term hyperglycemia in most cases results in various microvascular, macrovascular, and neural complications leading to conditions such as stroke, heart, kidney, and eye problems [2]. Diabetic neuropathy (DN) is one of the most common of these complications, and about 60% of diabetic men have varying degrees of nerve injury manifested by neuropathic pain, numbness, and tingling, particularly in the legs and feet. Diabetic neuropathic pain has been linked to local neuroinflammation and the activation of glial cells, where astrocytes and microglia become activated and release inflammatory mediators, which contribute to the regulation of pain signal transmission [3].

Diabetic-induced hyperalgesia is a condition where individuals with diabetic neuropathy experience an increased sensitivity to pain. This can manifest as spontaneous pain or heightened responses to normally non-painful stimuli. The mechanisms behind hyperalgesia are complex and involve factors such as the formation of advanced glycation end products, pro-inflammatory cytokines, and oxidative stress [4].

Flavonoids are natural compounds with diverse phenolic structures, commonly found in fruits, vegetables, grains, roots, stems, bark, tea, and wine. These natural products have many health benefits as they contain biologically active phytochemical constituents [5].

Flavonoids have been used in cosmetics, anti-wrinkle skin care products [6], and in natural dyes [7]. However, the most notable applications of flavonoids are in the medical field. They have been extensively used as antioxidants, anticancer, antiangiogenic, neuroprotective, antimicrobial, antiviral, and antiproliferative agents [8]. They also prevent cardiometabolic disorders [9] and have been shown to maintain better cognitive performance with age [10].

Over 6000 different classes and subclasses of flavonoids have been identified to date and are primarily synthesized by a variety of plants. Structurally, flavonoids have a 15-carbon skeleton, featuring 2 benzene rings connected by a three-carbon chain, hence classified as C6-C3-C6 compounds [11]. Depending on the carbon of the C ring to which the B ring is attached, as well as the degree of oxidation and unsaturation of the C ring, flavonoids can be split into a variety of distinct subgroups: *Isoflavones, Neoflavonoids, Typical flavonoids* (including flavones, flavonols, flavanones, flavanonols, catechins, and anthocyanins), and *chalcones* [5].

Naringin is a flavanone glycoside, scientifically known as 4',5,7-trihydroxyflavanone-7-rhamnoglucoside, found in grapes and citrus fruits (the major flavonoid of grapefruit). Many studies have reported several biological effects of naringin such as anti-inflammatory, antioxidant, antiviral, antihypertensive, hepatoprotective, nephroprotective, immunomodulatory, and anticancer activities, but some studies have also shown naringin related side effects and drug interactions [12].

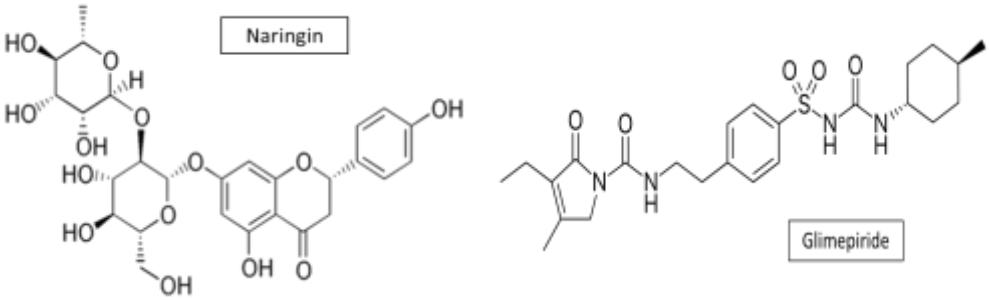
In accordance with the above, the main objective of the present work was to evaluate the potential antidiabetic, lipid-lowering, anti-inflammatory and antioxidant effects of naringin, which may alleviate diabetic peripheral sensory neuropathy in rats, and compare them with that of standard drug glimepiride.

Materials and methods

The animal experiments were conducted following the guidelines and recommendations of the Institutional Animal Care and Use Committee (IACUC), after being approved by the Ethical Review Committee of the Faculty of Medicine, Al-Azhar University, Damietta, Egypt (IRB 00012398, 11 March 2024).

Drugs and chemicals:

Naringin and STZ were obtained from Sigma-Aldrich Chemical Co., Burlington, MA, USA. Glimepiride was purchased from Sanofi-Aventis, Egypt. All other chemicals and reagents were of analytical quality and were purchased commercially at the purest grade available.



Chemical structure of *Naringin*	Chemical Structure of *Glimepiride*
Molecular formula: $C_{27}H_{32}O_{14}$	Molecular formula: $C_{24}H_{34}N_4O_5S$
Molecular weight: *580.53 g/mol*	Molecular weight: *490.62 g·mol*$^{-1}$

Figure 1. Chemical structures, molecular weights and molecular formulas for naringin and glimepiride.

Animals:

Adult male, healthy, Sprague-Dawley rats (n = 40, aged 9 – 12 weeks, weighing 150 - 200 g) were purchased from the National Research Centre (NRC) in Giza, Egypt. The animals were maintained under standard laboratory conditions in clean polypropylene cages (5 rats/cage), temperature (22 ± 2°C), humidity (30% - 50% RH), and a 12-hour light/dark cycle. They had free access to water and standard rat chow throughout the adaptation period. Experimental procedures were conducted in the animal house of Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt.

Induction of diabetes and animal grouping:

After two weeks adaptation period, diabetes was induced in 30 rats after 16 hours fast by a single intraperitoneal (i.p.) injection of STZ (45 mg/kg BW) dissolved in citrate buffer (0.1 M, pH 4.5) [13]. Blood samples were obtained from rat lateral tail veins 36 hours after STZ injection and the fasting blood glucose (FBG) levels were measured by a glucose strip test in a glucometer (Easy Gluco Blood Glucose Monitoring system, Infopia, Korea). Rats with FBG ≥ 250 mg/dl from at least 3 samplings were considered diabetic. Diabetic rats were fed a high-fat diet (HFD) instead of standard rat chow, for 4 weeks until the duration of the experiment to induce diabetic complications such as diabetic neuropathy [14]. The rats were then divided into four groups as follows: *Group 1: Normal control (NC)*- 10 non-diabetic rats received citrate buffer (pH 4.5) and normal drinking water. *Group 2: Diabetic Control (DC)*- 10 diabetic rats received normal drinking water. *Group 3: Naringin-treated diabetic rats (NGN-D)*- 10 diabetic rats, treated with naringin (100 mg/kg BW/day) by intra-gastric gavage for 4 weeks [15]. *Group 4: Glimepiride-treated diabetic rats (GD-D)*- 10 diabetic rats received glimepiride (0.5 mg/kg BW/day) orally for 4 weeks [16].

Initial body weight (BW) was measured at the beginning of the experiment and final BW was recorded at the end of the experiment. The next day after treatment, hyperalgesia was assessed by several behavioral tests, blood samples were collected from retro-orbital veins, and the rats were then lightly anesthetized with ether and killed by decapitation. The animal's brains were removed, washed with ice-cold saline after removing the adhering, and stored on ice. The animal experiments were approved by the Ethical Review Committee of the Faculty of Medicine, Al-Azhar University, Damietta, Egypt (IRB 00012398, 11 March 2024).

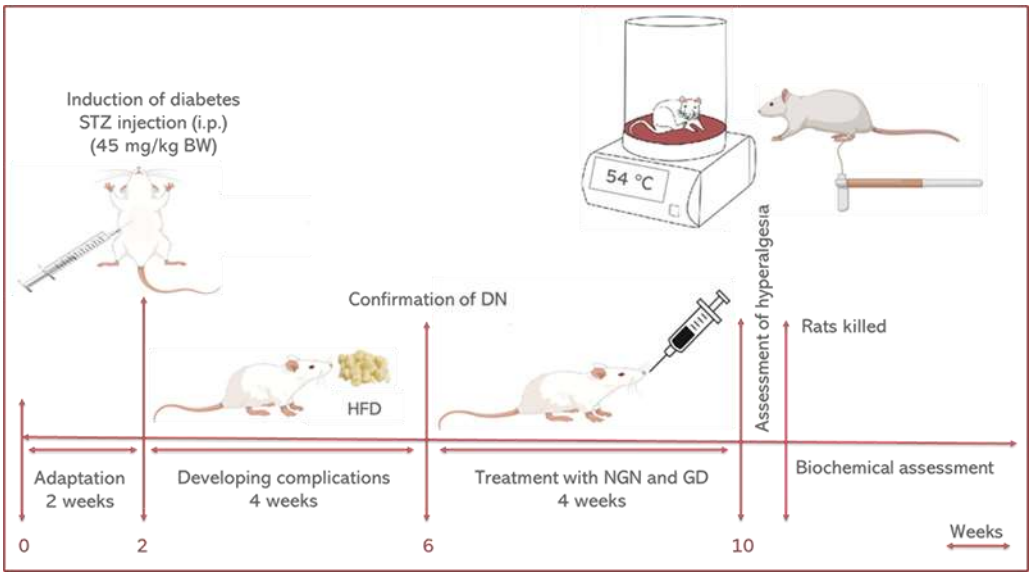


Figure 2. Graphical experimental design.

Preparation of plasma, serum, and brain homogenate samples

For measurement of insulin, IL-6 and TNF-α, plasma was separated by collecting a portion of the blood into heparinized tubes and centrifuging it at 600×g for 15 minutes. While another portion of the blood was centrifuged at 3000×g for 15 minutes to separate the serum for determination of glucose, TG, t-cholesterol, HDL-C, LDL-C, and NO.

On the other hand, the stored brain tissues were rinsed with ice-cold saline, dried, sectioned into small pieces and then homogenized in ice-cold buffer (10 mM KH2PO4; 20 mM EDTA; 30 mM KCl). The resulting homogenate was centrifuged at 4°C (1000×g for 10 min), and the isolated supernatant was used to determine brain oxidants and antioxidant defense markers.

Biochemical assay in serum and brain homogenate

Serum glucose, TG, t-cholesterol, LDL-C, HDL-C, proinflammatory cytokine including TNF-α, and IL-6 were measured by enzymatic methods using commercially available kits (RayBiotech), following the manufacturer's recommendations.

Oxidative stress markers

Brain level of Malondialdehyde (MDA), was measured following the method described by Ohkawa et al., [17]. Superoxide dismutase (SOD) activity was measured by the conventional technique of Marklund and Marklund [18]. Nitric oxide (NO) and Catalase (CAT) enzyme activity were calorimetrically assessed according to Montgomery and Dymock [19] and Aebi [20] respectively. Glutathione peroxidase (GPx) enzyme activity was assessed following the method of Paglia and Valentine [21] and Factor et al., [22] by measuring NADPH oxidation at 340 nm in the presence of glutathione.

Assessment of diabetes-induced hyperalgesia

Diabetes-induced hyperalgesia was evaluated in the diabetic rats by observing pain thresholds and pain perception using behavioral tests including hot plate, tail immersion, and mechanical sensitivity (von Frey) tests [23].

In the *hot plate test,* rats were placed individually on a hot plate (54 ± 0.1°C), and the latency to front and hind paw licking, rubbing, or jumping to avoid the heat was determined. To avoid tissue damage, a cutting time of 30 seconds was set [24].

The *tail immersion test* was carried out by immersing the rat's tail in a hot water bath maintained at 42 ± 0.5 °C and determining the duration before tail withdrawal (the shortness of which indicates hyperalgesia) [25].

In *Mechanical sensitivity (von Frey) test,* rats were placed individually in mesh-floored boxes and allowed to adapt for 20 minutes. Calibrated von Frey filaments were pressed perpendicularly on the plantar surface of the hind paw with a sufficient force to bend the filament for 6 seconds. With animals that didn't give a response, filaments of the next-greater force were applied, while in presence of a response, filaments of the next-lower force were used. Each animal was tested 4 - 5 times with 5-minute intervals, and the mean values were used [26].

Statistical analysis:

The results were statistically analyzed using the Statistical Packages for Social Science (SPSS) program (version 7.5.1, SPSS Inc, Chicago, Illinois, 1996). Quantitative data were presented as means ± SEM for groups of 10 animals each. A one-way analysis of variance (ANOVA) was employed to compare more than two means, and Tukey's Post Hoc test was used for multiple comparisons among different variables. Differences between groups were considered significant at $P \leq 0.05$.

Results

Effect of treatment on body weight and glycemic status of diabetic rats:

As shown in **Table 1**, there were no significant differences ($p > 0.05$) in the BW between the studied groups at the beginning of the experiment. While at the end of the experiment, diabetic rats showed a decrease in the final BW, with a significantly reduced weight gain ($P < 0.01$) compared to the normal control (NC) rats. Treatment with naringin or glimepiride resulted in significant weight gain and recovery of the reduced BW compared with the diabetic control (DC) rats ($p < 0.01$). **Table 1** also shows that naringin and glimepiride significantly modulate the glycemic status of diabetic rats by decreasing plasma glucose and increasing insulin levels compared with DC rats ($P < 0.01$).

Table 1: Effect of treatment on body weight and the glycemic status of diabetic rats**.**

	Groups				
	NC	**DC**	**NGN-D**	**GD-D**	**p-Value**
Initial BW (g)	161.07 ± 1.89	169.15 ± 1.33	165.36 ± 1.81	167.17 ± 1.48	p > 0.05
Final BW (g)	200.15 ± 2.29	146.41 ± 2.45[a]	209.4 ± 2.31[b]	206.94 ± 3.84[b]	p < 0.01
Weight gain	41.08 ± 0.41	-23.74 ± 2.12[a]	44.04 ± 0. 5[b]	39.77 ± 2.36[b]	p < 0.01
Glucose (mg/dl)	68.47 ± 5.12	229.17 ± 8.13[a]	72.08 ± 4.13[b]	74.91 ± 4.41[b]	p < 0.01
Insulin (µU/ml)	17.44 ± 2.19	7.17 ± 0.81[a]	19.08 ± 1.54[b]	21.11 ± 2.53[b]	p < 0.01

Data are expressed as means ± SEM of 10 rats/group.
Initial and final body weight (BW) were measured, and weight gain was calculated.
NC, Normal Control; DC, Diabetic Control; NGN-D, Naringin-treated Diabetic rats; GD-D, Glimepiride-treated Diabetic rats.
[a]Significantly different from the NC group ($P < 0.01$).
[b]Significantly different from the DC group ($P < 0.01$).

Effect of treatment on pain threshold:

The results of hot-plate and tail-immersion tests revealed a significantly decreased pain threshold in diabetic control rats compared to the NC rats ($p < 0.01$). Both tests revealed a significant increase in nociceptive threshold in both naringin-treated and glimepiride-treated diabetic rats as compared to DC rats ($p < 0.01$), with a more pronounced response in the tail-immersion test to naringin compared to glimepiride ($p < 0.05$) **(Figure 3).**

Also, the results of the mechanical sensitivity (von Frey) test revealed a significant reduction in the tactile withdrawal threshold in DC rats compared with the normal controls ($p < 0.01$), which is significantly corrected when diabetic rats were treated with either naringin, or glimepiride **(Figure 3).**

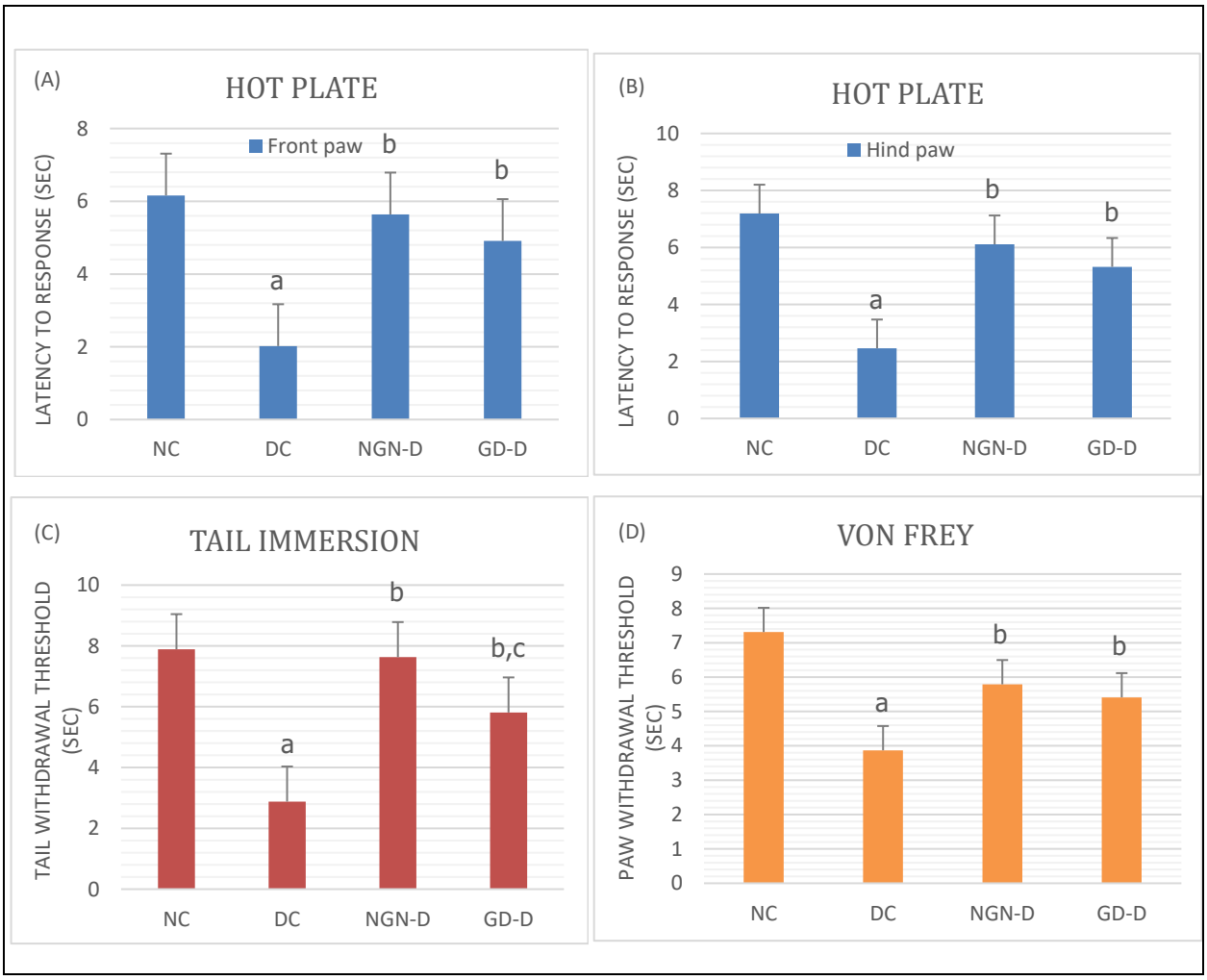


Figure 3. Effect of naringin or glimepiride on pain threshold in hot plate (front paw) (A), hot plate (hind paw) (B) tail immersion (C) and von Frey (D) tests in diabetic rats.

Data are expressed as means ± SEM of 10 rats/group.
NC, Normal Control; DC, Diabetic Control; NGN-D, Naringin-treated Diabetic rats; GD-D, Glimepiride-treated Diabetic rats.
[a]Significantly different from the NC group ($P < 0.01$).
[b]Significantly different from the DC group ($P < 0.01$).
[c]Significantly different from the NGN-D group ($P < 0.05$).

Naringin or glimepiride significantly increased response latency in hot plate and tail immersion tests, and tactile withdrawal threshold in von Frey test, with a more pronounced response in the tail-immersion test to naringin compared to glimepiride.

Effect of treatment on lipid profile and inflammatory cytokines in diabetic rats:

As shown in **Table 2**, diabetic rats reported significantly higher levels of TG, t-cholesterol, LDL-C, TNF-α, and IL-6 with a significant reduction in HLD-C compared with the NC rats. Interestingly, the naringin and glimepiride-treated diabetic groups experienced ameliorated alterations in NO, serum lipids, as well as plasma IL-6 and TNF-α with almost the same degree of efficacy for all observed parameters except for the proinflammatory cytokines, where the anti-inflammatory effect of naringin was more pronounced than that of glimepiride ($p < 0.05$).

Table 2: Effect of treatment on lipid profile and proinflammatory cytokines in diabetic rats

	Groups				
	NC	**DC**	**NGN-D**	**GD-D**	**p-Value**
TG (mg/dl)	75.02 ± 1.4	131.17 ± 2.31[a]	69.15 ± 4.42[b]	71.66 ± 5.37[b]	p < 0.01
t-Cholesterol (mg/dl)	81.15 ± 4.72	125.42 ± 3.88[a]	76.91 ± 3.21[b]	79.77 ± 5.81[b]	p < 0.01
LDL-C (mg/dl)	34.62 ± 3.52	79.74 ± 2.52[a]	32.71 ± 3.65[b]	35.55 ± 3.67[b]	p < 0.01
HDL-C (mg/dl)	38.14 ± 3.49	16.32 ± 2.38[a]	40.12 ± 3.71[b]	37.28 ± 2.46[b]	p < 0.01
TNF-α (ng/ml)	0.74 ± 0.19	2.31 ± 0.08[a]	0.76 ± 0.15[b]	1.02 ± 0.14[bc]	p < 0.01
IL-6 (pg/ml)	311.4 ± 7.06	612.1 ± 27.18[a]	309.2 ± 11.4[b]	357.6 ± 32.2[bc]	p < 0.01

Data are expressed as means ± SEM of 10 rats/group.
NC, Normal Control; DC, Diabetic Control; NGN-D, Naringin-treated Diabetic rats; GD-D, Glimepiride-treated Diabetic rats; TG, triglyceride; t-Cholesterol, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.
[a]Significantly different from the NC group (p < 0.01).
[b]Significantly different from the DC group (p < 0.01).
[c]Significantly different from the NGN-D group (P < 0.05).

Effect of treatment on oxidants and antioxidant defense markers:

Table 3 shows the effect of treatment with naringin and glimepiride on brain oxidants and antioxidant defense markers in diabetic rats, where the DC rats had a significant increase in plasma NO level and brain content of MDA, with a significant decrease in brain GSH, GPx, SOD, and CAT compared to the NC rats. In contrast, the naringin-treated and glimepiride-treated diabetic rats showed nearly normalized brain levels of MDA, GSH, GPx, SOD, and CAT, with no significant difference between the two drugs.

Table 3. Effect of treatment on oxidative stress biomarkers in diabetic rats.

Brain content (mg pr)	Groups				
	NC	**DC**	**NGN-D**	**GD-D**	**p-Value**
NO (µmols/L)	5.47 ± 1.23	19.18 ± 3.53[a]	7.06 ± 2.62[b]	7.97 ± 1.41[b]	p < 0.01
MDA (nmol/mg pr)	1.15 ± 0.29	5.42 ± 0.48[a]	1.43 ± 0.31[b]	1.81 ± 0.21[b]	p < 0.01
GSH (µg/mg pr)	7.17 ± 1.41	2.74 ± 0.12[a]	6.14 ± 0.75[b]	5.73 ± 1.06[b]	p < 0.01
GPx (U/mg pr)	28.14 ± 3.19	16.21 ± 2.13[a]	25.12 ± 3.41[b]	24.28 ± 2.34[b]	p < 0.01
SOD (units/mg pr)	5.41 ± 0.51	2.21 ± 1.48[a]	4.72 ± 1.39[b]	3.35 ± 1.15[b]	p < 0.01
CAT (µmols of H_2O_2)	5.53 ± 1.51	3.18 ± 0.46[a]	5.62 ± 1.01[b]	6.39 ± 1.33[b]	p < 0.01

Data are expressed as means ± SEM of 10 rats/group.
NC, Normal Control; DC, Diabetic Control; NGN-D, Naringin-treated Diabetic rats; GD-D, Glimepiride-treated Diabetic rats; NO, nitric oxide; MDA, malondialdehyde; GSH, reduced glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, Catalase.
[a]Significantly different from the NC group (P < 0.01).
[b]Significantly different from the DC group (P < 0.01).

Discussion

Diabetic neuropathy is difficult to study because of the complex multiple causes of diabetic nerve damage including long-term hyperglycemia, dyslipidemia, inflammation, oxidative stress, growth factor deficiency, and accumulation of advanced glycation end products (AGEs) due to exposure of protein and fat to sugars [27].

The precise mechanisms underlying the STZ diabetes-induced neurodegeneration are also complex and multifactorial, most notably increased production of reactive oxygen species and other free radicals due to oxidative stress to which nerve cells are particularly vulnerable due to their greater reliance on oxidative phosphorylation as compared with other cells [28]. Hyperglycemia also causes endoplasmic reticulum stress, leading to various neuronal apoptotic processes [29]. Additional mechanisms include dyslipidemia, impaired nerve perfusion, disrupted redox status, chronic low-grade neuroinflammation, and disturbed calcium homeostasis [30].

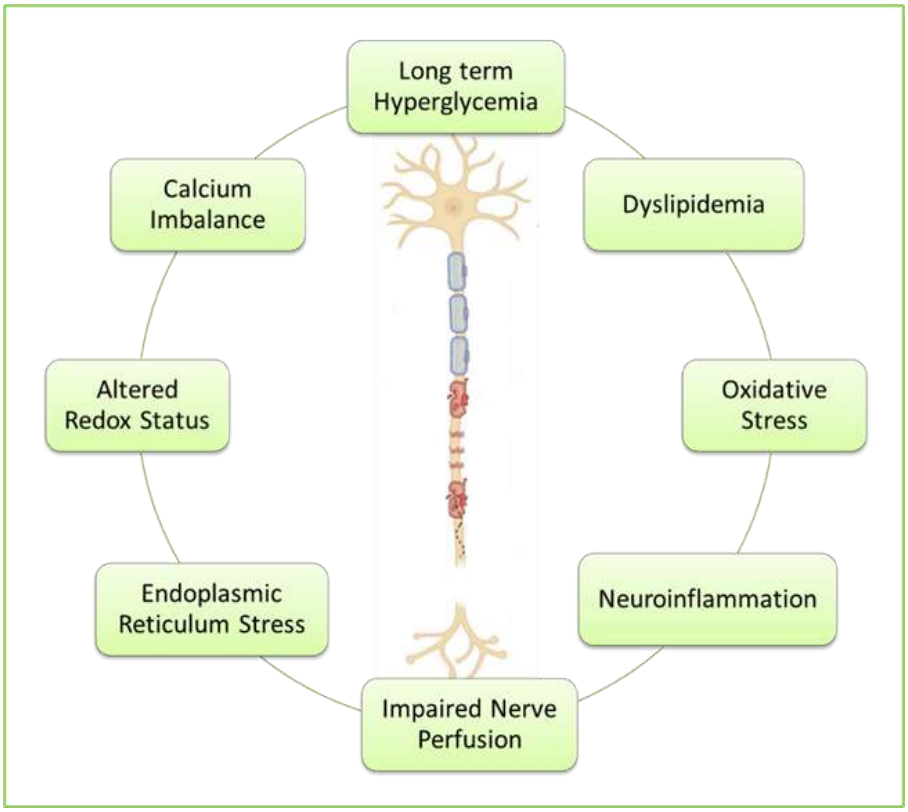


Figure 4. Key mechanisms underlying diabetes-induced neurodegeneration

The present study investigated the neuroprotective properties of naringin (a citrus flavonoid) on STZ-induced peripheral diabetic neuropathy in rats by investigating its hypoglycemic, hypolipidemic, antioxidant and anti-inflammatory effects and compared them with the effects of glimepiride (a standard antidiabetic drug).

The results showed a significant increase in serum levels of BG, TG, t-cholesterol, LDL-C, and NO, accompanied by a decrease in HDL-C and BW secondary to induction of diabetes in rats. Diabetic rats also showed a significant decrease in brain antioxidants (GSH, GPx, SOD, and CAT) with a concomitant increase in brain MDA. Furthermore, diabetic rats showed a significant decrease in plasma insulin levels while the levels of proinflammatory cytokines (IL-6 and TNF-α) were significantly increased. These results align with other studies that have observed increased serum levels of blood glucose, TG, t-cholesterol, and LDL-C, accompanied with a decreased serum HDL-C, elevated brain MDA, and reduced brain antioxidant enzyme activities, as well as significantly increased plasma levels of TNF-α and IL-6, with significantly decreased plasma insulin levels in diabetic rats [31,23,33].

In this study, treatment of diabetic rats with naringin or glimepiride resulted in significant weight gain and nearly normalized plasma glucose and insulin levels. These results are consistent with previous studies showing that naringin can increase insulin secretion and reduce blood glucose levels by modulating key enzymes involved in glucose metabolism [34,35,36].

Results of the current study also demonstrated that naringin or glimepiride reduced pain hypersensitivity and improved hyperalgesia in diabetic rats, as evidenced by increased response latency in tail immersion and hot plate

tests, and tactile withdrawal threshold in von Frey test, with naringin showing a more pronounced effect in the tail-immersion test suggesting its stronger anti-inflammatory and antioxidant properties [37]. This is supported by other research suggesting that naringin can reduce neuroinflammation and oxidative stress, which are key contributors to neuropathic pain [14]. These results are also similar to previous studies that observed a reduced pain threshold in animals after diabetes induction and a dose-dependent improvement after treatment with naringin [38,39].

Elevated levels of TG, t-cholesterol, and LDL-C, along with reduced HDL-C, are common in diabetes and contribute to cardiovascular risk. Inflammatory cytokines have often been linked to the induction of neuropathic pain, increased excitability of afferent sensory nerves, demyelination and altered permeability of the blood-nerve barrier leading to peripheral neuronal degeneration [40,41]. This study showed that naringin or glimepiride significantly improved the lipid profile and reduced inflammatory cytokines in diabetic rats, with naringin showing a more pronounced anti-inflammatory effect. This highlights their role in alleviating diabetic dyslipidemia and inflammation, which are critical factors in the development of diabetic complications. These findings are consistent with the observation that naringin reduces hypercholesteremia in rats fed a HFD by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) resulting in lower plasma LDL-C and TG levels, without impacting HDL-C levels [42]. Hu and Zhao [43], Tsai et al. [44] and Bai et al. [45] also observed that chronic treatment of diabetic rats with naringin, significantly inhibited formation and release of these inflammatory mediators.

Oxidative stress plays a central role in the pathogenesis of diabetes and its complications. This study found that diabetic rats exhibited increased levels of MDA and decreased brain levels of GSH, GPx, SOD, and CAT. Treatment with naringin or glimepiride attenuated oxidative stress and improved antioxidant capacity in the brains of diabetic rats, indicating their potential to protect against oxidative damage. This is consistent with other studies showing that naringin has strong antioxidant properties via decreasing lipid peroxidation and buildup of reactive oxygen species (ROS) [46], lowering SOD, GSH, GPx, and CAT activities [47].

Comparative Efficacy

While both naringin and glimepiride demonstrated positive effects in managing diabetes and its complications, naringin appeared to have a more pronounced impact on reducing neuroinflammation and oxidative stress. This indicates that naringin could be a more effective treatment option, especially for diabetic neuropathy. The study's findings are supported by other research showing that naringin can modulate various biochemical pathways involved in glucose and lipid metabolism, inflammation, and oxidative stress [48].

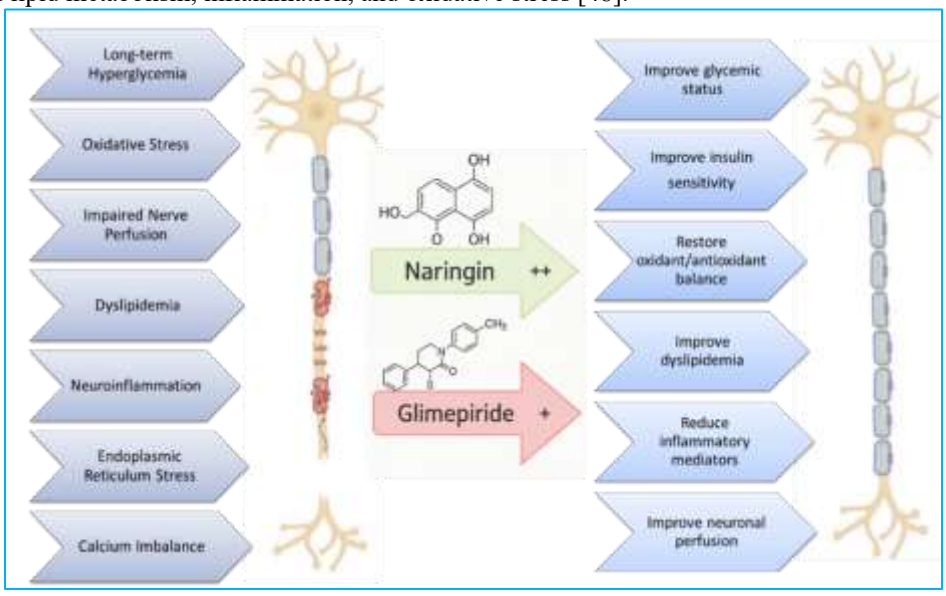


Figure 5. Scheme summarizing some of the molecular mechanisms of diabetes-induced neuronal damage, and how treatment with naringin or glimepiride improves it

Conclusion

In summary, the study highlights the therapeutic potential of naringin in the management of diabetes and its complications, comparing it with glimepiride. Both compounds were effective in improving body weight, glycemic status, pain sensitivity, lipid profile, neuroinflammation, and oxidative stress in diabetic rats. Notably, naringin exhibited more significant anti-inflammatory and antioxidant effects. These findings suggest that naringin could be a

valuable addition to diabetes treatment, offering benefits beyond glycemic control, such as protection against neuropathy, dyslipidemia, and oxidative damage. A limitation of this study is that it didn't thoroughly investigate all the molecular mechanisms of naringin's mitigating effects in diabetic neuropathy, more in-depth research on its detailed mechanisms of action is needed.

Authors' Contributions: OMM, AE, and BHE contributed to the conceptualization and design of the study, generated, curated, and analyzed the data. OMM, AE, BHE, MGMH, MRE, AMH, NMG and AIS interpreted the results, wrote the original draft and the final manuscript. OMM, AE, and BHE edited and revised the manuscript. All Authors revised and approved the final version submitted for publication

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