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The effects of taxifolin on neuropathy related with hyperglycemia and neuropathic pain in rats: A biochemical and histopathological evaluation

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Abstract

Background. Hyperglycemia can be considered a determining factor in the development of diabetic neuropathy as well as neuropathic pain. There is a relationship between the excessive production of reactive oxygen species (ROS) and the pathogenesis of diabetic neuropathic pain. Taxifolin, on the other hand, is a flavonoid that has been documented to inhibit ROS production.

Objectives. To investigate the effects of taxifolin, which has antioxidant and neuroprotective effects, on alloxan-induced hyperglycemia-induced neuropathy and neuropathic pain, biochemically and histopathologically.

Materials and methods. The albino Wistar male rats were divided into 3 groups: healthy group (HG), only alloxan group (AXG) and alloxan+taxifolin group (ATG). Hyperglycemia in animals was caused through intraperitoneal injection of alloxan at a dose of 120 mg/kg. Paw pain thresholds of animals were measured using Basile algesimeter. Sciatic nerve tissues were examined biochemically and histopathologically in order to evaluate neuropathy.

Results. Our experimental results revealed that taxifolin significantly prevented the increase of plasma glucose concentration level with alloxan administration, the decrease of the paw pain threshold related to hyperglycemia, the change of oxidant–antioxidant balance in the sciatic nerve tissue in favor of oxidants, and the deterioration of tissue morphology in animals.

Conclusions. Our experimental results indicate that taxifolin alleviates alloxan-induced hyperglycemia-related neuropathy and neuropathic pain.

Key words: hyperglycemia, rats, neuropathic pain, neuropathy, taxifolin

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Background

Peripheral nerve damage is usually caused by compression, various traumas, and ischemic and metabolic disorders.[1] Hyperglycemia, which is the main symptom of diabetes mellitus (DM), a metabolic disease, occurs due to the absence of insulin secreted by pancreatic cells, or the decrease in the sensitivity of target cells to insulin.[2] The risk of developing chronic peripheral neuropathy has been recorded as 30–50% in diabetic patients. Peripheral neuropathy is a significant complication of DM which can cause foot ulcers and lower extremity amputation.[3] Hyperglycemia is a determining factor in the development of diabetic neuropathy. In DM, it may lead to the deterioration of motor and sensory nerve conduction velocity.[4] Hyperglycemia has been reported to cause neuropathy also in individuals without DM.[5] It has also been described to have a role in the formation of neuropathic pain in both animal models and diabetic patients.[6] Nearly 30% of patients with DM develop chronic neuropathic pain.[2] Even though many scientific studies related to diabetic neuropathy exist, its pathogenesis is still not fully explained. Former studies have suggested that excessive mitochondrial glucose loading increases electron transfer to oxygen and the production of reactive oxygen species (ROS).[7,8] Reactive oxygen species facilitate the production of toxic products and lead to the oxidation of cell membrane lipids.[9] Lipid peroxidation (LPO) caused through increased ROS as a result of hyperglycemia, has been shown to be important in the development of DM complications.[10] Moustafa et al. reported that the amount of malondialdehyde (MDA), one of the LPO end products, increased significantly in the sciatic nerve tissue of rats with DM.[11] Galeshkalami et al. have shown that hyperglycemia, which causes diabetic neuropathy, leads to the generation of ROS in neurons and their subsequent death. In addition, they stated that increasing total antioxidant status (TAS) and glutathione (GSH) in neurons, and preventing the increase of ROS and LPO are associated with neuroprotection.[12] Solanki and Bhavsar revealed that the pain threshold decreased in diabetic rats having high oxidant levels and low antioxidant levels.[13] It has also been revealed that neuropathy developing in diabetic rats causes significant hyperalgesia.[14] The acquired information suggest that antioxidants may be useful in the treatment of diabetic neuropathy and neuropathic pain. In neuropathic pain, anti-inflammatory drug treatments are recommended to control neuroinflammation.[15] Today, opioids, tricyclic antidepressants and anticonvulsants, which have significant side effects, are used in the treatment of neuropathic pain. Therefore, research has focused on identifying alternative treatments with fewer side effects.[16]

Taxifolin (dihydroquercetin) is an antioxidant flavonoid which has been tested against diabetic neuropathy and neuropathic pain in this study.[17] Taxifolin has numerous pharmacological effects, including antioxidant, anti-inflammatory, antiviral, antibacterial, anticancer, as well as neuroprotective activities. Moreover, taxifolin was documented to inhibit the production of ROS, which suggests that taxifolin may be useful in the treatment of hyperglycemia-induced neuropathy and neuropathic pain.[18] In the literature, there is no information about the protective effect of taxifolin against hyperglycemia-related neuropathy and neuropathic pain.

Objectives

The aim of this study was to biochemically and histopathologically investigate the effect of taxifolin on alloxan-induced hyperglycemia-induced neuropathy and neuropathic pain in rats, as well as to examine the relationship of hyperglycemia-related neuropathy and neuropathic pain with oxidative stress, and to assess the benefits of the antioxidant therapy.

Materials and methods

Animals

A total of 18 albino Wistar male rats weighing from 235 g to 247 g were used for the experiment. The animals were obtained from Ataturk University Medical Experimental Application and Research Center (Erzurum, Turkey). Prior to the experiment, the animals were housed and fed for 1 week at normal room temperature (22°C) in the appropriate laboratory environment. The protocols and procedures were approved by the local Ataturk University Animal Experimentation Ethics Committee (meeting No. 2020/06, March 6, 2020).

Chemicals

Sodium thiopental (1 g solution for injection) utilized within the experiment was purchased from IE Ulagay (Istanbul, Turkey), while alloxan (Cat. No. A7413, 25 g, >98% purity) was provided by Sigma (St. Louis, USA). Taxifolin, each tablet containing 25 mg of dihydroquercetin, was obtained from Evalar (Biysk, Russia).

Experimental groups

The animals utilized in our study were divided into 3 groups (6 animals in each group): healthy group (HG), only alloxan group (AXG) and alloxan+taxifolin group (ATG).

Inducing diabetes

Alloxan dissolved in distilled water was injected intraperitoneally in rats at a dose of 120 mg/kg for 3 consecutive days in order to induce hyperglycemia. Fasting plasma glucose concentration was measured in blood samples taken from the tail veins of rats at the end of the 3rd month following alloxan

administration. A commercially available blood meter was employed for plasma glucose concentration measurement. Animals having plasma glucose concentration of 250 mg/dL and above were included in the study in line with our study. As it is commonly known, the animals having plasma glucose concentration above 250 mg/dL are considered diabetic.[19]

Experiment procedure

Taxifolin (50 mg/kg) was given orally to the ATG. The same volume of distilled water as a solvent was applied in the same way to the AXG and HG. The cited procedure was repeated once a day for 3 months. Paw pain thresholds of all animal groups were measured using the Basile algesimeter at the 1st, 2nd and 3rd h after the last dose of taxifolin was administered.[20] Immediately following the measurement at the 3rd h, the rats were sacrificed with high-dose thiopental anesthesia and their sciatic nerve tissues were removed. Malondialdehyde, total glutathione (tGSH), total oxidant status (TOS) and TAS capacity were measured in sciatic nerve tissue samples, which were removed later. Furthermore, tissues were examined histopathologically.

Biochemical analyzes

Determination of glucose concentration in plasma

Accu-Chek Performa Nano (Roche, Istanbul, Turkey) glucometer was used to determine the glucose concentration in the blood samples taken from the tail vein.

Preparation of samples

An amount of 0.2 g of nerve tissue was taken from rats thoroughly washed with NaCl (0.9%). Tissues were homogenized in ice cold with a high speed homogenizer. After the homeogenization process, 2 mL of 1.15% potassium chloride buffer solution (pH 7.4) was centrifuged at 10,000 × g at 4°C for 15 min. The obtained supernatants were used for biochemical analyzes, including MDA, tGSH, TOS, and TAS levels.

Measurements of tissue MDA and tGSH

Malondialdehyde measurements are based on the method utilized by Ohkawa et al., which includes spectrophotometric measurement of the absorbance of the pink-colored complex formed by thiobarbituric acid (TBA) and MDA.[21] Total glutathione measurement was performed, according to the method described by Sedlak and Lindsay.[22]

Measurements of tissue TOS and TAS

Tissue homogenate TOS and TAS levels were determined using a novel automated measurement method and commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey), both developed by Erel.[23,24] The TAS method is based on the bleaching of a characteristic color of a more stable ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The measurements are performed at 660 nm. The results are expressed as nmol H_2O_2 (hydrogen peroxide) equivalent/L. In the TOS method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically at 530 nm, was related to the total amount of oxidant molecules present in the sample. The results are expressed as μmol Trolox equivalent/L. The percentage ratio of TOS to TAS was used as the oxidative stress index. Oxidative stress index was calculated as TOS divided by 100 × TAS.

Histopathological examination

All of the tissue samples were first identified in a 10% formaldehyde solution for light microscope assessment. Following the identification process, tissue samples were washed under tap water in cassettes for 24 h. Samples were then treated with conventional grade of alcohol (70%, 80%, 90%, and 100%) to remove the water within tissues, which were then passed through xylol and embedded in paraffin. Four-to-five micron sections were cut from the paraffin blocks and hematoxylin and eosin (H&E) staining was administered. Their photos were taken following the Olympus DP2-SAL firmware program (Olympus® Inc., Tokyo, Japan) assessment. Histopathological assessment was carried out by the pathologist blinded for the study groups.

Statistical analysis

The Shapiro–Wilk test was used to determine whether the data obtained from the groups were normally distributed. One-way analysis of variance (ANOVA) test was applied to normally distributed data. In the follow-up, Tukey's or Games–Howell test was performed, according to the results of Levene's test as post hoc test. The Kruskal–Wallis test was applied to the data that did not show normal distribution and Dunn's test was applied as post hoc test. Since the histopathological data were discrete variables, the evaluation was done with the Kruskal–Wallis test. The results from the experiments were expressed as mean value ± standard deviation (X ±SD) or median 1st quartile–3rd quartile (Q1–Q3). Paw pain threshold was evaluated using repeated measures ANOVA. Sphericity was confirmed using Mauchly's test of sphericity. When sphericity had been violated, the Greenhouse–Geisser correction was used. The effect of time and groups were shown as a line chart. All statistical operations were performed with the SPSS v. 22 software (IBM Corp., Armonk, USA), and a value of $p < 0.05$ was considered statistically significant.

Results

Analysis results of glucose concentration in plasma

Three months after alloxan administration, the mean fasting glucose concentration in plasma was 287.8 ±8.1 mg/dL in the AXG, while it was 144 ±4.6 mg/dL and 86.3 ±5 mg/dL in the ATG and HG, respectively. At the end of the 3rd month of taxifolin administration, a statistically significant decrease in the glucose concentration in plasma was achieved in the ATG compared to the AXG ($p < 0.001$) (Table 1, Supplementary Table 1).

Test results of paw pain threshold

As can be seen from Table 2 and Supplementary Table 2, taxifolin significantly prevented the reduction of the pain threshold in animal foot claws with hyperglycemia in the 1st, 2nd and 3rd h. When the AXG was compared to the HG, the pain threshold was statistically significantly decreased ($p < 0.001$). When the ATG was compared with the AXG, the pain threshold increased significantly ($p < 0.001$). Paw pain threshold was evaluated between groups using repeated measures ANOVA. According to the Mauchly's test of sphericity, sphericity had been violated ($\chi^2 = 10.203$, $p = 0.006$) and therefore, the Greenhouse–Geisser correction was used. There has been a significant effect of time on paw pain threshold, $F(1.318, 19.769) = 14.125$, $p = 0.001$. At the same time, the between-group effect was statistically significant ($p < 0.001$). While there was no statistically significant difference between the 1st and 2nd h paw pain threshold measurements of the animals, there was a statistical difference in terms of the 1st and 3rd h measurements and the values measured at the 2nd and 3rd h. Intra-group time-dependent comparisons of paw pain threshold measurements in the HG, AXG and ATG are presented in Table 3 and Supplementary Table 3. The levels of paw pain threshold are shown in Fig. 1. Taxifolin has decreased neuropathic pain in rats with hyperglycemia in the 1st, 2nd and 3rd h by 70.9%, 75.8% and 82%, respectively.

Biochemical findings

MDA and tGSH analysis results

As can be seen from Table 4 and Fig. 2, the development of hyperglycemia in the sciatic nerve tissue of animals, an increase in MDA and a decrease in tGSH created a statistically significant difference in the AXG when compared to the HG ($p < 0.001$). In the values obtained after

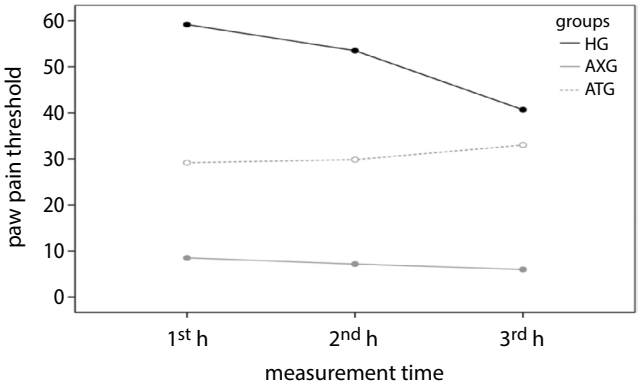

Fig. 1. Evaluation of time-dependent repeated paw pain threshold measures between groups using analysis of variance (ANOVA)

HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group.

Table 1. Analysis of variance (ANOVA) test results and post hoc p-values for group comparisons in plasma glucose concentration analysis

Plasma glucose concentration measurement times	Plasma glucose concentration (mg/dL) X ±SD			ANOVA results		Post hoc test p-values		
	HG	AXG	ATG	F (2,15)	p-value	HG vs AXG	HG vs ATG	AXG vs ATG
Before alloxan*	80.7 ±4.2	79.3 ±2.6	84.2 ±3.9	2.840	0.090	0.803	0.248	0.086
Third month after alloxan*	86.3 ±5.0	287.8 ±8.1	144.0 ±4.6	1742.708	0.001	0.001	0.001	0.001
The difference*	5.7 ±2.1	208.5 ±7.5	59.8 ±6.7	1867.067	0.001	0.001	0.001	0.001

HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; X ±SD – mean value ± standard deviation. As the post hoc test, *Tukey's honestly significant difference (HSD) test was performed after ANOVA (F (2,15)).

Table 2. Analysis of variance (ANOVA) test results and post hoc p-values for group comparisons, and paw pain threshold and analgesic activity values of the groups

Measurement time	ANOVA results		Post hoc test p-values			Paw pain threshold [g] (X ±SD)			Analgesic effect (%)		
	F (2,15)	p-value	HG vs AXG	HG vs ATG	AXG vs ATG	HG	AXG	ATG	HG	AXG	ATG
1st h*	1141.63	0.001	0.001	0.001	0.001	59.2 ±2.1	8.5 ±1.0	29.2 ±2.1	85.7	–	70.9
2nd h**	137.57	0.001	0.001	0.001	0.001	53.5 ±7.5	7.2 ±0.8	29.8 ±3.7	87.0	–	75.8
3rd h**	254.35	0.001	0.001	0.008	0.001	40.7 ±2.9	6.0 ±0.9	33.0 ±3.7	85.3	–	82.0

HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; X ±SD – mean value ± standard deviation. As the post hoc test, *Tukey's honestly significant difference (HSD) test or **Games–Howell test was performed after ANOVA (F (2,15)).

Table 3. The effect of time on the paw pain thresholds measured in the study groups

ANOVA results (repeated measures)						Paw pain threshold [g] (X ±SD)		
F (1.32,19.77)		p-value	p-value					
			1st h vs 2nd h	1st h vs 3rd h	2nd h vs 3rd h	1st h	2nd h	3rd h
All groups	14.13	0.001	0.350	0.001	0.037	32.28 ±0.4	30.17 ±1.1	26.56 ±0.7
HG	–	–	0.063	0.001	0.001	59.2 ±2.1	53.5 ±7.5	40.7 ±2.9
AXG	–	–	1.000	0.073	1.000	8.5 ±1.0	7.2 ±0.8	6.0 ±0.9
ATG	–	–	1.000	0.005	0.513	29.2 ±2.1	29.8 ±3.7	33.0 ±3.7

ANOVA – analysis of variance; HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; X ±SD – mean value ± standard deviation. Since sphericity was violated according to the Mauchly's test of sphericity, the evaluation was made using the Greenhouse–Geisser correction.

taxifolin application, a decrease in MDA and an increase in tGSH were found in the ATG compared to the AXG ($p < 0.001$).

TOS and TAS analysis results

As can be seen from Table 4 and Fig. 3, taxifolin significantly prevented the increase in TOS and decrease in TAS associated with hyperglycemia in the sciatic nerve tissue. When the HG and AXG were compared for TOS and TAS values, a statistically significant difference was observed ($p < 0.01$). There was no significant difference between HG and ATG in terms of TOS values ($p = 0.324$). When the TAS values of the AXG and ATG were compared, a significant difference was observed ($p < 0.001$).

Histopathological findings

As described in Table 5, histological examination of the sciatic nerve of the HG revealed that the nerve structure was normal, axons were surrounded by myelin sheaths and were located centrally, and Schwann cell nuclei and blood capillaries were normal (Fig. 4A). In the AXG, myelinated nerve fibers were swollen, pale and with histopathological changes, while myelinated nerve fibers showed degenerative appearance. Also,

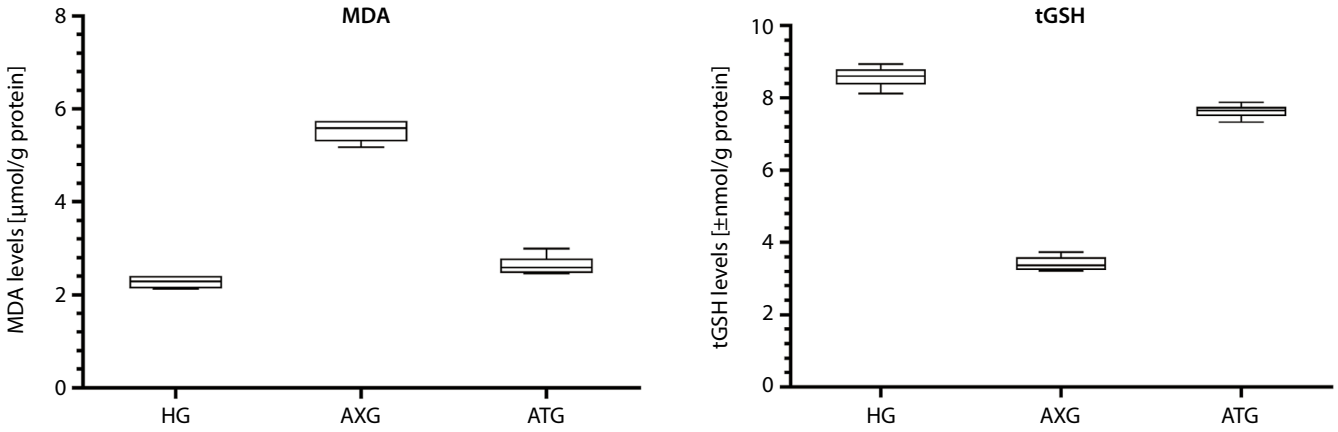

Fig. 2. MDA and tGSH levels in the sciatic nerve tissue of the study groups

Q – quartile; MDA – malondialdehyde; tGSH – total glutathione; HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; horizontal line – median; bottom line of the box – Q1 (25th); topline of the box – Q3 (75th); whiskers – minimum and maximum observation.

Table 4. Analysis of variance (ANOVA) or Kruskal–Wallis test results and post hoc p-values of group comparisons for MDA, tGSH, TOS, and TAS values

Biochemical parameters	Mean value ± standard deviation or median (Q_1–Q_3)			ANOVA or KW		Post hoc test p-values		
	HG	AXG	ATG	F (2,15) or KW	p-value	HG vs AXG	HG vs ATG	AXG vs ATG
MDA*	2.28 ±0.13	5.53 ±0.23	2.64 ±0.20	517.270	0.001	0.001	0.014	0.001
tGSH*	8.58 ±0.28	3.41 ±0.21	7.63 ±0.18	898.772	0.002	0.001	0.001	0.001
TOS**	15.5 (14.8–17.8)	37.5 (35.3–39.3)	19.0 (16.0–25.3)	11.996	0.001	0.002	0.324	0.192
TAS*	22.83 ±2.48	10.95 ±1.32	19.83 ±1.60	65.663	0.001	0.001	0.035	0.001

Q – quartile; HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; MDA – malondialdehyde; tGSH – total glutathione; TOS – total oxidant status; TAS – total antioxidant status; KW – Kruskal–Wallis test. As the post hoc test, *Tukey's honestly significant difference (HSD) test was performed after ANOVA (F (2,15)); **Kruskal–Wallis test was used and Dunn's test was performed as post hoc test.

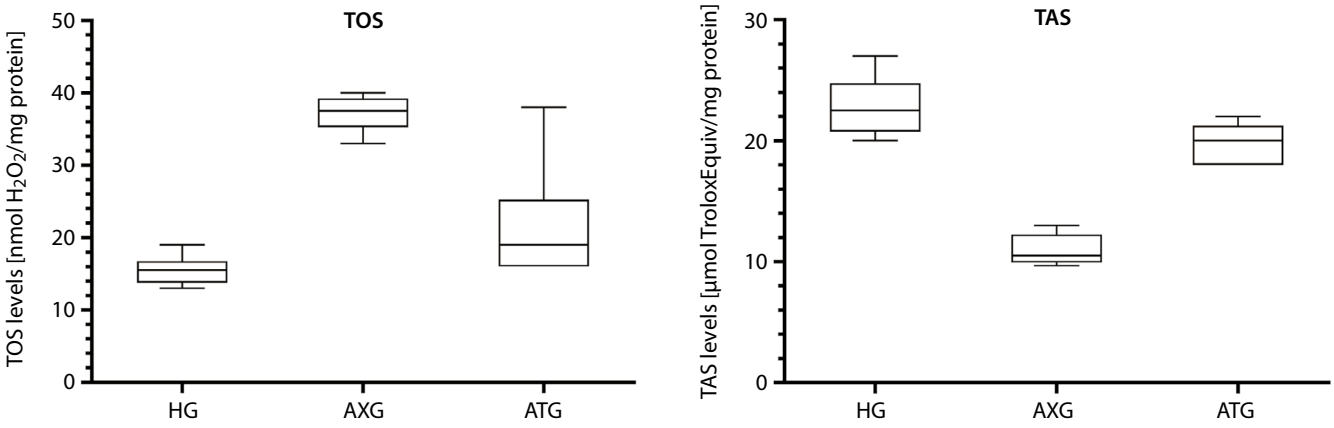

Fig. 3. TOS and TAS levels in the sciatic nerve tissue of the study groups

Q – quartile; TOS – total oxidant status; TAS – total antioxidant status; HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; horizontal line – median; bottom line of the box – Q1 (25^{th}); topline of the box – Q3 (75^{th}); whiskers – minimum and maximum observation.

myelin sheath that surrounded axons lost its central position. Schwann cells showed hypertrophy and hyperplasia. Locally, myelin sheath degeneration, disorganization and loss were detected. Blood capillaries were dilated and congested (Fig. 4B). When the AXG was compared with the HG, there was a statistically significant difference in terms of myelinated axon degeneration, Schwann cell degeneration and congestion ($p < 0.001$). In rats treated with ATG, myelinated nerve fibers were generally normal in sight and axons were located centrally. Schwann cells were normal in shape, degeneration of myelin sheaths decreased and blood capillaries were also normal (Fig. 4C). When the ATG was compared with the AXG, there was a statistically significant difference in terms of myelinated axon degeneration, Schwann cell degeneration and congestion ($p < 0.001$). There was no significant difference between HG and ATG in terms of congestion ($p = 0.217$).

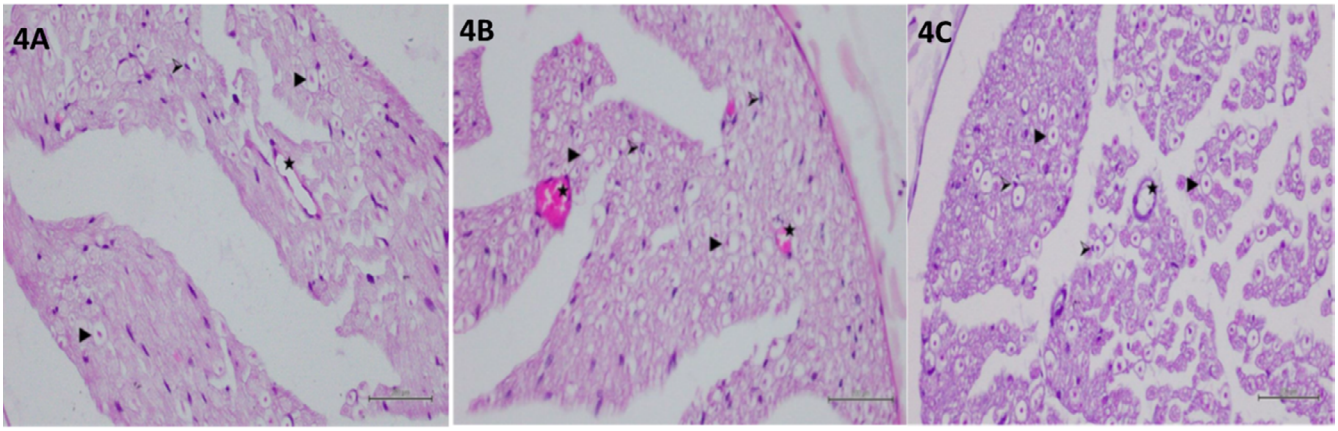

Fig. 4. Histopathological appearance of sciatic nerve tissue in the study groups. A. Hematoxylin and eosin (H&E) staining in sciatic nerve tissue in the healthy group; ▶ – myelinated axon; ➢ – Schwann cell nucleus; ★ – normal blood capillary; ×400 magnification; B. H&E staining in sciatic nerve tissue in the alloxan group; ▶ – swollen and pale degenerative myelinated axon; ➢ – hypertrophic and hyperplastic Schwann cell nucleus; ★ – dilated and congested blood capillary; ×400 magnification; C. H&E staining in sciatic nerve tissue in the alloxan+taxifolin group; ▶ – myelinated axon; ➢ – normal Schwann cell nucleus; ★ – normal blood capillary; ×400 magnification

Table 5. Kruskal–Wallis test results and post hoc p-values for group comparisons in histopathological evaluation

Histopathological change	Median (Q_1–Q_3)			KW results		Post hoc test p-values		
	HG	AXG	ATG	KW	p-value	HG vs AXG	HG vs ATG	AXG vs ATG
Myelinated axon degeneration*	0(0–0)	3(2–3)	1(0–1)	88.954	0.001	0.001	0.009	0.001
Schwann cell degeneration*	0(0–0)	3(2–3)	1(0–1)	90.129	0.001	0.001	0.010	0.001
Congestion*	0(0–0)	2(2–3)	0(0–1)	90.483	0.001	0.001	0.217	0.001

Q – quartile; HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; KW – Kruskal–Wallis test; *Kruskal–Wallis test was used and Dunn's test was performed as post hoc test.

Discussion

The effect of taxifolin on alloxan-induced hyperglycemia-related neuropathy and neuropathic pain in rats was investigated in this study. Hyperglycemia in rats was performed by applying the method utilized before.[25] Pain is an important manifestation of hyperglycemia-associated neuropathy in animal models and patients with DM.[6] The paw pain threshold of the animals in the AXG, whose glucose concentration in plasma was above 250 mg/dL for 3 months, was observed to be significantly lower compared to the HG and ATG. The reason why we chose the paw of neuropathic pain in animals for the evaluation is because the first symptoms of neuropathy are observed in this area.[26] It was determined that neuropathy developing in diabetic rats caused a significant hyperalgesia in previous studies.[14] Najafi et al. demonstrated the role of LPO in the pathogenesis of diabetic neuropathy.[27] Moustafa et al. reported that the amount of MDA as the last product of LPO increased significantly in the sciatic nerve tissue of rats with DM.[11] In the study of Ince et al., it was reported that the increase in the amount of MDA is directly proportional to the decrease in the pain threshold.[28] In another study, it was shown that MDA has a role in the pathogenesis of nondiabetic neuropathic pain.[29] The literature supports the finding that the MDA value was higher in the AXG compared to the HG and ATG, and the pain threshold was lower.

The role of tGSH in the pathogenesis of neuropathic pain was also investigated in our study. Glutathione is an endogenous antioxidant molecule. It has very important roles in protecting cells from oxidative damage and maintaining redox homeostasis.[30] As it is commonly known, GSH is a tripeptide which contains glutamate, cysteine and glycine. Decreased GSH level triggers the development of neurodegenerative illnesses.[31] It has been reported that the content of GSH and other enzymatic antioxidants decreases significantly in alloxan-induced diabetic pain.[32] The tGSH amount and the paw pain threshold of the AXG was lower compared to the HG and ATG. The TOS and TAS levels were measured to evaluate the relationship between oxidative stress and neuropathic pain in the sciatic nerve tissue in more detail. The TOS and TAS reflect the total effects of all oxidants and antioxidants in tissues.[23,24] The low paw pain threshold in the AXG with high TOS levels and low TAS levels confirm the fact that our experimental results are compatible with the literature information.

Taxifolin significantly decreased the glucose concentration in plasma level increased by alloxan in our study. These findings are in line with the literature. Taxifolin has been shown to decrease carbohydrate absorption by inactivating some of the enzymes involved in carbohydrate metabolism.[33] Rehman et al. revealed that taxifolin inhibits α-amylase enzyme in diabetic animal models and prevents postprandial hyperglycemia.[34] Taxifolin, which suppresses the rise of glucose concentration in plasma caused by alloxan administration, also prevented the decrease of paw pain threshold of rats. No studies investigating the effect of taxifolin on diabetic neuropathy with pain were found in the literature. However, various flavonoids have been reported to alleviate the peripheral neuropathic pain state in different animal species.[35] As it is known, patients with diabetic neuropathy experience burning in their feet or hands in addition to various forms of pain.[35,36]

As stated above, the relation has been determined between diabetic neuropathic pain and oxidant–antioxidant levels.[13] The fact that taxifolin prevented the production of MDA, a product of LPO, from increasing with alloxan, and the decrease of GSH in the sciatic nerve tissue suggests that taxifolin provides a treatment for the pathogenesis of diabetic neuropathic pain. In the experiment, the results of which are in line with our study, an increase of the LPO as well as a decrease of the GSH and other nonenzymatic antioxidants in the diabetic neuropathic pain have been revealed.[18]

In our study, sciatic nerves were examined histopathologically for hyperglycemic neuropathy. The sciatic nerve is widely used in the evaluation of diabetic neuropathy.[32] In addition, the most common site of diabetic neuropathy is peripheral nerve tissues in the lower extremity.[3] As can be seen from our experimental results, taxifolin attenuated the development of myelinated nerve fibers damage (swelling, myelin sheath degeneration, disorganization and loss), hypertrophy and hyperplasia in Schwann cells, vasodilation and congestion. Recent studies have documented degeneration and the presence of demyelinating fibers in the sciatic nerves of diabetic animals.[37] Again, there are studies showing irregular myelin structure and sheath detachment in diabetic rats.[38] It has been reported that various histopathological symptoms such as atrophy of sciatic nerve axons, edema, myelin damage, and loss of myelin develop in diabetic neuropathy.[39] The change in the oxidant–antioxidant balance in the nervous tissue in favor of oxidants indicates that oxidative stress is an important factor in the pathogenesis of diabetic neuropathy.[37] This shows that all our experimental results obtained with taxifolin are in agreement with the literature.

Limitations

Our study has some limitations. In order to clarify the pathogenesis of the treatment of hyperglycemia-related neuropathy and neuropathic pain with taxifolin, proinflammatory cytokine levels, as well as enzymatic antioxidant activity levels such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase, should be investigated.

Conclusions

Alloxan-induced hyperglycemia significantly lowered the paw pain threshold of animals. Hyperglycemia caused oxidative stress in paw tissue. It has been shown

histopathologically that neuropathy develops in the sciatic nerve tissue. Taxifolin prevented the reduction of alloxan-induced hyperglycemia-related paw pain threshold, and the oxidant–antioxidant balance in the sciatic nerve tissue changed in favor of oxidants. Furthermore, taxifolin alleviated the morphological disorders developing in the sciatic nerve. Our experimental results indicate that taxifolin may be useful in the treatment of hyperglycemia-associated neuropathy and neuropathic pain. To clarify the mechanism of treatment with taxifolin, more detailed studies, such as investigations of the proinflammatory cytokines and enzymatic antioxidant activity levels (catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase), may be useful in providing a treatment for the etiopathogenesis of neuropathic pain in the future.

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References

1. Gugliandolo E, D'Amico R, Cordaro M, et al. Effect of PEA-OXA on neuropathic pain and functional recovery after sciatic nerve crush. *J Neuroinflammation*. 2018;15(1):264. doi:10.1186/s12974-018-1303-5
2. Nicodemus JM, Enriquez C, Marquez A, Anaya CJ, Jolivalt CG. Murine model and mechanisms of treatment-induced painful diabetic neuropathy. *Neuroscience*. 2017;354:136–145. doi:10.1016/j.neuroscience.2017.04.036
3. Marshall SM, Flyvbjerg A. Prevention and early detection of vascular complications of diabetes. *BMJ*. 2006;333(7566):475–480. doi:10.1136/bmj.38922.650521.80
4. Ward JD, Barnes CG, Fisher DJ, Jessop JD, Baker RW. Improvement in nerve conduction following treatment in newly diagnosed diabetics. *Lancet*. 1971;1(7696):428–430. doi:10.1016/s0140-6736(71)92415-9
5. Marfella R, Verrazzo G, Acampora R, et al. Glutathione reverses systemic hemodynamic changes induced by acute hyperglycemia in healthy subjects. *Am J Physiol*. 1995;268(6 Pt 1):E1167–E1173. doi:10.1152/ajpendo.1995.268.6.E1167
6. Thye-Rønn P, Sindrup SH, Arendt-Nielsen L, Brennum J, Hother-Nielsen O, Beck-Nielsen H. Effect of short-term hyperglycemia per se on nociceptive and non-nociceptive thresholds. *Pain*. 1994;56(1):43–49. doi:10.1016/0304-3959(94)90148-1
7. Wentholt IM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. *Diabetologia*. 2008;51(1):183–190. doi:10.1007/s00125-007-0842-6
8. Gadjeva VG, Goycheva P, Nikolova G, Zheleva A. Influence of glycemic control on some real-time biomarkers of free radical formation in type 2 diabetic patients: An EPR study. *Adv Clin Exp Med*. 2017;26(8):1237–1243. doi:10.17219/acem/68988
9. Tosun M, Olmez H, Unver E, et al. Oxidative and pro-inflammatory lung injury induced by desflurane inhalation in rats and the protective effect of rutin. *Adv Clin Exp Med*. 2021;30(9):941–948. doi:10.17219/acem/136194
10. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc Diabetol*. 2005;4:5. doi:10.1186/1475-2840-4-5
11. Moustafa PE, Abdelkader NF, El Awdan SA, El-Shabrawy OA, Zaki HF. Liraglutide ameliorated peripheral neuropathy in diabetic rats: Involvement of oxidative stress, inflammation and extracellular matrix remodeling. *J Neurochem*. 2018;146(2):173–185. doi:10.1111/jnc.14336
12. Galeshkalami NS, Abdollahi M, Najafi R, et al. Alpha-lipoic acid and coenzyme Q10 combination ameliorates experimental diabetic neuropathy by modulating oxidative stress and apoptosis. *Life Sci*. 2019;216:101–110. doi:10.1016/j.lfs.2018.10.055
13. Solanki ND, Bhavsar SK. An evaluation of the protective role of *Ficus racemosa* Linn. in streptozotocin-induced diabetic neuropathy with neurodegeneration. *Indian J Pharmacol*. 2015;47(6):610–615. doi:10.4103/0253-7613.169579

Supplementary Table 1. Normality assumption evaluated using The Shapiro–Wilk test

Measurement	Group	Shapiro–Wilk		
		statistic	df	Sig.
Before alloxan	HG	0.874	6	0.245
	AXG	0.979	6	0.945
	ATG	0.899	6	0.368
3rd month after alloxan	HG	0.894	6	0.339
	AXG	0.944	6	0.692
	ATG	0.899	6	0.366
The difference	HG	0.915	6	0.473
	AXG	0.956	6	0.789
	ATG	0.970	6	0.890

The blood glucose measurements in the groups before alloxan, 3 months after alloxan and the difference values showed normal distribution. Analysis of variance (ANOVA) was performed. HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; df – degrees of freedom.

Supplementary Table 2. Homogeneity of variances assumption

Measurement	Levene's statistic	df1	df2	Sig.
Before alloxan	0.423	2	15	0.662
3rd month after alloxan	0.239	2	15	0.790
The difference	2.215	2	15	0.144

Tukey's honestly significant difference (HSD) test was applied post hoc as the homogeneity of variances before alloxan and 3 months after alloxan, and the difference values were met.

Supplementary Table 3. Normality assumption evaluated using Shapiro–Wilk test

Measurement	Group	Shapiro–Wilk		
		statistic	df	Sig.
1st h	HG	0.890	6	0.317
	AXG	0.960	6	0.820
	ATG	0.892	6	0.331
2nd h	HG	0.963	6	0.841
	AXG	0.866	6	0.212
	ATG	0.920	6	0.503
3rd h	HG	0.958	6	0.804
	AXG	0.853	6	0.167
	ATG	0.898	6	0.362

The 1st, 2nd and 3rd h values were normally distributed in groups. Analysis of variance (ANOVA) was performed. HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; df – degrees of freedom.

Supplementary Table 4. Homogeneity of variances assumption evaluated for 1st, 2nd and 3rd h values

Measurement	Levene's statistic	df1	df2	Sig.
1st h	1.947	2	15	0.177
2nd h	4.677	2	15	0.026
3rd h	4.643	2	15	0.027

Tukey's honestly significant difference (HSD) test was applied post hoc as the homogeneity of variances for 1st h values were met. For 2nd h and 3rd h values, homogeneity of variances assumption was not met; therefore, the Games–Howell test was applied as post hoc test.

Supplementary Table 5. Normality assumption evaluated using Shapiro–Wilk test

Parameter	Group	Shapiro–Wilk		
		statistic	df	Sig.
MDA	HG	0.859	6	0.186
	AXG	0.890	6	0.320
	ATG	0.879	6	0.265
tGSH	HG	0.976	6	0.931
	AXG	0.928	6	0.563
	ATG	0.972	6	0.907
TOS	HG	0.945	6	0700
	AXG	0.957	6	0.794
	ATG	0.700	6	0.006
TAS	HG	0.957	6	0.794
	AXG	0.885	6	0.295
	ATG	0.908	6	0.425

Malondialdehyde (MDA), total glutathione (tGSH) and total antioxidant status (TAS) were normally distributed in groups. Analysis of variance (ANOVA) was performed. Total oxidant status (TOS) did not met normality assumption; therefore, the Kruskal-Wallis test was chosen. HG – healthy group; AXG – alloxan group; ATG – alloxan+taxifolin group; df – degrees of freedom.

Supplementary Table 6. Homogeneity of variances assumption was evaluated for malondialdehyde (MDA), total glutathione (tGSH) and total antioxidant status (TAS)

Parameter	Levene's statistic	df1	df2	Sig.
MDA	0.877	2	15	0.436
tGSH	0.533	2	15	0.597
TAS	0.919	2	15	0.420

Tukey's honestly significant difference (HSD) test was applied post hoc as the homogeneity of variances for MDA, tGSH and TAS were met.

14. Kishore L, Kaur N, Singh R. Effect of kaempferol isolated from seeds of *Eruca sativa* on changes of pain sensitivity in streptozotocin-induced diabetic neuropathy. *Inflammopharmacology*. 2018;26(4):993–1003. doi:10.1007/s10787-017-0416-2
15. Peritore AF, Siracusa R, Fusco R, et al. Ultramicronized palmitoylethanolamide and paracetamol, a new association to relieve hyperalgesia and pain in a sciatic nerve injury model in rat. *Int J Mol Sci*. 2020;21(10):3509. doi:10.3390/ijms21103509
16. D'Amico R, Impellizzeri D, Cuzzocrea S, Di Paola R. ALIAmides Update: Palmitoylethanolamide and its formulations on management of peripheral neuropathic pain. *Int J Mol Sci*. 2020;21(15):5330. doi:10.3390/ijms21155330
17. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med*. 1996;20(7):933–956. doi:10.1016/0891-5849(95)02227-9
18. Cai C, Liu C, Zhao L, et al. Effects of taxifolin on osteoclastogenesis in vitro and in vivo. *Front Pharmacol*. 2018;9:1286. doi:10.3389/fphar.2018.01286
19. Jaouhari JT, Lazrek HB, Jana M. The hypoglycemic activity of *Zygophyllum gaetulum* extracts in alloxan-induced hyperglycemic rats. *J Ethnopharmacol*. 2000;69(1):17–20. doi:10.1016/s0378-8741(99)00064-1
20. Cadirci E, Suleyman H, Hacimuftuoglu A, Halici Z, Akcay F. Indirect role of beta2-adrenergic receptors in the mechanism of analgesic action of nonsteroidal antiinflammatory drugs. *Crit Care Med*. 2010;38(9):1860–1867. doi:10.1097/CCM.0b013e3181e8ae24
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351–358. doi:10.1016/0003-2697(79)90738-3
22. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem*. 1968;25(1):192–205. doi:10.1016/0003-2697(68)90092-4
23. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem*. 2005;38(12):1103–1111. doi:10.1016/j.clinbiochem.2005.08.008
24. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem*. 2004;37(2):112–119. doi:10.1016/j.clinbiochem.2003.10.014
25. Icel E, Icel A, Uçak T, et al. The effects of lycopene on alloxan induced diabetic optic neuropathy. *Cutan Ocul Toxicol*. 2019;38(1):88–92. doi:10.1080/15569527.2018.1530258
26. Perry MC. *The Chemotherapy Source Book*. 4th ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2008.
27. Najafi R, Hosseini A, Ghaznavi H, Mehrzadi S, Sharifi AM. Neuroprotective effect of cerium oxide nanoparticles in a rat model of experimental diabetic neuropathy. *Brain Res Bull*. 2017;131:117–122. doi:10.1016/j.brainresbull.2017.03.013
28. Ince I, Aksoy M, Ahiskalioglu A, et al. A comparative investigation of the analgesic effects of metamizole and paracetamol in rats. *J Invest Surg*. 2015;28(3):173–180. doi:10.3109/08941939.2014.998798
29. Kuyrukluyıldız U, Küpeli İ, Bedir Z, et al. The effect of anakinra on paclitaxel-induced peripheral neuropathic pain in rats. *Turk J Anaesthesiol Reanim*. 2016;44(6):287–294. doi:10.5152/tjar.2016.02212
30. Forman HJ, Zhang H, Rinna A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med*. 2009;30(1–2):1–12. doi:10.1016/j.mam.2008.08.006
31. Matsumura N, Kinoshita C, Aoyama K. Mechanism of glutathione production in neurons. *Nihon Yakurigaku Zasshi*. 2021;156(1):26–30. doi:10.1254/fpj.20068
32. Yi Z, Shao-Long Y, Ai-Hong W, et al. Protective effect of ethanol extracts of *Hericium erinaceus* on alloxan-induced diabetic neuropathic pain in rats. *Evid Based Complement Alternat Med*. 2015;2015:595480. doi:10.1155/2015/595480
33. Yoon KD, Lee JY, Kim TY, et al. In vitro and in vivo anti-hyperglycemic activities of taxifolin and its derivatives isolated from pigmented rice (*Oryzae sativa* L. cv. Superhongmi). *J Agric Food Chem*. 2020;68(3):742–750. doi:10.1021/acs.jafc.9b04962
34. Rehman K, Chohan TA, Waheed I, Gilani Z, Akash MSH. Taxifolin prevents postprandial hyperglycemia by regulating the activity of α-amylase: Evidence from an in vivo and in silico studies. *J Cell Biochem*. 2019;120(1):425–438. doi:10.1002/jcb.27398
35. Basu P, Basu A. In vitro and in vivo effects of flavonoids on peripheral neuropathic pain. *Molecules*. 2020;25(5):1171. doi:10.3390/molecules25051171
36. Kaur S, Pandhi P, Dutta P. Painful diabetic neuropathy: An update. *Ann Neurosci*. 2011;18(4):168–175. doi:10.5214/ans.0972-7531.1118409
37. Ostovar M, Akbari A, Anbardar MH, et al. Effects of *Citrullus colocynthis* L. in a rat model of diabetic neuropathy. *J Integr Med*. 2020;18(1):59–67. doi:10.1016/j.joim.2019.12.002
38. Ling Q, Liu M, Wu MX, et al. Anti-allodynic and neuroprotective effects of koumine, a Benth alkaloid, in a rat model of diabetic neuropathy. *Biol Pharm Bull*. 2014;37(5):858–864. doi:10.1248/bpb.b13-00843
39. Huang Y, Hu B, Zhu J. Study on the use of quantitative ultrasound evaluation of diabetic neuropathy in the rat sciatic nerve. *Australas Phys Eng Sci Med*. 2016;39(4):997–1005. doi:10.1007/s13246-016-0448-8