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Original Article

The effect of lutein on paclitaxel-induced neuropathy and neuropathic pain in rats

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Abstract: Background: Paclitaxel is a drug used to treat cancer, but it causes neuropathic pain and peripheral neuropathy. One of the causes of these effects of paclitaxel on nerve tissue is oxidative damage. Lutein has antioxidant effects. In this study, it was investigated the influence of lutein whether decrease oxidative damage of paclitaxel on nerve tissue or not. Methods: Pain threshold (PT) in paw of paclitaxel-only group (PC), paclitaxel+lutein group (PL), and control group (CG) rats were measured using a Basile algesimeter. The sciatic nerve tissue of experimental animals was used to conduct biochemical analyses. Analyses of malondialdehyde (MAD), total glutathione (GT), myeloperoxidase (MP), interleukin 1 beta (IL-1β) were performed. Histopathologic examination was performed in all groups. The CG and the other groups were compared. Results: PT was lowest in the PC group. However considerable increase in pain threshold in paw was observed in the PL group with respect to the PC group. As a result, the usage of lutein increased PT in paw. The PC group had signs of oxidative damage. With the use of lutein, paclitaxel was prevented from causing oxidative damage. Histopathological examination revealed that paclitaxel caused damage to myelin sheath and axon of sciatic nerve tissue. However, lutein was found to prevent this damage to the myelin sheath and axon. Paclitaxel causes neuropathic pain and peripheral neuropathy. One of the causes is the oxidative damage of paclitaxel in nerve tissue. Conclusion: Lutein, which has an antioxidant effect, prevents the damage of paclitaxel on nerve tissue.

Keywords: Paclitaxel, lutein, nerve tissue, antioxidants

Introduction

Paclitaxel is a natural anticancer drug obtained from the bark of *Taxus brevifolia* trees [1]. It is commonly used In the treatment of many cancers [2]. Paclitaxel has been shown to be more effective against these types of cancer at high doses [3]. However, peripheral neuropathy develops at high doses [4], causing pain and sensory loss in patients, and adversely affecting the quality of their life [5]. These side effects can even lead to discontinuation of treatment [6]. To decrease neuropathic pain, antidepressants, anti-histamines, anti-convulsants, non-steroidal anti-inflammatory drugs, and opioids are used. Yet, these drugs are only partially effective and the desired results are often not achieved [7]. Many studies showed that neuropathic pain may persist for up to one year after cessation of medication [8, 9].

It has been thought that oxidative stress is one of the causes of paclitaxel-induced neuropathic pain [10]. In many studies, the cause of neuropathic pain is related to nerve damage because of increasing levels of reactive oxygen species (ROS) [11]. In one study, paclitaxel-treated rats experienced a decrease in pain threshold. It was determined that malondialdehyde (MAD) and myeloperoxidase (MP) levels were increased and total endogenous glutathione (GT) levels decreased in the paw tissue of experimental animals [12]. In another study, paclitaxel was reported to cause neuropathic pain by creating oxidative stress and increasing proinflammatory interleukin-1 beta (IL-1β) production [13]. These reports show that anti-inflammatory and antioxidant therapy is effective in preventing the development of neuropathy and neuropathic pain caused by the use of paclitaxel. In the present study, lutein ($C_{40}H_{56}O_2$), a tetraterpe-

noid [14], was investigated with regards to its effect on paclitaxel-induced neuropathic pain. Lutein has been reported to provide antioxidant activity by inhibiting lipid peroxidation and preventing the reduction of GT levels [15]. To date, no studies investigating the influence of lutein on paclitaxel-induced neuropathy and neuropathic pain have been conducted. For this reason, in this research the influence of lutein on paclitaxel-induced peripheral neuropathic pain and its effect on sciatic neuropathy via histological and biochemical analyses were investigated.

Materials and methods

Before experimental study, the ethic permission was taken from related institution of the Atatürk University (The Ethical Committee of Ataturk University, Number: 1800314836, Dated: 01.11.2018). In addition, same university's Guidelines for the Use and Care of Laboratory were adopted as a standard to perform live animal experiments.

Experimental animals

In the present study, 30 albino Wistar male rats (weights between 285-297 g) were used in the experiment. Experimental animals were supplied by Medical Experimental Application and Research Center of Atatürk University. All rats in groups were kept in the Laboratory of Pharmacology under standard convenient conditions.

Chemicals

In the experiments, the following reagents such as paclitaxel, sodium thiopental and lutein were provided by Actavis (Istanbul, Turkey), IE Ulagay (Istanbul, Turkey) and Solgar (Leonia, USA), respectively.

Experimental groups

The rats were grouped as follows: a paclitaxel-only group (PC), a paclitaxel+lutein group (PL), and a control group that received saline vehicle (CG).

Paclitaxel-induced pain model

In the course of the experiment, the PC (n=10) and PL (n=10) groups were administered a single dose of paclitaxel (2 mg/kg) intraperitoneally (i.p) at two-day intervals for a total of four doses [16]. Prior to the administration of paclitaxel, PL animals were administered lutein by oral gavage at a dose of 1 mg/kg once daily for eight days. CG animals were administered an equivalent volume of normal saline (0.9% NaCl) vehicle. PT (gram) of PC, PL, and CG rats were measured using a Basile algesimeter one hour after the last dose of lutein and saline [17]. Immediately after measurement, rats were euthanized with high-dose sodium thiopental anesthesia and sciatic nerve tissues were extracted. MAD, MP, GT, and IL-1β levels were measured from extracted nerve tissues. The excised nerve tissue was examined histopathologically. Attained results from the experiment were compared with the CG group.

Biochemical analyses

MAD analysis: For MAD (μmol/g protein) measurement, we used the technique described previously by Ohkawa et al [18]. According to this technique, the absorbance (at 532 nm) of the pink complex formed by MAD with thiobarbituric acid (TBA) at a high temperature (95°C) was measured spectrophotometrically. The homogenates were centrifuged at 5000 g for 20 minutes and the supernatants were used for MAD quantification. Specifically, 250 μL of homogenate, 100 μL of 8% sodium dodecyl sulfate (SDS), 750 μL of 20% acetic acid, 750 μL of 0.08% TBA, and 150 μL of purified water were vortexed in capped test tubes. The mixture was allowed to incubate at 100°C for 60 min, after which 2.5 mL of n-butanol was added and spectrophotometric measurements were performed. The absorbance of the resulting red solutions was read at 532 nm using 3 mL cuvettes and the MAD concentrations of the specimens were obtained by taking the dilution coefficients into account using a standard curve generated using the previously prepared MAD stock solution.

MP analysis: Potassium phosphate buffer (pH 6) containing 0.5% hekzadesil trimetil ammonium bromide (HDTMAB) was prepared for MP (unit/g protein) tissue homogenates. Then, it was centrifuged for 15 min at 10000 rpm at 4°C. The supernatant was used for analysis. For the determination of MP activity, an oxidation reaction was performed with MP-mediated

H_2O_2 oxidation using 4-amino antipyrine/phenol solution as the substrate [19].

GT analysis: The amount of GT (nmol/g protein) in the prepared samples was evaluated in compliance with modified technique used by Sedlak and Lindsay [20]. First of all, prepared specimens were weighted and homogenized in the solution compose of 2 mL of 50 mmol/L Tris-HCl buffer containing 20 mmol/L EDTA (ethylene diamine tetra acetic acid) and 0.2 mmol/L sucrose at pH 7.5. 0.1 ml of 25% trichloroacetic acid was added to the homogenate and centrifuged at 4200 rpm for 40 min at 4°C, and then the resulting precipitate was removed. GT level was determined using the supernatant. A total of 1500 µL of measurement buffer (200 mmol/L Tris-HCl buffer containing 0.2 mmol/L EDTA at pH 7.5), 500 µL supernatant, 100 µL 5,5-Dithiobis (2-nitrobenzoic acid) (DTNB) (10 mmol/L) and 7900 µL methanol were added to a tube and vortexed and incubated for 30 min at 37°C. DTNB has created a yellow complex by means of the sulfhydryl groups it contains and has enabled it to be used as a chromogen. Spectrophotometer (412 nm) was used to measure the absorbance (Beckman DU 500, USA). The stock solution of GT was used to obtain the standard curve.

IL-1β analysis: A rat-specific sandwich ELISA (enzyme-linked immunosorbent assay) kit was used to measure tissue-homogenate IL-1β (picogram/ml) concentrations (Cat no: YHB06-16Ra, Shanghai LZ). The analysis was made taking into account the manufacturers' suggestions. The solution containing antibodies specific for rat IL-1β was added to the microplates and then pipetted by the tissue homogenate, standards, biotinylated monoclonal antibody and streptavidin-horseradish peroxidase (HRP)-conjugated antibodies, incubated at 37°C for 60 min. After washing, chromogen reagents A and B were added, producing a colored production with the HRP enzyme. In order to attain reaction, the plate was incubated at 37°C for 10 min and then the stop solution was added. The intensity of this colored product has direct relation with the concentration of rat IL-1β present in the original specimen. A microplate reader (450 nm) was used to read the plates (Bio-Tec, USA). The absorbance of the samples was estimated with formulas that used standard curves generated from the standards supplied by the manufacturer.

Histopathologic examination

To perform light microscopic observation, prepared specimens were fixed in a 10% formaldehyde solution. Subsequently, tissue specimens were washed with water for 24 h. Alcohol (70%, 80%, 90%, and 100%) was used to remove the water inside the tissues. Tissues were then passed through xylol and embedded in paraffin.

The paraffin blocks were sectioned as 4-5 µm thickness subsequently stained with hematoxylin-eosin staining. The Olympus DP2-SAL firmware program was used to attain images of the sections (Olympus® Inc., Tokyo, Japan). Histopathological examination was conducted by a pathologist blinded to the study groups.

Statistical analysis

Statistical analysis was performed using the IBM SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). For continuous variables as MAD, MP, GT and IL-1β results were presented as mean ± standard deviation. The normality of distribution for continuous variables was confirmed with the Shapiro-Wilk normality test. While comparing IL-1β, MAD and GT between groups Kruskal-Wallis test was used and Dunn's test performed as a post-hoc. For MP and Paw pain threshold which were normally distributed, ANOVA was used for comparison. Homogeneity of variances was confirmed with Levene's test. For MP the homogeneity assumption was provided, Tukey's HSD was used as a post-hoc test after ANOVA. While comparing Paw pain threshold between groups Games-Howell test was used as a post-hoc test. A *p*-value <0.05 was considered statistically significant for all tests.

Results

PT were evaluated in the study groups and the pain threshold was lower in the PC compared to CG (P<0.001). The PL group had a significantly higher pain threshold than the PC group (P<0.001) (**Figure 1**).

In terms of biochemical results, MAD, MP, IL-1β, GT were compared among the groups. When PC group was compared with CG group, MAD, MP, IL-1β levels were higher and GT level was considerably lower (P<0.001, P<0.001, p<0.001, P<0.001, respectively). It was not obtained st-

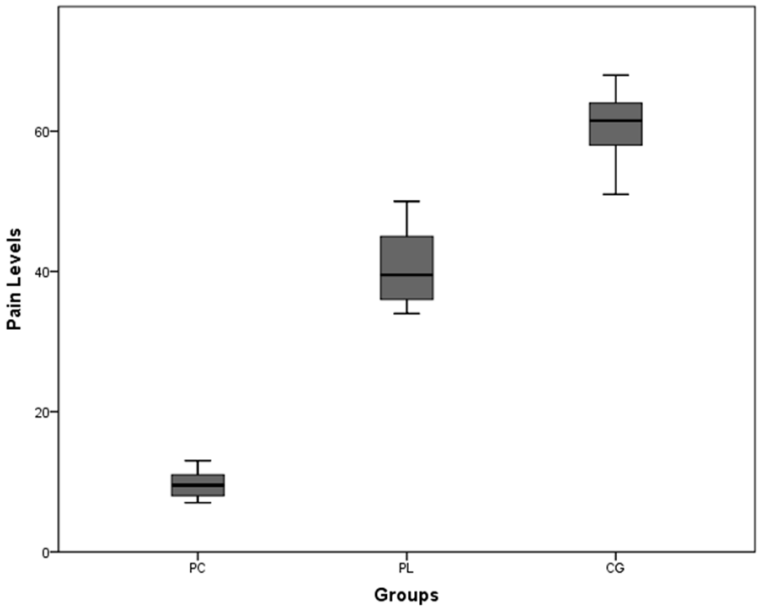

Figure 1. Paw pain threshold values between groups.

Table 1. Biochemical results in study groups

	PC	PL	CG	p
MAD	77.2±5.4[a]	29.3±4.8[b]	22.6±3.3	<0.001*
MP	30.1±4.3[a]	13.4±2.7[b]	10.1±1.5	<0.001+
GT	11.9±1.9[a]	27.7±3.4[b]	33.6±4.1	<0.001*
IL-1β	8.2±0.7[a]	3.4±0.5[b]	3.1±0.5	<0.001*

Variables were shown as mean ± standard deviation. *Kruskal Wallis test or +ANOVA was used. Statistically significant (P<0.05) when compared with a: CG group, b: PC group.

atistically significant difference between PL and CG (P=0.170, P=0.055, P=0.891, P=0.179, respectively). When PC group was compared with PL group, MAD, MP, IL-1β levels were higher and GT level was considerably lower (P=0.013, P=0.010, P=0.003, P=0.012, respectively) (**Table 1**).

In the CG, histological examination of the sciatic nerve revealed that the nerve structure was normal. Axons were located centrally and surrounded by myelin sheaths and Schwann cell nucleuses were normal in shape (**Figure 2A**). In the PC group, myelinated nerve fibers were swollen, myelin sheath surrounded axons lost their central position, and Schwann cells were hypertrophic and hyperplastic. Locally, myelin sheath degeneration and loss were detected; blood vessels were also dilated and congested (**Figure 2B**, **2C**). In PL rats, myelinated nerve fibers were partially swollen but generally normal in appearance and axons were located centrally. Schwann cells had normal morphology, the degeneration of myelin sheaths was decreased, and blood vessels were also normal (**Figure 2D**).

Discussion

Paclitaxel is anticancer drug that is effective during cell division by inhibiting mitosis. It exerts effects on microtubules which are necessary for the transport of chromosomes during cell division [21]. Unfortunately, paclitaxel also causes neurodegeneration of peripheral nerve endings, leading to the development of painful neuropathy. Paclitaxel causes an increase in intracellular ROS production, which has a toxic effect on the microtubules within the cell and causes the release of proinflammatory cytokines. Therefore, the equilibrium between oxidation and antioxidant mechanisms in the cell is disrupted. Increased ROS also causes calcium accumulation in the cell, which opens the pores of the mitochondria and causes edema, resulting in disrupted mitochondrial function. This, in turn, leads to mitochondria-induced neuroinflammation and neurodegeneration [22].

Lutein is found in green leafy vegetables and eggs, and is a fat-soluble carotenoid with strong antioxidant properties [23]. ROS can alter the expression of genes or proteins by affecting intracellular signaling pathways. Lutein has anti-inflammatory, anti-apoptotic, and antioxidant effects, reduces the effects of ROS, and prevents ROS formation [24].

In the present study, the PT of PC group rats decreased significantly. It was observed a significant increase in pain threshold in paw in the PL compared to the PC. Hyperalgesia occurs as a result of paclitaxel administration and manifests as a lower pain threshold [25], which we observed. In one study, it was found that lutein administration prevents the development of hyperalgesia [26], while others have reported

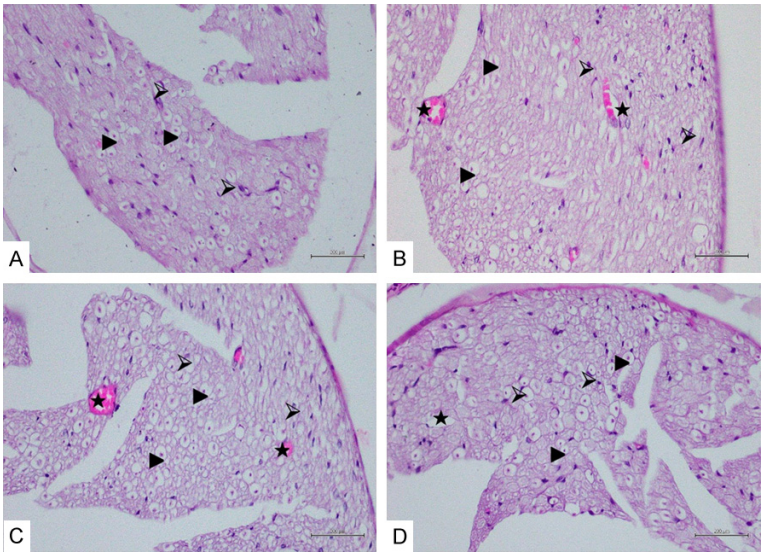

Figure 2. A. Hematoxylin-eosin staining in sciatic nerve tissue in the CG group. ►: myelinated axon, ➢: Schwann cell nucleus, ×400. B. Hematoxylin-eosin staining in sciatic nerve tissue in the PC group. ►: degenerated and swollen axon, ➢: hypertrophic and hyperplasic Schwann cell nucleus, ★: dilated and congested blood vessel, ×400. C. Hematoxylin-eosin staining in sciatic nerve tissue in the PC group. ►: demyelinated and swollen, ➢: hypertrophic and hyperplasic Schwann cell nucleus, ★: dilated and congested blood vessel, ×400. D. Hematoxylin-eosin staining in sciatic nerve tissue in the PL group. ►: myelinated axon, ➢: normal Schwann cell nucleus, ★: normal blood vessel, ×400.

lutein also increases the pain threshold [27]. In the present study, our findings are compatible with previous studies.

An increase in MAD levels was observed in experimental animals after paclitaxel administration. MAD occurs as a result of lipid peroxidation, which causes ROS formation. The level of MAD in tissues is one of the most useful indicators of oxidative stress [28]. We observed that the PL group experienced a significant decrease in MAD levels. In one study, it was observed that the MAD levels of experimental animals increased after paclitaxel administration [29]. In another study, MAD levels decreased after lutein administration. It was concluded that lutein reduced MAD levels and ROS production [30]. In the present study, MP levels increased significantly after paclitaxel administration, which was abrogated by lutein treatment. In one study, it was observed that MP levels increased after paclitaxel administration [22], which is important given that increased levels of MP indicate increased oxidative stress [31]. In another study, it was observed that lutein administration reduced MP levels and prevented oxidative stress [32].

In this study, attained results showed that IL-1β levels increased significantly in the PC group. Conversely, the IL-1β levels of the PL group were similar to the control group. Elevation of IL-1β increases oxidative stress [33], and this was observed after paclitaxel administration. Increased levels of IL-1β are involved in the formation of oxidative stress [34]. Lutein decreases ROS formation by reducing IL-1β levels [35].

In this study, attained results showed that GT levels decreased considerably in the PC. In the PL group, GT levels were similar to the control group. The measurement of total glutathione levels is a good method of quantifying oxidative stress. In one study, it was observed that glutathione levels decreased in paclitaxel-administrated animals [36]. In another study, total glutathione levels decreased and oxidative stress increased in paclitaxel-administrated animals [37]. It has also been observed that lutein increased total glutathione levels and reduced oxidative stress [38]. In another study, GT levels increased significantly in rats after lutein administration and this GT reduced oxidative stress [39].

In histopathological examinations, PC animals showed signs of edema, myelin sheath loss, and axon damage in myelinated nerve fibers. Lutein was found to reduce paclitaxel-induced damage on myelin sheaths and prevent axon damage. In previous studies, paclitaxel-administrated animals exhibited similar signs of axonal degeneration, myelin sheath demyelination, and destruction [40]. In one study, paclitaxel was found to cause demyelination and axonal degeneration [41]. In experimental studies, lutein has been shown to have protective effects on neurons [42]. In one study, lutein has been shown to prevent myelin damage and axonal degeneration [43].

Conclusion

After paclitaxel administration, neuropathic pain and a decrease in pain threshold were observed. Our biochemical analyses showed that paclitaxel increased ROS production and caused neuronal damage by creating oxidative stress. Histopathological examinations revealed that paclitaxel caused myelin sheath damage and axonal degeneration. It was observed that lutein administration protected nerves from oxidative stress caused by paclitaxel. From histopathologically analyses, we noted that paclitaxel-induced myelin and axonal damage were prevented by lutein administration. Ultimately, obtained results suggest that lutein may be a new option for preventing paclitaxel-induced neuropathy and neuropathic pain, a condition for which there are currently no effective treatments.

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Disclosure of conflict of interest

None.

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