Effect of thiamine pyrophosphate on oxidative damage to the oropharyngeal, nasal and cochlear tissues induced by doxorubicin in Guinea pigs

Article *in* LATIN AMERICAN JOURNAL OF PHARMACY · January 2017

CITATIONS
0

READS
136

8 authors, including:

Fatih Turan
Erzincan University
12 PUBLICATIONS **36** CITATIONS
SEE PROFILE

Suat Terzi
Recep Tayyip Erdoğan Üniversitesi
67 PUBLICATIONS **452** CITATIONS
SEE PROFILE

Metin Celiker
Rize Education and Training Hospital
59 PUBLICATIONS **258** CITATIONS
SEE PROFILE

Renad Mammadov
Erzincan University
63 PUBLICATIONS **281** CITATIONS
SEE PROFILE

Experimental Animals
JALAS
Japanese Association for Laboratory Animal Science

The Effect of Thiamine and its Metabolites on Peripheral Neuropathic Pain Induced by Cisplatin in Rats.

Journal:	*Experimental Animals*
Manuscript ID	EA-17-0090
Manuscript Type:	Original Paper
Date Submitted by the Author:	02-Aug-2017
Complete List of Authors:	Onk, Didem; Erzincan University, Faculty of Medicine, Department of Anesthesiology and Reanimation MAMMADOV, Renad; Department of Pharmacology, Faculty of Medicine, Erzincan University, Erzincan-Turkey Suleyman, Bahadir Keskin Cimen, Ferda; Department of Pathology, Mengucek Gazi Education and Research Hospital, Erzincan, Turkey. Cankaya, Murat; Department of Biology, Faculty of Arts and Sciences, Erzincan University, Erzincan- Turkey. Gul, Vahdet; , Department of Psychiatry ,Faculty of Medicine, Erzincan University Erzincan, Turkey. Altuner, Durdu; Pharmacology Senol, Onur; Department of Analytical Biochemisry, Faculty of Pharmacy, AtaturkUniversity, Erzurum, Turkey Kadioglu, Yucel; Department of Analytical Biochemisry, Faculty of Pharmacy, AtaturkUniversity, Erzurum, Turkey. Malkoc, Ismail; Department of Anatomy Faculty of Medicine, Ataturk University, Erzurum, Turkey. Süleyman, Halis; Erzincan University, Pharmacology
Keywords:	cisplatin, peripheral neuropathy, pain, rat, thiamine.
Category:	Pharmacology

Pharmacology

The Effect of Thiamine and its Metabolites on Peripheral Neuropathic Pain Induced by Cisplatin in Rats.

Running head: Effect of Thiamine on Peripheral Neuropathic Pain.

Didem Onk[1)], Renad Mammadov[2)], Bahadir Suleyman[2)], Ferda Keskin Cimen[3)], Murat Cankaya[4)], Vahdet Gul[5)], Durdu Altuner[2)], Onur Senol[6)], Yucel Kadioglu[6)], Ismail Malkoc[7)], Halis Suleyman[2)*].

Article type: Original paper.

Affiliations:

[1)]Department of Anesthesiology, Faculty of Medicine, Erzincan University, Erzincan, 24030 Turkey.

[2)]Department of Pharmacology, Faculty of Medicine, Erzincan University, Erzincan, 24030 Turkey.

[3)]Department of Pathology, Mengucek Gazi Education and Research Hospital, Erzincan, 24030 Turkey.

[4)]Department of Biology, Faculty of Arts and Sciences, Erzincan University, Erzincan, 24030 Turkey.

[5)]Department of Psychiatry ,Faculty of Medicine, Erzincan University, Erzincan, 24030 Turkey.

[6)]Department of Analytical Biochemisry, Faculty of Pharmacy, AtaturkUniversity, Erzurum, 25240 Turkey.

[7)] Department of Anatomy Faculty of Medicine, Ataturk University, Erzurum, 25240 Turkey.

[*]Corresponding author

Halis Suleyman, MD, PhD.

Department of Pharmacology, Faculty of Medicine, Erzincan University, 24030, Erzincan, Turkey. Cell Phone: +90 5309211909, E-mail: halis.suleyman@gmail.com

Pharmacology

Abstract

Thiamine pyrophosphate (TPP) is the active metabolite of thiamine. This study aimed to investigate the effects of thiamine and TPP on cisplatin-induced peripheral neuropathic pain (PNP) in rats. It also examines whether cisplatin-induced PNP is associated with blood serum TPP deficiency.

Animals were divided into groups (n=6) that received 2 mg/kg cisplatin (CIS), 2 mg/kg cisplatin+25 mg/kg thiamine (CTM), 2 mg/kg cisplatin+25 mg/kg TPP (CTPP) and distilled water administered healthy group (HG) intraperitoneally. Thiamine, TPP and distilled water were given once a day, and cisplatin was administered once every two days for 8 days, then were measured with Basile Algesimeter to evaluate analgesic activity. Blood samples were taken from the tail veins of the rats for determination of the pro-inflammatory interleukin 1Beta (IL-1Beta), malondialdehyde (MDA), total glutathione (tGSH), thiamine and TPP. Histopathological examinations were performed on removing sciatic nerves from animals.

Thiamine did not increase paw pain threshold suppressed by cisplatin, but TPP significantly increased. Increased production of IL-1Beta and MDA by cisplatin was inhibited by TPP, while not being inhibited by thiamine. Conversion of thiamine to TPP significantly decreased in the CIS group. Histopathological and biochemical investigations have demonstrated, hyperalgesia and sciatic nerve damage developed in the CIS and CTM groups with low TPP levels These results indicate that cisplatin inhibits the formation of TPP from thiamine leading to severe PNP. This finding suggests that TPP may be more beneficial than thiamine for the treatment of cisplatin-induced PNP.

Keywords: cisplatin, peripheral neuropathy, pain, rat, thiamine.

Introduction

Pain that is caused by impairment of the peripheral nervous system or by impairment of function or sensation has been described by the International Association for the Study of Pain as peripheral neuropathic pain (PNP) [5,37]. PNP is the most common side effect of chemotherapy [3,8], occurring in 80–90% of the patients undergoing this treatment [19]. This PNP side effect is therefore a serious pathological event that can lead to cessation of chemotherapy treatment; consequently, the treatment and pathogenesis of chemotherapy-induced PNP are of considerable scientific interest. Chemotherapy-induced PNP models are now used for the discovery of drugs that show fewer side effects and greater effectiveness against the elicitation of PNP.

The platinum-derived anticancer drug, cisplatin, is a known cause of PNP in animals and has been used to generate an experimental chemotherapy-induced PNP model [4,7,24]. The mechanism of chemotherapy-induced PNP is not yet well understood [24], but many studies indicate a role for interleukin-1β (IL-1β) in the formation of PNP [12,38]. Some studies also suggest that neuropathic pain induced by platinum-derived anticancer drugs is associated with oxidative stress [25]. An association may also exist between cisplatin neurotoxicity and oxidative stress. In this context, thiamine itself has no protective effect, whereas thiamine pyrophosphate (TPP) has a beneficial effect in the treatment of oxidative brain damage induced by cisplatin [34].

Doxorubicin causes TPP deficits in oxidative heart damage by inhibiting the thiamine pyrophosphokinase enzyme, which converts thiamine to TPP in rats. TPP, in turn, is believed to protect cardiac tissue from doxorubicin toxicity [28], and a thiamine deficiency was possible in the tissue despite its normal levels in blood [30]. Cisplatin treatment may also lead to oxidative PNP by inhibiting the formation of TPP from thiamine in the body. In addition, TPP may be beneficial in the treatment of cisplatin-induced PNP.

Pharmacology

In this study; our aim was to study the effects of TPP against cisplatin-induced PNP. TPP, an active metabolite of thiamine, is also known as vitamin B1. It is the best indicator of thiamine activity [29] and is formed in the liver by phosphorylation of thiamine by the thiamine pyrophosphokinase enzyme [29, 33]. No evidence has been recorded in the literature to indicate that cisplatin-induced PNP is caused by TPP deficiency. Therefore, the aim of our study was to investigate the effects of thiamine and TPP on cisplatin-induced PNP in rats. The association between the severity of cisplatin-induced PNP and the degree of thiamine deficiency was also further assessed.

Material and Methods

Animals

Rats were obtained from Ataturk University Medical Experimental Application and Research Center. The experiment was carried out using a total of 24 male albino Wistar rats weighing 235–245 grams. The animals were housed and fed in groups under appropriate conditions at normal room temperature (22 ^{o}C) in the Pharmacology Laboratory for 7 days. Animal experiments were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the local animal ethics committee of Ataturk University, Erzurum, Turkey (Ethics Committee Number: 7/144, Dated: 04.11.2016)

Chemical Substances

Cisplatin (50 mg/100 ml; Cisplatin; Ebewe) was provided by Liba (Turkey), thiamine and TPP were provided by Biopharma (Russia), and thiopental sodium was obtained from IE Ulagay (Turkey).

Experimental groups

Rats were divided into four groups: cisplatin (CIS) treated (n=6), cisplatin + thiamine (CTM) treated (n=6), cisplatin + TPP (CTPP) treated (n=6), and untreated healthy controls (HG) (n=6).

Experimental procedure

The normal paw pain thresholds of all rat groups were measured using a Basile Algesimeter before drug administration. The animals were then intraperitoneally (ip) administered 25 mg/kg of thiamine (CTM group), 25 mg/kg TPP (CTPP group), or the same volume of distilled water (CIS and HG groups). Five minutes after drug administration, 2 mg/kg cisplatin was administered ip to all rat groups except the HG group. The thiamine, TPP, and distilled water treatments were repeated once a day for 8 days. Cisplatin was administered once every two days for a total of four doses.

After the treatment period, blood samples were taken from the tail veins for analysis of interleukin 1β (IL-1β), malondialdehyde (MDA), total glutathione (tGSH), thiamine, and TPP. The paw pain thresholds of all rat groups were measured in the same way 8 days after drug administration. The analgesic effects of the drugs were determined by comparing the results of the CTM, CTPP, or HG groups with those of the CIS groups. The percent analgesic effect was calculated using the following formula: analgesic effect (%) = (1 − D/C) × 100, where D represents the difference in the pain threshold for the CTM, CTPP, or HG groups before and after drug administration, and C represents the difference in the pain threshold for the CIS group before and after cisplatin administration[6]. The rats were subsequently killed with a high dose of thiopental sodium, and the sciatic nerves were removed for histopathological examinations.

Biochemical analysis

Preparation of sera

Blood samples were taken from all rats and collected into separation gel vacutainer serum tubes. All blood samples were incubated for 15 min at room temperature, and then the sera were separated by centrifugation at 1500 ×g for 10 min. All serum samples were stored at −80°C until biochemical analysis.

MDA analysis in serum

MDA measurements were based on a previous method involving spectrophotometric measurement of absorbance of the pink-colored complex formed by thiobarbituric acid. The serum sample (0.1 mL) was added to a solution containing 0.2 ml of 80 g/L sodium dodecyl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL distilled water. The mixture was incubated at 95 ℃ for 1 h. Upon cooling, 5mL of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm. A standard curve was generated using 1,1,3,3-tetramethoxypropane[26].

Serum tGSH analysis

According to a previously defined method, 5,5'-dithiobis [2-nitrobenzoic acid] disulfide (DTNB) was used as the chromogen in the medium, as it is reduced easily by sulfhydryl groups. The yellow color produced during the reduction was measured spectrophotometrically at 412 nm. For measurement, a cocktail solution was prepared (5.85 mL 100 mM Na-phosphate buffer, 2.8 mL 1 mM DTNB, 3.75 mL 1 mM NADPH, and 80 µL 625 U/L glutathione reductase). Before measurement, 0.1 mL meta-phosphoric acid was added to 0.1 mL serum and centrifuged for 2 min at 2000 rpm to deproteinize the sample. A 0.15 mL volume of cocktail solution was added to 50 µL of supernatant. A standard curve was generated using GSSG [31].

IL-1β analysis in serum

Serum IL-1β concentrations were measured using a rat-specific sandwich enzyme-linked immunosorbent assay (ELISA) rat interleukin 1β kit (Cat no: YHB0616Ra, Shanghai LZ) and a rat tumor necrosis factor α ELISA kit (Cat no: YHB1098Ra, Shanghai LZ). Analyses were performed according to the manufacturers' instructions. Briefly, monoclonal antibodies specific for rat IL-1β and TNF-α were coated onto the wells of microplates. The serum

samples, standards, and biotinylated specific monoclonal antibodies and streptavidin-HRP were pipetted into the wells and incubated at 37 °C for 60 min. After washing, chromogen reagent A and chromogen reagent B were added to produce a color upon reaction with the bound enzyme. After incubation at 37 °C for 10 min, a stop solution was added. The intensity of this colored product is directly proportional to the concentration of rat IL-1β present in the original specimen. The concentrations of the colored product in the well plates were read at 450 nm with a microplate reader (Bio Tek, USA). The absorbance of the samples was estimated with formulas using standard curves.

Measurement of thiamine and TPP levels in serum samples

Whole blood samples were stored at −80 °C and then 10% trichloracetic acid solution was added at a 1:1 ratio to extract thiamine and TPP. After 5 minutes of vortexing and centrifugation at 5000 rpm for 10 minutes, the extract was reacted in basic medium containing $K_3(FeCN)_6$ and 20% NaOH to form thiochromes. The reaction mixture was applied to an HPLC column, separated with mobile phase components, and thiamine and TPP were detected using a fluorescence detector (Agilent Technologies, Germany) at 375 nm wavelength for excitation and 435 nm wavelength for emission. The mobile phases were 74% KH_2PO_4 buffer (pH 6.2) and 26% methanol. Thiamine and TPP peaks eluted at the 7.9 and 2.8 minutes, respectively.

Histopathological examination

The removed sciatic nerve tissues of rats were fixed in 10% formalin solution for 24 hours. Sections (4 μm thick) were obtained from paraffin blocks after routine tissue processing and stained with hematoxylin & eosin. All sections were evaluated under a light microscope (Olympus BX 52, Tokyo, Japan) by a pathologist following a blind allocation of samples.

Statistical Analysis

Pharmacology

The results of the experiments were expressed as "mean value ± standard error" (x ± SEM). The significance level between the groups was determined using one-way ANOVA. A Tukey test was performed as a post hoc analysis. All statistical procedures were performed using the "SPSS Statistics Version 18" statistical program A value of $p < 0.05$ was accepted as statistically significant.

Results

Pain test

Table 1 shows that the paw pain threshold in the CIS group was 28.2 ± 1.4 g lower after cisplatin administration than before cisplatin administration. The paw pain threshold difference before and after drug administration was 22.2 ± 2.2 g in the CTM group ($P > 0.05$). This indicated that thiamine produced a 21.3% analgesic effect in animals receiving cisplatin. The difference in paw pain threshold before and after drug administration was 4.8 ± 0.4 g in the CTPP group ($P < 0.0001$). This suggests that TPP reduced cisplatin-induced pain by 82.9%. In the HG group, the pain threshold difference before and after distilled water was evaluated as 1.3 ± 0.2 g ($P < 0.0001$).

Biochemical findings

MDA levels

As shown in Fig.1, the MDA level in sera of the HG group was 1.5 ± 0.2 µmol/g protein. The MDA level in the serum samples of the CIS group was increased to 4.2 ± 0.2 µmol/g protein ($p < 0.001$, versus the HG group). The serum level of MDA in the CTM group was 4.6 ± 0.2 µmol/g protein ($p > 0.05$, versus the CIS group). The serum level of MDA in the CTPP group was 1.8 ± 0.1 µmol/g protein, ($p < 0.001$, versus the CIS group)

tGSH levels

The amount of tGSH in the sera of the HG group was 7.0 ±0.3 nmol/g protein. However, the amount of tGSH in the serum of the CIS group rats given cisplatin was 2.1 ± 0.1 nmol/g

protein (p <0.0001, versus the HG group). The serum level of tGSH in the CTM group was 2.4 ± 0.2 nmol/g protein (p> 0.05, versus the CIS group). The amount of tGSH in the CTTP group was 6.6 ± 0.3 nmol/g protein (p <0.0001, versus the CIS group) (Fig.1).

IL-1β levels

The amount of serum IL-1β in the HG group was 1.7 ± 0.1 pg/ml, and this value increased to 5.3 ± 0.2 pg/ml in the CIS group (p <0.0001, versus the HG group). The serum level of IL-1β in the CTM group was 4.8 ± 0.3 pg/ml, (p> 0.05, versus the CIS group). The amount of serum IL-1β in the CTPP group was 2.0 ± 0.2 pg / ml (p <0.0001, versus the CIS group) (Fig.2).

The Thiamine and TPP levels in serum

The serum thiamine level was higher in the CTM group than in the CIS group (p <0.001). No significant difference was noted in the thiamine levels in the serum samples of the CTPP and HG groups (p> 0.05, versus the CIS group). However, cisplatin caused a decrease in TPP in the serum of the CIS group animals (p <0.0001, versus the HG group), whereas the TPP level was increased in the sera of the CTM and CTPP groups rats (p> 0. 0001, versus the CIS group) (Fig.3).

Histopathological findings

Histopathologically normal structures were observed for the epineurium (line arrow), vessels (circle arrow), fat tissue (smooth arrow), perineurium (square arrow), and nerve fascicles (bilateral arrow) in the sciatic nerves of the HG group (Fig.4). Increased dilated congested blood vessels were seen in the sciatic nerve epineurium layers of the CIS group (Fig.5a). The nerve fascicles showed destruction and edema in the CIS group (Fig.5b). The S-100 (Fig.5c) and trichrome dye (Fig.5d) results also confirmed the development of destruction of the sciatic nerve fascicles in the CIS group. The CTM group treated with thiamine showed fasciculus injury (round arrow), edema (line arrow), and dilated congested vessels (smooth

arrow) in the sciatic nerve (Fig.6). The CTPP group treated with TPP showed only edema (straight arrow) (Fig.7).

Discussion

This study investigated the effects of thiamine and TPP on cisplatin-induced PNP in rats. We also investigated whether cisplatin-induced PNP correlates with serum thiamine and TPP deficiency. Our experimental results showed that cisplatin reduced the paw pain threshold in the HG and TPP groups, but cisplatin insignificantly reduced the pain threshold in the CTM group. In the literature, the reduction in the pain threshold is considered to represent hyperalgesia, whereas elevation indicates analgesia [20] .

PNP is one of the most common side effects of chemotherapy. For this reason, chemotherapy-induced PNP models have gained importance when they are directed toward the prevention of the side effects of cancer drugs. In recent years, the paw withdrawal test has been widely used as a method of pain evaluation [2, 20]. In particular, the reason for choosing the paw withdrawal test to assess chemotherapy-induced PNP is that neuropathic pain first appears in this region [27]. The paw withdrawal test is also used to generate experimental PNP with cisplatin [24]. Our results suggest that TPP is effective in decreasing pain associated with cisplatin in rat paws, while thiamine is ineffective.

Cisplatin, which reduced the threshold of paw pain, increased the amount of MDA in the serum of the animals and decreased the amount of tGSH. MDA is used to estimate lipid peroxidation, and tGSH is used for determination of antioxidant activity [11]**.** Increases in MDA were reported in the cisplatin-induced peripheral neurotoxicity model, whereas tGSH levels decreased [32]. Recent studies have also suggested a significant link between pain/analgesia and oxidant/antioxidant parameters [2,9]. Another study reported that MDA levels increased in the rat paw in proportion to the decrease in the pain threshold, whereas

tGSH levels decreased [2]. In the present study, the amounts of IL-1β and MDA were increased and tGSH was decreased in the blood serum of the rats given cisplatin.

Previous studies have also suggested that IL-1β plays a role in the development of painful peripheral neuropathy [36].

Chemotherapy-induced PNP is associated with increased IL-1β [17]. Stimulation of IL-1β in the spinal dorsal horn also plays a critical role in the development of painful peripheral neuropathy [23]. This finding supports our experimental results with cisplatin.

In this study, we observed that thiamine did not prevent the increase in MDA and IL-1β or the decrease in tGSH induced by cisplatin, but TPP did prevent these responses. However, these effects of thiamine and TPP on chemotherapy-induced PNP were not found in some other studies. Some reports indicate that TPP protects tissues from oxidative damage. TPP inhibits the increase in MDA and the decrease in tGSH induced by chemotherapeutic drugs in the liver [14]. TPP was effective at inhibiting cisplatin-induced oxidative damage in kidney tissue, whereas thiamine was ineffective [35]. TPP also has an inhibitory effect on proinflammatory IL-1β, as well as antioxidant activity [10]. This finding is compatible with literature reports showing that TPP is able to maintain the levels of serum MDA, IL-1β, and tGSH at physiological levels in rats receiving cisplatin, whereas thiamine does not.

The CTPP group had a high paw pain threshold and high tGSH levels, whereas the MDA and IL-1β levels were low, and the serum TPP levels were close to those of the HG group. This suggests that cisplatin may inhibit the in vivo formation of TPP from the thiamine and may have given rise to PNP. Other studies also support this hypothesis; for example, the use of doxorubicin in chemotherapy prevented the formation of TPP, which is the active form of thiamine [28], in agreement with an earlier study [18]. TPP is a known cofactor of the transketolase enzyme that participates in the synthesis of natural antioxidants such as

NADPH and GSH. TPP may therefore play a very important role in energy production in heart, muscles, and brain and in the vision and nervous systems [13,16, 26].

Our study also showed histopathological findings that were consistent with the biochemical results. The histopathological examinations revealed dilated congested blood vessels, edema, and destruction of nerve fascicles in the CIS and CTM groups, which also contained high levels of MDA and IL-1β and low levels of tGSH. However, no pathological findings were observed other than edema in the CTPP group, which had low levels of MDA and IL-1β and high levels of tGSH.

Numerous studies that have investigated cisplatin effects support our histopathological findings on the sciatic nerve tissue. For example, cisplatin caused destructive damage to the sciatic nerve [21] and was reported to cause pathological changes, such as sciatic axonal degeneration, axonal connective tissue loss, and edema [15]. The amount of serum MDA was high and the amount of tGSH was low in the cisplatin-induced neurotoxicity model [1].

Conclusions

Biochemical and histopathological studies on cisplatin confirmed that it produces oxidative stress in the sciatic nerve tissue of rats. TPP, but not thiamine itself, is effective against cisplatin-induced PNP. The lack of thiamine efficacy suggests that the cisplatin effects may involve an inhibition of the formation of TPP from thiamine. Therefore, administration of TPP may be more beneficial than thiamine as a treatment for cisplatin-induced PNP.

Pharmacology

Reference:

1. Akman, T., Akman, L., Erbas, O., Terek, M.C., Taskiran, D., and Ozsaran, A. 2015. The preventive effect of oxytocin to cisplatin-induced neurotoxicity: an experimental rat model. *Biomed. Res. Int.* 2015: 167235.

2. Aksoy, M., Ahiskalioglu, A., Ince, I., Celik, M., Dostbil, A., Kuyrukluyildiz, U., Altuner, D., Kurt, N., and Suleyman, H. 2015. The relation between the effect of a subhypnotic dose of thiopental on claw pain threshold in rats and adrenalin, noradrenalin and dopamine levels. *Exp. Anim.* 64: 391.

3. Aley, K. O., Reichling, D. B., and Levine, J, D. 1996. Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. *Neuroscience.*73: 259-265.

4. Authier, N., Fialip, J., Eschalier, A., and Coudoré, F. 2000. Assessment of allodynia and hyperalgesia after cisplatin administration to rats. *Neurosci. Lett.* 291: 73-76.

5. Beydoun, A. 2003. Neuropathic pain: from mechanisms to treatment strategies. *J. Pain. Symptom. Manage.* 25: S1-3.

6. Cadirci, E., Suleyman, H., Hacimuftuoglu, A., Halici, Z., and Akcay, F. 2010. Indirect role of β2-adrenergic receptors in the mechanism of analgesic action of nonsteroidal antiinflammatory drugs. *Crit. Care. Med.* 38: 1860-1867.

7. Cavaletti, G., Petruccioli, M. G., Tredici, G, Marmiroli, P., Barajon, I., Fabrica, D., and Di Francesco, A. 1991. Effects of repeated administration of low doses of cisplatin on the rat nervous system. *Int. J. Tissue. React.* 13: 151-157.

8. Cavaletti, G., Tredici, G., Braga, M., and Tazzari, S. 1995. Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. *Exp. Neurol.* 33: 64-72.

9. Cetin, N., Suleyman, B., Kuyrukluyildiz, U., Nalkiran, H. S., Kiran, A., Gencoglu, S., Duzgun, A., Kurtoglu, I. Z., Yarali, O., Gul, m.A., and Suleyman, H. 2016. Investigation

of mucus obtained from different fish species on the acute pain induced with scalpel incision in paw of rats. *Exp. Anim.* 65: 77.

10. Cinici, E., Mammadov, R., Findik, H., Suleyman, B., Cetin, N., Calik, I., Balta, H., Tas, I.H., Sener, E., and Altuner, D. 2017. The protective effect of thiamine pyrophosphate against sugar-induced retinal neovascularisatıon in rats. *Int. J. Vitam. Nutr. Res.* 1–7 https://doi.org/10.1024/0300-9831/a000xxx.
11. Coskun, A. K., Yigiter, M., Oral, A., Odabasoglu, F., Halici, Z., Mentes, O., Cadırcı, E., Atalay, A., and Suleyman, H. 2011. The Effects of Montelukast on Antioxidant Enzymes and Proinflammatory Cytokines on the Heart, Liver, Lungs., and Kidneys in a Rat Model of Cecal Ligation and Puncture–Induced Sepsis. *Scientific. World. Journal*. 11: 1341-1356.
12. Costa, G. M. F., de Oliveira, A. P., Martinelli, P. M., Camargos, E. R, Arantes, R. M., and de Almeida-Leite, C.M. 2016. Demyelination/remyelination and expression of interleukin-1β, substance P, nerve growth factor, and glial-derived neurotrophic factor during trigeminal neuropathic pain in rats. *Neurosci. Lett*. 612: 210-218.
13. Lima, L. F., Leite, H. P., and Taddei, J. A. 20011. Low blood thiamine concentrations in children upon admission to the intensive care unit: risk factors and prognostic significance. *Am. J. Clin. Nutr.* 93: 57-61.
14. Demiryilmaz, I., Sener, E., Cetin, N., Altuner, D., Suleyman,B., Albayrak, F., Akcay, F., and Suleyman, H. 2012. Biochemically and histopathologically comparative review of thiamine's and thiamine pyrophosphate's oxidative stress effects generated with methotrexate in rat liver. *Med. Sci. Monit.* 18: BR475-BR481.
15. Erken, H. A., Koç, E. R., Yazıcı, H., Yay, A., Önder, G. Ö., and Sarıcı, S. F. 2014. Selenium partially prevents cisplatin-induced neurotoxicity: A preliminary study. *Neurotoxicol.* 42: 71-75.

16. Gangolf, M., Czerniecki, J., Radermecker, M., Detry, O., Nisolle, M., Jouan, C., Martin, D., Chantraine, F., Lakaye, B., Wins, P., Grisar, T., and Bettendorff, L. 2010. Thiamine status in humans and content of phosphorylated thiamine derivatives in biopsies and cultured cells. *PLoS. One*. 5: e13616.

17. Guindon, J., Deng, L., Fan, B., Wager-Miller, J., and Hohmann, A. G. 2014. Optimization of a cisplatin model of chemotherapy-induced peripheral neuropathy in mice: use of vitamin C and sodium bicarbonate pretreatments to reduce nephrotoxicity and improve animal health status. *Mol. Pain*. 10: 1.

18. Hanninen, S. A., Darling , P. B., Sole, M.J., Barr, A., and Keith, M. E. 2006. The prevalence of thiamin deficiency in hospitalized patients with congestive heart failure. *J. Am. Coll. Cardiol.* 47: 354-361.

19. Hoke, A. 2012. Animal models of peripheral neuropathies.The journal of the American Society for Experimental NeuroTherapeutics. *Neuro. Therap.* 9: 262-269.

20. Ince, I., Aksoy, M., Ahiskalioglu, A., Comez, M., Dostbil, A., Celik, M., Yilmaz, I., Dogan, H., Ozgermen, B, B., and Altuner, D. 2015. A Comparative Investigation of the Analgesic Effects of Metamizole and Paracetamol in Rats. *J. Invest. Surg.* 28: 173-180.

21. Kamisli, S., Ciftci, O., Kaya, K., Cetin, A., Kamisli, O., and Ozcan, C. 2015. Hesperidin protects brain and sciatic nerve tissues against cisplatin-induced oxidative, histological and electromyographical side effects in rats.*Toxicol. Ind. Healt.* 9: 841-51.

22. Kopelman, M. D., Thomson, A. D., Guerrini, I., and Marshall, E. J. 2009. The Korsakoff syndrome: clinical aspects, psychology and treatment. *Alcohol. Alcohol.* 44: 148-154

23. Li, Z. Y., Zhang, Y. P., Zhang, J., Li, D., Huang, Z. Z., and Xin, W. J. 2016. The possible involvement of JNK activation in the spinal dorsal horn in bortezomib-induced allodynia: the role of TNF-α and IL-1β. *J. Anesth.* 30: 55-63.

373 24. Lin, H., Heo, B. H., and Yoon, M. H. 2015. A New Rat Model of Cisplatin-induced
374 Neuropathic Pain. *Korea. J. Pain.* 28: 236-243.
375 24. Naji-Esfahani, H., Vaseghi, G., Safaeian, L., Pilehvarian, A. A., Abed, A., and Rafieian-
376 Kopaei, M. 2016. Gender differences in a mouse model of chemotherapy-induced
377 neuropathic pain. *Lab. Anim.* 50: 15-20.
378 25. Nassini, R., Gees, M., Harrison, S., De siena, G., Materazzi, S., Moretto, N., Failli, P.,
379 Preti, D., Marchetti, N., Cavazzini, A., Mancini, F., Pedretti, P., Nillus, R., Patacchini, R.,
380 and Gepetti, P. 2011. Oxaliplatin elicits mechanical and cold allodynia in rodents via
381 TRPA1 receptor stimulation. *Pain.* 152: 1621-1631.
382 26. Ohkawa, H., Ohishi, N., and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by
383 thiobarbituric acid reaction. *Anal. Biochem.* 95: 351-358.
384 27. Perry, M, C. 2008. The chemotherapy source book: Lippincott Williams & Wilkins.
385 28. Polat, B., Suleyman, H., Sener, E., and Akcay, F. 2015. Examination of the effects of
386 thiamine and thiamine pyrophosphate on Doxorubicin-induced experimental
387 cardiotoxicity. *J. Cardiovasc. Pharmacol. Ther.* 20: 221-229.
388 29. Rindi, G., Patrini, C., Laforenza, U., Mandel, H., Berant, M., Viana, M.B., Poggi, V., and
389 Zarra, A. N. 1994. Further studies on erythrocyte thiamin transport and phosphorylation in
390 seven patients with thiamin-responsive megaloblastic anaemia. *J. Inherit. Meta. Dis.* 17:
391 667-677.
392 30. Sasaki, T., Yukizane, T., Atsuta, H., Ishikawa, H., Yoshiike, T., 2010. [A case of
393 thiamine deficiency with psychotic symptoms--blood concentration of thiamine and
394 response to therapy]. *Seishin. Shinkeigaku. Zasshi.*112: 97-110
395 31. Sedlak, J., and Lindsay, R. H. 1968. Estimation of total, protein-bound, and nonprotein
396 sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* 25: 192-205.

Pharmacology

32. Sharawy, N., Rashed, L., Youakim, M, F. 2015. Evaluation of multi-neuroprotective effects of erythropoietin using cisplatin induced peripheral neurotoxicity model. *Exp Toxicol. Pathol.* 67: 315-322.

33. Sica, D. A. 2007. Loop diuretic therapy, thiamine balance, and heart failure. *Congest Heart. Fail.* 13: 244-247.

34. Turan, M. I., Cayir, A., Cetin, N., Suleyman, H., Siltelioglu, I., and Tan, H. 2014. An investigation of the effect of thiamine pyrophosphate on cisplatin-induced oxidative stress and DNA damage in rat brain tissue compared with thiamine: thiamine and thiamine pyrophosphate effects on cisplatin neurotoxicity. *Hum. Exper. Toxicol.* 33: 14-21.

35. Turan, M. I, Siltelioglu T. I., Mammadov., R., Altınkaynak, K., Kisaoglu, A.2013. The effect of thiamine and thiamine pyrophosphate on oxidative liver damage induced in rats with cisplatin. *Biomed. Res. Int.* 2013: 783809. doi: 10.1155/2013/783809.

36. Watkins, L.R., and S. F. Maier. 2003. Glia: a novel drug discovery target for clinical pain. *Nat. Rev. Drug. Dicov*. 2: 973-985.

37. White, S. 2004. Assessment of chronic neuropathic pain and the use of pain tools. *Br. J. Nurs.* 7: 372-378.

38. Whitehead, K., Smith, C., Delaney, S., Curnow, S. J., Salmon, Hughes, J. P, and Chessell, I. P. 2010. Dynamic regulation of spinal pro-inflammatory cytokine release in the rat in vivo following peripheral nerve injury. *Brain. Behav. Immun.* 24: 569-576.

Figure legends

Fig.1. Serum levels of MDA and tGSH levels in the CIS, CTM, CTPP, and HG rat groups.

* P<0.0001, versus the CIS rat group.

Fig.2. Serum IL-1β levels in the CIS, CTM, CTPP, and HG rat groups.

* P<0.0001, according to the CIS group.

Fig.3. Serum thiamine and TPP levels in the CIS, CTM, CTPP, and HG rat groups.* P<0.0001, versus the CIS group.

Fig.4. Normal structure of the sciatic nerve, epineurium, vessels, adipose tissue, perineurium, and nerve fascicles in the HG rat groups.

Fig.5. 5a; Dilated and congested blood vessels in the epineurium layer of the sciatic nerve tissue in the CIS rat group. **5b;** Destruction and edema in the nerve fascicles of the CIS rat group. **5c;** Fascicule destruction determined by S-100 of the sciatic nerve in the CIS rat group. **5d;** Fascicule destruction determined by trichrome staining of the sciatic nerve in the CIS rat group.

Fig.6. Fasciculus injury, edema, and dilated congested vessels structure in the CTM rat group.

Fig.7. Edema in sciatic nerve tissue of the CTPP rat group.

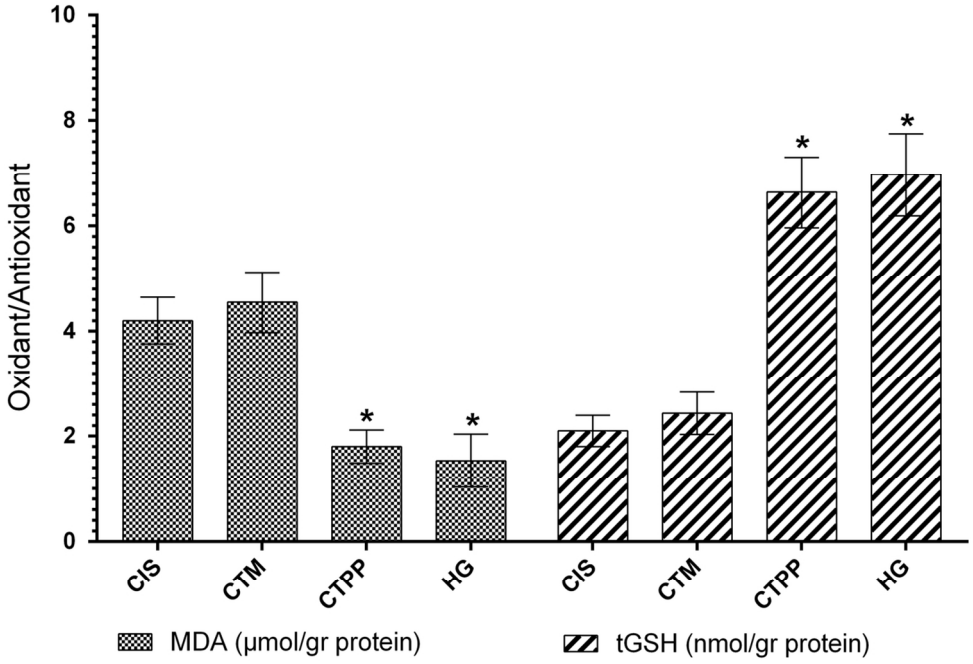

Fig.1. Serum levels of MDA and tGSH levels in the CIS, CTM, CTPP, and HG rat groups.
* P<0.0001, versus the CIS rat group.

116x82mm (300 x 300 DPI)

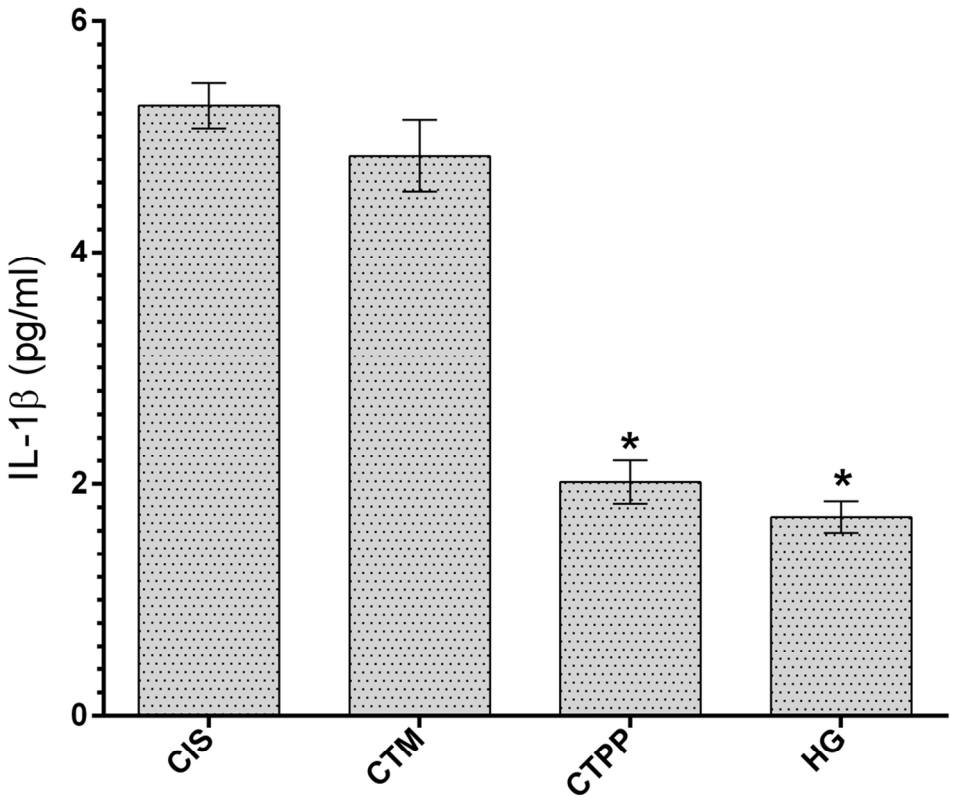

Fig.2. Serum IL-1β levels in the CIS, CTM, CTPP, and HG rat groups.
* P<0.0001, according to the CIS group.

108x95mm (300 x 300 DPI)

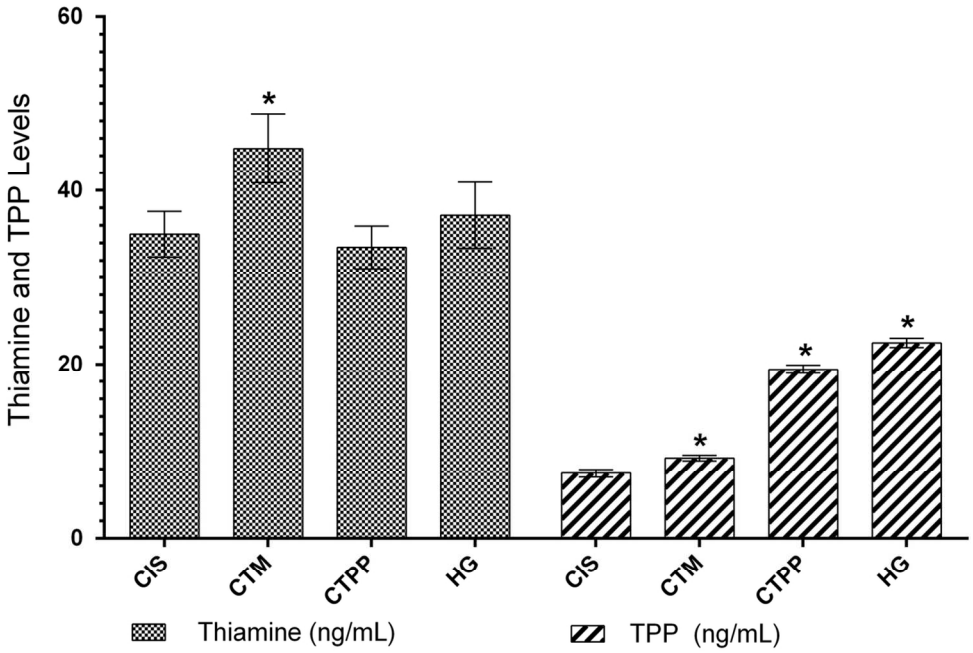

Fig.3. Serum thiamine and TPP levels in the CIS, CTM, CTPP, and HG rat groups.* P<0.0001, versus the CIS group.

115x80mm (300 x 300 DPI)

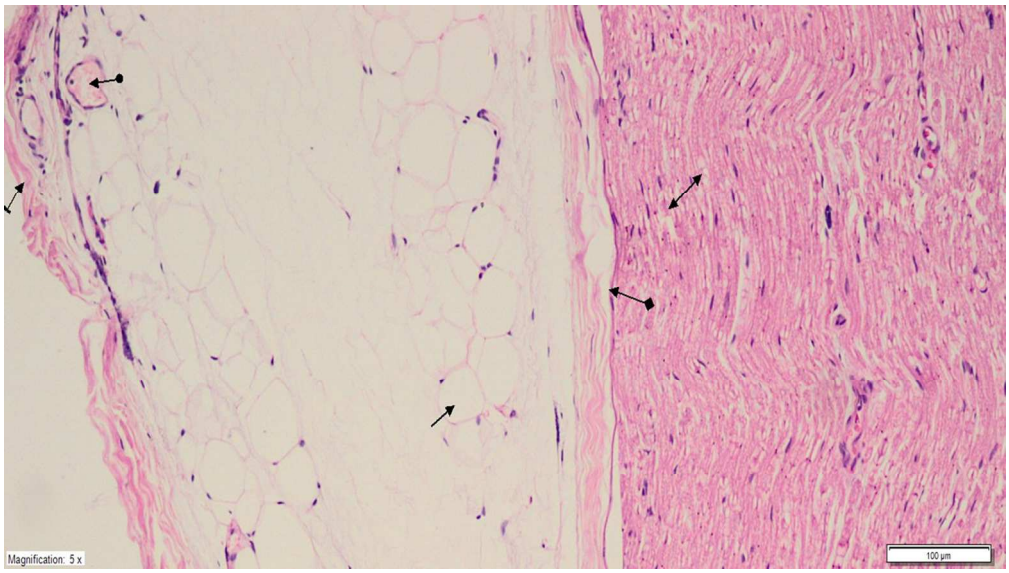

Fig.4. Normal structure of the sciatic nerve, epineurium, vessels, adipose tissue, perineurium, and nerve fascicles in the HG rat groups.

190x107mm (300 x 300 DPI)

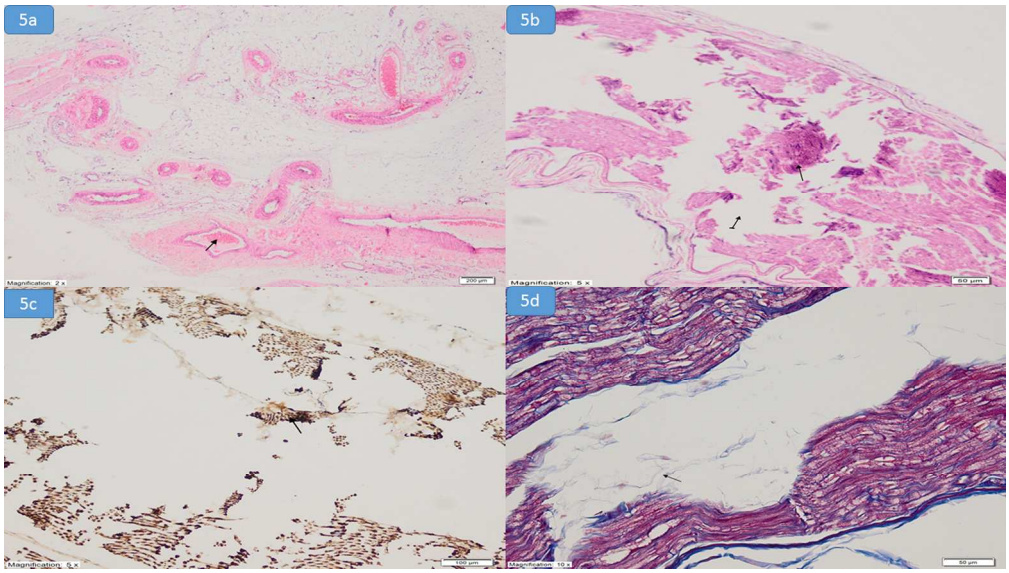

Fig.5. 5a; Dilated and congested blood vessels in the epineurium layer of the sciatic nerve tissue in the CIS rat group. 5b; Destruction and edema in the nerve fascicles of the CIS rat group. 5c; Fascicule destruction determined by S-100 of the sciatic nerve in the CIS rat group. 5d; Fascicule destruction determined by trichrome staining of the sciatic nerve in the CIS rat group.

190x107mm (300 x 300 DPI)

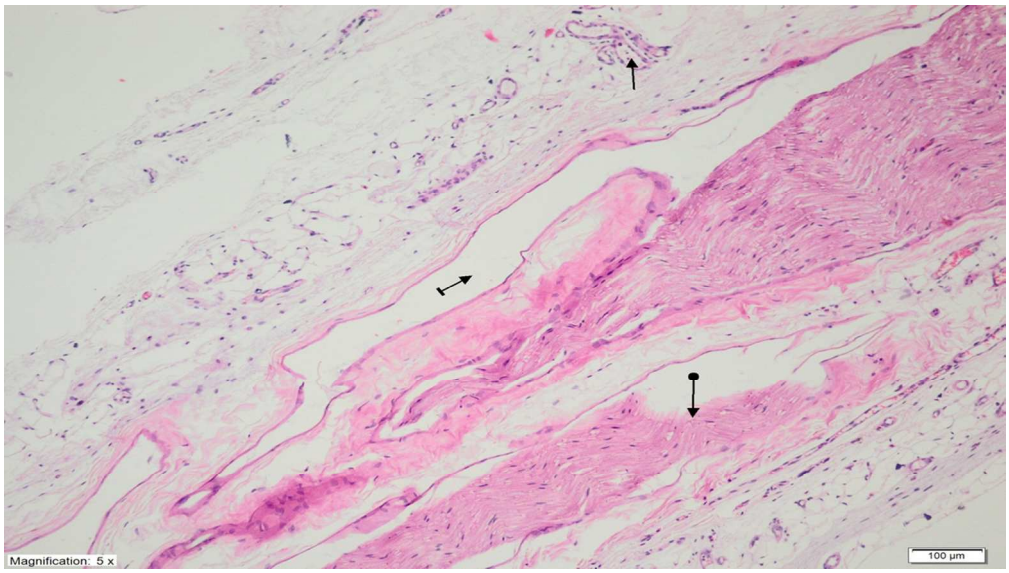

Fig.6. Fasciculus injury, edema, and dilated congested vessels structure in the CTM rat group.

190x107mm (300 x 300 DPI)

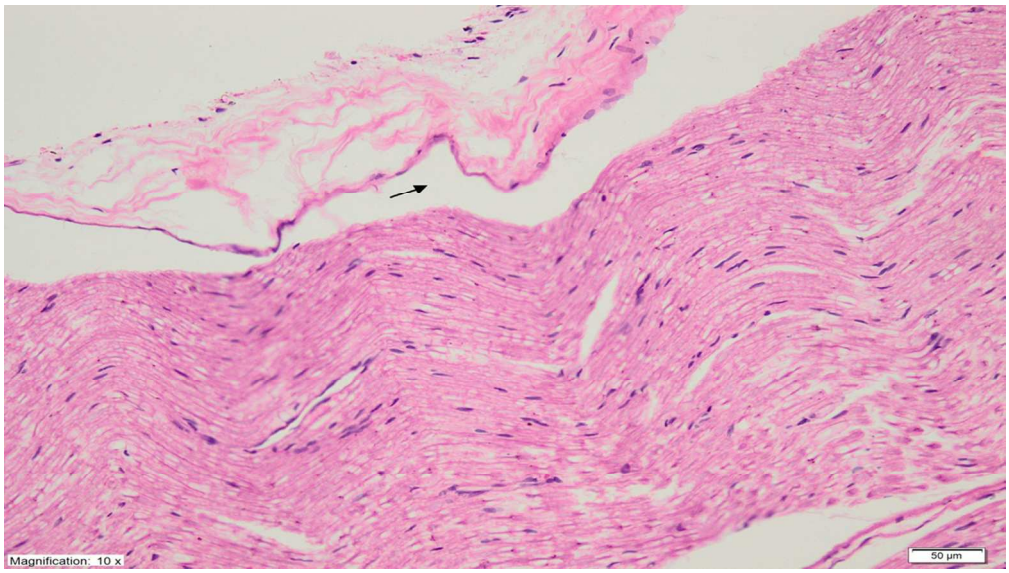

Fig.7. Edema in sciatic nerve tissue of the CTPP rat group.

190x107mm (300 x 300 DPI)