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# Post-injection sciatic neuropathy: Effects of intramuscular and intraneural administration of thiocolchicoside in mice

Alizade A[1] & Terzi MY[2,3]*

[1]Department of Pharmacology, Faculty of Medicine, Van Yüzüncü Yıl University, Van, Türkiye

[2]Department of Medical Biology, Faculty of Medicine, [3]Department of Molecular Biochemistry and Genetics, Graduate School of Health Sciences, Hatay Mustafa Kemal University, Hatay, Türkiye

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We aimed to investigate whether neuropathic pain post-gluteal injections in mice occurs due to sciatic nerve damage by injector or neurotoxicity of thiocolchicoside (Thio) itself. Acute pain till 48 h post-gluteal injection was analyzed with rot-a-rod, tail-flick, cold-plate, Von-Frey, and paw-grip-endurance tests in no-injection-received control group and intramuscular (IM) and intraneural (IN) Thio or vehicle injection-received mice. Histopathological examination was performed with hematoxylin-eosin staining. Behavioral tests showed that nociceptive parameters and motor functions of mice in IN-injected groups were significantly lower than in IM-injected ones and control group. Behavioral tests performed post-24-h showed no significant difference between control and IM-injected groups. However, IN-injected groups showed marked and consistent differences until 48-h compared to control. The difference between IM-vehicle and IM-Thio groups at early time points revealed partial, temporary neurotoxic effect of Thio. The histopathologic analyses measured degeneration severity and revealed that IN-Thio injections caused the most severe degeneration which aligned with the behavioral tests. We observed mild, temporary pathologic effect of Thio by diffusion on sciatic nerve. IN-injections caused insistent and severe sciatic nerve damage due to mechanical impact. Our results suggest that the prevention of injection-triggered neuropathy requires appropriate injection methods/agents performed by experienced medical personnel.

**Keywords**: Injection neuropathy, Sciatic nerve injury, Thiocolchicoside, Mouse model

Intramuscular (IM) administration of drugs is a commonly used way amongst parenteral administration routes which provides the delivery of drugs into large muscle bulks[1]. Intramuscular drug administration can readily be performed into quadriceps, deltoid, and gluteal muscles, of the latest is preferred more frequently[2]. Despite correct practices, sciatic nerve neuropathy can occur after intramuscular gluteal injections[3]. Sciatic nerve injection injury (SNII) refers to the post-injection injury on sciatic neurites. Among the reasons of SNII, malpractice due to unexperienced health personal and the drug complications *e.g.*, irritations on the sciatic nerves can be listed[4]. Following SNII, some collateral damages can emerge namely apsis, tissue irritation, and chronic pain[5]. The most commonly injured tissue is sciatic nerve during gluteal intramuscular injections and the most prevalent complaints post-injection are sensorial or motor impairments together with gluteal neuropathic pain[6]. The pathogenesis of injection-triggered injury can vary depending on the location and type of agent. While extrafascicular injection does not lead to any nerve damage, intrafascicular injection can cause nerve injury at various levels from mild to severe which depends on the type and dose of the agent[7]. Among the drugs administered via the intramuscular route, antibiotics and analgesics most frequently cause sciatic neuropathy[8,9]. In a previous study conducted with rats, intrafascicular lidocaine administered to the sciatic nerve was shown to cause neuropathy and severe nerve damage[10]. Besides, analgesics, namely diclofenac, tramadol, and metamizole, were previously associated with the SNII[11]. Even extrafascicular injection of some agents such as dexamethasone, midazolam, and nonsteroidal anti-inflammatory drugs can result in SNII due to their high level of neurotoxicity[12].

Thiocolchicoside (Thio) is a muscle relaxative, anti-inflammatory, and analgesic agent and commonly used against muscle spasms in clinical practice[13]. Thio is a semisynthetic derivative of colchicoside, which is an analogue form of colchicine

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*Correspondence:
Phone: +90 326 245 51 14
E-mail: menderesyusufterzi@gmail.com

that has been shown to induce experimental epilepsy in rats[13]. It mostly remains unclear that the pain and complaints by patients after intramuscular gluteal administration of Thio arise from the neurotoxic effect of the drug or the direct damage on the sciatic nerve caused by the injector. In the present study, we aimed to compare the effect of intraneural and intramuscular Thio administration on the sciatic nerve in a mouse model. For this purpose, we evaluated the paralysis and pain symptoms post-injections with several behavioral tests and examined the sciatic nerve tissue with histopathological staining.

## Materials and Methods

### Experimental animals

This study was approved by the Çukurova University Experimental Animals Ethics Committee (Approval date/no: 15-04-2021/2). Eight-week-old Swiss Albino mice (20-30 g) were obtained from Çukurova University Health Sciences Experimental Application and Research Center. All animal experiments were conducted by following ARRIVE guideline to minimize suffering of animals and to restrict total animal numbers (3R policy)[14]. The mice were kept under standard laboratory conditions (12-h light/dark cycle, at 22 ± 2°C) and fed *ad-libitum*. The surgical procedures on animals were performed under aseptic conditions in behavioral pharmacology laboratory.

### Groups and experimental protocol

Forty Swiss albino male mice were used in total and grouped randomly in 5 different cages (n=8) as described previously[15]. No inclusion/exclusion criteria were utilized for selection of animals. No animals/data were excluded throughout the experimental procedures. In order to calculate the dosage per animal, all mice were weighed before the experiments. Mice were treated with ketamine (80 mg/kg, Keta-Control, Doğa İlaç) and xylazine (10 mg/kg, Control 10%, Doğa İlaç) intraperitoneally for general anesthesia before sciatic nerve injection. Gluteal region (the caudal aspect of the femur) was laterally incised after shaving and cleaning the area with antiseptic (betadine) solution. Muscle tissues were cleared with dissection and the sciatic nerve was exposed. The mice in 5 groups were treated as follows: (A) Control; no injection, (B) SN+Vehicle-IM; intramuscular 0.9% saline injection, (C) SN+Thio-IM; intramuscular thiocolchicoside (4 mg/kg/0.2 mL body weight) injection[16], (D) SN+Vehicle-IN; intraneural 0.9% saline injection, (E) SN+Thio-IN; intraneural thiocolchicoside (4 mg/kg/0.2 mL) injection. For groups (C) and (E), 4 mg/kg body weight Thio was used[17]. All the injections were administered by intra peritoneal route by using 26G needle[16]. The experimental animals were simultaneously randomized to the groups without bias or any other variable by a researcher who was blind to the groups. After experimental procedures, gluteal muscles and skin tissues were sutured. The behavioral tests were initiated to analyze the injection-mediated neuropathic pain 30 min after all their normal vital functions were restored (n=8). After last behavioral tests were ended, the mice were sacrificed via decapitation 45 min after deep anesthesia via intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg)[18]. Then the nerve tissues were dissected for histological examination (n=8). During the experimental procedures, no strategy was used to control confounders. All experiments were conducted by a blind researcher to the experimental groups.

### Behavioral tests

#### *Rotarod*

Rotarod was performed to test the effect of injections on the motor functions of animals as described previously with minor modifications[19]. Rotarod device (Rota-Rod treadmill for mice, Ugo Basile 7600, Varese, Italy) was used for the tests pre-injections (0), and 0.5, 1, 3, 6, 24, and 48 h post-injections. Cut-off time was set to 180 s and the mice were located onto the rotating rod at a constant speed of 18 rpm for 3 min long. The mice were scored based on their fall latencies (s).

#### *Paw grip endurance (PAGE)*

In order to analyze paw muscle capacity, the PAGE test was conducted at time points (0), 1, 3, 6, 24, and 48 h post-injections[20]. During the test, each mouse was placed on a 15 cm × 15 cm wire mesh held 40 cm above the ground. The mesh was then slowly inverted upside down, and the duration of paw grip was recorded. Each mouse underwent three tests.

#### *Tail-flick*

Tail-flick test was performed to analyze the effect of injections on acute pain as described previously[21]. Briefly, tail-flick latency duration was recorded using tail flick analgesia meter (Harvard, Edenbridge, Kent, and UK) which was measured as the duration between the heat exposure of the tail and tail retraction. The tests were conducted pre-injections (0) and 1, 3, 6, 12, 24, and 48 h post-injections. The cut-off duration for radiant heat exposure was restricted to 10 s to prevent any tissue damage on tail.

### *Cold plate*

The effect of Thio injection on sciatic nerve injury was analyzed with cold hyperalgesia cold plate test. Pre-injections (0) and 1, 4, 8,12, 24, and 48 h post-injections, cold allodynia was measured with a cold plate apparatus (Ugo Basile, Comerio, Italy)[22]. The temperature of the plate was kept at 5±0.2°C, the animals were located on the cold plate for 30 s as the cut-off time and the latency was recorded until paw retraction or jump reactions of the animals as a response to the cold hyperalgesia.

### *Von Frey*

Mechanical allodynia was measured with von Frey test to analyze the effect of Thio injection by recording the paw reactions (up-down movements) against point impulses[23]. On the day of the experiments, each animal was habituated inside a transparent box for 15 min before the tests. Then, a Von Frey filament was pressed vertically to the plenary surface of the right hind paw of each animal till it slightly bent. The scale of Von Frey filaments was between 0.04, 0.07, 0.16, 0.4, 1, 2, 4, 6, and 8 g with approximately equal logarithmic increments (Aesthesio, User Manual, USA). Each filament was applied for 5 times and in case there was no paw reflex within a 3 s period, the response to the filament was recorded as negative and the filament was replaced with a more rigid one. Paw retraction, licking, bite, tremor, and quick retraction of paws more than 3 times were recorded as positive response. The tests were performed at time 0 (zero, pre-injections), and at times 1, 4, 8, 12, 24, and 48 h post-injections.

### Histology

The histological stating was performed as described earlier[24]. Briefly, excised sciatic nerve tissue samples were fixed in a 10% neutral formalin solution. Next, they underwent dehydration using an ascending series of ethanol and absolute xylene before being embedded in paraffin blocks to obtain 5 μm thick tissue slices. These tissue sections were stained with hematoxylin and eosin (H&E) and semi-qualitatively analyzed under a light microscope (Novex, Holland) to identify Schwann cell and axonal and endoneural degeneration of the sciatic nerve. In our study, H&E-stained sciatic nerve sections were evaluated using a semi-quantitative scoring system ranging from 0 to 4 based on histopathological severity. This scoring method is adapted from established literature as follow[25]: 0 = Normal histology, 1 = Minimal changes, 2 = Moderate alterations, 3 = Severe degeneration and 4 = Extensive or diffuse tissue damage.

Each tissue section was evaluated independently by two blinded researchers to ensure consistency and objectivity in the scoring of axonal and endoneural degeneration, as well as fascicular symmetry. The focus on Schwann cells was based on their critical role in peripheral nerve integrity and regeneration. Schwann cells are the principal glial cells of the peripheral nervous system and serve as early indicators of nerve injury. Morphological changes in Schwann cells, such as swelling, nuclear hyperchromasia, or disorganization were noted and qualitatively described to supplement the semi-quantitative evaluation. However, these observations did not contribute to the numerical scoring itself[26,27].

### Statistical analysis

All statistical analyses were performed using Graphpad Prism v.8.0.2. Non-categorical continuous variables were analyzed for normality with Shapiro-Wilk test. Parametric data in multiple groups were compared with one-way ANOVA test and then post-hoc Tukey test was performed for comparison of two groups. For non-parametric data, Kruskal Wallis-H test was applied following Mann-Whitney U test to compare two groups. The data were presented either mean standard deviation or *P* values less than 0.05 were considered as statistically significant.

## Results

We analyzed the acute neuropathy triggered by sciatic nerve injury after intraneural or intramuscular Thio injection in Swiss albino mice using behavioral tests (Fig. 1, Supple. Tables 1-5) and histological examination (Fig. 2). During behavioral tests (rotarod, tail-flick, cold plate, Von Frey, and PAGE), we observed significant alterations between the mice that received intramuscular and intraneural injections. The behavioral tests at time 0 (zero, pre-injections) did not show any significant alterations between the groups ($P$>0.05).

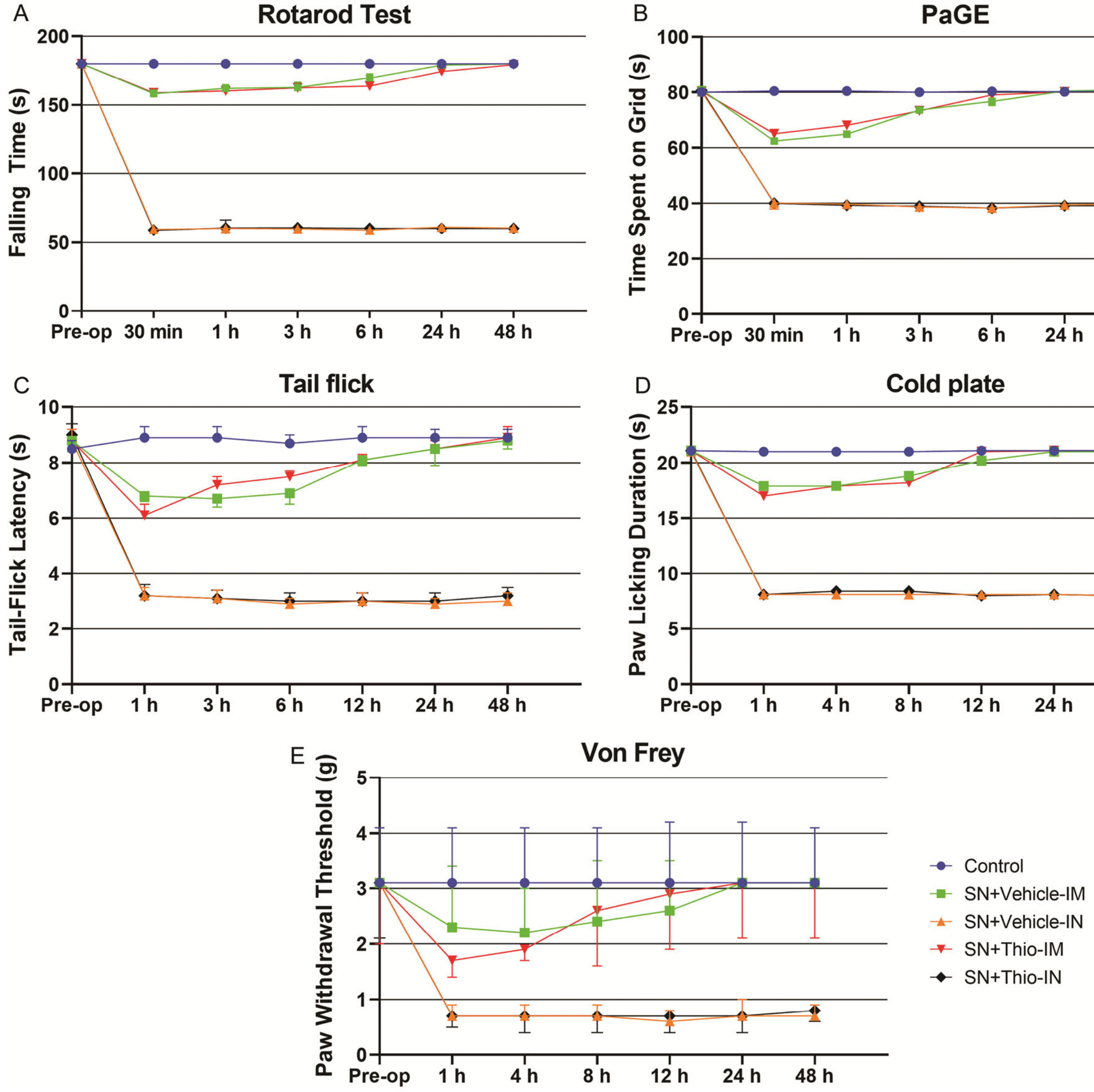

Fig. 1 — Line plots representing the results of behavioral tests. (A) Rotarod (30 min - 48 h), (B) PAGE (30 min - 48 h), (C) Tail-flick (1 h - 48 h), (D) Cold plate (1 h - 48 h), (E) Von Frey (1 h - 48 h) in control (dot). [SN+Vehicle-IM (square), SN+Thio-IM (reverse triangle), SN+Vehicle-IN (triangle), and SN+Thio-IN (rhombus). PAGE: Paw grip endurance, SN: Sciatic nerve, IM: Intramuscular, IN: Intraneural, Thio: Thiocolchicoside].

**Rotarod**

Rotarod tests were applied to each mouse pre-injections (time 0) and 0.5, 1, 3, 6, 24, and 48 h post-injections (Fig. 1A). There was a significant difference in falling times between the control and IN-injected groups at each time point ($P<0.05$). Falling times were significantly different between the control and IM-injected groups at 0.5, 1, 3, and 6 h time points ($P<0.05$). At 24 h and 48 h, there was no significant difference in falling times between control and SN+Vehicle-IM groups ($P>0.05$). Whereas the falling time at 24 h significantly shortened in SN+Thio-IM group compared to control ($P<0.05$), at 48 h, there was no significant difference between these groups ($P>0.05$).

Among injection groups, the falling times in IM-injected group at each time point were significantly longer than the ones in IN-injected group ($P<0.05$). There was no significant difference between SN+Vehicle-IN and SN+Thio-IN groups at any time point ($P>0.05$). At 6 and 24 h, the falling times in SN+Vehicle-IM were significantly longer than the ones in SN+Thio-IM group ($P<0.05$) while there was no significant difference at other time points ($P>0.05$).

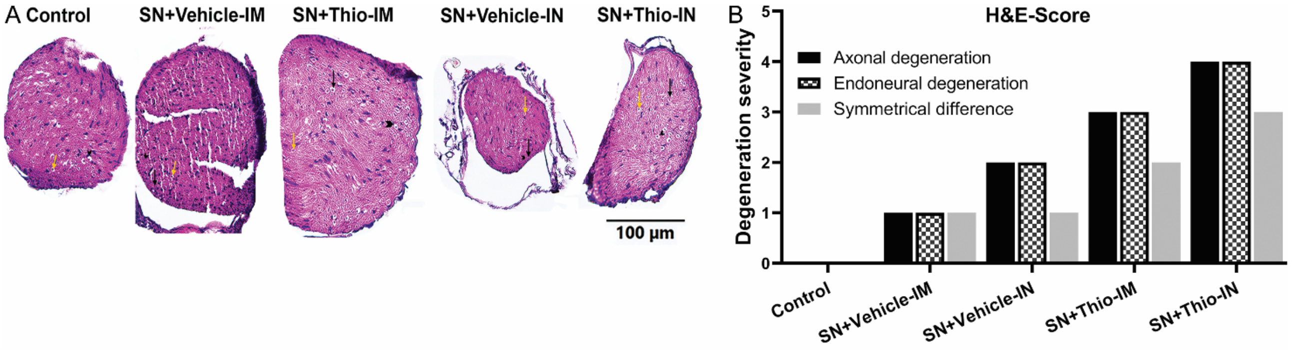

Fig. 2 — (A) Representative microscopic images of HE-stained sciatic nerve sections. Control, SN+Vehicle-IM, SN+Thio-IM, SN+Vehicle-IN, and SN+Thio-IN. Normal axons were depicted with yellow arrows, degenerated axons with black arrows, Schwann cells with arrowhead. (B) Semi-quantitative analysis of the degeneration severity- 0-4 grading system for evaluation of axonal and neuronal degenerations and symmetrical difference. [Scale bar=100 µm. SN: Sciatic nerve, IM: Intramuscular, IN: Intraneural, Thio: Thiocolchicoside].

**Paw grip endurance (PAGE)**

The effect of gluteal injections on paw muscle capacity was analyzed with PAGE test pre-injections (0) and 0.5, 1, 3, 6, 24, and 48 h post-injections (Fig. 1B). There was a significant difference in time spent on grid between the control and IN-injected groups at each time point ($P<0.05$). There was a significant difference between the control and IM-injected groups at 0.5, 1, and 3 h ($P<0.05$). At 6, 24, and 48 h, there was no significant difference between control and IM-injected groups ($P>0.05$).

Among injection groups, the time spent on grid in IM-injected group at each time point were significantly longer than the ones in IN-injected group ($P<0.05$). There was no significant difference between both SN+Vehicle-IN / SN+Thio-IN and SN+Vehicle-IM / SN+Thio-IM groups at any time point ($P>0.05$).

**Tail-flick**

Tail-flick test was performed to observe nociceptive effect on gluteal region pre-injections (0) and 1, 3, 6, 12, 24, and 48 h post-injections (Fig. 1C). There was a significant difference in tail-flick latency times between the control and IN-injected groups at each time point ($P<0.05$). Latency times were significantly different between the control and IM-injected groups at 1, 3, 6, and 12 h time points ($P<0.05$). At 24 h and 48 h, there was no significant difference in falling times between control and IM-injected groups ($P>0.05$).

Among injection groups, the latency times in IM-injected group at each time point were significantly longer than the ones in IN-injected group ($P<0.05$). There was no significant difference between SN+Vehicle-IN and SN+Thio-IN groups at any time point ($P>0.05$). At 1, 3, and 6 h, the latency times were significantly different between SN+Vehicle-IM and SN+Thio-IM groups ($P<0.05$) while there was no significant difference at other time points ($P>0.05$).

**Cold plate**

Cold plate test was performed to analyze thermal nociceptive pain threshold on gluteal region of the mice pre-injections (0) and 1, 4, 8, 12, 24, and 48 h post-injections (Fig. 1D). There was a significant difference in paw licking duration times between the control and IN-injected groups at each time point ($P<0.05$). There was a significant difference between the control and IM-injected groups at 1, 4, and 8 h ($P<0.05$). At 12, 24, and 48 h, there was no significant difference between control and IM-injected groups ($P>0.05$) except control and SN+Vehicle-IM at 12 h ($P<0.05$).

Among injection groups, the paw licking duration times in IM-injected group at each time point were significantly longer than the ones in IN-injected group ($P<0.05$). There was no significant difference between SN+Vehicle-IN and SN+Thio-IN groups at any time point ($P>0.05$) except 8 h ($P<0.05$). At 1, 8, and 12 h, the paw licking duration times were significantly different between SN+Vehicle-IM and SN+Thio-IM groups ($P<0.05$) while there was no significant difference at other time points ($P>0.05$).

**Von Frey**

The effect of gluteal injections on the pain threshold was analyzed with Von Frey test pre-injections (0) and 1, 4, 8, 12, 24, and 48 h post-injections (Fig. 1E). There was a significant difference in paw withdrawal threshold between the control and IN-injected groups at each time point (*P*<0.05). There was no significant difference between the control and IM-injected groups at any time point (*P*>0.05) except control and SN+Thio-IM at 1 and 4 h (*P*<0.05).

Among injection groups, the paw withdrawal threshold in IM-injected group at each time point was significantly higher than the ones in IN-injected group (*P*<0.05). There was no significant difference between both SN+Vehicle-IN / SN+Thio-IN and SN+Vehicle-IM / SN+Thio-IM groups at any time point (*P*>0.05).

### Histopathological evaluation

The histological structures/cells post-injections were qualitatively evaluated with HE staining with light microscopy as depicted in Fig. 2A & B. Normal axons were spotted with yellow arrow, degenerated axons with black arrow, and Schwann cells with arrowhead. In control group, normal histologic structure of sciatic nerve tissue section was observed. In IM-injected groups, the sciatic nerve structure exhibited more axonal and endoneural degeneration, along with slight symmetrical differences in axons, compared to control group. Endoneural degeneration in SN+Thio-IM group was more progressive compared to control and SN+Vehicle-IM groups. In SN+Vehicle-IN group, the axonal and endoneural degeneration was stronger compared to IM-injected groups accompanying more symmetrical differences in sciatic nerve axons. The tissue structure in SN+Thio-IN group was the most degenerated one compared to other groups.

## Discussion

The World Health Organization (WHO) declared that unsafe injections are closely associated with morbidity and mortality. They also acknowledged that intramuscular gluteal injections can result in sciatic nerve deformation and neuropathy, defined as SNII[4]. SNII can cause serious clinical conditions, namely paresthesia and neurological symptoms[28]. Injuries due to injections to the nerve or surrounding tissues can arise from allergic reactions caused by drug composition or direct nerve fiber damage[29]. In case of SNII that does not come up due to direct physical damage of injector, the toxic effect of the injected drug in interfascicular region can cause sciatic nerve damage after its epineural accumulation around the sciatic nerve[30]. Based on the clinical case records of Türkiye Supreme Council of Health regarding sciatic nerve injury complaints due to intramuscular gluteal injections, Thio has been noted as one of the most prevalent agents being injected intramuscularly[31]. Thio is utilized as a muscle relaxant, anti-inflammatory, and analgesic agent in clinical practice, and among its adverse effects, vasovagal reactions such as nausea, inertia, and allergic reactions can be listed[32]. After IM Thio injection to dorsogluteal region, sciatic neuropathy, paralysis cases, and other emergency situations are commonly experienced beside local pain complaints[8,9]. Post-injection injuries start to become evident within the first 30 min and are characterized with prevalent axon and myelin degeneration[33]. We aimed to investigate the effect of Thio injection on acute neuropathy in the gluteal region between 30 min and 48 h in the present study, using several behavioral tests and histopathological examinations. There are similar studies in the literature focusing on neuropathic pain caused by sciatic nerve injection[31] however, best to our knowledge, there is no study comparing the effect of Thio injection on sciatic nerve via intramuscular or intraneural route.

We used rotarod test to analyze the effect of neuropathic pain on motor functions of animals. We observed a significant difference between control and both IM- and IN-injected groups at the time points between 30 min and 6 h, suggesting that both IM and IN injections can cause motor function deficits. Moreover, IN injections worsened motor functions more notably till 48 h unlike IM injections. The reduced motor function after IM Thio injection compared to vehicle between 6-24 h also shows the neurotoxic effect of Thio whereas negative effects of IN injections arise substantially from injector-mediated physical damage beside Thio′s partial toxic effect.

Nociceptive effect of gluteal injections on sciatic nerves was assessed with tail flick test and we observed that both IM and IN injections between 1-12 h caused a marked decrease in nociceptive reflex compared to control group. At later time points, the effect of IM injections disappeared more likely due to healing of Thio-induced toxicity on sciatic nerve, despite the consistent impact of IN injections that led to more severe damages than IM injections due to physical contact of the injector to the nerve fibers. Additional nociceptive tests, called cold plate and

Von Frey tests, were conducted to measure thermal and mechanical pain thresholds respectively. Similar to tail flick test, both IM and IN injections resulted in loss of pain perception compared to control animals at earlier time points. Later on, this effect had been restored in IM injected animals after healing of Thio induced toxicity whereas IN-injected animals could not be recovered.

PAGE test was used to analyze paw muscle capacity of animals after injections. IM and IN injections decreased the endurance of animals compared to control at early time point. After 6 h, despite the recovery of IM-injected animals, the damage on the muscle capacity of IN-injected animals lasted till 48 h. Basically all behavioral tests revealed common outcomes suggesting that, IN injections resulted in heavier and insistent damages on sciatic nerves regarding both pain perception and motor components due to its mechanical impact on nerve fibers compared to temporary toxic effect of IM Thio injection-related impairments on sciatic nerves.

We observed after histopathologic analyses that consistent with the outcomes of behavioral tests, IM and IN injections bring about endoneural degenerations at different levels. Axonal degeneration was more substantial in IN-injected animals compared to IM-injected ones due to the deeper damage of injector on nerves by mechanical impact. IM Thio injections caused minimal degenerations on nerve fibers meaning that Thio could exert neurotoxic effect by diffusion around the sciatic nerve tissue after IM injection. IN Thio injection led to the most severe axonal and endoneural degeneration pattern among other groups, which demonstrates the synergistic effect of both drug toxicity and mechanical impact of the needle on nerve fibers.

Injection is of critical importance as one of the iatrogenic reasons of sciatic nerve injury etiopathology in the world. Some of the factors increase the collateral risk level of IM injections that can seem to be a safe clinical practice and endangers patients' health such as inaccurate choice of the injection location, lack of anatomical knowledge and practical skills, side effects of the injected drug. Sciatic nerve damage is one of the most prevalent types of injury caused by intragluteal injections[3]. Gluteus medius muscle is the most preferred region for IM injections in adults. Serious complications can occur at this location due to the transition of sciatic nerves throughout the inner side of the gluteal muscle. In case of mechanical traumas post-injection, nerve fiber is directly damaged by the injector and sciatic nerve lesion can be in form of demyelinization injury, axonal degeneration, and partial or complete nerve rupture[7]. In ischemic and toxic mechanisms, as a result of intra- or epi-injections of chemical stimuli to vasa venorum or sciatic nerve can lead to wound formation on sciatic nerve[34]. The type of drug is another critical factor in injection-related neuropathy beside the injection location. Analgesics, antibiotics, and anti-inflammatory agents are among the most prominent toxic drugs that lead to toxic neuropathy[35].

Thio is primarily used to treat conditions such as fibromyalgia, lumbar pain, lumbago, tension-type headache, myofascial pain, and muscle spasms. However, patients have common pain complaints after gluteal Thio injections. In the present study, we aimed to determine the probable toxic effect of Thio, commercially known as Muscoril, on the sciatic nerve. Specifically, we investigated whether the source of pain is chemical or mechanical.

The most common side effects after Thio injection are mild nausea and vomiting; however, oral uptake of the drug rarely causes side effects[36]. In an *in vivo* rat model comparing the effects of extraneural and intraneural injections of Meloxicam and diclofenac on sciatic nerve, Meloxicam caused milder toxic effects compared to diclofenac[37]. In the same study, the authors underlined that the sciatic nerve injury developed particularly when the injector targeted the nerves. Parallel with these findings, we demonstrated with histological examinations that IN Thio injections caused axonal degeneration at levels similar to IN vehicle injection, suggesting that sciatic nerve injury resulted primarily from the mechanical impact of the injector rather than the toxic effect of the agent. Additionally, behavioral test results indicated that IM Thio injection led to acute, temporary neuropathy at early time points, establishing the mild neurotoxicity of the agent. These findings align with another *in vivo* study which found that Thio exerted strong convulsion activity in rats due to an antagonistic interaction with a cortical subunit of the GABAA receptor[38]. Additionally, Thio is used to treat muscle pain, relaxant with anti-inflammatory and analgesic effects by suppressing osteoclastogenesis induced by RANKL and tumor cells via the NF-κB signaling pathway and reduced reactive oxygen species (ROS) generation[39,40]. Overall, our study suggests that the neurotoxic effect of Thio is temporary while sustained neuropathy in IN injection groups indicates that neuropathy progression in mice results from sciatic nerve injury due to physical contact with the injector.

There are certain limitations of the present study. First, immunohistochemistry analyses are missing, which could provide information concerning the expression levels of inflammatory, apoptotic, and other pain-related biomarkers on sciatic nerve tissue. Additionally, quantitative protein assays such as ELISA and/or Western blot could be conducted to reach more conclusive rational results for interpretation.

## Conclusion

Thio is commonly used in emergency services owing to its spasmolytic activity and thus, injection-induced neuropathy complaints can increase due to its neurotoxic nature just like diclofenac. Based on our findings, IN-Thio injections caused the most severe endoneural and axonal degenerations partly due to Thio itself while mostly due to physical impact of the needle. Thio injections should be administered to gluteal regions of the patients with great care and attention. Additionally, SNII related neuropathies triggered by Thio injection can be prevented by favoring ventrogluteal region injections over dorsogluteal ones due to fewer large vessels and nerve bundles as well as less subcutaneous fat composition in ventrogluteal area.

## Ethical statement

Ethical approval was obtained from Çukurova University Animal Experiments Local Ethics Committee (Approval date-no: 15/04/2021-2).

## Conflict of interest

The authors declare that they have no known competing financial interests.

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