ORIGINAL ARTICLE

*Veterinary Research Forum. 2025; 16 (2): 89 - 96*
doi: 10.30466/vrf.2024.2025053.4204
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

# Thymoquinone attenuates paw incision-induced spontaneous and evoked pain through anti-oxidative and anti-inflammatory mechanisms in rats

**Seyed Siavash Ghoreishi[1], Saeed Azizi[2]*, Esmaeal Tamaddonfard[3], Safiyeh Aghazadeh[3]**

*[1] DVSc Candidate, Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [2] Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [3] Department of Basic Sciences, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.*

**Article Info**

**Article history:**

Received: 16 March 2024
Accepted: 07 May 2024
Available online: 15 February 2025

**Keywords:**

Celecoxib
Incision
Pain
Rats
Thymoquinone

## Abstract

Surgery commonly causes post-operative pain that should be alleviated to prevent complications. In addition to the use of synthetic drugs, there has been a widespread desire to use medicinal plants for surgical pain management. Thymoquinone (TQ), a constituent of *Nigella sativa* black seeds, exhibits a potent anti-oxidant property. Celecoxib (CLX), a potent non-steroidal anti-inflammatory drug, is widely used in pain management. In the present study, the effects of TQ and CLX on pain caused by hind paw surgical incision were compared. Fifty-six rats were divided into four groups of 14 rats as intact, vehicle, TQ, and CLX groups. In each group, six rats were planned to record pain-related behaviors on days 1 - 10 and eight rats were designed for determination of serum biochemical alterations on days 1 (four rats) and 3 (four rats) after surgery. Oral administrations of TQ and CLX at a same dose of 10.00 mg $kg^{-1}$ alleviated paw lifting number (spontaneous pain) and paw withdrawal threshold evoked by von Frey filaments on metal mesh floor, improved the decreased contents of serum total anti-oxidant capacity and superoxide dismutase, and restored the increased levels of serum malondialdehyde and tumor necrosis factor-alpha. The results suggested that TQ by employing anti-oxidant and anti-inflammatory mechanisms, might relieve the pain induced by hind paw plantar incision, being comparable with CLX.



## Introduction

Surgeries commonly cause post-operative pain, which should be alleviated in order to prevent complications, such as depression, anxiety, cognitive impairment, and transition from acute to chronic condition.[1,2] Due to the recruitment of multiple mechanisms at the local peripheral, spinal, and supra-spinal levels, various therapeutic approaches, such as the use of opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and neurotransmitter receptor antagonists are available in the management of acute post-operative pain.[3] The possible side effects of NSAIDs on the body tracts, such as digestive, cardiovascular, and renal systems are known, and despite the effective analgesic effects of these drugs, their usage should be considered with cautiousness.[4] Although opioids are the first line of treatment for pain, they cause side effects, including sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance, and respiratory depression.[5] Searching for alternative treatments with very few side effects is one of the goals of pain management.[6] The use of medicinal plants and their biologically active components has been established for the management of acute surgical pain treatment.[7,8] In this context, oral administration of standardized extract of *Ginkgo biloba* increased paw withdrawal latency in the hind paw incisional model of pain in rats.[9]

Black cumin (*Nigella sativa L.*) and its main bioactive component, thymoquinone (TQ; 2-isopropyl-5-methyl-1, 4-benzoquinone) with anti-oxidative and anti-inflammatory properties, exhibit beneficial protective effects against cardiovascular, renal, digestive, and neurological disorders.[10,11] Laboratory animals studies suggest anti-nociceptive effects of TQ in the acute and chronic pain models. For example, intra-plantar and intracerebroventricular injections of TQ caused anti-nociceptive effects in both early and late phases of

***Correspondence:**
Saeed Azizi. DVM, DVSc
Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
**E-mail:** s.azizi@urmia.ac.ir

formalin-induced pain through L-arginine/NO/cGMP/$K_{ATP}$ (nitric oxide (NO), 3', 5'-cyclic guanosine monophosphate (cGMP) and potassium (K+) channels, ATP-sensitive K+ channels) channel pathway.[12] In addition, intra-peritoneal injection of TQ alleviated behavioral signs and improved biochemical outcomes induced by chronic constrictive injury of sciatic nerve in rats.[13]

Inflammation due to tissue injury is the main outcome of acute post-operative pain.[14] To overcome inflammation and pain, NSAIDs, including both the cyclooxygenase (COX)-1 and COX-2 inhibitors (ibuprofen, diclofenac, and indomethacin), and selective COX-2 blockers (celecoxib [CLX] and rofecoxib) are frequently used in a short-term post-operative period.[15] In this context, it has been reported that oral administrations of indomethacin, CLX, and naproxen alleviate mechanical hyperalgesia and tactile allodynia in the rat model of hind paw incisional pain.[16]

As far as we know, there has been no report regarding the effect of TQ and its possible mechanisms in acute surgical pain. Therefore, in the present study, the effect of short-term oral administration of TQ on spontaneous and evoked pain was investigated in the hind paw incision model of surgical pain. Possible mechanisms of TQ effect were followed by biochemical determination of oxidative and inflammatory markers. Also, the effects of TQ were compared with CLX.

## Materials and Methods

**Animals.** Adult male *Wistar* rats (180 - 200 g) were used throughout the study. The animals were maintained in standard breeding conditions (temperature: 22.00 ± 0.50 ˚C, humidity: 60.00 - 70.00%, and 12 hr dark-light cycles) with free access to food and water. Pain-related behavior recording was done between 10.00 AM - 15.00 PM. Veterinary Ethics Committee of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, approved the study protocol (Ethical Code: IR-UU-AEC-3/83).

**Drugs and kits.** The TQ and CLX were purchased from Sigma-Aldrich, St. Louis, USA. Both chemical compounds were dissolved in Tween 80.00% and diluted by normal saline, until the concentration of Tween was reached to 5.00%. Therefore, in the present study, Tween 5.00% was used as a vehicle. Chemical solutions were prepared 30 min before use. All the analytical chemicals, including sodium dodecyl sulphate, acetic acid, thiobarbituric acid (TBA), n-butanol, pyridine, 2,4,6-tripyridyl-S-triazine, and ferric chloride hexahydrate were purchased from Merck Chemical Company (Darmstadt, Germany). Navand Salamat Company (Urmia, Iran) supplied malondialdehyde (MDA), superoxide dismutase (SOD), and total anti-oxidant capacity (TAC) assay kits. Moreover, ELISA kit of tumor necrosis factor-alpha (TNF-α; Diaclone, Besançon, France) was purchased.

**Study protocol.** During this study, in order to be compatible with the test conditions, the rats were placed on metal mesh floor for three consecutive days once every day for one hr. Then, baseline pain data were recorded on two consecutive days. Later, they underwent plantar incision surgery.[17] Thereafter, on days 1 - 10 after surgery, spontaneous and evoked pains were recorded after placing animals on a metal mesh floor. Pain behaviors were recorded 1 hr after drug treatment. Some of the rats (as follows) were euthanized on days 1 and 3 after surgery, and serum samples were prepared to determine biochemical alterations.

**Animal grouping.** In the present study, 56 rats were divided into four groups of 14 rats in each as follows:

Group 1 (intact group)**:** This group did not receive any kind of anesthetic or treatment, and no surgery was performed; Group 2 (vehicle group): This group received TQ and CLX vehicle (Tween 5.00%; 2.00 mL $kg^{-1}$) after surgery; Group 3 (TQ group): This group received 10.00 mg $kg^{-1}$ TQ after surgery; Group 4: This group was treated with 10.00 mg $kg^{-1}$ CLX after surgery. The drug dosages used in the current study are consistent with other studies,[18,19] and our preliminary experiments.

**Plantar incision surgery.** Plantar incision surgery was performed according to the previously described method.[20] Under anesthesia using 80.00 mg $kg^{-1}$ ketamine (Alfasan, Woerden, The Netherlands) and 8.00 mg $kg^{-1}$ xylazine (Alfasan) via intra-peritoneal injection, the plantar surface of the left hind paw was cleaned and prepared for surgery. A 1.50 cm longitudinal midline incision was made on the skin, starting from one-half inch from the proximal end of the heel. The underlying fascia was incised and using blunt dissection, the plantaris muscle was exposed and a longitudinal incision was made on the belly of the muscle. Subsequently, the wound was closed with two 4-0 nylon (Supa, Tehran, Iran) horizontal mattress sutures as the knots placed on the lateral side of the incision. Finally, the rat was transferred to a warm recovery chamber. Different stages of the rat plantar incision surgery are shown in Figure 1.

**Oral administration.** The TQ and CLX were dissolved in 80.00% Tween and diluted with normal saline until the Tween concentration reached to 5.00%. The prepared drug solutions were orally administered by gavage at the constant volume of 2.00 mL $kg^{-1}$.[18] The TQ and CLX were administered at 4 hr, and days 1 and 2 after plantar incision surgery.

**Spontaneous pain assessment.** Spontaneous pain behavior was measured 2 days before, and at days 1 - 10 after surgery. For this purpose, the animals were placed on a metal mesh floor, and after the 20-min adaptation period, the number of paw lifting was counted for 15 min using a laboratory manual counter.[21]

**Evoked pain assessment.** Evoked pain was assessed by determining the paw withdrawal threshold (PWT) after

application of von Frey filaments in the heel of the left hind leg. Eleven von Frey filaments (IITC-Life Science Instruments, Woodland Hill, USA) were chosen (von Frey numbers: 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, being equivalent to 0.40, 0.80, 1.00, 1.20, 1.50, 2.50, 3.60, 4.00, 8.00, 10.00, and 20.00 g, respectively). Animals were placed in a transparent plastic box (25.00 ×15.00 × 20.00) with a metal wire mesh floor. After a 20-min adaptation period, the middle filament (number 8, being equivalent to 2.50 g) in the series was repeatedly applied over a 10-sec time interval from below and perpendicular to the heel for approximately 5-7 sec. If there was a withdrawal response to the filament stimulation (positive), the next lower force was delivered. If the response was negative, the next higher force was applied. After recording of positive and negative responses, PWT 50.00% was calculated using the following formula:

$$PWT\ 50.00\% = 10^{(Xf+kd)}$$

where, *Xf* is the value of the final von Frey filament used (in log units), *k* is the tabular value for the pattern of positive/negative responses, and *d* is the mean difference between stimuli in log units (0.155). This method of determining evoked pain and the corresponding formula have been used in other studies regarding paw incision pain.[22,23]

**Serum biochemical assay.** On days 1 and 3 after surgery, four rats from each group were anesthetized using ketamine (100 mg $kg^{-1}$) and xylazine (10.00 mg $kg^{-1}$), blood samples were taken directly from the heart and then, the rats were euthanized by intra-cardiac injection of 1.00 mL ketamine. Serum samples were obtained by blood centrifuging and stored at – 20.00 ˚C for further assay of TAC, SOD, MDA, and TNF-α. The serum biochemical evaluation time was chosen based on the Wang *et al*., study and our preliminary experiment results.[24]

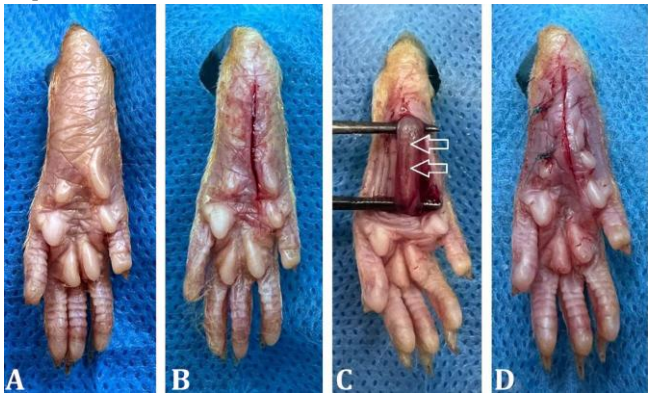


**Fig. 1.** Photographs of the different stages of the plantar incision surgery. **A)** Surgical preparation of the intact left plantar hind paw; **B)** A 1.50-cm longitudinal skin incision is seen; **C)** Exposing and longitudinal incision (white arrows) of the plantaris muscle are shown; and **D)** Closing the skin incision with two horizontal mattress stitches is observed.

Samples TAC levels were determined using TAC assay kit according to the manufacturer's instruction. Briefly, TAC was measured based on the ferric reducing ability of plasma method. The anti-oxidant power was estimated based on the reduction of $Fe^{3+}$-tripyridyltriazine (TPTZ) to an intense blue color $Fe^{2+}$-TPTZ complex with an absorption maximum at 593 nm (UV-975; Jasco, Tokyo, Japan) at low pH, and the final results were presented as mmol $L^{-1}$. The serum SOD activity was analyzed by SOD assay kit based on the manufacturer's protocol. Briefly, the principle of SOD enzyme assay is inhibition of pyrogallol oxidation. Pyrogallol is a compound being rapidly auto-xidized in the presence of molecular oxygen in an alkaline environment. As a result of autoxidation of pyrogallol, an intermediate compound called semiquinone radical is produced, followed by pyrogallol-quinone production, and the last compound can be measured at 420 nm. The higher the activity of the enzyme, the less pyrogallol-quinone compound is produced. Finally, the activity of SOD is expressed as U $L^{-1}$. To evaluate serum MDA level, a commercial MDA assay kit was used. Briefly, estimation of MDA is based on reaction with TBA and generation of the MDA-TBA adduct. The MDA-TBA adduct can be simply quantified colorimetrically at the wavelength of 532 nm by a spectrophotometer. The findings are expressed as nmol $mL^{-1}$. Serum TNF-α contents were evaluated using ELISA kit pursuant to the manufacturing company's instruction. Briefly, rat TNF-α standards and samples were bounded to the coated antibodies, followed by binding of the biotinylated anti-rat TNF-α secondary antibody. Next, horseradish peroxidase conjugate solution was added to the wells. Then, for color visualization, chromogen substrate was added to the wells and after blue color development, it was stopped by the addition of acid. The strength of the produced color was directly proportional to the amount of TNF-α being present in the samples. Finally, the optical density of the color of each well was measured and plotted against concentration. The concentration of rat TNF-α in samples was estimated using standard curve and presented as pg $mL^{-1}$.

**Statistical analysis.** Data were statistically analyzed using GraphPad Prism (version 8.2; GraphPad Software Inc., San Diego, USA). The time-point results were analyzed using two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. Serum biochemical alterations were analyzed by one-way ANOVA followed by Tucky's *post hoc* test. Data are presented as mean ± SEM. A *p* value less than 0.05 was considered statistically significant for all results.

## Results

Figure 2 shows the effects of TQ and CLX on spontaneous pain (paw lifting) after paw incision. Intact group exhibited no signs of spontaneous pain.

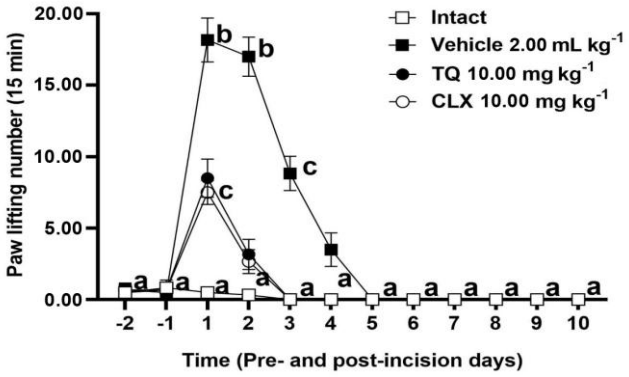


**Fig. 2.** The effects of oral administrations of thymoquinone (TQ), celecoxib (CLX), and the vehicle on the paw lifting number following plantar incision surgery. Intact group was used to compare the effects of TQ with CLX. The TQ and CLX were orally administered at 4 hr, and days 1 and 2 after surgery.
[abc] Non-similar letters show significant differences among groups (a *vs* b: $p < 0.001$, a *vs* c: $p < 0.01$, and b *vs* c: $p < 0.01$).

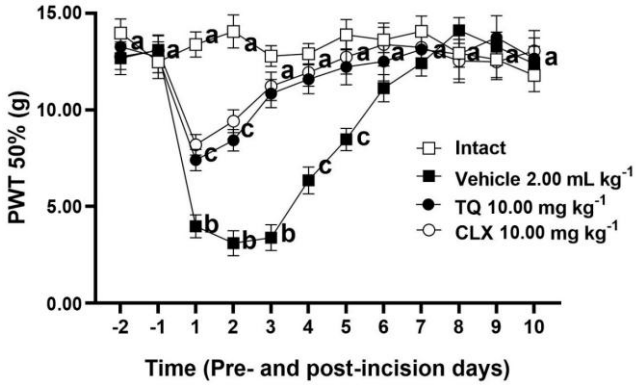


**Fig. 3.** The effects of oral administrations of thymoquinone (TQ), celecoxib (CLX), and the vehicle on the percentage of paw withdrawal threshold (PWT 50.00%) following plantar incision surgery. Intact group was used to compare the effects of TQ with CLX. The TQ and CLX were orally administered at 4 hr, and days 1 and 2 after surgery.
[abc] Non-similar letters show significant differences among groups (a *vs* b: $p < 0.001$, a *vs* c: $p < 0.01$, and b *vs* c: $p < 0.01$).

Compared to the intact group, the vehicle-receiving group showed maximal paw lifting on the first (18.17 ± 1.54; $p < 0.001$) and second (17.01 ± 1.37; $p < 0.001$) days after surgery. On the third day, a significant difference (8.83 ± 1.21; $p < 0.01$) was also observed between the intact and vehicle-receiving groups. From the fourth day to the end of the experiment, these two groups did not show any significant differences. Oral administrations of TQ and CLX at a similar dose of 10.00 mg $kg^{-1}$ significantly reduced the numbers of paw lifting at day 1 (TQ: 8.49 ± 1.34 and CLX: 7.47 ± 0.85; $p < 0.01$), and with no significant differences with intact group, reached them to 3.17 ± 1.05 and 2.67 ± 0.84 at day 2 after surgery, respectively. No significant effects were expressed between TQ and CLX regarding the paw lifting.

Figure 3 shows the effects of TQ and CLX on evoked pain (mechanical allodynia) after paw incision. Intact group showed PWT 50.00% as 13.21 ± 0.51 g. Compared to intact group, vehicle-receiving group showed significant minimum PWT 50.00% on days 1 (3.97 ± 0.59 g; $p < 0.001$), 2 (3.11 ± 0.64 g; $p < 0.001$), and 3 (3.39 ± 0.66 g; $p < 0.001$) after surgery. Significant elevations of PWT 50.00% were also observed between intact and vehicle-receiving groups on day 4 (6.35 ± 0.69 g; $p < 0.01$) and day 5 (8.47 ± 0.57 g; $p < 0.05$) after surgery. From the sixth day to the end of the study, these two groups did not show significant differences in PWT 50.00%. Oral administrations of TQ and CLX at a same dose of 10.00 mg $kg^{-1}$ significantly increased PWT 50.00% at days 1 (TQ: 7.41 ± 0.55 g and CLX: 8.19 ± 0.53 g; $p < 0.01$) and 2 (TQ: 8.42 ± 0.56 g and CLX: 9.41 ± 0.51 g; $p < 0.01$) after surgery. From day 3 to the end of the experiment, the effects of TQ and CLX showed no significant differences in comparison with the intact group. There were no significant differences between the effects of TQ and CLX on PWT 50.00%.

Figure 4 shows the effects of TQ and CLX on serum biochemical alterations after paw incision injury.

Serum TAC levels (Fig. 4A) were 1.53 ± 0.08 mmol $L^{-1}$ at day 1 and 1.52 ± 0.05 mmol $L^{-1}$ at day 3 in intact group. These values significantly decreased to 0.93 ± 0.08 mmol $L^{-1}$ at day 1 ($p < 0.01$) and 1.35 ± 0.07 mmol $L^{-1}$ at day 3 after surgery. The incision-induced reductions of TAC levels were significantly improved by 10.00 mg $kg^{-1}$ TQ and CLX treatments at day 1 ($p < 0.01$).

Serum activities of SOD were 76.09 ± 3.94 U $L^{-1}$ and 71.39 ± 3.93 U $L^{-1}$ at days 1 and 3 in intact rats, respectively. The hind paw incision significantly reduced SOD activity at day 1 (47.54 ± 3.49 U $L^{-1}$; $p < 0.01$), but insignificantly at day 3 (67.19 ± 4.38 U $L^{-1}$). The elevated SOD activities induced by hind paw incision were significantly restored by 10.00 mg $kg^{-1}$ TQ and CLX treatments at day 1 ($p < 0.01$).

Serum levels of MDA were 3.02 ± 0.15 nmol $mL^{-1}$ and 3.52 ± 0.19 nmol $mL^{-1}$ at days 1 and 3 in intact group, respectively. The MDA levels were significantly increased at day 1 (6.49 ± 0.19 nmol $L^{-1}$; $p < 0.01$), but inconsiderably at day 3 (4.38 ± 0.21 nmol $L^{-1}$) after hind paw incision. These increased serum MDA levels were attenuated by 10.00 mg $kg^{-1}$ TQ and CLX treatments at day 1 ($p < 0.01$) after surgery.

Serum levels of TNF-α were 48.75 ± 5.68 pg $mL^{-1}$ and 51.37 ± 4.11 pg $mL^{-1}$ at days 1 and 3 in intact rats, respectively. The levels of TNF-α were significantly increased at day 1 (92.38 ± 5.21 pg $mL^{-1}$; $p < 0.01$), but insignificantly at day 3 (63.93 ± 5.76 pg $mL^{-1}$) after hind paw incision. These increased serum levels of TNF-α were decreased by 10.00 mg $kg^{-1}$ TQ and CLX treatments at day 1 ($p < 0.01$). No significant differences were observed between the effects of TQ and CLX on serum contents of TAC, SOD, MDA, and TNF-α (Figs. 4A-D).

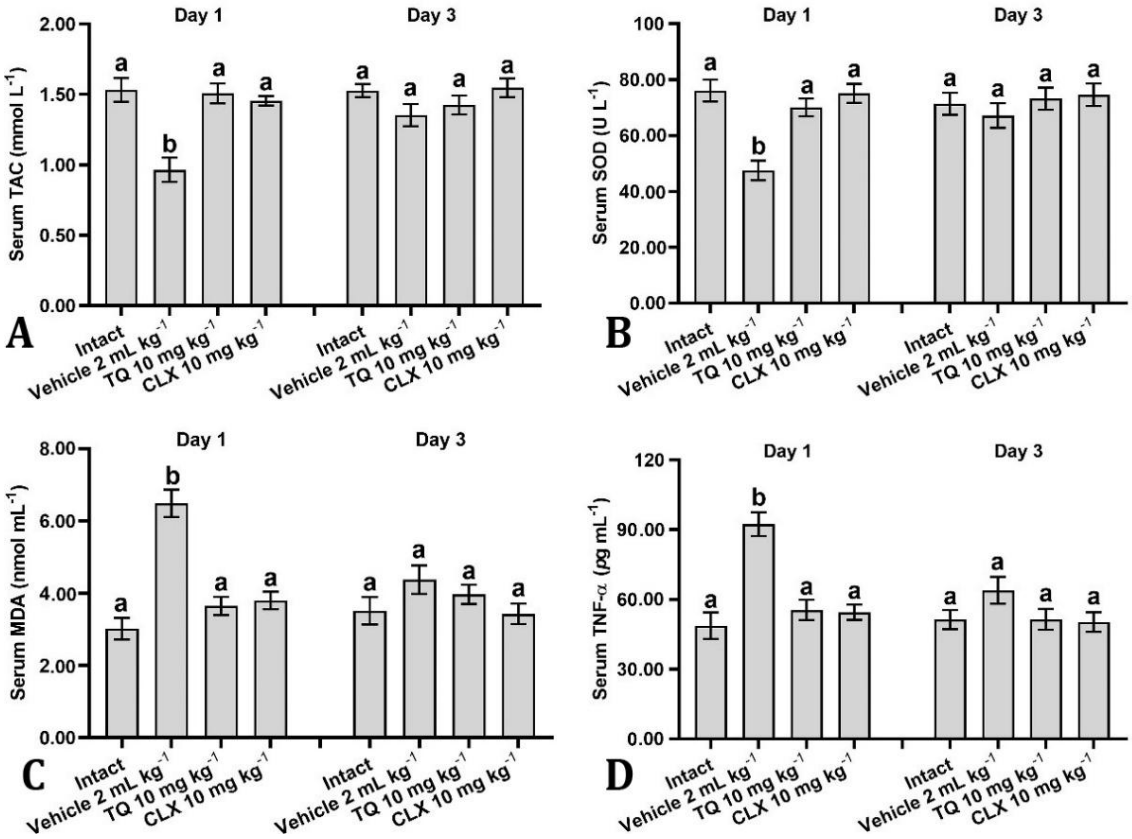


**Fig. 4.** The effects of oral administrations of thymoquinone (TQ), celecoxib (CLX), and the vehicle on serum biochemistry at days 1 and 3 after plantar incision surgery. **A)** Total anti-oxidant capacity (TAC); **B)** Superoxide dismutase (SOD); **C)** Malondialdehyde (MDA); and **D)** Tumor necrosis factor-α (TNF-α). Intact group was used to compare the effects among the experimental groups. The TQ and CLX were orally administered at 4 hr, and days 1 and 2 after surgery. Similar letters indicate no significant differences. Non-similar letters show significant differences among groups (a *vs* b: $p < 0.01$).

## Discussion

The results of the current study showed that spontaneous pain with the characteristic of paw lifting occurs after surgical incision. This pain behavior had the highest intensity on the first and second days after surgery, decreased on the third day, and reached the intact level on the fourth day. Using aluminum mesh, it has been explained that paw lifting starts 3 hr after plantar skin and muscle incisions and continues until the fourth day after surgery.[21] By measuring the weight bearing of the incised and non-incised paws to determine spontaneous pain, maximum pain intensity has been reported on the first day after surgery.[25] Therefore, our study results might support the above-mentioned findings. In the present study, evoked pain was induced using plantar surface ascending-descending application of von Frey filaments and the resulting behavior, paw withdrawal, was recorded and calculated as PWT 50.00%. The PWT 50.00% was at its lowest on days 1 - 3 after surgery. On the fourth and fifth days, the PWT 50.00% elevated towards the intact PWT 50.00%, reached the intact level on the sixth day, and remained at this level to the end of the recording period. By application of von Frey filaments at the heel of the foot with a plantar incision, it has been determined that the reduction of the PWT starts from 2 hr after surgery, decreases more on days 1 to 4, and from day 5 onward returns to the pre-surgery level.[20,21] It has been reported that the peripheral sensitized C- and Aδ-fibers contribute to non-evoked pain, as well as mechanical hyperalgesia and allodynia in the early days after incisions of skin and muscle.[26,27] It has been demonstrated that this sensitization occurs due to the release of various signal molecules, such as histamine, serotonin, interleukin-beta, and TNF-α from neutrophils and mast cells called to the surgical area.[14]

The TAC is believed to be the cumulative effect of all anti-oxidants in a tissue, blood, or body fluids, and SOD is one of the major parts of anti-oxidant defense enzymatic system in all cells exposed to $O_2$.[28] The MDA is a molecule being generated as a result of membrane polyunsaturated fatty acid lipid peroxidation and hence, its amount is considered as a primary indicator of lipid peroxidation and oxidative damage in a tissue.[29] The TNF-α is a cytokine being identified as a major regulator of inflammatory responses.[30] The decrease in the serum levels of TAC and

SOD, and increase in the serum levels of MAD and TNF-α at the first, but not third day after surgery, observed in our study, might indicate that acute surgical pain suppresses the anti-oxidative system, while the oxidative and inflammatory systems are activated. Although there are no reports showing serum level alterations of TAC, SOD, MDA, and TNF-α following plantar incision surgery in rats, the changes of these biomarkers in hind paw tissues and spinal dorsal root ganglion in relation to the incisional pain have been investigated. For example, it has been reported that the level of MDA increases and total glutathione content decreases in plantar tissue on the first day after plantar incision surgery.[31] In addition, plantar incision surgery caused increased MDA and decreased SOD contents in the dorsal root ganglion.[32] Moreover, in a rat model of post-operative pain, the concentrations of TNF-*α* at the site of injury increased within 24 to 48 hr of surgery and remained elevated for 4 to 8 days after surgery.[33] Having said that, the serum oxidative, anti-oxidative, and inflammatory biomarkers alterations observed in the current study are consistent with pain intensity on the first, but not on the third day, and the alterations on the first day after surgery might be related not only with the entry of biomarkers into the blood circulation but also with the acute stress caused by surgery.[34]

In the present study, TQ attenuated post-operative pain and improved the serum alterations of the studied biomarkers. In other words, TQ might have created an analgesic effect through anti-oxidative, anti-inflammatory, and anti-oxidant enhancing mechanisms. There are no reports describing the effect of TQ on surgical pain. Although the effect of TQ on surgical pain has not been reported so far, its beneficial analgesic effects in other pain models and possible mechanisms have been investigated to some extent. For example, in the model of pain caused by spinal cord injury, TQ increased the threshold of mechanical withdrawal and latency of thermal withdrawal by reducing the serum levels of nitric oxide, MDA, and TNF-α.[35] By inhibiting the analgesic action of TQ after intra-plantar and intra-cerebroventricular injections of L-NAME (a nitric oxide synthase inhibitor), glibenclamide (a blocker of KATP channel), and tetraethylammonium (a $K_V$ channel blocker), a role of the L-arginine/NO/cGMP/KATP channel signaling pathway in local and central analgesic effects of TQ has been suggested in the chronic constriction model of neuropathic pain.[12] In addition, it has been reported that central cannabinoid and α2-adrenergic receptors are involved in the anti-nociceptive effect of TQ in acetic acid-induced visceral pain.[18]

In the present study, CLX attenuated surgical pain and restored the serum changes associated with oxidative and inflammatory systems. The NSAIDs exert anti-inflammatory and analgesic effects by inhibiting COX-2, an enzyme responsible for causing up-regulation of prostaglandins and other pro-inflammatory markers.[36] Coxibs, including rofecoxib, valdecoxib, and CLX, have similar efficacy to non-selective NSAIDs and superiority in the post-operative setting because of reduced adverse events.[37] In this context, CLX effectively reversed mechanical hyperalgesia and tactile allodynia in the incised rat hind paw.[16] In addition, in a rat plantar incision post-operative pain model, CLX produced anti-hyperalgesic effect by reducing mechanical and thermal hyperalgesias.[38] The CLX attenuated oxaliplatin-induced peripheral neuropathic pain by reducing sciatic nerve levels of COX-2, prostaglandin E2, TNF-α, and MDA and increasing Bcl-2 and TAC contents.[39] Therefore, CLX exerts its pain-reducing action *via* several mechanisms, including anti-oxidative and anti-inflammatory mechanisms.

Based on the analgesic, anti-oxidative, and anti-inflammatory functions identified in the present study, it could be suggested that TQ and CLX produce almost similar effects. In different models of pain, including surgical pain, the analgesic effects of CLX have been compared with other synthetic and natural substances with analgesic effects. This comparison was made to use new drugs with less side effects. For example, almost similar effects have been reported in the analgesic effects of diclofenac, acetaminophen, and CLX after minor oral surgery.[40] It has been found that *Salvia officinalis* extracts produce similar or even better anti-oxidant, anti-inflammatory, and analgesic effects compared to CLX.[41] Therefore, the results of the present study might be considered as the first report on comparing the anti-oxidative, anti-inflammatory, and anti-nociceptive effects of CLX with the active ingredient of a medicinal plant, *N. sativa*, black seeds.

Based on the present results, hind paw incision causes spontaneous and evoked pains, as well as serum biochemical alterations in rats. Oral administrations of TQ and CLX attenuate paw lifting (spontaneous pain) and PWT 50.00% (evoked pain). This anti-nociceptive effect accompanies with the serum level changes of TAC, MDA, SOD, and TNF-α. The effects of TQ and CLX were approximately similar. Therefore, TQ and CLX might produce anti-nociceptive effects by employing anti-oxidative and anti-inflammatory mechanisms.

## Acknowledgments

This work was financially supported by Office of Vice Chancellor for Research of Urmia University, Urmia, Iran (Thesis No. 18538/1402-07-29).

## Conflict of interest

No financial or other conflicts of interest are declared by the authors.

## References


1. Ghoneim MM, O'Hara MW. Depression and post-operative complications: an overview. BMC Surg 2016; 16: 5. doi: 10.1186/s12893-016-0120-y.
2. Chapman CR, Vierck CJ. The transition of acute postoperative pain to chronic pain: an integrative overview of research on mechanisms. J Pain 2017; 18(4):359.e1-359.e38. doi: 10.1016/j.jpain.2016.11.004.
3. Small C, Laycock H. Acute postoperative pain management. Br J Surg 2020; 107(2): e70-e80. doi: 10.1002/bjs.11477.
4. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications J Pharm Pharm Sci 2013; 16(5): 821-847.
5. Benyamin R, Trescot AM, Datta S, et al. Opioid complications and side effects. Pain Physician 2008; 11(2 Suppl): S105-S120.
6. Mitra S, Carlyle D, Kodumudi G, et al. New advances in acute postoperative pain management. Curr Pain Headache Rep 2018; 22(5): 35. doi: 10.1007/s11916-018-0690-8.
7. Park KM, Kim JH. Herbal medicine for the management of postoperative pain: a protocol for the systematic review of randomized controlled trials. Medicine (Baltimore). 2019; 98(1): e14016. doi: 10.1097/MD.0000000000014016.
8. Arruda APN, Ayala AP, Lopes LC, et al. Herbal medications for surgical patients: a systematic review protocol. BMJ Open 2017; 7(7): e014290. doi: 10.1136/bmjopen-2016-014290.
9. Biddlestone L, Corbett AD, Dolan S. Oral administration of *Ginkgo biloba* extract, EGb-761 inhibits thermal hyperalgesia in rodent models of inflammatory and post-surgical pain. Br J Pharmacol 2007; 151(2): 285-291.
10. Hannan MA, Rahman MA, Sohag AAM, et al. Black cumin (*Nigella sativa* L.): a comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients 2021; 13(6): 1784. doi: 10.3390/nu13061784.
11. Pottoo FH, Ibrahim AM, Alammar A, et al. Thymoquinone: review of its potential in the treatment of neurological diseases. Pharmaceuticals (Basel) 2022; 15(4): 408. doi: 10.3390/ph15040408.
12. Parvardeh S, Sabetkasaei M, Moghimi M, et al. Role of L-arginine/NO/cGMP/$K_{ATP}$ channel signaling pathway in the central and peripheral antinociceptive effect of thymoquinone in rats. Iran J Basic Med Sci 2018; 21(6): 625-633.
13. Amin B, Taheri MM, Hosseinzadeh H. Effects of intraperitoneal thymoquinone on chronic neuropathic pain in rats. Planta Med 2014; 80(15): 1269-1277.
14. Pogatzki-Zahn EM, Segelcke D, Schug SA. Postoperative pain-from mechanisms to treatment. Pain Rep 2017; 2(2): e588. doi: 10.1097/PR9.0000000000000588.
15. Gupta A, Bah M. NSAIDs in the treatment of postoperative pain. Curr Pain Headache Rep 2016; 20(11): 62. doi: 10.1007/s11916-016-0591-7.
16. Whiteside GT, Harrison J, Boulet J, et al. Pharmacological characterization of a rat model of incisional pain. Br J Pharmacol 2004; 141(1): 85-91.
17. Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain 1996; 64(3): 493-502.
18. Naderi S, Tamaddonfard E, Nafisi S, et al. Effect of thymoquinone on acetic acid-induced visceral nociception in rats: role of central cannabinoid and α2-adrenergic receptors. Vet Res Forum 2024; 15(3): 131-138.
19. Craft RM, Hewitt KA, Britch SC. Antinociception produced by nonsteroidal anti-inflammatory drugs in female vs male rats. Behav Pharmacol 2021; 32(2&3): 153-169.
20. Kumar R, Gupta S, Gautam M, et al. Diverse characters of Brennan's paw incision model regarding certain parameters in the rat. Korean J Pain 2019; 32(3): 168-177.
21. Kabadi R, Kouya F, Cohen HW, et al. Spontaneous pain-like behaviors are more sensitive to morphine and buprenorphine than mechanically evoked behaviors in a rat model of acute postoperative pain. Anesth Analg 2015; 120(2): 472-478.
22. Ying YL, Wei XH, Xu XB, et al. Over-expression of P2X7 receptors in spinal glial cells contributes to the development of chronic postsurgical pain induced by skin/muscle incision and retraction (SMIR) in rats. Exp Neurol 2014; 261: 836-843.
23. Chen H, Jiang YS, Sun Y, et al. p38 and interleukin-1 beta pathway via toll-like receptor 4 contributed to the skin and muscle incision and retraction-induced allodynia. J Surg Res 2015; 197(2): 339-347.
24. Wang Y, Liu Y, Liu J, et al. Coadministration of curcumin and hydromorphone hydrochloride alleviates postoperative pain in rats. Biol Pharm Bull 2022; 45(1): 27-33.
25. Joksimovic SL, Lamborn N, Jevtovic-Todorovic V, et al. Alpha lipoic acid attenuates evoked and spontaneous pain following surgical skin incision in rats. Channels (Austin) 2021; 15(1): 398-407.
26. Hämäläinen MM, Gebhart GF, Brennan TJ. Acute effect of an incision on mechanosensitive afferents in the plantar rat hind paw. J Neurophysiol 2002; 87(2): 712-720.
27. Pogatzki EM, Gebhart GF, Brennan TJ. Characterization of Adelta- and C-fibers innervating the plantar rat hind paw one day after an incision. J Neurophysiol 2002; 87(2): 721-731.

28. Silvestrini A, Meucci E, Ricerca BM, et al. Total antioxidant capacity: biochemical aspects and clinical significance. Int J Mol Sci 2023; 24(13): 10978. doi: 10.3390/ijms241310978.
29. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 2014: 360438. doi: 10.1155/2014/360438.
30. Varfolomeev E, Vucic D. Intracellular regulation of TNF activity in health and disease. Cytokine 2018; 101: 26-32.
31. Bedir Z, Ozkaloglu Erdem KT, Doymus O, et al. Effects of benidipine, paracetamol, and their combination on postoperative and normal tissue pain thresholds. Front Pharmacol 2024; 14: 1326128. doi: 10.3389/fphar.2023.1326128.
32. Lv CC, Xia ML, Shu SJ, et al. Attenuation of remifentanil-induced hyperalgesia by betulinic acid associates with inhibiting oxidative stress and inflammation in spinal dorsal horn. Pharmacology 2018; 102(5-6): 300-306.
33. Loram LC, Themistocleous AC, Fick LG, et al. The time course of inflammatory cytokine secretion in a rat model of postoperative pain does not coincide with the onset of mechanical hyperalgesia. Can J Physiol Pharmacol 2007; 85(6): 613-620.
34. Finnerty CC, Mabvuure NT, Ali A, et al. The surgically induced stress response. JPEN J Parenter Enteral Nutr 2013; 37(5 Suppl): 21S-29S.
35. Celik F, Göçmez C, Karaman H, et al. Therapeutic effects of thymoquinone in a model of neuropathic pain. Curr Ther Res Clin Exp 2014; 76: 11-16.
36. Chang RW, Tompkins DM, Cohn SM. Are NSAIDs safe? Assessing the risk-benefit profile of nonsteroidal anti-inflammatory drug use in postoperative pain management. Am Surg 2021; 87(6): 872-879.
37. Moore RA, Derry S, McQuay HJ, et al. Single dose oral analgesics for acute postoperative pain in adults. Cochrane Database Syst Rev 2011; 9: CD008659. doi: 10.1002/14651858.CD008659.pub2.
38. Merlos M, Portillo-Salido E, Brenchat A, et al. Administration of a co-crystal of tramadol and celecoxib in a 1:1 molecular ratio produces synergistic antinociceptive effects in a postoperative pain model in rats. Eur J Pharmacol 2018; 833: 370-378.
39. Abd-Elmawla MA, Abdelalim E, Ahmed KA, et al. The neuroprotective effect of pterostilbene on oxaliplatin-induced peripheral neuropathy via its anti-inflammatory, anti-oxidative and anti-apoptotic effects: comparative study with celecoxib. Life Sci 2023; 315: 121364. doi: 10.1016/j.lfs.2022.121364.
40. Hanzawa A, Handa T, Kohkita Y, et al. A comparative study of oral analgesics for postoperative pain after minor oral surgery Anesth Prog 2018; 65(1): 24-29.
41. Vieira SF, Ferreira H, Neves NM. Antioxidant and anti-Inflammatory activities of cytocompatible *Salvia officinalis* extracts: a comparison between traditional and soxhlet extraction. Antioxidants (Basel) 2020; 9(11): 1157. doi: 10.3390/antiox9111157.