Iranian Journal of Pharmaceutical Sciences
2025: 21 (1): 249- 258
https://journals.sbmu.ac.ir/IJPS

Research Article

Analgesic effects of Iranian oak extract (*Quercus brantii*) mediated by the opioid system under stressful situations in female mice

Hossein Pourkhosravi[a], Mahnaz Kasmati[a], Mozhgan Torabi[a]*

[a] *Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.*

Received: February 8, 2025 **Last Revision:** April 12, 2025 **Accepted:** May 06, 2025 **Available online:** July 09, 2025.

Abstract

This study investigated the mechanism of the analgesic effect of oak fruit extract in interference with an opioidergic system in female mice under acute stress induction. Adult female mice were divided into groups of control, oak extract 1, 10, and 100 mg/kg, morphine and naloxone 1 mg/kg, morphine or naloxone/oak 1 and 100 mg/kg, restraint stress (60 minutes), stress/ naloxone 1 mg/kg and stress/ naloxone/oak 1 or 100 mg/kg. All mice were co-cycled before the beginning of the study; the formalin test induced inflammatory pain. Components were injected after stress induction, and formalin tests were done 30 minutes after injections. Oak fruit hydroalcoholic extract was obtained using the maceration method. Serum total oxidant status (TOS) and total antioxidant capacity (TAC) were measured. Oak extract 100 mg/kg induced analgesia in the acute phase of pain, and all doses induced analgesia in the chronic phase. Morphine improved the analgesic effect of oak extract 100 mg/kg in the acute phase. Naloxone in the stressed mice induced analgesia in the acute phase and improved the analgesic effect of Oak 100 mg/kg in the chronic phase. In the stressed mice, the TOS level was increased by the oak 1 mg/kg and decreased by the oak 100 mg/kg. Probably part of the analgesic effect of oak is due to the activity of the opioid system in acute stress situations. In addition, the change in the ratio of oxidant/antioxidant compounds in this process indicates that the mechanism of action of oak fruit in modulating pain and inflammation is multifactorial.

Keywords: Mouse; Oak; Opioid system; Pain; Stress.

1. Introduction

Pain is an unpleasant experience and is commonly categorized as acute (short-term) or chronic (long-term) [1, 2]. Non-opioid analgesic agents such as steroid drugs, non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants, antiepileptic medications, local and general anesthetics, and opioid drugs such as morphine are among the most commonly used drugs for pain management [3, 4].

Opioid drugs have traditionally been used for the treatment of acute and chronic pain [5]. The opioid receptor family includes three receptors: mu, delta, and kappa, which are widely expressed throughout the peripheral and central nervous systems [5]. One of the problems with currently available opioid analgesics is their unintended and nonselective activation of opiate receptors located throughout the central and peripheral regions [6]. Opioid use is often associated with side

*** Corresponding Author**:
Mozhgan Torabi, Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran. E-mail: m.torabi@scu.ac.ir.

Cite this article as: Pourkhosravi H., Kasmati M., Torabi M. Analgesic effects of Iranian oak extract (*Quercus brantii*) mediated by the opioid system under stressful situations in female mice. Iran. J. Pharm. Sci., 2025, 21 (1): 249- 258.

DOI: https://doi.org/10.22037/ijps.v21i1.47529

effects or clinical problems such as respiratory depression, constipation, dependence, and euphoria. Additionally, the development of tolerance is a significant problem, as it requires increasing doses to achieve the same therapeutic effect [7]. Gradually increasing doses of the drug can induce or exacerbate opioid-induced hyperalgesia, which, in turn, increases the tolerance of behavioral analgesic responses [8].

Combining addictive opioids with non-narcotic analgesics may reduce their side effects, such as addiction and tolerance [9].

Herbal medicine for pain management has been used from ancient times to today, and herbal products or their extracts are common worldwide, especially in Asia [10-12]. *Quercus brantii* (*Q. brantii*, Oak) belongs to the Fagaceae family, and it is a large tree with a height of 20 meters and a large spherical crown. The leaves of this plant are generally egg-shaped with a serrated margin. This tree was found in the Zagros Mountain range of Iran [13, 14].

Oak fruit extract has numerous medicinal properties due to its tannic acid, gallic acid, ellagic acid, and flavonoids such as catechin, quercetin, and naringin. Oak fruit with antioxidant properties prevents and treats gastric ulcers, and can inhibit the growth of pathogens [15-18]. Flavonoids can inhibit pain perception by targeting the transient receptor potential cation channel receptor subfamily 1 (TRPV 1) and inhibiting cytokine release as well as inhibiting the nuclear factor kappa (NF-kB) pathway, which is a pathway responsible for the inflammation [19].

Anti-inflammatory effects of *Q. brantii* extract have been confirmed in rats [17]. In addition, it has been shown that the extract of *Quercus infectoria* fruit hulls (Jaft-E-Baloot) alleviated inflammatory pain in mice [20, 21].

Our previous study has shown that the hydroalcoholic extract *of Q. brantii* fruit improved amnesia induced by morphine in mice and increased serum total antioxidant capacity (TAC) [14]. Ellagic acid can enhance morphine analgesia and prevent dependence in mice. It is while naloxone, as an opioid receptor antagonist, causes tolerance to the combination of morphine/ ellagic acid in both central and peripheral systems [21].

On the other hand, stress causes behavioral changes and disrupts physiological homeostasis in the human and animal body [22-24]. Stress disrupts the coordination between mind and body by altering biochemical reactions, including enzymes [25]. Restraint stress significantly increases oxidative status and lipid peroxidation, which are indicators of oxidative stress [26]. It has been shown that acute stress can activate mu-opioid receptors on GABAergic neurons in the hippocampus of mice [27].

Acute and chronic stress have different effects on the pain perception, such that acute immobility stress causes analgesia, but chronic immobility stress causes hyperalgesia in mice [28, 29]. Also, acute restraint stress can induce analgesia by activation of mu-opioid receptors in the medial prefrontal cortex of mice [30].

As mentioned above, stress and *Q. brantii* extract can affect opioid system activity [14, 20, 30]. So that in this study, their interfering effects with the opioid system, as a main system involved in pain perception, were evaluated in a model of inflammatory pain induced by the formalin test.

2. Materials and Methods

2.1. Animals and Treatments

Adult female NMRI mice (22-28 g) were obtained from the laboratory animal reproduction center of Jundishapur University of Medical Sciences, Ahvaz, Iran. Animals were kept in standard laboratory conditions at a temperature of 24±2 C° and in a light-dark cycle (12/12 hours) without limited access to water and food. Behavioral tests were done between 8 AM and 12 PM.

To minimize hormonal influence in all groups, animals of each group were kept in a cage for 2 weeks before the beginning of the experiments. Mice were randomly divided into groups of control (recived saline 0.9%), hydroalcoholic extract of Oak fruit (Oak) (1, 10 and 100 mg/kg), naloxone (1 mg/kg), morphine (1 mg/kg), naloxone or morphine 1 mg/kg with Oak (1 and 100 mg/kg), stress of 60 min + saline, stress 60 min+ naloxone (1 mg/kg), stress 60 min+ Naloxone (1 mg/ kg) + Oak (1 and 100 mg/kg). All components were dissolved in saline 0.9% and injected intraperitoneally (i.p.) (5 *mL/kg)*. The number of animals in each group was 6. A formalin test was done 30 minutes after the Oak injection or stress induction. Morphine or naloxone was injected 15 min before the Oak injection. In stressed groups, components were injected immediately after stress induction. The interval between injections and the formalin test was selected based on previous studies on the effects of Oak fruit on pain perception [20]. **Figure 1** shows the study protocol.

All experiments complied with the animal care guidelines approved by the Ethical Committee of the Shahid Chamran University of Ahvaz, Iran (Permission number: IR.SCU.REC.1402.044).

2.2. Oak fruit hydroalcoholic extraction

Oak fruit was collected from Khorramabad in Lorestan province, where the only Oak species in these areas is *Q. brantii*. Extraction was done by the maceration method. Oak powder was stirred frequently in ethanol at room temperature for three days. The mixture was filtered, and the solution was poured at room temperature. After evaporation, a dried powder was used for further experiments.

2.3. Formalin test and stress induction

Formalin (2.5%, 40 µL/mouse) was injected in the right plantar site of the mouse, and the licking time of the foot was calculated in acute (0- 5 min) and chronic phases (15-25 minutes) after formalin injection. This test used a 10-minute interval between acute and chronic phases [31].

The Plexiglas restrainer induced acute restraint stress. The mouse was immobilized in the restrainer for 60 minutes [30].

2.4. Oxidant/antioxidant analysis

The whole blood of animals was taken after a formalin test, centrifuged, and TAC and *total oxidant status (TOS)* were measured in the serum (**Figure 1C**).

TAC was evaluated by mixing serum (10 µL) with FRAP (ferric reducing antioxidant power) reagent (250 µL), which contained 2,4,6-tris(2-pyridyl)-striazine (TPTZ)10 mM in HCl 40 mM and FeCl320 mM in acetate buffer 0.1 M (pH=3.6). Then, the mixture was added with distilled water up to 1 mL, and after incubation, it was centrifuged, and the absorbance of the TPTZ complex was read at 593 nm.

TOS was measured using the Erel (2005) method. In this way, 225 µl of reagent 1, composed of 150 µM xylenol orange, 140 mM NaCl, and 1.35 M glycerol (PH 1.75), *was mixed with 35 µL of sample and* read at 560 and 800 nm after 3 min. Then, mixed with reagent two, 5 mM ferrous ammonium sulfate and 10 mM O-dianisidine dihydrochloride, and read at 560 and 800 nm after 3 min. The assay is calibrated with hydrogen peroxide (H2O2) 200 Um [32].

Oxidant status index (OSI) was calculated using the following formula: OSI (arbitrary units) = [(TOS, mmolH_2O_2 Eq./mL)/ (TAS, mmol Vit C Eq./ mL) × 100].

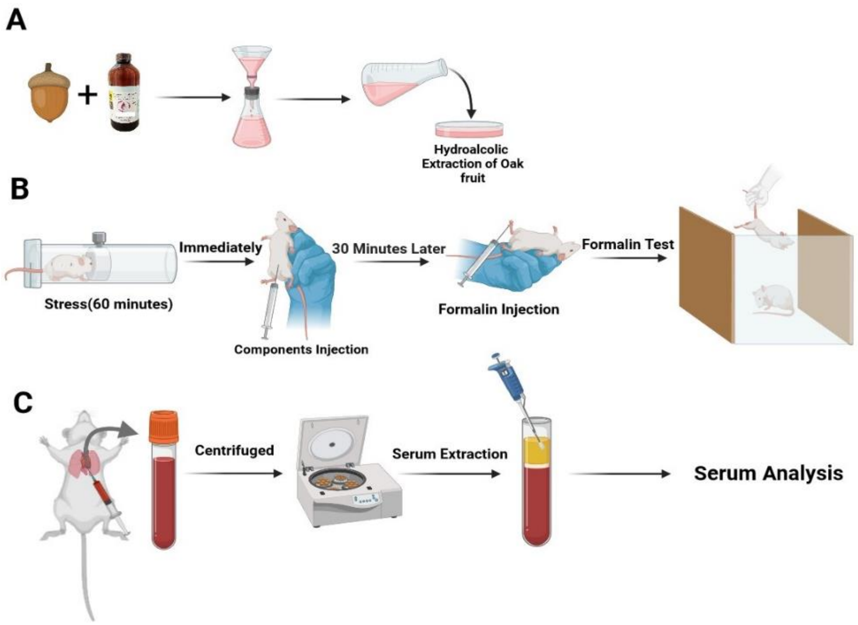

Figure 1. Schematic the study diagram. A: hydroalcoholic extraction of Oak fruit. B: stress induction (60 minutes), components (Oak extract, morphine, and naloxone) injection, and formalin test. C: *TAC* and *TOS measurement in the serum of the selected group.*

2.5. Data Analysis

Instat 3 software was used for statistical analysis. The one-way analysis of variance (ANOVA) was used to analyze, followed by Tukey's post hoc test. In all statistical investigations, p<0.05 was considered a significant difference. Data were presented as mean±standard error of means (SEM).

3. Results and Discussion

3.1. Effects of Oak fruit extract on inflammatory pain

Injection of Oak 100 mg/kg significantly improved pain in the acute phase of the formalin test (p<0.001). Also, Oak 1, 10, and 100 mg/kg injections significantly induced analgesia in the chronic test phase (p<0.001). These results indicate that 100 mg/kg of Oak extract could induce an analgesic effect in the formalin test (**Figure 2**).

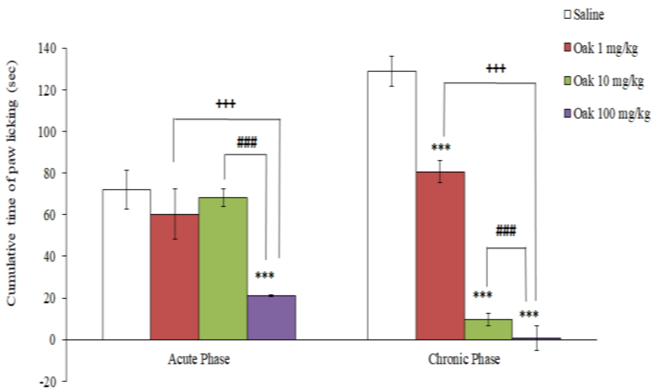

Figure 2. Effects of Oak fruit extract on inflammatory pain. ***p<0.001 is compared to the saline group at the same pain phase. +++p<0.001 is compared to the Oak 1 mg/kg at the same pain phase. ###p<0.001 is in comparison to the Oak 10 mg/kg at the same phase of pain.

Mokhtari and Khabbaz (2010) have shown that the extract of leaves of *Q. brantii* induced analgesia in the formalin test in rats [33]. Also, Ismail et al. (2017) have demonstrated that Oak leaf extract plays a role in anti-inflammatory activity by inhibiting histamine release [34]. Taib et al. (2020) showed that polyphenolic compounds in different Oak species can reduce pain by inhibiting inflammatory pathways [35]. Yang et al. (2023) have shown that gallic acid can induce analgesic effects in rats by regulating the P2X purinoceptor 7 - Reactive oxygen species (ROS) signaling pathway [36]. It has been confirmed that ellagic acid has anti-inflammatory and analgesic properties [21, 37].

Therefore, the possible mechanism of the analgesic effects of Oak fruit extract could be due to the presence of phenolic compounds such as gallic acid, tannins, and flavonoids, which components can reduce inflammation and chronic pain by inhibiting the ROS production [15, 33, 38-40].

Also, the difference in pain reduction in different phases of the formalin test could be due to differences in pain mechanisms in the acute and chronic phases, the accumulation of active metabolites of the extract in the tissue over time, and differences in the immune and inflammatory response to the extract in different phases [41].

3.2. Effects of morphine/ naloxone on the analgesic effect of Oak fruit extract

Morphine 1 mg/kg significantly induced analgesia in both phases of the formalin test (p<0.05 (acute) and p<0.001 (chronic)). Thus, morphine 1 mg/kg was co-injected with Oak 1 and 100 mg/kg and could maintain an analgesic effect of Oak 100 mg/kg (p<0.001) in the acute and chronic phase of the formalin test (**Figure 3A** and **D**).

Naloxone 1mg/kg could not change the formalin test's cumulative paw licking time (**Figure 3B**). This dose of naloxone, as an ineffective dose of opioidergic receptor antagonist, was co-injected with Oak 1 and 100 mg/kg.

Even though co-injection of naloxone with Oak 1 and 100 mg/kg could maintain the analgesic effect of Oak (p<0.05 and p<0.001), analgesia was specifically decreased in the chronic phase of pain (**Figure 3B** and **C**).

From these results, it can be concluded that co-injection of morphine with the high dose of Oak had synergistic effects in inducing analgesia, and co-injection of naloxone with Oak, especially in the chronic phase, reduced the analgesic effect of Oak. Therefore, one of the routes of the analgesic effect of the Oak extract was probably through the opioid system.

Taghi Mansouri et al. (2013) have demonstrated that Oak ellagic acid exerts its analgesic effect through the opioid system [21]. Ismail et al. (2017) have shown that oak leaves can produce an analgesic effect, but this effect is less than morphine [15, 34]. Saeed et al. (2016) observed that the analgesic effect of oak fruit is blocked by naloxone, indicating that the analgesic effect of oak extract is due to the stimulation of opioid receptors [15].

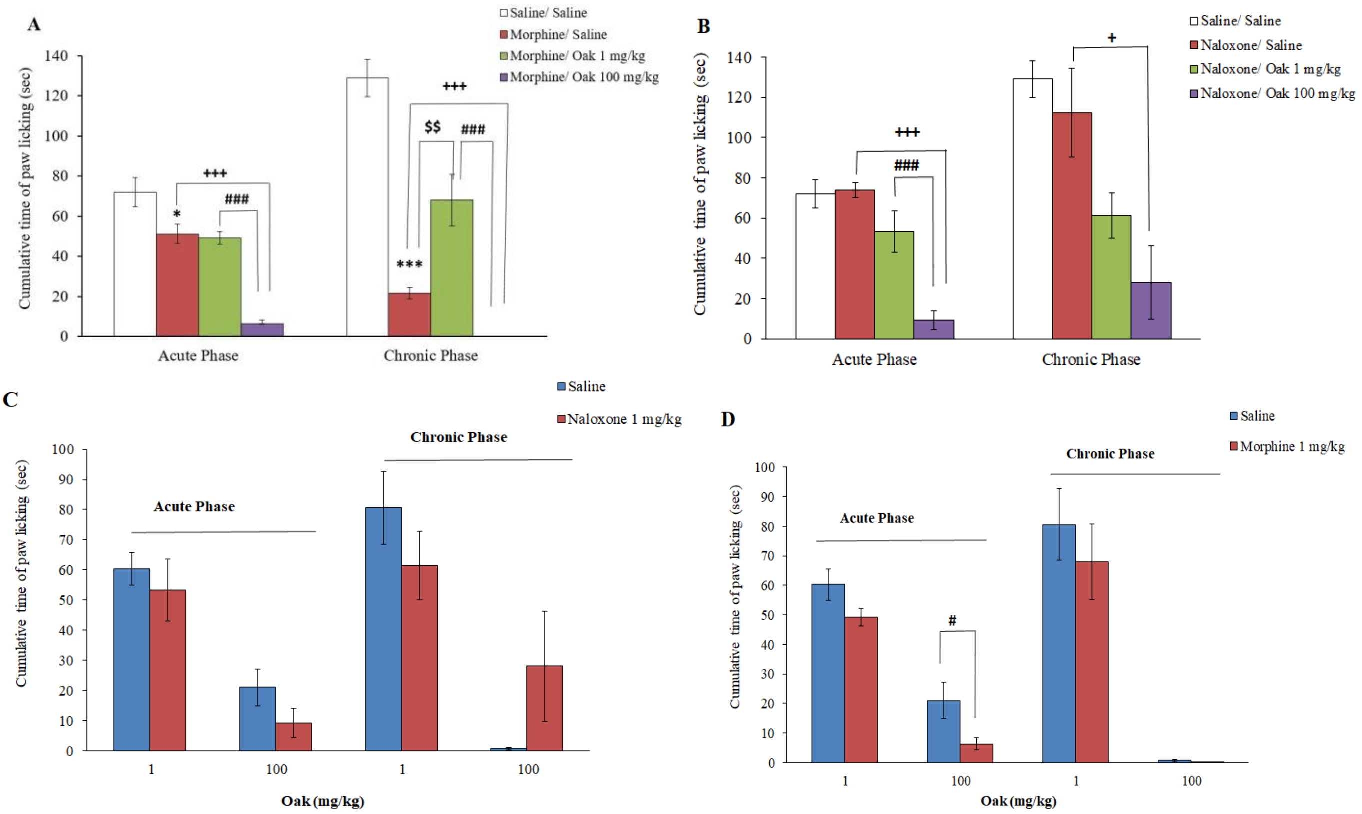

Figure 3. Effects of morphine 1mg/kg and naloxone 1mg/kg on the analgesic effect of Oak fruit extract. *p<0.05 and ***p<0.001 are in comparison to the saline group at the same phase of pain. +P <0.05 and +++p<0.001 are in comparison to the morphine 1 mg/kg or naloxone 1 mg/kg at the same phase of pain. ###p<0.001 is in comparison to the morphine or naloxone +Oak 100 mg/kg at the same phase of pain. $$p<0.01 is in comparison to the morphine+ Oak 1mg/kg at the same phase of pain.

3.3. Effects of stress and naloxone on the analgesic effect of Oak fruit extract

Induction of acute stress for 60 minutes could not change pain perception in both acute and chronic phases of pain (Figure 4A). The analgesic effect of Oak 1 and 100 mg/kg was not changed following stress induction in the acute phase of formalin tests, in comparison analgesic effect of Oak 1 mg/kg increased compared to Oak 1 mg/kg alone in the chronic phase (p<0.001) (**Figure 4A** and **C**).

These results indicated that stress induction could improve the analgesic effect of a low dose of Oak (**Figure 4C**).

In stressed mice, injection of naloxone 1mg/kg could induce analgesia in the acute phase test (p<0.01). In these mice, injection of Oak 1 and 100 mg/kg significantly induces analgesia in the chronic pain phase (**Figure 4B** and **D**).

In this study, induction of acute stress alone did not affect the analgesic effect of Oak, and induction of acute stress with injection of a low dose of Oak produced significant analgesic effects in the chronic phase of pain. Also, the analgesic effect of the Oak in the presence of stress remains even after the opioid system activity was removed by the naloxone (**Figure 4**).

It has been shown that acute restraint stress can cause analgesia in rats [19]. The endogenous opioidergic system can mediate stress effects on nociception pathways [42]. Thus, the analgesic effect of the oak fruit extract in a stressful situation was probably not affected by the opioid system.

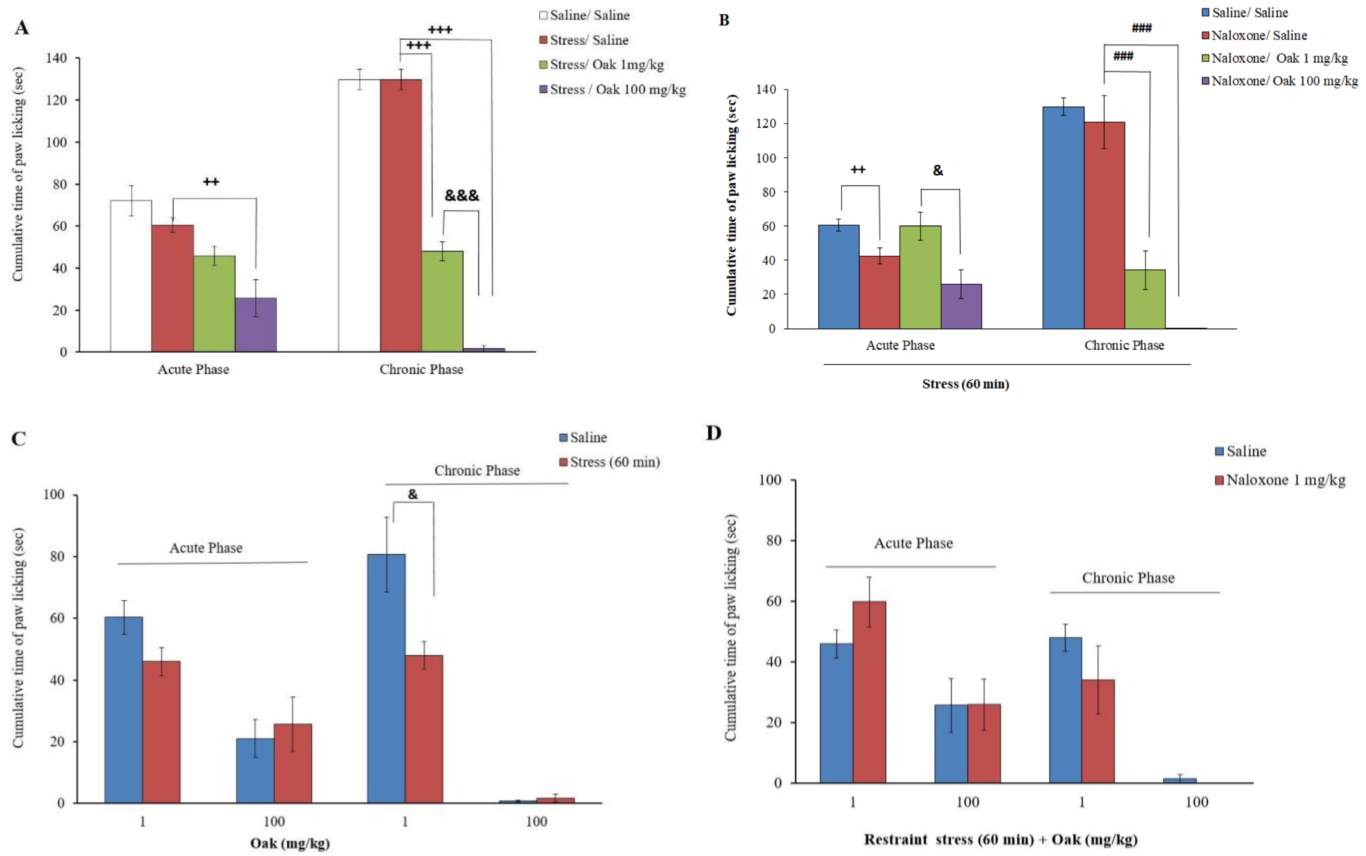

Figure 4. Effects of stress and naloxone 1 mg/kg on the analgesic effect of Oak fruit extract. ++p<0.01 and +++p<0.001 are in comparison to the stress group at the same phase of pain. ###p<0.001 is in the comparison to the stress+ naloxone at the same phase of pain. &p<0.05 and &&&p<0.001 are in the comparison to the stress or naloxone+ Oak 1mg/kg at the same phase of pain.

3.4. Analysis of oxidant/antioxidant parameters

Data in **Table 1** show that Oak 1 and 100 mg/kg as non-analgesic and analgesic doses, respectively, and restraint stress of 60 min could not change TAC and TOS serum levels and TOS/TAC*100 ratio. In stressed mice, Oak 1 and 100 mg/kg did not change the TAC level. Injection of Oak 1 mg/kg in the stressed group significantly decreased the TAC level compared to the injection of Oak 1 mg/kg alone ($p<0.001$) (**Table 1**). In the stressed groups, TOS level increased in Oak 1 mg/kg treated mice ($p<0.05$) and decreased in Oak 100 mg/kg treated mice ($p<0.01$) (**Table 1**).

It has been shown that the hydroalcoholic extract of acorns of *Q. brantii*, by increasing TAC level in the serum of mice, induced antioxidant activity [43]. Our previous study has shown that chronic injection of the hydroalcoholic extract of Oak fruit increased serum TAC in male mice [14]. However, the current study indicates that acute injection of Oak extract does not affect these evaluated oxidant/antioxidant factors.

However, under stress conditions, treatment with Oak fruit extract 1 mg/kg significantly decreased TAC and increased TOS level, while TOS level was decreased with Oak fruit extract 100 mg/kg (**Table 1**). Also, the decrease in the TOS/TAC ratio in the Oak fruit extract 100 mg/kg treated group could indicate the inhibition of inflammatory pathways, which was increased in the low dose. This effect is probably mediated by reducing the production of proinflammatory cytokines and inhibiting NF-κB [44]. Modulation of oxidative stress can lead to a decrease in the sensitivity of pain receptors [45, 46]. These findings could be important in determining the appropriate dose of the extract in different clinical conditions.

Table 1: TAC and TOS levels in the serum

Groups		TAC (mmol Vit C Eq/mL)	TOS (mmol H2O2 Eq/mL)	TOS/TAC*100
Control (saline %0.9)		1.5562± 0.2005	4.3080± 0.3954	339.0973± 97.65
Oak 1 mg/kg		1.8854± 0.0344$^{\#\#\#}$	3.6588± 0.06795$^{\#\#\#}$	194.7227± 7.31$^{\#\#}$
Oak 100 mg/kg		1.6063± 0.1738	4.20927± 0.3427^{+++}	291.9065 ±56.52^{+}
Restraint stress (60 min)	Saline	1.8789± 0.2526	3.6717± 0.4981	221.9939± 49.67
	Oak 1 mg/kg	0.9824± 0.1543^{+}	5.43951± 0.3042$^{*\ +++}$	618.937± 107. 30$^{**\ +++}$
	Oak 100 mg/kg	2.1210±0.2891	1.7088± 0.2708**	94.0910±25.90

*p<0.05 and **p<0.01are in comparison to restrain stress group
##p<0.01 and ###p<0.001 is in comparison to restrain stress+ Oak 1 mg/kg group
+p<0.05 and +++p<0.001 are in comparison to restrain stress+ Oak 100 mg/kg group

Conclusion

According to the above results, it seems that part of the analgesic effect of Oak fruit is due to the activation of the opioid system. In contrast, its analgesic effect in acute stress situations was not affected in the absence of the opioid system. This interaction between stress and the absence of the opioid system in maintaining the analgesic effect of the extract can be considered as a new therapeutic strategy. Also, acute application of Oak fruit extract did not affect the TOS/TAC system. However, under stress conditions, different effects on this parameter depend on the dose, which indicates the importance of the dose used in stressful conditions.

Ethics approval

All experiments were conducted in compliance with the animal care guidelines established by the institution and approved by the Local Ethical Committee at the Shahid Chamran University of Ahvaz, Iran (Permission number: IR.SCU.REC.1402.044).

Acknowledgment

This study was supported morally and financially by the Shahid Chamran University of Ahvaz. The authors are sincerely grateful to the Avin Stem Gen Bio Health Inc., Ltd, Ahvaz for technical supporting performing assistance.

Conflict of interest

No conflict of interest.

Data availability

All relevant data are included within the article.

Athors Contributions

H.P, investigation and writing the original draft; M. K, Conceptualization, supervision, project administration, validation, funding acquisition, and draft editing; M.T, data curation, formal analysis, writing original draft preparation, writing review and editing draft.

Athors Orcid numbers:

Hossein Pourkhosravi: 0009-0006-8560-7056

Mahnaz Kesmati: 0000-0002-0168-6441

Mozhgan Torabi: 0000-0002-3085-0036

Funding

This study was funded by the Shahid Chamran University of Ahvaz, and under gran number of SCU.SB1402.760 (webpage of the grant number: http://scu.ac.ir/ scu.sb1402.760).

Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

References

1. Calvino B, Grilo RM. Central pain control. Joint Bone Spine. Joint Bone Spine (2006);73(1):10-6. https://doi.org/10.1016/j.jbspin.2004.11.006.
2. Dydyk AM, Conermann T. Chronic Pain. 2024 May 6. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. PMID: 31971706.
3. Sall JL, Sandbrink F, Murphy JL, Spevak C, Edens E. The Use of Opioids in the Management of Chronic Pain. Ann Intern Med (2023)176(9):eL230225. https://doi.org/10.7326/L23-0225
4. Queremel Milani DA, Davis DD. Pain Management Medications. [Updated 2023 Jul 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560692.
5. Nafziger AN, Barkin RL. Opioid Therapy in Acute and Chronic Pain. J Clin Pharmacol (2018); 58(9):1111-1122. https://doi.org/10.1002/jcph.1276. Epub 2018 Jul 9.
6. Stein C. New concepts in opioid analgesia. Expert Opin Investig Drugs (2018) ;27(10):765-75. https://doi.org/10.1080/13543784.2018.1516204
7. Grim TW, Acevedo-Canabal A, Bohn LM. Toward directing opioid receptor signaling to refine opioid therapeutics. Biol psychiatry (2020) 87(1):15-21. https://doi.org/10.1016/j.biopsych.2019.10.020.
8. Mercadante S, Arcuri E, Santoni A. Opioid-induced tolerance and hyperalgesia. CNS drugs (2019) 33:943-55. https://doi.org/10.1007/s40263-019-00660-0.
9. Wang X, Bao C, Li Z, Yue L, Hu L. Side Effects of Opioids Are Ameliorated by Regulating TRPV1 Receptors. International Journal of Environmental Research and Public Health (2022) 19(4):2387. https://doi.org/10.3390/ijerph19042387.
10. Boyd A, Bleakley C, Hurley DA, Gill C, Hannon-Fletcher M, Bell P, McDonough S. Herbal medicinal products or preparations for neuropathic pain. Cochrane Database Syst Rev (2019) 4(4):CD010528. https://doi.org/ 10.1002/14651858.CD010528.pub4.
11. 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. https://doi.org/ 10.1097/MD.0000000000014016.
12. Jo HG, Seo J, Lee D. Clinical evidence construction of East Asian herbal medicine for inflammatory pain in rheumatoid arthritis based on integrative data mining approach. Pharmacol Res (2022)185:106460. https://doi.org/ 10.1016/j.phrs.2022.106460. Epub 2022 Sep 21.
13. Soheili F, Abdul-Hamid H, Almasi I, Heydari M, Tongo A, Woodward S, Naji HR. How Tree Decline Varies the Anatomical Features in Quercus brantii. Plants (Basel) (2023) 12(2):377. https://doi.org/ 10.3390/plants12020377.
14. Fazelian SA, Kesmati M, Torabi M. The Biochemical Effects of Hydroalcoholic Extract of Quercus brantii Fruit on Morphine-Induced Amnesia in the Presence and Absence of Cholinergic Muscarinic Receptors in Adult Male Mice. Zahedan J Res Med Sci (2024) 26(3):e148029. https://doi.org/10.5812/zjrms-148029.
15. Saeed M, Gul F, Zakiullah Z, Gilani SN, Rehman YU, Khan I, Muhammad N, Khan H. Antinociceptive and anti-inflammatory properties of methanol fruit extract of Quercus incana in rat and mice models. Tropical Journal of Pharmaceutical Research. (2016) 15(8):1691-1696. https://doi.org/10.4314/tjpr.v15i8.14.
16. Jafari Barmak M, Dehghan Menshadi A, Mahmoudi R, Bardania H. Evaluation of the effect of the internal layer of oak fruit (jaft) extract on the prevention of gastric ulcers caused by stress in male rats. J Med Life (2018) 11(3):225-230. https://doi.org/10.25122/jml-2017-0025.
17. Alizade Naini M, Mehrvarzi S, Zargari-Samadnejadi A, Tanideh N, Ghorbani M, Dehghanian A, Hasanzarrini M, Banaee F, Koohi-Hosseinabadi O, Irajie C, Iraji A. The Antioxidant and Anti-Inflammatory Effects of Quercus brantii Extract on TNBS-Induced Ulcerative Colitis in Rats. Evid Based Complement Alternat Med (2021) 2021: 3075973. https://doi.org/ 10.1155/2021/3075973.
18. Altememy D, Patra I, Hussam F, Jabr HS, Alwan NH, Dawood AH, Abdelamir DF, Hamad DA, Shokri S, Darvishi M. Determination and evaluation of total antioxidant capacity of methanolic extracts of Quercus brantii, Thymbra spicata, Citrullus colocynthis. Adv Life Sci (2022) 9:372-9. http://www.als-journal.com/9320-22/.
19. Pinho-Ribeiro FA, Hohmann MS, Borghi SM, Zarpelon AC, Guazelli CF, Manchope MF,

Casagrande R, Verri Jr WA. Protective effects of the flavonoid hesperidin methyl chalcone in inflammation and pain in mice: role of TRPV1, oxidative stress, cytokines and NF-κB. Chem Biol Interact (2015) 228:88-99. https://doi.org/10.1016/j.cbi.2015.01.011.

20. Karimian A, Nafari A, Sarvestani A, Zamani A, Behnaminia N, Mohammadi R, Almasian M. The Effect of the Hydroalcoholic Extract of Quercus infectoria Fruit Hulls (Jaft-E-Baloot) on Formalin-Induced Inflammation and Pain in Male Mice. Herbal Med J (2023) 8(1):26-33. https://doi.org/ 10.22087/hmj.v8i1.1011.
21. Taghi Mansouri M, Naghizadeh B, Ghorbanzadeh B, Farbood Y. Central and peripheral antinociceptive effects of ellagic acid in different animal models of pain. Eur J Pharmacol (2013) 707(1-3):46-53. https://doi.org/10.1016/j.ejphar.2013.03.031. Epub 2013 Mar 23.
22. Kumar A, Garg R, Prakash AK. Effect of St. John's Wort (Hypericum perforatum) treatment on restraint stress-induced behavioral and biochemical alteration in mice. BMC Complement Altern Med (2010) 10:18. https://doi.org/10.1186/1472-6882-10-18.
23. Morey JN, Boggero IA, Scott AB, Segerstrom SC. Current directions in stress and human immune function. Curr Opin Psychol (2015) 5:13-7. https://doi.org/10.1016/j.copsyc.2015.03.007.
24. Travers G, Kippelen P, Trangmar SJ, González-Alonso J. Physiological Function during Exercise and Environmental Stress in Humans-An Integrative View of Body Systems and Homeostasis. Cells (2022) 11(3):383. https://doi.org/10.3390/cells11030383.
25. Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic Stress and Oxidative Stress as Common Factors of the Pathogenesis of Depression and Alzheimer's Disease: The Role of Antioxidants in Prevention and Treatment. Antioxidants (Basel) (2021)10(9):1439. https://doi.org/10.3390/antiox10091439.
26. Pal G, Mishra HP, Suvvari TK, Tanwar A, Ghosh T, Verma P, Pal A, Patial K, Mahapatra C, Amanullah NA, Shukoor SA, Kamal S, Rohil V. Oxidative Stress in Wistar Rats Under Acute Restraint Stress and Its Modulation by Antioxidants and Nitric Oxide Modulators. Cureus (2023)15(8):e43333. https://doi.org/10.7759/cureus.43333.
27. Fan KM, Qiu LJ, Ma N, Du YN, Qian ZQ, Wei CL, Han J, Ren W, Shi MM, Liu ZQ. Acute Stress Facilitates LTD Induction at Glutamatergic Synapses in the Hippocampal CA1 Region by Activating μ-Opioid Receptors on GABAergic Neurons. Front Neurosci (2019) 13:71. https://doi.org/10.3389/fnins.2019.00071.
28. Costa A, Smeraldi A, Tassorelli C, Greco R, Nappi G. Effects of acute and chronic restraint stress on nitroglycerin-induced hyperalgesia in rats. Neurosci Lett (2005) 383(1-2):7-11. https://doi.org/10.1016/j.neulet.2005.03.026. Epub 2005 Apr 18.
29. Torabi M, Kesmati M, Galehdari H, Varzi HN, Pourreza N. MgO and ZnO nanoparticles anti-nociceptive effect modulated by glutamate level and NMDA receptor expression in the hippocampus of stressed and non-stressed rats. Physiol Behav (2020) 214: 112727. https://doi.org/ 10.1016/j.physbeh.2019.112727.
30. Du Y, Zhao Y, Zhang A, Li Z, Wei C, Zheng Q, Qiao Y, Liu Y, Ren W, Han J, et al. The Role of the Mu Opioid Receptors of the Medial Prefrontal Cortex in the Modulation of Analgesia Induced by Acute Restraint Stress in Male Mice. Int J Mol Sci (2024) 25(18):9774. https://doi.org/10.3390/ijms25189774.
31. Jahangiri L, Kesmati M, Najafzadeh H. Evaluation of analgesic and anti-inflammatory effect of nanoparticles of magnesium oxide in mice with and without ketamine. Eur Rev Med Pharmacol Sci (2013) 17(20):2706-10. PMID: 24174350.
32. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem (2005) 38(12):1103-11. https://doi.org/10.1016/ j.clinbiochem. 2005. 08. 008. Epub 2005 Oct 7.
33. Mokhtari M, Khabbaz Z. Evaluation of Anti-nociceptive and Anti-inflammatory Effects of Hydroalcoholic Extract of Leaves of Oak, Quercus brantii by Formalin Test and CarraggeenanModel in Rat. Nat Environ Pollut Technol (2010) 9(2): 371-378.
34. Ismail H, Rasheed A, Haq IU, Jafri L, Ullah N, Dilshad E, Sajid M, Mirza B. Five Indigenous Plants of Pakistan with Antinociceptive, Anti-Inflammatory, Antidepressant, and Anticoagulant Properties in Sprague Dawley Rats. Evid Based Complement Alternat Med (2017) 2017:7849501.

https://doi.org/ 10.1155/2017/7849501. Epub 2017 Nov 23.

35. Taib M, Rezzak Y, Bouyazza L, Lyoussi B. Medicinal uses, phytochemistry, and pharmacological activities of Quercus species. Evid Based Complement Alternat Med. 2020;2020:1920683. Evid Based Complement Alternat Med (2020) 2020:1920683. https://doi.org/ 10.1155/2020/1920683.
36. Yang R, Shi L, Si H, Hu Z, Zou L, Li L, Xu X, Schmalzing G, Nie H, Li G, Liu S, Liang S, Xu C. Gallic Acid Improves Comorbid Chronic Pain and Depression Behaviors by Inhibiting P2X7 Receptor-Mediated Ferroptosis in the Spinal Cord of Rats. ACS Chem Neurosci (2023) 14(4):667-676. https://doi.org/10.1021/acschemneuro.2c00532. Epub 2023 Jan 31.
37. Cornélio Favarin D, Martins Teixeira M, Lemos de Andrade E, de Freitas Alves C, Lazo Chica JE, Artério Sorgi C, Faccioli LH, Paula Rogerio A. Anti-inflammatory effects of ellagic acid on acute lung injury induced by acid in mice. Mediators Inflamm (2013)2013:164202. https://doi.org/10.1155/2013/164202. Epub 2013 Feb 27.
38. Nili-Ahmadabadi H, Emami MH, Omidifar N. Effectiveness of Quercus brantii hydroalcoholic extract on dyspepsia: A randomized, double blind clinical trial. J Edu Health Pro (2017) 6(1):62. https://doi.org/10.4103/jehp.jehp_31_17.
39. Youn SH, Kwon JH, Yin J, Tam LT, Ahn HS, Myung SC, et al. Anti-inflammatory and anti-urolithiasis effects of polyphenolic compounds from Quercus gilva Blume. Molecules (2017) 22(7):1121. https://doi.org/10.3390/molecules22071121.
40. Raichurkar AP, Reddy SGH. Evaluation of analgesic activity of the methanol extract from the galls of Quercus infectoria alone and as an adjuvant in wistar rats. International Journal of Basic & Clinical Pharmacology (2020) 9(5):1. http://dx.doi.org/10.18203/2319-2003.ijbcp20201521.
41. Fan SH, Ali NA, Basri DF. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of Quercus infectoria (Olivier) in Rats. Evid Based Complement Alternat Med (2014) 2014:976764. https://doi.org/10.1155/2014/976764. Epub 2014 Aug 31.
42. Ferdousi M, Finn DP. Stress-induced modulation of pain: Role of the endogenous opioid system. Prog Brain Res (2018) 239:121-177. https://doi.org/10.1016/bs.pbr.2018.07.002. Epub 2018 Aug 23.
43. Soleimanzadeh A, Kian M, Moradi S, Malekifard F. Protective effects of hydroalcoholic extract of Quercus brantii against lead-induced oxidative stress in the reproductive system of male mice. Avicenna J Phytomed. 2018 ;8(5):448-456.
44. Sung M, Lim S, Park S, Choi Y, Kim S. Anti-inflammatory effects of phytosphingosine-regulated cytokines and NF-kB and MAPK mechanism. Cell Mol Biol (Noisy-le-grand) (2024) 70(9):22-30. https://doi.org/10.14715/cmb/2024.70.9.3.
45. Makhlouf FZ, Squeo G, Barkat M, Trani A, Caponio F. Antioxidant activity, tocopherols and polyphenols of acornoil obtained from Quercus species grown in Algeria. Food Res Int (2018)114:208-213. https://doi.org/ 10.1016/j.foodres.2018.08.010. Epub 2018 Aug 4.
46. Othón-Díaz ED, Fimbres-García JO, Flores-Sauceda M, Silva-Espinoza BA, López-Martínez LX, Bernal-Mercado AT, Ayala-Zavala JF. Antioxidants in Oak (*Quercus* sp.): Potential Application to Reduce Oxidative Rancidity in Foods. *Antioxidants.* (2023) 12(4):861. https://doi.org/10.3390/antiox12040861.