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Original Article

Investigating the Analgesic Effects of the *Chenopodium Botrys* L. Hydroalcoholic Extract Using Behavioral Tests on Mice

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Abstract

Regarding the unpleasant pain sensation and the public's desire to use traditional herbs, the present study aimed to assess the analgesic and anti-inflammatory effect of *Chenopodium botrys* L. The present research was divided into two main sections to assess the analgesic and anti-inflammatory effect of *Chenopodium botrys* L. in male mice. To investigate the analgesic effect of *Chenopodium botrys* L., thirty-six male mice were randomly classified into six groups vehicle (receiving normal saline), experimental groups received morphine (a well-known opioid, 1 mg/kg), hydroalcoholic extracts of *Chenopodium botrys* L. (30, 100, and 300 mg/kg), and naloxone (opioid receptor antagonist) with the highest dose of *Chenopodium botrys* L. concurrently for one week. Thirty mice were divided into five groups to receive normal saline, various doses of extract (30, 100, and 300 mg/kg), and dexamethasone (a well-known anti-inflammatory drug, 10 mg/kg) to assess the anti-inflammatory effect of *Chenopodium botrys* L. In both sections, after the last doses of the treatment, the animals got ready for behavioral study related to pain. Overall, the highest doses of *Chenopodium botrys* L. demonstrated much better results than other doses, which were comparable to opioids and dexamethasone, a well-known analgesic and anti-inflammatory medicines, respectively. *Regarding present findings, the Chenopodium botrys L. plant can be a new candidate as an analgesic agent, which needs more investigation* in the search and pharmaceutical development.

Keywords: *Chenopodium botrys*; analgesia; pain; mice; opioid.

1. Introduction

Pain, as a protective mechanism associated with the body's response against harmful stimuli or tissue damage, is known as the most common disease symptom; however, it is an unpleasant sensation accompanying us from an early age [1, 2]. Even though pain has been considered an individual's defense mechanism, in this condition, the causes of pain are not removed, leading to pain persistence and generating anxiety and other related disorders [3].

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Pain can be mainly caused by pain receptor stimulation depending on various factors, including the strength of the stimulus, individual susceptibility, or psychogenic pain, which refers to a patient without tissue damage [4, 5]. Although pain is a physiological process for avoiding injuries and consequently for survival, it can significantly reduce quality of life. Two different types of fibers, Aδ and C, transfer the noxious stimulus, generating sharp and chronic pain, respectively [6, 7]. In experimental settings, analgesic medication is more effective in pain inhibition than sharp or fast pain [8].

Numerous animal studies have been conducted on the pivotal role of pro-inflammatory cytokines such as histamine, substance P, bradykinin, acetylcholine, leukotrienes, and prostaglandins in generating acute and chronic pain [9]. In addition, inflammation is considered an immune response to infection and injury, which has been implicated in the pathogenesis of various diseases, including arthritis, cancer, neurodegenerative disorders, and inflammatory pain [10, 11].

In this regard, several studies demonstrated the antinociceptive effect of endogenous opioid peptides in inflamed paws through the activation of local opioid receptors, including mu (μ), delta (δ), and kappa (K) located in pain pathways by which the pain response can be modulated and these receptors also are stimulated by synthetic opioids such as morphine, codeine, oxycodone [12, 13]. Opioid drugs and endogenously released opioid peptides can modulate signal transduction mechanisms and intracellular processes, resulting in alterations in protein phosphorylation and gene expression; however, the mechanism is not fully understood [14, 15].

Although opioids are one of the most widely prescribed medications in the world as strong and potent pain-relieving therapeutics, there are increasing concerns about adverse effects followed by long-term usage of them mainly, dependency and tolerance, which can lead to addiction and disturb the individual's regular life over time. Therefore, these findings may provide a rationale for the development of alternatives to opioids [16, 17]. Regarding increasing public interest in herbs, traditional medicine can be considered complementary and alternative medicine [18, 19]. Many clinical observations provided strong evidence of the beneficial effect of herbal medicine in alleviating cancer pain with the least adverse effect. In this regard, *Chenopodium botrys* L. is a native herb discovered in Europe, Asia, and North America; it has been widely used for medicinal purposes and demonstrated a wide variety of pharmacologic therapeutic effects, including anthelmintic, antifungal, sedative, and pain-relieving, which mostly attributed to its major constituents such as terpineol, iso-ascaridole, and p-cymene [20, 21]. These data and related research studies suggest that Chenopodium botrys L. may be an interesting candidate for treating inflammatory pain. Thus, the present study aimed to investigate the analgesic and anti-inflammatory effects of *Chenopodium botrys* hydroalcoholic extract in male mice.

2. Materials and Methods

2.1. Animal and experimental protocol

The male mice (25 ± 5 g) were prepared from the animal house of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were kept at room temperature (23 ± 2 C) and 50% ± 5% moisture, and a 12 h dark/light cycle. The animals had access to feed and drink freely. The experimental setting was categorized into two sections to investigate the antinociceptive and anti-inflammatory effects of *Chenopodium botrys* L.

Thirty-six male mice were randomly divided into six groups including vehicle (received normal saline, i.p), morphine (received morphine (1 mg/kg, i.p), naloxone (received naloxone (1 mg/kg, i.p) [22], treatment (received different dosage of hydroalcoholic extract of *Chenopodium botrys* L. (30, 100, 300 mg/kg) via gavage to assess antinociceptive effect of *Chenopodium botrys* L.

When the test was completed, the animals were subjected to a behavioral study to assess the analgesic effect of *Chenopodium botrys* L., including writhing time, tail flick, and formalin test.

Thirty male mice were randomly divided into five groups, including the vehicle (received normal saline, i.p), dexamethasone (received dexamethasone (10 mg/kg) [22] via gavage), and treatment (received different dosage of hydroalcoholic extract of *Chenopodium botrys* L. (30, 100, 300 mg/kg) via gavage to assess anti-inflammatory effect of *Chenopodium botrys* L.

All procedures described have been approved by the Institutional Animal Care and Use Committee at Mashhad University of Medical Sciences, IR.MUMS.MEDICAL.REC.1400.477.

2.2. Extraction and analysis of the Chenopodium botrys L.

The *Chenopodium botrys* was collected from the Zarrin-kuh Protected Area, Razavi Khorasan Province (NE Iran), and identified by Dr. M.S Amiri (No. 681). First, the plant was dried and soaked for three consecutive days at room temperature in a hydroalcoholic solution (70%). The extract was evaporated under vacuum using a rotary till dried. The dried extract was dissolved in distilled water and administered orally to prepare the desired concentration.

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the *Chenopodium botrys* extract. It was mixed in dry potassium bromide (KBr) and pressed at 6 bars within 2 min to form a KBr thin disc. Then, the disc was placed in a diffuse reflectance accessory sample cup. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer.

2.3. Behavioral Pain Study in Animal Models

2.3.1. Acetic Acid-Induced Writhing in Mice

As a preliminary assessment for measuring anti-nociceptive activity, the writing test was conducted using a chemically noxious substance. The animals were treated with three different doses of Chenopodium *botrys* L. for seven consecutive days, and fifteen minutes after the last treatment, the animals received acetic acid (1%, 1mL/kg) as a noxious substance to activate the nociceptor receptors due to inflammation response and five minutes after injection of acetic acid, some abdominal contractions recorded the writhing response. As

expected, the contraction frequency must be diminished using analgesic medication [22].

2.3.2. Tail flick test

The tail flick is a basic pain test in animal research studies to measure the analgesic effect of compounds, and it was first described by D'Amour and Smith in 1941. The rodent's tail was exposed to radiant heat from the lamp at the specified spot. As expected, when the level of heat reaches a point of discomfort, the rodent will withdraw the tail. The tail-flick latency, referring to the time taken to respond to the heat stimulation (in a range of 46–55 °C, 10 seconds), is directly associated with the effectiveness of analgesics. The mean value of tail-flick latency was reported after the test was repeated thrice [22].

2.3.3. Hot plate test

The hot plate device is a plate heated by an electric current. In our experiment, all mice are placed on this plate heated to 55 degrees Celsius before injection, and the start time (zero) is determined. As soon as the hands start to lick or there is a specific change in the mice's gait, the tolerance level of the animal's base is recorded. After that, according to the injection groups, saline and drug are injected. Then, about 30 minutes after the injection, their tolerance level is measured and compared with the baseline tolerance level. The maximum time considered for the endurance level of mice is 40 seconds [22].

2.3.4 Formalin test

One of the satisfactory models to screen the effectiveness of analgesics is the formalin test, which measures either acute or long-lasting pain. To induce the pain, 50 µL of formalin 2.5% was injected sub continuously (SC) into the animal's right paw thirty minutes after the last treatment doses. Flexion, tonic flexion, and licking of the injected limb responses were divided into two main phases: acute (the first five minutes), lasting five to ten minutes post-injection, and chronic phase lasting roughly 40-60 minutes[22].

2.3.5. Xylene-Induced Ear Edema

Xylene was used as an inflammatory agent to induce edema and inflammation. Xylene (50 µL) was smeared on the surface of the right ear about one hour after the last treatment. The animals were sacrificed one hour after the smear, and then two ears were cut off and weighed. The inhibition rate was calculated according to the weight difference of two ears [22].

2.4. Statistical analysis

All statistical analysis was determined using SPSS 19.0 statistical software and one-way analysis of variance (ANOVA) in Duncan's test. $P<0.05$ was considered statistically significant and $P<0.01$ very significant. The data were expressed as mean value ± standard deviation (M ± SD).

3. Results and Discussion

3.1. FTIR analysis

As shown in **Figure 1**, the peak of 3397 cm-1 created in the FTIR record confirms the presence of hydroxyl groups in the structure of alcoholic and phenolic compounds of the extract. Also, the

2851 cm-1 and 2924 cm-1 peaks correspond to the tensile vibrations of C-H bonds. The 690 to 900 cm-1 and 1340 to 1470 cm-1 peaks indicate the presence of aliphatic groups of C-H in alkanes and aromatic rings of phenolic compounds. The peak with the wave number 1630 cm-1 is also probably due to the presence of C=O or C=C bands in the structure of the extract compounds.

3.2. Writhing time

As shown in **Figure 2**, the animals treated with morphine as a strong and potent opioid analgesic significantly reduced the writhing number as compared with the vehicle (P≤0.001), which means morphine was able to reduce the inflammatory effect induced by acetic acid. Concerning previous and present findings, the extract of *Chenopodium botrys* L. at doses of 100 and 300 mg/kg remarkably reduced the writhing number (P ≤ 0.001 for both), while the lowest dose (30 mg/kg) demonstrated an insignificant difference with the vehicle. Despite the effectiveness of the highest doses of *Chenopodium botrys* L. when administered alone, the group received a concurrent dosage of naloxone as a potent opioid antagonist with the highest dose of *Chenopodium botrys* L.

Notably diminished occurred in the analgesic effect of *Chenopodium botrys* L. that demonstrated insignificant difference with the vehicle.

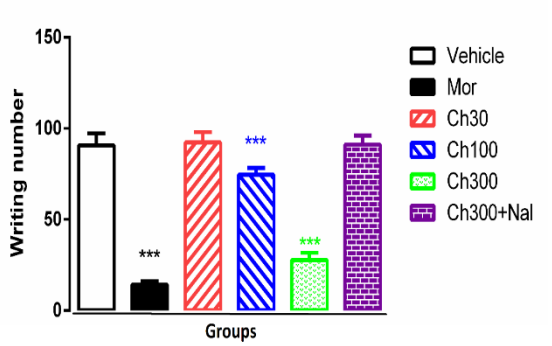

Figure 2. The writing numbers in treated mice with different Chenopodium botrys L. extract doses. Data presented as Mean±SD. ***p<0.001 compared to the vehicle.

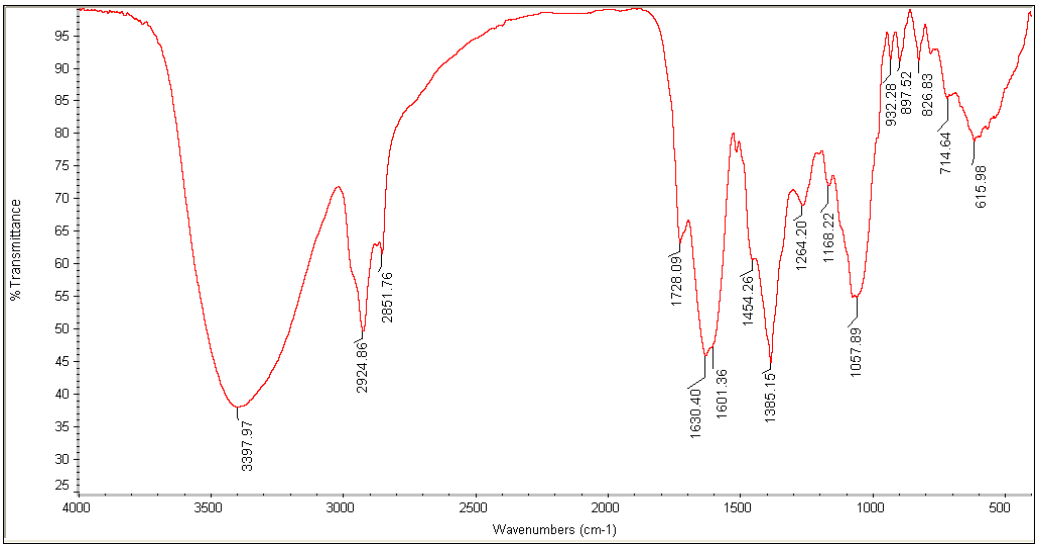

Figure 1. The Fourier transform infrared (FTIR) record of the *Chenopodium botrys* L. extract.

3.3. Tail flick test

The animals treated with morphine demonstrated significant tolerance to heat exposure within 90 minutes of the experiment study (P≤0.001; **Fig. 3**). The data demonstrated a notable analgesic effect at doses of 100 and 300 mg/kg within 45 minutes after the heat exposure (P≤0.001, and P≤0.05 respectively) to assess the analgesic effect of *Chenopodium botrys* L.; however, the lowest doses of *Chenopodium botrys* L. (30 mg/kg) demonstrated insignificant difference with the vehicle. Interestingly, concurrent treatment with naloxone and *Chenopodium botrys* L. (300 mg/kg) exhibited insignificant differences with the vehicle.

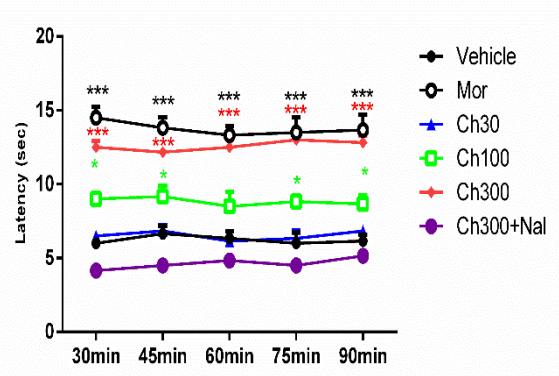

Figure 3. The latency time in treated mice with different Chenopodium botrys L. extract doses in the tail flick test. Data presented as Mean±SD. *p<0.05, ***p<0.001 compared to the vehicle.

3.4. Formalin test

As expected, the animals treated with both morphine as a potent opioid and *Chenopodium botrys* L. (100 and 300 mg/kg) demonstrated significant reduction in licking time in second phase (P≤0.001 for all), while the animals treated with the lowest doses of *Chenopodium botrys* L. and the groups received concurrent dose of naloxone and *Chenopodium botrys* L. (300 mg/kg) exhibited insignificant difference with the vehicle (**Fig. 4**).

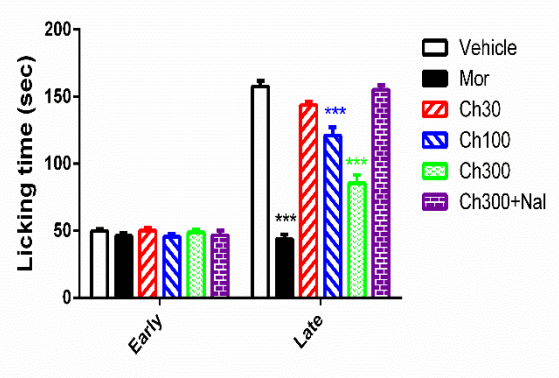

Figure 4. The licking time in treated mice with different Chenopodium botrys L. extract doses. Data presented as Mean±SD. ***p<0.001 compared to the vehicle.

3.5. Xylene-Induced Ear Edema

As shown in **Figure 5**, the animals received dexamethasone as a potent anti-inflammatory drug belonging to the glucocorticosteroids family and *Chenopodium botrys* L. at doses of 100, 300 mg/kg demonstrated significant difference with vehicle (P≤0.001). In contrast, the animals receiving the lowest extract dose exhibited insignificant differences.

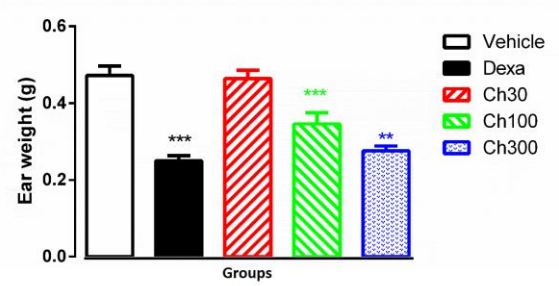

Figure 5. The ear weight in treated mice with different Chenopodium botrys L. extract doses. Data presented as Mean±SD. ***p<0.001 compared to the vehicle.

3.6. Hot plate test

As shown in **Figure 6**, the latency time in the hot plate in groups treated with morphine and *Chenopodium botrys* L. (100 and 300 mg/kg) significantly increased (P<0.05, and P<0.001), whereas the animals received the lowest dose of extract exhibited insignificant difference.

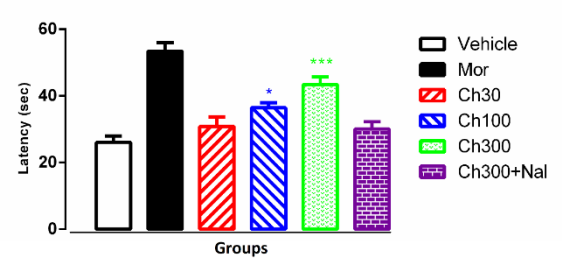

Figure 6. The latency time in treated mice with different Chenopodium botrys L. extract doses in the hot plate test. Data presented as Mean±SD. *p<0.05, ***p<0.001 compared to the vehicle.

The present findings confirmed the null hypothesis about the effectiveness of *Chenopodium botrys* L. on pain induced by inflammatory agents. The various doses of *Chenopodium botrys* L., including 30, 100, and 300 mg/kg, were gavaged for one week, and after that, the related behavioral tests, including writhing test, tail flick, and formalin test, along with xylene-induced inflammation test was applied. In an animal behavioral study related to pain, *Chenopodium botrys* L. at 100 and 300 mg/kg doses demonstrated much better results than the lowest dose. The results were comparable with morphine as a potent analgesic belonging to the opioid family. Interestingly, when the highest dose of *Chenopodium botrys* L. was administered with naloxone concurrently, the analgesic effect significantly vanished, which means the other possible mechanisms of *Chenopodium botrys* L. probably is through opioid receptors that were blocked with naloxone as an opioid receptor antagonist.

Moreover, in xylene-induced inflammation, the highest doses of *Chenopodium botrys* L. significantly diminished the inflammation, and the results were comparable with dexamethasone as a potent and well-known anti-inflammatory drug belonging to the glucocorticosteroid family. In this regard, several studies demonstrated a broad pharmacologic therapeutic effect of *Chenopodium botrys* L., including analgesic, anti-inflammatory, antimicrobial, antioxidant, and neuroprotective. Recorded FTIR shows the presence of phenolic compounds and flavonoids in the extract, which can confirm it. This extract also possesses monoterpenes, which are anti-inflammatory agents. The essential oils of this extract and two of their major components, namely 1,8-cineole and limonene, are believed to contribute to the anti-inflammatory activity of these species, in which 1,8-cineole has been shown to cause a partial potentiation of the anti-inflammatory action exhibited by limonene [23]. Also, Flavonoids are known to have analgesic, anti-inflammatory, and antioxidant properties [24]. These effects are related to the inhibition of NF-κB-dependent pro-inflammatory cytokines, VEGF, ICAM-1, STAT3, and activation of antioxidant transcription factor Nrf2 [25]. However, much evidence implicated several pharmacologic effects related to *Chenopodium botrys* L. Few studies investigate the anti-inflammatory or analgesic effect of that in detail. To the best of the authors' knowledge, the present findings concerning investigating the analgesic effect of *Chenopodium botrys* L. are rarely established. However, it needs further investigation. In fact, Chenopodium botrys L. is

one of the famous plant species belonging to a large family of Chenopodiaceae, which is a rich source of medicinal plants in Iran. Furthermore, there is some evidence of the positive effect of *Chenopodium botrys* L., which is mainly attributed to ascaridole as a main and effective constituent of *Chenopodium botrys* L. Regards to the present findings and related similar studies, it is clear that this plant needs to be further investigated for the isolation and characterization of new and effective phytochemicals. This study could be a precursor for future studies.

4. Conclusion

Based on the findings, the *Chenopodium botrys* L. plant can be introduced as a new candidate in herbal medicine to treat pain and inflammation comparable with morphine and dexamethasone as potent analgesic and anti-inflammatory drugs. However, it required further investigation to identify the exact compounds responsible for these effects.

Acknowledgments

None.

Conflict of interest

The authors declare to have no conflict of interest.

References

[1] Świeboda P, Filip R, Prystupa A, Drozd M. Assessment of pain: types, mechanism and treatment. Pain (2013) 2(7).

[2] Belmonte C. Pain, dryness, and itch sensations in eye surface disorders are defined by a balance between inflammation and sensory nerve injury. Cornea (2019) 38:S11-S24.

[3] Linton SJ. A transdiagnostic approach to pain and emotion. Journal of Applied Biobehavioral Research (2013) 18(2):82-103.

[4] Shraim MA, Masse-Alarie H, Hall LM, Hodges PW. Systematic review and synthesis of mechanism-based classification systems for pain experienced in the musculoskeletal system. The Clinical journal of pain (2020) 36(10):793-812.

[5] Zielińska A, Włodarczyk M, Makaro A, Sałaga M, Fichna J. Management of pain in colorectal cancer patients. Critical Reviews in Oncology/Hematology (2021) 157:103122.

[6] Shimo K, Ogawa S, Niwa Y, Tokiwa Y, Dokita A, Kato S, et al. Inhibition of current perception thresholds in A-delta and C fibers through somatosensory stimulation of the body surface. Scientific Reports (2022) 12(1):1-6.

[7] Talagas M, Lebonvallet N, Berthod F, Misery L. Cutaneous nociception: role of keratinocytes. Experimental Dermatology (2019) 28(12):1466-9.

[8] D'Amato T, Martorelli F, Fenocchio G, Simili V, Kon E, Di Matteo B, et al. Tapentadol vs oxycodone/naloxone in the management of pain after total hip arthroplasty in the fast track setting: an observational study. Journal of Experimental Orthopaedics (2019) 6(1):1-7.

[9] Korbut R, Guzik TJ. Inflammatory Mediators and Intracellular Signalling. Nijkamp and Parnham's Principles of Immunopharmacology (2019):139-63.

[10] Wang RX, Zhou M, Ma HL, Qiao YB, Li QS. The Role of Chronic Inflammation in Various Diseases and Anti- inflammatory Therapies Containing Natural Products. ChemMedChem (2021) 16(10):1576-92.

[11] Fatima Shad K, Soubra W, Cordato DJ. The role of thymoquinone, a major constituent of Nigella sativa, in the treatment of inflammatory and infectious diseases. Clinical and Experimental Pharmacology and Physiology (2021) 48(11):1445-53.

[12] Trento MMS, Moré AOO, Duarte ECW, Martins DF. Peripheral receptors and neuromediators involved in the antihyperalgesic effects of acupuncture: a state-of-the-art review. Pflügers Archiv-European Journal of Physiology (2021) 473(4):573-93.

[13] Khan A, Khan S, Kim YS. Insight into pain modulation: nociceptors sensitization and therapeutic targets. Current drug targets (2019) 20(7):775-88.

[14] Mohammadkhani A, Borgland SL. Cellular and behavioral basis of cannabinioid and opioid interactions: implications for opioid dependence and withdrawal. Journal of Neuroscience Research (2022) 100(1):278-96.

[15] Kaczmarski P, Karuga FF, Szmyd B, Sochal M, Białasiewicz P, Strzelecki D, et al. The role of inflammation, hypoxia, and opioid receptor expression in pain modulation in patients suffering from obstructive sleep apnea. International Journal of Molecular Sciences (2022) 23(16):9080.

[16] Gazelka HM, Leal JC, Lapid MI, Rummans TA, editors. Opioids in older adults: indications, prescribing, complications, and alternative therapies for primary care. Mayo Clinic Proceedings; 2020: Elsevier.

[17] Han Y, Yan W, Zheng Y, Khan MZ, Yuan K, Lu L. The rising crisis of illicit fentanyl use, overdose, and potential therapeutic strategies. Translational psychiatry (2019) 9(1):282.

[18] James PB, Kaikai AI, Bah AJ, Steel A, Wardle J. Herbal medicine use during breastfeeding: a cross-sectional study among mothers visiting public health facilities in the Western area of Sierra Leone. BMC complementary and alternative medicine (2019) 19(1):1-11.

[19] Dehghan M, Namjoo Z, Bahrami A, Tajedini H, Shamsaddini-Lori Z, Zarei A, et al. The use of complementary and alternative medicines, and quality of life in patients under hemodialysis: A survey in southeast Iran. Complementary Therapies in Medicine (2020) 51:102431.

[20] Nasim S, Shah M, Shah SWA, Ahmed MN, Ahmad M, Anwar N, et al. Activity guided isolation and mechanistic approach towards analgesic potential of Chenopodium mediated through opioidergic pathway. Pakistan Journal of Pharmaceutical Sciences (2021) 34.

[21] SEZER ENŞ, UYSAL T. Phenolic screening and biological activities of Chenopodium botrys L. extracts. Anatolian Journal of Botany (2021) 5(2):78-83.

[22] Fallahzadeh AR, Mohammadi S. Assessment of the antinociceptive, anti-inflammatory, and acute toxicity effects of solanum dulcamara essential oil in male mice. Journal of Babol University of Medical Sciences (2020) 22(1):162-8.

[23] de Cássia da Silveira e Sá R, Andrade LN, de Sousa DP. A review on anti-inflammatory activity of monoterpenes. Molecules (2013) 18(1):1227-54.

[24] Verri Jr WA, Vicentini FTMC, Baracat MM, Georgetti SR, Cardoso RDR, Cunha TM, et al. Flavonoids as anti-inflammatory and analgesic drugs: mechanisms of action and perspectives in the development of pharmaceutical forms. Studies in natural products chemistry (2012) 36:297-330.

[25] Ferraz CR, Carvalho TT, Manchope MF, Artero NA, Rasquel-Oliveira FS, Fattori V, et al. Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. Molecules (2020) 25(3):762.