Razi Med J. 2026;2(1):58-62
https://doi.org/10.69667/rmj.26108
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Original article

Analgesic Effect of Ethanolic Buthus occitanus Tail Extract in Albino Mice

Malak Eljafari*, Ebtihal Franda

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya.
Corresponding email: *M.jafari@uot.edu.ly*

Abstract
Scorpion powders have been used in traditional Chinese medicine for centuries to treat inflammation, pain, and cancer. Buthus martensii Karsch is the most frequently investigated scorpion species, whereas the Libyan species of B. occitanus remains pharmacologically unexplored. To study the potential analgesic effect of a Buthus occitanus ethanolic tail extract (BOETE) collected from Tarhona, Libya, in albino mice. Adult male and female albino mice (weighing 19–30 g, n = 7 per group) received intraperitoneal injections of either vehicle (0.9 % saline), BOETE (300 or 1000 mg/kg), or tramadol HCl (20 mg/kg). Thirty minutes post-injection, thermal nociception was quantified with the hot-plate (55 ± 1 °C) and tail-immersion (50 ± 1°C) tests; cut-off latencies were set at 30 s, respectively, to prevent tissue damage. Both doses of BOETE significantly increased reaction times compared to vehicle in the hot-plate assay ($p < 0.05$). In contrast, the extract at either dose did not prolong latency in the tail-immersion test compared to the saline group, and tramadol hydrochloride produced the largest analgesic effect during tail immersion. A promising analgesic effect was obtained by BOETE against thermal stimulation in mice, supporting its traditional use as an analgesic. These findings warrant further studies to isolate the bioactive components and elucidate the underlying mechanisms of action.
Keywords. *Analgesic, Scorpion, Buthus Occitanus, Ethanolic Extract.*

Received*: 19/11/25*
Accepted*: 17/01/26*
Published*: 25/01/26*

Introduction

Scorpions have been used for centuries as animal-derived medicinal agents in traditional Chinese medicine, as documented in numerous ancient medical books [1]. Historically, scorpion preparations were employed in the treatment of a wide range of conditions, including stroke, epilepsy, rheumatism, and hernia [2]. In the most recent edition of the Chinese Pharmacopoeia (2020), several scorpion-based formulations, such as Naoxintong capsules and Dianxianping tablets, are officially listed and clinically used for the management of rheumatism, muscle spasms, convulsions, hemiplegia, palpitations, cerebral infarction, coronary heart disease, angina pectoris, and related disorders [3].

Recently, modern pharmacological research has begun to substantiate these traditional applications. Scorpion-derived compounds, particularly venoms and venom-associated peptides, have been shown to possess diverse biological activities, including analgesic, anti-inflammatory, immunomodulatory, antidiabetic, and antitumor effects [4–8]. To date, scorpion venoms have been considerably more extensively characterized than whole-body scorpion preparations or powders [9-11], with the Chinese scorpion *Buthus martensii* Karsch (BmK) being among the most thoroughly studied species. Notably, more than 2,200 scorpion species have been identified worldwide, indicating substantial interspecies diversity with respect to pharmacological potential [12]. *Buthus occitanus*, commonly known as the yellow scorpion, belongs to the family Buthidae and is widely distributed across North Africa, the Middle East, and parts of southern Europe. Adult specimens typically measure 6–8 cm in length and are yellow to yellow-brown in color. The venom of *B. occitanus* contains several bioactive toxins, including BotIT6, with toxicity known to vary geographically [13]. Despite its wide distribution, particularly in Libya and other regions of North Africa, the pharmacological and biological properties of *B. occitanus* remain poorly characterized.

Limited studies from the Maghreb region have investigated specific aspects of *B. occitanus*, such as chemical composition analysis and antivenom efficacy [14,15]. However, these investigations have largely focused on isolated toxicological or immunological endpoints rather than providing a comprehensive pharmacological evaluation. Consequently, substantial gaps remain in our understanding of the biological activities of *B. occitanus*, particularly with respect to its potential therapeutic applications. Considering all of the previous, the present study was designed to study the potential analgesic effect of Libyan *Buthus occitanus* ethanolic tail extract (BOETE) using thermal experimental pain models in albino mice.

Razi Med J. 2026;2(1):58-62
https://doi.org/10.69667/rmj.26108
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Materials and Methods

Scorpion Sources and Identification

Approximately 57 adult scorpions used in this study were collected in April 2025 from the Sidi Alsaid area, Tarhoona, Libya, during the active harvesting season, which extends from April to October. The collected scorpions were transported to the Zoology Department, University of Tripoli, where they were maintained alive in plastic containers under appropriate laboratory conditions. Species identification and taxonomic classification were conducted by a specialized taxonomist, Prof. Masoud Moamer, who confirmed the specimens as *Buthus occitanus*.

Preparation of Extracts

Identified scorpions were extracted using the hot-dip method (Appendix XA) in accordance with the alcohol-soluble extract assay described in the Chinese Pharmacopoeia (2005 edition) [3]. Briefly, the scorpions were crushed and accurately weighed, and a 3.0166 g sample was transferred to a 250-mL Erlenmeyer flask. The sample was mixed with 100 mL of 75% (v/v) diluted ethanol and allowed to stand for 1 hour. Extraction was then carried out under heat reflux for 1 hour, after which the mixture was cooled and reweighed. Any loss in weight was compensated for by the addition of diluted ethanol. The extract was subsequently filtered, and a 25-mL aliquot of the filtrate was evaporated to dryness and dried to constant weight. Final dehydration was performed in a water bath or oven at 105 °C for 3 hours. The dried samples were then cooled in a desiccator for 30 minutes and accurately weighed.

Animals

Pharmacological experiments were conducted using healthy Swiss albino mice of both sexes, weighing 19–30 g. The animals were housed in the animal facility of the Faculty of Pharmacy, University of Tripoli. Mice were maintained in polyacrylic cages under standard laboratory conditions, randomly assigned to experimental groups, and kept on a 12 h light/12 h dark cycle. Food and water were provided *ad libitum*. All animals were allowed to acclimatize to the laboratory environment before the experiment. The animals were handled in accordance with current guidelines for the care of laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals. All efforts were made to minimize the number of animals used and their suffering.

Drugs and chemicals

Tramadol hydrochloride (TRAMADIS©, Tunisia) in the form of solution for injection, all other chemicals were of analytical or medical grade.

Experimental Design

A total of twenty-eight mice were randomly assigned to four experimental groups, with seven animals in each group. Group I served as the negative control and received normal saline as the vehicle. Group II was administered B. occitanus tail extract at a dose of 300 mg/kg via intraperitoneal injection, [16] while Group III received the same extract at a higher dose of 1000 mg/kg intraperitoneally. [17] Group IV acted as the positive control and was treated with tramadol at a dose of 20 mg/kg administered intraperitoneally.

Mice were fasted for 12 h before the experiment, with free access to water. All treatments were administered 30 minutes before nociceptive testing (hot plate and tail immersion tests).

Hot Plate Test

The hot-plate test was employed to assess thermal nociceptive response, following the method of Eddy and Leimbach [18]. Each mouse was placed on a hot-plate apparatus maintained at 55±1 °C, and the latency to a pain response was defined as (paw licking or jumping), the reaction time was recorded in seconds. To ensure consistent baseline sensitivity, mice were pre-screened for responsiveness several days before the experiment, and only animals exhibiting response latencies of less than 30 seconds were included in the study.

Tail-Flick Test

The analgesic activity of the extract was evaluated by the tail-flick method described [19]. About 5 cm from the distal end of the tail of each mouse was immersed in a warm water bath maintained at 50±1°C. The reaction time (in seconds) was the time taken by the rat to flick its tail due to pain.

Razi Med J. 2026;2(1):58-62
https://doi.org/10.69667/rmj.26108
Razi Medical Journal
https://razi.edu.ly/rmj/index.php/hm

Statistical analysis

The raw data that were obtained from the experiment were expressed as mean ± SEM (standard error of the mean). The results were statistically analyzed using Welch-ANOVA followed by the Games-Howell Post Hoc Test for multiple comparisons to compare results among groups, and the results were considered significant at $p < 0.05$ (SPSS version 22 software was used for data processing).

Results

Effect of Buthus occitanus tail extract on thermal pain response (Hot Plate Test)

The hot-plate test results (Figure 1) demonstrated a significant difference in analgesic response latency among the experimental groups ($p < 0.05$). Administration of *Buthus occitanus* ethanolic tail extract (BOETE) at both tested doses significantly prolonged response latency compared with the negative control group. The tramadol-treated group exhibited the greatest increase in latency; however, no statistically significant difference was observed between the tramadol-treated and BOETE-treated groups.

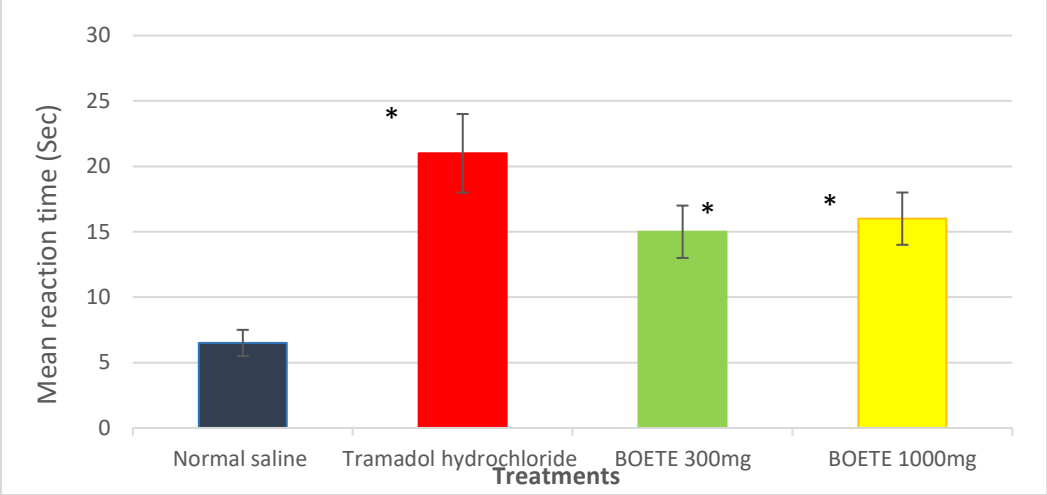

Figure 1. Effect of BOETE on reaction time in thermally induced pain stimulus in mice (Hot plate method) *P<0.05 shows a significant statistical difference compared with the negative control, (N=7; value is expressed as Mean ± SEM). BOETE= Buthus occitanus ethanolic tail extract

Effect of Buthus occitanus tail extract on thermal pain response (Tail immersion test)

As shown in (Figure 2), tramadol hydrochloride produced a marked and statistically significant increase in latency compared with all other groups, while treatment with BOETE at either dose did not produce a statistically significant change in tail-withdrawal latency compared with the (normal saline) negative control group ($p > 0.05$).

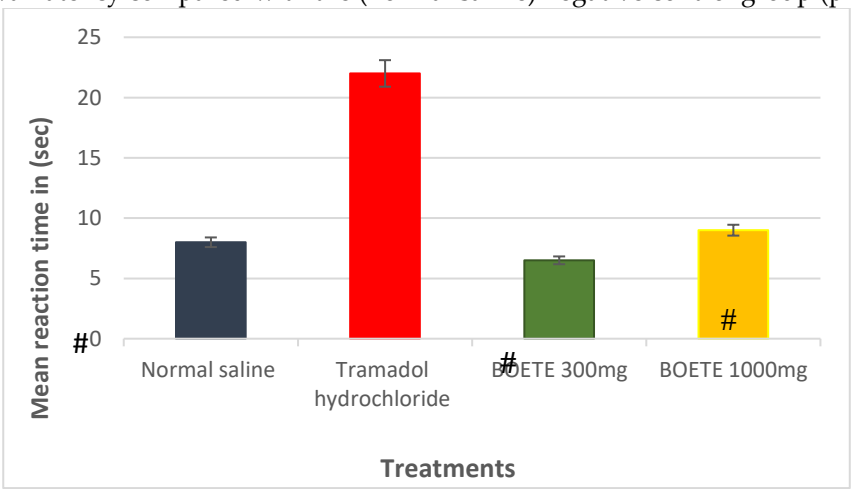

Figure 2. Effect of BOETE on reaction time in thermally induced pain stimulus in mice (Tail immersion method) P<0.05 shows a significant statistical difference compared with the Tramadol hydrochloride (N=7; value is expressed as Mean ± SEM). BOETE= Buthus occitanus ethanolic tail extract.

Discussion

In the current study, BOETE demonstrated a significant analgesic effect in the hot-plate test, particularly in groups treated with the high dose of the extract, indicating a reduction in pain sensitivity and confirming its analgesic potential. These results are consistent with previous studies, such as the work on the β-type scorpion neurotoxin Syb-prII-1, isolated from the Chinese Scorpio BmK venom, which significantly alleviated pain behaviors in a rat model of trigeminal neuralgia, likely through modulation of voltage-gated sodium channels and inhibition of mitogen-activated protein kinase (MAPK) signaling pathways, which are known to mediate pain sensitization and inflammatory responses [20]. Similarly, Shao and colleagues reported the purification and identification of an analgesic peptide from BmK, yielding substantial amounts of natural analgesic peptides (BmK AS) with high analgesic activity [21]. In contrast, the tail-immersion test did not show a significant prolongation of latency in mice treated with BOETE at either low or high doses compared to the normal saline group, and both BOETE-treated groups exhibited significantly lower latency than the tramadol-treated group ($P < 0.05$). The differences observed between the two thermal assays may be related to the distinct pain pathways they target: the tail-immersion test primarily reflects spinal reflex responses to nociceptive stimuli, whereas the hot-plate test measures supraspinal responses to pain [22,23]. Overall, the preliminary findings of this study indicate that BOETE exhibits promising analgesic activity, particularly against thermally induced nociception using the hot-plate method. These results provide a pharmacological basis for further investigation of Libyan-harvested *Buthus occitanus* as a potential source of analgesic agents.

Limitations of the study

This study represents an initial pharmacological assessment of BOETE using thermal pain models. Future investigations involving inflammatory and neuropathic pain models, together with mechanistic and chemical analyses, are necessary to identify and characterize the specific bioactive constituents responsible for the observed activity. Broad pharmacological evaluations will be essential to support the therapeutic development of BOETE.

Conclusion

The findings of this study demonstrate that Buthus occitanus ethanolic tail extract (BOETE) exerts significant analgesic activity in the hot-plate test, particularly at higher doses, suggesting modulation of supraspinal pain pathways. In contrast, BOETE did not produce significant effects in the tail-immersion test, indicating limited influence on spinal reflex responses. These results highlight the potential of Libyan-harvested Buthus occitanus as a source of natural analgesic compounds and provide a pharmacological basis for further studies aimed at isolating and characterizing its active constituents. Continued investigation is warranted to clarify its mechanisms of action and explore its therapeutic applicability in pain management.

Acknowledgment

We sincerely acknowledge Professor Masoud Moamer, taxonomist at the Zoology Department, Faculty of Science, University of Tripoli, for his invaluable assistance and cooperation in accurately identifying the scorpion species used in this study.

References

1. Zheng Y, Wen Q, Huang Y, Guo D. The significant therapeutic effects of Chinese scorpion: modern scientific exploration of ion channels. Pharmaceuticals (Basel). 2024 Dec 22;17(12):1735. doi:10.3390/ph17121735. PMID:39770577; PMCID:PMC11678150.
2. Zhang K, Zhang Y, Yang C, Fu J, Li Y, J, et al. Research progress on processing history evolution, chemical constituents and pharmacological action of Scorpio. China J Chin Mater Med. 2024;49:868-883. doi:10.19540/j.cnki.cjcmm.20230814.202.
3. Chinese Pharmacopoeia Commission. Chinese Pharmacopoeia. 2020 ed. Vol. 1. Beijing (China): China Medical Science Press; 2020.
4. Mao Q, Ruan J, Cai X, Lu W, et al. Antinociceptive effects of analgesic-antitumor peptide (AGAP), a neurotoxin from the scorpion Buthus martensii Karsch, on formalin-induced inflammatory pain through a MAPKs-dependent mechanism in mice. PLoS One. 2013;8:e78239. doi:10.1371/journal.pone.0078239.
5. Liu Y, Li Y, Zhu Y, Zhang L, Ji J, Gui M, et al. Study of anti-inflammatory and analgesic activity of scorpion toxins DKK-SP1/2 from Buthus martensii Karsch. Toxins (Basel). 2021 Jul 17;13(7):498. doi:10.3390/toxins13070498. PMID:34357970; PMCID:PMC8310270.

6. Haddad L, Chender A, Roufayel R, Accary C, et al. Effect of Hottentotta judaicus scorpion venom on nociceptive response and inflammatory cytokines in mice using experimental hyperalgesia. Molecules. 2025;30(13):2750. doi:10.3390/molecules30132750.
7. Xie W, Zhao Y, Gu D, Du L, et al. Scorpion in combination with gypsum: novel antidiabetic activities in streptozotocin-induced diabetic mice by up-regulating pancreatic PPARγ and PDX-1 expressions. Evid Based Complement Alternat Med. 2011;2011:683561. doi:10.1093/ecam/neq031. PMID:21799688; PMCID:PMC3136920.
8. Srairi-Abid N, Othman H, Aissaoui D, BenAissa R. Anti-tumoral effect of scorpion peptides: emerging new cellular targets and signaling pathways. Cell Calcium. 2019;80:160-174. doi:10.1016/j.ceca.2019.05.003.
9. Nasr S, Borges A, Sahyoun C, Nasr R, et al. Scorpion venom as a source of antimicrobial peptides: overview of biomolecule separation, analysis and characterization methods. Antibiotics (Basel). 2023;12(9):1380. doi:10.3390/antibiotics12091380.
10. Cid-Uribe JI, Veytia-Bucheli JI, Romero-Gutierrez T, Ortiz E, et al. Scorpion venomics: a 2019 overview. Expert Rev Proteomics. 2020;17(1):67-83. doi:10.1080/14789450.2020.1705158.
11. Thanasarakul S, Yodsiri P, Mongmonsin U, Saengkun Y, et al. Pharmacological activity of scorpion venom: updating and application. Thai J Toxicol. 2021;36(2):28-47.
12. Graham MR. Scorpions of the world. 2011. doi:10.1636/0161-8202-39.1.166.
13. Martin-Eauclaire MF, Bosmans F, Céard B, Diochot S, Bougis PE. A first exploration of the venom of the Buthus occitanus scorpion found in southern France. Toxicon. 2014 Mar;79:55-63. doi:10.1016/j.toxicon.2014.01.002. PMID:24418174; PMCID:PMC3952629.
14. Darkaoui B, Hilal I, Khourcha S, Lafnoune A, et al. Development and efficacy of the antivenom specific to severe envenomations in Morocco and North Africa: advancements in scorpion envenomation management. Toxins (Basel). 2024;16(5):214. doi:10.3390/toxins16050214.
15. Daoudi K, El Ayeb M, Srairi-Abid N, et al. Mass spectrometry-based top-down and bottom-up approaches for proteomic analysis of the Moroccan Buthus occitanus scorpion venom. FEBS Open Bio. 2021;11:1867-1892. doi:10.1002/2211-5463.13143.
16. Abdel-Rahman MA, Mohammed AK, Ahmed SH, Binnaser YS, Abdel-Nabi IM. Antidiabetic effect of the scorpion Scorpio maurus palmatus body extract using alloxan-induced diabetic mice model. J Taibah Univ Sci. 2019;13(1):504-513. doi:10.1080/16583655.2019.1599184.
17. Li L, Ge S, Wang Y, et al. Untargeted metabolomics reveal the corrective effects of scorpion on epileptic mice. Sci Rep. 2025;15:937. doi:10.1038/s41598-024-84028-5.
18. Eddy NB, Leimback D. Synthetic analgesic II. Diethlenyl butenyl and dithienyl butylamines. J Pharmacol Exp Ther. 1953;107:385-393.
19. Ramabadran K, Bansinath M, Turndorf H, Puig MM. Tail immersion test for the evaluation of a nociceptive reaction in mice: methodological considerations. J Pharmacol Methods. 1989;21(1):21-31. doi:10.1016/0160-5402(89)90019-3.
20. Bai F, Song Y, Cao Y, Ban M, Zhang Z, Sun Y, et al. Scorpion neurotoxin Syb-prII-1 exerts analgesic effect through Nav1.8 channel and MAPKs pathway. Int J Mol Sci. 2022;23(13):7065. doi:10.3390/ijms23137065.
21. Shao J, Kang N, Liu Y, Song S, Wu C, Zhang J. Purification and characterization of an analgesic peptide from Buthus martensii Karsch. Biomed Chromatogr. 2007 Dec;21(12):1266-1271. doi:10.1002/bmc.882. PMID:17604360.
22. Arslan R, Bektas N. Evaluation of the centrally acting mechanisms of some nonsteroidal anti-inflammatory drugs. Asian J Pharm Health Res. 2015;3(6).
23. Türkmen NB, Aydın S. The possible action mechanisms of central analgesic effect of protocatechuic acid. Int J Neuropsychopharmacol. 2016;19(Suppl 1):35-36. doi:10.1093/ijnp/pyw044.644. PMCID:PMC561697.