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Review Article

Muscimol as a treatment for nerve injury-related neuropathic pain: a systematic review and meta-analysis of preclinical studies

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ABSTRACT

Background: Muscimol's quick onset and GABAergic properties make it a promising candidate for the treatment of pain. This systematic review and meta-analysis of preclinical studies aimed at summarizing the evidence regarding the efficacy of muscimol administration in the amelioration of nerve injury-related neuropathic pain.

Methods: Two independent researchers performed the screening process in Medline, Embase, Scopus and Web of Science extracting data were extracted into a checklist designed according to the PRISMA guideline. A standardized mean difference (SMD [95% confidence interval]) was calculated for each. To assess the heterogeneity between studies, I^2 and chi-square tests were utilized. In the case of heterogeneity, meta-regression and subgroup analyses were performed to identify the potential source.

Results: Twenty-two articles met the inclusion criteria. Pooled data analysis showed that the administration of muscimol during the peak effect causes a significant reduction in mechanical allodynia (SMD = 1.78 [1.45–2.11]; $P < 0.0001$; $I^2 = 72.70\%$), mechanical hyperalgesia (SMD = 1.62 [1.28–1.96]; $P < 0.0001$; $I^2 = 40.66\%$), and thermal hyperalgesia (SMD = 2.59 [1.79–3.39]; $P < 0.0001$; $I^2 = 80.33\%$). This significant amendment of pain was observed at a declining rate from 15 minutes to at least 180 minutes post-treatment in mechanical allodynia and mechanical hyperalgesia, and up to 30 minutes in thermal hyperalgesia ($P < 0$ .0001).

Conclusions: Muscimol is effective in the amelioration of mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia, exerting its analgesic effects 15 minutes after administration for up to at least 3 hours.

Keywords: Analgesia; Gamma-Aminobutyric Acid; Hyperalgesia; Meta-Analysis; Muscimol; Neuralgia; Pain; Peripheral Nerve Injuries; Spinal Cord Injuries.

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INTRODUCTION

Pain refers to the unpleasant emotional and sensory experience generated by noxious stimuli. It is a complex and multifaceted experience, and the most common symptomatic complaint in medicine [1]. The classifications of pain exhibit variations in scientific literature, leading to different estimates of prevalence, and treatment strategies [2–4]. Chronic pain is one of the most debilitating complications of trauma to the nervous system with a prevalence of around 68% in people with spinal cord injuries [5].

The International Association for the Study of Pain describes chronic neuropathic pain (NP) as "*chronic pain caused by a lesion or disease of the somatosensory nervous system*" [6,7]. NP is categorized into central and peripheral, with recent evidence suggesting that a majority of patients with traumatic nerve injuries are affected [8–10]. This type of pain is extremely hard to treat due to its complex and heterogeneous etiologies. NP is often severe and resistant to treatment, making management challenging for clinicians [11–13]. In light of that, it should be noted that the current management strategies express moderate efficacy, leading to low quality of life and high costs of care [14]. While acetaminophen, nonsteroidal anti-inflammatory drugs, and opioids have traditionally been the go-to medications for pain management, there has been an urgent need for safer and more effective alternatives mostly due to the side effects that limit their use, including the high potential of addiction and tolerance [15]. In recent years, remedies such as some derivatives of mushrooms have emerged as promising sources of analgesics. Muscimol, a compound found in the Amanita muscaria mushroom, has been identified as having analgesic properties because of its ability to activate gamma-aminobutyric acid (GABA) receptors [16].

$GABA_A$ receptors are ligand-gated ion channels that mediate the majority of inhibitory nerve transmission in the central nervous system. It is believed that by binding selectively to the $GABA_A$ receptors at the same site as GABA, muscimol increases GABA's affinity for the receptor, which enhances neuronal inhibition and causes a subsequent reduction in pain sensation [17]. A hypothesis put forth was thatmuscimol demonstrated greater efficacy than GABA, producing approximately 120%–140% of GABA's maximal efficacy [18].

Along with its analgesic properties, muscimol has been found to possess antioxidant and anti-inflammatory effects [19]. Moreover, another key advantage of muscimol as a potentialpain medication is its relatively short half-life. The effect of muscimol peaks around 3 hours after administration [20]. This demonstrates that muscimol is rapidly metabolized and excreted from the body, reducing the risk of accumulation and toxicity.

Muscimol's quick onset and GABAergic properties make it a promising candidate for the management of pain. Its ability to selectively bind to specific $GABA_A$ receptor subtypes may also provide opportunities for the development of more targeted pain therapies with fewer side effects. Although muscimol has shown promising properties for alleviating pain, different studies have yielded variable results and conclusions, highlighting the need for a systematic review. The primary objective of this systematic review and meta-analysis is to gain a comprehensive understanding of muscimol's potential as a treatment for alleviating nerve injury-related NP.

MATERIALS AND METHODS

1. Study design and search strategy

The present systematic review and meta-analysis aimed at summarizing the evidence regarding the efficacy of muscimol administration in the amelioration of nerve injury-related NP. For this purpose, the keywords related to muscimol, pain, and nerve injury were selected from a comprehensive search in the MeSH database of Medline, Emtree of Embase, and recommendations from experts in the field. The keywords were assembled in a search strategy designed exclusively for each database with appropriate tags and Boolean operators. An extensive search was conducted in Medline, Embase, Scopus, and Web of Science by May 1, 2023, to find related articles. Also, a manual search in the grey literature (Google and Google Scholar) was directed to avoid missing any articles. **Table 1** presents our search strategies in each database.

2. Selection criteria

PICO in this study was defined as Population (P) being animals with nerve injury-associated NP, Intervention (I) being the administration of muscimol, Comparison (C) being made with a control group, and Outcomes (O) being alterations in different scales of NP measurements. Review studies, studies without a traumatic nerve injury induction method, studies evaluating chemically-induced or inflammatory pain, studies not reporting a desired outcome, studies that did not use muscimol, studies without a valid control group, studies without an

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Table 1. Quality assessment of the included articles

		Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Overall
Dias	2016	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Gwak	2016	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hama	2012	Unclear	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Low
Hosseini	2014	Yes	Yes	No	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hosseini	2020	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hwang	1997	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Jeon	2006	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Jiang	2014	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
LaGraize	2007	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Lee	2010	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Low
Lee	2015	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Low
Moon	2016	Unclear	Yes	Yes	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Low
Moon	2017	Unclear	Yes	Yes	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Low
Nasirinezhad	2019	Unclear	Yes	Yes	Yes	Unclear	Unclear	Yes	Unclear	Yes	Yes	Low
Pedersen	2007	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Low
Rashid	2002	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Rode	2005	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes	Low
Sadeghi	2021	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Seno	2018	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Wei	2009	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Yowtak	2013	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low
Zarrindast	2001	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Low

1. Was the allocation sequence adequately generated and applied?
2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?
3. Was the allocation adequately concealed?
4. Were the animals randomly housed during the experiment?
5. Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?
6. Were animals selected at random for outcome assessment?
7. Was the outcome assessor blinded?
8. Were incomplete outcome data adequately addressed?
9. Are reports of the study free of selective outcome reporting?
10. Was the study apparently free of other problems that could result in high risk of bias?

immediate post-intervention follow-up evaluation, and abstracts were excluded.

3. Data collection and quality assessment

The results of the systematic search were integrated into the Endnote 20.0 software and duplicate records were removed. In the initial screening process, two independent researchers screened the titles and abstracts of all obtained articles. If an article was considered potentially relevant, the full text was attained and all full texts were reviewed in the secondary screening process. By implementing the inclusion criteria, the final included articles were selected. If an article's full text was unavailable, we contacted the corresponding author at least twice by email. If an article was in a language other than English, it was translated by a researcher fluent in both languages. The data from the included articles were extracted into a checklist designed based on the PRISMA guideline. Data included information regarding the study's first author, year of publication, studied animals' characteristics, nerve injury method, time interval to muscimol administration, muscimol dose, route of muscimol administration, assessment timelines, assessment sites for pain detection, and the outcome tests.

The quality of the included studies was evaluated based on the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias assessment tool. This tool evaluates the overall methodology and potential risk of bias in pre-clinical studies by answering the questions in 10 major domains. In general, the adequate generation, blinding, and application of the allocation sequence, the blinding of the research conductors, caregivers, and outcome assessors, the avoidance of selective outcome reporting, and random housing and outcome assessments are investigated. In the case of a disagreement, the dispute was resolved through discussions with a third researcher.

4. Certainty of evidence

The certainty of the evidence was assessed by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework [21].

5. Statistical analyses

Statistical analyses were done using STATA 17.0 (StataCorp LLC). The included studies were classified based on the reported outcome. A standardized mean difference (SMD) with a 95% confidence interval (95% CI) was calculated for each sample and they were pooled to calculate an overall effect size. If a study used a scale in which a higher efficacy was observed with a lower score on the index scale, the absolute SMD value was inserted into the analysis. It should be noted that meta-analysis was only performed if data were reported by at least three separate analyses. A Galbraith plot was used to assess outlier studies. If we observed an outlier in a reported outcome, we did not include the data in the pooled analysis. A random or fixed effect model was chosen based on the presence or absence of heterogeneity. To assess the heterogeneity between studies, I^2 and chi-square tests were utilized. In the case of heterogeneity, meta-regression was performed to identify the potential source. Additionally, publication bias was reported with a Funnel Plot using Egger's test.

RESULTS

1. Study characteristics and flow

Finally, the data from 22 articles were included in the present meta-analysis (**Fig. 1**) [22–43]. Nineteen articles employed rats and 3 articles used mice. Nine studies used the chronic compression injury model and 5 studies carried out sciatic nerve injury/sciatic nerve ligation (SNL) for pain induction. Pain induction was established by spinal cord injury (SCI) in 6 studies. One study used a caudal trunk nerve cut to cause pain and another study induced pain during two separate experiments of SNL and SNL + SCI models.

The administered doses in the included studies ranged from 0.1 ng to 450,000 ng. In 12 studies, the administered dose was less than or equal to 100 ng in at least one experiment. Noticeably, the range of administered doses varied greatly. Therefore, the dose was entered into the analysis in the logarithm of 10. The method of administration was intrathecal in 12 studies, inside the brain nuclei in 7 studies, intraperitoneal in 2 studies, subcutaneous in one, and intraplantar in one. Mechanical allodynia was the investigated outcome in 21 studies, mechanical hyperalgesia in 5 studies, and thermal hyperalgesia in 2 studies. **Table 2** shows a summary of the included articles.

2. The effect of muscimol administration on mechanical allodynia

Data from 20 studies evaluated mechanical allodynia.

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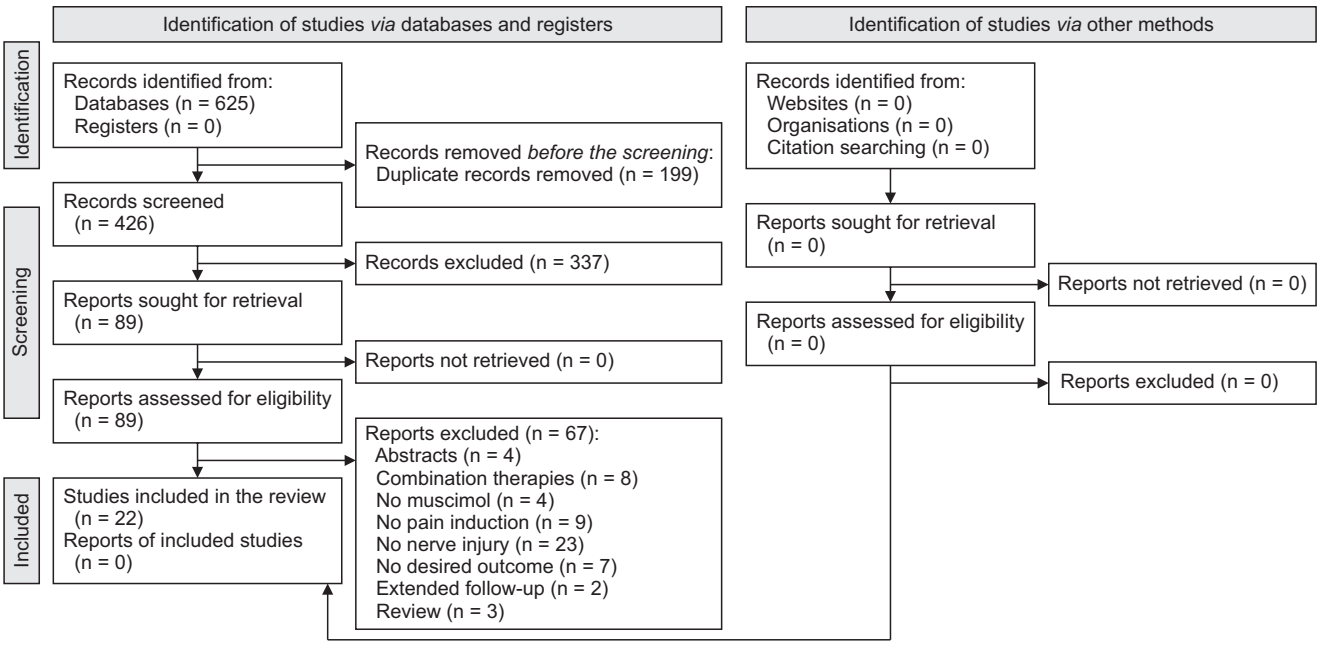

Fig. 1. PRISMA flow diagram of the article selection process.

Galbraith's plot demonstrated that 2 experiments were outliers. Therefore, Lee et al. [27] was omitted from the pooled analysis and data from 19 studies comprising 49 separate experiments were included in the present analysis. Pooled data analysis showed that the administration of muscimol during the peak effect caused a significant reduction in mechanical allodynia (SMD = 1.78; 95% CI: 1.45, 2.11, $P < 0.0001$; $I^2 = 72.70\%$) (**Fig. 2**). Subgroup analysis was performed to find the origin of the observed moderate heterogeneity. The analysis showed that the administration of muscimol ameliorated pain with a central origin (SMD = 2.47; 95% CI: 1.82, 3.11; $P < 0.0001$; $I^2 = 72.59\%$) and with a peripheral origin (SMD = 1.47; 95% CI: 1.13, 1.82; $P < 0.0001$; $I^2 = 66.05\%$). It was also found that the site of administration was not the source of heterogeneity. Moreover, different routes of administration including intrathecal (SMD = 2.18; 95% CI: 1.74, 2.62; $P < 0.0001$; $I^2 = 68.46\%$), intracerebral (SMD = 1.18; 95% CI: 0.61, 1.75; $P < 0.0001$; $I^2 = 71.94\%$), and systemic (SMD = 1.43; 95% CI: 0.75, 2.11; $P < 0.0001$; $I^2 = 67.80\%$) were all significantly effective in the amelioration of mechanical allodynia (**Table 3**). We conducted a meta-regression analysis to investigate the effect of the administered muscimol dose on its effectiveness in the amendment of mechanical allodynia. Meta-regression showed that the increase in dose had no significant effect on the efficacy of muscimol in the amelioration of this type of pain (Coef. = 0.035; 95% CI: –0.73, 0.14; $P = 0.525$). In other words, the evidence demonstrates that muscimol ameliorated mechanical allodynia in all reported doses (**Fig. 3A**). As an additional analysis, the effect of follow-up time on the efficacy of muscimol in mechanical allodynia was investigated. This analysis showed that mechanical allodynia was significantly improved 15 minutes after the treatment (SMD = 2.13, 95% CI: 1.58, 2.68; $P < 0.0001$; $I^2 = 75.02\%$) and lasted for up to 180 minutes (SMD = 1.00, 95% CI: 0.63, 1.37; $P < 0.0001$; $I^2 = 45.44\%$) (**Table 3**). Yet, the effectiveness of muscimol in mechanical allodynia decreases over time (Coef. = –0.006; 95% CI: –0.009, –0.003; $P < 0.0001$) (**Fig. 3B**). Since the amount of heterogeneity in some classes was reduced by performing this subgroup analysis, it seems that the cause of the observed heterogeneity was due to the difference in the follow-up time.

3. The effect of muscimol administration on mechanical hyperalgesia

In the assessment of the efficacy of muscimol in mechanical hyperalgesia, data from 8 articles and 18 separate analyses were included. Pooled analysis showed that muscimol significantly reduced mechanical hyperalgesia (SMD = 1.62; 95% CI: 1.28, 1.96; $P < 0.0001$; $I^2 = 40.66\%$) (**Fig. 4A**). Subgroup analysis showed that the administration of muscimol was effective both in pain with a central origin (SMD = 1.79; 95% CI: 1.47, 2.10; $P < 0.0001$; $I^2 = 0.00\%$) and in pain with a peripheral origin (SMD = 1.27;

Table 2. Characteristics of the included studies

Study	Animal species, sex, weight (g)	Model	Injury to muscimol administration (days)	Dose (ng)	Administration site	Treatment to pain	Pain type	N Treated[a]	N Non-treated
Dias and Prado [39]	Rat, Wistar, M, 140–160	SNL, SNL and SCI	2, 7	300	Intrathecal	15, 30, 60, 90	Mechanical allodynia	5	5
Gwak et al. [40]	Rat, SD, M, 200–250	SCI	21	1,000	Intrathecal	30, 120, 180	Mechanical allodynia	5	5
Hama and Sagen [22]	Rat, SD, M, 100–150	SCI	21	0.1, 0.3, 1, 3	Intrathecal	30, 60, 90, 120	Mechanical allodynia	7	7
Hosseini et al. [23]	Rat, Wistar, M, 140–160	SCI	21	10, 100, 1,000	Intrathecal	15, 60, 180	Thermal hyperalgesia, mechanical hyperalgesia, mechanical allodynia	10	10
Hosseini et al. [24]	Rat, Wistar, M, 140–160	SCI	24	10	Intrathecal	15, 60	Mechanical allodynia, mechanical hyperalgesia	10	10
Hwang and Yaksh [41]	Rat, SD, M, 120–150	SNL	7	100, 300, 1,000	Intrathecal	15, 30, 45, 60, 120, 180	Mechanical allodynia	5-6	5-6
Jeon et al. [25]	Rat, SD, N/R, 200–250	CCI	14	100, 300, 1,000, 1.71, 3.42, 1,711	Intrathecal, Intraplantar	15, 30, 45, 60, 75, 90, 105, 120	Mechanical allodynia	6	6
Jiang et al. [31]	Rat, SD, M, 150–180	SNL	7	25	Intra-CeA	30	Mechanical allodynia	14	14
LaGraize and Fuchs [42]	Rat, SD, M, 3–4 mo	SNL	3	1, 100, 500	Intra rostral anterior cingulate cortex	35	Mechanical allodynia	8	10
Lee et al. [26]	Rat, SD, M, 150–200	Caudal trunk nerve cut	14	1,000	Intrathecal	30	Mechanical allodynia	7	5
Lee et al. [27]	Rat, SD, M, 180–200	CCI	7	570	Intrathecal	30, 60, 90, 120, 180	Mechanical allodynia	8	10
Moon et al. [28]	Rat, SD, M, 250–300	SCI	14	570, 1,140, 1,700	ZI	60	Mechanical hyperalgesia	10	10
Moon and Park [29]	Rat, SD, M, 250–300	CCI	10	285, 2,830	ZI	120	Mechanical allodynia	10	10
Nasirinezhad et al. [43]	Rat, Wistar, M, 140–160	SCI	24	10, 100, 1,000	Intrathecal	15, 60, 180	Mechanical hyperalgesia, thermal hyperalgesia, mechanical allodynia	8	8
Pedersen et al. [30]	Rat, SD, M, 180–200	CCI	14	20, 50	CeA	30	Mechanical allodynia, mechanical hyperalgesia	8	6
Rashid and Ueda [32]	Mice, ddY, M, 25–30	CCI	0	3.42, 11.4, 34.2	Intrathecal	60	Mechanical allodynia	6	6
Rode et al. [33]	Rat, SD, M, 250	SNI	0		Sub-cutaneous	30, 60, 90, 120, 150	Mechanical allodynia, mechanical hyperalgesia	6	6

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Table 2. Continued

Study	Animal species, sex, weight (g)	Model	Injury to muscimol administration (days)	Dose (ng)	Administration site	Treatment to pain	Pain type	N Treated[a]	N Non-treated
Sadeghi et al. [34]	Rat, Wistar, M, 200–250	CCI	14	112,500, 225,000, 450,000	Intra-peritoneal	30	Thermal hyperalgesia, mechanical allodynia	8	8
Seno et al. [35]	Rat, Wistar, M, 180–200	CCI	14	500	CeA, BLA	30	Mechanical hyperalgesia, mechanical allodynia	8	5
Wei et al. [36]	Rat, Hannover-Wistar, M, 180–250	CCI	4	100, 300	Hypothalamus A11	15, 30, 60	Mechanical allodynia, mechanical hyperalgesia, thermal hyperalgesia	5-6	6
Yowtak et al. [37]	Mice, GAD67-EGFP, M, N/R	CCI	4	50, 100	Intrathecal	30, 60, 90, 120	Mechanical allodynia	8	8
Zarrindast and Mahmoudi [38]	Mice, albino NMRI, M, 20–25	SNI	14	10,000, 20,000, 40,000	Intra-peritoneal	75	Thermal hyperalgesia	8	8

SNL: sciatic nerve ligation, SCI: spinal cord injury, CCI: chronic compression injury, SNI: sciatic nerve injury, CeA: central nuclei of the amygdala, ZI: zona incerta, BLA: basolateral nuclei of the amygdala, N/R: not recorded.
[a]The number of animals was reported as number of animals per each treated group. Some studies had several experimental groups according to dose of muscimol, site of administration and model of pain induction.

95% CI: 0.58, 1.95; $P < 0.0001$; $I^2 = 57.51\%$). It was also found that the route of administration was not the source of heterogeneity. Both the intrathecal administration of muscimol (SMD = 2.07; 95% CI: 1.68, 2.46; $P < 0.0001$; $I^2 = 0.00\%$) and the intracerebral administration (SMD = 1.21; 95% CI: 0.74, 1.67; $P < 0.0001$; $I^2 = 35.25\%$) significantly improved mechanical hyperalgesia (**Table 3**). To investigate the effect of the administered muscimol dose on its efficacy in mechanical hyperalgesia, meta-regression was performed. Meta-regression showed that an increase in the administered dose had no meaningful effect on the effectiveness of muscimol in the amelioration of mechanical hyperalgesia (Coef. = –0.16; 95% CI: –0.33, 0.006; $P = 0.059$). In other words, the evidence shows that muscimol improved mechanical hyperalgesia in all reported doses (**Fig. 3C**). Moreover, the effect of follow-up time on the effectiveness of muscimol in mechanical hyperalgesia was investigated. This analysis showed that mechanical hyperalgesia was improved 15 minutes (SMD = 2.07, 95% CI: 1.68, 2.46; $P < 0.0001$; $I^2 = 0.00\%$) and up to 180 minutes after the administration of muscimol (SMD = 1.15, 95% CI: 0.64, 1.66; $P < 0.0001$; $I^2 = 49.55\%$) (**Table 3**). Although the effectiveness of muscimol decreases over time, this decrease was not statistically significant (Coef. = –0.003; 95% CI: –0.008, 0.003; $P = 0.070$) (**Fig. 3D**).

4. The effect of muscimol administration on thermal hyperalgesia

Regarding the effect of muscimol on thermal hyperalgesia, data from 5 articles and 13 separate experiments were included. Pooled data analysis with high heterogeneity showed that muscimol significantly reduced thermal hyperalgesia (SMD = 2.59; 95% CI: 1.79, 3.39; $P < 0.0001$; $I^2 = 80.33\%$) (**Fig. 4B**). Subgroup analysis demonstrated that the administration of muscimol was effective both in pain of a central origin (SMD = 1.87; 95% CI: 1.43, 2.32; $P < 0.0001$; $I^2 = 0.00\%$) and in pain of a peripheral origin (SMD = 3.33; 95% CI: 1.87, 4.80; $P < 0.0001$; $I^2 = 84.89\%$). Since the amount of heterogeneity in pain with a central origin was equal to zero, the origin of pain may be one of the main causes of heterogeneity. Additionally, it was also found that the site of administration may also be a source of heterogeneity. Intrathecal muscimol administration (SMD = 1.87; 95% CI: 1.43, 2.32; $P < 0.0001$; $I^2 = 0.00\%$) and systemic administration (SMD = 3.82; 95% CI: 2.75, 4.90; $P < 0.0001$; $I^2 = 61.01\%$) were both effective in the amelioration of thermal hyperalgesia (**Table 3**). To investigate the effect of the administered dose on its efficacy in the improvement of thermal hyperalgesia, meta-regression

Study	Dose (ng)	Time (min)	Treatment N	Treatment Mean	Treatment SD	Control N	Control Mean	Control SD	Hedges's g with 95% CI	Weight (%)
Dias and Prado [39]	300	15	5	36	4.79	5	18.25	3.76	3.72 [1.74, 5.70]	1.42
Dias and Prado [39]	300	15	5	36.3	4.86	5	18.91	2.92	3.92 [1.87, 5.97]	1.37
Dias and Prado [39]	300	15	5	33.91	3.89	5	19.13	2.92	3.88 [1.84, 5.92]	1.38
Dias and Prado [39]	300	15	5	27.12	10.95	5	18.25	3.76	0.98 [−0.22, 2.18]	2.10
Gwak et al. [40]	1,000	30	5	32.78	14.8	5	50.2	17.14	0.98 [−0.22, 2.18]	2.10
Hama and Sagen [22]	1	30	7	7.96	4.81	7	2.45	.74	1.50 [0.37, 2.63]	2.17
Hama and Sagen [22]	.3	30	7	3.59	1.65	7	2.45	.74	0.83 [−0.19, 1.86]	2.27
Hama and Sagen [22]	3	30	7	9.5	4.51	7	2.45	.74	2.04 [0.80, 3.28]	2.06
Hama and Sagen [22]	.1	30	7	3.59	1.95	7	2.45	.74	0.72 [−0.29, 1.74]	2.28
Hosseini et al. [23]	100	15	10	14.6	.95	10	9.3	2.21	2.98 [1.74, 4.23]	2.05
Hosseini et al. [23]	1,000	15	10	14.9	.32	10	8.5	2.53	3.40 [2.05, 4.75]	1.96
Hosseini et al. [23]	10	15	10	14.75	.79	10	8.8	1.74	4.22 [2.66, 5.77]	1.77
Hosseini et al. [24]	10	15	10	12.16	1.62	10	7.74	2.57	1.97 [0.93, 3.01]	2.26
Hosseini et al. [24]	10	15	10	11.96	2.28	10	7.74	2.57	1.66 [0.68, 2.65]	2.31
Hwang and Yaksh [41]	100	30	6	6.39	6.55	6	1.63	1.94	0.91 [−0.20, 2.02]	2.19
Hwang and Yaksh [41]	300	60	5	8.44	6.26	5	1.63	1.77	1.34 [0.07, 2.60]	2.04
Hwang and Yaksh [41]	1,000	60	5	13.2	3.83	5	1.63	1.77	3.50 [1.60, 5.40]	1.48
Jeon et al. [25]	1.71	60	6	7.21	2.19	7	1.9	1.58	2.62 [1.19, 4.06]	1.88
Jeon et al. [25]	100	15	6	11.46	5.96	6	2.93	.97	1.84 [0.57, 3.12]	2.02
Jeon et al. [25]	300	15	6	11.81	4.74	6	2.93	.97	2.40 [0.98, 3.81]	1.89
Jeon et al. [25]	3.42	60	8	11.88	3.09	7	1.9	1.58	3.74 [2.10, 5.39]	1.69
Jeon et al. [25]	1,711	60	6	2	1.34	7	1.9	1.58	0.06 [−0.95, 1.08]	2.28
Jiang et al. [31]	25	30	14	5.77	3.83	14	1.68	.68	1.44 [0.63, 2.26]	2.48
LaGraize and Fuchs [42]	500	60	11	−313.24	14.63	10	−298.53	37.2	0.49 [−0.35, 1.33]	2.46
LaGraize and Fuchs [42]	100	60	11	−298.53	29.27	10	−298.53	37.2	0.00 [−0.82, 0.82]	2.47
LaGraize and Fuchs [42]	1	60	8	−300	37.43	10	−298.53	37.2	0.04 [−0.85, 0.92]	2.41
Lee et al. [27]	1,000	30	7	12.68	3.76	5	.89	1.3	3.60 [1.81, 5.38]	1.57
Moon and Park [28]	285	120	10	19.62	1	10	17.06	1.28	2.13 [1.07, 3.20]	2.23
Moon and Park [28]	2,850	120	10	27.5	3.83	10	17.06	1.28	3.50 [2.13, 4.87]	1.93
Nasirinezhad et al. [43]	1,000	15	8	14.9	.28	8	8.5	2.26	3.76 [2.16, 5.36]	1.73
Nasirinezhad et al. [43]	100	15	8	14.6	.85	8	9.3	1.98	3.29 [1.82, 4.76]	1.84
Nasirinezhad et al. [43]	10	15	8	14.8	.85	8	8.8	1.7	4.22 [2.49, 5.95]	1.61
Pedersen et al. [30]	50	30	4	11	9.6	6	2.3	.24	1.34 [0.05, 2.62]	2.02
Pedersen et al. [30]	20	30	8	7.6	5.66	6	2.3	.24	1.15 [0.07, 2.23]	2.22
Rashid and Ueda [32]	11.4	60	6	37.65	4.9	6	30.5	4.9	1.35 [0.17, 2.52]	2.12
Rashid and Ueda [32]	3.42	60	6	35	1.47	6	30.5	4.9	1.15 [0.01, 2.29]	2.16
Rashid and Ueda [32]	34.2	60	6	44.3	1.71	6	30.5	4.9	3.47 [1.73, 5.21]	1.61
Rode et al. [33]	.	60	6	28.49	33.06	6	0	6.01	1.11 [−0.03, 2.24]	2.17
Rode et al. [33]	.	60	6	11.01	7.51	6	0	6.01	1.49 [0.29, 2.70]	2.10
Rode et al. [33]	.	60	6	1.8	4.51	6	0	6.01	0.31 [−0.74, 1.36]	2.25
Sadeghi et al. [34]	225,000	30	8	35.92	21.55	8	7.62	4.55	1.72 [0.62, 2.82]	2.20
Sadeghi et al. [34]	450,000	30	8	45.95	26.39	8	7.62	4.55	1.91 [0.77, 3.05]	2.16
Sadeghi et al. [34]	112,500	30	8	15.35	11.06	8	7.62	4.55	0.86 [−0.11, 1.84]	2.32
Seno et al. [35]	500	30	8	6.5	2.83	5	1.3	2.24	1.84 [0.58, 3.10]	2.05
Seno et al. [35]	500	30	8	5.9	4.53	5	1.8	.67	1.05 [−0.07, 2.16]	2.18
Wei et al. [36]	100	30	5	.15	.52	6	.13	.31	0.04 [−1.04, 1.13]	2.21
Wei et al. [36]	300	30	6	3.11	1.86	6	.13	.31	2.06 [0.73, 3.39]	1.97
Yowtak et al. [37]	100	30	8	35.77	35.61	8	79.48	15.83	1.50 [0.44, 2.56]	2.24
Yowtak et al. [37]	50	30	8	65.14	22.75	8	79.48	15.83	0.69 [−0.27, 1.65]	2.34
Overall									**1.78 [1.45, 2.11]**	

Heterogeneity: τ^2 = 0.96, I^2 = 72.70%, H^2 = 3.66
Test of $\theta_i = \theta_j$: Q(48) = 162.97, P = 0.00
Test of θ = 0: z = 10.63, P = 0.00

Random-effects REML model

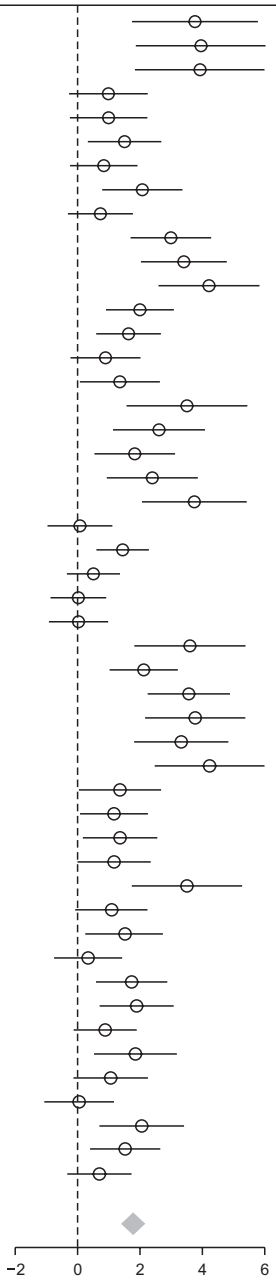

Fig. 2. The effect of muscimol administration on nerve injury-related mechanical allodynia in observed peak effect time. SD: standard deviation, 95% CI: 95% confidence interval.

was performed. Meta-regression showed that the efficacy of muscimol in thermal hyperalgesia increased with the dose (Coef. = 0.24; 95% CI: 0.033, 0.440; P = 0.023). In other words, the evidence shows that muscimol improved thermal hyperalgesia in all reported doses and this effect was dose-dependent (**Fig. 3E**). Moreover, the effect of follow-up time on the effectiveness of muscimol in thermal hyperalgesia was investigated. This analysis showed that thermal hyperalgesia was improved 15 minutes after the administration of muscimol (SMD = 1.87, 95% CI: 1.43, 2.32; P < 0.0001; I^2 = 0.00%) and 30 minutes post-treatment (SMD = 4.74; 95% CI: 3.32, 6.17; P < 0.0001; I^2

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Table 3. Subgroup analysis for the effect of muscimol on nerve injury-related neuropathic pain

Subgroups	n experiments	SMD	95% CI	*P* value	I^2 (%)	*P* value
Mechanical allodynia						
Time of follow-up (min)						
15	22	2.13	1.58, 2.68	0.000	75.02	< 0.0001
30	33	1.44	1.15, 1.73	0.000	48.63	< 0.0001
60	46	1.49	1.20, 1.78	0.000	64.47	< 0.0001
90	26	1.03	0.63, 1.42	0.000	66.79	< 0.0001
120	28	0.99	0.62, 1.36	0.000	67.05	< 0.0001
180	15	1.00	0.63, 1.37	0.000	45.44	0.024
Pain origin						
Central	15	2.47	1.82, 3.11	0.000	72.59	< 0.0001
Peripheral	34	1.47	1.13, 1.82	0.000	66.05	< 0.0001
Administration site						
Intrathecal	28	2.18	1.74, 2.62	0.000	68.46	< 0.0001
Intracerebral	12	1.18	0.61, 1.75	0.000	71.94	< 0.0001
Systemic	9	1.43	0.75, 2.11	0.000	67.80	0.003
Mechanical hyperalgesia						
Time of follow-up (min)						
15	8	2.07	1.68, 2.46	0.000	0.00	0.850
30	6	1.50	1.01, 1.99	0.000	1.23	0.389
60	14	0.87	0.57, 1.16	0.000	23.95	0.118
90	2			Lack of sufficient data		
120	2			Lack of sufficient data		
180	8	1.15	0.64, 1.66	0.000	49.55	0.050
Pain origin						
Central	11	1.79	1.47, 2.10	0.000	0.00	0.466
Peripheral	7	1.27	0.58, 1.95	0.000	57.51	0.028
Administration site						
Intrathecal	8	2.07	1.68, 2.46	0.000	0.00	0.850
Intracerebral	8	1.21	0.74, 1.67	0.000	35.25	0.142
Systemic	2			Lack of sufficient data		
Thermal hyperalgesia						
Time of follow-up (min)						
15	6	1.87	1.43, 2.32	0.000	0.00	0.416
30	3	4.74	3.32, 6.17	0.000	39.21	0.195
60	7	−0.31	−0.66, 0.03	0.077	0.00	0.700
90	2			Lack of sufficient data		
180	6	−0.13	−0.49, 0.23	0.478	0.00	0.989
Pain origin						
Central	6	1.87	1.43, 2.32	0.000	0.00	0.416
Peripheral	7	3.33	1.87, 4.80	0.000	84.89	< 0.0001
Administration site						
Intrathecal	6	1.87	1.43, 2.32	0.000	0.00	0.416
Intracerebral	1			Lack of sufficient data		
Systemic	6	3.82	2.75, 4.90	0.000	61.01	0.029

SMD: standardized mean difference, 95% CI: 95% confidence interval.

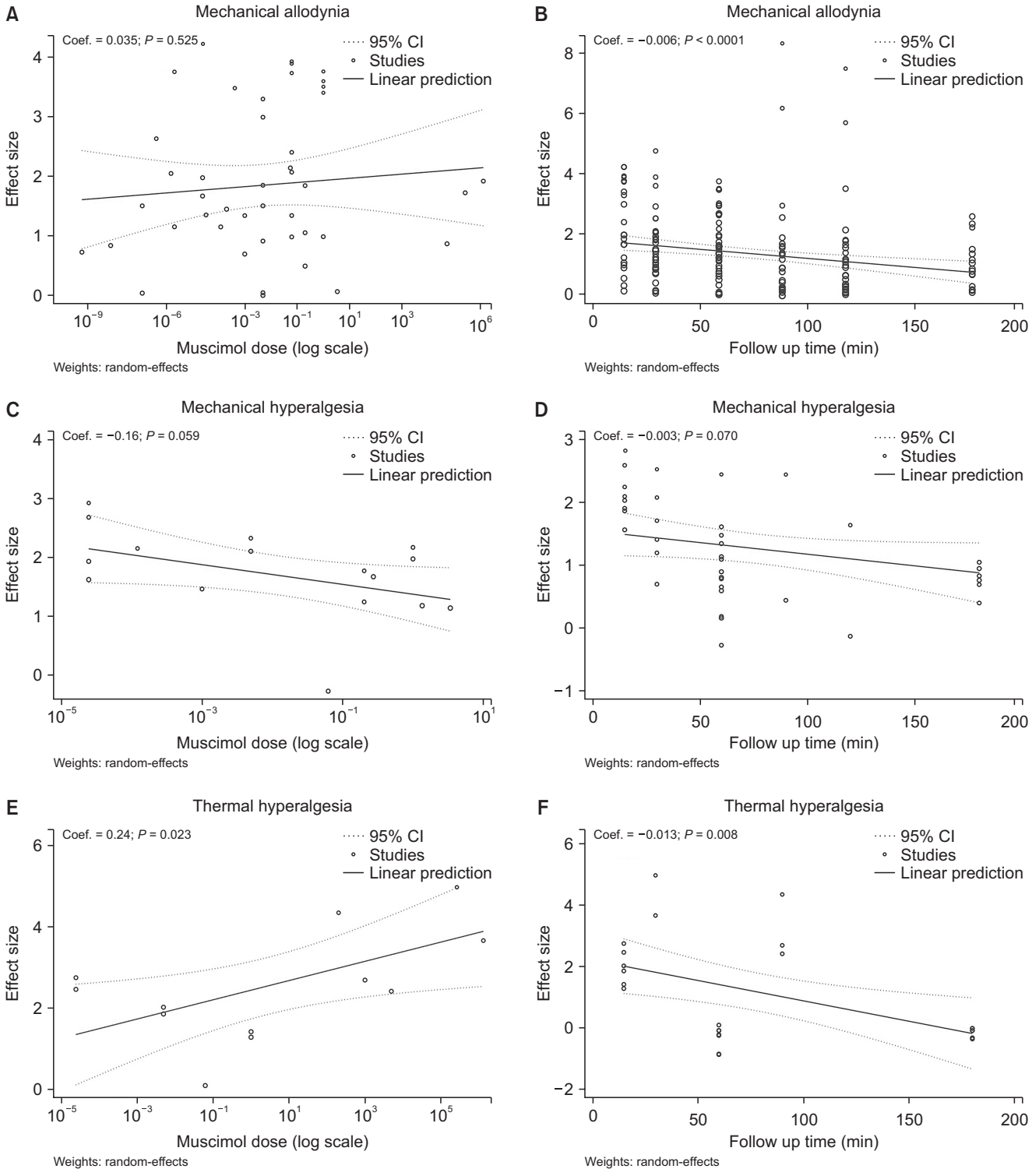

Fig. 3. Meta-regression for the assessment of dose-effect and follow-up duration on muscimol efficacy in nerve injury-related mechanical allodynia (A, B), mechanical hyperalgesia (C, D), and thermal hyperalgesia (E, F). 95% CI: 95% confidence interval.

= 39.21%). Nevertheless, the administration of muscimol had no meaningful effect on the improvement of thermal hyperalgesia from 60 to 180 minutes post-treatment (**Table 3**). In other words, the effectiveness of muscimol on thermal hyperalgesia decreased over time (Coef. = –0.013; 95% CI: –0.023, –0.003; $P = 0.008$) (**Fig. 3F**).

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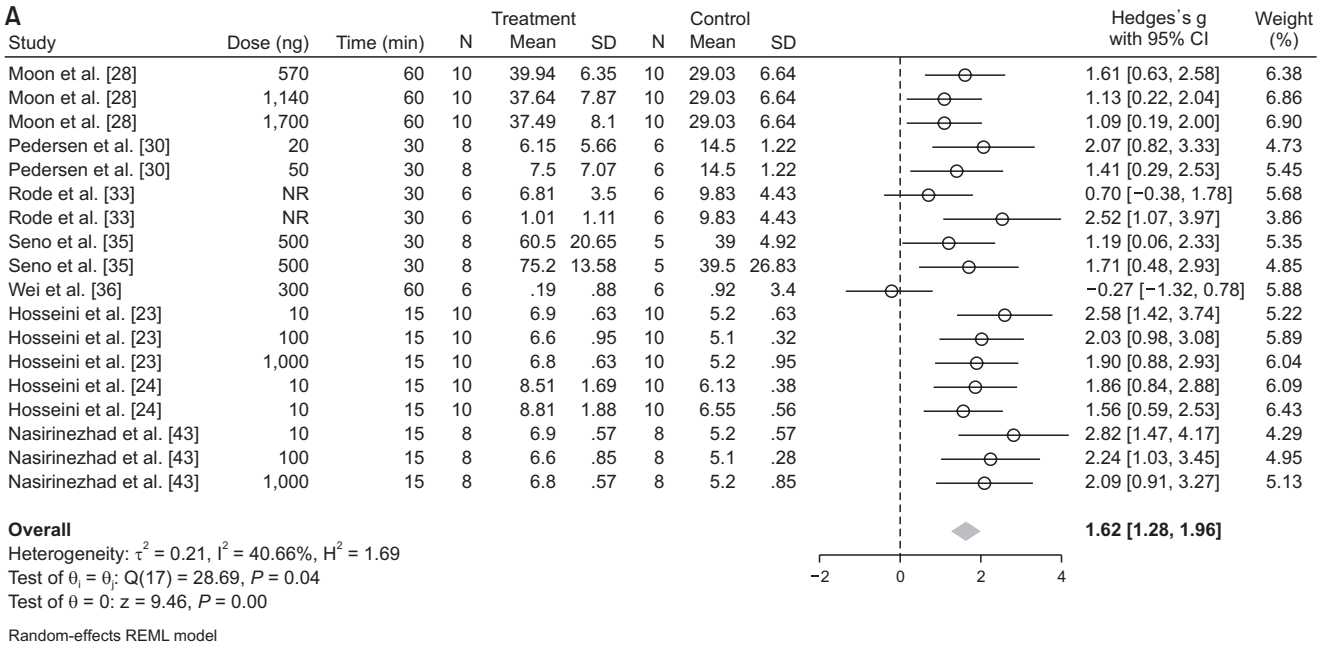

Fig. 4. The effect of muscimol on nerve injury-related mechanical hyperalgesia (A) and thermal hyperalgesia (B) in the observed peak effect time. SD: standard deviation, 95% CI: 95% confidence interval.

5. Quality control and certainty of evidence

We evaluated the methodology and the overall risk of bias in our included pre-clinical studies according to SYRCLE's risk of bias tool. The allocation sequence was adequately generated and applied in only two articles. All included articles used animals similar at baseline. The allocation concealment was clearly disclosed in 4 articles, and in one article no concealment was reported. Random housing during the experiment was reported in 4 articles. Investigators and outcome assessors were blinded in 4 and 8 articles, respectively. A random selection for outcome assessment was unclear in all articles, except for one in which no randomization was observed. There was no incomplete outcome data or other factors that could potentially cause bias. Conclusively, the overall quality of the included articles was considered low (**Table 1**).

In the assessment of the certainty of evidence based on the GRADE framework, the level of evidence was downrated one grade due to the serious risk of bias for all included outcomes. The overall level of evidence was considered moderate (**Table 4**).

Table 4. The certainty of evidence based on the GRADE framework

Outcome	Number of experiments	Risk of bias	Imprecision	Inconsistency	Indirectness	Publication bias	Judgment	Level of evidence
Mechanical allodynia	49	Serious	No serious imprecision	No serious inconsistency[a]	No serious indirectness	No publication bias	Level of evidence was downrated one grade due to possible risk of bias	Moderate
Mechanical hyperalgesia	18	Serious	No serious imprecision	No serious inconsistency[a]	No serious indirectness	No publication bias	Level of evidence was downrated one grade due to possible risk of bias	Moderate
Thermal hyperalgesia	13	Serious	No serious imprecision	No serious inconsistency[a]	No serious indirectness	No publication bias	Level of evidence was downrated one grade due to possible risk of bias	Moderate

GRADE: Grading of Recommendations Assessment, Development, and Evaluation.
[a]There is no serious inconsistency since the sources of heterogeneity were identified.

6. Publication bias

Egger's test demonstrated that there was no publication bias in the reports of mechanical allodynia (P = 0.672), mechanical hyperalgesia (P = 0.440), and thermal hyperalgesia (P = 0.664) (**Fig. 5**).

DISCUSSION

Our findings indicate that muscimol, an agonist for the $GABA_A$ receptor, was able to significantly alleviate pain in its peak effect, determined by the amelioration of behavioral responses to stimuli for mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia. Although this efficacy is dose-independent in mechanical allodynia and mechanical hyperalgesia, the observed effect increases with dose in the evaluation of thermal hyperalgesia.

The underlying mechanisms of pain are fundamentally different from one another and their precise pathways are yet unknown [44]. It is believed that pain following nerve injury mostly incorporates the peripheral activation of previously non-nociceptive neurons into nociceptors, alterations in neuronal excitability in pain pathways, inflammation, axonal loss due to injury, and various subsequent dysfunctions of the supraspinal regions that are responsible for pain perception [45,46]. GABAergic neurons are important mediators of pain and therefore, dysregulation in their signaling has been shown to play a pivotal role in the development of pain [47].

Muscimol, a $GABA_A$ receptor agonist, exerts its analgesic effects through multiple pathways. For instance, this mushroom-derivative compound exhibits antioxidant properties that potentially halt the reactive oxygen species in inflammatory cascades of the injured tissue [48]. Moreover, current evidence suggests that muscimol improves the plasticity in the posterior horn of the spinal cord as the central terminal of the afferent pain pathways, which is affected by both central and peripheral nerve injuries [49,50].

In this literature review, we demonstrated that as a relatively short-acting GABA analog [51], muscimol begins to exert its analgesic effects 15 minutes after administration. While decreasing in efficacy, this effect lasts for up to 180 minutes in the improvement of mechanical allodynia and hyperalgesia. Conversely, muscimol seems to be effective in thermal hyperalgesia only from 15 to 30 minutes post-treatment. These findings are in alignment with previous evidence which suggests a different response to musci-

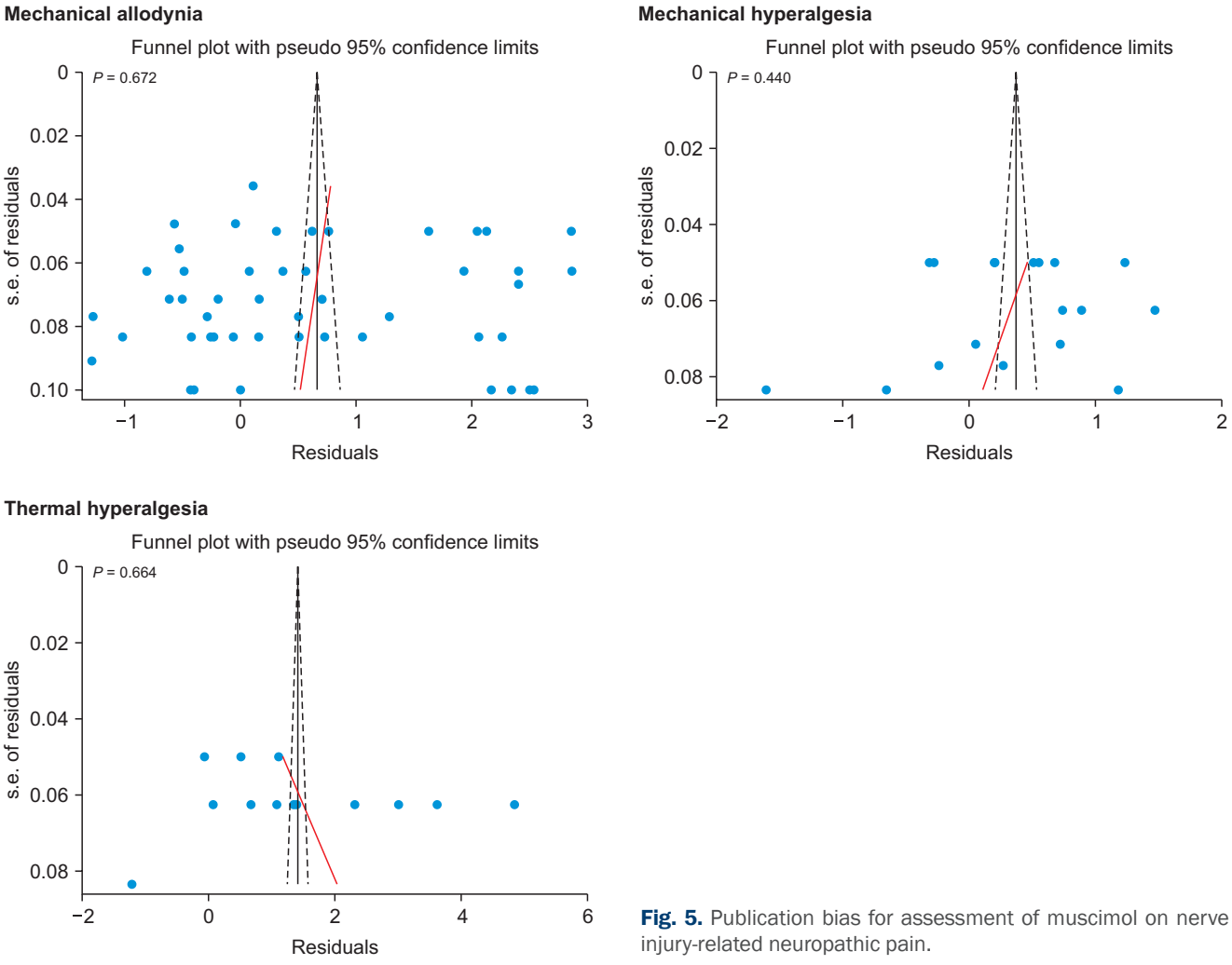

Fig. 5. Publication bias for assessment of muscimol on nerve injury-related neuropathic pain.

mol in thermal hyperalgesia than allodynia. Conspicuously, Sadeghi et al. [34] concluded that thermal hyperalgesia is more sensitive to muscimol than allodynia, while Hosseini et al. [24] disclosed that muscimol is effective in mechanical allodynia and hyperalgesia, but not effective in thermal hyperalgesia.

Moreover, we demonstrated that different routes of muscimol administration are all effective in the amelioration of both the peripheral and central origins of pain, which could be due to muscimol's ability to cross the blood-brain barrier through an active transport system, and the vast distribution of its receptors throughout the nervous system [52–54]. However, it should be considered that this broad dispersion of the target receptors holds a liability for potential adverse effects [55].

A limitation of our study is the broad differences in the administered doses of muscimol. Although different routes of administration require different administration doses for optimal efficacy, this study demonstrated the need for a more comprehensive approach for selecting the muscimol administration route and dose in future prospective studies.

Also, it has recently been argued that due to the lack of concordance between guidelines for conducting preclinical studies and guidelines for their quality assessment, some domains might be at high risk of bias, solely due to the fact that the authors did not document them in their articles, even though those recommendations might have been followed during the experiment [56]. The overall level of evidence was downrated only due to the serious risk of bias. Therefore, the advancement of research into clinical trials could be taken into consideration.

Moreover, although chronic NP is most often considered to be simultaneous and non-evoked in humans, evoked pain perception is the target of research in most preclinical studies [57]. Therefore, the findings of our study should be interpreted in terms of the potential efficacy of muscimol in the symptomatic management of NP

for future clinical research [58].

Conclusively, muscimol is effective in the amelioration of mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia. Muscimol exerts its analgesic effects 15 minutes after administration, and this effect is observed for up to at least 3 hours post-administration.

DATA AVAILABILITY

The datasets supporting the finding of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Hamzah Adel Ramawad: Data gathering, Manuscript drafting, Critical revision; Parsa Paridari: Data gathering, Manuscript drafting, Critical revision; Sajjad Jabermoradi: Data gathering, Manuscript drafting, Critical revision; Pantea Gharin: Data gathering, Critical revision; Amirmohammad Toloui: Data analysis, Manuscript drafting, Critical revision; Saeed Safari: Study design, Data analysis, Critical revision; Mahmoud Yousefifard: Study design, Data analysis, Manuscript drafting, Critical revision.

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