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Experimental Research Article

# Beneficial effect of metformin on tolerance to analgesic effects of sodium salicylate in male rats

**Elham Akbari[1,2], Dawood Hossaini[1,2], Farimah Beheshti[1], Mahdi Khorsand Ghaffari[3], Nastran Roshd Rashidi[3], and Masoumeh Gholami[3,1]**

[1]Department of Physiology, School of Medicine, Torbat Heydariyeh University of Medical Sciences, Torbat Heydariyeh, Iran
[2]Department of Biology and Microbiology, School of Medical Laboratory Technology, Khatam Al-Nabieen University, Kabul, Afghanistan
[3]Department of Physiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran

ABSTRACT

**Background:** Tolerance to the analgesic effects of opioids and non-steroidal anti-inflammatory drugs (NSAIDs) is a major concern for relieving pain. Thus, it is highly valuable to find new pharmacological strategies for prolonged therapeutic procedures. Biguanide-type drugs such as metformin (MET) are effective for neuroprotection and can be beneficial for addressing opioid tolerance in the treatment of chronic pain. It has been proposed that analgesic tolerance to NSAIDs is mediated by the endogenous opioid system. According to the cross-tolerance between NSAIDs, especially sodium salicylate (SS), and opiates, especially morphine, the objective of this study was to investigate whether MET administration can reduce tolerance to the anti-nociceptive effects of SS.
**Methods:** Fifty-six male Wistar rats were used in this research (weight 200–250 g). For induction of tolerance, SS (300 mg/kg) was injected intraperitoneally for 7 days. During the examination period, animals received MET at doses of 50, 75, or 100 mg/kg for 7 days to evaluate the development of tolerance to the analgesic effect of SS. The hot plate test was used to evaluate the drugs' anti-nociceptive properties.
**Results:** Salicylate injection significantly increased hot plate latency as compared to the control group, but the total analgesic effect of co-treatment with SS + Met50 was stronger than the SS group. Furthermore, the effect of this combination undergoes less analgesic tolerance over time.
**Conclusions:** It can be concluded that MET can reduce the analgesic tolerance that is induced by repeated intraperitoneal injections of SS in Wister rats.

**Keywords:** Analgesic Effect; Anti-Inflammatory Agents, Non-Steroidal; Drug Tolerance; Metformin; Nociception Tests; Pain; Rats; Sodium Salicylate.

## INTRODUCTION

The prolonged use of opioid medications can lead to tolerance, ultimately reducing the analgesic effect and limiting its clinical application [1]. Therefore, a combination therapy that uses different analgesics can be a viable alternative for pain management. One typical instance is the co-administration of non-steroidal anti-inflammatory drugs (NSAIDs) with opioid agonists for the treatment of severe and persistent pain [2].

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Correspondence: Masoumeh Gholami
Department of Physiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran
Tel: +98-86-33838607, Fax: +98-86-31473521, E-mail: ma.gholami@arakmu.ac.ir

Studies have shown that inflammatory mediators like bradykinin, prostaglandins, tumor necrosis factor-α (TNF-α), and interleukin-1β induce pain and increase neuronal sensitivity [3]. Thus, an anti-inflammatory therapeutic strategy has the potential to improve pain symptoms. For example, acetaminophen and L-carnosine were found to alleviate neuropathic pain through the nuclear factor kappa B (NF-κB) pathway and antioxidant properties in a model of chronic constriction injury [4]. It's important to mention that the development of tolerance to analgesic effects is not limited to opioids. Non-opioid painkillers can also lead to tolerance, either individually or through cross-tolerance with opioids [5,6]. For instance, chronic use of NSAIDs such as lornoxicam, sodium salicylate (SS), ketorolac, diclofenac sodium, and dipyrone is associated with a gradual decrease in the analgesic effects [7–10]. SS, a metabolite of aspirin, is an effective treatment for inflammation-related illnesses. The pharmacological effects of salicylate include inhibiting cyclooxygenases and the NF-κB signaling pathway. These actions contribute to the anti-inflammatory and analgesic properties associated with salicylate [11]. To treat moderate to severe pain, this medication is frequently used either by itself or in conjunction with opioids. Nevertheless, long-term usage of these can lead to adverse effects such as tolerance to their analgesic effects. Biguanide-class drugs such as metformin (MET) are effective as a first-line treatment for type 2 diabetes, providing robust glucose-lowering effects, a well-established safety profile, and a cost-effective option for patients [12]. MET has been found to penetrate the blood-brain barrier in prior investigations, enabling it to exert numerous beneficial effects within the central nervous system, including anti-inflammatory and neuroprotective properties [13,14]. Several studies have demonstrated the anti-inflammatory effects of MET in various experimental models of inflammation. Additionally, several studies have demonstrated the neuroprotective effects of MET. These studies suggest that MET protects against neuronal damage by reducing oxidative stress, inflammation, and apoptosis [15]. These studies indicate that MET's anti-inflammatory effects are likely mediated by inhibition of NF-κB signaling [16]. Pan et al. [1] reported that MET has the potential to reduce morphine tolerance in mice by inhibiting microglial activation and suppressing central sensitization in the spinal cord. The findings suggest that MET could be useful for managing opioid tolerance in chronic pain treatment. However, further studies are required to understand its mechanisms and determine its clinical applicability. Thus, the present study aimed to investigate the possible effect of acute and chronic administration of MET on tolerance to anti-nociceptive effects of SS in male rats.

## MATERIALS AND METHODS

### 1. Animals

Fifty-six male Wistar rats were used in this research (weight 200–250 g). Rats were randomly divided into 8 groups (n = 7). All the rats were housed in special Plexiglas cages with dimensions of 30 cm × 40 cm × 15 cm and in a situation with controlled conditions in repeated periods of 12 hours of light and 12 hours of darkness (the light cycle starts at 7 a.m.). Food and water were freely available, and the temperature was kept at 23°C ± 2.0°C. The instructions for the care and use of laboratory animals were followed throughout all experimental procedures (National Academic Press, Washington D.C, 2010). All experimental protocols were approved by the ethics committee at Torbat Heydariyeh University of Medical Sciences (IR.THUMS.REC.14000.027). All animals were handled cautiously to minimize undesired stress.

### 2. Drugs and experimental groups

SS (Sigma-Aldrich), and MET (Merck) were each injected intraperitoneally after being completely dissolved in saline (0.9%). The rats were divided into the following 8 groups: (1) the control, which received saline (as a vehicle) daily for 7 days; (2) SS, which received salicylate (300 mg/kg) daily for 7 days; (3) Met50, which received MET (50 mg/kg) for 7 days; (4) Met75, which received MET (75 mg/kg) for 7 days; (5) Met100, which received MET (100 mg/kg) for 7 days; (6) SS + Met50, which received salicylate (300 mg/kg) and MET (50 mg/kg) for 7 days; (7) SS + Met75, which received salicylate (300 mg/kg) and MET (75 mg/kg) for 7 days; and (8) SS + Met100, received salicylate (300 mg/kg) and MET (100 mg/kg) for 7 days. The drug doses were chosen based on studies evaluating the analgesic effects of intraperitoneally injected SS and MET in rats [17–20].

### 3. Hot plate test

A study demonstrated that tolerance to NSAID's antinociceptive effects is mediated by the endogenous opioid system, potentially involving descending pain modulatory systems [21]. The hot plate test is used to assess supraspinal pain pathways in rats, as it was shown that

hind limb withdrawal does not occur in rats with spinal transection [22]. Therefore, to assess the anti-nociceptive effects of the drugs the hot plate test (Borj Sanat Azma) was performed. For this procedure, the heat was adjusted to a constant temperature of 50°C ± 2°C. Individual rats were placed on a hot plate, and their response latency (the time taken to withdraw a hind paw, lick, or jump) was recorded in seconds. A cut-off duration of 50 seconds was specified to prevent tissue injury. To assess acute drug response, the mean reaction time was recorded at 15, 30, 45, and 60 minutes after drug administration.

### 4. Statistical analysis

All data were presented as mean ± standard error of the mean. One-way analysis of variance (ANOVA) test and Tukey's *post hoc* test were used for statistical analysis of data with a single independent variable. Two-way ANOVA was performed for data with numerous independent variables, followed by Tukey's *post hoc* test. The GraphPad Prism software (version 8.0) was used to perform statistical analyses and prepare figures. Statistical significance for differences between means was considered as $P < 0.05$.

## RESULTS

### 1. Response time evaluation of acute treatments

To determine of best response time, after the first injection of the treatments, hot plate latencies were recorded each 15 minutes until 60 minutes. As shown in **Fig. 1** most responses to the treatments were 45 minutes after injection.

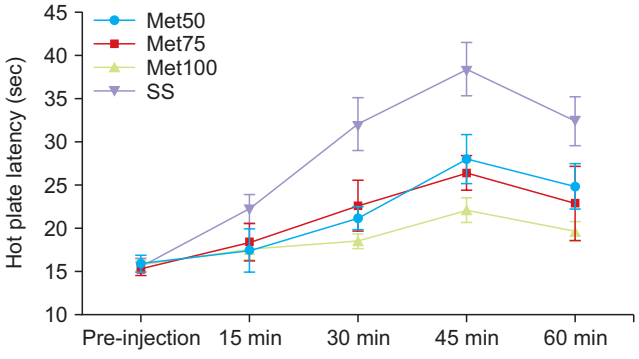


**Fig. 1.** Line chart of hot plate latencies after injection of the treatments. Data were presented as mean ± standard error of the mean, n = 7. Met50: received MET (50 mg/kg) for 7 days, Met75: received MET (75 mg/kg) for 7 days, Met100: received MET (100 mg/kg) for 7 days, SS: received salicylate (300 mg/kg) daily for 7 days, MET: metformin.

### 2. The analgesic effect of MET

Different doses of MET significantly increased hot plate latency on the first day after injection as compared to the control group (Met50, $P = 0.048$; Met75, $P = 0.014$). Moreover, the Met50 group still showed a significant effect compared with the control group on the second day after injection ($P = 0.025$; **Fig. 2A**). Analysis of the line chart

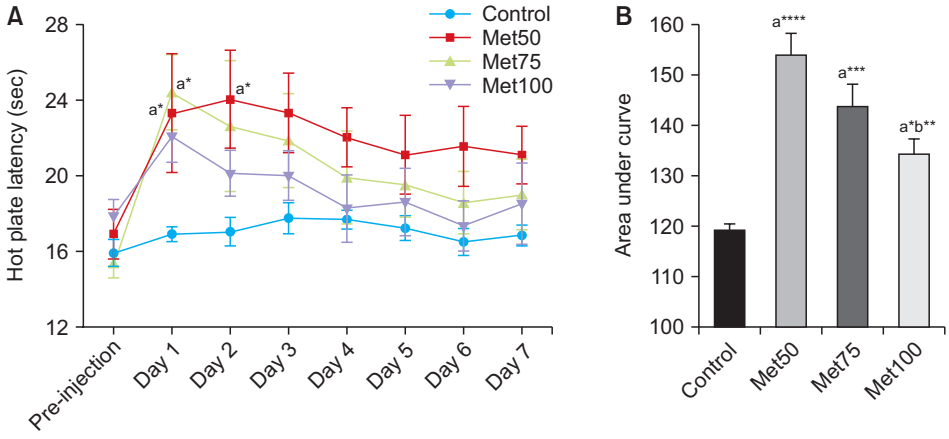


**Fig. 2.** Effect of different doses of metformin (MET) on hot plate latencies. (A) Line chart of hot plate latencies after MET injection at different doses over time. (B) Area under curve of the line chart. Data were presented as mean ± standard error of the mean, n = 7, and analyzed by ANOVA followed by Tukey's multiple comparison tests. (a) Compared to the control group, (b) Compared to the Met50 group. Met50: received MET (50 mg/kg) for 7 days, Met75: received MET (75 mg/kg) for 7 days, Met100: received MET (100 mg/kg) for 7 days. $^{****}P < 0.0001$; $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$.

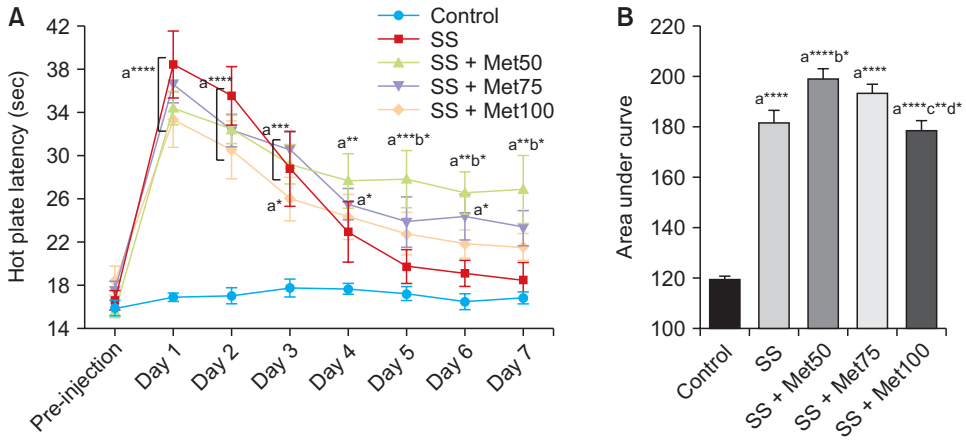


**Fig. 3.** Effect of salicylate and metformin (MET) co-treatment on hot plate latencies. (A) Line chart of the groups hot plate latencies thorough the days. (B) Area under curve of the line chart. Data were presented as mean ± standard error of the mean, n = 7, and analyzed by ANOVA followed by Tukey's multiple comparison tests. (a) Compared to the control group, (b) Compared to the SS group, (c) Compared to the Met50 group, (d) Compared to the Met75 group. Met50: received MET (50 mg/kg) for 7 days, Met75: received MET (75 mg/kg) for 7 days, Met100: received MET (100 mg/kg) for 7 days, SS: received salicylate (300 mg/kg) daily for 7 days. ****$P$ < 0.0001; ***$P$ < 0.001; **$P$ < 0.01; *$P$ < 0.05.

area under the curve (AUC) was shown MET-treated groups have significantly higher hot plate latencies than the control group (Met50, Met75, $P$ < 0.001; Met100, $P$ = 0.019). Also, the Met100 group had a significantly lower AUC as compared to the Met50 group ($P$ = 0.002; **Fig. 2B**).

### 3. The effect of MET co-treatment on salicylate tolerance

Salicylate injection significantly increased hot plate latency as compared to the control group ($P$ < 0.001) but this latency decreased through the days after injection, and after day 4, salicylate injection did not significantly increase the latency, which indicated tolerance development to the salicylate. SS + Met co-treatment somehow prevented this tolerance development and the latency remained significant after day 4 (SS + Met50, $P$ = 0.002; SS + Met75, $P$ = 0.025). Moreover, after day 5 the SS + Met50 group latency became significant to the SS group ($P$ = 0.018; **Fig. 3A**). Analysis of the line chart AUC showed that the salicylate salicylate-treated groups have significantly higher hot plate latency than the control group ($P$ < 0.001). Co-treatment of SS + Met50 had a stronger analgesic effect as compared to the SS group ($P$ = 0.015; **Fig. 3B**).

## DISCUSSION

In the present study, the authors investigated the MET effect on salicylate tolerance development. MET is a well-known drug and widely used to treat patients with type 2 diabetes. It was shown that MET activates AMP-activated protein kinase (AMPK), which plays a role in nociceptive processing. Therefore, it is expected to have an effect on nociception [23,24]. Studies show that MET reduces neuropathic pain in rodents by suppressing aberrant translation pathways in primary afferent neurons and inhibiting neuronal excitability [25]. Moreover, MET has the potential to prevent the development of pain and thermal hyperalgesia in inflammatory pain induction models [26,27]. In agreement with previous studies, it was found that administration of the MET induced analgesic effects, as evidenced by the increased hotplate latency observed in the MET-treated groups (**Fig. 2**). A study reported that MET significantly increased nociceptive response latency in the hot-plate model [28]. A dose-response curve study of MET has revealed that the efficacy of the drug is biphasic and reduces at both very low and high doses [29]. Another study demonstrated that MET at 50 mg/kg attenuates IL-1β and TNF-α levels better than high doses of 100 and 200 mg/kg, and also this dose has stronger analgesic efficacy than higher doses in the spinal cord injury model [19]. The authors' results, in accordance with the mentioned studies, indicate that MET at the dose of 50 mg/kg had the highest analgesic effect, and the Met100 group had significantly lower AUC than the Met50 group (**Fig. 2B**). NSAIDs are widely used for mild pain relief and produce their effects by inhibiting cyclo-oxygenase, a key enzyme in the production of prostaglandins which potentiate the pain caused by other mediators, *e.g.*, histamine,

serotonin, and bradykinin [30]. Studies have shown that NSAIDs could inhibit spinal cord nociceptive neurons [31]. It is confirmed that the usual analgesics, like lysine-acetylsalicylate, induce antinociception through the activation of neurons in opioid-related brain structures [32]. Also, another study showed that a morphine antagonist, naloxone can block the analgesic effects of NSAIDs [5]. This evidence suggests that the analgesic effect of NSAIDs may be related to the endogenous opioid system. In this study, after injecting salicylate, the hot plate latency increased. However, after repeated administration, this effect was reduced and on day 5, the latency reached the same level as the control group (**Fig. 3A**). Consistent with the authors' observations, a study showed that administering diclofenac prolongs the response time of the hot plate in rats [33]. Another study reports that systemic injections of NSAIDs attenuate tail-flick and hot plate responses and repeated treatment leads to tolerance development. Moreover, this confirms that the tolerance was related to an opiate-mediated mechanism [34]. In the present study, it was found that co-treatment of MET with salicylate could prevent tolerance development as evidenced by hot plate response persistence in the co-treatment groups (**Fig. 3A**). Also, the SS + Met50 group had a significantly higher analgesic effect than the SS group (**Fig. 3B**). It was reported that MET reduced the development of analgesic tolerance resulting from repeated intraperitoneal injections of morphine in mice [35]. A study found that MET could enhance the analgesic effects of ibuprofen and aspirin in an inflammatory pain model, and this effect is not related to pharmacokinetic interactions [20]. MET activates AMPK, a kinase present in all cells that regulates various functions. In neural cells, it inhibits mitogen-activated protein kinase and mammalian target of rapamycin kinase pathways, which are important for pain plasticity and sensitization [36,37]. As tolerance to the analgesic effects of NSAIDs is related to the endogenous opioid system, and a recent study showed MET could prevent opioid-related tolerance, it is possible that MET, through AMPK activation, affects the opioid system and reduces salicylate tolerance development.

This study did not have sufficient tests to confirm this possibility, and further research is needed to explore the mechanism. Specifically, changes in the excitability of the ascending and descending pain pathways must be investigated by membrane potential recording. In addition, examining changes in gene expression and receptor density could help identify the mechanism behind this effect. Unfortunately, due to budget and device limitations, these changes were not studied. From the obtained results it could be concluded that MET has the potential to reduce analgesic tolerance induced by repeated intraperitoneal injections of SS in Wister rats. This suggests that MET could be a new approach to preventing NSAID analgesic tolerance.

## DATA AVAILABILITY

The datasets generated and/or analyzed in the current study are available from the corresponding author upon reasonable request.

## CONFLICT OF INTEREST

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

## FUNDING

No funding to declare.

## AUTHOR CONTRIBUTIONS

Elham Akbari: Investigation; Dawood Hossaini: Investigation; Farimah Beheshti: Investigation; Mahdi Khorsand Ghaffari: Writing/manuscript preparation; Nastran Roshd Rashidi: Writing/manuscript preparation; Masoumeh Gholami: Project administration.

## ORCID

Elham Akbari, https://orcid.org/0000-0001-7856-8948
Dawood Hossaini, https://orcid.org/0000-0001-7873-7617
Farimah Beheshti, https://orcid.org/0000-0003-1524-2339
Mahdi Khorsand Ghaffari, https://orcid.org/0000-0001-6165-4989
Nastran Roshd Rashidi, https://orcid.org/0000-0002-5570-9805
Masoumeh Gholami, https://orcid.org/0000-0002-6746-0488

## REFERENCES

1. Pan Y, Sun X, Jiang L, Hu L, Kong H, Han Y, et al. Metformin reduces morphine tolerance by inhibiting microglial-mediated neuroinflammation. J Neuroinflammation 2016; 13: 294.

2. Déciga-Campos M, López UG, Reval MI, López-Muñoz FJ. Enhancement of antinociception by co-administration of an opioid drug (morphine) and a preferential cyclooxygenase-2 inhibitor (rofecoxib) in rats. Eur J Pharmacol 2003; 460: 99-107.
3. Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. J Anesth 2019; 33: 131-9.
4. Owoyele BV, Bakare AO, Olaseinde OF, Ochu MJ, Yusuff AM, Ekebafe F, et al. Corrigendum: synergistic interaction between acetaminophen and L-carnosine improved neuropathic pain via NF-κB pathway and antioxidant properties in chronic constriction injury model. Korean J Pain 2022; 35: 488. Erratum for: Korean J Pain 2022; 35: 271.
5. Pernia-Andrade AJ, Tortorici V, Vanegas H. Induction of opioid tolerance by lysine-acetylsalicylate in rats. Pain 2004; 111: 191-200.
6. Wideman GL, Keffer M, Morris E, Doyle RT Jr, Jiang JG, Beaver WT. Analgesic efficacy of a combination of hydrocodone with ibuprofen in postoperative pain. Clin Pharmacol Ther 1999; 65: 66-76.
7. Sunshine A, Olson NZ, O'Neill E, Ramos I, Doyle R. Analgesic efficacy of a hydrocodone with ibuprofen combination compared with ibuprofen alone for the treatment of acute postoperative pain. J Clin Pharmacol 1997; 37: 908-15.
8. Christie MJ, Vaughan CW, Ingram SL. Opioids, NSAIDs and 5-lipoxygenase inhibitors act synergistically in brain via arachidonic acid metabolism. Inflamm Res 1999; 48: 1-4.
9. Tsiklauri N, Gurtskaia G, Tsagareli M. Study of non-opioid analgesics tolerance in young and adult rats. Georgian Med News 2008; 158: 40-4.
10. Trujillo KA, Akil H. Opiate tolerance and dependence: recent findings and synthesis. New Biol 1991; 3: 915-23.
11. Bao W, Luo Y, Wang D, Li J, Wu X, Mei W. Sodium salicylate modulates inflammatory responses through AMP-activated protein kinase activation in LPS-stimulated THP-1 cells. J Cell Biochem 2018; 119: 850-60.
12. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocr Rev 2021; 42: 77-96.
13. Liu SN, Liu Q, Sun SJ, Hou SC, Wang Y, Shen ZF. [Metformin ameliorates β-cell dysfunction by regulating inflammation production, ion and hormone homeostasis of pancreas in diabetic KKAy mice]. Yao Xue Xue Bao 2014; 49: 1554-62. Chinese.
14. Zhou C, Sun R, Zhuang S, Sun C, Jiang Y, Cui Y, et al. Metformin prevents cerebellar granule neurons against glutamate-induced neurotoxicity. Brain Res Bull 2016; 121: 241-5.
15. Bonnefont-Rousselot D, Raji B, Walrand S, Gardès-Albert M, Jore D, Legrand A, Peynet J, et al. An intracellular modulation of free radical production could contribute to the beneficial effects of metformin towards oxidative stress. Metabolism 2003; 52: 586-9.
16. Bułdak Ł, Machnik G, Bułdak RJ, Łabuzek K, Bołdys A, Okopień B. Exenatide and metformin express their anti-inflammatory effects on human monocytes/macrophages by the attenuation of MAPKs and NFκB signaling. Naunyn Schmiedebergs Arch Pharmacol 2016; 389: 1103-15.
17. Houser VP, Paré WP. Analgesic potency of sodium salicylate, indomethacin, and chlordiazepoxide as measured by the spatial preference technique in the rat. Psychopharmacologia 1973; 32: 121-31.
18. Sadegh M, Fathollahi Y, Naghdi N, Semnanian S. Morphine deteriorates spatial memory in sodium salicylate treated rats. Eur J Pharmacol 2013; 704: 1-6.
19. Afshari K, Dehdashtian A, Haddadi NS, Haj-Mirzaian A, Iranmehr A, Ebrahimi MA, et al. Anti-inflammatory effects of Metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats: introduction of an alternative therapy. Spinal Cord 2018; 56: 1032-41.
20. Pecikoza UB, Tomić MA, Micov AM, Stepanović-Petrović RM. Metformin synergizes with conventional and adjuvant analgesic drugs to reduce inflammatory hyperalgesia in rats. Anesth Analg 2017; 124: 1317-29.
21. Tsiklauri N, Pirkulashvili N, Nozadze I, Nebieridze M, Gurtskaia G, Abzianidze E, et al. Antinociceptive tolerance to NSAIDs in the anterior cingulate cortex is mediated via endogenous opioid mechanism. BMC Pharmacol Toxicol 2018; 19: 2.
22. Giglio CA, Defino HL, da-Silva CA, de-Souza AS, Del Bel EA. Behavioral and physiological methods for early quantitative assessment of spinal cord injury and prognosis in rats. Braz J Med Biol Res 2006; 39: 1613-23.
23. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia 2017; 60: 1577-85.
24. Asiedu MN, Han C, Dib-Hajj SD, Waxman SG, Price TJ, Dussor G. The AMPK activator A769662 blocks voltage-gated sodium channels: discovery of a novel pharmacophore with potential utility for analgesic

development. PLoS One 2017; 12: e0169882.
25. Baeza-Flores GDC, Guzmán-Priego CG, Parra-Flores LI, Murbartián J, Torres-López JE, Granados-Soto V. Metformin: a prospective alternative for the treatment of chronic pain. Front Pharmacol 2020; 11: 558474.
26. Russe OQ, Möser CV, Kynast KL, King TS, Stephan H, Geisslinger G, et al. Activation of the AMP-activated protein kinase reduces inflammatory nociception. J Pain 2013; 14: 1330-40.
27. Bullón P, Alcocer-Gómez E, Carrión AM, Marín-Aguilar F, Garrido-Maraver J, Román-Malo L, et al. AMPK phosphorylation modulates pain by activation of NLRP3 inflammasome. Antioxid Redox Signal 2016; 24: 157-70.
28. Augusto PSA, Braga AV, Rodrigues FF, Morais MI, Dutra MMGB, Batista CRA, et al. Metformin antinociceptive effect in models of nociceptive and neuropathic pain is partially mediated by activation of opioidergic mechanisms. Eur J Pharmacol 2019; 858: 172497.
29. Riddle M. Combining sulfonylureas and other oral agents. Am J Med 2000; 108 Suppl 6a: 15S-22S.
30. Burian M, Geisslinger G. COX-dependent mechanisms involved in the antinociceptive action of NSAIDs at central and peripheral sites. Pharmacol Ther 2005; 107: 139-54.
31. Vanegas H, Schaible HG. Prostaglandins and cyclooxygenases [correction of cycloxygenases] in the spinal cord. Prog Neurobiol 2001; 64: 327-63. Erratum in: Prog Neurobiol 2001; 65: 609.
32. Vanegas H, Tortorici V. Opioidergic effects of nonopioid analgesics on the central nervous system. Cell Mol Neurobiol 2002; 22: 655-61.
33. Elberry AA, Sharkawi SMZ, Wahba MR. Antinociceptive and anti-inflammatory effects of N-acetylcysteine and verapamil in Wistar rats. Korean J Pain 2019; 32: 256-63.
34. Tsiklauri N, Viatchenko-Karpinski V, Voitenko N, Tsagareli MG. Non-opioid tolerance in juvenile and adult rats. Eur J Pharmacol 2010; 629: 68-72.
35. Shirooie S, Esmaeili J, Sureda A, Esmaeili N, Mirzaee Saffari P, Yousefi-Manesh H, et al. Evaluation of the effects of metformin administration on morphine tolerance in mice. Neurosci Lett 2020; 716: 134638.
36. Ji RR, Gereau RW 4th, Malcangio M, Strichartz GR. MAP kinase and pain. Brain Res Rev 2009; 60: 135-48.
37. Lisi L, Aceto P, Navarra P, Dello Russo C. mTOR kinase: a possible pharmacological target in the management of chronic pain. Biomed Res Int 2015; 2015: 394257.