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ORIGINAL ARTICLE

Asprosin, a novel therapeutic candidate for painful neuropathy: an experimental study in mice

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Abstract

Recent studies indicate presence of a strong link between adipokines and neuropathic pain. However, the effects of asprosin, a novel adipokine, on neuropathic pain have not been studied in animal models.

Mouse models were employed to investigate the antinociceptive effectiveness of asprosin in the treatment of three types of neuropathic pain, with metabolic (streptozocin/STZ), toxic (oxaliplatin/OXA), and traumatic (sciatic nerve ligation/CCI [chronic constriction nerve injury]) etiologies, respectively. Changes in nociceptive behaviors were assessed relative to controls using thermal (the hot plate and cold plate tests, at 50 °C and 4 °C respectively) and mechanical pain (von Frey test) tests after intraperitoneal (i.p.) administration of asprosin (10 µg/kg) and gabapentin (50 mg/kg) in several times intervals. Besides, possible effect of asprosin on the motor coordination of mice was assessed with a rotarod test. Serum level of asprosin was quantified by ELISA.

In neuropathic pain models (STZ, OXA, and CCI), asprosin administration significantly reduced both mechanical and thermal hypersensitivity, indicating that it exhibits a clear-cut antihypersensitivity effect in the analyzed neuropathic pain models. The most effective time of asprosin on pain threshold was observed 60 min after its injection. Also, asprosin displayed no notable effect on the motor activity. Asprosin levels were significantly lower in neuropathic pain compared to healthy group ($p < 0.05$). The results yielded by the present study suggest that asprosin exhibits an analgesic effect in the neuropathic pain models and may have clinical utility in alleviating chronic pain associated with disease and injury originating from peripheral structures.

Keywords Asprosin · Neuropathic pain · *Hot plate* test · *Cold plate* tests · *Von Frey* test

Introduction

Neuropathic pain is a severe pathology of the nervous system that offers no adaptable benefit. The neuropathic pain has recently received much attention due to the alarming increases in the prevalence of obesity, diabetes, and cancer (Yawn et al., 2009; Treede, 2018). In addition, further evidence also suggests people with obesity are more pain sensitive (Stone and Broderick, 2012). Although neuropathic pain etiology is not fully understood, available evidence points to an association with obesity, as excessive levels of adipose tissue hormones and adipokines affect neuropathic pain. Numerous adipokines may play an important role in pain sensitivity and transmission, and may thus be one of the possible targets in the treatment of pain (Pottie et al., 2006; Sharma, 2018).

When asprosin, a hormone released from white fat cells, reaches the liver, it causes rapid glucose release into the circulation. Asprosin is encoded by the fibrillin 1 (FBN1) gene and comprises 140 amino acids, with a weight of about 30 kDa (Romere et al., 2016). Its levels may change in obesity, diabetes, and metabolic syndrome associated with insulin resistance (Romere et al., 2016; Ugur and Aydin, 2019; Wang et al., 2019; Zhang et al., 2020). Asprosin dysfunction is caused by immunological or genetic factors, and results in

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a significant decrease in glucose and insulin concentrations. Administration of asprosin also increases blood glucose levels in healthy mice (Romere et al., 2016), but has no effects on diabetic mice (Hekim et al., 2021). Prior investigations further indicate that plasma asprosin crosses the blood–brain barrier and activates orexigenic AMP-dependent-aqouti-related neuropeptide (AgRP) neurons in the hypothalamus. This signaling results in inhibition of anorexigenic pre-opiomelanocortin (POMC) neurons in a GABA-dependent manner (Greenhill, 2016; Duerrschmid et al., 2017; Beutler and Knight, 2018; Li et al., 2018; Ozcan et al., 2020). While activation of AgRP and POMC neurons has been shown to influence pain as well as appetite (Cerritelli et al., 2016; Alhadeff et al., 2018), it is not known whether asprosin levels change in mice models for painful neuropathy. There is the possible association between impaired glucose tolerance and neuropathy (Rajabally, 2011). In addition, the effects of glycogenic hormone asprosin on neuropathic pain that cause hyperalgesia in mice models are also unknown.

Given that animal models can mimic the changes in nociceptive behaviors in toxic, metabolic, and traumatic neuropathic pain in humans, the aim of the present study was to investigate the effects of asprosin on changes in nociceptive behaviors in diabetes, chemotherapy, and sciatic nerve injury–induced neuropathic pain using mouse models (Le Bars et al., 2001). Our further objective was to ascertain whether asprosin level changes in mouse models for painful neuropathy.

Materials and methods

Animals

All experiments performed 125 adult male Balb/C mice, 6–8 weeks old, weighing 25 to 30 g, and adhered fully to the guidelines of the ARRIVE (Animal in Research: In Vivo Experiments) as well as the principles of the International Association for the Study of Pain (IASP) Research and Ethics Committee (Zimmermann, 1983). This study was carried out with the approval of Firat University Animal Experiments Local Ethics Committee (dated 16.01.2019, protocol number 2019/06 with the decision number 2019/09). The animals were housed for 1 week before starting the experiments to acclimatize them and were maintained under standard laboratory conditions on a 12-h light/dark cycle at a constant temperature (23 ± 2 °C) and humidity (60 ± 5%) with food and water supplied ad libitum.

Behavioral tests were performed on all groups in a quiet room at the same time by the same researchers blind to the randomization, thus minimizing the influence of environmental factors and researcher effects. Tests and exercises were conducted between 9.00 am and 12.00 am and all treatments were delivered intraperitoneally (i.p) (Fig. 1).

Healthy mice

Thirty-five healthy animals were randomly divided into five subgroups: control, morphine group (10 mg/kg), asprosin (1 µg/kg), asprosin (10 µg/kg), and asprosin (30 µg/kg) ($n = 7$, each group). We first evaluated the effectiveness of asprosin (1, 10, and 30 µg/kg, i.p.) and morphine (10 mg/kg) on pain behavior in healthy animals, which were individually acclimatized to the environment and test setup, respectively. The effective dose of asprosin used in this study was calculated as LogEC50 = 0.9281.

Streptozotocin-induced diabetes model in mice

Streptozotocin (STZ) was dissolved in 0.4 mL (0.1 M) Na^+ citrate buffer (pH 4.5). Normoglycemic mice were administered STZ i.p. at a dose of 150 mg/kg. Three days later, blood glucose was measured with manual glucometer (Optima, Taiwan) (all measurements were made between 9.00 and 10.00 am). Only mice with glucose levels above 250 mg/dL in blood taken from the tail vein were retained. From this group, mice that lost ≥ 10% of body weight before starting the experiment, exhibited decreased activity or hair erection, were also excluded. The STZ-induced diabetic neuropathic pain group was randomly divided into three subgroups: STZ (diabetic control), STZ + asprosin (10 µg / kg), and STZ + gabapentin (50 mg/kg) ($n = 10$, each group). The control group was injected i.p. with the solvent sodium citrate buffer. Behavioral tests were conducted 3 weeks after STZ administration to mice.

Induction of neuropathy: chronic constriction nerve injury

The chronic construction of the sciatic nerve was performed using clamps as previously described (Salvat et al., 2018). First, mice were anesthetized with a ketamine (68 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and the right sciatic nerve was exposed after dissection. Next, 2-mm PE-20 polyethylene tube (Harvard Apparatus, 59–8323, Les Ulis, France) was placed at around 5 mm distance from the trifurcation of the sciatic nerve, after which the wound was closed by suturing muscle (chromic catgut) and skin (3.0 silk). The neuropathic pain group with sciatic nerve chronic constriction injury (CCI) was divided into three subgroups: CCI (sham control), CCI + asprosin (10 µg /kg), and CCI + gabapentin (50 mg/kg) ($n = 10$, each group). The sham groups were operated as described above, but without a polyethylene tube (cuff implantation).

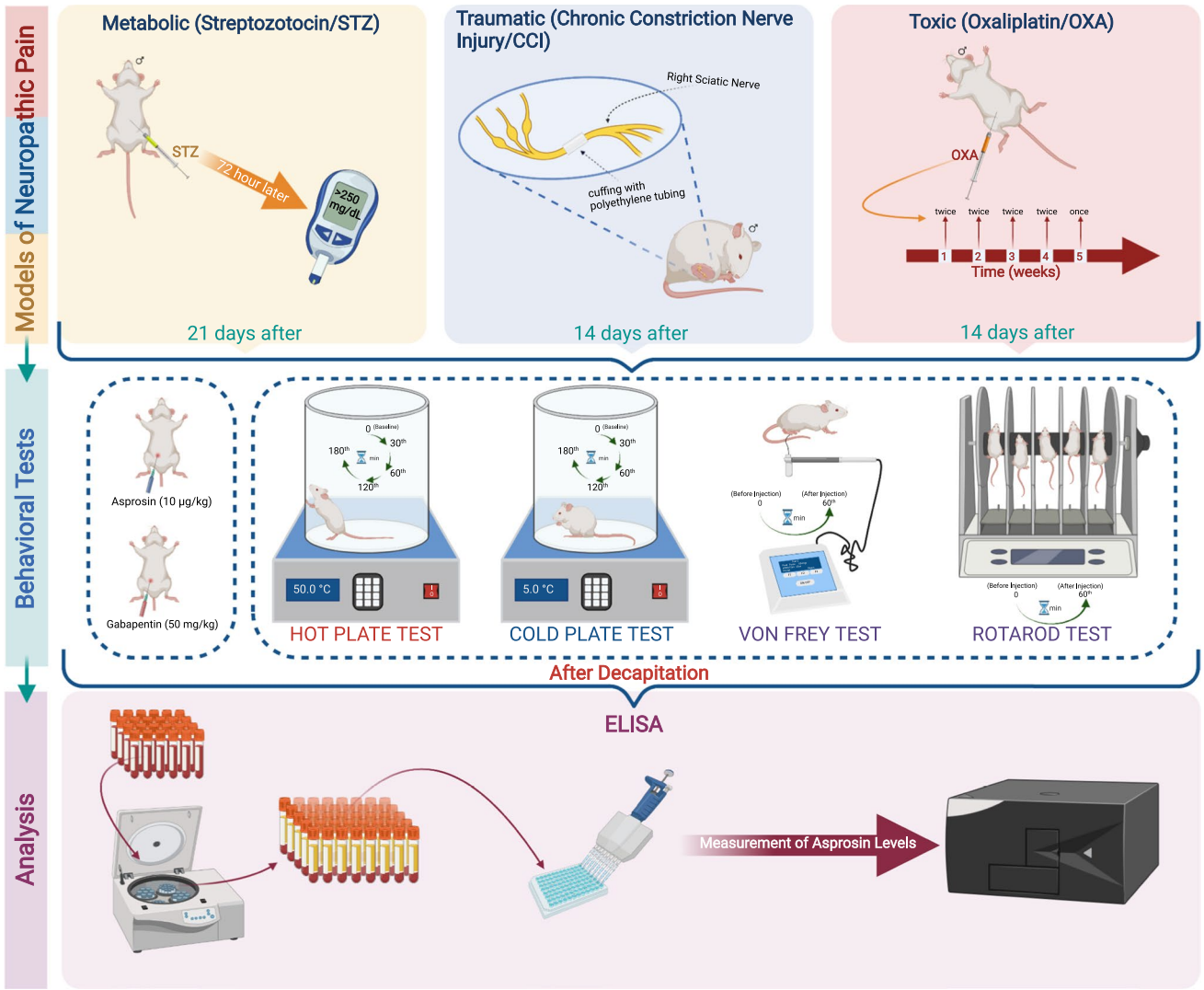

Fig. 1 Flowchart of experimental design

Animals with CCI were subjected to behavioral tests after 2 weeks of waiting for their wounds to heal.

Oxaliplatin-induced neuropathy

Oxaliplatin (OXA) was prepared at a concentration of 3 mg/mL in a 5% dextrose solution according to the weight of each animal and was administered i.p. at a dose of 3 mg/kg twice a week for 4.5 weeks. The control group was injected with 5% dextrose solution. The neuropathic pain group after oxaliplatin (OXA) use was also divided into three subgroups, denoted as OXA (control), OXA + asprosin (10 µg /kg), and OXA + gabapentin (50 mg/kg) ($n = 10$, each group).

Animals were subjected to behavioral tests 2 weeks after the last injection of the drug OXA.

Nociceptive test procedures

Groups were determined by randomly selecting mice. Before commencing the behavioral tests, the animals assigned to all groups were numbered by marking their tails. Behavioral tests were performed on all groups in a quiet room between 9.00 am and 12.00 pm by the same researchers that were blind to randomization, thus minimizing the influence of environmental factors and researcher effects. Gabapentin (50 mg/kg, i.p.) was used as positive control. Three nociceptive tests (hot plate, cold plate, and von Frey) were performed 3 days interval to avoid animal suffering.

Hot plate test

The *hot plate* test was performed by placing the animals on a warm floor within a clear plexiglass cylinder of 15 cm

diameter and 22.5 cm height to prevent them from coming out. The hot plate analgesimeter table was set to 50 ± 0.5 °C (Deuis et al., 2017). Pain behavior was determined as the time (in seconds, with a 40-s cut-off) that lapsed from placing the animal on the floor to its visible reaction to pain (quickly pulling, licking, or contracting its extremities). At the baseline, pain threshold values were determined before any injection was given to the mice (the time of injection was accepted as 0 min), and then measurements were performed 30, 60, 120, and 180 min after the injection.

Cold plate test

Cold hyperalgesia was evaluated using a *cold plate* at the optimum temperature of 5 ± 0.2 °C (Cerles et al., 2017; Luiz et al., 2019). The sum of paw lifts and jumps during the 5-min measurement interval (while excluding movement-related behaviors and coordinated movements of all four extremities) was considered a response to cold hyperalgesia. As with the *cold plate* test, the time of injection was accepted as 0 min, and further measurements were performed at 30, 60, 120, and 180 min after injection.

von Frey test

Mechanical allodynia was assessed using von Frey filaments as previously described (Yalcin et al., 2014). Briefly, 10 von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (von Frey numbers equivalent to 0.04, 0.07, 0.16, 0.4, 1, 2, 4, 6, 8, and 10 g). On the day of the test, animals were kept in the boxes for 15 min without any treatment, after which each von Frey filament was pressed 5 times perpendicularly against the plantar surface of the right hind paw until slight buckling. The filament that produced the same response in at least three instances was defined as the result. These measurements were performed at baseline (i.e., before the mice were given any injections) and the time of injection was accepted as 0 min. The test was repeated 1 h after injection. This procedure was applied to all groups.

Motor activity

Prior to testing, mice were acclimated to the environment and the test protocol by placing them on the fixed bar for 5 min for two consecutive days and then on the rotating bar while increasing its speed from 4 to 40 rpm. Following the acclimation period, mice were placed on accelerating rotating rods to determine the effect of asprosin on motor activity and the time taken for the animals to fall off the rod was recorded (cut-off time = 300 s) (Spooren et al., 2000). As with other tests, the baseline measurements were performed before the mice were given any injections and the time of injection was accepted as 0 min. The test was repeated 1 h after the injection, as this interval corresponds to the highest pain threshold. This procedure was applied to all groups.

Asprosin measurements

At the end of all experimental protocols, blood samples were collected from all mice by decapitation between 9:00 am and 10:00 am. After the blood samples were centrifuged at 3500 rpm for 10 min, their serums were stored at − 80 °C until the experiment was conducted. Analyses were performed using the mouse asprosin ELISA Kit (USCN catalog number: SEA332Mu) following the manufacturer's protocol. The amount of asprosin antigen in the serum was determined using a microplate coated with the asprosin antibody included in the kit. The color change that occurs at the end of the experiment indicates the presence of antigen and the measuring range of the kit spans from 78 to 5000 pg/mL, while its sensitivity is ≤ 32 pg/mL, with the inter-assay and intra-assay %CV (coefficient of variation) values of ˂ 12% and ˂ 10%, respectively. The experimental protocol included in the ELISA kit was used to determine the serum asprosin level.

Drugs

Gabapentin, STZ, and OXA were purchased from Sigma. Gabapentin was prepared by dissolving in saline, while the recombinant asprosin (Elabscience Company, USA) was dissolved in phosphate-buffered saline (PBS). All drugs were freshly prepared on the day of the experiment.

Statistical analyses

The analyses were performed using GraphPad Prism version 9.3.0 for macOS, GraphPad Software, San Diego, CA, USA, and Fig. 1 is created with biorender.com. Conformity to normal distribution was evaluated via the Shapiro–Wilk test and one-way analysis of variance was performed to compare the values of quantitative variables between groups when there were three or more groups. When a significant difference was identified, multiple comparisons were conducted using Dunnet's test and Tukey's test, with $p < 0.05$ indicating statistical significance.

Results

Effects of asprosin administration on pain behavior in healthy mice

The effects of asprosin on thermal and mechanical nociceptive pain threshold were evaluated using hot plate,

cold plate, and von Frey tests. We first tested effects of asprosin in healthy mice at different times (30, 60, 120, and 180 min) after injection. Asprosin (1 µg/kg, 10 µg/kg, and 30 µg/kg i.p.) increased pain threshold compared to control group in healthy mice. Moreover, the antinociceptive effect caused by asprosin was similar to morphine (10 mg/kg) (Fig. 2).

Effects of asprosin administration on pain behavior in diabetic mice

There were significant decreases in nociceptive behavior latencies in diabetic mice compared with the healthy animals. Asprosin (10 µg/kg) treatment was significantly decreased mechanical and thermal hypersensitivity in diabetic animals. Gabapentin (50 mg/kg) caused significant increases in pain threshold compared with the vehicle group (10 mg/kg) in diabetic mice. This effect of gabapentin was similar to asprosin treatment group in diabetic mice (Fig. 3).

Effects of asprosin administration on neuropathic pain behavior after oxaliplatin use in mice

The OXA-administered mice developed a significant hyperalgesic activity. Asprosin (10 µg/kg) treatment caused significant increases in pain threshold compared with the vehicle group (10 mg/kg) in OXA-administered mice. Moreover, the antinociceptive effect caused by asprosin was found to be similar to gabapentin (50 mg/kg) in OXA-administered animals (Fig. 4).

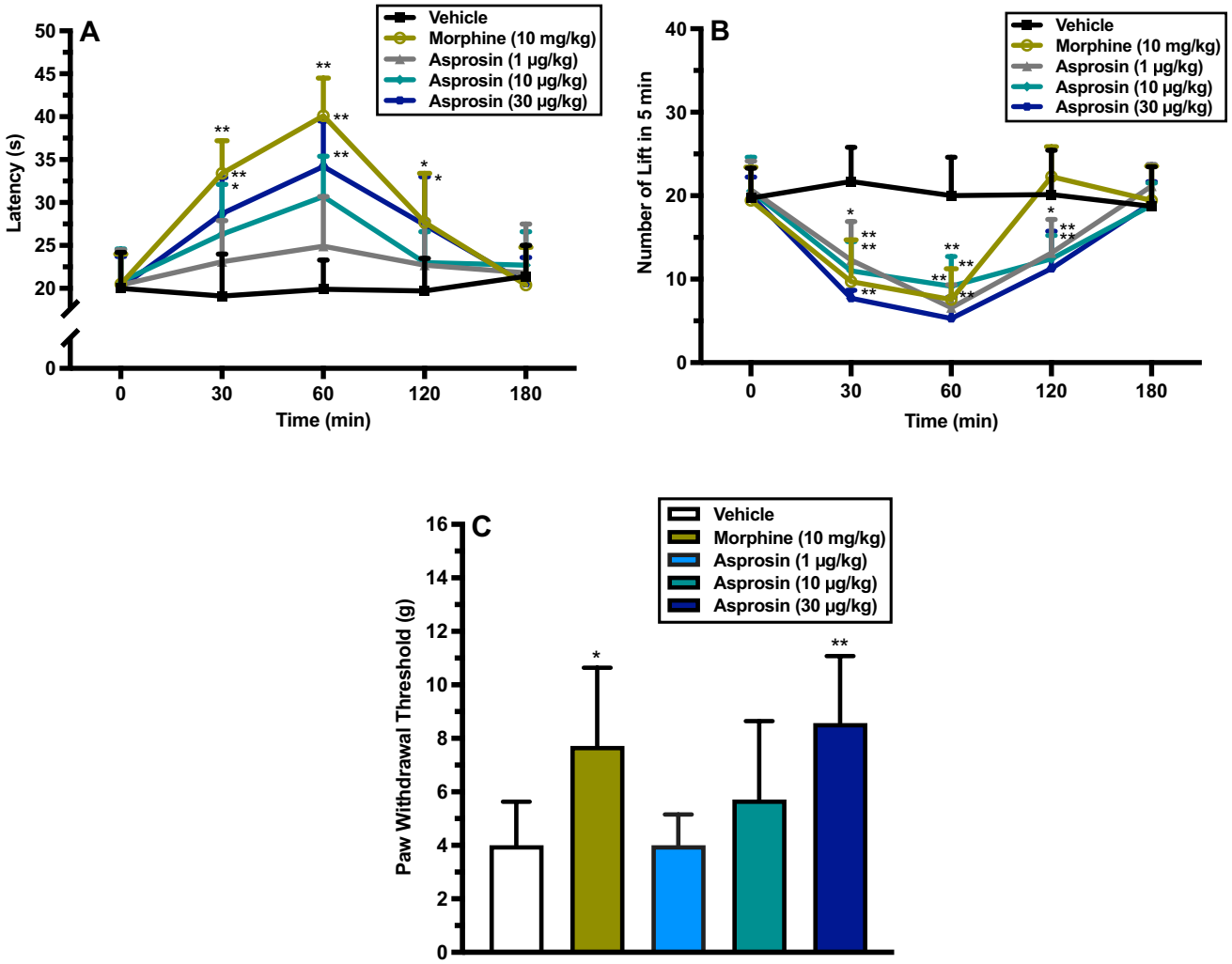

Fig. 2 Effect of asprosin (1, 10, and 30 µg/kg, ip) on pain threshold in healthy mice. Mean ± SD values of mouse response to thermal and mechanical stimulus in healthy mice. Asprosin administered groups were compared with the control group using Dunnet's test $^{*}p < 0.05$, $^{**}p < 0.01$. **A** The mean ± SD values of the time-dependent response of healthy mice with the *hot plate* test, **B** cold plate test, and **C** von Frey test

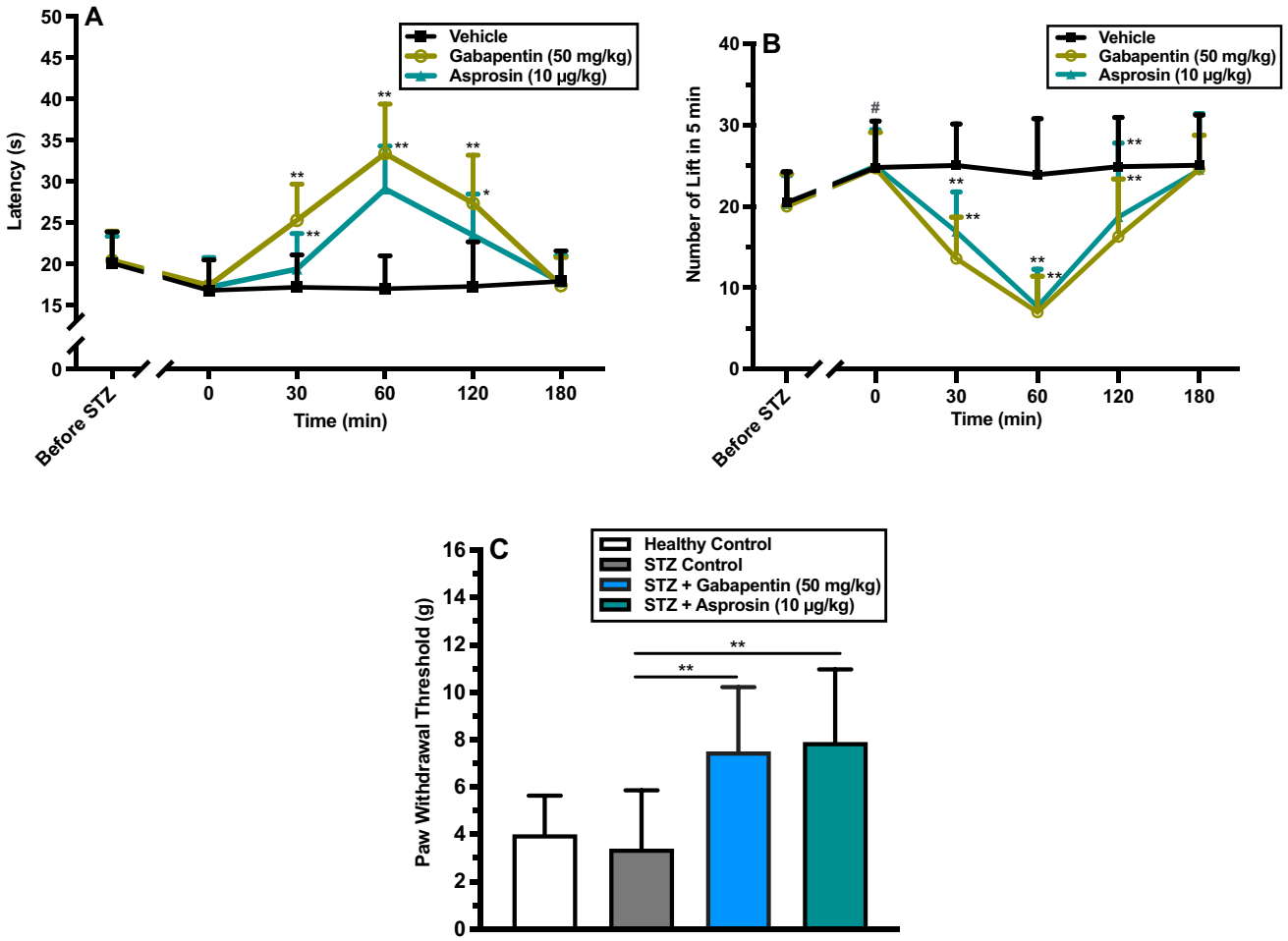

Fig. 3 Effect of asprosin (10 µg/kg, ip) and gabapentin (50 mg/kg, ip) on pain threshold in diabetic mice. Asprosin and gabapentin administered groups were compared with the diabetic control group $^{*}p<0.05$, $^{**}p<0.01$, and healthy control group $\#p < 0.05$. **A** The mean ± SD values of the time-dependent response of diabetic mice with the *hot plate* test, **B** *cold plate* test, and **C** von Frey test (one-way analysis of variance followed by a post-hoc Dunnet's test)

Effects of asprosin administration on neuropathic pain behavior after sciatic nerve injury in mice

There was significant difference in the response time (or in the von Frey test results) between CCI and healthy animals. Asprosin (10 µg/kg) treatment was significantly decreased mechanical and thermal hypersensitivity in CCI animals. However, the antinociceptive effect caused by asprosin (50 mg/kg) was lower than the antinociceptive effect caused by gabapentin in CCI animals (Fig. 5).

Effect of asprosin administration on motor activity

There were no effects of asprosin treatment on motor coordination in neuropathic pain–induced animals. However, CCI caused to significantly decrease in motor activity in mice according to healthy, diabetic, and OXA-administered mice ($p<0.05$) (Fig. 6).

Asprosin levels in healthy mice and those experiencing induced neuropathic pain

Asprosin level in healthy mice was measured at 54.5 ± 8.9 ng/mL. In order to determine whether asprosin levels change in neuropathic pain conditions, the same measurements were performed in toxic (oxaliplatin), metabolic (STZ), and traumatic (sciatic nerve construction injury) neuropathic pain groups. The findings revealed that asprosin level decreased in animals with neuropathic pain, as 30.5 ± 7.1 ng/mL was obtained for animals with type 1 diabetes, 23.1 ± 7.3 ng/mL for animals with neuropathy after OXA use, and 22.0 ± 6.9 ng/mL for animals with neuropathy after CCI (Fig. 7).

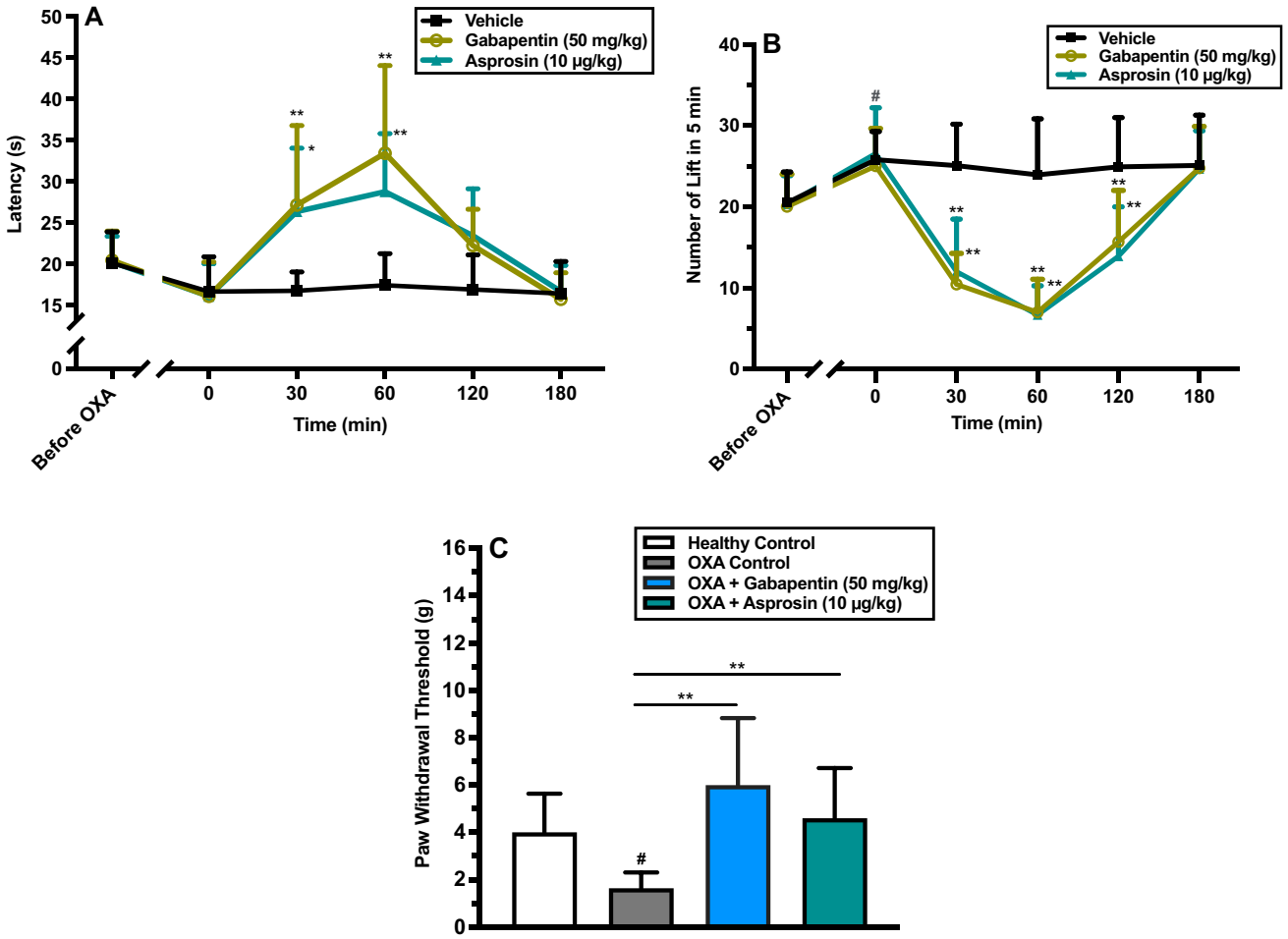

Fig. 4 The effect of asprosin (10 µg/kg, ip) andgabapentin (50 mg/kg, ip) on neuropathic pain behavior after oxaliplatin use in mice. Asprosin and gabapentin administered groups were compared with the OXA control group $*p<0.05$, $**p<0.01$, and healthy control group $\#p < 0.05$. **A** The mean ± SD values of the time-dependent response of OXA induced mice with the *hot plate* test, **B** *cold plate* test, and **C** von Frey test (one-way analysis of variance followed by a post-hoc Dunnet's test)

Discussion

The findings yielded by the present study indicate that asprosin administration significantly reduced mechanical and thermal hypersensitivity in mouse models for different painful neuropathies (toxic, metabolic, and traumatic). Moreover, asprosin levels in the neuropathic pain–induced group were significantly lower than in healthy mice.

Hunger and pain are two competing signals that individuals must respond to for their survival. Yet, the neural processes that prioritize these conflicting survival needs are poorly understood (Alhadeff et al., 2018). Findings yielded by extant studies indicate that some orexinergic/anorexinergic peptides influence nociceptive behaviors. Leptin, ghrelin, and orexin were demonstrated to inhibit the development of neuropathic pain in rodents (Vergnano et al., 2008; Chiou et al., 2010; Guneli et al., 2010; Li et al., 2013). However, kisspeptin has been shown to exert lower pain threshold and enhance nocifensive behavior in mice (Elhabazi et al., 2013; Kelestimur et al., 2021). Many studies have been published regarding the possible association between impaired glucose tolerance and neuropathy, but the actual responsibility of impaired glucose tolerance as cause of neuropathy remains still uncertain (Pottie et al., 2006; Rajabally, 2011; Sharma, 2018). In the present investigation based on a mouse model, we focused on asprosin (a glycogenic peptide), and found that it induces changes in nociceptive behaviors in neuropathic pain. Our findings are consistent with those yielded by previous research, indicating that pain may be affected not just by appetite hormones, but also by the projections of orexinergic nerves and distribution of orexinergic receptors in the body.

It has been determined that the spinal cord carries primary afferent nociceptive and thermoreceptive information from orexinergic neurons and sends projections to the superficial dorsal horn (lamina I and II) (van den Pol,

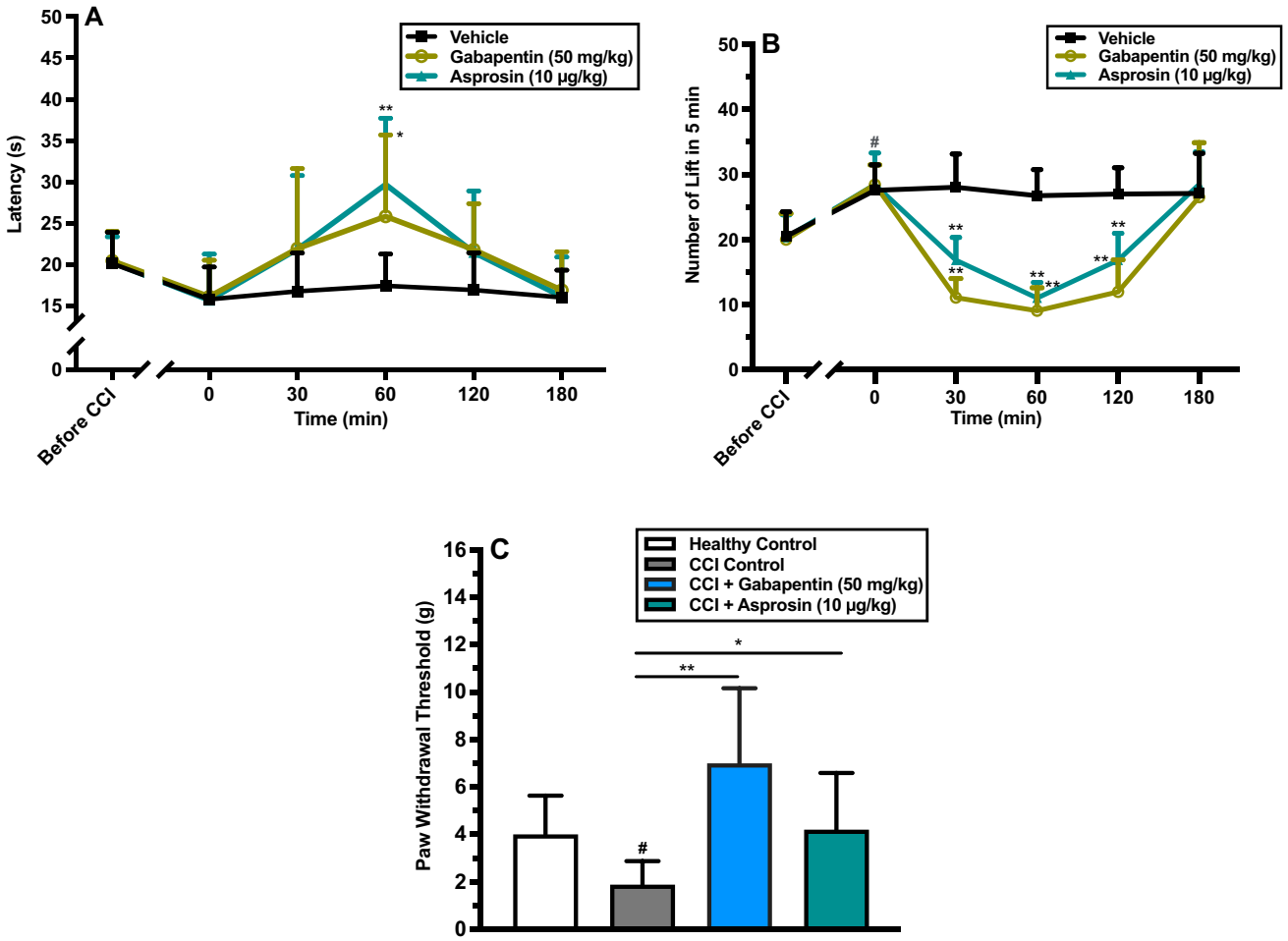

Fig. 5 Effect of asprosin (10 µg/kg, ip) and gabapentin (50 mg/kg, ip) on neuropathic pain behavior after sciatic nerve construction injury in mice. Asprosin and gabapentin administered groups were compared with the CCI control group $*p<0.05$, $**p<0.01$, and healthy control group $\#p<0.05$. **A** The mean ± SD values of the time-dependent response of CCI induced mice with the *hot plate* test, **B** *cold plate* test, and **C** von Frey test (one-way analysis of variance followed by a post-hoc Dunnet's test)

1999; Berrendero et al., 2018). Studies in rat superficial dorsal horn cells similarly show that anorexinergic/orexinergic peptides play a role in arousal, nociception, pain, and temperature sensations (Grudt and Perl, 2002). The activity of orexigenic AgRP neurons in the hypothalamus has been shown to inhibit both thermal acute pain and inflammatory pain (Alhadeff et al., 2018). Available findings further indicate that activation of anorexigenic POMC neurons in the brain stem produces opioidergic analgesia (Cerritelli et al., 2016). Peripheral asprosin, crossing the blood–brain barrier and activating AgRP and POMC neurons in the hypothalamic feeding circuitry (Duerrschmid et al., 2017; Beutler and Knight, 2018). As asprosin increases appetite via AgRP and POMC neurons, changes in nociceptive behaviors following asprosin administration in this study may be attributed to the activation of AgRP and POMC neurons. The association between asprosin and orexigenic/anorexigenic neurons (including neuropathic pain processes) should thus be examined in more detail to confirm or refute this assertion.

Although there is a positive association with obesity and pain threshold, the mechanisms underpinning this link are not fully understood (McCarthy et al., 2009; Eslick, 2012; Stone and Broderick, 2012). According to the prior studies in this field, asprosin level increases in obesity, type 2 diabetes, and metabolic syndrome. Several authors are of view that asprosin level increases in parallel with the increase in adipose tissue in obese and diabetic individuals and animals (Romere et al., 2016; Ugur and Aydin, 2019; Wang et al., 2019; Zhang et al., 2020). However, Long et al. (2019) observed significantly lower asprosin levels in obese children. At the same time, studies demonstrated that there is no association between plasma asprosin concentration and body mass in polycystic ovary syndrome (PCOS) and elderly women with metabolic disorders (Chang et al., 2019; Long et al., 2019; Wiecek

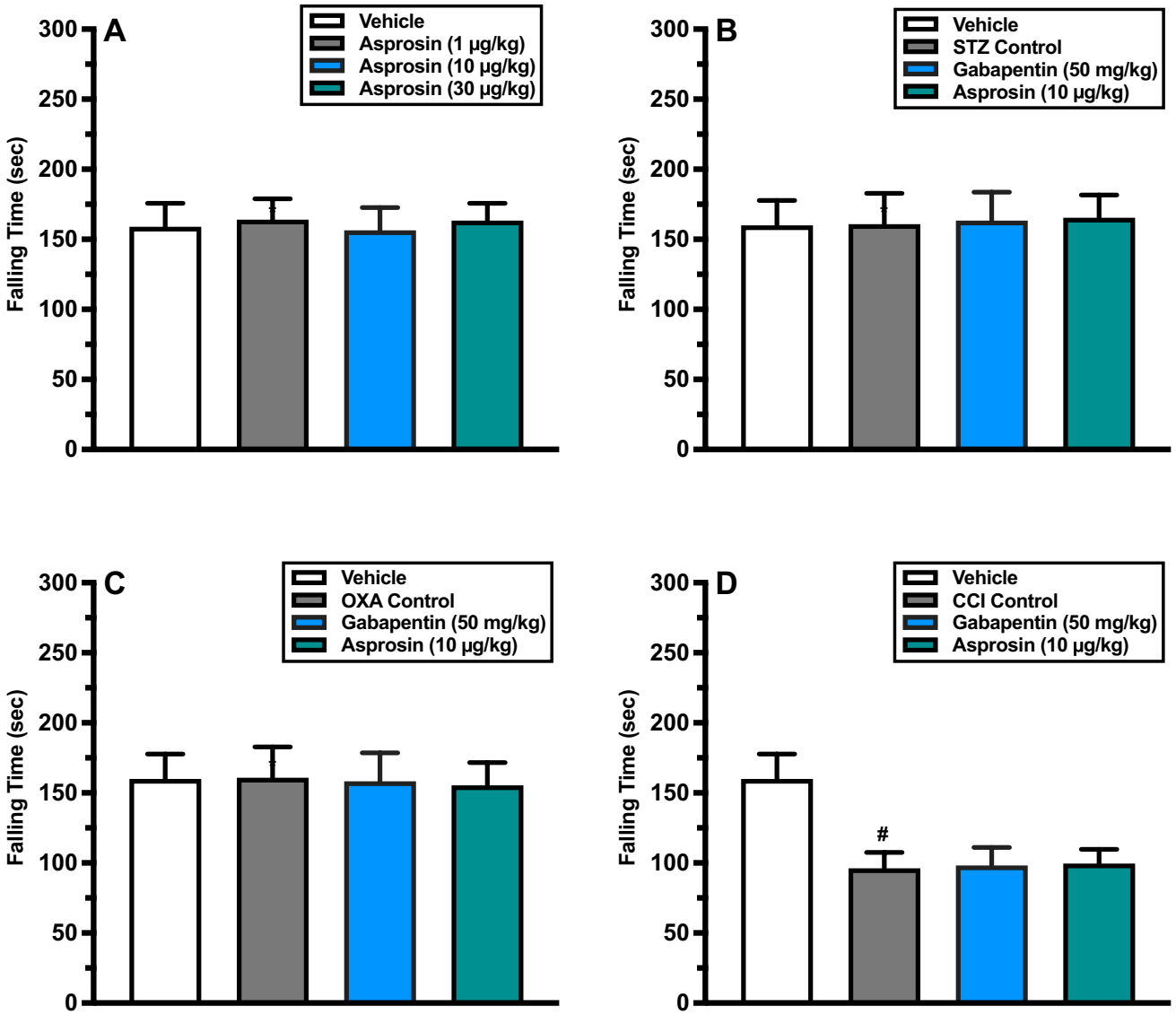

Fig. 6 Mean ± SD values of the response to falling from a spinning rod in mice undergoing the rotarod test. Comparisons were made using the Tukey HSD test #$p < 0.05$. **A** Evaluation of motor activities of mice after administration of asprosin at doses of 1, 10, 30 µg/kg in healthy animals. **B** Evaluation of motor activities of diabetic mice after administration of asprosin (10 ug/kg) and gabapentin (50 mg/kg). **C** Evaluation of motor activities of mice with neuropathy after oxaliplatin administration as a result of administration of asprosin (10 µg/kg) and gabapentin (50 mg/kg). **D** Evaluation of the motor activities of mice with sciatic nerve chronic construction injury with asprosin (10 µg/kg) and gabapentin (50 mg/kg)

et al., 2019). Another study reported by our study group indicates that serum asprosin levels in obese female rats are similar with healthy female rats (Ozcan et al., 2020). The excess adipose tissue in obesity has been linked to the risk of developing insulin resistance and metabolic syndrome. In individuals with type 1 diabetes that develop hypoglycemia, lower asprosin response is associated with insulin resistance. The patients with type 1 diabetes may have a blunted response to asprosin (Groener et al., 2019). The degree of diabetes control may also be an important factor influencing asprosin levels (Chen et al., 2005; Paisley and Serpell, 2016; Callaghan et al., 2020). At the same time, the duration and severity of diabetes may play an important role in pain formation, as this condition can cause hyperalgesia and allodynia, as well as loss of sensation (hypoalgesia) (Chen et al., 2005; Paisley and Serpell, 2016). In the current study, the decrease in asprosin levels in the neuropathic pain–induced groups may be a helpful aid for understanding painful neuropathy. Further research is needed to elucidate the exact mechanisms of asprosin analgesic action.

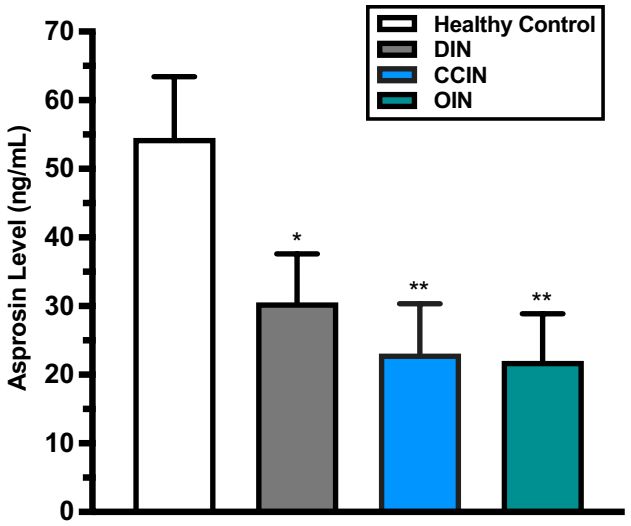

Fig. 7 Asprosin levels in different models of neuropathic pain with animals ($n=7$ for healthy control and $n=10$ neuropathic pain with mice for each group). Comparisons between groups were made using the Tukey test. When animals with neuropathic pain were compared with healthy animals, $*p<0.01$, $**p<0.001$; DIN diabetes-induced neuropathy, CCIN chronic constriction–induced neuropathy, OIN oxaliplatin-induced neuropathy

Conclusion

In summary, these results clearly demonstrate, for the first time, that asprosin exerts an antinociceptive effect on neuropathic pain of different etiologies in mice. We also provide critical preliminary evidence suggesting that asprosin treatment and serum asprosin levels may be a novel therapeutic candidate for painful neuropathy.

Author contribution S.O., M.O.: designed the study, contributed to the analysis of data and preparation of manuscript. S.C., O.B.: designed the study and contributed to experimental study. M.M.K., M.G.H., F.B., B.B.: the experiments perform. S.O., M.O.: manuscript preparation and critical revision. All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The data presented in this study are available on request from the corresponding author.

Code availability Not applicable.

Declarations

Ethics/animal welfare approval All experiments adhered fully to the guidelines of the ARRIVE (Animal in Research: In Vivo Experiments) as well as the principles of the International Association for the Study of Pain (IASP) Research and Ethics Committee. This study was carried out with the approval of Firat University Animal Experiments Local Ethics Committee (dated 16.01.2019, protocol number 2019/06 with the decision number 2019/09).

Consent to participate Not applicable.

Consent for publication.
Not applicable.

Conflict of interest The authors declare no competing interests.

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