Alleviation of capsaicin-induced mechanical allodynia by *Arbutus andrachne* L. methanolic leaf extract in male rats

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Abstract

Pain is one of the major health problems that impose huge social and economic burdens all over the world. Mechanical allodynia is a symptom of pain defined as a painful sensation resulting from innocuous stimuli such as touch. Currently available drugs have several side effects urging the need for new natural sources that alleviate mechanical allodynia. This study investigated the effect of *Arbutus andrachne* L. (a medicinal plant growing in Jordan) on capsaicin (Caps)-induced mechanical allodynia in male rats and the mechanism that underlie its effect. Caps injection decreased paw withdrawal threshold (PWT) significantly compared to control group. Mechanical allodynia was detected 30, 90 and 150 min after Caps injection to the plantar surface of the left hind paw of rats. The intraplantar (ipl) injection of 100 µg (but not 50 or 200 µg) of *A. andrachne* extract, ipsilaterally, prior to Caps injection increased PWT at all-time points similar to the anti-nociceptive effect in the positive group that was treated with 2.5% diclofenac sodium prior to Caps injection. The ipsilateral pre-treatment with the antagonists of transient receptor potential vanilloid-1 (TRPV1), cannabinoid receptor 1 (CB1) and peroxisome proliferator-activated receptor alpha (PPARα) reversed the activity of the leaf extract in contrast to the antagonists of PPARγ and α2-adrenergic receptors. In all groups, no effect was detected on the contralateral hind paw of animals at any time point. In conclusion, *A. andrachne* can be exploited as an anti-nociceptive agent.

Keywords: *Arbutus andrachne,* TRPV1, mechanical allodynia, PPAR, CB1, α2-adrenergic

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تخفيف تأثير الألم الميكانيكي المحفز عن طريق كابسيسين بواسطة المستخلص الميثانولي لأوراق نبات القيقب في ذكور الجرذان

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ملخص

يعتبر الألم أحد المشاكل الصحية الرئيسية التي تفرض أعباء اجتماعية واقتصادية ضخمة في جميع أنحاء العالم. الألم الميكانيكي هوعرض من أعراض الألم والذي يعرف بالألم الناتج من محفزات غير ضارة مثل اللمس. هناك العديد من الآثار الجانبية للأدوية المتوفرة حاليًا مما يزيد الحاجة لمصادر طبيعية جديدة تخفف من الألم الميكانيكي.

بحثت هذه الدراسة تأثير نبات القيقب (نبات طبي ينمو في الأردن) على الألم الميكانيكي المحفز بواسطة كابسيسين في ذكور الجرذان وآلية عمله. إن حقن كابسيسين قلل حد الوزن المسبب لرفع القدم بشكل ملحوظ مقارنة مع المجموعة المرجعية. تم الكشف عن الألم الميكانيكي بعد 30 و 90 و 150 دقيقة من حقن كابسيسين على السطح الأخمصي للقدم الخلفية اليسرى. إن حقن 100 ميكروغرام من المستخلص الميثانولي لأوراق نبات القيقب (و ليس ٥٠ أو 200 ميكروغرام) قبل حقن كابسيسين في نفس القدم زاد حد الوزن المسبب لرفع القدم في جميع النقاط الزمنية المقاسة. كان تخفيف الألم الميكانيكي مشابها لتأثير المسكن الذي ظهر في المجموعة ذات التأثير الإيجابي والتي عولجت بنسبة 2.5٪ من ديكلوفيناك الصوديوم قبل حقن كابسيسين. عكست المعالجة المسبقة بمضادات المستقبلات CB1, TRPV1 و PPARα على نفس القدم تأثير مستخلص الأوراق على الألم الميكانيكي المحفز بواسطة كابسيسين بشكل ملحوظ على النقيض من مضادات المستقبلات PPARγ وadrenergic−α2. لم يتم الكشف عن أي تأثير على القدم اليمنى وغير المحقونة في أي نقطة زمنية. الإستنتاج ، مستخلص أوراق نبات القيقب يمكن استغلاله كعامل مضاد للألم.

الكلمات الدالة: Arbutus andrachne, TRPV1, آلام ميكانيكية, α2- , PPAR, CB1, adrenergic,

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1. Introduction:

Pain is one of the major health problems that lead to disability, mortality and the need for health care services (Brooks and Tracey, 2005). Transient receptor potential vanilloid-1 (TRPV1) is a key receptor involved in the detection of different painful stimuli including mechanical allodynia (Caterina et al., 1997; Gilchrist et al., 1996). TRPV1 is a non-selective cation channel that is activated by noxious heat (> 43°C), anadamide, protons as well as endogenous activators 'endovanilloids' (Caterina et al., 1997; Brandt et al., 2012). It is also activated by the potent and highly selective agonist capsaicin (Caps), the active ingredient in chilli peppers (Caterina et al., 1997). According to Caterina and Julius (2001), the deletion of TRPV1 gene in mice attenuated paw swelling in Caps-treated animals. Thus, TRPV1 is considered a promising target for pain control (Jara-Oseguera et al., 2008).

Previous studies confirmed an interaction between TRPV1 channel and other receptors in pain modulation. These receptors include cannabinoid receptor 1 (CB1) (O'Sullivan, 2016), alpha 2 (α2)-adrenergic receptor (Filippi et al., 2016) and peroxisome proliferator-activated receptors α and gamma (PPAR α and γ) (Ambrosino et al., 2014, O'Sullivan, 2016). Noteworthy, CB1 receptor is expressed in peripheral sensory neurons and epidermal layers in human skin (Caterina, 2014). CB receptors play a crucial role in itch and pain responses through several mechanisms (Caterina, 2014). Also, α2-adrenergic receptor is among the receptors that contribute to TRPV1 functionality (Dessaint et al., 2004; Matsushita et al., 2018). It was documented that the application of yohimbine (an antagonist for α2-adrenergic receptor) inhibited the firing of dorsal root ganglia (DRG) neurons, induced by Caps (Dessaint et al., 2004; Matsushita et al., 2018). With respect to PPAR receptors, Alsalem et al. (2016) reported that TRPV1 channel can be modulated by a dual agonist for PPARγ and PPARα receptors.

Notably, natural compounds from various sources can modulate TRPV1 channel. In this regard, (Abbas, 2020) reviewed 137 natural products that

affect TRPV1 activity in different assays highlighting that natural sources, including medicinal plants, can be good sources for analgesics. According to (Oran, 2014), there are 363 medicinal plants in Jordan including *Arbutus andrachne* L. (Qaiqab or Qatlab in Arabic) which is a medicinal plant growing in Ajloun, Irbid, Amman, Jarash and Salt (Al-Eisawi, 1998; Oran, 2015). It belongs to Ericaceae family (Oran, 2015) and Arbutoideae sub-family (Tenuta et al., 2018). It is a laurel-like genus described as long evergreen shrub that reaches 2-4 m (Tenuta et al., 2018; Al-Eisawi, 1998; Oran, 2015). This plant is edible and has many uses in traditional medicine (Oran, 2015). Several studies were conducted to determine the biological effects of this medicinal plant. However, there is lack in the studies that evaluate the anti-nociceptive effect of *A. andrachne* in pain models induced by mechanical stimuli. The aim of this study was to determine the effects of different doses of *A. andrachne* leaf extract on Caps-induced mechanical allodynia in rats. Also, to explore the roles of TRPV1, CB1, PPARα, PPARγ, and α2-adrenergic receptors in mediating the effect of *A. andrachne.*

2. Materials and methods:

2.1. Drugs

GW9662, GW6471, yohimbine, capsazepine, Caps and SR141716A were from Tocris Bioscience, UK. Diclofenac sodium was purchased from Novartis, Switzerland. Olive oil was brought from the Department of Agriculture, the University of Jordan, Amman, Jordan. Ethanol and methanol were purchased from Scharlau, Spain. Syringes (1 ml) were from Becton, Dickinson and Company, United States. Caps was dissolved in olive oil while other drugs were freshly prepared from stock in sterile saline.

2.2. Collection and identification of *A. andrachne* leaves

The leaves of *A. andrachne* were collected in March 2019 from Jarash in Jordan and were authentically identified by Prof. Sawsan Oran, a plant taxonomist at the Department of Biological Sciences, the University of Jordan, Amman, Jordan. A voucher specimen was placed at the herbarium in the same department.

2.3. Preparing the methanolic extract from *A. andrachne* leaves

The leaves of *A. andrachne* were washed, dried, ground with a blender, then were soaked in methanol (100 g in 1000 ml) at 10:1 v/w ratio for 72 hrs at room temperature with continuous shaking (Pandey and Tripathi, 2014) followed by filtration using Whatman filter paper and evaporation of methanol by rotary evaporator at 45 °C. Successive extractions were used to prepare the methanolic extract. The extract was kept in a container at -20 °C.

2.4. Experimental animals

The procedures in all experiments were approved by the ethical committee at the University of Jordan (ethical approval number 235/2020/19). Male Wistar rats (200-250 g) were used in the experiments. The animals were kept in the animal house, the University of Jordan in controlled conditions and had free access to food and water.

2.5. Measurement of Caps-induced mechanical allodynia

Each group included 6 animals. The rats were allowed to acclimatize for 1 hr in inverted cages covered with perforated lids placed on an elevated wire mesh surface. Baseline withdrawal thresholds were determined to ensure that the animals have equivalent baseline readings before being divided into different groups. The animals were assigned into control and experimental groups. Mechanical allodynia was induced by the intraplantar (ipl) administration of 30 µg Caps (dissolved in olive oil) into the left hind paw of animals. Control group received ipl injection of olive oil. Other groups received different doses of *A. andrachne* extract (50, 100 or 200 µg extract/paw) 30 min prior to Caps injection. Positive control group received 2.5% diclofenac sodium 30 min prior to Caps injection (according to Nozadze et al., 2016). The dose of the extract that reversed Caps-induced mechanical allodynia (100 µg) was selected to determine the effect of pre-treatment with different antagonists on the anti-allodynic activity of the leaf extract. Different antagonists were administered ipsilaterally to the dorsal side of the hind paw, 30 min prior to extract injection. The dorsal side of the hind paw was chosen for the injection of antagonists to allow local administration of drugs while minimizing the effects of multiple injections to the plantar surface of the hind paw (Ibrahim et al., 2005). The following doses were used for the antagonists: 50 µg GW9662, a PPARγ antagonist

(Alsalem et al., 2016), 1 µg capsazepine, a TRPV1 antagonist (Alsalem et al., 2016), 0.01 µg SR141716A, a CB1 antagonist (Sagar et al., 2004), 50 µg GW6471, a PPARα antagonist (Alsalem et al., 2016) and 100 µg yohimbine, an α2-adrenergic receptor antagonist (Lee et al., 2013). The volume injected to the paws was 50 µl/injection in all groups.

Paw withdrawal thresholds (PWT) of the ipsilateral and contralateral hind paws were determined 30, 90 and 150 min after the injection of Caps or its vehicle. Mechanical allodynia was measured using von Frey anesthesiometer (Gilchrist et al., 1996). Briefly, thin filament was used to press against the plantar surface of the hind paw of animal till the animal lifts its paw. The number of grams (gm) that caused paw withdrawal was recorded. A cut-off mass unit of 80 g was used in this experiment as in Ferrier et al. (2016).

2.6. Statistical analysis

Normality test for all groups was performed using Shapiro–Wilk test**.** Kruskal-Wallis non-parametric test was used to examine the statistical differences between groups followed by Dunn's post hoc test. The tests were performed using GraphPad Prism version 6. $p < 0.05$ was considered significant. Data were presented as Means±standard error of means (SEM).

3. Results:

The ipl injection of 30 µg Caps to the left hind paw was used to induce mechanical allodynia in male Wistar rats. Caps injection decreased PWT in the left hind paw significantly compared to control group after 30, 90 and 150 min of injection (figures 1A, 1B and 1C, respectively). No effect was detected on the contralateral hind paw at any time point (figures 2A, 2B and 2C). Pre-injecting the animals with 100 µg *A. andrachne* methanolic leaf extract alleviated Caps-induced mechanical allodynia at all measured time points similar to the effect that was observed in the positive control group treated with 2.5% diclofenac sodium/Caps (figures 1A, 1B and 1C). Two doses of *A. andrachne* (50 and 200 µg) did not show significant effect in reversing or decreasing the mechanical allodynia that was evoked by Caps (figures 1A, 1B and 1C).

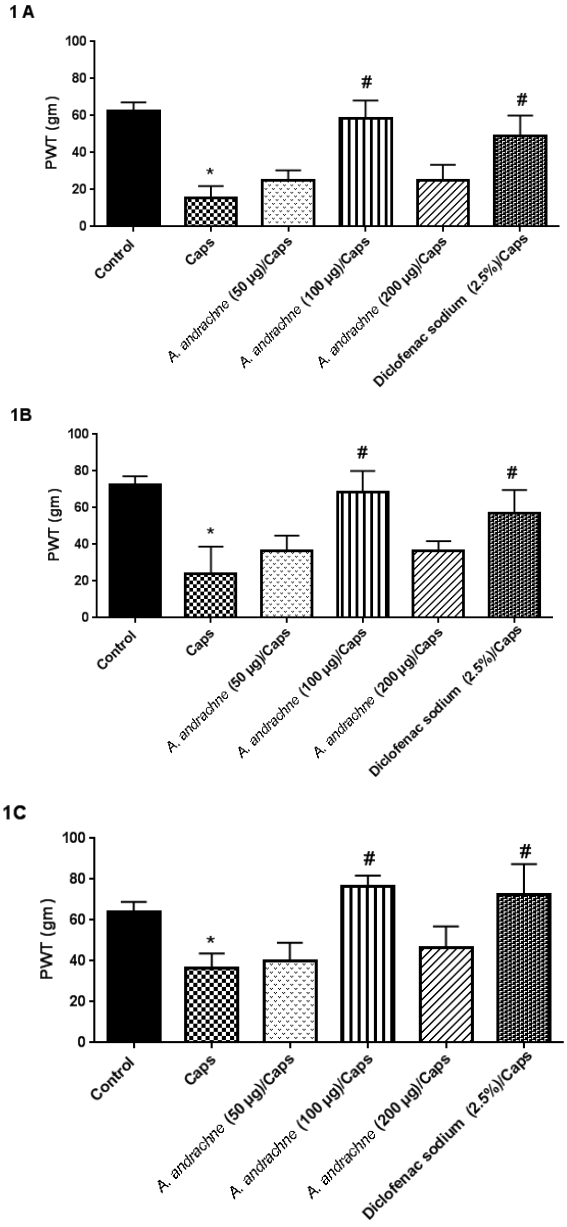


Figure 1: PWT of the injected left hind paw measured 30 min (1A), 90 min (1B) and 150 min (1C) after the ipl injection of vehicle (control), Caps, *A. andrachne*/Caps or diclofenac sodium/Caps.

* Significant compared to control group, # Significant compared to Caps-treated group ($p<0.05$).

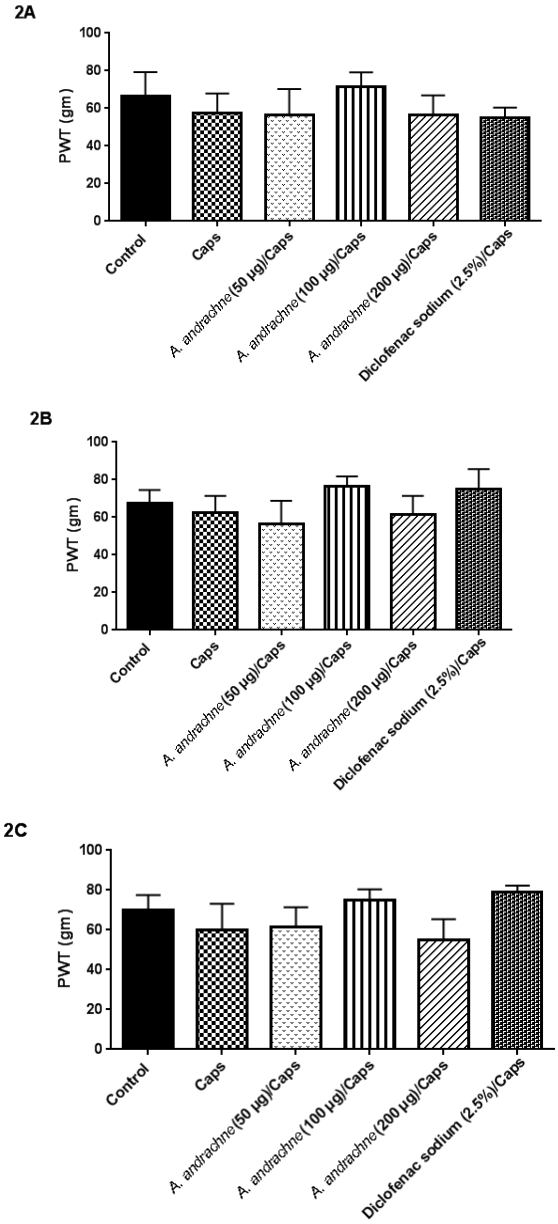
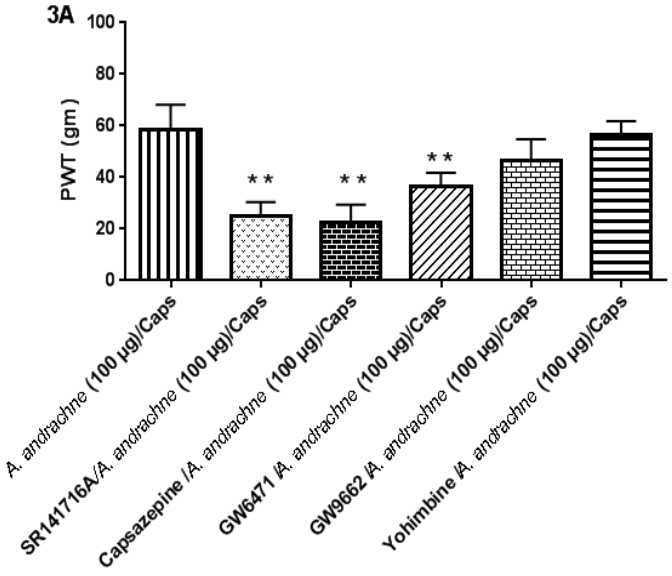


Figure 2: PWT of the right hind paw (contralateral to the site of injections) measured 30 min (2A), 90 min (2B) and 150 min (2C) after the ipl injection of vehicle (control), Caps, *A. andrachne*/Caps or diclofenac sodium/Caps.

Figures 3A, 3B and 3C present PWT measured at 30, 90 and 150 min, respectively (after Caps injection) for animals treated with different antagonists prior to *A. andrachne*/Caps. The anti-nociceptive effect of *A. andrachne* in this mechanical allodynia model decreased when the animals were injected with 0.01 µg SR141716A, 1 µg capsazepine or 50 µg GW6471 prior to *A. andrachne/*Caps at all-time points (after 30, 90 and 150 min of Caps injection). Generally, the animals that had low PWT displayed guarding behaviour for their paw. No effect was detected in the groups that were pre-treated with 50 µg GW9662 or 100 µg yohimbine. Also, no effect was found on the contralateral hind paw in any group at any time point (figures 4A, 4B and 4C).



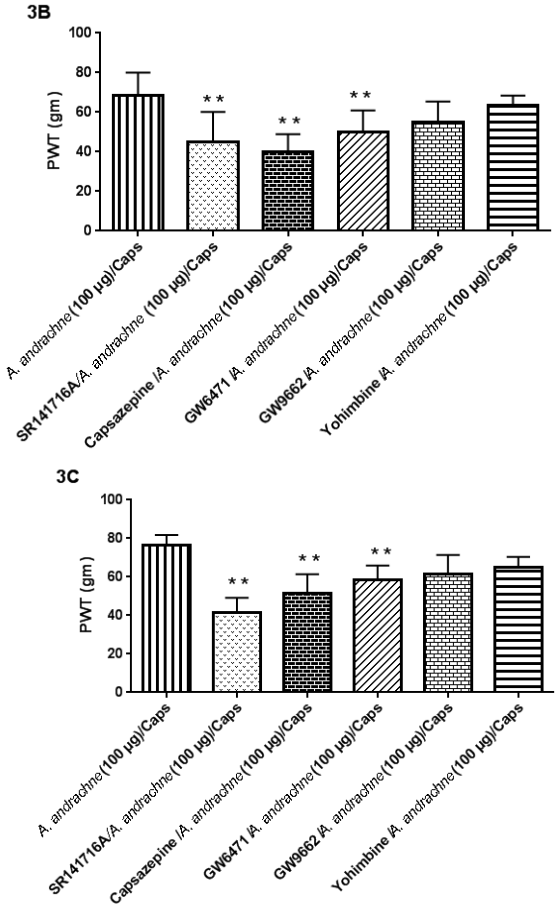


Figure 3: PWT of the injected left hind paw measured 30 min (3A), 90 min (3B) and 150 min (3C) after the ipl injection of *A. andrachne* (100 µg)/Caps or different antagonists prior to *A. andrachne/*Caps injection.

** Significant compared to *A. andrachne* (100 µg)/Caps ($p<0.05$)

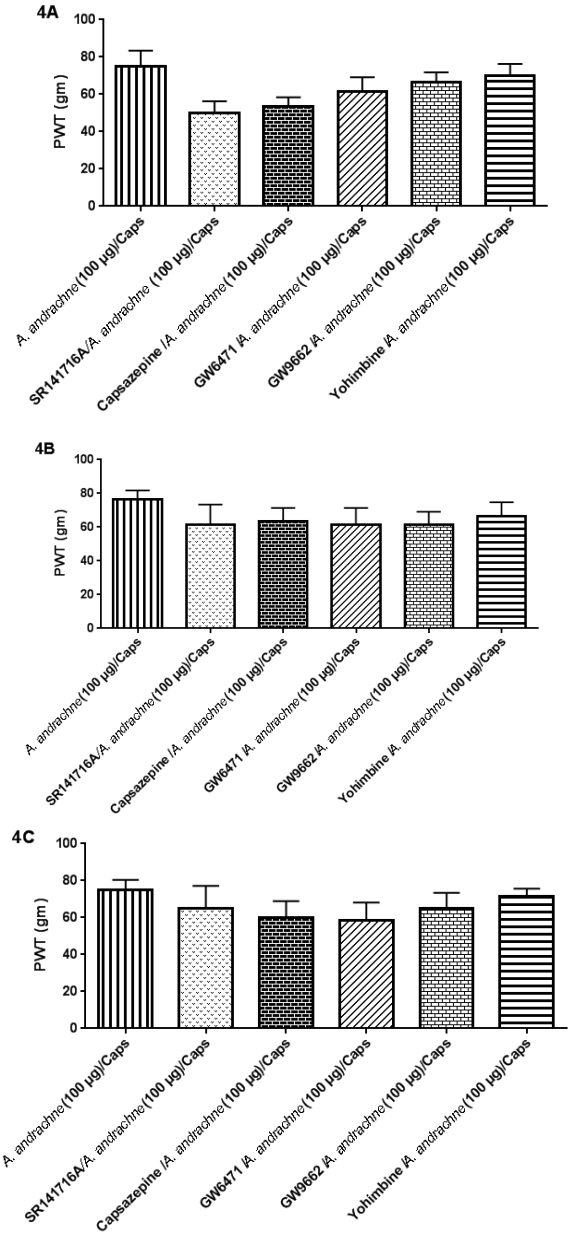


Figure 4: PWT of the right hind paw (contralateral to the site of injections) measured 30 min (4A), 90 min (4B) and 150 min (4C) after the ipl injection of *A. andrachne* (100 µg)/Caps or different antagonists prior to *A. andrachne*/Caps injection.

4. Discussion:

The present study sheds the light on the effect of *A. andrachne* methanolic leaf extract on Caps-induced mechanical allodynia in rats and highlights the involvement of key receptors in its effect.

A. andrachne methanolic extract (100 µg per paw) increased PWT in animals that received Caps injection. The lower dose of the leaf extract (50 µg) didn't inhibit Caps-induced mechanical allodynia and this can be attributed to the faster inactivation or clearance of this dose (Andersen, 1981). Other scenario for explaining this finding can be that the lower dose of *A. andrachne* was not sufficient to activate receptors that are likely correlated with this response. On the other hand, the lack of effect in the higher dose of *A. andrachne* leaf extract (200 µg) can be attributed to an *in vivo* pharmacokinetic mechanism of drug clearance and excretion to avoid toxicity (Andersen, 1981). Other explanation is that the high dose of the leaf extract caused off-target effects that antagonized the anti-allodynic effect of the extract. No influence was detected on the contralateral hind paw confirming the local effect of *A. andrachne*. This is the first study that examined the effect of *A. andrachne* on the alleviation of Caps-evoked mechanical allodynia. Moreover, the ipsilateral pre-treatment with SR141716A, GW6471 or capsazepine reversed the anti-allodynic effect of *A. andrachne* reflecting that CB1, PPARα and TRPV1 receptors contribute to the effect of the leaf extract in this model. In contrast, no effect was found for the antagonists of PPARγ or α2-adrenergic receptors on the effect of *A. andrachne* in response to mechanical stimulus in this model.

Importantly, the activity of *A. andrachne* was similar to the effect of the non-steroidal anti-inflammatory drug (NSAID), diclofenac sodium. In fact, the action of the leaf extract on Caps-induced mechanical allodynia implies the pathway through which the anti-nociceptive effect was mediated. In more details, the neurons that transmit the signals from mechanoreceptors synapse at the superficial lamina of the spinal cord, while the signals from thermoreceptors synapse at the superficial and deep laminae of the spinal cord (Benarroch, 2016; Greenspon, et al., 2019). Of note, *A. andrachne* methanolic leaf extract exhibited anti-nociceptive effect in thermal and chemical models of pain (Jaffal et al., 2020). Furthermore, the extract had strong anti-inflammatory and antipyretic potential in carrageenan-induced paw edema model and yeast-evoked pyrexia models, respectively (Jaffal et al., 2021).

Based on the findings of this study and earlier reports, there is possibility that the injection of the leaf extract evoke the release of endogenous

cannabinoids that inhibit, at certain dose, neuropeptides release in the skin (Engel et al., 2011). In more details, previous studies showed that the stimulation of calcitonin gene-related peptide (CGRP) release by anandamide and tetrahydrocannabinol was antagonized by TRPV1 knockout and by using the antagonists of TRPV1 (BCTC) and CB1 implying the crosstalk between these two receptors (Engel et al., 2011; Ahluwalia et al., 2003). Additionally, anti-allodynic effects of the synthetic cannabinoids (WIN55212 and HU210) and tesaglitazar (a dual PPAR agonist) were reported in streptozotocin (STZ)-induced diabetic neuropathic pain model (Alsalem et al. 2019; 2020). Furthermore, Caterina (2014) reviewed the importance of CB and TRPV1 receptors in cutaneous sensation. Additionally, many reports revealed that TRP channels (including TRPV1) can be inhibited or activated by phytocannabinoids, synthetic cannabinoids and/or endocannabinoids (Caterina, 2014). In fact, the effects of cannabinoids on skin were attributed to the modulation of TRPV1 directly and indirectly (Caterina, 2014). At high doses, cannabinoids directly induce TRPV1 activation while at low doses; they suppress TRPV1 channel indirectly by CB1 receptors (Litwack, 2013; Caterina, 2014). On the other hand, the results of this study demonstrated that inhibiting CB1, TRPV1, PPARα receptors reversed the anti-allodynic effect of *A. andrachne* while the previous results revealed that the anti-nociceptive effect of *A. andrachne* in thermal models was inhibited by the antagonists of CB1, TRPV1 and PPARγ receptors (Jaffal et al., 2020). Accordingly, the modulation of TRPV1 channel by PPAR receptors *in vivo* depends on the type of stimulus and the used model of pain.

More importantly, our previous analysis of *A. andrachne* methanolic leaf extract indicates that the extract is rich in 17 active constituents (Jaffal et al., 2020). Many of these compounds exhibit anti-allodynic effects. For instance, previous studies demonstrated that the ipl injection of linalool attenuated mechanical allodynia in a mouse model of neuropathic pain (partial sciatic nerve ligation, PSNL) in a dose dependent manner (Kuwahata, et al., 2013). Also, the single and repetitive intraperitoneal (i.p) injection of 100 mg/kg body wt quercetin produced an anti-mechanical hypersensitivity in cancer-induced bone pain model in Sprague-Dawley rats (Liu, et al., 2018). Similar effect in attenuating mechanical allodynia was depicted after the i.p and intrathecal (i.t) injection of isoquercetin in diabetic rats (Resham, et al., 2020). Moreover, Azevedo, et al. (2013) reported that all doses of rutin and quercetin inhibited mechanical allodynia in a mouse model of oxaliplatin-induced neuropathy accompanied with a decrease in nitric oxide (NO) synthase immunostaining in the spinal cord (Azevedo, et

al., 2013). Moreover, kaempferol extracted from *Eruca sativa* produced an anti-allodyniac effect in STZ-induced diabetic neuropathy (Kishore, et al., 2018). The amelioration of mechanical alldoynia in different animal models was also mediated by linoleic acid (Shi, et al., 2019), gallic acid (Trevisan, et al., 2014), α-tocopherol (Tiwari, et al., 2009), ursolic acid (Bhat, et al., 2016), myricetin (Meotti , et al., 2006) and epigallocatechin-3-gallate (An, et al., 2014). These findings justify the results of this study and suggest that *A. andrachne* extract is a potential source of anti-allodynic compounds.

In conclusion, *A. andrachne* exhibited anti-nociceptive properties in Caps-induced mechanical allodynia in a mechanism that involves PPARα, CB1 and TRPV1. The use of this plant can open a gate towards finding promising therapeutics for mechanical allodynia which is a key symptom of many chronic pain conditions.

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Conflict of interest:

The authors confirm that there is no conflict of interest.

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