Medicinal Chemistry Research (2019) 28:992–999
https://doi.org/10.1007/s00044-019-02353-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Evidence for the involvement of opioid receptor in *Ajuga chamaepitys* action in chemical and thermal models of pain in BALB/c mice

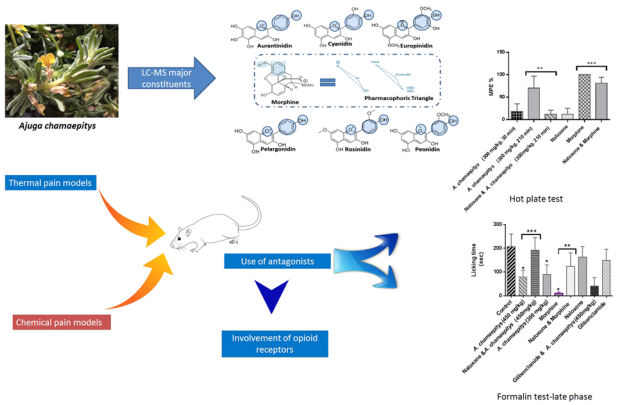
Sahar M. Jaffal[1] · Manal A. Abbas[2] · Mohammad Alsalem[3] · Belal O. Al-Najjar[2]

Received: 20 February 2019 / Accepted: 29 April 2019 / Published online: 6 May 2019

Abstract

Ajuga chamaepitys is a small herbaceous annual or biannual plant that belongs to Lamiaceae family. It grows in Europe and Eastern parts of the Mediterranean. One of the ethno-pharmacological uses of this plant is its use as a painkiller. In the present experimental work, the antinociceptive effect of the methanolic extract of *A. chamaepitys* collected from Jordan was investigated using chemical and thermal models of pain in mice. *A. chamaepitys* extract decreased significantly the number of writhes that were induced in mice by the injection of 1% acetic acid compared to negative control group. The inhibitory effect produced by 300 mg/kg of the extract given i.p was comparable to that of 300 mg/kg aspirin. The i.p administration of 450 mg/kg *A. chamaepitys* caused a remarkable decrease in paw licking time during the early and late phases of formalin test. Furthermore, the latency time increased in hot plate test but not in tail flick test in animals that were treated i.p with 300 mg/kg *A. chamaepitys* extract compared to control animals. The involvement of opioid receptor was proven in formalin and hot-plate tests by abolishing the effect of *A. chamaepitys* extract by pretreatment with naloxone, an opioid antagonist. LC-MS analysis resulted in the identification of 19 compounds. Isovitexin, orientin, flavonol, and cyanidin were the major compounds. Our results suggest that the methanolic extract of *A. chamaepitys* has pronounced antinociceptive effects, which provide the scientific basis of the traditional therapeutic use of *A. chamaepitys* in folk medicine.

Graphical Abstract



✉ Manal A. Abbas
m.abbas@ammanu.edu.jo

[1] Department of Biological Sciences, Faculty of Science, The University of Jordan, Amman 11942, Jordan

[2] Faculty of Pharmacy, Al-Ahliyya Amman University, Amman 19328, Jordan

[3] Department of Anatomy and Histology, Faculty of Medicine, The University of Jordan, Amman 11942, Jordan

Keywords *Ajuga chamaepitys* · Antinociceptive · Opioid · Writhing test · Formalin test

Introduction

Despite the progress in understanding the pathophysiology of pain, the demand for new analgesics for clinical use is still urgently needed. The alkaloid morphine extracted from the plant *Papaver somniferum* is still one of the principal analgesic medicines used clinically despite its well-known side-effects (Vij et al. 2014). The side effects of opioids such as constipation, dysphoria, respiratory depression (Lozama 2010) and associated risks like overdose and addiction necessitate finding suitable alternatives, especially when dealing with chronic pain (Volkow and McLellan 2016). This has renewed the interest in finding new alternatives from synthetic and natural sources such as plant-derived secondary metabolites. In a large survey conducted by Almeida et al. (2001), 210 medicinal plants, belonging to 79 families having analgesic activity were listed. Examples of such plants include *Salix species, Capsicum species*, and *Cannabis sativa* (Khalid et al. 2017). In fact, less than 10% of the biodiversity on earth has been tested for potential pharmacological activity. This provides an opportunity for discovering new natural lead compounds (Dias et al. 2012).

Ajuga chamaepitys is a small herbaceous grayish-green annual or biannual plant that belongs to Lamiaceae family (Venditti et al. 2016). It grows throughout Europe, Anatolia, and Eastern parts of the Mediterranean (Caliskan et al. 2017; Öztürk et al. 2016; Venditti et al. 2016). *A. chamaepitys* is commonly known as yellow bugle or ground pine because it gives off a characteristic smell of pine when crushed (Venditti et al. 2016).

In folk medicine, *A. chamaepitys* is used to regulate menstrual disorders, as astringent, antitussive, for treatment of hemorrhoids, diabetes, stomachache, inflammatory diseases such as gout and joint pain and as a painkiller (Caliskan et al. 2017; Fakir et al. 2016; Öztürk et al. 2016; Venditti et al. 2016). Many plants belonging to Lamiaceae family were reported to have antinociceptive activity such as *Mentha* species (Mogosan et al. 2017), *Salvia divinorum* (Simón-Arceoa et al. 2017), *Salvia lachnostachys* (Santos et al. 2017) and *Hyptis suaveolens* (Begum et al. 2016). Up to our best knowledge, no previous study has investigated this activity in *A. chamaepitys*. Therefore, this study was designed to investigate the antinociceptive action using chemical (acetic acid writhing and formalin tests) and thermal (hot-plate and tail flick) pain models in BALB/c mice. Furthermore, the role of opioid receptors in mediating the analgesic effect was investigated.

Materials and methods

Drugs

Naloxone was purchased from Tocris Bioscience (United Kingdom). Aspirin (acetylsalicylic acid) was from Bayer (Germany), glibenclamide (Glibil) from Hikma Pharmaceuticals (Jordan), MST continus morphine sulfate tablets were from Napp pharmaceuticals limited (Cambridge). All used drugs were freshly prepared before use by dissolving them in sterile normal saline.

Plant collection and identification

A. chamaepitys was collected from Irbid region, Jordan during April 2017. The collected plant was identified by Professor Barakat Abu-Irmaileh (The University of Jordan, Faculty of Agriculture) based on the morphology of fresh specimens. A voucher specimen was deposited at Al-Ahliyya Amman University-Laboratory of Graduate Studies (Voucher specimen Lam# 4-2017).

Methanolic extract of *A. chamaepitys* aerial parts was prepared by maceration of coarsely grinded dried flowering shoots in methanol 96% (Scharlau, Spain). Methanol was evaporated under reduced pressure using a rotary evaporator at temperature not exceeding 45 °C and kept at −20 °C until used. Methanolic extract was administered to animals after dissolving it in sterile normal saline.

Experimental animals

All procedures were approved by the ethical committee for the use of experimental animals at the University of Jordan. Female BALB/c mice were obtained from the animal house at Al-Ahliyya Amman University, Amman, Jordan. Animals were maintained at 23 ± 2 °C with 12 h light/dark cycle. Water and food pellets were available *ad libitum*. Mice were adapted to the laboratory for at least 2 h before tests. Different animals were used for each experiment.

Pretreatment with antagonists

In the following experiments, mice were treated i.p with either vehicle, glibenclamide (10 mg/kg) or naloxone (5 mg/kg) 30 min before the administration of vehicle, morphine sulfate (positive control) or *A. chamaepitys* extract (300 or 450 mg/kg). Subsequently, the response

was examined 30 min after the administration of vehicle or extract.

Acetic acid-induced writhing test

Writhing test was performed according to the method of Koster et al. (1959). Mice were divided randomly into 4 groups (8 animals each). Acetic acid solution (1%, 10 ml/kg) was i.p. administered 30 min after vehicle, *A. chamaepitys* extract (300 or 450 mg/kg) or acetylsalicylic acid (aspirin) administration. Animal behavior was video recorded and the number of writhes was counted 10 min after acetic acid injection for twenty min. A writhe was defined as a contraction of the abdominal muscles with extending the forelimbs and elongating the body.

Formalin test in mice

The formalin test was carried out as in Hunskaar et al. (1985). Mice were divided randomly into 4 groups (8 animals each) that received vehicle, *A. chamaepitys* extract (300 mg/kg or 450 mg/kg) or morphine. Thirty minutes later, formalin (2.5%, 20 µl) was injected intraplantarly to the right hind paw of the animal. Animal behavior was video recorded. The total time spent in licking and the flinching response was measured in 2 phases: early phase (0–5 min) and late phase (25–30 min) after formalin injection.

Hot-plate test

The hot-plate test was used to measure pain reaction latencies as in Woolfe and McDonald (1946). Mice were divided randomly into different groups (13 animals each) that received vehicle, *A. chamaepitys* extract (300 mg/kg) or morphine with or without antagonist. Thirty minutes later, each mouse was placed into a glass beaker on a hot-plate maintained at 55 ± 1 °C and the test was performed only once for each mouse. The time between the animal's placement and first jump was recorded as an index of pain reaction latency. A cutoff time of 60 sec was used to prevent tissue damage of the animal (Bannon and Malmberg 2007). Antinociceptive response was calculated as percentage of maximum possible effect (%MPE) as in (Alsharari et al. 2015), where %MPE = [(test value − control value)/(cutoff (60 s) − control value)] × 100.

Tail flick test

The tail flick test was assessed by immersing the tail in water bath at 55 ± 1 °C. Mice were divided randomly into different groups (13 animals each) that received vehicle, *A. chamaepitys* extract (300 mg/kg) or morphine with or without antagonist. After 30 min, the time from immersing the tail till producing the first flick was recorded. Cutoff time was considered 15 s. Antinociceptive response was calculated as percentage of MPE (%), where %MPE = [(test value − control value)/(cutoff (15 s) − control value)] × 100.

Liquid chromatography-mass spectrometry (LC-MS) analysis

Liquid chromatography-mass spectrometrometric separation was performed using Agilent Zorbax Eclipse XDB-C18 (2.1 × 150 mm × 3.5 µm) column, oven temperature 25 °C with the mobile phase containing solvent A and B in gradient, where A was 0.1% (v/v) formic acid in water and B was 0.1% (v/v) formic acid in acetonitrile, flow rate of 0.5 ml/min. The eluent was monitored by Shimadzu LC-MS 8030 with electrospray ion mass spectrometer (ESI-MS) under positive ion mode and scanned from 100 to 1000 *m/z*. ESI was conducted by using a fragmentor voltage of 125 V, skimmer 65 V. High-purity nitrogen (99.999%) was used as drying gas and at a flow rate of 10 L/min, nebulizer at 45 psi and capillary temperature at 350 °C. Shimadzu CBM-20A system controller mass detector, LC-30AD pump, SIL-30AC autosampler, and CTO-30 column oven were used.

Statistical analysis

For all tested parameters, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was done using GraphPad Prism version 7. The data were presented as mean ± SD. $P \leq 0.05$ was considered significant.

Results and discussion

Writhing test

In spite of the progress in drug industry nowadays, effective and potent analgesics are still highly needed, especially for the treatment of chronic pain. Natural products continue to provide unique structural diversity which presents opportunities for discovering mainly novel lead compounds (Fakir et al. 2016). In the present investigation, *A. chamaepitys* extract; dose-independently, decreased the number of writhings significantly compared to the control group. The effect of 300 mg/kg *A. chamaepitys* was comparable to the effect produced by the same dose of aspirin (Fig. 1). Similar results were obtained by Pal and Pawar (2011) in which an analgesic effect was observed at 200 and 400 mg/kg using water and chloroform extracts of *A. bracteosa* wall ex. Benth in writhing test (induced by acetic acid) in mice. The authors suggested that the action of *A.*

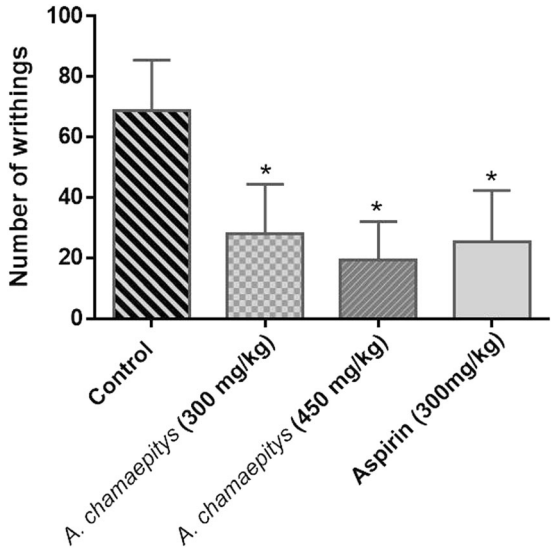


Fig. 1 Results of acetic acid-induced writhing test. Both doses of *A. chamaepitys* (300 mg/kg, 450 mg/kg), as well as aspirin, were significantly different from the control but not significantly different from each other at ($p \leq 0.05$)

bracteosa extracts is mediated by opioid receptor. Similarly, the evaluation of the analgesic activity of *A. iva* showed that the aqueous extract decreased the number of abdominal cramps in the test of writhing provoked by acetic acid. The aqueous extract of *A. iva* was more effective than ibuprofen (Chabane et al. 2012).

Formalin test in mice

Animals treated with *A. chamaepitys* extract showed a significant decrease in the time spent in paw licking in the second phase of formalin test (Fig. 2b). The high dose of *A. chamaepitys* extract (450 mg/kg) but not the low dose (300 mg/kg) inhibited licking in the first phase of formalin test (Fig. 2a). Khanavi et al. (2014) investigated the anti-nociceptive effect of *A. chamaecistus*. Administration of different fractions of the methanolic extract (200, 400, and 600 mg/kg) of *A. chamaecistus* had no effect in the early phase of the formalin test while hexane fraction (200 mg/kg) was effective in the late phase as well as the aqueous and diethyl ether extracts.

In formalin test, pretreatment with glibenclamide had no effect on *A. chamaepitys* action in early and late phases of formalin test (Fig. 2a, b). On the other hand, naloxone antagonized morphine antinociceptive action in early and late phases. Also, naloxone antagonized the action of *A. chamaepitys* (450 mg/kg) at late phase (Fig. 2a, b). Two distinct phases characterized by high licking activity can be observed in forrmalin test. The neurogenic phase (phase I) that starts immediately after intraplantar injecting formalin. This early phase is due to direct chemical activation of

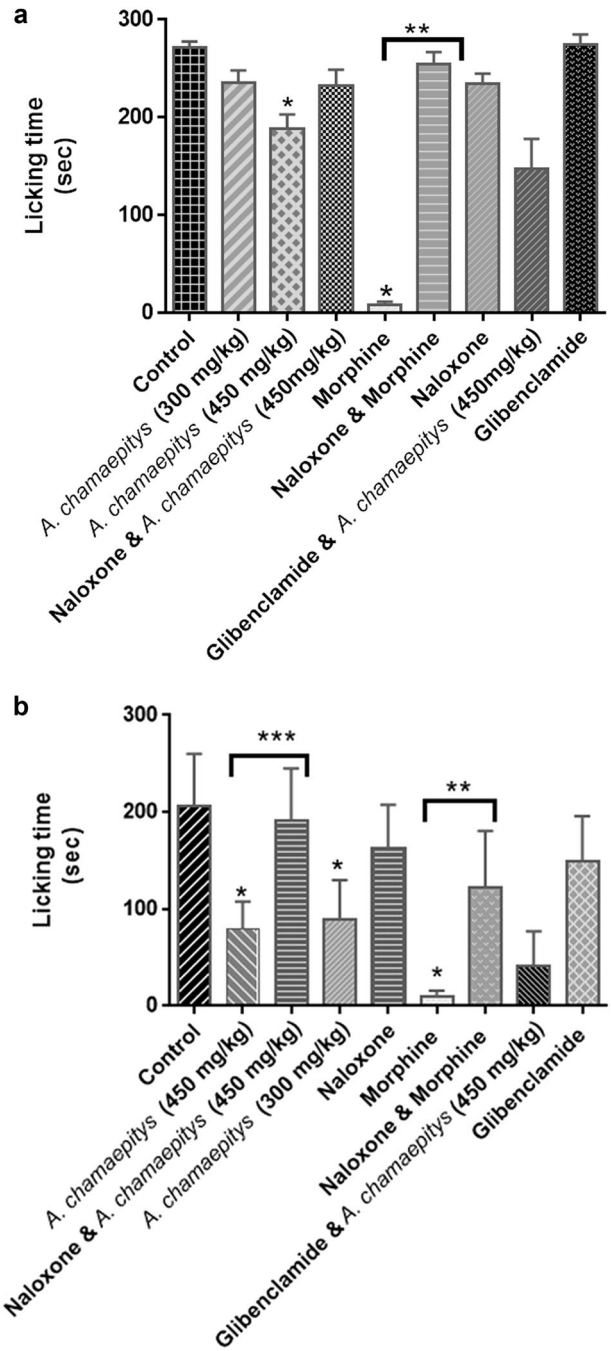


Fig. 2 Formalin test. **a** Phase I (early phase, 0–5 min). Morphine and the high dose of *A. chamaepitys* (450 mg/kg) were significantly different from the control. Naloxone reversed the action of morphine. **b** Phase II (late phase, 25–30 min). Morphine, as well as *A. chamaepitys* (300 mg/kg and 450 mg/kg), were significantly different from the control. Naloxone reversed the action of morphine as well as the action of *A. chamaepitys*. *Significant difference from the control ($p < 0.05$). **Significant difference between morphine (10 mg/kg) and naloxone (5 mg/kg) with morphine (10 mg/kg) ($p < 0.0001$). ***Significant difference between *A. chamaepitys* extract (450 mg/kg) and *A. chamaepitys* extract (450 mg/kg) with naloxone (5 mg/kg) ($p < 0.05$)

nociceptive sensory fibers. The inflammatory phase (phase II) lasts from 20 to 30 min after formalin injection. This late phase II is due to central sensitization of spinal cord circuits secondary to the inputs that occurred during phase I. In fact, formalin test is sensitive to various classes of analgesic drugs in which opioids inhibit both phases (Abotsi et al. 2016). The present work provided evidence for the frst time for the involvement of opioid receptor in the action of *A. chamaepitys* extract in paw licking test.

Hot-plate and tail flick tests

In hot-plate test, the latency of jumping responses increased 210 min (3.5 h) after *A. chamaepitys* extract (300 mg/kg) administration but not after 30 min. Pretreatment with naloxone abolished the effect of *A. chamaepitys* extract (Fig. 3). No significant difference was found between control and *A. chamaepitys* treated groups in tail flick test. The positive control morphine increased latency time while naloxone given 30 min before morphine abolished its action (Fig. 4).

In the present study, *A. chamaepitys* aerial parts extract increased the threshold of pain sensation in hot-plate test but not in tail flick test. An important distinction between hot-plate and tail flick test is that hot plate measures supraspinal response while tail flick is considered a reflexive nociceptive test (Gunn et al. 2011). The results of our study support the involvement of supraspinal (central mechanism) in *A. chamaepitys* action in hot plate as indicated by abolishing the effect of *A. chamaepitys* by pretreatment with naloxone, an opioid antagonist (Fig. 3). Similar results were obtained using methanolic extract of *A. bracteosa* aerial parts in hot-plate test in rats (Kayani et al. 2016).

LC-MS analysis

LC-MS analysis resulted in the identification of 19 compounds. Isovitexin, orientin, flavonol, and cyanidin were the major compounds (Table 1).

LC-MS analysis detected the presence of isovitexin, orientin, flavonol and cyanidin as major compounds. The anthocyanin cyanidin isolated from *A. chamaepitys* (Topcu et al. 2004) lowered nociceptive scores in formalin test in diabetic rats (Nasri et al. 2010). Other possible active constituents are the C-Glycosylated flavones such as orientin and isovitexin isolated from *A. chamaepitys* (L.) Schreb.

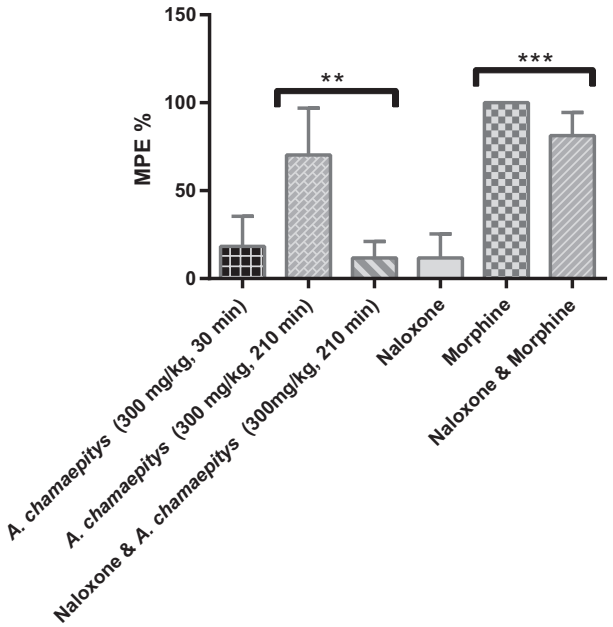


Fig. 3 Results of hot-plate test. Morphine and *A. chamaepitys* (300 mg/kg, 210 min) were significantly different from the control. Naloxone reversed the action of morphine and *A. chamaepitys*. *Significantly different from the control ($p < 0.05$). **Statistically significant difference between morphine (10 mg/kg) and naloxone (5 mg/kg) with morphine (10 mg/kg) ($p < 0.05$). ***Statistically significant difference between *A. chamaepitys* extract (300 mg/kg) after 210 min and naloxone (5 mg/kg) with *A. chamaepitys* extract (300 mg/kg) after 210 min ($p < 0.05$)

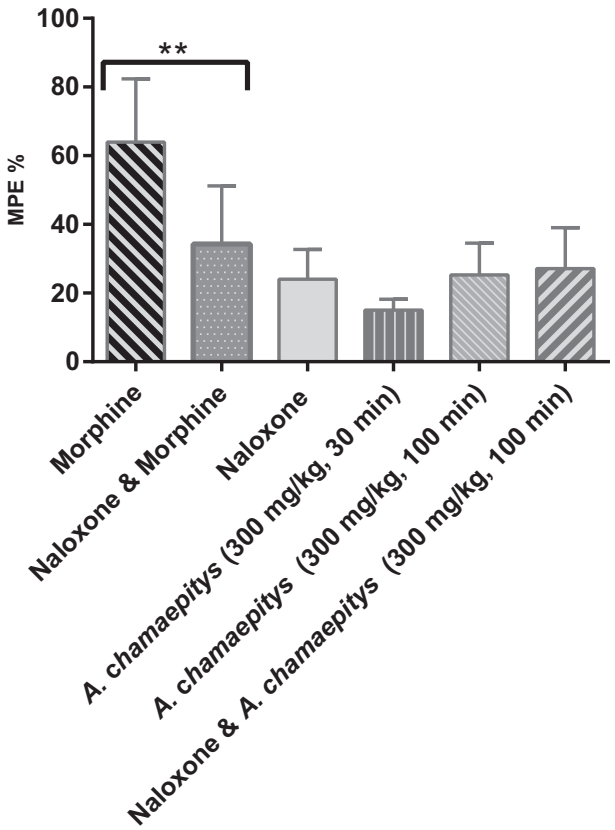


Fig. 4 Results of tail flick test. Only morphine increased latency time. Naloxone reversed the action of morphine. *Significant difference from the control ($p < 0.05$). **Significant difference between morphine (10 mg/kg) and naloxone (5 mg/kg) with morphine (10 mg/kg) ($p < 0.05$)

Table 1 Compounds detected by LC-MS from *A. chamaepitys* extract

Compound	%
Aurantinidin	3.1
Cyanidin	9.5
Diterpenoids	6.4
Europinidin	8.1
Flavone	1.5
Flavonol	10.1
Flavanone	3.3
Hydroxytyrosol	1.66
3-Hydroxyflavanone	5.4
Iridomyrmecin	6.3
Isovitexin	12.3
Monoterpenoids	1.92
Orientin	10.9
Pelargonidin	1.05
Peonidin	2.6
Rosinidin	2.3
Triterpenoids	7.1
Tyrosol	4.3
Verbascoside	2.3

subsp. chia var. ciliata (Merichi et al. 1994; Jakovljević et al. 2015). According to Da Silva et al. (2010), orientin is 20-fold more potent than acetylsalicylic acid and 3.5-fold more active than indomethacin. Also, it is likely that isovitexin exerts pharmacological activities similar to vitexin due to structural similarity (He et al. 2016). Investigations on the antinociceptive activity of pure vitexin demonstrated that the targets involve opioidergic δ, κ, and μ receptor subtypes and the nitrergic pathway through hot-plate, tail-clip, rota-rod, and writhing tests (Ozkay and Can 2013). Opioids differ in their binding affinity to different types of opioid receptors. This results in activation of μ, κ, and δ opioid receptors with variable degree depending on receptor binding affinities of the opioid. Therefore, different opioids vary in their pharmacokinetics, bioavailability, potency and side effects (Volkow and McLellan 2016).

Therefore, it is recommended to study the interaction of the compounds isolated from *A. chamaepitys* with different opioid receptor subtypes in vitro binding assays. Also, the possibility exists that several active constituents may act synergistically to perform the antinociceptive action of this plant.

Researchers have emphasized on the importance of at least three features: Phenolic hydroxyl group, aromatic ring and positively ionazable atom (Cheng et al. 2011; Singh et al.

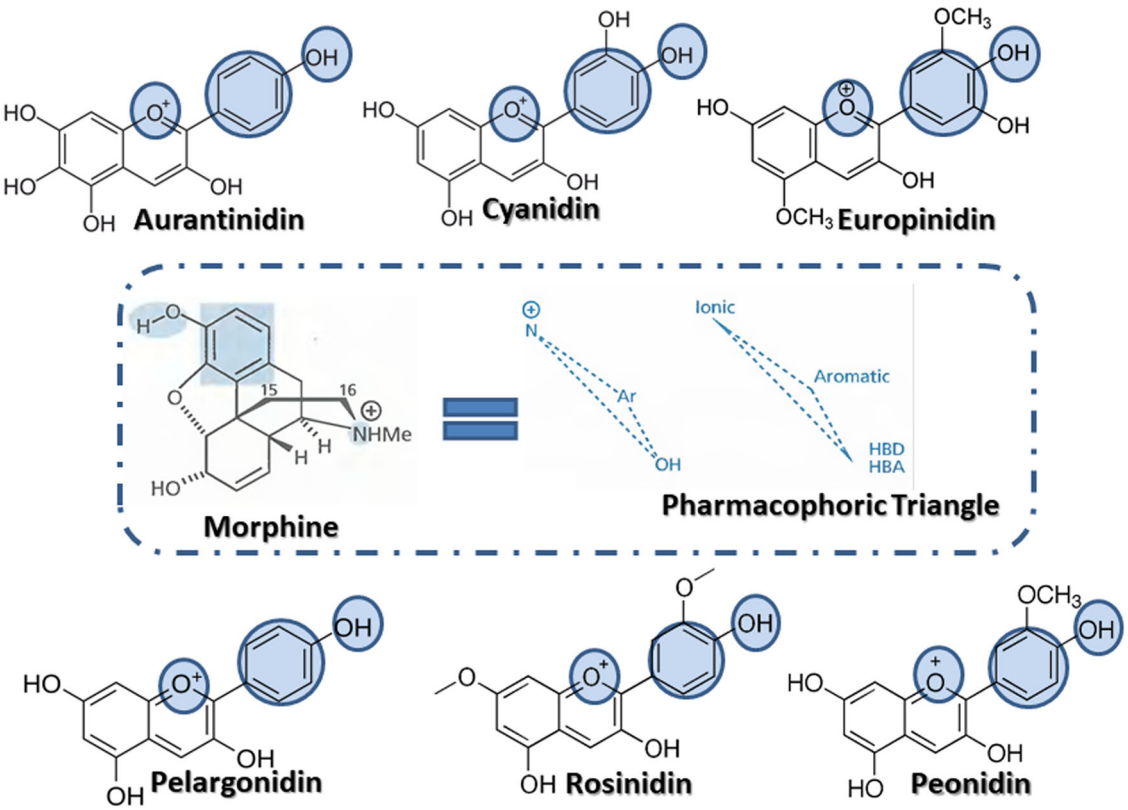


Fig. 5 Isolated compounds with possible binding affinity to opioid receptor based on structural similarity to morphine

2008; Yamaotsu et al. 2010). These features are responsible to perform hydrogen bond, aromatic and ionic interactions, respectively, with opioid receptors (pharmacophoric triangle). As shown in Fig. 5, these features are obviously found in the following compounds: Aurantinidin, Cyanidin, Europinidin, Pelargonidin, Rosinidin, and Peonidin. These preliminary expectations can be further tested in the future starting by creating a 3D-pharmacophore model followed by screening these compounds to find best fitting molecule. Selected molecules will be then tested in vitro.

Conclusion

The present research is the first one to report the anti-nociceptive effect of *A. chamaepitys* and the involvement of opioid receptor in its action. More detailed mechanistic study is needed in future. Such a study may lead to the development of new analgesics with limited side effects.

Acknowledgements This work was published with the support of Al-Ahliyya Amman University.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable guidelines for the care and use of animals at The University of Jordan were followed.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Abotsi WKM, Lamptey SB, Afrane S, Boakye-Gyasi E, Umoh RU, Woode E (2016) An evaluation of the anti-inflammatory, anti-pyretic and analgesic effects of hydroethanol leaf extract of Albizia zygia in animal models. Pharm Biol 55:338–348

Almeida RN, Navarro DS, Barbosa-Filho JM (2001) Plants with central analgesic activity. Phytomedicine 8:310–322

Alsharari SD, King JR, Nordman JC, Muldoon PP, Jackson A, Zhu AZ, Tyndale RF, Kabbani N, Damaj MI (2015) Effects of menthol on nicotine pharmacokinetic, pharmacology and dependence in mice. PLoS ONE 10:e0137070

Bannon AW, Malmberg AB (2007) Models of nociception: hot-plate, tail-flick, and formalin tests in rodents. Curr Protoc Neurosci 41 (1):8–9

Begum A, Sama V, Dodle JP (2016) Study of antinociceptive effects on acute pain treated by bioactive fractions of *Hyptis suaveolens*. J Acute Dis 5(5):397–401

Cheng J, Liu G, Zhang J, Xu Z, Tang Y (2011) Insights into subtype selectivity of opioid agonists by ligand-based and structure-based methods. J Mol Model 17(3):477–493

Caliskan UK, Aka C, Oz MG (2017) Plants used in Anatolian traditional medicine for the treatment of hemorrhoid. Records in Natural Products 11(3):235–250

Chabane D, Saidi F, Rouibi A, Azine K (2012) Étude comparative de l'activité antispasmodique de l'extrait aqueux d'Ajuga iva L. et de l'ibuprofène chez les souris. Afrique Science 8(2):131–137

Da Silva RZ, Yunes RA, de Souza MM, Delle Monache F, Cechinel V (2010) Antinociceptive properties of conocarpan and orientin obtained from *Piper solmsianum* C. *DC. var. solmsianum (Piperaceae)*. J Nat Med 64:402–408

Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. Metabolites 2:303–333

Fakir H, Korkmaz M, Icel B (2016) Medicinal plants traditionally used for pain alleviation in Antalya province, Turkey. Studies on Ethno-Medicine 10(3):314–324

Gunn A, Bobeck EN, Weber C, Morgan MM (2011) The Influence of non-nociceptive factors on hot plate latency in rats. JPain 12 (2):222–227

He M, Min J-W, Kong W-L, He X-H, Li J-X, Peng B-W (2016) Review on the pharmacological effects of vitexin and isovitexin. Fitoterapia 115:74–85

Hunskaar S, Fasmer OB, Hole K (1985) Formalin pain in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods 14:69–76

Jakovljević DZ, Vasić SM, Stanković MS, Čomić LR, Topuzović MD (2015) Secondary metabolite content and *in vitro* biological effects of *Ajuga chamaepitys* (L.) Schreb. Subs P. *chamaepitys*. Arch Biol Sci 67(4):1195–1202

Kayani WK, Dilshad E, Ahmed T, Ismail H, Mirza B (2016) Evaluation of *Ajuga bracteosa* for antioxidant, anti-inflammatory, analgesic, antidepressant and anticoagulant activities. BMC Complement Altern Med 16:375–388

Khalid M, Bilal M, Hassani D, Zaman S, Huang D (2017) Characterization of ethno-medicinal plant resources of karamar valley Swabi, Pakistan. J Radiat Res Appl Sci 10:152–163

Khanavi M, Davoodipoor AM, Sadati SN, Ardekani MRS, Sharifzadeh M (2014) Antinociceptive effect of some extracts from Ajuga chamaecistus Ging. spp. tomentella (Boiss.) Rech. f. aerial parts. DARU. J Pharm Sci 22(1):1–6

Koster, R, Anderson, M, & De Beer, EJ (1959) Acetic acid for analgesic screening. Federation Proceedings 18: 412–421.

Lozama, A (2010). Preparation of neoclerodane diterpenes as probes for the opioid receptor system. PhD thesis, University of Iowa.

Merichi F, Merichi AH, Korkmaz-Alp Y, Yilmaz F (1994) Volatile and phenolic compounds of *Ajuga chamaepitys* subsp. chia var. ciliata. Acta Pharmaceutica Turcica 36:17–19

Mogosan C, Vostinaru O, Oprean R, Heghes C, Filip L, Balica G, Moldovan RI (2017) A comparative analysis of the chemical composition, anti-inflammatory, and antinociceptive effects of the essential oils from three species of Mentha cultivated in Romania. Molecules 22:263

Nasri S, Roghani M, Tourandokht B, Balvardi M, Rabani T (2010) Antinociceptive effect of chronic administration of the anthocyanin cyanidin in diabetic rats: Behavioral evidence. Kowsar Med J 15(3):135–140

Ozkay UD, Can OD (2013) Anti-nociceptive effect of vitexin mediated by the opioid system in mice. Pharmacol Biochem Behav 109:23–30

Öztürk M, Altay V, Mert Gönenç T (2016) Herbals from the High Mountains in the East Mediterranean. In: Bhojraj S, Talas-Ogras T, Adam S, Madhunapantula SRV (Eds) Drug Discovery From Herbs-Approaches and Applications, Chapter 24. DAYA Publishing Houser, India, p 327–367

Pal A, Pawar RS (2011) A study on *Ajuga bracteosa* wall ex. Benth for analgesic activity. Int J Cur Biol Medi Sci 1(2):12–14

Santos JA, Piccinelli AC, Formagio MD, Oliveira CS, Santos EP, Alves Stefanello ME, Lanza Junior U, Oliveira RJ, Sugizaki MM, Kassuya CA (2017) Antidepressive and antinociceptive effects of

ethanolic extract and fruticuline A from *Salvia lachnostachys* Benth leaves on rodents. PLoS ONE 12:e0172151

Simón-Arceoa K, González-Trujanob ME, Coffeena U, Fernández-Masc R, Mercadod F, Almanzad A, Contrerasa B, Jaimesa O, Pellicera F (2017) Neuropathic and inflammatory antinociceptive effects and electrocortical changes produced by *Salvia divinorum* in rats. J Ethnopharmacol 206:115–124

Singh N, Nolan TL, McCurdy CR (2008) Chemical function-based pharmacophore development for novel, selective kappa opioid receptor agonists. J Mol Graph Model 27(2):131–139

Topcu G, Koekdil G, Tuerkmen Z, Voelter W, Adou E, Kingston DGI (2004) A new clerodane diterpene and other constituents from *Ajuga chamaepitys* ssp. Laevigata. Naturforsch 59:584–588

Venditti A, Frezza C, Maggi F, Lupidi G, Bramucci M, Quassinti L, Giuliani C, Cianfaglione K, Papa F, Serafini M, Bianco A (2016) Phytochemistry, micromorphology and bioactivities of *Ajuga chamaepitys* (L.) Schreb. (Lamiaceae, Ajugoideae): Two new harpagide derivatives and an unusual iridoid glycosides pattern. Fitoterapia 113:35–43

Vij VV, Sharma V, Kumar P, Deshmukh R (2014) Herbs as positive modulator in neuropathic pain and their antinociceptive effect. Innovations in Pharmaceuticals and Pharmacotherapy 2 (3):378–387

Volkow ND, McLellan AT (2016) Opioid Abuse in Chronic Pain - Misconceptions and Mitigation Strategies. N Engl J Med 374 (13):1253–1263

Woolfe G, McDonald AD (1946) Analgesic action of pethidine derivatives and related compounds. Br J Pharmacol 1:4–14

Yamaotsu N, Fujii H, Nagase H, Hirono S (2010) Identification of the three-dimensional pharmacophore of κ-opioid receptor agonists. Bioorg Med Chem 18(12):4446–4452