Physiology and Pharmacology 26 (2022) 322-332
Original Article

Antinociceptive and antioxidant effects of *Onosma platyphyllum riedl* extract

Kamran Ranjbar[1], Alireza Moulazadeh[2*], Amin Dakhili Ardestani[1], Mohammad Soleimanian[2], Zakaria Meshkibaf[3], Mohammad Hassan Meshkibaf[4*]

1. Student Research Committee, Fasa University of Medical Sciences, Fasa, Iran
2. Noncommunicable Disease Research Center, Fasa University of Medical Sciences, Fasa, Iran
3. DIPSAR, Delhi Pharmaceutical Sciences and Research University, New Delhi, India
4. Department of Clinical Biochemistry, Fasa University of Medical Sciences, Fasa, Iran

ABSTRACT

Introduction: Pain is an unpleasant and annoying sensation caused by tissue damage and inflammatory stimuli. Due to the side effects of the conventional drugs, the present study was conducted to investigate the antinociceptive effects of *Onosma platyphyllum* (OP) and its antioxidant activity.

Methods: The OP hydroethanolic extract was evaluated by gas chromatography–mass spectrometry (GC-MS) analysis. The phenolic and flavonoid content respectively were determined by Folin-Ciocalteu and aluminum chloride assays. The antioxidant activity of the extract was evaluated by FRAP and DPPH methods. Formalin test was also used to evaluate the herbal antinociceptive effects on various doses (50, 100, 150 and 200mg/kg).

Results: The content of β-phenylethyl butyrate, 2-pentanone,4-hydroxy-4-methyl, 3'-acetyllycopsamine and indolizine analysed by GC-MS were 25.9%, 8.86%, 8.5% and 5.52% of the extract respectively. The phenolic and flavonoid content of the extract were 45.20±2.75 µgGAE/mg and 184.40±26.23 µgQE/mg. The monovalent reducing activity was 382.35±34.83 µMFe^{2+}. The radical scavenging activity of the extract in the dose of 500µg/mL was also 13.71±3.67. The acute and chronic pain score was significantly reduced in treatment with OP extract. The chronic antinociceptive effects of the extract were higher than acute effects. The highest antinociceptive effect was related to 200mg/kg concentration of the extract.

Conclusion: OP is effective in pain relieving, especially in the chronic phase probably by improving the oxidative stress. It's side effects and hepatotoxicity should be evaluated.

Keywords:
Pain
Nociceptive
Antioxidants
Plant extracts
Onosma platyphyllum

Introduction

Pain is an unpleasant sensation that is caused by pressure, extreme temperatures or damage and inflammatory mediators. Almost everyone has experienced pain in their life time (Izadpanah et al., 2020). Acute pain occurs following direct damage to various tissues and

* Corresponding authors: Mohammad Hassan Meshkibaf, Meshkibaf.m@fums.ac.ir
Alireza Moulazadeh, a.moulazadeh@fums.ac.ir

Received 20 February 2021; Revised from 21 September 2021; Accepted 25 September 2021

Citation: Ranjbar K, Moulazadeh A, Dakhili Ardestani A, Soleimanian M, Meshkibaf Z, Meshkibaf M.H. Antinociceptive and antioxidant effects of Onosma platyphyllum riedl extract. Physiology and Pharmacology 2022; 26: 322-332. http://dx.doi.org/10.52547/phypha.26.4.10

FIGURE 1. The species of *Onosma platyphyllum riedl* collected from Tang-e Karam mountains, Fasa, Fars province, Iran.

organs. The pain severity should be reduced. Otherwise, it will lead to a chronic phase. The chronic phase of pain is accompanied by increasing the multiple inflammatory mediators, neurotransmitters and neurotrophins. The pain-related gene expression increases in the posterior horn of the spinal cord. And finally, the activation threshold of the peripheral pain receptors reduces (Humble et al., 2015; Mollayeva et al., 2017).

Pain can be controlled by various methods and natural remedies in the form of medicine. Such as narcotic analgesics (encephalin, endorphins, morphine and methadone) and non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin and acetaminophen are used. The use of narcotic analgesics is commonly used in the treatment of severe pain, such as surgical pain (Luque-Suarez et al., 2019). NSAIDs are used to reduce inflammatory pain. Previous studies indicate that these drugs cause many side effects such as anaphylactic shock, kidney failure, gastrointestinal and heart problems (Gandhi et al., 2011). Other antinociceptive therapies include blocking the neural pathways and multimodal antinociceptive, which are associated with many clinical complications (Hozack et al., 2019). Therefore, it is important to find new drugs with more effectiveness and lesser clinical complications. Medicinal plants are one of the rich and potential sources in the production of pain-reducing agents that are widely used in traditional medicine in the treatment of inflammatory diseases and chronic pain relief (de Sá Coutinho et al., 2018).

Onosma is a plant family native to Asia and the Mediterranean that grows in rocky mountainous areas and is used in some rural area for chronic pain treatment. Phytochemical studies of *Onosma* family have shown that these plants contain strong metabolites such as phenolic and flavonoid compounds (Sen et al., 2010; Stebler et al., 2019). Phenolic and flavonoid compounds have antioxidant activity and is used in improving oxidative stress, acute and chronic pain (Binzet, 2016). To the best of our knowledge, there is no study on the effectiveness of *Onosma platyphyllum* (OP) extract on pain; though there is limited studies on the other species of *Onosma* species. The antioxidant effects of plants can be effective against chronic pain by reducing oxidants and inflammatory mediators (Kumar et al., 2013). The present study was conducted to investigate the phenolic and flavonoid content, antioxidant activity and antinociceptive effect of OP hydroethanolic extract.

Material and methods

Collection and extraction of plant

The aerial parts of the plant were collected from Tang-e Karam Mountains, Fasa, Fars province, Iran (Figure 1). The collected plants were submitted to Shiraz School of Pharmacy and identified as *Onosma platyphyllum riedl* by experts. The voucher number of N.739 was assigned for the plant in the herbarium of Shiraz School of Pharmacy. Extraction was done by hydroethanolic maceration method. For this purpose, the plant powder (100g) was immersed in ethanol (70% V/V) and kept at room temperature and darkness for one week. The solid and insoluble particles were removed by passing the extracts on a filter paper. The excess solvent was also evaporated at 50°C (Moulazadeh et al., 2021). The concentrated extract was incubated at 50°C for 24h to dry. The yield of the extraction was 3.18%. The dried extract was placed at -20°C.

TABLE 1: The scoring of the motion responses to painful stimuli after formalin (2.5% v/v) injection.

The motion response	Score
keeps its weight evenly on both feet	0
limps while walking	1
held its paws up	2
began to lick and bite its paws	3

The gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS analysis of the OP hydroethanolic extract was carried out using a SHIMADZU QP2010 system and gas chromatograph interfaced to a mass spectrometer equipped with Elite-1 fused silica capillary column (length: 30.0m, diameter: 0.25 mm, film thickness: 0.25µm composed of 100% dimethyl poly siloxane). GC-MS spectroscopic detection, an electron ionization system with ionization energy of 70eV was used. Pure helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min. The oven temperature was programmed from 40°C to 170°C at 5°C/min, from 170 C to 290°C at 10°C/min then held isothermal for 10min. Diluted samples (1/100 v/v in methanol) of 1µl was injected in the split mode with a split ration 120:1. The relative percentage of the chemical constituents in the OP crude extract was expressed as percentage by peak area normalization (Krone et al., 2010).

Measurement of phenolic content

The phenolic content of the OP extract was measured using the Folin− Ciocalteu method. Accordingly, 500 µL of Folin− Ciocalteu reagent (10% v/v) was added to 100 µL of the extract (1mg/mL) and incubated for 5min at room temperature and darkness. Then 400 µL of sodium carbonate (7.5% w/v) was added to the sample and the resulting solution was kept at room temperature and darkness for 60min. Finally, absorbance of the samples was measured at 765nm by Synergy HTX multi-mode reader. Gallic acid was also used as standard and the phenol content of the extracts was reported in micrograms gallic acid equivalent (GAE) per milligram of dry weight (µgGAE/mg) (Moulazadeh, 2021). All measurements were done in duplicate.

Measurement of flavonoid content

Aluminum chloride method was used to measure the flavonoid content of the extract. Accordingly, 50 µL of aluminum chloride (10% w/v) and 50 µL of sodium nitrite (5% w/v) were added to 200 µL of the OP extract (1mg/mL). The solution was incubated 6min at room temperature and darkness, then 700 µL of sodium hydroxide (4% w/v) was added and the total volume of the solution was 1 mL. After complete stirring, the resulting solution was again incubated at room temperature and darkness for 15min and the absorbance of the solution was read at 510nm using a Synergy HTX multi-mode reader. Quercetin was also used as standard and the flavonoid content of the extracts was reported in micrograms quercetin equivalent (QE) per milligram of dry weight (µgrQE/mgr) (Moulazadeh, 2021). All measurements were done in duplicate.

Evaluation of antioxidant activity

In the present study, FRAP and DPPH methods were used to compare the antioxidant power of the OP extract. The FRAP assay was used to investigate the potency of monovalent antioxidants that capable of reducing Fe^{3+} to Fe^{2+} ions. Whereas in the DPPH method, the total radical scavenging activity is determined by reducing the stable nitrogen radical (DPPH radical) (Legault et al., 2011).

Evaluation of the monovalent reducing power

As mentioned, the FRAP assay measures the antioxidant capacity of the extracts by reducing the Fe^{3+} ions (present in the Fe-TPTZ complex) to Fe^{2+} ions, so it's called monovalent reduction. Working solution of FRAP assay is not stable and is recommended to be prepared just before testing. Therefore, the following solutions (A, B and C) are mixed at a ratio of 1–1–10, respectively. A: 10mM TPTZ solution in 40mM hydrochloric acid; B: FeCl3 solution (20mM) and C: acetate buffer (300mM, pH=3.6).

In the following, 1.5mL of the prepared FRAP work-

ing solution was poured into a test tube and 50µL of the OP extract (1mg/mL) were added and mixed. After 10min incubation at 37° C, the absorbance of the colored solution was read at 593nm. Serial dilution of $FeSO_4$ solution (1mM) was used as standard and the reducing power of the extract was reported in µmolFe^{2+}/g (Moulazadeh et al., 2021).

Determination of radical scavenging activity

Total radical scavenging activity of the extract was determined by DPPH assay. The DPPH radicals has a purple color that turns to yellow after reduction by antioxidants in the herbal extracts. The intensity of colorlessness indicates the radical scavenging activity in different concentration. Accordingly, serial dilution of plant extracts (10, 50, 100, 200 and 500) was prepared using ethanol 70%. In the following 40µL of the extract was added to 160µL of DPPH radicals (0.3mM) and kept at room temperature and darkness for 30min. Finally, the optical absorption changes of the samples were determined at 517nm using Synergy HTX multimode reader (Makoolati et al., 2022). All measurements were done in duplicate. Ascorbic acid as a reference antioxidant was used for comparing the results. The antioxidant activity was calculated as percentage of inhibition relative to the control using the following equation:

Antioxidant power= [(Optical absorption of control group-Optical absorption of experimental group)/ Optical absorption of control group]× 100

Experimental animals

While observing the ethical principles of working with laboratory rodents and receiving the ethics code of IR.FUMS.REC.1396.258 from the ethics committee of Fasa University of Medical Sciences, Male Wister rats (200-220g) were obtained from the animal facility. All animals were left for a week at experimental conditions for acclimatization. Rats were housed under standard laboratory conditions (room temperature: 22±2°C; relative humidity: 55±5%) at a 12h light/dark cycle and given free access to standard rat pellet and tap water. All animal experiments and animal care procedures were approved by the Animal Ethical and Welfare Committee of Fasa University of Medical Sciences. The rats were divided into five groups of five including: 1. control groups (normal saline), 2. OP extract (50mg/kg), 3. OP extract (100mg/kg), 4. OP extract (150mg/kg) and 5. OP extract (200mg/kg).

Formalin test

Formalin test was used to measure the antinociceptive effect of the OP extract based on movement behavioral changes due to pain. According to preliminary study and toxicity evaluation, the doses of 50, 100, 150 and 200mg/kg of the OP extract (1mL) were selected for formalin test. The different doses of the extract were administered orally by gavage for two weeks. Normal saline (0.9% w/v, 1mL) was also gavaged in the control group for two weeks. The rats were transferred to a formalin test box and kept one hour for adaptation. The box was made of glass (30×30×30cm) with a lower mirror for better monitoring. Painful stimuli was induced by injection of formalin (50µl, 2.5% v/v) subcutaneously in the right foot of the rats. The rats were returned to the box and the motion response to formalin was scored every 15 seconds according to the Table 1 in two intervals of 0 to 5min (acute pain) and 5 to 60min (chronic pain). In fact, the motion responses of mice to formalin were evaluated 240 times (once every 15 seconds). The first 20 times (0-5min) were related to the acute phase and the next 220 times (5-60min) were related to the chronic phase. According to the Table 1, the motion response to painful stimuli in every evaluation was scored as 0, 1, 2 and 3. Therefore, the total score of the acute pain was reported by calculating the total scores of the motion responses in the first 5min divided by 20. The total score of chronic pain was also reported by calculating the total scores of the motion responses in the next 55min divided by 220. The total score of the acute and chronic pain were in the range of 0-3, which were converted to the percentage (multiplied by 33.33) (Dubuisson and Dennis, 1977).

Statistical analysis

Statistical analysis of data was performed using t-test and ordinary one-way ANOVA in GraphPad Prism 8.0.2 software. Significance level was considered less than 0.05 (P<0.05) and the data were expressed as mean±SD. IC50 also calculated by four parametric logistic regression.

Results

GC-MS analysis

Thirty-nine different compounds were identified in

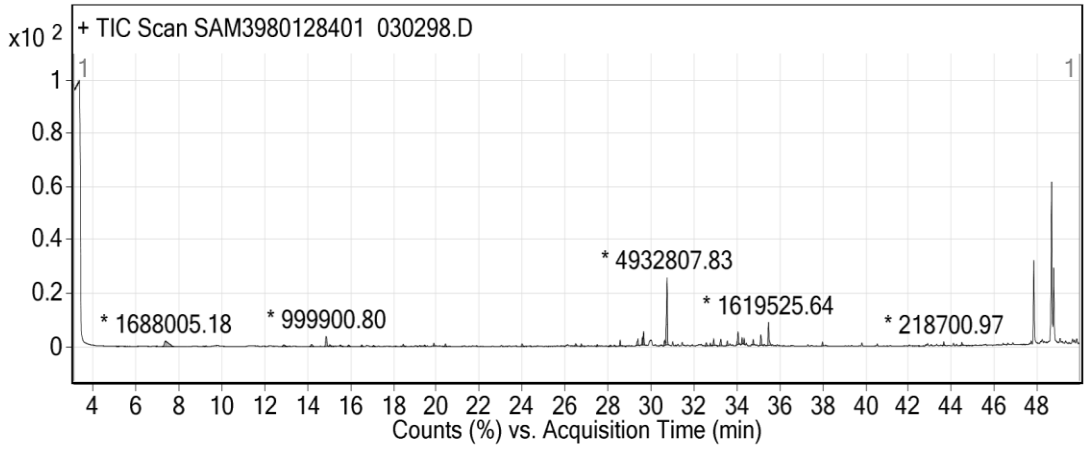

FIGURE 2. Chromatogram diagram of hydroethanolic extract of *Onosma platyphyllum* (OP) in the GC-MS analysis.

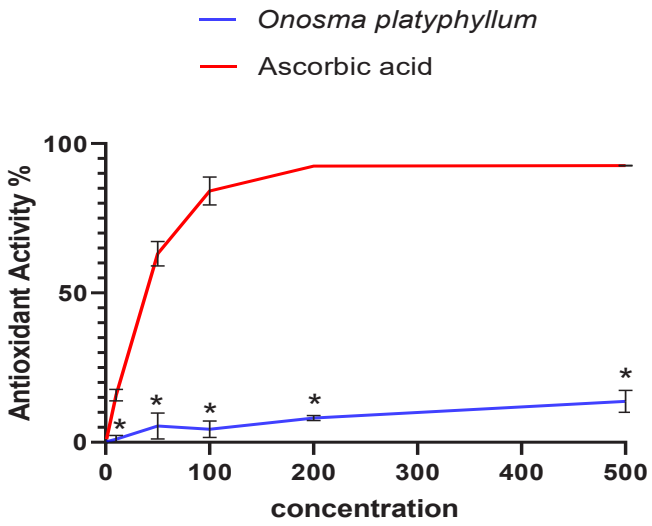

FIGURE 3. Comparison of the radical scavenging activity of *Onosma platyphyllum* (OP) extract and Ascorbic acid. Data are expressed as mean±SD. The bullet of (*) indicates a statistically significant difference between OP and ascorbic acid by t-test as *P*-value <0.05.

the OP hydroethanolic extract by GC-MS analysis (Table 2). According to the Figure 2 and Table 2, the more abundant ingredients in the extract was β-phenylethyl butyrate which contain 25.9% of the total extract. 2-pentanone, 4-hydroxy-4-methyl and 3'-acetyllycopsamine and indolizine also accounted for 8.86%, 8.5% and 5.52% of the total extract, respectively.

Phenolic and flavonoid content

The phenolic content of the OP hydroethanolic extract was 45.20±2.75 µgGAE/mg using Folin-Ciocalteu method. The flavonoid content of the OP extract was also calculated by aluminium chloride method as 184.40±26.23 µgQE /mg.

Antioxidant activity

The monovalent reducing activity of the OP hydroethanolic extract was measured by FRAP method as 382.35±34.83 µMFe^{2+}. According to the Table 3 and Figure 3, the radical scavenging activity of the OP extract was significantly (P<0.0001) lower than ascorbic acid as a strong antioxidant. 13.71% of free radicals were inhibited by the OP extract in the concentration of 500µg/mL.

Antinociceptive effect of the extract

According to the Table 4 and Figure 4, the acute pain score was significantly reduced in treatment with 150mg/kg and 200mg/kg doses of the OP extract, respectively by 23.75% (P=0.0002, F=21.40) and 32.08 %

TABLE 2: Chemical composition of *Onosma platyphyllum* (OP) extract using GC-MS analysis.

	The Components	Retention time	Area %
1	2-Pentanone, 4-hydroxy-4-methyl	7.39	8.86
2	Cyclohexene, 1-methyl-4-(1-methylethylidene)	12.89	1.22
3	γ-Terpinene	14.18	1.21
4	Indolizine	14.86	5.25
5	3-Carene	15.04	0.70
6	cis-p-mentha-1(7),8-dien-2-ol	15.51	0.54
7	Carveol	15.91	0.63
8	Cyclohexene, 3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl	16.52	0.55
9	Ethanone, 1-(1,4-dimethyl-3-cyclohexen-1-yl)	17.07	0.38
10	Dodecane	18.46	0.71
11	3-Cyclohexene-1-methanol, 2-hydroxy-α,α,4-trimethyl	19.45	0.42
12	2-Thiazolidinimine, 3-methyl	19.88	1.00
13	cis-Verbenol	20.41	0.83
14	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone	23.98	1.17
15	(Hexahydropyrrolizin-3-ylidene)-acetaldehyde	26.49	1.14
16	Phenol, 2,4-bis(1,1-dimethylethyl)	26.75	0.71
17	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	27.48	0.52
18	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl	28.11	0.54
19	Furan, 3-(4,8-dimethyl-3,7-nonadienyl)	28.29	0.38
20	Megastigmatrienone	28.56	1.68
21	Isoaromadendrene epoxide	29.38	2.76
22	Stevioside	29.59	2.55
23	Megastigmatrienone	29.65	4.32
24	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo [4.1.0] heptan-2-ol	30.63	1.54
25	β-Phenylethyl butyrate	30.75	25.90
26	2-Acetoxy-1,1,10-trimethyl-6,9-epidioxydecalin	32.56	1.35
27	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	32.76	0.79
28	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	32.92	2.04
29	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	33.25	3.02
30	2,6,10-Dodecatrien-1-ol, 12-acetoxy-2,6,10-trimethyl	33.55	1.51
31	β-Eudesmol	34.05	4.94
32	Epiglobulol	34.24	1.90
33	Echinatine	34.34	1.66
34	Rivularine	34.76	1.59
35	Hexanoic acid, 2-phenylethyl ester	35.12	3.82
36	3'-Acetyllycopsamine	35.48	8.50
37	Maltose	37.99	1.13
38	Verrucarol	43.64	1.16
39	Isoaromadendrene epoxide	44.48	1.08
	The sum of components (%)		**98.92**

(P<0.0001, F=21.40). Chronic pain score was also significantly reduced in treatment with 100mg/kg (P=0.02, F=20.34), 150mg/kg (P<0.0001, F=20.34) and 200mg/kg (P<0.0001, F=20.34) doses of the OP extract, respectively by 16.25%, 28.25% and 37.71%.

TABLE 3: The radical scavenging activity of different concentrations of *Onosma platyphyllum* (OP) extract and ascorbic acid by DPPH assay.

Concentration (µg/mL)	OP		Ascorbic acid		*P-value*
	Mean	IC50	Mean	IC50	
10	1.05±1.20		15.79± 1.83		<0.0001
50	5.42± 4.32		63.12± 4.12		<0.0001
100	4.37± 2.74	>500	84.10± 4.68	30.99	<0.0001
200	8.15± 0.83		92.42± 0.03		<0.0001
500	13.71± 3.67		92.62± 0.05		<0.0001

Data are expressed as mean±SD. *P*-value indicate the significant differences between the groups by t-test analysis. IC50 values of total radical scavenging activity were calculated by four parametric logistic regression.

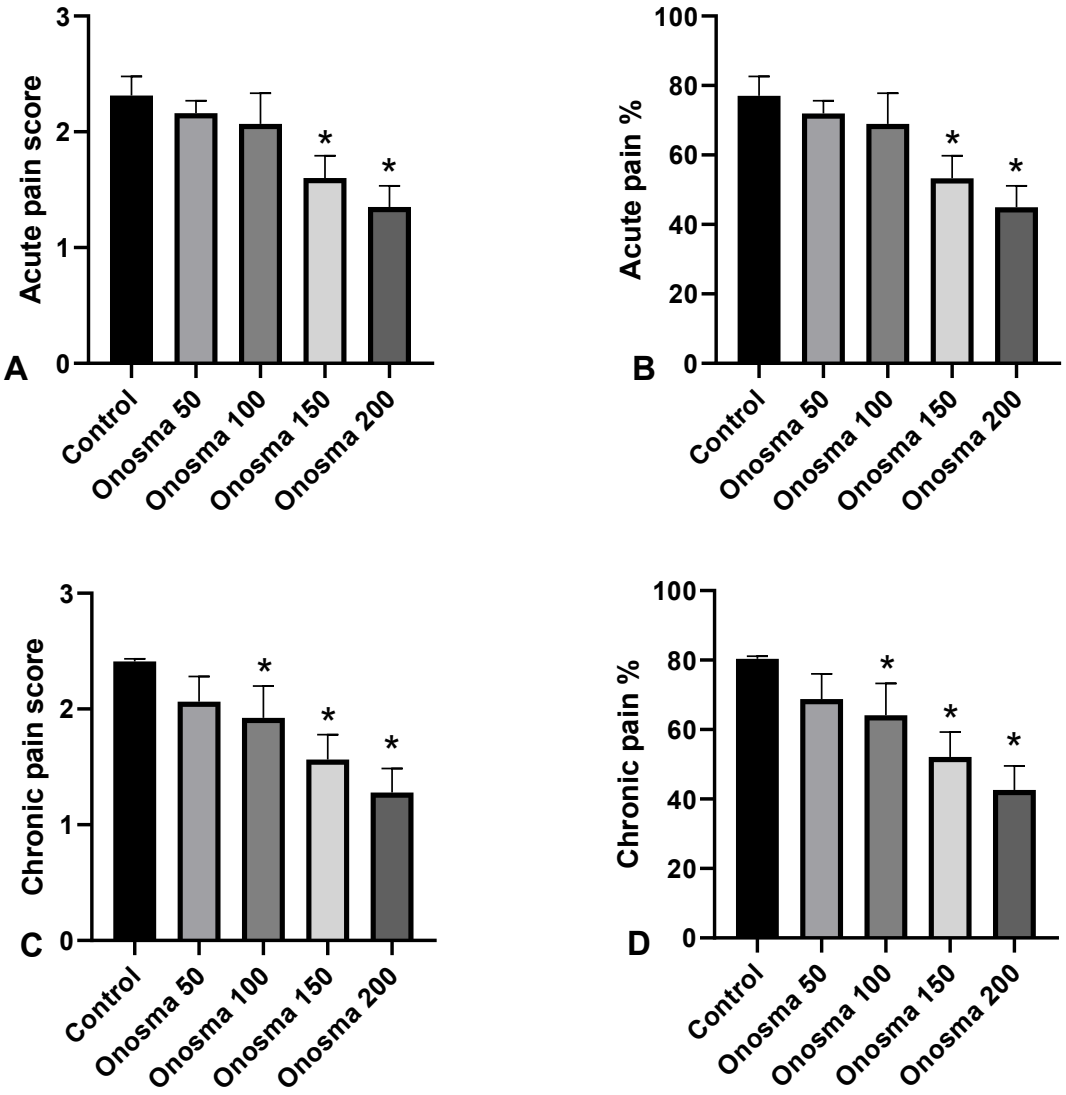

FIGURE 4. The pain score of control and experimental groups treated with different doses of *Onosma platyphyllum* (OP) extract in the acute (A, B) and chronic (C, D) phases. The pain score (0-3) and pain percentage (0-100) are expressed as mean±SD. Statistical analysis was investigated by ordinary one-way ANOVA. The bullet of (*) means the significant difference compare to control group as $P<0.05$.

TABLE 4: Acute and chronic pain score of control and experimental groups treated with different doses of *Onosma platyphyllum* (OP) extract.

Groups		Acute Pain					Chronic Pain				
		Total score (0-3)		% Total score		*P-value*	Total score (0-3)		% total score		*P-value*
		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Control		2.31	0.16	77.08	5.50	-	2.41	0.02	80.37	0.70	-
Onosma extract (mg/kg)	50	2.16	0.10	71.99	3.61	0.75	2.06	0.21	68.80	7.22	0.16
	100	2.07	0.26	68.99	8.78	0.35	1.98	0.27	64.12	9.15	**0.02**
	150	1.6	0.19	53.33	6.45	**0.0002**	1.56	0.21	52.12	7.14	**<0.0001**
	200	1.35	0.18	44.99	6.12	**<0.0001**	1.28	0.20	42.66	6.90	**<0.0001**

Data are expressed as mean±SD. *P*-value indicate the significant differences between the groups by ordinary one-way ANOVA analysis.

Discussion

Acute pain is induced following direct tissue damages. The pain severity is reduced by damage repair. But in the presence of pain stimulants, accumulation of oxidants and inflammatory mediators, the pain becomes chronic. The present study for the first time showed that the OP hydroethanolic extract has a high potential in inhibiting acute and chronic pain. The extract probably reduces the stimulatory threshold of peripheral pain receptors and posterior horn neurons in the spinal cord, which leads to reduce acute and chronic pain. The antinociceptive effects of the OP extract increases by a dose dependent manner, this increase appeared by more than 25% on the 200mg/kg doses of OP extract in chronic and acute condition as compared with 50mg/kg.

Moreover, the antinociceptive effects of the OP extract were more effective on chronic pain than the acute. The chronic pain score was reduced by 11.57% and 16.25% after treatment with 50 and 100mg/kg of the extract, respectively. However, the acute pain score was reduced just by 5.09% and 7.09% respectively. In fact, the antinociceptive effects of the OP extract were approximately 2.3 times higher in the chronic phase than the acute. In addition, the antinociceptive effect of the150 and 200mg/kg doses of the OP extract were approximately 1.3 times higher in the chronic phase compared to the acute. Therefore, it is appeared that the OP extract has a stronger effect on the chronic pain.

Traditionally, the *Onosma* species has been used as antinociceptive, antioxidant and anti-inflammatory remedy to treat stomach ulcers, burn wound healing, rheumatism and bladder pain (Safavi et al., 2019). As the best of our knowledge, few studies have been performed on the antinociceptive effects of the *Onosma* species in the acute and chronic phases. Tosun et al. (2008) were reported the antinociceptive and anti-inflammatory effects of *Onosma isauricum*, *Onosma sericeum*, *Onosma tauricum* and *Onosma tauricum* on painful abdominal contractions. The chloroform extract of *Onosma aucheranum* and hydroethanolic extract of *Onosma sericeum* showed the highest antinociceptive effect. Shoaib et al. (2019b) were also reported the neuroprotective activity of *Onosma echioides* in the animal model of diabetic neuropathy. Diabetic neuropathy is a deceptive and symmetrical degenerative disorder of peripheral nerves, which affects sensory, motor and autonomic neurons. In diabetic neuropathy, sensitivity to touch and pain decreases due to changes in neurotransmitters related to hyperalgesia. they were reported that the *Onosma echioides* extract regulates the pain sensitivity by neuroprotective activity in a dose-dependent manner. The neuroprotective activity of the *Onosma echioides* extract includes the myelination and decreasing the axonal swelling of nerve fibers. Shoaib et al. (2019a) were also reported in another study that the n-hexane extract of the *Onosma echioides* has the therapeutic properties for diabetic neuropathy in human neuroblastoma SHSY5Y cell line.

In addition to neuroprotective effects, several studies have shown the anti-inflammatory effects of *Onosma* species (Dong et al., 2017; Hemmati et al., 2018; Safavi et al., 2019). Dong et al. (2017) were reported the anti-inflammatory effects of four new naphthoquinones isolated from the methanol extract of the *Onosma paniculatum*. The isolated naphthoquinones significantly inhibited the nitric oxide production in murine macrophage RAW 264.7 cells. Safavi et al. (2019) were reported the anti-inflammatory effect of *Onosma dichroantha* extract on nitric oxide production and wound

healing (Safavi et al., 2019). Hemmati et al. (2018) were also reported the anti-inflammatory and healing effects of the *Onosma bulbotrichum* cream (5%) in second degree burns. Cadirci et al. (2007) were reported the anti-inflammatory effects of *Onosma armeniacum* on ethanol-induced stomach ulcers by improving oxidative stress. There is the direct association between the inflammatory mediators and pain perception (Ronchetti et al., 2017). Therefore, it is appeared that the *Onosma* species withe the anti-inflammatory properties probably show the antinociceptive effects, too.

The antinociceptive effects of the *Onosma* species are probably due to the antioxidant and antinociceptive compounds of the extract. About the OP extract, the most important and abundant antinociceptive compound was β-phenylethyl butyrate. The β-phenylethyl butyrate comprises 25.9% of the total extract. It is present in cigarettes and tobacco too and reduces the sensitivity of involved receptors by cell membrane manipulation (Nosova et al., 2020). The 2-pentanone, 4-hydroxy-4-methyl is another antinociceptive compound in the OP extract that shows its antinociceptive effects on central nervous system, inhalation of this compound causes dizziness and anesthesia and has narcotic effects in high concentrations. (El-Shazly and Wink, 2014). The 3'-acetyllycopsamine of the extract is also used in pain related to burns and rheumatoid arthritis (Attalah et al., 2020; Poma et al., 2018). Indolizine and megastigmatrienone also have antioxidant, anti-inflammatory and antinociceptive effects (Inchab et al., 2019; Kumar et al., 2013; Tatipamula et al., 2019). The antinociceptive effects of this family are probably due to the presence of antioxidant compounds such as phenols, flavonoids, shikonins and alkannins (Safavi et al., 2019).

The antioxidant compounds reduce inflammatory pain by reducing oxidants and inflammatory mediators. The OP extract shows its radical scavenging activity in a dose dependent manner. The radical scavenging activity of the extract increased almost 13-fold at 500 µg/mL compared to 50 µg/mL. Consistent with the present study, numerous studies were reported the antioxidant and radical scavenging activity of different species of *Onosma* such as *O. hispidum* (Kumar et al., 2017), *O. lycaonica* and *O. papillosa* (Saravanakumar et al., 2021), *O. isauricum* (Zengin et al., 2019), *O. isaurica* and *O. bracteosa* (Saravanakumar et al., 2019), *O. sericea* and *O. stenoloba* (Stanković et al., 2020) and *Onosma pulchra* (Sarikurkcu et al., 2020). About the phenolic and flavonoic content of the *Onosma* species, also there are several studies in the literature. For example, Demir et al. (2021) was reported the phenolic and flavonoid content of *O. armeniacum* respectively as 26.1±0.4 mgGAE/g and 13.7±0.9 mgQE/g. Kumar et al. (2017) was reported the phenolic content of the *O. hispidum* extract as 110.46±12.86 mgGAE/g. Saravanakumar et al. (2021) were reported the phenolic and flavonoid content of *O. lycaonica* respectively as 43.5±1.5 mgGAE/g and 26.0±0.5 mgQE/g. They were also reported the phenolic and flavonoid content of *O. papillosa* respectively as 33.9±0.4 mgGAE/g and 32.9±0.3 mgQE/g.

Conclusion

It appears that the OP extract has an appropriate capacity to control the acute and chronic pain by improving the oxidative stress. For comprehensive conclusion about the antinociceptive effects of the OP extract, it is necessary to compare with conventional antinociceptive drugs such as morphine or NSAIDs. It is one of the limitations of the present study and should be considered in the future studies. The side effects and hepatotoxicity of the OP extract in animal models should be investigated. So, alarm should be given to avoid using the plant by indigenous peoples due to lack of sufficient study related to the side effects of OP extract and possible toxicity. In addition, it is also recommended that the antinociceptive and antioxidant effects of their fractions be investigated in the future studies.

Acknowledgments

The authors are grateful to the authority from Fasa University of Medical Sciences for the support and providing facilities and laboratory equipment for this research. The research was supported by a grant kindly provided by Research deputy of Fasa University of Medical Sciences (NO. 96033).

Conflict of interest

The authors have no conflict of interest

References

Attalah K, Abdalla A, Aslam A, Ahmed M, Abourehab M, ElSawy N, et al. Ethyl benzoate bearing pyrrolizine/indolizine moieties: Design, synthesis and biological evaluation of anti-inflammatory and cytotoxic activities.

Bioorg Chem 2020;94:1-21. https://doi.org/10.1016/j.bioorg.2019.103371

Binzet R. Onosma anatolica, a new species of Boraginaceae from Turkey. PhytoKeys 2016;69:39-49. https://doi.org/10.3897/phytokeys.69.8360

Cadirci E, Suleyman H, Aksoy H, Halici Z, Ozgen U, Koc A, et al. Effects of Onosma armeniacum root extract on ethanol-induced oxidative stress in stomach tissue of rats. Chem Biol Interact 2007; 170:40-8. https://doi.org/10.1016/j.cbi.2007.06.040

Coutinho D, Pacheco M, Frozza R, Bernardi A. Anti-inflammatory effects of resveratrol: mechanistic insights. Int J Mol Sci 2018;19:1-25. https://doi.org/10.3390/ijms19061812

Demir S, Demir EA, Turan I, Ozgen U. Evaluation of cytotoxic effect of Onosma armeniacum extract on various cancer cells. KSU J Agric Nat 2021;24:252-9. https://doi.org/10.18016/ ksutarimdoga.vi. 729814

Dong M, Liu D, Li Y, Chen X, Luo K, Zhang Y, et al. Naphthoquinones from Onosma paniculatum with potential anti-inflammatory activity. Planta Med 2017;83:631-5. https://doi.org/10.1055/s-0043-100122

Dubuisson D, Dennis S. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. pain 1977;4: 161-74. https://doi.org/10.1016/0304-3959(77)90130-0

El-Shazly A, Wink M. Diversity of pyrrolizidine alkaloids in the Boraginaceae structures, distribution, and biological properties. Diversity 2014;6:188-282. https://doi.org/10.3390/d6020188

Gandhi K, Heitz J, Viscusi E. Challenges in acute pain management. Anesthesiol clin 2011;29: 291-309. https://doi.org/10.1016/j.anclin.2011.04.009

Hemmati A, Namjuyan F, Yousefi S, Housmand G, Haghighian H, Rezaei A. The healing effect of n-hexan-dichloromethane extract root Onosma bulbotrichum in second degree burns. World J Plast Surg 2018;7:25-33.

Hozack B, Abboudi J, Gallant G, Jones C, Kirkpatrick W, Liss F, et al. Prospective evaluation of opioid consumption following cubital tunnel decompression surgery. HAND 2019; 14: 42-47. https://doi.org/10.1177/1558944718800732

Humble S, Dalton A, Li L. A systematic review of therapeutic interventions to reduce acute and chronic post-surgical pain after amputation, thoracotomy or mastectomy. Eur J Pain 2015;19:451-65. https://doi.org/10.1002/ejp.567

Inchab K, Khonsung P, Chiranthanut N, Kunanusorn P, Panthong A, Sireeratawong S. Evaluation of anti-inflammatory, analgesic, and antipyretic activities of Pseuderanthemum palatiferum. Chiang Mai Univ J Nat Sci 2019;18:393-407. https://doi.org/10.12982/CMUJNS.2019.0027

Izadpanah E, Saei E, Abdollahi O, Ahmadi A, Moloudi M. Analgesic effect of safranal in animal model of acute pain; possible role of the GABAergic pathway. Sci J Kurd Univ Med Sci 2020;25:76-83. https://doi.org/10.52547/sjku.25.1.76

Krone N, Hughes B, Lavery G, Stewart P, Arlt W, Shackleton C. Gas chromatography/mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography tandem mass spectrometry (LC/MS/MS). J Steroid Biochem 2010;121:496-504. https://doi.org/10.1016/j.jsbmb.2010.04.010

Kumar N, Kumar R, Kishore K. Onosma L.: A review of phytochemistry and ethnopharmacology. Pharmacogn Rev 2013;7:140-51. https://doi.org/10.4103/0973-7847.120513

Kumar N, Singh A, Sharma D, Kishore K. In-Vitro anti inflammatory and antioxidant activity of Onosma hispidum (Ratanjot) Roots. IJPBS 2017;3:30-5.

Legault J, Perron T, Mshvildadze V, Girard-Lalancette K, Perron S, Laprise C, et al. Antioxidant and anti-inflammatory activities of quercetin 7-O-β-D-glucopyranoside from the leaves of Brasenia schreberi. J Med Food 2011;14:1127-34. https://doi.org/10.1089/jmf.2010.0198

Luque-Suarez A, Martinez-Calderon J, Falla D. Role of kinesiophobia on pain, disability and quality of life in people suffering from chronic musculoskeletal pain: a systematic review. Br J Sports Med 2019;53:554-9. https://doi.org/10.1136/bjsports-2017-098673

Makoolati Z, Bahrami H, Zamanzadeh Z, Mahaldashtian M, Moulazadeh A, Ebrahimi L, et al. Efficacy of Ficus carica leaf extract on morphological and molecular behavior of mice germ stem cells. Anim Reprod 2022;19:1-18. https://doi.org/10.1590/1984-3143-AR2022-0036

Mollayeva T, Cassidy J D, Shapiro C, Mollayeva S, Colantonio A. Concussion/mild traumatic brain injury-related chronic pain in males and females: a diagnostic modelling study. Medicine 2017;96:1-14. https://doi.org/10.1097/MD.0000000000005917

Moulazadeh A, Ranjbar R, Dakhili Ardestani A, Najafipour S. Evaluation of antioxidant activity and cytotoxic effects of Hypnea musiformis on MCF7 and MDA-MB-231 cell lines. Iran J Pharm Sci 2021;17:33-46. https://doi.org/10.22034/IJPS.2021.521195.1763

Moulazadeh A, Ranjbar R, Dakhili Ardestani A, Keshavarzi A, Karimzadeh F, Rahnavard M, et al. Evaluation of Phe-

nolic Content, Antioxidant Activity and Cytotoxic Effects of Ulva lactuca and Hypnea musiformis Marine Algae on MDA-MB-468 Cell Line. J Adv Biomed Sci 2021;11:3921-8. https://doi.org/10.18502/jabs.v11i3.8785

Moulazadeh A, Ranjbar R, Ardestani AD, Hekmat M, Azarnia S, Najafipour S. Antioxidant activity, phenolic and flavonoid content of Lawsonia inermis and Haplophyllum vermiculare. Physiol Pharmacol 2021;25:261-9. https://doi.org/10.52547/ppj.25.3.261

Moulazadeh A, Ranjbar R, Hekmat M, Sedaghat F, Yousefzadi M, Najafipour S. Comparison the cytotoxic effects of Ulva fasciata and Ulva lactuca on the MCF-7 and MDA-MB-231 breast cancer cell lines. Physiol Pharmacol 2021;25:373-83. https://doi.org/10.52547/phypha.25.4.2

Nosova N, Sokolov A, Gein O, Gein V, Yankin A, Danilov S, et al. Synthesis and analgesic activity of N, 6-diaryl-4-hydroxy-4-methyl-2-oxocyclohexane-1-carboxamides and their dehydration products. Russ J Gen Chem 2020;90:1581-90. https://doi.org/10.1134/S1070363220100011

Poma P, Labbozzetta M, Notarbartolo M, Bruno M, Maggio A, Rosselli S, et al. Chemical composition, in vitro antitumor and pro-oxidant activities of Glandora rosmarinifolia (Boraginaceae) essential oil. PLoS One 2018;13:1-11. https://doi.org/10.1371/journal.pone.0196947

Ronchetti S, Migliorati G, Delfino D. Association of inflammatory mediators with pain perception. Biomed Pharmacother 2017;96:1445-52. https://doi.org/10.1016/j.biopha.2017.12.001

Safavi F, Farimani M, Golalipour M, Leung P, Lau K, Kwok H, et al. Investigations on the wound healing properties of Onosma dichroantha Boiss root extracts. S Afr J Bot 2019;125:344-52. https://doi.org/10.1016/j.sajb.2019.08.005

Saravanakumar K, Sarikurkcu C, Sahinler S, Sarikurkcu R, Wang M. Phytochemical composition, antioxidant, and enzyme inhibition activities of methanolic extracts of two endemic Onosma species. Plants 2021;10:1-10. https://doi.org/10.3390/plants10071373

Saravanakumar K, Sarikurkcu C, Sarikurkcu R T, Wang M-H. A comparative study on the phenolic composition, antioxidant and enzyme inhibition activities of two endemic Onosma species. Ind Crop Prod 2019;142:111878. https://doi.org/10.1016/j.indcrop.2019.111878

Sarikurkcu C, Sahinler S, Ceylan O, Tepe B. Onosma pulchra: Phytochemical composition, antioxidant, skin-whitening and anti-diabetic activity. Ind Crop Prod 2020;154:112632. https://doi.org/10.1016/j.indcrop.2020.112651

Sen S, Chakraborty R, De B, Ganesh T, Raghavendra H, Debnath S. Analgesic and anti-inflammatory herbs: a potential source of modern medicine. Int J Pharm Sci Res 2010; 1: 32-44.

Shoaib A, Dixit R, Badruddeen, Rahman M, Bagga P, Kaleem S, et al. Cure of human diabetic neuropathy by HPLC validated bark extract of Onosma echioides L. root. Nat Prod Res 2019a;33:2699-703. https://doi.org/10.1080/14786419.2018.1460838

Shoaib A, Dixit R, Ganash M, Barreto G, Ashraf G, Siddiqui H. Beneficial effects of n-hexane bark extract of Onosma echioides L. on diabetic peripheral neuropathy. J Cell Biochem 2019b;120:16524-16532. https://doi.org/10.1002/jcb.28912

Stanković J, Ceylan R, Zengin G, Matić S, Jurić T, Diuzheva A, et al. Multiple biological activities of two Onosma species (O. sericea and O. stenoloba) and HPLC-MS/MS characterization of their phytochemical composition. Ind Crop Prod 2020;144:112053. https://doi.org/10.1016/j.indcrop.2019.112053

Stebler K, Martin R, Kirkham K, Lambert J, De Sede A, Albrecht E. Adductor canal block versus local infiltration analgesia for postoperative pain after anterior cruciate ligament reconstruction: a single centre randomised controlled triple-blinded trial. Br J Anesth 2019;123:e343-9. https://doi.org/10.1016/j.bja.2019.04.053

Tatipamula V, Kolli M, Lagu S, Paidi K, Reddy R, Yejella R. Novel indolizine derivatives lowers blood glucose levels in Streptozotocin-induced diabetic rats: A histopathological approach. Pharmacol Rep 2019;71:233-42. https://doi.org/10.1016/j.pharep.2018.11.004

Tosun A, Akkol E, Bahadır Ö, Yeşilada E. Evaluation of anti-inflammatory and antinociceptive activities of some Onosma L. species growing in Turkey. J Ethnopharmacol 2008;120:378-81. https://doi.org/10.1016/j.jep.2008.09.007

Zengin G, Mahomoodally M, Picot-Allain C, Cakmak Y, Uysal S, Aktumsek A. In vitro tyrosinase inhibitory and antioxidant potential of Consolida orientalis, Onosma isauricum and Spartium junceum from Turkey. S Afr J Bot 2019;120:119-23. https://doi.org/10.1016/j.sajb.2018.01.010