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Effects of *Sonchus asper* and apigenin-7-glucoside on nociceptive behaviors in mice

[Efectos de *Sonchus asper* y apigenina-7-glucósido sobre comportamientos nociceptivos en ratones]

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Abstract

Context: *Sonchus asper* is an important herbal medicine that traditionally used to treatment of digestive system infections and heart disease.

Aims: To evaluate of anti-inflammatory and antinociceptive effects of *Sonchus asper* hydroalcoholic leaf extract (SALE) and one of its major constituent, apigenin-7-glucoside (Ap7G), in male mice.

Methods: In this experimental studies were used nociceptive assessment tests, which include writhing, tail-flick, and formalin-, and glutamate-induced paw licking tests. In addition, xylene test was used for evaluating of anti-inflammatory effect of SALE and Ap7G.

Results: In tail-flick, writhing and glutamate-induced paw licking tests, application of a dose of 300 mg/kg of extract showed significantly ($p < 0.01$) antinociceptive effect compared to the control group. In the formalin test, treatment with a dose of 100 mg/kg of extract reduced the pain scores in the tonic phase compared with the control group ($p < 0.05$). In formalin model, also naloxone (an opioid non-selective antagonist) plus the extract (300 mg/kg) reduced licking and biting in mice. Moreover, the use of morphine decreased the nociceptive activity in all assessment tests. In addition, in xylene test, treatment with dose of 100 mg/kg of SALE increased the inhibition (49%) comparing to the control group. The Ap7G showed significant antinociceptive and anti-inflammatory effects in all tests.

Conclusions: SALE and Ap7G have both antinociceptive and anti-inflammatory effects under the experimental conditions performed in this study. The modulation of the glutamatergic system by opioid receptors could be involved, at least in part, in these effects.

Resumen

Contexto: *Sonchus asper* es una planta medicinal que tradicionalmente se utiliza para el tratamiento de infecciones del sistema digestivo y enfermedades del corazón.

Objetivos: Evaluar los efectos antiinflamatorios y antinociceptivos del extracto hidroalcohólico de hoja de *Sonchus asper* (SALE) y uno de sus componentes principales, apigenin-7-glucósido (Ap7G), en ratones machos.

Métodos: En estos estudios experimentales se usaron pruebas de evaluación nociceptiva, que incluyeron pruebas de contorsiones, retirada de la cola y tiempo de lamido de la pata inducido por formalina y glutamato. Además, se utilizó la prueba de xileno para evaluar el efecto antiinflamatorio de SALE y Ap7G.

Resultados: En las pruebas de retirada de cola, contorsiones y tiempo de lamido inducido por glutamato, la aplicación del extracto (300 mg/kg) mostró, significativamente ($p < 0.01$), efecto antinociceptivo comparado al grupo control. En la prueba de la formalina, el tratamiento con el extracto (100 mg/kg) redujo la escala de dolor en la fase tónica comparado con el control ($p < 0.05$). En este modelo, también naloxona (un antagonista no selectivo de opioides) más el extracto (300 mg/kg) redujeron el lamido en ratones. Además, el uso de morfina decreció la actividad nociceptiva en todas las pruebas. En la prueba de xileno el tratamiento con SALE (100 mg/kg) exhibió una inhibición del 49% comparado con el grupo control. Ap7G mostró efectos antinociceptivo y anti-inflamatorio en todas las pruebas.

Conclusiones: SALE y Ap7G tienen efectos antinociceptivos y antiinflamatorios bajo las condiciones experimentales realizadas en este estudio. La modulación del sistema glutamatérgico por los receptores opioides podría estar implicada, al menos en parte, en estos efectos.

Keywords: hypernociception; inflammation; medicinal plants; pain.

Palabras Clave: dolor; hipernocicepción; inflamación; plantas medicinales.

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INTRODUCTION

Pain is a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components (Williams and Craig, 2016). There are two categories of pain according to pain management guideline: acute and tonic pain. Inflammation is a common symptom of many diseases that weakens the immune system. Since pain is a cardinal sign of the inflammation, several chemical mediators are common in some molecular pathways of biological process. Inflammatory nociceptive pain is one of the chronic pain type that resulting from inflammatory process mediated by potassium, bradykinin, P substance, prostaglandins, serotonin, and histamines, which can stimulate nociceptors (Weiner, 2001).

Nowadays, drugs used to pain relief either are opioid generally, nonopioid drugs as nonsteroidal anti-inflammatory drugs, corticosteroids, antidepressants, and anticonvulsants (which have analgesic mechanisms particularly in neuropathic pain independently of these primary effects). Using opioids in short and long terms can cause side effects such as decrease the activity of the digestive system, nausea, urinary retention, as well as dependence. Subsequently, developing of novel efficient analgesic drugs with a major safety profiles are necessary (Garrido et al., 2001).

Medicinal plants are an important source of new chemicals with potent therapeutic effects (Golshani et al., 2015). However, in most cases the origin and the mechanism of the therapeutic effects are unknown. Because 80% of world people live in developing countries, in which synthetic drugs are very expensive or even inaccessible, the quest for medicinal plants gets even more serious (Zarei et al., 2015).

Sonchus asper is one of the most important medicinal plants that belong to Asteraceae family. It can be used as a supplementary drug for colds, and tonsil pain, as well as treating burns, and anemia (Zargari, 1995). Also, it can be used in treating various other diseases as well like stomach disorders, skin sores and intestine disease (Koche et al., 2008). Kidney, liver problems, and impotency are also treated by all parts of the plant. Moreover, it is

one of the plants known for its use in treating asthma, digestive system infections, and heart diseases (Sabeen and Ahmad, 2009).

There are phytochemical compounds in the plant, such as flavonoids, carotenoids, sesquiterpenes, and phenolic compounds (Hussain et al., 2010). One of major constituents that exist in this plant is apigenin-7-glucoside (Ap7G) that belongs to flavonoids (Balasundram et al., 2006). There are numerous documents on the effects of flavonoids compounds on treating pain and inflammation (Khan and Ahmed, 2009). Studies have shown that another species of this genus such as *Sonchus oleraceus* has anti-nociceptive, anti-inflammatory, and anti-febrile effects as well (Vilela et al., 2010).

Considering the chemical compounds that exist in the plant, such as flavonoids or phenolic compounds, and the intense relationship of these components with anti-inflammatory, tranquilizing and analgesic effects, and considering the fact that this valuable plant's anti-nociceptive effects have not been experimented on mice yet, we decided to show and analyze the anti-nociceptive and anti-inflammatory effects of *Sonchus asper* leaf extract (SALE) and one of its major constituent in mice.

MATERIAL AND METHODS

Plant collection and preparation of the leaf extract

For this experimental study, in July 2016 four kilograms of the fresh leaves of the *Sonchus asper* were collected from the mountainsides of mount Alvand, Hamadan (34° 47' 59.99" N, 48° 30' 59.99" E), and was then taken to BuAli Sina University to be approved by a botanist (Dr. Mahtab Asgari Nematian). A specimen (No. 17,328) was retained in the herbarium of the Bu-Ali Sina University, Hamadan, Iran for identification. After separating the petioles, the plant's leaves were dried in shadow at room temperature (25°C). Then, the dried leaves were turned into powder by a mechanical mill (particle size of 1.18 mm). Subsequently, 200 g of the powder was placed in 1 L of methanol 80% for 72 h. The solution was filtered (qualitative Whatman filter papers, degree 1, thickness of 0.20 mm) and then the liquid phase was concentrated under

reduced pressure in a rotary evaporator (N-1300S-W 115V, Clarkson Laboratory & Supply Inc., California, USA), then it was put under hood in a dish for one week to be re-dried and stored in the darkness at a temperature of 20°C until analysis.

Animals

Male albino Swiss mice (20-30 g) were purchased from Pasteur Institute of Iran and were kept in animals' room under standard conditions 12 h/12 h of dark/light cycle (light beginning at 7 a.m.) and temperature conditions of $22 \pm 1^\circ\text{C}$. The animals had free access to water and food in their metal cages. They were accustomed to the laboratory conditions at least two hours before experiments. The experiments were carried out between 8 a.m. and 12 p.m. Animal care and research protocols were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985), and also it has been approved with local Ethics Committee of Hamadan University of Medical Sciences.

Drugs and administration routes

All injections were performed by intraperitoneally (i.p.) route 30 min before the procedures. The agonist and antagonist drugs and doses were selected according to the previous studies (Mahmoodi et al., 2016) and on pilot experiments in our laboratory. The SALE in all experimental tests was prepared in normal saline 0.9% (control or vehicle) except in glutamate induced licking test in which dimethyl sulfoxide (DMSO) 5% was used as a solvent agent (control or vehicle). Morphine sulfate, naloxone, and dexamethasone were purchased from Daroopakshsh (Iran). Acetic acid, formalin, xylene, and DMSO from Merck (Germany). Apigenin-7-glucoside isolated from *Sonchus asper* was purchased from Sigma Chemicals Company, St. Louis (USA).

Lethal dose 50 (LD₅₀)

LD₅₀ was examined on the basis of the previous model (Lorke, 1983). Different doses of the extract were given to male mice intraperitoneally in separate injections. The numbers of fatalities for the

next 72 h were counted and thus the LD₅₀ of the plant's extract was determined.

Inflammation test

In this test (xylene-induced ear swelling), the animals were divided into six groups (n=5). A total of 30 µL of xylene in 100 µL of DMSO was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (DMSO, 45 µL). SALE (10, 50, 100 mg/kg) and AP7G (30 mg/kg) were administered intraperitoneally 35 min before xylene application. Two control groups were used: a control group with the application of xylene on the right ear and administered with saline 0.9% (10 mL/kg, i.p.), and the reference group was treated with dexamethasone (15 mg/kg, i.p.).

Two hours after the application of xylene, animals were killed by cervical dislocation and a 6 mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight. This difference in weight between right treated and left untreated sections showed the amount of inflammation (Atta and Alkofahi, 1998). Inhibition percentage was expressed as a reduction in weight with respect to the control group.

$$\% \text{ Inhibition} = 100 \left(\text{VC} - \frac{V_t}{V_c} \right)$$

Where,

Vc = ear weight difference, control group

Vt = ear weight difference in groups treated by extract or standard drug

Nociceptive tests

Tail flick test

Mice were divided into eight groups (n=5) that contained control group (saline 0.9%, 10 mL/kg, i.p.), morphine group (1 mg/kg, i.p.), experimental groups treated with SALE (30, 100 and 300 mg/kg, i.p.), AP7G (30 mg/kg, i.p.), morphine plus naloxone (1 mg/kg, i.p.) and also the group treated with SALE (300 mg/kg, i.p.) plus naloxone (1 mg/kg, i.p.). The treatment was administered 30 min before of tail flick test. This test was carried out by a tail

flick meter (LE7106 Harvard apparatus, USA). The animal's tail was positioned on a slot of adjustable width equipped with a groove that guarantees a correct placement. A photo beam with adjustable sensitivity detects the tail flick and the latency automatically is presence on a digital display on the control unit. Measurements of reaction time are given with a 0.1 precision. A cut-off time can be set to avoid tissue damage (by default 20 s) (D'Amour and Smith, 1941). Thus if the animal did not pull its tail for 15 s after the start of photo beam, it was cut in order to avoid tissue damage. The delay in pulling the tail was measured 20 min after injecting saline and the extract in three times (at 15 min intervals until 2 h) and then the average of them was as delay time.

Writhing test

Mice were divided into eight groups (n=5) that contained control group (saline 0.9%, 10 mL/kg, i.p.), morphine group (1 mg/kg, i.p.), experimental groups that treated with SALE (30, 100 and 300 mg/kg, i.p.), AP7G (30 mg/kg, i.p.), morphine plus naloxone (1 mg/kg, i.p.) and also the group that treated with SALE (300 mg/kg, i.p.) plus naloxone (1 mg/kg, i.p.). Animals were placed in a translucent plastic box for 30 min before running the experiments in order to get used to laboratory conditions. SALE doses were administrated 20 min before i.p. injection of acetic acid 0.6% in a dose volume of 10 mL/kg body-weight. After injection, their responses (writhing) were counted during continuous observation for 2, 5, or 30 min. It is also necessary to mention that each animal was used only once (Collier et al., 1968).

Formalin test

Mice were divided into eight groups (n=5) that contained control group (saline 0.9%, 10 mL/kg, i.p.), morphine group (1 mg/kg, i.p.), experimental groups that treated with SALE (30, 100 and 300 mg/kg, i.p.), AP7G (30 mg/kg, i.p.), morphine plus naloxone (1 mg/kg, i.p.) and also the group that treated with SALE (300 mg/kg, i.p.) plus naloxone (1 mg/kg, i.p.). One hour before the test, the animals were transferred into the special box (32 x 32 x 32 cm) of formalin test in order to get used to the experiment condition. Positioned in 45°; a mirror was inserted below the box and in front of the

observer to observe the animal's behaviors more clearly. Twenty minutes after the i.p. injection of the drugs, there was a subcutaneous injection of 50 μ L of formalin 2.5% solution into the plantar surface of the left hind paw; then the animal was placed to the test special box again. Every 15 seconds the physical response to pain was rated 0, 1, 2, or 3 in the accompanying way: 0 was used when the animal had complete balance while moving, its weight shared between the legs; 1 was given when the animal did not endure its weight on the leg that had taken the injection, or when it took care of the leg; 2 was used when the animal raised the painful leg and did not touch the floor of the container, and 3 indicated an animal that licked, chewed or jerked the painful foot. The average initial 5 min of the test was considered as the first phase of the formalin test (acute phase) and the average 15-60 min of the test as the second phase (tonic phase) (Dubuisson and Dennis, 1978).

Glutamate-induced paw licking test

The animals were divided into five groups (n=5). The control group (treated with DMSO), experimental groups treated with SALE (30, 100 and 300 mg/kg, 1 mL/kg, i.p.) and AP7G (30 mg/kg, i.p.). To explore the possible activity of this extract on the modulation of the glutamatergic system in the analgesic action, the study described by Beirith et al. (2002) was used. Mice were pretreated intraperitoneally by DMSO or SALE 60 min prior to glutamate injection. An amount of 20 μ L of glutamate (10 μ mol/paw) was injected using intraplantarly (i.pl.) pathway in the right hind paw of rats. Immediately after the application of the phlogistic agent, the rats were placed in a transparent observation chamber and were observed separately from 0 to 15 min, after the glutamate injection. The amount of time spent licking the injected paw was timed with a wristwatch and was considered as indicative of nociception.

Statistical analysis

Data was presented as mean \pm standard error of mean (SEM) and one-way analysis of variance (ANOVA), followed by the Tukey's multiple comparison test. P values less than 0.05 ($p < 0.05$)

were considered as indicative of significance. SPSS software was used for data analysis.

RESULTS

Acute toxicity test

In this experimental model, after seventy-two hours of injection of the different dosage of extract and apigenin-7-glucoside no fatalities were recorded (Table 1).

Xylene test

According to the results (Table 2), using 50 and 100 mg/kg doses of extract with $p < 0.05$ and $p < 0.01$ and with the inhibition percentages of 34.2% and 47.9% respectively, compared to the control group, it caused decrease of inflammation. However, using 10 mg/kg dose of extract did not show significant difference compared to the control group.

Tail flick test (TFT)

In TFT, using the 300 mg/kg dose of the extract showed a significant difference ($p < 0.01$) in compa-

risson to control group and the TFL of the mice was raised from amount of 2.8 ± 0.67 to 5.8 ± 0.79 seconds. Whereas using the 30 and 100 mg/kg doses of the extract did not show a significant difference in anti-nociceptive effects compared to control group. This study shows that using naloxone and a high dose of the extract at once reversed the analgesic effect of extract. Morphine injection led to increase in TFL, from 2.8 ± 0.59 s in control group to 8.1 ± 2.1 s in morphine group ($p < 0.001$) (Fig. 1A-B).

Writhing test

All of SALE doses were effective [30 and 100 mg/kg ($p < 0.05$) and 300 mg/kg ($p < 0.01$)] in decreasing writhings compared to the control group. Naloxone plus the higher dose of the extract caused the reversion of the pain relief the effects of the SALE. Morphine also decreased the abdominal constriction responses compared to the control group ($p < 0.001$). On the other hand, using the 300 mg/kg dose of the extract, in comparison to morphine group, showed a significant difference ($p < 0.05$) (Fig. 2A-B).

Table 1. Effects of *Sonchus asper* hydroalcoholic leaf extract (SALE) and apigenin-7-glucoside (Ap7G) on acute toxicity test.

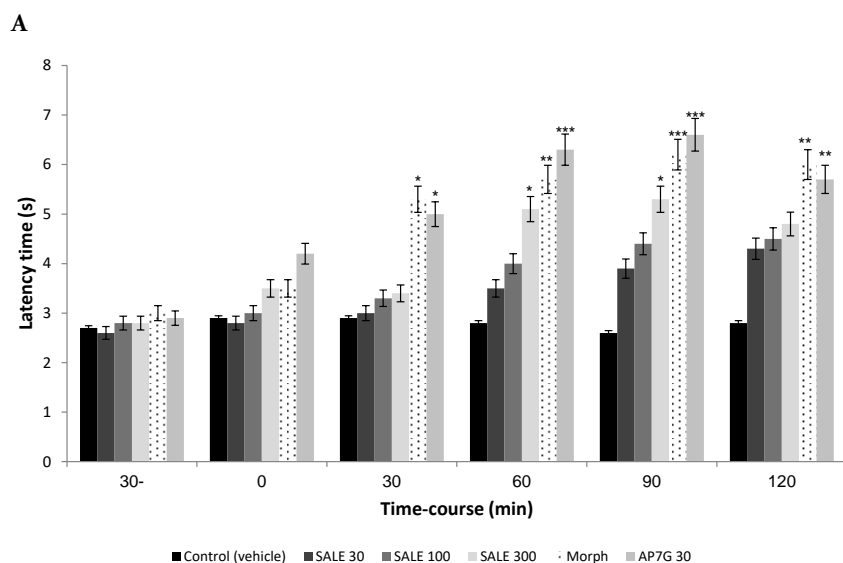
1 st part of investigation			2 nd part of investigation		
Substances	Doses (mg/kg)	Mortality ¹	Substances	Doses (mg/kg)	Mortality ¹
SALE	10	0/3	SALE	1600	0/11
	100	0/3		2900	0/11
	1000	0/3		5000	0/11
Ap7G	10	0/3	Ap7G	1600	0/11
	100	0/3		2900	0/11
	1000	0/3		5000	0/11
LD ₅₀ >5000 mg/kg					

¹Number of animals which died/Number of animals used.

Table 2. The effects of *Sonchus asper* hydroalcoholic leaf extracts (SALE), apigenin-7-glucoside (AP7G), and dexamethasone on inflammation induced by xylene in mice.

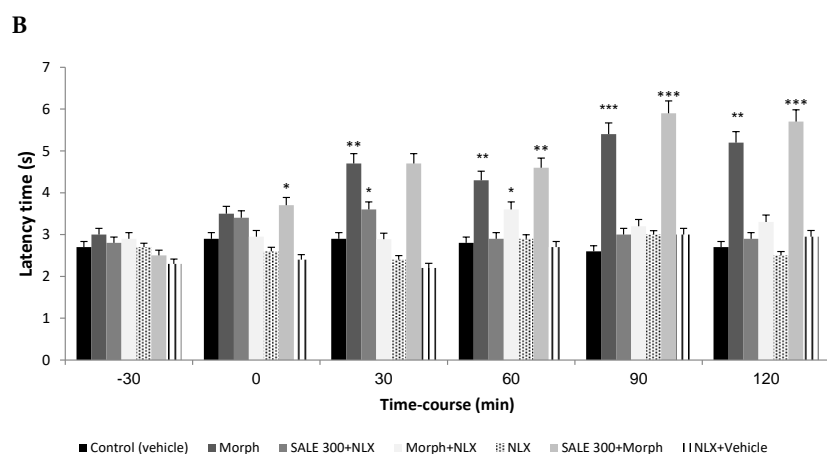
Group	Dosage (mg/kg)	Ear edema (mg)	Inhibition (%)
Control	10 mL/kg	7.6 ± 0.4	-
SALE	10	7.1 ± 0.1	6.0
	50	4.1 ± 0.8*	34.2
	100	3.8 ± 0.1**	47.9
AP7G	30	2.9 ± 0.6***	60.8
Dexamethasone	15	3.2 ± 0.3***	56.2

Each value represents the mean ± SEM of five mice. *P<0.05, **p<0.01 and ***p<0.001 as significant difference respect to the control group.

**Figure 1.** Antinociceptive effect of hydroalcoholic leaf extract of *Sonchus asper* in the tail flick test.

A. The effect dose-related of *Sonchus asper* hydroalcoholic leaf extract (SALE, 30, 100, 300 mg/kg, i.p.) in the tail-flick test with respect to control treated with vehicle (saline 0.9%, 10 mL/kg, i.p.) and included morphine (Morph, 1 mg/kg, i.p.) as reference compound and apigenin (AP7G, 30 mg/kg) as main compound. Each column represents the reactivity time of 5 animals per group as mean ± SEM. *P<0.05, **p<0.01 and ***p<0.001 as significant difference to control group.

B. The influence of the pre-treatment with naloxone (NLX, 1 mg/kg) on the anti-nociceptive effect of the *Sonchus asper* hydroalcoholic leaf extract (SALE, 300 mg/kg) in the tail-flick test. Morph (morphine, 1 mg/kg, i.p.), NLX (naloxone, 1 mg/kg, i.p.). Each column represents the reactivity time of 5 animals per group as mean ± SEM. *P<0.05 and ***p<0.001 as significant difference respect to control treated with vehicle.



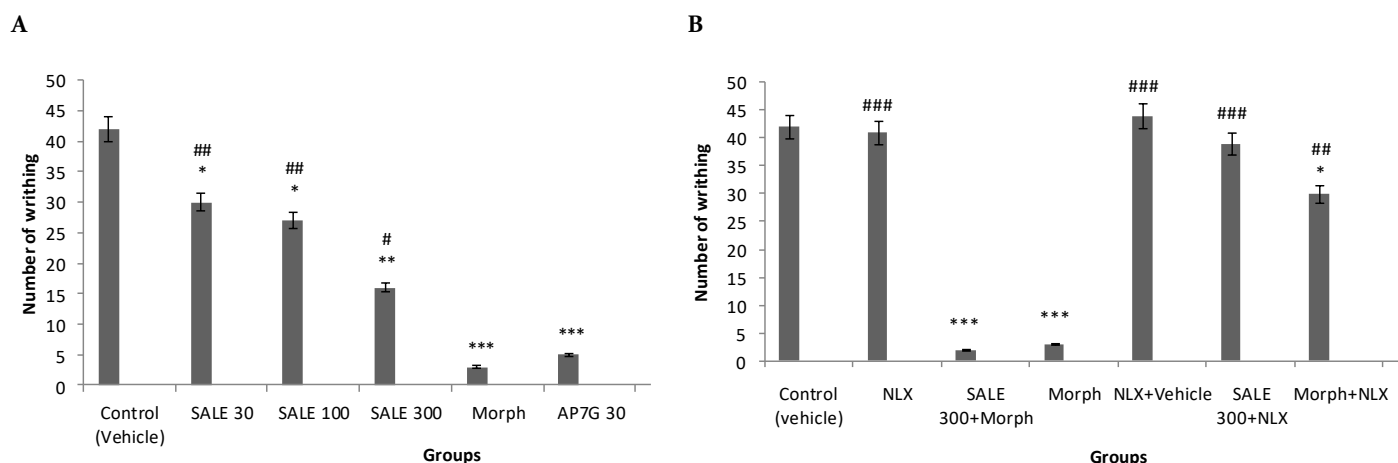


Figure 2. Antinociceptive effect of hydroalcoholic leaf extract of *Sonchus asper* in the writhing test.

A. The effect dose-related of *Sonchus asper* hydroalcoholic leaf extract (SALE, 30, 100, 300 mg/kg, i.p.) in the writhing test with respect control treated with vehicle (saline 0.9%, 10 mL/kg) and included morph (Morphine, 1 mg/kg, i.p.) as reference and apigenin (AP7G, 30 mg/kg, i.p.) as main compound. Each column represents the reactivity time of 5 animals per group as mean \pm SEM. * $P < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as significant difference to respect control treated with vehicle. # $P < 0.05$ and ## $p < 0.01$ as significant difference to morphine group.

B. The influence of the pre-treatment with naloxone (NLX) on the anti-nociceptive effect of the *Sonchus asper* hydroalcoholic leaf extract (SALE, 300 mg/kg, i.p.) in the writhing test. Morphine (Morph, 1 mg/kg, i.p.), naloxone (NLX, 1 mg/kg, i.p.). Each column represents the reactivity time of 5 animals per group as mean \pm SEM. * $P < 0.05$ and *** $p < 0.001$ as significant difference to respect control treated with vehicle. ### $P < 0.01$ and #### $p < 0.001$ as significant difference to morphine group.

Formalin test

According to results of this study (Fig. 3A-B), injection of a dose of 100 mg/kg of the extract in the tonic phase had a significant antinociceptive effect with $p < 0.05$ compared to control group. Injection of the dose of 300 mg/kg of the extract in both tonic and acute phases showed a significant difference in anti-nociceptive effects in comparison to control group ($p < 0.01$). Additionally, using morphine, like the 300 mg/kg dose of the extract, showed a substantial analgesic effect compared to control group in both tonic and acute phases ($p < 0.01$). Using naloxone and a high dose of the extract led to return the analgesic effects of the extract and injection of the dose of 30 mg/kg of the extract in both tonic and acute phases no showed anti-nociceptive effect in comparison to control group.

Glutamate-induced paw licking test

Figure 4 shows the analgesic effect of SALE in the glutamate-induced paw licking test. The measurements of combined behavioral reaction time

(licking time) for i.p. injection of both SALE 100 ($p < 0.05$) and 300 mg/kg ($p < 0.01$), and Ap7G ($p < 0.001$) were altogether shorter than control (DMSO-vehicle) group.

DISCUSSION

For the first time, this study reported the anti-nociceptive effect, in addition to the anti-inflammatory activity, of *Sonchus asper* hydroalcoholic extract as well as the contribution of one of its major component (apigenin-7-glucoside).

In a study carried out by Barros et al. (2006) the analgesic effect of *Pluchea quitoc* was proven in the acetic acid test. In the present study as well the SALE was proven to prevent abdominal constrictions caused by acetic acid, thus we speculate that the products systemically administered could modulate several targets peripheral and centrally.

These results show that SALE has anti-hypernociceptive effect in a model of viscerosomatic acute inflammation (McMahon et al., 2013).

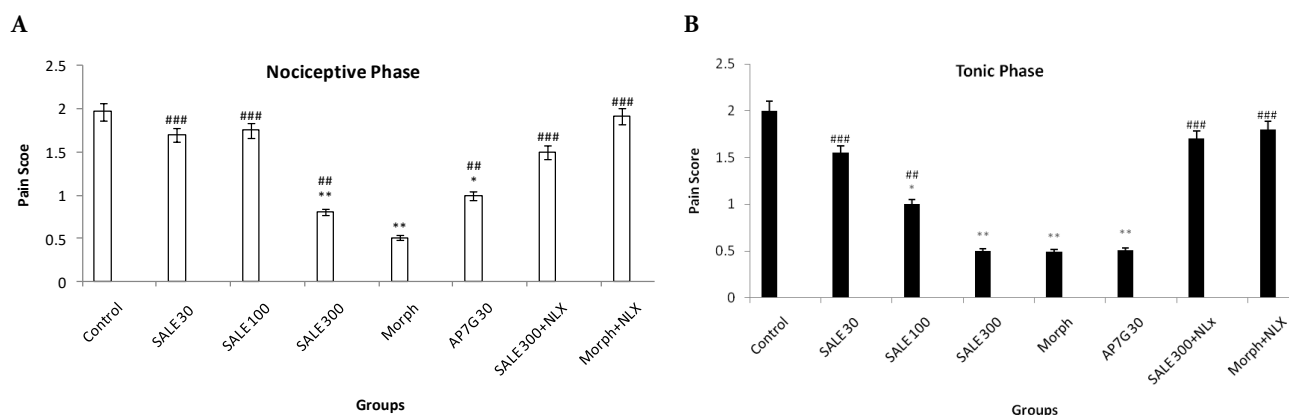


Figure 3. Antinociceptive effect of hydroalcoholic leaf extract of *Sonchus asper* in the formalin test.

A. Comparing the effects of *Sonchus asper* hydroalcoholic leaf extract (SALE, 30, 100, 300 mg/kg, i.p.), morphine (Morph, 1 mg/kg, i.p.), SALE 300+naloxone (NLX, 1 mg/kg, i.p.), morphine (Morph) + naloxone (NLX), apigenin-7-glucoside (AP7G, 30 mg/kg, i.p.) on nociceptive phase (phase I) of formalin test in mice. Each column represents the 5 animals per group as mean \pm SEM. * $P < 0.05$ and ** $p < 0.01$ as significant difference to control group. ## $P < 0.01$ and ### $p < 0.001$ as significant difference to morphine group.

B. Comparing the effects of *Sonchus asper* hydroalcoholic leaf extract (SALE, 30, 100, 300 mg/kg), morphine (Morph, 1 mg/kg, i.p.), SALE 300 mg/kg + naloxone (NLX, 1 mg/kg, i.p.), morphine (Morph) + naloxone (NLX), apigenin-7-glucoside (AP7G, 30 mg/kg, i.p.) on tonic phase (phase II) of formalin test in mice. Each column represents the 5 animals per group as mean \pm SEM. * $P < 0.05$ and ** $p < 0.01$ as significant difference to control group. ## $P < 0.01$ and ### $p < 0.001$ as significant difference to morphine group.

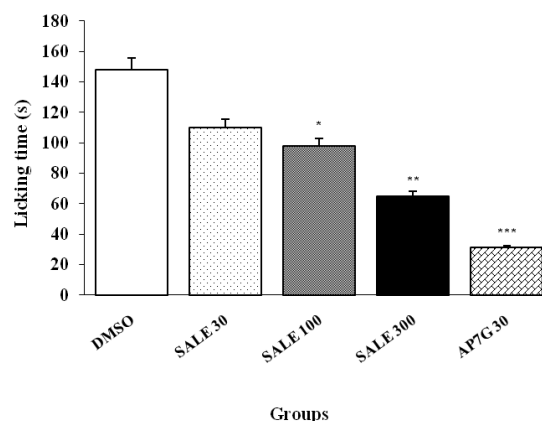


Figure 4: Effects of *Sonchus asper* hydroalcoholic leaf extract (SALE, 30, 100, 300 mg/kg, i.p.) and apigenin-7-glucoside (AP7G, 30 mg/kg, i.p.) on glutamate-induced paw licking model in mice.

Each column represents the reactivity time of 5 animals per group as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ represents the statistical difference between treated groups and control (vehicle or DMSO).

Various studies have attempted to assess the pain relief effects of different plants' extracts using thermal analgesia test; for instance, Mahmoodi et al. (2016) in which it was shown that the extract of *Rhus coriaria* can relieve pain in large doses. The results of the present study show that injection of SALE leads to decrease the thermal nociception.

Because the tail flick test is used to analyze spinal cord reflexes and identifying the central analgesic route (D'Amour and Smith, 1941) therefore, we could claim that the SALE has effect directly on neural system, according to these results. The positive results in TFT suggest that SALE could modulate nociceptive pathways, at least in part through of opioid receptors, but is impossible in this experiments elucidate the localization of action.

The formalin test has two phases, the first is the neurogenic (acute) phase that is brought about around active neurons pain under the direct effect of formalin and the second is the inflammatory (tonic) phase and is caused by sensitization of the dorsal horn of the spinal cord (Dubuisson and Dennis, 1978). In a study performed by Vilela et al. (2010) the anti-nociceptive effect of *Sonchus oleraceus* was proven by formalin test in which was shown that it can decrease pain in tonic phase and these effects are mainly because of flavonoids and alkaloids extant in the extract of the plant. The results of the present study show that the SALE has inhibitory effects on pain, and this effect is stronger on the tonic rather than the acute phase pain. The inhibition effect on the tonic phase pain in formalin test can be a result of inflammations that cause the release of compounds such as E2 and F2

prostaglandins that, at least in certain amounts, can lead to the sensitization of central neurons pain (Abbott et al., 1995). On the other hand, the positive results in phase II of formalin test and the other tonic models that utilized chemical irritants, which induces acute inflammation and sensitization, suggest that SALE could have a favorable action in conditions of pathological pain, particularly inflammation (alterations of nociceptive processing).

To evaluate interaction between the opioid system and the pain relief effect of the SALE, the naloxone, an opioid system antagonist, was used to prevent opioid receptors from being activated (Vaccarino et al., 1989). The results of the present study showed that naloxone reverse the analgesic effect of SALE. The effect is mediated at least in part by opioid receptors, because, several target are implicated in these model. Previously, it has been reported that AP7G is the major component of SALE. In this investigation is demonstrated that AP7G probably could decrease pain by means of the action on opioid system.

In try to explain the performance of glutamatergic system in the modulation by SALE antinociception, the extract was undergoing the glutamate-induced paw-licking test (Coderre, 1993). Experimental reports have manifested that the glutamate and glutamatergic receptors (both ionotropic and metabotropic glutamate receptors) are compatible in the peripheral, spinal, and supraspinal nociceptive neurotransmission, which is profoundly mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Haley et al., 1990). In debut, activation of glutamate receptors furthermore have been declared to underwrite to the assistance of peripheral nociceptive processes that are associated by all of inflammatory pain, which is concurrent with report that administration of glutamate receptor antagonist restricted the inflammatory, nonetheless not neurogenic phases of the formalin test (Petrenko et al., 2003). Based on our findings, glutamatergic system did impede the modulation of antinociception induced by SALE and AP7G.

The xylene test is one of the most useful models developed for assessment the anti-inflammatory candidates. After administration of xylene, the

expansion of arteries happens that leads to acute skin edema. According to the results of this study, the weight gain in mice ears were inhibited dependently of dose after injection of the extract. These results reveals the anti-inflammation effects of this extract and as mentioned, there are important chemical compounds in this plant, such as flavonoids, monoterpenes, sesquiterpenes, and diterpenes, that could be involve in the anti-inflammatory effects of SALE (Hussain et al., 2010).

In a study carried out by Shimizu et al. (1989) it was shown that the sesquiterpenoid compounds present in the plant cause a reduction in nitric oxide amounts. According to the results of the present research, the SALE caused a decrease in inflammation. One of the anti-inflammatory mechanisms could probably be through of the inhibition of nitric oxide, an inflammatory mediator.

As mentioned earlier, the *Sonchus asper* has important phytochemical compounds such as flavonoids (Guil-Guerrero et al., 1998). Some flavonoids have numerous anti-nociceptive and anti-inflammatory effects. In this study, the flavonoid AP7G showed significant analgesic and anti-inflammatory effects. Since flavonoids cause reduction in intracellular Ca^{2+} by inhibition of NMDA receptor that could decrease the presynaptic release of glutamate and neuropeptides and reduce post-synaptic excitability of the glutamatergic neurons. In addition, in another mechanism flavonoids activate of opioid receptors (especially μ opioid receptors) and finally could be affected its analgesic effects. The ideal analgesic drug restored the balance between excitatory inputs and inhibitory inputs on nociceptive pathways (Mahmoodi et al., 2016).

CONCLUSIONS

These results suggest that SALE and AP7G decrease nociceptive behaviors and inflammation in these algesimetric tests. A possible modulation of glutamatergic systems by opioid receptors at least in part, could be involved in the effect of extract. In addition, it can help resist pain and reduce sensitivity in the tonic phase as well as acute pain causing a reduction in pain through the inhibition of inflammatory mediators.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Zarei M	Mohammadi S	Shahidi S	Fallahzadeh AR
Concepts or ideas	X	X		
Design	X	X		
Definition of intellectual content	X	X		
Literature search		X		
Experimental studies		X		
Data acquisition		X		
Data analysis			X	
Statistical analysis		X		
Manuscript preparation	X			
Manuscript editing				X
Manuscript review		X		X

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