Antinociceptive & anti-inflammatory effects of *Berberis vulgaris* L. root's hydroalcoholic extract and determination of it's possible antinociceptive mechanism in male mice

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ABSTRACT

Berberis vulgaris L. (*B. vulgaris*) and specially its root have been used for a long time as a plant medicine in many countries including Iran. This plant is native in different parts of the world and is native in north areas of Iran like Khorasan. Recent research on this plant has shown its different therapeutic effects, Alkaloids especially Berberine has the most therapeutic usage among its compounds. This research is about the effects of *Berberis vulgaris* L. root's hydroalcoholic extract has done in two parts: its antinociceptive and anti-inflammatory effects was determined by Formalin and Xylene test respectively after determination of mortal plant dosage. Their dosage was 20, 40, 80 and 160 mg/kg. The three drugs include Naloxone 2 mg/kg, Dextromethorphan 20 mg/kg and L-NAME 10 mg/kg interact with more significant dosage of *B. vulgaris.* The results showed antinociceptive and anti-inflammatory effects of root's extract of *B. vulgaris* in acute and chronic phases of Formalin test. The extract efficiency was analyzed in part two through Formalin test by three drugs individually and also by root's extract. Conclusion: due to reduction of extract's antinociceptive effect in both acute and chronic phases after Naloxone injection, it may concluded that the extract shows its signs through opioid receptors.

Keywords: Formalin test; Xylene test; Naloxone; Dextromethorphan; L-NAME; hydroalcoholic extract; *Berberis vulgaris* L.; male mice

INTRODUCTION

Human being has been living in nature for accessing his feed and garment for centuries. Unfortunately, by industrial evolution, people have forgotten nature and it has made several problems for them. Now a day, returning toward nature has been improved. Removing and relief pain especially for chronic or irreversible pain was vital for human kind all the time. Chronic pain has made different problems for patient such as reducing concentration and abilities, reducing communication, depression, sleeping disorder and suicide [13]. Sometimes, acute pain in critical disease or cancer is so tiresome and doing operation to relief it may disconnect pain fibers [19]. Therefore using antinociceptive and anti-inflammatory drugs is obligate. Chemical anti-inflammatory and antinociceptive drugs divided to opioid antinociceptive (Enkephalin, Endrophin, Morphine, and Methadone, etc) and NSAIDs or nonsteroidal anti-inflammatory and antinociceptive drugs (Aspirin , Acetaminophen, etc) [18]. Meanwhile there are multiple narcotic drugs, their side effects threat patient in long term using. Some of these side effects are reducing drug efficiency, addiction, hormonal changes, increasing pain sensitivity and immunological disorders [5].

Herbal Medicines have created biological equilibrium due to their natural effective materials and accompanying with other compounds, which inhibit drug toxin accumulation in body. People, researchers and industries consider herbal

medicines a lot due to their low or non-side effects [1]. Because of the importance of antinociceptive and anti-inflammatory drugs, the emergency of more researches on herbal medicines for producing drugs that are more efficient could be understood. Meanwhile using herbal medicines is not completely without risk, their best usage should consider by more researches [6].

Berberis vulgaris L. is a member of Berberidaceae family, *Berberis vulgaris* L. is a shrub, compound leaves with stipules, bisexual flowers and bacciform fruit [31], yellow wood that its colour will be clear after cutting [7].

However, its fruit is used as condiment in Iran and has important role in the agricultural economy in Khorasan (Iran), its herbal usage is familiar for people too. Some of its remedy effects are healing diarrhea, reducing bilious fever, haemorrhage, gum swelling, sore throat as well as using it as poultice on wound and stroked spots [4].

Its dried leaves was used as vessels dilator, mouth wash and treatment of ascorbic disease but its root has more efficient function as blood purificator, declining melancholy, healing hepatitis, curing painful menstruation, diarrhea, heart arrhythmia, rheumatism, arthritic, nephritis and antipyretic [15]. As mentioned before, this investigation has done due to extensive treatment effects of hydroalcoholic extract of *B. vulgaris* roots as anti-inflammatory and antinociceptive by two experimental tests include Formalin and Xylene test and its antinociceptive mechanisms has been examined because of its importance as pain killer.

MATERIALS AND METHODS

Plant Collection and Desiccation

The root of *Berberis vulgaris* was collected from Kalat Naderi areas in north east of Iran, then desiccated in standard condition (without light, microbe infection and humidity) [12], thereafter identified as 527 ID number in department of Pharmacognosy, Faculty of Pharmacy, Medical Science of Shaheed Beheshti University.

Plant Extraction

The dried root milled first, then extraction done by maceration method. In this method after watering plant in 70% alcoholic solvent for 48 hours and filtering, the produced solution was desiccated in 40˚C and the extraction relative humidity was determined as 31.12 [8].

Animals

In this research, 260 male mice of NMRI with weighting 20-25 gr have used. They have kept in standard condition like light, temperature, free access to food and water. Mice were grouped incidentally: Formalin test group to examine extract antinociceptive effect, Xylene test group to measure extract anti-inflammatory effect, different groups related to drugs interaction with plant extract.

Determination of Lethal Dosage

Lethal dosage was determined to assign injection dosages. Lethal dosage or LD50 is the injection dosage of extract that kill 50% of animals. To assign this dosage, *B. vulgaris* hydroalcoholic root extract was injected to 10 groups each one with 10 members in 10 dosages: 1000, 833, 694, 578, 482, 401, 334, 279, 232, 193 mg/kg with arithmetical progression ½. The rate of killing was calculated by percentage after 24 hours. Lethal dosage was determined 400 mg/kg and by One tenth of the Lethal dosage in the geometric progression with ratio two, the extract injection dosage was assigned 20, 40, 80, 160 mg/kg [23].

Nociceptive and Inflammatory Tests

Formalin Test: this test used to determine antinociceptive effects. Plant extract injected in different dosage and 20mg/kg Morphine used as positive control. 0.02 ml of 1% Formalin solution injected subcutaneous to right palms of mice. Then mice were put in pain box. The box was 30×30×30 with a mirror in 45 degree position in bottom of box and in front of observant. There was a glass page in the box with a loss opening funnel on it. The animals observed 0-5 min for licking palm in acute phase [24] and 15-30 min for chronic phase in Formalin test. [9].

Xylene Test: this test used to produce swelling on mouse ear via Xylene. In this test, the sample animal was killed 2 hours after Xylene administration to frontal and dorsal area of its right ear. Then both right and left ears were cut and created 7mm slices on both ears, the difference between their weight which is an index for measuring inflammatory was cleared after weighting. Dexamethasone 15 mg/kg used as positive control [10].

Usable drugs for determining probable mechanism of extract antinociceptive function

Used drugs include: Dextromethorphan 20 mg/kg as NMDA receptors antagonist, Naloxone 2 mg/kg as opioid receptors antagonist, L-NAME 10 mg/kg as nitric oxide synthase enzyme inhibitor [11] which was injected to animal via intra peritoneum and 30 min before extract injection.

B. vulgaris root extract 160 mg/kg dosage was injected to animals half hour before test intraperitoneally [22].

Statistical Analysis

Data presented as the mean±SEM. The result was tested by One-Way ANOVA and Tukey post test. P-value < 0.05 was considered statistically significant.

RESULTS

***B. vulgaris* root extract effect on acute & chronic pain in Formalin test**

Comparison current results with positive control groups showed significant effects of high extract dosage specially 160 mg/kg in reducing acute & chronic pain(P<0.001).

There were not any significant differences between morphine receiving group and 160 mg/kg dosage experimental group (Table-1).

Table 1. Formalin Test : The effects of *B. vulgaris* hydroalcoholic root extract intraperitoneum injection on acute and chronic pain by Formalin test

Drug(mg/kg)	nociceptive Phase	Licking time(s)	Inhibition of nociception(%)
Sham (normal saline)	Acute	**147.12±2.96**	**-**
	Chronic	**82.87±4.04**	**-**
Morphine(20)	Acute	**39.12±2.55[a]**	**73.40**
	Chronic	**19.62±0.75[a]**	**76.32**
Extract (20)	Acute	**146.63±2.15**	**0.33**
	Chronic	**82.12±1.39**	**0.9**
Extract (40)	Acute	**123.75±5.64[a]**	**15.88**
	Chronic	**76.25±2.79**	**7.98**
Extract (80)	Acute	**61.12±3.53[a]**	**58.45**
	Chronic	**43.25±1.81[a]**	**47.80**
Extract (160)	Acute	**42.25±1.42[a]**	**71.28**
	Chronic	**22.75±0.81[a]**	**72.54**

a: difference with Sham (p<0.001)

The result of *B. vulgaris* Extract injection on inflammatory in Xylene test

B. vulgaris root hydroalcoholic extract injected to desired samples, especially by 20-40-80-160 mg/kg, reduced Xylene inflammatory effects in comparison to control and positive control groups. The results showed significant differences between right and left ears' weight in treatment groups which this differences was considerable in 160 mg/kg dosage (P<0.001). There weren't any significant differences between Dexamethasone receiver and 160 mg/kg dosage receiver group(Table-2).

Table 2. Xylene test : The effects of *B. vulgaris* hydroalcoholic root extract intraperitoneum injection on inflammatory by Xylene method to induce ear swelling.

Drug(mg/kg)	Weight difference of two ears (mg)	Inhibition of edema(%)
Sham (normal saline)	**6.07±0.21**	**-**
Dexamethasone (15)	**0.87±0.08[a]**	**85.66**
Extract (20)	**5.48±0.27**	**9.71**
Extract (40)	**3.5±0.67[a]**	**42.33**
Extract (80)	**2.61±0.13[a]**	**57.00**
Extract (160)	**0.73±0.08[a]**	**87.97**

a: difference with Sham (p<0.001)

The effect of NMDA receptors antagonist on antinociceptive arising from *B. vulgaris* root extract in Formalin test

Dextromethorphan was used to examine inhibition of NMDA receptors on painless resulting from *B. vulgaris*' root extract. The results showed any significant differences between extract and drug receiver group and extract receiver group individually. There were significance differences between drug receivers with Sham group in both phases but there were not any significance differences with extract receiver (Table 3).

Table 3. Dextromethorphan interaction by extract: The Effects of Drug on painless arising from *B. vulgaris* hydroalcoholic root extract (160 mg/kg) intraperitoneum injection on acute and chronic pain by formalin test.

Material(mg/kg)	Pain Phase	Licking time(s)	Inhibition of pain(%)
Sham (normal saline)	Acute	**147.12±2.96**	**-**
	Chronic	**82.87±4.04**	**-**
Extract(160)	Acute	**42.25±1.42^{A}**	**71.28**
	Chronic	**22.75±0.81^{A}**	**72.54**
Dextromethorphan(20)	Acute	**136.12±2.18^{Ba}**	**7.47**
	Chronic	**75.75±2.05^{Ba}**	**8.59**
Dextromethorphan(20) & Extract(160)	Acute	**46.12±1.46^{A}**	**68.65**
	Chronic	**26±1.3^{A}**	**68.62**

A: difference with Sham group ($p<0.001$).
a: difference with Sham group ($p<0.05$).
B: differences with drug and extract receiver group ($p<0.001$).

Antagonist effects of opioid receptors on antinociceptive arising from *B. vulgaris* Root extract in Formalin test

Naloxone used in this research. The result showed that using Naloxone with extract significantly causes reducing and prevention of extract painless effects in both acute and chronic phases. There was significance difference between extract receiver with extract and drug receiver group .There were not significance differences between drug and extract receiver with Sham group in both phases but there were significance differences with extract receiver (Table 4).

Table-4. Naloxone interaction by extract : The effects of drug on painless arising from *B. vulgaris* hydroalcoholic root extract (160 mg/kg) intraperitoneum injection on acute and chronic pain by Formalin test.

Material(mg/kg)	Pain Phase	Licking time(s)	Inhibition of pain(%)
Sham (normal saline)	Acute	**147.12±2.96**	**-**
	Chronic	**82.87±4.04**	**-**
Extract(160)	Acute	**42.25±1.42^{AB}**	**71.28**
	Chronic	**22.75±0.81^{AB}**	**72.54**
Naloxone(2)	Acute	**139.38±1.87**	**5.26**
	Chronic	**77.37±1.33**	**6.63**
Naloxone(2) & Extract(160)	Acute	**138.88±2.31**	**5.60**
	Chronic	**79.25±1.64**	**4.36**

A: difference with Sham group ($p<0.001$).
B: differences with drug and extract receiver group($p<0.001$).

Effects of Nitric Oxide synthesis enzyme inhibitor (L-NAME) on antinociceptive arising from *B. vulgaris* Root extract in formalin test

L-NAME(N-Nitro-L-Arginine Methyl Ester) was used in this research. There weren't any significance differences between drug and extract injection with extract injection individually. This result has been observed in acute and chronic phase. There were significance differences between drug and extract receiver with Sham group in both phases but there weren't any significance differences with extract receiver alone (Table5).

Table-5. L-NAME interaction by extract: The Effects of Drug on painless arising from *B. vulgaris* hydroalcoholic root extract (160 mg/kg) intraperitoneum injection on acute and chronic pain by formalin test.

Material(mg/kg)	Pain Phase	Licking time(s)	Inhibition of pain(%)
Sham (normal saline)	Acute	**147.12±2.96^{B}**	-
	Chronic	**82.87±4.04^{B}**	-
Extract(160)	Acute	**42.25±1.42**	**71.28**
	Chronic	**22.75±0.81**	**72.54**
L-NAME(10)	Acute	**139.62±1.83^{B}**	**5.09**
	Chronic	**75.62±1.11^{B}**	**8.74**
L-NAME (10) & Extract(160)	Acute	**39±1.36^{A}**	**73.49**
	Chronic	**21±0.75^{A}**	**74.65**

A: difference with Sham group ($p<0.001$).
B: differences with drug and extract receiver group($p<0.001$).

DISCUSSION

Berberis vulgaris L. specially its root has been used for a long time as plant medicine in many countries like Iran. In some other countries like Bulgaria, its root used as healing chronic hepatitis, gall bladder, nephritis and arthritic. It has been used due to its Berberine alkaloid for healing leukopenia arising from chemotherapy and radiotherapy in some countries like China and Japan since 1970s [16]. Root chemical analysing by HPLC showed 1.24% and 2.5% Berberine and Berbamin respectively [20]. In this research, the most effective curing of *B. vulgaris* attributed to Berberine. In another investigation, the rate of Berberine in root was determined 3% which confirm the importance of root in herbal medicine [21].Other than alkaloids which are the most abundant compounds in this plant.There are some other chemical materials in lower concentration. *B. vulgaris*' root and shoot have sugar, resin, mucilage, pectin as well as vitamin C in root's epidermis [17], Myristic acid (resin by astringent flavour), garlic acid, talic acid and essential oils can be found in *B. vulgaris* too [2].Current research has been done in two phases. In first part antinociceptive and anti-inflammatory effects of *Berberis vulgaris* L. hydroalcoholic root extract was examined through Formalin test (for measuring antinociceptive effects) and Xylene test (for determining anti-inflammatory effects). In second part due to positive effects of antinociceptive and anti-inflammatory *B. vulgaris* root extract, the interaction between the extract with 3 drugs include Dextromethorphan, Naloxone and L-NAME was used to realize its mechanism. In first part antinociceptive and anti-inflammatory effects of extract was completely obvious. In Formalin test higher extract dosage (80 and 160 mg/kg) showed antinociceptive effect in both acute and chronic phases ($P<0.001$). Response accession occurs in both acute and chronic phases, which is one the Formalin test characters and is due to its mechanism. The first phase starts immediately after Formalin injection and continues for 5 minutes via presence of substance P, bradykinin and chemical stimulus of nociceptors [29].

In second phase, which started 15-40 minutes after Formalin injection, histamine, serotonin and prostaglandins act on inflammatory process, therefore chronic pain spreading may be due to inflammatory function. Compounds like Morphine, which effects on central nervous receptors can inhibit both phases, but some drugs such as Aspirin effects on peripheral nervous system then can inhibit the second phase [27]. In fact, inflammatory drugs restrict the pain which arising from swelling by inhibition of prostaglandin producing [3].

Anti-inflammatory effect of *B. vulgaris* was shown in all dosage especially in 160mg/kg in Xylene test ($P<0.001$). Xylene is a compound that cause neuroma and release special compounds from the end of sensory neurons which cause inflammatory [28]. In this inflammatory mechanism some materials like neuropeptide release from terminal of sensory neurons which are the most important inflammatory promoters [26].

In second part of research, opioid's receptors antagonist with extract cease antinociceptive effect in both acute and chronic phase, but the

extract antinociceptive effects don't change by nitric oxide enzyme and NMDA receptor antagonist. Because of nitric oxide inhibitors, namely L-NAME [30] couldn't improve extract antinociceptive effects, it can be prospect that probably extract induces its antinociceptive effect through another mechanism.

Opioid receptors affect directly on spinal and Supraspinal Nervous System, therefore general prescription of these receptors'antagonists namely Naloxone inhibit antinociceptive drugs like Morphine and even induce algesia [25], probably *B. vulgaris*' root extract acts through these receptors. This result is equal in two phases due to inhibition of extract antinociceptive effect by Naloxone, suggested that *B. vulgaris*' root extract induce its antinociceptive effect through opioid receptors. As mentioned before chronic phase of Formalin test was due to inflammatory mechanism activation by Formalin test and inhibited by no steroid drugs like Aspirin and Indomethacin [14].

Opioid antagonist induce analgesia through junction to specific receptors, which are joined to G proteins, these receptors are mainly in parts of brain and spinal, which are related in pain transition. Opioid receptors are divided in three groups: μ, κ and δ. Opioid receptors form a protein group in molecular level which are joined to G protein and effect on opening ion channels and regulate intra cellular transition and protein phosphorylation. General antagonist of these receptors namely Naloxone is able to inhibit antinociception arising from opioid receptors. This drug has high affinity to μ and lower to δ and κ receptors. Receptors blocking prevent receptors antagonist function like Morphine [18] and probably *B. vulgaris*' root extract.Because of non-significant differences between Dextromethorphan (NMDA receptors antagonist) accompanied by extract injection in comparison to extract injection individually, it may concluded that extract antinociceptive route is different from NMDA receptors. It is applicable for L-NAME too. This drug (which inhibits nitric oxide synthase enzyme) could not induce antinociception in extract function so the extract route may be different. As a consequence of this research, The antinociceptive and anti-inflammatory effects of *B. vulgaris*' hydro alcoholic root was recognized and probable root 'extract antinociceptive function was determined as opioid receptor, therefore more exploitation of *B. vulgaris* is expected due to its expanded usage.

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