Analgesic and anti-inflammatory effects of methanol extracts of aerial parts Artemisia aucheri in mice (Balb/c).

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Abstract: Considering the fact that Analgesic and anti-inflammatory drugs have a wide range of side effects, Therefore, research on the medicinal properties of plants to explore the effects of drugs with similar or even better than chemical drugs with fewer side effects is of great importance. Therefore, the present study was carried out to study the anti-inflammatory and Analgesic properties of methanolic extract Aerial parts of Artemisia aucheri. In this Experimental study formalin test and xylene test were used to study the anti-inflammatory and Analgesic properties of this plant. In each test 30 Balb/c mice which their weight ranged from 20 to 25 gram were randomly divided into 5 groups each including 6 mice, namely the negative control group (receives phosphate buffered saline and Tween 80%), the positive control group in formalin test (receives 10 mg/Kg morphine), the control group in xylene test (receives 15 mg/Kg Dexamethasone), and 3 experimental groups. The methanolic extract of Aerial parts of Artemisia aucheri Boiss was prepared and different doses (100, 200, 300 mg/Kg) were intraperitoneally injected into the mice of the experimental groups. The results data were analyzed by statistical tests. Results showed that, Compared to the negative control group, three different concentrations of the methanolic extract of Artemisia aucheri Boiss (100, 200, 300 mg/Kg) significantly reduced acute pain, and its highest concentration (300 mg/Kg) reduced the inflammation resulted from xylene while none of these extracts were effective in chronic phase. Considering the finding, In formalin test the methanolic extract of Artemisia aucheri Boiss has anti-inflammatory and Analgesic in acute phase which seems to be related to the sterol, flavonoids and camphor.

Keywords: Analgesic, anti inflammation, Artemisia aucheri, methanolic extract

Introduction
Pain and Edema are form main symptoms of inflammatory diseases (Tekieh et al. 2012). Pain is the most common symptom which forces people to visit doctors (Howard & Mrtin. 1998) which can be caused by various reasons such as destruction and damage to a tissue due to physical factors such as heat, impact, tear, elongation and electricity or chemical reasons such as poisons, caustic, neurotransmitter or pathological processes such as ischemia, necrosis, inflammation, spasms or organic disease such as neuropathy, and bone fracture (Goldman & Bennett. 2000).

Pain is felt in two major types of acute - fast and slow chronic (Zamanigandomani & Forouzandeh.2014, Morshed et al. 2010). In fact, pain is used as protective against tissue damages (Yazdian & Irani. 2015, repeat. 2014). Depending on whether pain is fast or slow, the place of interpretation is different: fast pains are interpreted in brain cortex whereas chronic slow pains are interpreted in thalamus (Azarm et al. 1997).

Inflammation is a dynamic complex process which begins in response to tissue damage or infection (Bishop-Bailey & Bystrom, 2009). It has also acute and chronic forms.

Analgesics and anti-inflammatory chemical drugs are divided into narcotic analgesics (enkephalins, endorphins, morphine, methadone, etc.), and Non-steroidal anti-inflammatory and analgesic (aspirin, acetaminophen, etc.) (Yazdian & Irani. 2015).

Extant analgesics and anti-inflammatory drugs cause wide range of side effects (Kiakojouri et al. 2013). For example, steroidal anti-inflammatory drugs enforce their effects by controlling cyclooxygenase enzyme. Cox-1 is produced mainly in physiological situation of body and inhibiting it can lead to unwanted side effects (Haghirebrahimabadi & Irannejad. 1996). But Cox-2 is created by the induction of cytokines and plays a role in the development of inflammation (Hauber Gameir et al. 2008) and inhibiting it controls the inflammation. Primary anti-inflammatory drugs inhibit both forms of the enzyme non-specifically and therefore have side effects of controlling Cox-1. But in recent years, new derivatives have been produced which control cox-2 specifically and do not have side effects of controlling Cox-1 (Haghirebrahimabadi & Irannejad. 1996).
Undesired effects of these drugs on digestion system may limit using them. Opioids leads to nausea and constipation and are addictive in long-term use (Dashtiratmatabadi. 1999).

These issues encouraged scientists to find inexpensive, widely available drugs without side effects. In this regard, medicinal plants have been considered a lot. medicinal plants have fewer side effects than chemical drugs due to existence of attachment compounds. It is believed that most natural ingredients especially herbs can be the source of new compounds because most of famous current compounds such as aspirin, atropine, morphine and cocaine have been produced from analgesic plants (Zamanigandomani & Forouzandeh.2014).

Artemisia is from the Compositae family which has 200-400 species worldwide in various climatic regions. In Iran, 34 species of this plant exist (Massry et al.2002).

Mountain Artemisia (Artemisia aucheri) is a shrub plant with 25 to 50 cm height, with many stalks, oval or nearly round leaves, yellow flowers and capitulum inflorescence and dried nut. This plant is found in northern Iran (Ghahreman.2000). The goal of this study was investigating anti-inflammatory and analgesic effects of Aerial parts of Artemisia aucheri aucheri as herbal source of traditional medicine.

Materials and methods
Preparation of methanol extract

Maceration method was used to extract Aerial parts of Artemisia aucheri. Mountains Artemisia aucheri were prepared from Kashan countries and dried flowers were powdered using electrical mill. Taxonomic identification was confirmed by herbarium department of Islamic Azad university (Falavarjan Branch).

Thirty grams of powder was dissolved in 250ml of solvent (80cc of Merck methanol and 20 cc of twice distilled water- Ghatranshimi) in an erlen. The erlen was covered with aluminum foil and placed on shaker at room temperature for 72 hours. After that, solution was filtered and herbal part was isolated from alcohol. Methanol extract was poured in plates to dry and was refrigerated.

Animals

Sixty mice (Balb/C) from 20-25g weight range were selected and kept in a room at 22±2°C and 12:12 hours photoperiod. All Principles of Neuroscience Research Center Ethics Committee relating to the use of laboratory animals were considered such as free access to food and water, preventing surgery pain, using standard methods of killing animals, no additional animals in each treatment group. Mice were kept for one week before experiment to adapt to environment. polycarbonate cages with stainless steel mesh lid.

Xylene anti-inflammatory test and formalin analgesic test were carried out on mice.

Both experiments had following groups, each group consisting of 6 mice which were randomly.

Negative control which received 0.2ml injection of phosphate buffered saline and Tween 80 % (Merck) (with a ratio of 1:4).

Positive control: for pain received 10mg/kg of morphine; for inflammation received 15mg/kg dexamethasone (Daroupakhsh. Iran).

Experimental groups which received 100, 200 and 300 mg/kg of methanol extract dissolved in phosphate buffered saline and Tween 80 %. All injections were intraperitoneal, single dose, and 15 minutes before formalin or xylene test (Nasri et al. 1998, Hosseinzadeh. 2000).

Formalin test

This test is one of the most unique tests of pain evaluating which can study can evaluate the analgesic effects in both acute or chronic stages (Zandiesfahan et al. 2014). In this test, two obvious pain stages are observed: in first phase (neurogenic pain) P matter and bradykinin and in second phase (environmental, inflammatory pain) histamines and prostaglandins play role which this can itself show inflammatory aspect of second stage (Hudger et al .2011).

In formalin test, a glass table was used with an animal holding box and a mirror with 45° angle Beneath it (for observing movements of animal). Before each experiment, the animals were weighed accurately. Injection was done intraperitoneal and animal was placed in observation box 15 minutes before test to adapt to new environment (Dashtirahmatabadi. 2001).

After that 0.02ml of 2.5% formalin (Ghatranshimi) was injected under the skin of right paw subcutaneously. animal was returned to test box again (Nasri et al. 2008) and its responses to painful stimulus were recorded for one hour (Dashtirahmatabadi.2009). To minimize observer errors, camcorder was used and then movies were observed precisely (Kiakojouri et al. 2013).

Licking and biting the leg during the 0-5 minutes (as the initial acute phase) and then 15-60 minutes (as secondary chronic phase) after injection of formalin were considered by scoring as an indicator of pain (Tekieh et al. 2012).
Scoring animal behavior

Every 15 seconds, pain was scored as 0, 1, 2, and 3 degrees according to Dubuisson Dennis method (Nasri et al. 2013):
Zero: animal sits or walks and walk in perfect balance without paying attention to injected leg.
One: animals has incomplete touch with and use its non-injected leg more.
Two: animal holds its painful toe up completely.
Three: animal licks, bites or severely shakes its injected toe because of severe pain.

Xylene test

Xylene test was used to investigate anti-inflammatory effects; Xylene is a stimulating substance that can cause inflammation locally. Inflammation was enforced on right ear of Syrian mice and the difference between weights of left and right ears considered as inflammation amount (Zandiesfahan et al. 2014). 15 minutes after mentioned intraperitoneal injections, 0.03 ml of xylene (Ghatranshimi) was injected in the frontal and dorsal right ear and two hours later animal was killed. Then, both ears were separated and 7 mm slices of ears were separated (from injecting places), weighed and weight difference was calculated. Higher weight difference shows higher inflammation (Nasri et al. 2008, Hudger et al. 2011).

Statistical analysis

Obtained data were analyzed using SPSS 18. Results were presented as average ± statistical deviation. One-way analysis of various, LSD test and Duncan test were used at five percent probability level.

Results

Acute pain (formalin test)

Variance analysis of data showed that extract in first five-minute reduced pain significantly in all doses in proportion to negative control (P<0.001). On the other hand, 200mg/kg dose did not show significant difference with morphine which shows this dose has been effective in reducing pain. The highest analgesic effect of extract was related to 200 mg/kg dose(graph1).

Chronic pain (formalin test)

Results showed that extract in 100, 200 and 300mg/kg doses of methanol extract could not affect chronic phase of pain (graph 2).

Inflammation (xylene test)

Injection of various doses of Artemisia extract and comparing with control group showed that 300mg/kg group reduced inflammation significantly (P<0.05) and was significantly different from control group but not from positive control group (graph 3).

Graph 1. Comparison of acute pain intensity in treatment groups .
Methanol extract significantly reduced acute pain in proportion to control group, this effect was more obvious in 200 mg/kg dose(***: P<0.001).
Graph 2. Comparison of chronic pain intensity in treatment groups. Methanol extract could not reduce chronic pain in proportion to control group (***: P<0.001, *: P<0.05).

Graph 3. Comparison of inflammation in treatment groups. Methanol extract in 300 mg/kg dose significantly reduced inflammation in proportion to control group (*: P<0.05).

Discussion

The ability of Artemisia aucheri in controlling pain and inflammation was investigated in current study. According to results, extract of this plant reduced acute pain and the highest analgesic effect was observed in 200mg/kg dose and also 300mg/kg dose had the highest anti-inflammation effect but chronic pain was not affected by this plant.

Main compounds of Artemisia are various flavonoids such as quercetin and rotenoide with the cleaning power of oxygen free radicals and antioxidant properties and also santonian and coumarin compounds (Rao et al. 2008), sterols, polyacetylenes, tannins, glycosides, saponins, bitter substances, lipids and carbohydrates are other compounds of this plant (Arjmand. 2013, Jafaridinani et al. 1996). Flavonoids are natural polyphenols in plants with analgesic, anti-inflammation effects (Balasubramanian & Eckert. 2006).

Flavonoids are from nitric oxide synthase enzyme inhibitors which reduce Intracellular calcium by controlling the activity of N-methyl D-aspartate (NMDA) receptors. Following that, the activity of nitric oxide synthase and calcium-
dependent phospholipase A2 is reduced and therefore by reducing nitric-oxide and prostaglandin amounts, analgesic effects of plant are appeared. Actually, prevent production of prostaglandin E from arachidonic in response to inflammatory stimuli by controlling Cox-2 enzyme (Hudger et al. 2011).

Controlling effects of flavonoids on acute and chronic inflammation is because of the effect on signaling pathways, including the activity of nuclear factor kappa B and MAP kinase phosphorylation. Furthermore, flavonoids reduce acute and chronic inflammation by reducing accumulation of fluid lipids that are essential for signaling pain. Therefore, flavonoids reduce chronic and acute inflammation by inhibiting the aggregation of receptors and signaling cascade (Yazdian and Irani. 2015).

Nasri et al. (2013) announced that analgesic and anti-inflammatory effects of root and stem of Scrophularia striata Boiss was related to its flavonoids especially quercetin which act via controlling phospholipase A2, Lipoxygenase and nitric oxide synthase. Yan (2008) reported that methanol extract of artemisia vestita had inflammatory activity which was due to existence of flavone derivations including apigenin. Therefore, probably analgesic and anti-inflammatory effects of Artemisia aucheri are because of extract flavonoid including quercetin and apigenin.

In a study about headache treatment, using traditional medicine of Iran, announced that camphor has been used in ancient medicine of Iran as a sedative in the treatment of one-sided headache locally or rectal (Gorji et al. 2003). Also, Hoaxing et al. In their study (2005) reported that camphor led to insensitivity of (TRPV1) channels in heat-sensitive receptors and reduced the Productive potential of them.

Nasri et al. (2008) reported that camphor amount of tanacetum partheniun plant in leaves and flowers was more than 35% which is the reason of its perfect effect as anesthetic and anti-inflammatory drug for treatment of headache, migraine and menstrual pain, rheumatism and arthritis. Maybe, analgesic and anti-inflammatory effects of Artemisia can be because of these compound.

In Ribas et al. study (2008), using pain induced by glutamate was found that scopoletin (a coumarin) and alpha-Spinasterol (a type of plant sterols) in extract are responsible for analgesic effects of herbal products. It was also found that this effect is enforced through blocking glutamate receptors and partial inhibition of proinflammatory cytokines (IL-1β, TNF α). According to results of this study a sterol was responsible for analgesic effects and its probable mechanism was disruption in glutamate pathway and calcium moves out of the cell membranes of neurons (Ribas et al. 2008). So, sterol can be another factor of analgesic and anti-inflammatory activity of Artemisia aucheri although in spite of its small amounts.

Probably, there are also compounds in Artemisia extract which prevent analgesic effect in chronic phase and anti-inflammatory effect in 100 and 200 mg/kg doses.

Conclusion
Methanol extract of aerial parts Artemisia aucheri, reduced acute pain in formalin test significantly. It also had anti-inflammatory effects in proportion to negative control group. It seems that compounds such as flavonoids, camphor and sterol are reasons of its analgesic and anti-inflammatory properties.

To determine analgesic and anti-inflammatory mechanisms, and their effects, more studies are necessary. Also, more studies with other pain and inflammation evaluating tests seems necessary. Also, it seems that more studies with other pain and inflammation evaluating tests are necessary to determine effective dose of Artemisia plant.

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References


1 - Interleukin-1

2 - tumor necrosis factor alpha
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