Antinociceptive Properties of Hydro-Alcoholic Extract of Calendula Officinalis in Rat

Siamak Shahidi[1], Minoo Mahmoodi[2], Noushin Farahmandlou[3*]

1. Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamedan, Iran.
2. Department of Biology, Islamic Azad University, Hamedan Branch, Hamedan, Iran.

Article info:
Received: 24 March 2012
First Revision: 22 April 2012
Accepted: 17 September 2012

Key Words:
Calendula Officinalis,
Antinociception,
Writhing,
Tail Flick,
Rat.

ABSTRACT

Calendula Officinalis (Asteraceae) is widely used in traditional medicine as an anti-inflammatory agent and has also been reported to have anti-bacterial, anti-fungal and anti-viral activities. Sesquiterpene glycosides, saponins, triol, triterpenes and flavonoids are observed in its composition. The present study was designed to evaluate the antinociceptive effect of hydro-alcoholic extract of Calendula Officinalis in male rats. The animals were treated intraperitoneally with different doses of the Calendula Officinalis flower extract (100, 150 and 250 mg/kg body weight). On the basis of the previous report the dose of 150 mg/kg is most effective. The analgesic activity was tested by tail flick and acetic acid-induced writhing tests. Data between experimental groups were compared by one way analysis of variance (ANOVA) followed by tukey's as post hoc test. All doses of the extract and also naloxone + extract (150mg/kg) significantly increased the tail flick latency compared to the control group. The extract of Calendula officinalis significantly reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid. Naloxone + extract (150mg/kg) significantly increased the number of writhing. From the results it could be concluded that the Calendula Officinalis extract exhibits anti-nociceptive activity. Analgesic effects of Calendula Officinalis have the same pathway as opioids, but just in the peripheral test (acetic acid-induced writhing test).

1. Introduction

Pain is a major symptom in many medical conditions, and it is the most common reason for medical consultation. Approximately one-half of all licensed drugs that were registered worldwide in the 25 years period prior to 2007 were natural products or their synthetic derivatives (David et al., 2011). More than 100 plants are known to have pain-relieving properties, but some are really outstanding (Mohammed, 2009). Calendula Officinalis, belonging to the family of Asteraceae, commonly known as English marigold or Pot Marigold is an aromatic herb which is used in traditional medicine (Chakraborthy et al., 2010). It has been reported to possess many pharmacological activities, which include antioxidant (Preethi et al., 2006), anti-inflammatory (Della et al., 1994; Zitterl et al., 1997), antibacterial (Dumenil et al., 1980), antifungal (Kasiram et al., 2000; Iauk et al., 2003) and antiviral (Barbour et al., 2004). This plant is rich in many pharmaceutical active ingredients like carotenoids, flavonoids, glycosides, steroids and sterols which have some modulatory role on nociception. This study was undertaken to evaluate the anti- nociceptive effect of Calendula Officinalis hydro-alcoholic extract by using acetic acid induced writhing test and tail flick response in the Wistar male rats.

**** Corresponding Author:***
Noushin Farahmandlou, PhD
Department of Biology, Islamic Azad University, Hamedan Branch Hamaden, Iran
Tel: +988118249463
E-mail: nfarahmandlou@yahoo.com

2. Methods

2.1. Preparation of the Extract

Fresh flowers of Calendula Officinalis were collected from the campus of the herbal garden in Iran –Hamedan, identified and authenticated. The flower parts were cleaned, dried under shade and powdered by a mechanical grinder. The powdered flower was soaked with ethanol and water (80: 20) for 72 hours. The resultant extract was filtered. The filtered extract was concentrated to dryness in a rotary evaporator under reduced pressure at a constant temperature of 50°C. The dried mass was stored in a refrigerator and considered as the extract.

2.2. Animals and Experimental Groups

Wistar male rats (250-300g) were purchased from the Pasteur institute of Iran. They were housed in standard polypropylene cage and kept under controlled room temperature (20 ± 20C; relative humidity 40- 60%) in a 12 hours light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Each rat was used only once. All procedures for the treatment of animals were approved by the research committee of the Islamic Azad University, Hamedan branch and were done according to the guide for care and use of laboratory animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1985). The rats were randomly divided into six experimental groups: control group; second, third, and fourth groups received intraperitoneal (i.p) injection of Calendula Officinalis extract at doses of 100 (cal100), 150 (cal150) and 250 (cal250) mg/kg, respectively; fifth group received morphine (1 mg/kg, i.p); and the sixth group received naloxone and extract of Calendula Officinalis at doses of 150 mg/ kg. The volume of injection was set at 10 mL/kg.

2.3. Nociceptive Testing

2.3.1. Tail Flick Test (TFT)

Nociception was assessed using the radiant heat tail flick test (Dogrul et al., 2007). The rat tail was marked with a pen about 2 cm from the tip and the light beam was focused on this marked site (Dogrul et al., 2007). Baseline tail flick latency for each rat was determined. The intensity of light was adjusted so that baseline latencies were 2-3 seconds, with a cutoff time of 10 seconds to prevent tissue damage. Animals were restrained and the drugs, extract or normal saline were injected intraperitonealy (i.p). Tail flick latencies were measured before and 20 min after drug, extract or saline injections.

2.3.2. Writhing Test

The method of Santos (2005) was used to measure writhing response (Santos et al., 2005). Visceral nociceptive response was induced by i.p injection of 0.06% solution of acetic acid. The numbers of writhing were recorded during the first 30 minutes after acetic acid injection. Each writhe consists of contractions of the abdominal musculature followed by extension of the posterior limbs. A reduction in the writhing numbers as compared to the control group was considered as the evidence for analgesia. Experimental groups were the same as in the tail flick test.

2.4. Statistical Analysis

The data of the tail flick latency was analyzed within each group using the paired t-test. Comparison of data between experimental groups of tail flick or writhing tests were analyzed by one way analysis of variance (ANOVA) followed by tukey's as post hoc test for multiple comparisons. P-values less than 0.05 were considered as statistically significant. Results were presented as mean ± SEM.

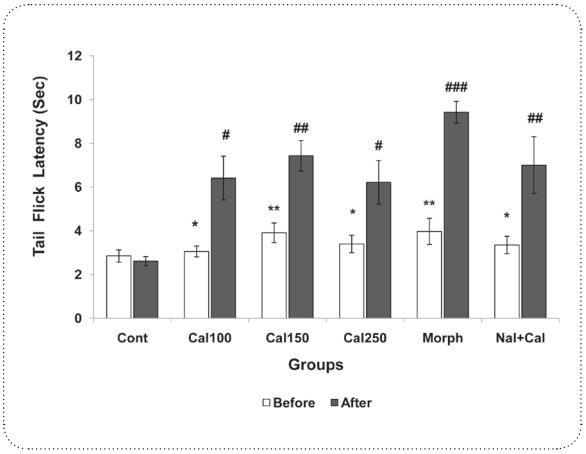
3. Results

3.1. Tail flick

Statistical analysis using one-way ANOVA indicated that all doses of the extract and naloxone + extract, significantly (cal 100 and cal 250, $p<0.05$; cal 150, $p<0.01$; nal+cal 150, $p<0.05$) increased the TFT time compared to the control. There was no significant difference in the tail flick latency between cal 150 and cal 150 + naloxone treated groups. The result of tail flick test is shown in figure 1.

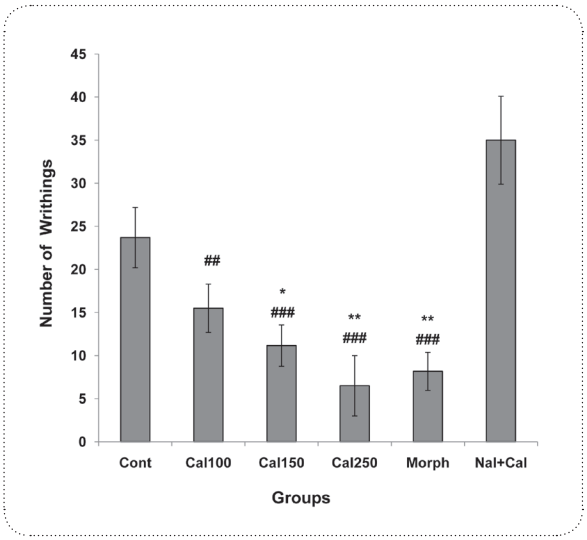
3.2. Writhing Test

Calendula Officinalis (cal 150; $p<0.05$, cal 250; $p<0.01$) significantly reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid (cal 100; $p>0.05$) (Figure 2). The hydro-alcoholic extract of the Calendula Officinalis (250 mg/kg i.p) exhibited greater activity, which was comparable with the standard drug morphine. The decreased number of writhes by cal 150 was reversed by naloxone.



NEUROSCIENCE

Figure 1. Rat tail-flick response before and 20 minutes after intraperitoneal administration of morph, nal+cal 150 and various doses of the extract of calendula officinalis (100,150,250 mg/kg). n=6 for each group. Values represent the mean ±S.E.M. *p<0.05 and **p<0.01 significantly difference in compared to after injection in the same group. #p<0.05, ##p<0.01 and### p<0.001 significantly different compared to control group. cont ;control , cal 100; calendula officinalis 100mg/kg , cal150; calendula officinalis 150mg/kg , cal250; calendula officinalis 250mg/kg , morph; morphine , nal+cal ; naloxan+ calendula officinalis 150mg/kg.



NEUROSCIENCE

Figure 2. Acetic acid- induced writhing response. The numbers of writhes during 30 minutes after acetic acid induced writhing response was shown. N=6 for each group. Values represent the mean ±S.E.M. *p<0.05 and **p<0.01 significantly difference in compared to after injection in the same group. ##p<0.01 and### p<0.001 significantly different compared to control group. cont ;control , cal 100; calendula officinalis 100mg/kg , cal150; calendula officinalis 150mg/kg , cal250; calendula officinalis 250mg/kg , morph; morphine , nal+cal ; naloxan+ calendula officinalis 150mg/kg.

4. Discussion

According to Woolf (2010), there are three classes of pain: nociceptive pain, inflammatory pain which is associated with tissue damage and the infiltration of immune cells, and pathological pain which is a disease state caused by damage to the nervous system (neuropathic pain) or by its abnormal function (dysfunctional pain). The results of the present study indicate that hydro-alcoholic extract of Calendul Officinalis exert anti-nociceptive effects both in tail flick and writhing test. These methods are used to detect central and peripheral analgesics. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia, and tail flick test is most sensitive to centrally acting analgesics. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE2 and PGF2α and their levels were increased in the peritoneal fluid of the acetic acid induced rat (Deraedt et al., 1980). The nociceptive is short lasting and it is well accepted that agonists of μ -opioid receptors produce analgesia in acute pain models. Therefore, it is believed that substances that are effective in tail flick exert their effects predominantly through μ -opioid receptors (Shuanglin et al., 2000; Chandana et al., 2010). The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptors to prostaglandins (Shekhawat et al., 2010). Therefore it is possible that the extracts exert their analgesic effect probably by inhibiting the synthesis or action of prostaglandins. The active components of calendula's anti-inflammatory activity are thought to be the triterpenoids, particularly resin. Resin is a novel highly specific inhibitor of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis (Chandana et al., 2010). Leukotriene as well as peptidoleukotrienes result in an increase in vascular permeability and chemotaxis of polymorphonuclear leucocytes as well as release of mediators from leucocytes, which sensitize nociceptors (Jain et al., 2001 ; Boden , 2001). The presence of alkaloid in the plant extract supports the claim that this compound has anti-nociceptive property, since alkaloids, flavonoids and saponins also have been found in the hydro-alcoholic extract of the calendula officinalis. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception (Preethi et al., 2009; Wang et al., 2006). Flowers are also found to contain lycopene and β-carotene. The ingredients of C. Officinalis, like lutein, possess chemopreventive potential

(Nishino et al., 2002) and β-carotene has been reported to suffocate reactive oxygen species (Bohm et al., 1993). Lycopene consistently reduces transcript levels of proinflammatory cytokines. From the results it could be concluded that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s).

Acknowledgments

This research was supported financially by grant from the Isalamic Azad University, Hamedan branch.

References

Boden, S.E., Schweizer, S., Bertsche ,T., Dufer, M., Drews, G& Safayhi H(2001). Stimulation of leukotriene synthesis in intact polymorphonuclear cells by 5-lipoxygenase inhibitor 3-oxo- tirucallic acid. Mol Pharmacol , 60,267- 273.

Bohm ,F., Haley ,J., Truscott ,T.G& Schalch, W(1993). Cellular bound beta-carotene quenches singlet oxygen in man. J Photochem Photobiol, 21, 219-221.

Calixto, J.B., Beirith. A., Ferreira, J., Santos, A.R., Cechinel Filho ,V& Yunes, R.A(2000). Naturally occurring anti-nociceptive substances from plants. Phytother Res, 14, 401-418.

Chakraborthy ,G.S., Arora , R& Majee , C (2010). Anti- diabetic and anti-hyperlipidemic effect of hydro –alcoholic extract of Calendula officinalis. IRJP, 2 (1), 61-65.

Chandana, C.B ., Archana ,T., Shameem, A., Lalit, C., Dilip, K., Debesh, C & Probodh, B (2010). Antinociceptive activity of methanolic extract of leaves of achyranthes aspera Linn. Indian Journal of Experimental Biology ,48,817-821.

David, O., Kennedy., Emma, L., Wightman & AdvNutr(2011). Herbal extracts and Phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv Nutrvol ,2, 32-50.

Della ,L., Tubaro, A., Sosa, S., Becker, H., Saar, S& Issac ,O(1994). The role of triterpenoids in the topical anti-inflammatory activity of Calendula flowers. Planta Med ,60,516–520.

Iauk ,L., Lo Bue , A.M., Milazzo, I., Rapisarda, A& Blandino, G(2003). Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. Phytother Res, 17(6),599-604.

Jain, N.K., Kulkarni ,S.K& Singh, A(2001). Role of cystenyl leukotrienes in nociception and inflammatory conditions in experimental animals. Eur J harmacol, 423,85-92.

Kasiram, K., Sakharkar ,P.R& Patil ,A.T(2000). Antifungal activity of Calendula officinalis. Indian J Pharm Sci, 6,464–466.

Mohammed, G (2009).The Best Herbs for Pain Relief, A look at some of the plants known to have pain-relieving properties. The herb companion (E-newsletter: http://www.herbcompanion.com/health/the-best-herbs-for-pain-relief.aspx#ixzz1RuhZURcX), 1-4.

Nishino, H., Murakosh ,M., Ii, T., Takemura, M., Kuchide, M., Kanazawa ,M., et al (2002). Carotenoids in cancer chemoprevention. Cancer Metastasis Rev , 21, 257-264.

Preethi ,K.C., Kuttan, G& Kuttan, R(2006). Antioxidant potential of Calendula officinalis flowers in vitro and in vivo. Pharmaceutical Biol ,44(9),691–697.

Preethi k ., Kuttan G ., Kuttan R., (2009). Anti-inflammatory activity of flower extract of Calendula officinalis Linn and its possible mechanism of action, Ind J Exp Biol, 47, 113-120.

Santos , F.A., Jeferson, F.A., Santos , C.C., Silveira , E.R& Rao , V.S. (2005). Antinociceptive effect of leaf essential oil from Croton sonderianus in mice. Life Sci, 77,2953-2963.

Shekhawat,N.,Vijayvergia,R,(2010).Investigation of anti-inflammatory,analgesic and antipyretic properties of madhuca indica GMEL. Int J mol med & adv sci,6(2),26-30.

Shuanglin , H., Osamu , T& Hiroshi , I(2000) . Intrathecal endomorphin-I produces antinociceptive modulated by alpha 2- adrenoceptors in the rat tail flick, tail pressure and formalin test. Life Sci, 6, 204.

Wang X., Richard J., Traub ., Anne Z., (2006). Murphy., Persistent pain model reveals sex difference in morphine potency , AJP - Regu Physiol, 291(2),300-306.

Woolf CJ. What is this thing called pain? (2010) J Clin Invest. 120(11), 3742–3744.