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Anti-Inflammatory Effect of *Launaea Acanthodes* Gum

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Abstract

Introduction:Due to the use of Launaea acanthodes in relieving the inflammation among the native population of the region and the importance of alternative chemical drugs with herbal, and despite the widespread use of this plant among the local people, a quantitative study were carried out on Launaea acanthodes properties, especially about its anti-inflammatory ones. This study evaluated the anti-inflammatory effect of Launaea acanthodes gum on the process of inflammation in rats.Methods: A total of 49 male mice, randomly divided into 7 groups, containing 7 rats for each group, in standard group (positive control), aspirin was taken in Placebo group, distilled water was injected and in treatments group, gum extract was injected and in a control group not received any treatment. Intraperitoneal injection were administered for the treatments group using the *Launaea acanthodes* gum extract at doses of 25, 50, 100 and 200 mg/per kg. Then to examine the anti-inflammatory test, the inflammation-making method was carried out through carrageenan 0.1 mL from 1% CAR suspension (w/v) dissolved into distilled water and 30 minutes after injecting the *Launaea acanthodes* extract, the standard drug of synthetic in standard group and distilled water in placebo group were injected into the in right hind paw of the rats subcutaneously. The control groups not receive any treatment but like previous groups, CAR injected. The edema was measured immediately before and after injecting CAR, at the time of zero and also at intervals 30 minutes, 1, 2, 3, 4, 5 and 6 hours by Plethysmometer machine; Mark IITC, made in USA.Results: The results showed that the mean ± SD edema in the right hind paw at various times after carrajeenan injection in the control group, placebo group, treatment groups with extract in dose of 25, 50, 100 and 200 mg/kg and oral aspirin group, were 0.809±0.104, 0.810±0.103, 0.782±0.084, 0.758±0.069, 0.747±0.059, 0.722±0.047 and 0.733±0.055 respectively. Comparison between the groups using one-way variance analysis in all times showed that there is a significant relationship between the control group with the groups which receved 25, 50, 100 and 200 mg/kg dose of extract (p=0.001) and group with the oral aspirin (P=0.001).Conclusion: The results showed that *Launaea acanthodes* has the anti-inflammatory effects and these effects have been more effective in doses of oral aspirin. Anti-inflammatory effect of *Launaea acanthodes* may be via the existence of flavanoids, phenols as well as terpens compouned, however, due to the lack of exact cause of anti- inflammatory effects and the exact mechanism of *Launaea acanthodes* function on the cells, it is necessary to be done more studies.

Keywords: *Launaea acanthodes*, Inflammation, Wistar rats, Carrageenan.

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Introduction

Excessive and inappropriate use of anti-inflammatory drugs and synthetic analgesic ones have caused the side effects, addiction to drugs and drug resistance among the patients. Therefore, the medicinal herbals can be a good choice for treatment. Meanwhile, these drugs have natural origin and are produced in an easy process. Study of pharmacological effects of medicinal plants in laboratories is the most important parts of ethnopharmacology in studies in the world. *Launaea acanthodes* is of the family of Asteraceae that has 3 local names: Charkhan, Charkhak or Shekarlouleh. It is a one-year plant, shrub and grows in desert with thick tentacles and hairless legs; split with white fluid being left is changed into a yellow like-glass material (Ghahreman, 1986; Mahmodi & Yasa, 1995). The plant gum is taken orally or locally on the skin. It is traditionally administratered to relieve the kinds of pain such as back pain, low back pain and leg pain gastroentritis disorders and diabetes (Wamegh et al., 1986; Piazzaet al., 2009). In extract of this plant, there are also materials such as flavonoids, terpenoids, saponins, alkaloids, tannins, polysaccharides and mono-saccharides; arabinose, mannose and acid derivatives. However, gloroniccho has also been recognized Wamegh et al., 1986; Ghaderian SM, Baker, 2007). There are a few studies on this plant, and its phamaceutical properties of are unknown and only a few reports have been

published about its medicinal properties. In these reports, anti-diabetic activity attribute to a kind of glycoside called Bengalnozid (Augusti , 1975) or the use of any *L. arborescens* to treat the diabetes in traditional medicine in Morocco (Bnouham et al., 2002), have been mentioned. The beneficial effects of ethanol extract of *L. acanthodes* in hypoglycemia (Rassouliet al., 2011) and to improve the liver function by preventing the progressive increase in liver indices; in diabetic rats tested and showed the useful effects (Jalali et al., 2012), and the gum in the traditional medicine and among the native peoples the gum of this plant that extracted from its stem is used as an effective drug in treating diseases such as neurological disorders, pain and especially in the local, joint inflammations, digestive disorders such as stomach ulcers and duodenal ulcer in desert areas (Mahmodi & Yasa, 1995 Piazza et al., 2009; Karimidokht et al., 2009). The aim of this study was to survey the anti-inflammatory activity of *Launaea acanthodes* (LA) Gum aqueous extract; experimentally induced inflammation on mice.

Materials and Methods:

Drugs and Chemical agents

The following drugs and reagents were used: Carrageenan (CAR), Morphine and Aspirin, which were purchased from Sigma (Sigma Chemical Company, St. Louis, USA); and formaldehyde that was from Merck (Darmstadt, Germany). Acetic acid was purchased from Sinopharm Chemical Reagent Co.

Extract preparation

In this study, after pharmacognosy and botanical investigation and approving the plant species by experts of pharmaceutical plants in research section at the Center of Agricultural Applied Sciences in Semnan province, the gum of *L. acanthodes* was collected in the southern areas in Semnan in summer and was stored for testing time at optimum environmental and thermal conditions (4°^{C}). The aqueous extract gum was prepared using purifying method in water and through soxhlet machine. The amount of 100 grams of powder prepared from this plant on filter paper through soxhlet machine for 3 hours at 30-40°^{C} was heated and the extract was separated via filtration. Then the additional water of extract was removed by rotary instrument and was changed into powder.

Experimental animals

Male Wistar rats (180 to 220 gr) were purchased from pasture institute (Tehran, Iran). The animals were kept in standard environmental conditions (21ºC, 60–70% humidity) with well-ventilated. The animals were housed in standard cages at a room temperature of 23±1ºC with a 12 h light/dark cycle with free access to standard diet (standard laboratory rodent's chow) and water. Male wistar rats were used for anti-inflammatory (CAR-induced mice hind paw edema) test; also female and male rats were used for acute toxicity assay. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (Winter et al., 1962)

Acute toxicity test

The median lethal dose (LD50), of the LA was evaluated in Rats according to the modified method (Otterness & Moore, 1988). The animals were handled in accordance with international principles guiding the use and handling of experimental animals (Swingle et al., 1969). All the animals were randomly divided into six groups; one control group and four treated groups, containing seven animals per group. The Rats in test groups were then allowed free access to food and water. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. They were observed for 24 hours for signs of toxicity. Also, animals in the different groups were observed for 2 h post treatment for immediate signs of toxicity and the mice in the test groups observed over a period of 7 days for signs of behavioral changes and the toxicity signs. The number of deaths within this period of time was recorded. Log–dose response plots are constructed for the agent, from which the median lethal dose (LD50) of the LA were determined.

Anti-inflammatory activity

In vivo anti-inflammatory activity was evaluated on the basis of inhibition of CAR-induced by the injection of CAR into the subplantar region of the right hind paw of the mice according to the method described by (Winter et al., 1962). CAR-induced edema model is widely used to study the inflammatory process as well as screening the anti-inflammatory agents (Otterness & Moore, 1988; Swingle et al., 1969). Before edematogenic agent injection, the average volume (three or four measurements) of the right paw of each animal was determined using a plethysmometer (V0). Rats were divided into six groups of 7 animals per group. Rats in groups 1 served as control group administered with saline, and rats in group 2 (standard group) received aspirin (300 mg/kg, p.o.) as the reference drug. Groups 3 to 6 were treated orally with LA extract (25, 50, 100 and 200 mg/kg) intraperitoneally as a test groups. One hour after oral administration of the various agents (LA, aspirin and saline) oedema was induced by an injection of 0.1ml of CAR (1%, w/v in saline). CAR solution by diluting the CAR (10 g/l) was prepared in distilled water one hour before testing (Fayyaz et al., 1992; Morán et al., 2002; Olajide et al., 2000). The paw volumes of these Rats were measured using a plethysmometer. The measures were determined at 0h (V0: before edematogenic agent injection) and 0.5,1, 2, 3, 4, 5 and 6 hr intervals later (VT). The difference between VT (1, 2, 3, 4, 5 and 6 hr) and V0 was taken as the edema value.

The percentages of edema were calculated according to the following formula:

$$\% \text{ edema} = (VT - V0) \times 100 / V0.$$

The percentages of inhibition were calculated according to the following formula:

$$\% \text{ inhibition} = ((VT - V0) \text{ control} - (VT - V0) \text{ treated group}) \times 100 / (VT - V0) \text{ control.}$$

Data Analysis:

The data were reported by Mean ± S.EM and to determine the relationship between variables, one-way Anova test was used. To analize the data, SPSS-version 16 with a significant level of 0.05 was used.

Result:

This study carried on 49 adult male Wistar rats (180 to 220 gr). They were randomly divided into 6 groups with 7 Rats in each group and were kept in the standard cages under the equal conditions and with the light cycle of 12 hours of light and 12 hours with darkness. They were free in access to water and food. The results showed that the mean ± SD edema in the right hind paw in before CAR injection and at various times after CAR injection which is shown in Table 1. The average of edema at six hour after CAR injection in the right hind paw in the control group, placebo group, treatment groups with extract in dose of 25, 50, 100 and 200 mg/kg and oral aspirin group, were 0.809±0.104, 0.810±0.103, 0.782±0.084, 0.758±0.069, 0.747±0.059, 0.722±0.047 and 0.733±0.055 respectively. Comparison between groups using one-way variance analysis in all times showed that there is a significant relationship between the control group with the groups which receved 50, 100 and 200 mg/kg dose of extract (P=0.001) and group with the oral aspirin (P=0.001). (Table 1, Figure 1 and 2).

Launaea acanthodes Gum ameliorate CAR-induced oedema test

The anti-inflammatory effects of LA on CAR-induced paw edema and average of edema in mice are shown in Table 1 and figure 2. LA has the high potential of anti-inflammatory at higer doses and significantly reduced CAR induced paw edema in selected times ($p < 0.001$). The maximum volume of paw edema in mice increased progressively and reached its maximum after 1h of CAR injection in control group. The highest anti-inflammatory activities were observed after 4 and 5 h for LA of CAR injection (Figuer 1). The LA, in doses of 50, 100 and 200 mg/kg, had a considerable anti-inflammatory effect in all time of test and this effect was maintained for 2h post injection of CAR. The highest inhibition of edema for aspirin (300 mg/kg) produced 5h after injection of CAR. The anti-inflammatory effect of 50, 100 and 200 mg/kg of LA were comparable to the standard drug (aspirin). In addition, the mean ± SD edema in the right hind paw in the control group, placebo group, treatment groups with extract in dose of 25, 50, 100 and 200mg/kg and oral aspirin group by the separation of times studied. The result show that, there is no any differences between control and placebo groups in all times (h0 – h6) but is tend to decrease edema which exposure to LA extract as well as asprine. To decreasing significantly is started from h1, also this is more in dose of 200 from h3 than asprin group (Figure1 and 2).

Table 1. Level of the right hind paws edema, before injecting carageeninan (V0) and immediately after carageeninan injection (0.0, 0.5, 1, 2, 3, 4, 5 and 6 hours)

	Groups	AV Before injection	SD	Compared with the control group	AV 0 h	SD	AV 0.5 hr	SD	AV 1 hr	SD	AV 2 hr	SD	AV 3 hr	SD	AV 4 hr	SD	AV 5 hr	SD	AV 6 hr	SD
1	Control	0.605	0.002	$_3$P-0.437 F-0.714	0.707	0.011	0.711	0.110	0.824	0.016	0.859	0.016	0.886	0.013	0.930	0.017	0.881	0.009	0.876	0.008
2	Distilled water (placebo)	0.607	0.003	$_3$P-0.496 F-0.538	0.707	0.011	0.711	0.900	0.834	0.015	0.863	0.015	0.890	0.012	0.927	0.015	0.881	0.009	0.870	0.008
3	Extract at the dose of 25 mg/ Kg	0.610	0.004	0.203-P_3 02.143-F	0.710	0.012	0.714	0.800	0.784	0.009	0.811	0.011	0.839	0.013	0.884	0.013	0.849	0.009	0.838	0.015
4	Extract at the dose of 50 mg/ Kg	0.606	0.004	0.777-P_3 0.089-F	0.710	0.012	0.714	0.800	0.757	0.007	0.779	0.007	0.801	0.011	0.843	0.011	0.811	0.011	0.803	0.011
5	Extract at the dose of 100 mg/ Kg	0.611	0.005	0.623-P_3 0.275-F	0.709	0.012	0.713	0.900	0.751	0.011	0.766	0.008	0.783	0.007	0.814	0.009	0.791	0.009	0.783	0.009
6	Extract at the dose of 200 mg/ Kg	0.604	0.004	0.604-P_3 0.306-F	0.706	0.013	0.710	0.100	0.730	0.010	0.743	0.009	0.753	0.009	0.764	0.009	0.750	0.010	0.740	0.010
7	Aspirin at the dose of 300 mg/Kg	0.603	0.004	$_3$P-0.437 F-0.714	0.706	0.013	0.710	0.800	0.732	0.008	0.729	0.008	0.769	0.009	0.793	0.007	0.773	0.011	0.760	0.008

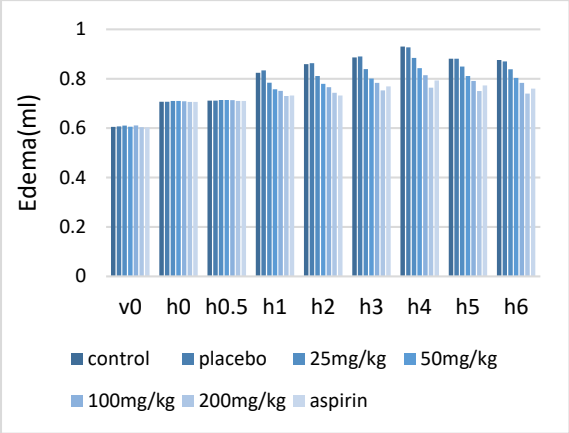

Figure 1: Bar chart of the average edema of the right hind paw in the study groups by the separation of times studied.

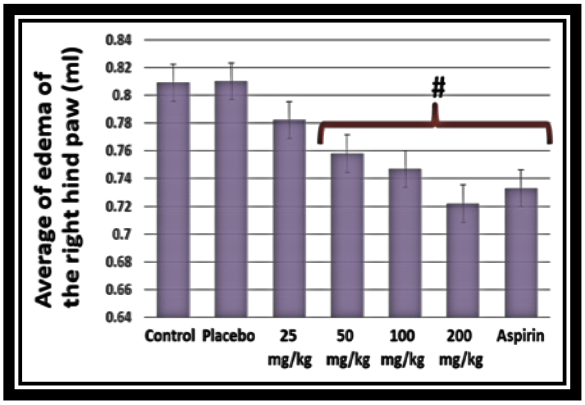

Figure 2: bar chart of the average edema of the right hind paw at various times after CAR injection in the study groups. # (P< 0.001)

Changing trend of the mean of severity of percentage edema from CAR injection to six hours after CAR injection in the control group, placebo group, treatment groups with extract in dose of 25, 50, 100 and 200 mg/kg and oral aspirin group, were 37.2 , 37.2 , 31.2 , 27.8 , 24.6 , 21.5 and 24; respectively. (Figures 3).

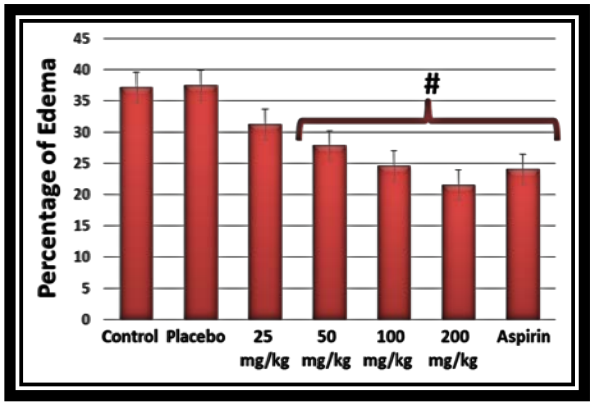

Figure 3: Bar chart of changing trend of the mean of severity of percentage Edema from CAR injection to six hours after Car injection, . # (P< 0.001).

Changing trend of the mean of severity of percentage inhibition from CAR injection to six hours after CAR injection in the control group, treatment groups with extract in dose of of 25, 50, 100 and 200 mg/kg and oral aspirin group, were 1.3 , 13.1 , 18.3 , 27.8 , 34.8 and 31.2; respectively. (Figures 4).

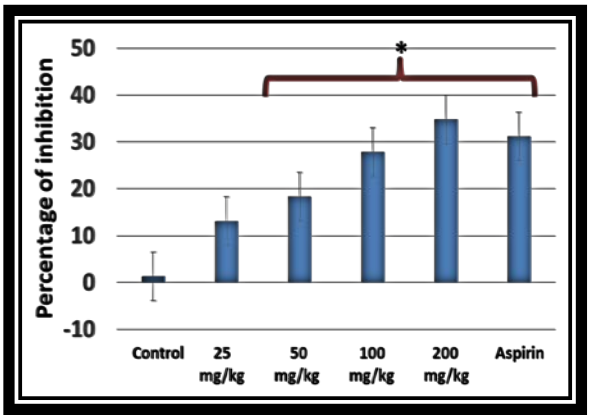

Figure 4: Bar chart of changing trend of the mean of severity of percentage inhibition from CAR injection time to six hours after the injection, # (P< 0.001).

Conclusion

Today the use of traditional medicine is increasing in industrialized countries and the World Health Organization has recommended the use of traditional medicine in health care systems (Omidbeigi, 2000; Shariat & Moatar, 2003). Thus the discovery of the beneficial effects of plants and their effective ingredients has been steadily growing by physicians, pharmacists and researchers (Zargari, 1989). On the other hand, inflammation is a protective response that the ultimate goal is to get rid of organism both for the primary cause of cell damage; bacteria, toxins, etc. and for the results of damge which leads to necrotic cells and tissues. The available anti-inflammatory drugs have many side effects that sometimes lead to discontinuation of the drug, on the other hand, excessive and increasing inappropriate use of anti-inflammatory and analgesic and synthetic drugs lead to side effects, addiction and drug resistance. Thus, access to newer drugs with fewer side effects is the researchers' aim. Regarding the nature of plants which are consistent with human body and due to having biological balance with fewer side effects which are considerable advantages compared to the chemical drugs (Mahdavi &, Moradi, 1995). Thus, according to the above, the medicinal plants can be the proper choice for treatment. Oral administration or topical application of the LA plant gum which is called Maghel and Malek by natives has been used traditionally to relieve or the treat back pain and the local pains and the muscular, gastro intestinal disorders and even diabetes has been used (Wamegh et al., 1986; Piazza et al., 2009). The results showed that the mean ± SD edema in the right hind paw at various times after CAR injection in the control group, the treatment groups with the extract in dose of 25, 50, 100 and 200 mg/kg and oral aspirin group, were deacresed significantly. . Comparison between groups using one-way variance analysis in

all times showed that there is a significant relationship between the control group with the groups which receved 25, 50, 100 and 200 mg/kg dose of extract (P=0.001) and the group with the oral aspirin (P=0.001). Changing trend of the mean of severity of percentage of edema from CAR injection to six hours after CAR injection in the control, treatments groups with extract in of 25, 50, 100 and 200 mg/kg and oral aspirin group, were decresed but changing trend of the mean of severity of percentage of inhibition from CAR increased significantly. Interestingly in which that the percentage of edema and inhibition of group in dose 200 mg/kg is more effective than asprin group. This study was the first resaerch which investigated the anti-inflammatory effects of *L. acanthodes*. However further study on this plant, particularly on molecular and structural scales is recommended to the researchers. In studies done in Iran, *L. acanthodes* is effective on pain relief, especially local inflammation and joint (Mahmodi & Yasa, 1995; 10]. How much LA is able to reduce the systemic inflammation is in ambiguity. The results of several studies carried out on the compounds of the LA showed that, in plant extract, in addition to materials such as flavonoids and terpenoids, saponins, alkaloids, tannins, there are polysaccharides and monosaccharides such as arabinose, mannose and acid derivatives (Wamegh et al., 1986; Ghaderian et al., 2007). They concluded that the type of L.A has the most anti-inflammatory effect in preventing the stomach ulcers and protection in stomach among this plant family. In 2013, Fallah Hosseini and colleagues stated that due to anti-inflammatory effects of the L. acanthodes and protection stomach ulcers caused by anti-PG which are found in this plant (Fallah et al., 2013). The involvement of *L. acanthodes* plant compounds to reduce the tumor necrosis factor of alpha type (TNF-α = Necrosis Factor Alpha Tumor) as an important factor in occurrence and development of inflammation (Takeuchi, 2012). Also, other studies have been published about its antioxidant effects and reducing of compounds such as H2O2 (Chattopadhyay et al., 2006). Further studies are required to clarify the complete pharmacological profile and mechanism of the anti-inflammatory activities of the LA in order to prepare it for safer and more effective usage. Also further phytochemical and biological tests are suggested to determine the active chemical constituent(s) of LA gun aqious extract responsible for these activities.

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