Anti-inflammatory Activity of Methanol Extract From *Inula graveolens L*.

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Abstract

In this study, the anti-inflammatory activity of methanolic extract of *Inula graveolens L.* was investigated. The effect of the extract was studied on formalin induced paw edema (chronic inflammation). The study was carried out using doses of 100, 200 & 400 mg/kg orally. The extract showed significant anti-inflammatory activity at the doses of 400mg/kg on the first day ($P < 0.05$), and the hind paw edema of rat disappeared after 6 days ($P < 0.01$) as compare to standard drug Diclofenac sodium (50mg/kg).

Introduction

Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules (Mate *et al*., 2008). Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and alzheimer's disease (Gupta *et al.,* 2006). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory

agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (Garcia *et al.,* 2004). Because exciting synthetic molecule like nonsteroidal anti-inflammatory drugs (NSAIDS) and selective Cox-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects (Chowdhury *et al.,* 2009). *Inula graveolens* L. is widely distributed in mediterranean region and middle east to west Pakistan. In Iraq-Basrah and lower Iraq. This plant is well known in Arabic and English system as ' Shuwaser, Suawaid ' and ' Stroing-smellind *Inula* ', respectively (Chakravarty, 1976). So, in order to overcome there is need to focus on the scientific exploration of herbal drugs having fewer side effects. The present study was designed to investigate the anti-inflammatory effects of *Inula graveolens* L. in chronic inflammation.

Materials and methods

Chemicals

All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), and solvents were from E. Merck (Darmstadt, Germany).

Plant material and extraction

Inula graveolens L. Plant, used in this study, was collected on october 2009 from Abu-Al-Khaseeb region (Southern of Basrah), Iraq. The plant was botanically authenticated and voucher specimens were deposited in the Herbarium of Basrah (Iraq, Basrah, College of Science, University of Basrah). A quantity (100 g) of powdered plant was extracted in a Soxhelet apparatus with 80% methanol, for 24 h. The methanolic extract was filtered and evaporated to dry under reduced pressure in a rotary evaporator. The yield was 9.47 g of dry extract.

Animals

Inbred colony of thirty adult rats (20-35g) of either sex (15 males and 15 females) were used for chronic anti-inflammatory activity. They were housed in polypropylene cages under a 12 h light:12 h dark cycle in a controlled temperature room (25±2 °C). All the animals were acclimatized to the laboratory conditions for a week before use. They had free access to food and water.

Chronic inflammation induced by formalin (formalin test)

This test was performed according to the method of (Sexena *et al.,* 1984) with minor modification. Rats were divided into five groups of six animals (3 males and 3 females), as follow:

Group I served as control received 2% w/v Tween 80 in normal salin,10mL/kg (P.O),

Group II received Diclofenac sodium, 10mg/kg (P.O),

Group III, IV and V received methanolic extract (100, 200 and 400) mg/kg (P.O) of *Inula graveolens* respectively.

The inflammation was produced by subaponeurotic injection of 0.1 mL of 2% formaldehyde in the right hind paw of the rats on the first and third day. The animals were treated daily with the extract or diclofenac post orally for 10 days. The daily changes in paw size were measured by wrapping piece of cotton thread round the paw and measuring the circumference with a meter rule.

Statistical analysis

The results are expressed as mean values ± SD and tested with analysis of variance followed by Student's *t*-test. P-values **<** 0.05, **<** 0.01 were considered to be statistically significant.

Result and Discussion

Table 1 and figure 1 shows the effects of the *Inula graveolens L*. methanol extract on formalin induced chronic anti-inflammation in rats. The activity was found to increase with increasing concentration of the extract. At the doses of 100, 200 and 400 mg/kg, the extract showed significant results (P **<** 0.05 and P **<** 0.01), respectively as compared to Diclofenac sodium (10mg/kg) and caused a significant inhibition in paw edema volume. In Diclofenac and the methanol extract (400mg/kg), groups, the hind paw edema of rat disappeared after 6 days (P **<** 0.01). Same groups, showed anti-inflammatory effect on day one (P **<** 0.05). On days 4 and 5, both diclofenac and the extract did not demonstrate anti-inflammatory activity.

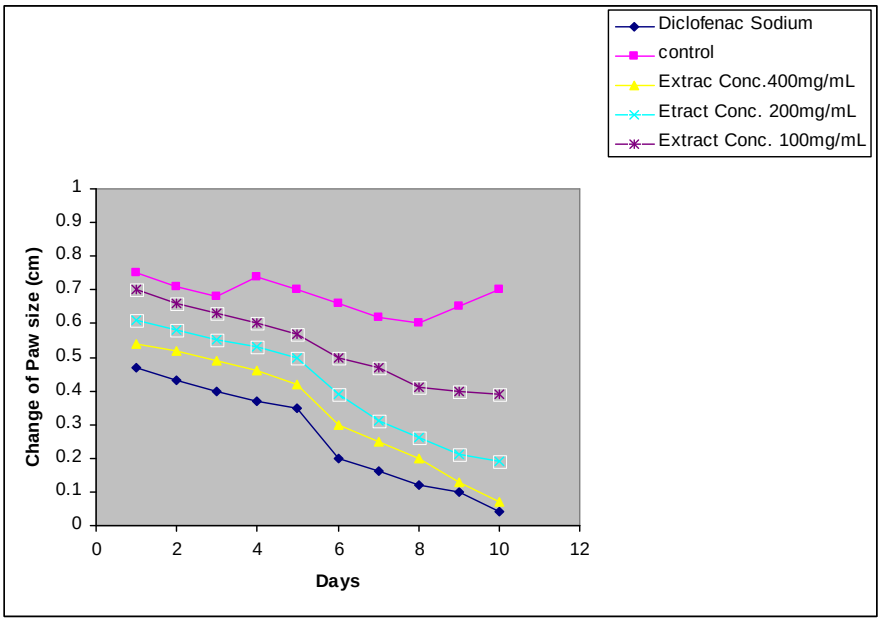

Figure 1 : Effect of the methanol extract of ***I. graveolens*** on formalin induced paw edema (chronic inflammation) in rats.

Table 1 : Effect of the methanol extract of ***I. graveolens*** on formalin induced paw edema (chronic inflammation) in rats.

Groups	Dose mg/kg	Change of paw size (cm)									
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
GroupI	Control	0.75 ± 0.15	0.71 ± 0.27	0.68 ± 0.12	0.74 ± 0.15	0.7 ± 0.14	0.66 ± 0.11	0.62 ± 0.21	0.6 ± 0.13	0.65 ± 0.15	0.7 ± 0.21
GroupII	Standard	0.47* ± 0.13	0.43 ± 0.17	0.4 ± 0.12	0.37 ± 0.19	0.35* ± 0.27	0.20** ± 0.15	0.16** ± 0.21	0.12** ± 0.14	0.1** ± 0.16	0.04** ± 0.17
GroupIII	100	0.7 ± 0.21	0.66 ± 0.24	0.63 ± 0.2	0.6 ± 0.17	0.57 ± 0.19	0.5 ± 0.2	0.47 ± 0.17	0.41* ± 0.11	0.4* ± 0.14	0.39* ± 0.23
GroupIV	200	0.61 ±	0.58 ±	0.55 ±	0.53 ±	0.5 ±	0.39* ±	0.31* ±	0.26** ±	0.21** ±	0.19** ±

		0.15	0.11	0.07	0.04	0.03	0.12	0.07	0.09	0.12	0.10
		0.54*	0.52	0.49	0.46	0.42	0.30**	0.25**	0.20**	0.13**	0.07**
GroupV	400	±	±	±	±	±	±	±	±	±	±
		0.35	0.40	0.32	0.36	0.31	0.31	0.30	0.34	0.35	0.30

N=6, values are mean ±SD, *P<0.05, ** P<0.01, as compared to control group.

The formalin test is believed to represent a more valid model for clinical pain (Tjolsen *et al.,* 1992). It is a very useful method, not only for assessing antinociceptive drugs, but also helping in the elucidation of the action mechanism (Fernanda *et al.,* 2002). The formalin produced a distinct biphasic response to pain stimulus and different analgesic compounds may act differently in the early and late phases of this test. The early phase is the result of direct chemical activation of nociceptive primary afferent fibers, while the factors that contribute the late phase are not well defined (Amanlou *et al.,* 2005). Zeashana *et al.* (2009) postulated that the neurogenic phase is probably a direct result of stimulation in the paw and reflects centrally mediated pain with release of substance P while the late phase is due to the release of histamine, serotonin, bradikynin and prostaglandins. Therefore, this test can be used to clarify the possible mechanisms of anti-nociceptive effect of a test compound (Hajhashemi *et al.,* 2002). Drugs that act primarily on the central nervous system, such as narcotics, inhibit both phases equally wile peripherally acting drugs such as anti-inflammatory non-steroidal (NSAID) and anti-inflammatory steroidal only inhibit the late phase (Radwan *et al.,* 2007). The effect of I. graveolens methanol extract on the first and second phases of formalin test suggests that its activity may be resulted from its peripheral action when compared with Diclofenac sodium activity in this respect to suggest any mediator merits further investigation.

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تقييم فعالية الخلاصة الميثانولية لنبات الشواصر *Inula graveolens L.* كمضاد للالتهاب

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ألخلاصه

درست فعالية الخلاصة الميثانولية لنبات الشواصر كمضاد للالتهاب المحدث بمادة الفورمالين على ألجرذان ألمختبريه، وذلك بتجريعها فمويا تراكيز مختلفة 100، 200، 400 ملغم/كغم من تلك الخلاصة. لوحظ أن فعاليه الخلاصة كمضاد للالتهابات عند الجرعة العالية 400 ملغم/ كغم بدأت منذ اليوم الأولى من المعالجة ($P < 0.05$) وبعد ستة أيام لوحظ اختفاء الورم تماما من كف الجرذان المعالجة وبفارق عالي المعنوية ($P < 0.01$) وقد قورنت النتائج مع عقار قياسي معروف هو صوديوم دكلوفيناك (50 ملغم/كغم) .