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Anti-inflammatory activities of triterpene lactones from *Lactuca sativa*

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Abstract

Lactuca sativa (lettuce) is a famous vegetable which is also used traditionally for management of inflammatory conditions. Aim of the study was to isolate and identify potential bioactive compounds that might be involved anti-inflammatory activity of *Lactuca sativa*. 3,14-Dihydroxy-11,13-dihydrocostunolide (compound **1**) and 8-Tigloyl-15-Deoxyl-actucin (compound **2**) were isolated from extract of *Lactuca sativa*. Both compounds showed substantial lipoxygenase inhibitory activity. Similarly, the isolated compounds revealed significant ($p<0.05$) *in-vivo* anti-inflammatory activity based on carrageenan induced paw edema model. Significant results of the isolated compounds indicate their considerable potential to be further studied at molecular and cellular level.

Keywords: triterpene lactones; lipoxygenase; anti-inflammatory activity; *Lactuca sativa*

Introduction

Lactuca sativa (lettuce, family Compositae) is a well-known vegetable as well as a medicinal plant is consumed globally. Traditionally it is famous for its use as folk remedy for inflammation, pain, stomach problems including indigestion and for lack of appetite (Sayyah et al., 2004). Considerable pharmacological studies have been conducted to evaluate therapeutic significance of the crude extracts of *Lactuca sativa*. *Lactuca sativa* showed Anticonvulsant, sedative-hypnotic, antioxidant, analgesic and anti-inflammatory activity (Sayyah et al., 2004). Substantial research work has been done to identify chemical constituents of *Lactuca sativa*. Various classes of natural products have been isolated from L. sativa, so far. These classes include sesquiterpene lactones (Mahmoud et al., 1986), phytols (Bang et al., 2002), carotenoids (Kimet et al., 2007), polyphenol oxidase and phenols (Altunkaya &

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Gökmen, 2008; Gawlik-Dziki et al., 2008), micronutrients (Nicolle et al., 2004) and proteins (Piero et al., 2002). In the present study, our aim was to identify potential compounds responsible for its use as folk remedy for pathological conditions associated with inflamemation.

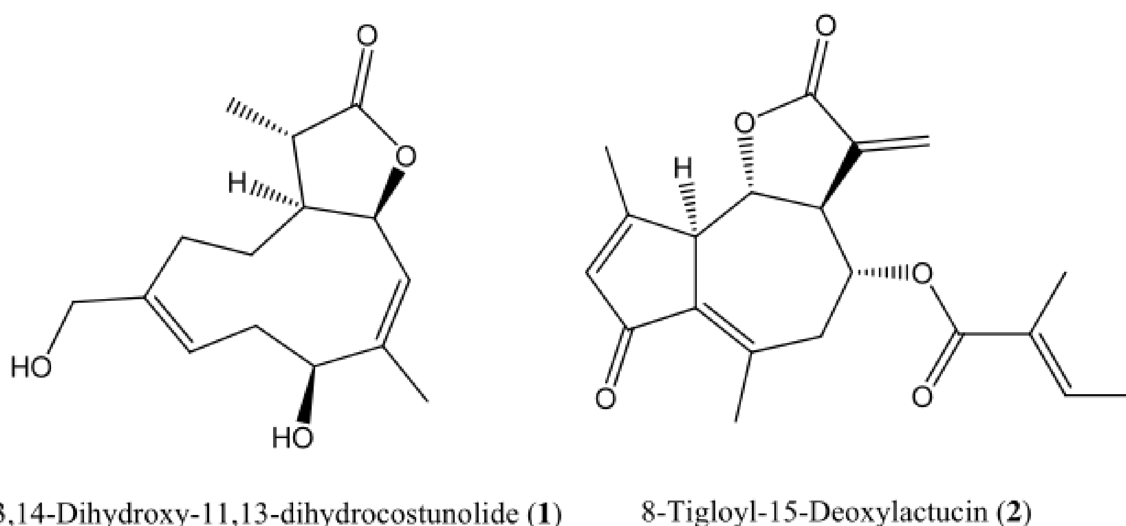
Materials & Methods

Plant material

The aerial parts of the plant were air-dried under shade for 5 consecutive weeks at room temperature. The dried plant material was later on chopped, finely ground and stored in a polyethylene bag under refrigeration for further experimentation.

Extraction and isolation

Dried and powdered Plant material (11 kg) was thoroughly extracted with methanol. The solvent was then removed under low pressure and the residue (814 g) was subjected to fraction. The aqueous solution was then extracted by petroleum ether, chloroform, ethyl acetate and n-butanol, respectively. The chloroform fraction was subjected to column chromatography on silica gel and eluted by a solvent mixture composed of chloroform and methanol with ratios changing from pure n-hexane-chloroform (50-50) gradient to methanol-chloroform (15-85) to yield 17 subfractions. Subfraction 4 and 5 were further purified over silica gel column chromatography (CC) developed with to afford compound **1** (158 mg) and **2** (271 mg). Structures of the isolated compounds were identified by comparing spectral data of the isolated compounds with the data in literature. Compound 1 and 2 were identified as 3,14-Dihydroxy-11,13-dihydrocostunolide (Nishimura et al., 1986) and 8-Tigloyl-15-Deoxylactucin (Okunade et al., 1994).



3,14-Dihydroxy-11,13-dihydrocostunolide (**1**) 8-Tigloyl-15-Deoxylactucin (**2**)

Figure 1. Structures of isolated triterpene lactones from *L. sativa*.

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Animals

Adult Wistar rats (160–240 g) of both sexes were kept in plastic cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed with standard diet and had access to tap water *ad libitum*. Their experimental usage was according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication (No. 83-23) revised (1978)).

In-vitro lipoxygenase inhibition assay

Enzyme inhibition assays were performed by using different concentrations of the isolated compounds. Lipoxygenase inhibitory activity was measured by slightly modifying the spectrometric method as developed by Tappel (1962). Lipoxygenase (EC 1.13.11.12) type I-B (Soybean) and linoleic acid were purchased from Sigma (St. Louis, MO) and were used without further purification. All other chemicals were of analytical grade and purchased from the same vendor i.e. Sigma (St. Louis, MO). 160µL of sodium phosphate buffer 0.1mM (pH 7.0), 10mL of the sample solutions (test compound) and 20µL of lipoxygenase solution were mixed and incubated for 5 min at 258 ◦C.

The reaction was initiated by the addition of 10µL linoleic acid substrate solution and the absorption change with the formation of (9Z,11E)-13S)-13-hydroperoxyoctadeca-9,11-dienoate was followed for 10 min. The test sample and the control were dissolved in 50% ethanol. All the reactions were performed in triplicate. Baicalein was used as positive control for lipoxygenase inhibition (Khan et al., 2009). The IC_{50} values were calculated using the EZFit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Carrageenan-induced paw oedema

Method of Winter et al (1962) was utilized to assess the anti-inflammatory potential of test sample via testing its ability to inhibit the carrageenan-induced hind paw oedema, as reported earlier (Khan et al., 2009; Arfan et al., 2010). Test samples and the control samples were administered orally in groups (n = 5) to rats. After I hour, acute inflammation at desired site was induced by subplantar injection of 1% suspension of carrageenan (0.1mL) using 2% gum acacia as a suspending agent in normal saline, in the right hind paw of the rats. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at '0' and 3 h after the carrageenan injection. Indomethacin 5mg/kg, p.o. suspended in 2% gum acacia was used as positive control. Percent inhibition of the inflammation was determined by applying statistics on raw data followed by the calculation of percent inhibition for each group by comparing with control group. The formula used for comparison was: $\%I=1-(dt/dc)\times100$, where "d*t*" is the difference in paw volume in the drug-treated group and "d*c*" is the difference in paw volume in control group. However, "*I*" stands for inhibition of inflammation.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and mortality was recorded.

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Data analysis.

Results of the study were expressed as mean ± SEM. Student's *t*-test was used to analyze data between the groups and analysis of variance (ANOVA) among groups followed by Dunnet's test for multiple comparisons. Values of $p \leq 0.05$ were considered significant in all cases.

Results

In-vitro lipoxygenase inhibition assay

Both showed promising inhibitory activity against lipoxygenase. IC_{50} values of 3,14 Dihydroxy-11,13-dihydrocost-unolide (**1**) and 8-Tigloyl-15-Deoxylactucin (**2**) were 59±0.21 and 14±0.19, respectively. IC_{50} value of the standard compound Baicalein was 22.1±0.03µM.

Carrageenan-induced oedema

Compound 1 and 2 exhibited significant ($p \leq 0.05$) reduction in oedema induced by carrageenan at a dose 5 and 10mg/kg (Figure 2). However compound **2** was slightly more active than compound **1.**

Acute toxicity

All the compounds were found safe after 48 hours of administration. Statistically, no

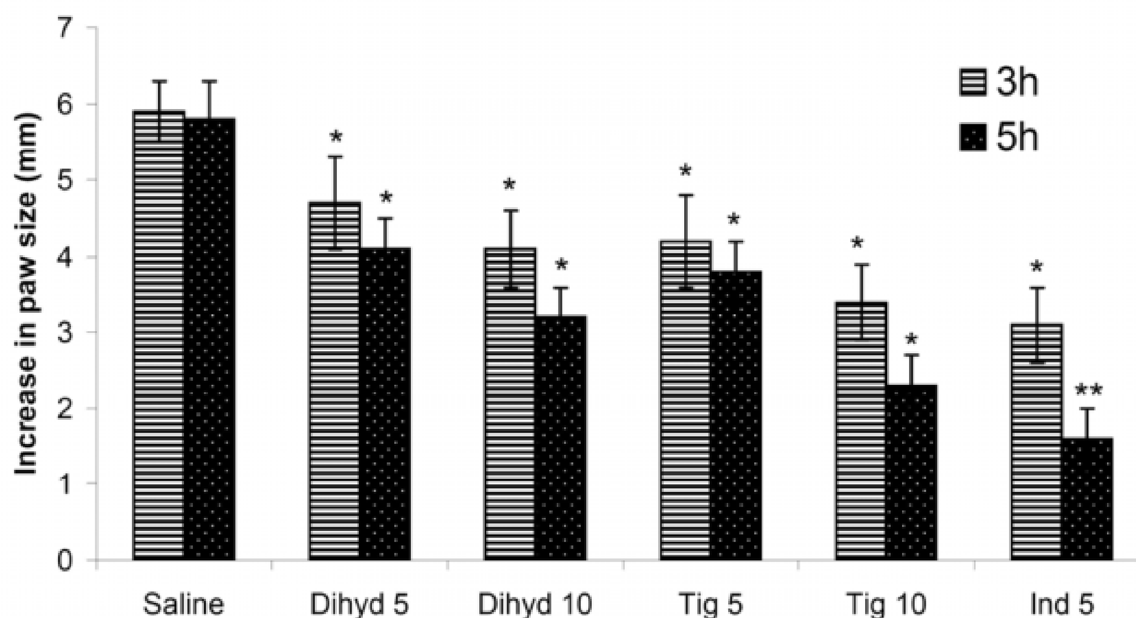

Figure. 2. Anti-inflammatory effect of the test compounds based on carrageenan-induced hind paw oedema model at 5, 10 and 20 mg/kg. Difference of means ± S.E.M of paw size (mm) between control and treatment values at different doses * = $p < 0.05$ (significant), ** = $p < 0.01$ (highly significant).

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considerable difference was observed between the negative control and other treatment groups both in terms of mortality and morbidity.

Discussion

Lipoxygenase (EC 1.13.11.12) constitutes a family of non-heme iron containing enzymes, as versatile biocatalysts are capable of catalyzing many reactions involved in xenobiotic metabolism (Khan et al., 2009). They are responsible for the metabolism of the fatty acids (FAs) and their metabolites eliciting inflammatory responses in the body. They also play a significant role in cancer cell growth (Rioux and Castonguay, 1998), metastasis, invasiveness, cell survival and induction of tumor necrosis factor (TNF) (Chan, 1995). Many COX-2 or 5-LOX inhibitors have been developed as drugs to treat inflammation (Viji and Helen, 2008). In this study both compounds exhibited significant inhibition of the lipoxygenase showing its strong potential to be developed as anti-inflammatory drug.

Carrageenan-induced paw edema being an *in-vivo* investigational model for acute inflammation which been extensively used to determine the anti-inflammatory effect of new investigational agents (Arfan et al., 2010). Both lactones (compound **1** and compound **2**) further established their significant ($p \leq 0.05$) anti-inflammatory potential in *in-vivo* study by controlling biphasic inflammatory events induced by carrageenan. The early phase (90–180 min) of the inflammation is due to the release of histamine, serotonin and similar substances. The later phase (270–360 min) of edema-induced by carrageenan is characterized by the highest volume of hind limb, during which the edema reaches its highest volume and by the presence of prostaglandins and other slowly acting inflammatory mediators which include kinin-like substances, i.e. prostaglandins, proteases and lysosome (Khan et al., 2009). These inflammation mediators are the main components responsible for swelling and edematous condition. Moreover, all the compounds were found safe after 48 hours of test compounds administration. Statistically, no considerable difference was observed between the negative control and other treatment groups both in terms of mortality and morbidity.

This significant data of 3,14 Dihydroxy-11,13-dihydrocostunolide (**1**) and 8-Tigloyl-15-Deoxylactucin (**2**) might share the same anti-inflammatory mechanism as in the case of indomethacin, which involves the inhibition of inflammation process initiated through carrageenan (Di Rosa et al., 1971). Current investigations regarding anti-inflammatory activities reveal the fact that isolated triterpene lactones should be extensively studied further in order to be developed as new lead compounds for treatment of inflammation.

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