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Review

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Anti-inflammatory sesquiterpenes from the root oil of *Ferula hermonis*

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9 *Ferula hermonis* Boiss. (Apiaceae), commonly known as ‘Shilsh-el-zallouh’,
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11 ‘Hashishat-al-kattira’ or ‘The Lebanese viagra’, is a small shrub that grows
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13 abundantly on the Hermon Mountain between Syria and Lebanon. The seeds and
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15 roots of this plant have long been used in the Middle East as an aphrodisiac, and
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17 for the treatment of frigidity and impotence for both men and women. The anti-
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19 inflammatory properties of three major daucane esters, ferutin (1) teferin (2)
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21 and teferidin (3), isolated from the root oil of *Ferula hermonis*, were assessed by
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23 the carrageenan-induced oedema model in rats. **The anti-inflammatory effect of**
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25 **both 1 and 2 was observed with the dose of 100 mg/kg, while compound 3 did not**
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27 **show any anti-inflammatory activity; conversely it produced a significant pro-**
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29 **inflammatory effect 2 and 3 h after carrageenan injection.**

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37 **Keywords:** *Ferula hermonis*; Apiaceae; ferutin; teferin; teferidin; anti-
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39 inflammatory; carrageenan
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INTRODUCTION

Ferula hermonis Boiss. (Apiaceae), commonly known as ‘Shilsh-el-zallouh’, ‘Hashishat-al-kattira’ or ‘The Lebanese viagra’, is a small perennial shrub that grows abundantly at more than 6000 feet on the high mountain areas of northern Lebanon, and on the biblical Mount Hermon in Southern Lebanon, at the joint borders of Syria and Israel (El-Taher et al., 2001; GRIN Taxonomy Database, 2010). Middle East herbalists have used the seeds and roots of this plant for centuries as a folk remedy as an aphrodisiac to treat frigidity in women, and erectile and sexual dysfunction in men by increasing blood flow to sexual organs (El-Taher et al., 2001; Lev and Amar, 2002; Said et al., 2002; Hadidi et al., 2003). The antimicrobial activity of the crude extract and the isolated compounds has been reported (Hadidi et al., 2003; Hilan et al., 2007). Previous phytochemical studies on this plant have revealed the presence of various sesquiterpenes, mainly of daucane ester type (Galal et al., 2001; Lhuillier et al., 2005; Auzi et al., 2008). As part of our continuing phytochemical and pharmacological studies on *F. hermonis* (Auzi et al., 2008; Elouzi et al., 2008), we now report on the anti-inflammatory properties of the main daucane sesquiterpenes, ferutinin (**1**) teferin (**2**) and teferidin (**3**), isolated from the root oil of *F. hermonis*, assessed by the carrageenan-induced oedema model in mice.

MATERIALS AND METHODS

General experimental procedures. UV spectra were obtained using a Hewlett-Packard 8453 UV/vis spectrophotometer in MeOH. IR spectra (KBr) were taken on a JASCO FTIR-4000/6000 Spectrometer. NMR spectra were recorded in CDCl₃ on a Bruker AMX 400 MHz NMR Spectrometer (400 MHz for ¹H and 100 MHz for ¹³C) using the residual solvent peaks as internal standard. MS analyses were performed on a Finnigan MAT95 spectrometer. Merck Silica gel 60 H was used for vacuum liquid chromatography (VLC). Silica gel 60G plates (0.5 mm thickness) were used for PTLC separation. HMBC spectra were optimized for a long-range J_{H-C} of 9Hz and NOESY experiment was carried out with a mixing time of 0.8s.

Plant material. The roots of *Ferula hermonis* Boiss. were collected from the Mount Hermon at a height of 2500 metres above the sea level close to the border between Lebanon and Syria in October 2002. The Plant material was identified at the Botany Department, Faculty of Sciences, University of Jordan, where a voucher specimen (FHS1999) has been maintained.

Extraction and isolation. The dried and powdered roots (500 g) of *F. hermonis* were macerated in a 1:1 mixture of hexane and ethyl acetate (EtOAc) for 24 h. The extract was concentrated under reduced pressure and a temperature not exceeding 50 °C to obtain an oil (10.5 g). A portion (4.7 gm) of the oil was fractionated by liquid-liquid partition using a mixture of immiscible solvents of increasing polarity (hexane:water:methanol - 4:3:1) to obtain two layers: a lipophilic portion (hexane layer)

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4 and hydrophilic portion (alcoholic layer). The solvents from these two layers were
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6 evaporated using rotary evaporator to obtain concentrated organic and aqueous extracts.
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9 The hexane layer was further subjected to both preparative thin layer chromatography
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11 (PTLC) and column chromatography (Auzi et al., 2008) yielding three major
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13 sesquiterpenes, ferutinin (**1**) (Saidkhodzhaev and Nikonov, 1979; Miski et al., 1983),
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15 teferin (**2**) (Khasanov et al., 1974) and teferidin (**3**) (Saidkhodzhaev and Nikonov, 1976;
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17 Miski et al., 1983).
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23 **Animals.** Wister rats weighing between 100-230 g of both sexes were used throughout
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25 this study for both test and control groups. The animals were obtained from the local
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27 animal house of the Pharmacology Department, Faculty of Pharmacy, El-Fatah
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29 University of Medical Sciences, Tripoli, Libya. The animals were obtained one week
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31 before the experiment. They were housed in cages of 5 animals each and were
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33 maintained under controlled laboratory conditions ($25^{\circ}\text{C} \pm 5$). Standard animal food
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35 and tap water were provided *ad libitum*. The animals were overnight fastened before
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37 each experiment. The studies involving rats were approved by the Ethical Review
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39 Committee, El-Fateh University, Libya, and the experiments were carried out strictly in
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41 accordance with the guidelines provided by the World Health Organization.
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50 **Carrageenan-induced rat paw oedema.** Three groups of overnight fasting Wister rats
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52 weighing (100-170 g) (n=5) received separately oral doses of 100 mg/kg of compounds
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54 solubilized in Tween 80 (5-10%) in water, and in 2% DMSO. A fourth group had
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56 received the vehicle only and served as a control and the fifth group was considered as
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58 the reference group where the animals were treated with aspirin (100 mg/kg) orally.
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Treatments were given one hour before carrageenan sub-plantar injection (1% in a volume of 0.1 mL) into the right hind paw of each rat (Winter and Risely, 1963; Falodun et al., 2006). The thickness of each hind paw of each animal was measured by means of a micrometer before any treatment (zero time, control values) and later one, two and three hours after carrageenan injection. The percentage increase in o was used for statistical comparison.

Statistical analysis. Data generated from the above studies were statistically analyzed by the SPSS, a computerized statistical program (version 10.0). Results were expressed as mean \pm S.E. The results were also analyzed for normality of distribution (i.e. if the results obtained are parametric or non-parametric), using Kolmogorove-Smirnov test (Fras et al., 2000). Paired and unpaired t-Tests were used when comparing two means. The one-way analysis of variance (ANOVA) was used for comparing more than two means of a parametric data followed by the LSD`s (Least Significant Difference) post hoc multiple comparisons to determine which population means were different. The differences between data are considered to be significant if the $P < 0.05$ and a highly significant if $P < 0.01$.

RESULTS AND DISCUSSION

A combination of column chromatography and preparative thin layer chromatography of the root oil of *Ferula hermonis* afforded three daucane esters sesquiterpenes, which were identified as ferutinin (**1**) teferin (**2**) and teferidin (**3**), (Figure 1) on the basis of their spectroscopic data, particularly NMR spectroscopy (Table 1) and also by comparison with respective published data.

Three sesquiterpenes (**1-3**), isolated from the root oil of *F. hermonis*, were assessed for the anti-inflammatory properties using the carrageenan-induced oedema model. The anti-inflammatory effect of both ferutinin (**1**) and teferin (**2**) was observed with the dose of 100 mg/kg, while teferidin (**3**) did not show any anti-inflammatory activity; conversely it produced a significant pro-inflammatory effect 2 and 3 hours after carrageenan injection (Figure 2). Ferutinin (**1**) significantly reduced the inflammation only at the initial phase (up to 2 hours after carrageenan injection), a phase that is said to be mediated by histamine and serotonin (Marsha-Lyn et al., 2002). Thus, it can be assumed that the anti-inflammatory effect of ferutinin (**1**) might have resulted from an antagonism of histamine and/or serotonin actions. It is noteworthy that the anti-inflammatory effect of **1** was significantly more potent than that produced by the selected dose of the positive control, aspirin, at this phase.

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4 On the other hand, when teferin (**2**), dissolved in 2% DMSO, was administered, it
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6 showed significant decrease in inflammation induced by carrageenan at both the initial,
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8 the intermediate and even the start of the third phase (3–6 hours after carrageenan
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10 injection) (Figure 3). This indicated that the anti-inflammatory effect of teferin (**2**)
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12 might have involved all inflammatory mediators including PGs, histamine, 5-
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14 hydroxytryptamine, bradykinin or nitric oxide, all of which have been reported in
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16 carrageenan-induced oedema (Marsha-Lyn et al., 2002). Teferidin (**3**) showed
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18 insignificant decrease in oedema only at the start of the initial phase, while it revealed
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20 significant pro-inflammatory effect at the later phases of inflammation.
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28 These results might be because of the structural differences in these compounds (**1-3**) in
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30 relation to the substitution of the benzene ring. Teferidin (**3**) has no substitution in its
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32 aromatic ring and lacks any anti-inflammatory effect. On the other hand, the mono-
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34 substituted benzene ring is present in ferutinin (**1**) and it displayed a considerable anti-
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36 inflammatory activity. Teferin (**2**), having a disubstituted benzene ring, proved to be the
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38 most potent anti-inflammatory agent among the three compounds. It is obvious that the
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40 degree of oxygenation on the benzene ring could be directly linked to the activity of
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42 these compounds. Substitution on the benzene ring is expected to affect the degree of
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44 ionization of the compound at biological fluids. It is well known that the ionization
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46 constants play a significant role in interpretation of the mechanisms of drug action. In
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48 particular ionization may influence the selectivity of drug action and the adsorption of
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50 drugs on the surface of receptors. The findings of the present study support the
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52 traditional use of the genus *Ferula* in the management of pains, e.g. headache and
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54 arthritis.
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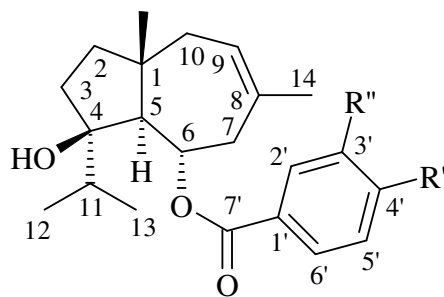
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Table 1. ^1H NMR (500 MHz, coupling constant J in Hz in parentheses) and ^{13}C NMR (125 MHz) data of daucane esters 1-3^a

Position	^1H NMR chemical shifts (δ_{H}) in ppm			^{13}C NMR chemical shifts (δ_{C}) in ppm		
	1	2	3	1	2	3
1	-	-	-	46.0	46.1	46.0
2	1.41 m; 1.62 m	1.40 m; 1.60 m	1.42 m; 1.61 m	41.5	41.6	41.6
3	1.95 m; 2.05 m	1.95 m; 2.05 m	1.95 m; 2.05 m	31.2	31.1	31.3
4	-	-	-	85.8	85.5	85.8
5	2.01 d (10.5)	2.00 d (10.5)	2.01 d (10.5)	52.2	52.3	52.3
6	5.89 m	5.90 m	5.92 m	71.1	71.2	71.1
7	2.54 m; 2.14 m	2.54 m; 2.11 m	2.52 m; 2.14 m	49.1	48.9	49.0
8	-	-	-	139.4	139.4	139.4
9	5.56 bt	5.57 bt	5.55 bt	126.3	126.3	126.3
10	2.22 bd	2.24 bd	2.28 bd	41.1	41.1	41.1
11	1.80 m	1.80 m	1.80 m	37.1	37.1	37.0
12	0.85 d (6.4)	0.85 d (6.4)	0.85 d (6.4)	17.4	17.5	17.5
13	0.96 d (6.4)	0.96 d (6.4)	0.96 d (6.4)	18.5	18.5	18.3
14	1.83 s	1.84 s	1.83 s	22.0	22.1	22.0
15	1.11 s	1.11 s	1.12 s	20.5	20.5	20.4
1'	-	-	-	122.8	122.6	130.5
2'	7.91 bd (8.6)	7.55 d (1.8)	8.06 m	132.9	112.1	130.0
3'	7.04 bd (8.6)	-	7.47 m	116.2	146.3	128.7
4'	-	-	7.60 m	160.8	150.6	133.4
5'	7.04 bd (8.6)	6.96 d (8.3)	7.47 m	116.2	114.5	128.7
6'	7.91 bd (8.6)	7.58 dd (8.3, 1.8)	8.06 m	132.9	124.5	130.0
7'	-	-	-	168.0	166.9	166.9
OMe	-	3.96 s	-	-	56.3	-

^aSpectra obtained in CDCl_3



Compounds	R'	R''
Ferutinin (1)	OH	H
Teferin (2)	OH	OMe
Teferidin (3)	H	H

Figure 1. Structures of ferutinin (1), teferin (2) and teferidin (3), isolated from *Ferula hermonis*

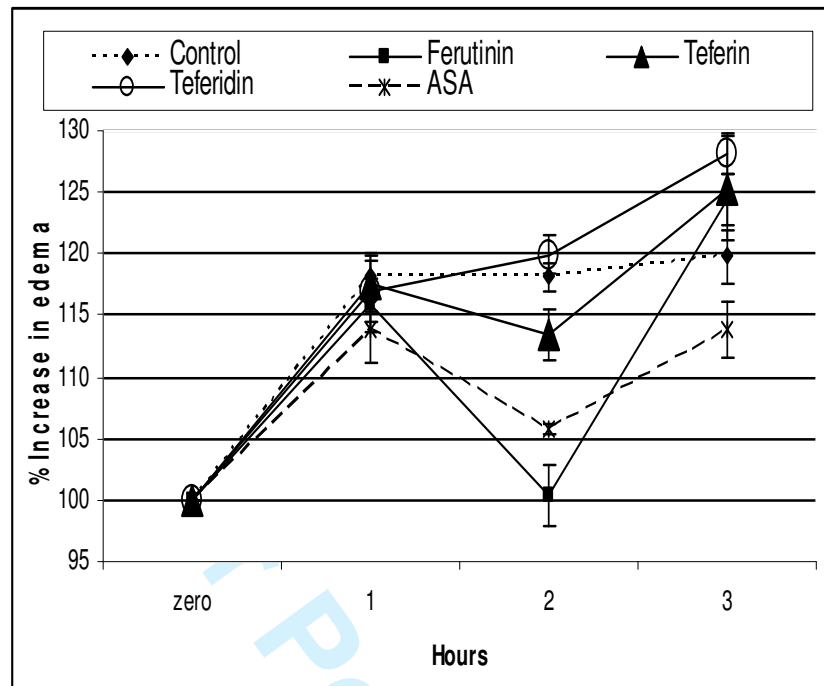


Figure 2. Anti-inflammatory effect of ferutinin (1), teferin (2) and teferidin (3) using Tween 80 (5%) at the dose (100 mg/kg) on the rat paw oedema compared with aspirin 1,2 and 3 hours after carrageenan injection.

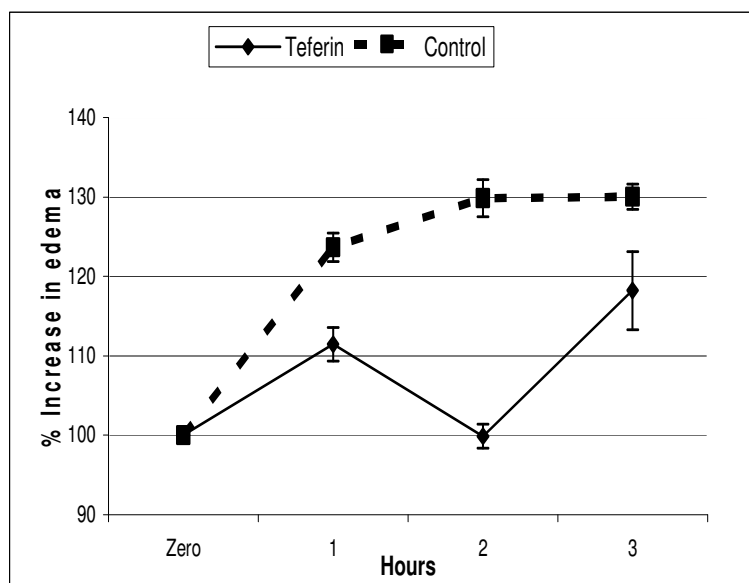


Figure 3. Anti-inflammatory effect of the compound teferin (**2**) (100 mg/kg) using 2% DMSO on the rat paw oedema 1, 2 and 3 hours after carrageenan injection.