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# Chemical constituents and antihepatotoxic effect of the berries of *Juniperus Phoenicea* Part II

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**Abstract** – Phytochemical investigation of the berries of *Juniperus phoenicea* led to the isolation of 4 compounds, namely; scutellarin, isoscutellarin, shikimic acid and the new palmitoyl lactone derivative 16-hydroxy palmitic-1, 16-olide. This is the first report for the occurrence of these compounds in the species grown in Libya. The identification of the isolated compounds was based on the application of different spectroscopic techniques. In addition, the antihepatotoxic effect of the aqueous decoction of the berries was investigated.
**Keywords** – *Juniperus phoenicea* berries, flavonoids, shikimic acid, 16-hydroxy palmitic acid-1, 16-olide, antihepatotoxic effect.

## Introduction

A wide variety of secondary metabolites such as terpenoids (Feliciano A.S. *et al.*, 1995), lignans (Feliciano *et al.*, 1989) and flavonoids (Maatooq *et al.*, 1998) were reported to occur in Juniper species. Several pharmacological activities were attributed to their active constituents e.g. cytotoxic (Ali A.M. *et al.*, 1996), antibacterial (Muhamed I. *et al.*, 1997), and antiplatelet aggregation (Kagawa *et al.*, 1993). In addition, some species were reported to be used as antidiabetic (Medina *et al.*, 1994) and antihepatotoxic (Jones S.M. *et al.*, 1998). Only few reports appeared in the literature regarding the phytochemical investigation of *Juniperus phoenicea*. The plant is well known in North Africa particularly in Libya. *Juniperus Phoenicea* sold in the Egyptian market is mainly imported from Libya.

In our previous study on the species grown in Libya we investigated the petroleum ether extract of both berries and needles, and the antidiabetic effect of the berries (Aboul Ela *et al.*, 2005). In continuation of our investigation of this plant, we wish here to report the isolation of two flavonoids namely; scutellarin and isoscutellarin, shikimic acid and 16-hydroxy palmitic-1, 16-olide for the first time from the berries of *Juniperus phoenicea*. The antihepatotoxic effect of the berries was also investigated.

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## Experimental

**General** – Melting points were determined on a Griffin melting point apparatus and were uncorrected. UV spectra were recorded on a Perkin Elmer double beam spectrophotometer Model 550S, attached to a Hitachi recorded Model 561. Shift reagents for UV spectral analyses are 5% $CH_3ONa$, 5% $AlCl_3$, conc. HCl. IR spectrum were determined on a Jasco infrared spectrophotometer, Model (FT/IR-300 E), in KBr pellets, NMR analyses were recorded on advance 300 MHz apparatus and mass spectra on a GC coupled with a Shimadzu 8080A, mass Spectrometer.

TLC was carried out on pre-coated plates (Silica gel 60 F-254, Merck) with absorbent layer thickness 0.25 mm. Detection was achieved by using ammonia vapour, UV light and/or spraying with anisaldehyde/ $H_2SO_4$ and heating at 105°C for 5 min.

**Plant material** – *Juniperus phoenicea* used in this study was purchased from a local herbalist in Alexandria, Egypt in the form of entire fruits and needles. The plant is very common in the flora of Libya mainly in the region of AL-Gabal Al-Akhdar. The second sample species obtained from a local herbalist in Aswan (Upper Egypt) who imported it from Saudi Arabia. The identity of the plant was kindly verified by Prof. Dr. Loutfy Boulous, Professor of Plant Taxonomy, National Research Center, EL-Dokki, Cairo, Egypt.

**Table 1.** The effect of daily oral administration of *Juniperus phoenicea* (J. Ph.) and Silymarin for six weeks, starting from the first day of thio-acetamide (TAA) administration on serum tumor necrosis factor-alpha (TNF-α), serum trnasforming growth factor-beta 1 (TGF-β1), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) in the rat (Mean ± SE, n = 10)

| Group | Serum tumor necrosis factor-alpha (TNF-α) (pg/ml) | Serum transforming growth factor-beta 1 (TG-F-$\beta_1$) (ng/ml) | Serum aspartate aminotrasferase (AST) (U/ml) | Serum alanine aminotransferase (ALT) (U/ml) |
|---|---|---|---|---|
| Group I (i.p. saline for 6 ws) | 14.68 ± 0.61 | 5.88 ± 0.32 | 36.22 ± 1.42 | 38.24 ± 1.86 |
| Group II (i.p. TAA for 6 ws) | 76.32 ± 2.56* | 25.65 ± 0.82* | 86.39 ± 2.92* | 81.86 ± 2.76* |
| Group III (silymarin 200 mg/kg/d orally for 6 ws) | 44.96 ± 1.36** | 18.86 ± 0.65** | 56.24 ± 2.24** | 59.26 ± 2.16** |
| Group IV (J. Ph. "Libya" 250 mg/kg/d orally, for 6 ws) | 46.52 ± 1.68** | 17.56 ± 0.81** | 58.18 ± 1.86** | 61.36 ± 2.94** |
| Group V (J. Ph. "Saudia" 250 mg/kg/d orally for 6 ws) | 47.36 ± 1.18** | 19.26 ± 0.96** | 59.24 ± 2.12** | 63.58 ± 2.76** |
| Group VI (control group for groups III-V) | 74.26 ± 2.82 | 27.48 ± 0.76 | 82.26 ± 2.58 | 85.36 ± 2.83 |
| P | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

* Significantly different (I ≠II)
** Significantly different (VI ≠III, IV & V)

**Investigation of the chloroformic and alcoholic extracts of the fruits** – The dried powdered fruits (104 g) after defatting with petroleum ether was extracted with chloroform then alcohol. The chloroform extract (4 g) was subjected to column chromatography (150 g silica gel Merck), using $CHCl_3$ with gradual increasing of polarity by MeOH. Fractions (22-25), eluted with 5% MeOH-$CHCl_3$, was concentrated under reduced pressure to give white residue (10 mg) and was designated as material 1.

The ethanol extract (5.3 g) was chromatographed on 180 g silica gel (Merck) column. Fractions 11-15 [15% MeOH in $CHCl_3$] afforded material 2 as yellow powder (50 mg). Fractions (17-21) [25% MeOH in $CHCl_3$] was subjected to pTLC ($CHCl_3$ : MeOH 8½ . 1½) to give material 3 in the form of yellow powder (8 mg) (MeOH). Fraction 26 (200 mg) eluted with 35% MeOH in $CHCl_3$ was rechromatographed on silica gel column Merck (60 g). The major spot was present in Fractions 1-4 eluted with (25-40% MeOH in $CHCl_3$) and isolated as pale yellow amorphous powder (MeOH, 20 mg); designated as material 4.

**Material (1)** – IR $\nu_{max}$ $cm^{-1}$, KBr, 2985, 2895, 1744, 1090, 850, 730. GCMS *m/z* ( % rel. abund. ), 256 (15), 238 (5), 201 (2), 167 (10), 121 (15), 83 (71), 57 (98).

$^1$H-NMR (300 MHz, $CDCl_3$) δ : 4.07 (2H, t, $CH_2$-O J = 6.7 Hz), 2.30 (2H, t, $CH_2$-CO, J = 7.5 Hz), 1.63 (m, 4H, $CH_2$-3, $CH_2$-14), 1-1.3 (m $(CH_2)_{11}$). $^{13}$C-NMR 75.0 MHz, ($CDCl_3$), d : 172.6 (C = O-O), 62.9 ($CH_2$-O), 32.9 ($CH_2$-C = O), 28.2~23.51 ($CH_2$-13).

**Material (2)** – Scutellarin, yellow powder, UV $\lambda_{max}$ MeOH; 244, 292, 330, 344 nm; NaOMe : 222, 312, 350, 388; $AlCl_3$ : 292, 312, 344, 360, 370, 410; $AlCl_3$/HCl : 292, 302, 344, 396. EIMS *m/z* ( % rel. abund.) 286 (3) ($M^+$; $C_{15}H_{10}O_6$), 168 (15), 138 (10), 118 (10), 112 (6); $^1$H-NMR (300 MHz, DMSO-$d_6$) 6.5 (1H, s, H-3), 6.79 (1H, s, H-8), 7.75 (2H, d, J = 8.5 Hz, H-2' and H-6'), 6.75 (2H, d, J = 8.5 Hz, H-3' and H-5'). $^{13}$C-NMR (75.0 MHz, DMSO-$d_6$, 162.0 (C-2), 102.0 (C-3), 176.0 (C-4), 158.0 (C-5), 144.0 (C-6), 160.5 (C-7), 97.0 (C-8), 161.0 (C-9), 105.9 (C-10), 118.0 (C-1'), 128.0 (C-2'), 114.0 (C-3'), 157.0 (C-4'), 114.2 (C-5'), 128.5 (C-6').

**Material (3)** – Isoscutellarin, dark yellow crystals, m.p. 300-301°C, UV $\lambda_{max}$ MeOH: 282, 305, 332, 350 nm; NaOMe : 230, 312, 350, 396; $AlCl_3$: 292, 348, 360, 400; $AlCl_3$/HCl; 286, 316, 347, 390. EI MS, (m/z, % rel. abund.) 286 (4) [$M^+$, $C_{15}H_{10}O_6$), 168 (8), 138 (12), 118 (8), 112 (5), $^1$H-NMR (300 MHz, $CD_3OD$), 6.4 (1H, s, H-3), 6.3 (1H, s, H-6), 7.22 (2H, d, J = 8.5 Hz, H-2' and H-6'), 6.64 (2H, d, J = 8.5 Hz, H-3' and H-5').

**Material (4)** – UV $\lambda_{max}$ MeOH 213, IR $\nu_{max}$ $cm^{-1}$, KBr, 3400, 2980, 2910, 2885, 1705, 1605,1500, 1000, 715. EI MS, (*m/z*, % rel. abund.) 174 (2) [$M^+$, $C_7H_{10}O_5$], 156 (4), 138 (40), 129 (15), 111 (50), 90 (10). $^1$H-NMR (300 MHz, $CD_3OD$), 2.19 (dd, J = 18.0, 5.3 Hz, H-6 eq), 2.80 (dd, J = 18.0, 7.2 Hz-$H_6$ax), 3.58 (dd, J = 8.6, 4.3 Hz, H-4), 3.94 (m, H-5), 4.33 (t, J = 4.3 Hz, H-3), 6.60 (br.s., H-2); $^{13}$C-NMR (75.0 MHz, $CD_3OD$), 174.90 (C = O-OH), 136.30 (C-1), 133.1 (C-2), 67.0 (C-3), 74.20 (C-4), 68.10 (C-5), 34.1 (C-6).

**Antihepatotoxic activity test** – The present study was conducted on 60 male albino rats weighing 150-200 g that were divided into the following groups, each of ten rats.

**Group I :** Rats in which liver injury to the degree of fibrosis was induced by intraperitoneal (i.p.) injection of thio-acetamide. (TAA) in 0.15 mol/L NaCl (40 mg TAA/ml NaCl) three times per week in a dose of 20 mg/100 g. b.wt. for six weeks (Nozu *et al.*, 1992).

**Group II:** Normal rats that were injected i.p. with 0.15

**Table 2.** The effect of daily oral administration of *Juniperus phoenicea* (J. Ph.) and Silymarin for six weeks, starting from the first day of thio-acetamide (TAA) administration on serum alkaline phosphatase (ALP), serum gammaglutamyl transferase (GGT), serum albumin and serum total bilirubin in the rat (Mean ± SE, n = 10)

| Groups | Serum alkaline phosphatase (ALP) (U/dl) | Serum gamma glutamyl transferase (GGT) (U/Ll) | Serum albumin (g/dl) | Serum total bilirubin (mg/dl) |
|---|---|---|---|---|
| Group I (i.p. saline for 6 ws) | 132.68 ± 3.67 | 4.86 ± 0.32 | 3.99 ± 0.04 | 0.19 ± 0.01 |
| Group II (i.p. TAA for 6 ws) | 261.28 ± 5.12* | 18.36 ± 0.39* | 2.82 ± 0.07* | 0.21 ± 0.02 |
| Group III (silymarin 200 mg/kg/d orally for 6 ws) | 190.52 ± 4.22** | 10.56 ± 0.28** | 3.66 ± 0.05** | 0.22 ± 0.01 |
| Group IV (J. Ph. "Libya" 250 mg/kg/d orally, for 6 ws) | 193.28 ± 4.16** | 11.28 ± 0.36** | 3.66 ± 0.06** | 0.18 ± 0.02 |
| Group V (JC. Ph. "Saudia" 250 mg/kg/d orally for 6 ws) | 196.39 ± 3.96** | 11.96 ± 0.42** | 3.56 ± 0.05** | 0.21 ± 0.01 |
| Group VI (control group for groups III-V) | 258.67 ± 4.96 | 17.46 ± 0.36 | 2.81 ± 0.06 | 0.22 ± 0.02 |
| P | < 0.001 | < 0.001 | < 0.001 | < 0.015 |

* Significantly different (I ≠II)
** Significantly different (VI ≠III, IV & V)

**Table 3.** The effect of daily oral administration of *Juniperus phoenicea* (J. Ph.) and Silymarin for six weeks, starting from the first day of thioacetamide (TAA) administration on serum malondialdehyde (MDA), liver reduced glutathione (GSH), liver glycogen and liver hydroxyproline (HPO) in the rat (Mean ± SE, n = 10)

| Group | Serum malondialdehyde (MDA) (nmol/ml) | Liver reduced glutathione (GSH) (mg/g wet tissue) | Liver glycogen (mg/g wet tissue) | Liver hydroxyproline (HPO) (mg/g wet tissue) |
|---|---|---|---|---|
| Group I (i,p, saline for 6 ws) | 2.32 ± 0.15 | 3.91 ± 0.09 | 16.12 ± 0.48 | 0.30 ± 0.01 |
| Group II (i.p. TAA for 6 ws) | 5.86 ± 0.13* | 1.96 ± 0.04* | 7.22 ± 0.17* | 0.66 ± 0.02* |
| Group III (silymarin 200 mg/kg/d orally for 6 ws) | 2.86 ± 0.14** | 3.56 ± 0.08** | 10.36 ± 0.38** | 0.44 ± 0.03** |
| Group IV (J. Ph. "Libya" 250 mg/kg/d orally, for 6 ws) | 3.37 ± 0.15** | 3.16 ± 0.07** | 10.92 ± 0.48** | 0.47 ± 0.02** |
| Group V (J. Ph. "Saudia" 250 mg/kg/d orally for 6 ws) | 3.56 ± 0.14** | 3.08 ± 0.08** | 11.12 ± 0.38** | 0.49 ± 0.02** |
| Group VI (control group for groups III-V) | 5.92 ± 0.14 | 1.97 ± 0.05 | 6.69 ± 0.16 | 0.71 ± 0.03 |
| P | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

* Significantly different (I ≠II)
** Significantly different (VI ≠III, IV & V)

mol/L NaCl three times per week in a dose of 0.5 ml/100 g.b.wt. for six weeks. This group served as a control group for group I.

**Group III:** Rats that received silymarin suspended in 2% gum acacia mucilage in a dose of 200 mg/kg b.wt./ day orally for six weeks starting from the first day of TAA administration.

**Group IV:** Rats that received a decoction of the powdered plant (*Juniperus phoenicea* L. which grows in Libya at 10% "w/v") dissolved in distilled water in a dose of 250 mg/kg b.wt./day orally for six weeks starting from the first day of TAA administration.

**Group V:** Rats that received a decoction of the powdered plant (*Juniperus phoenicea* which grows in Saudi Arabia) at 10% (w/v) dissolved in distilled water in a dose of 250 mg/kg b.wt./day orally for six weeks starting from the first day of TAA administration.

**Group VI:** Rats that received 2% gum acacia mucilage and distilled water daily orally for six weeks starting from the first day of TAA administration and served as a control group for groups III-V.

**Blood Samples** – At the end of the six weeks, blood samples were collected from the retro-obrital venous plexus of the rat (Riley 1960) and stored at -20°C until being analyzed. Serum samples were used for the determination of the following parameters:

1-Serum tumor necrosis factor-alpha-(TNF-α) concentration (Aukrust *et al.*, 1996).

2-Serum transforming growth factor-beta 1 (TGF-β1) concentration (Murawaki *et al.*, 1996)

3-Serum aspartate aminotransferase (AST) activity (Reitman and Frankel, 1957).

4-Serum alanine aminotransferase (ALT) activity (Reitman and Frankel, 1957).

5-Serum alkaline phosphatase (ALP) activity (Varley and Goweulock, 1980).

6-Serum gamma glutamyl transferase (GGT) activity (Rosalki and Tarlow, 1974).

7-Serum albumin level (Doumas and Biggs, 1972).

8-Serum total bilirubin (Doumas *et al.*, 1985).

9-Serum malondialdehyde (MDA) level as a marker of lipid peroxidation (Satoch, 1978).

**Tissue Samples** – After the blood samples were taken, animals were sacrificed by exsanguination; the liver isolated from each animal, and blotted by filter paper from blood. Homogenates were made from the liver using the specific homogenizing solutions and used for the determination of the following parameters :

1-Liver reduced glutathione (GSH) concentration as a marker of lipid peroxidation (Patterson and Lazarou 1995).

2-Liver hydroxyproline (HPO) concentration as a marker of liver fibrosis (Cheng 1969, Berman and Loxley 1963).

**Statistical Analysis** – Data were expressed as mean ± standard error (SE). One way analysis of variance (ANOVA) techniques were used to examine the studied parameters. For pairwise comparisons among group, the least significance difference (LSD) test was used.

## Results and Discussion

**Structure identification** – Compound **1** was isolated in the form of white amorphous powder. $^{1}$H-NMR, DEPT and $^{13}$C-NMR spectral data are in full agreement with the molecular formula $C_{16}H_{30}O_2$. GCMS analysis revealed the presence of a molecular ion peak at *m/z* 256, possibly corresponding to [$M^+$ + 2H], and another fragment at *m/z* 238 due to loss of $H_2O$. $^{1}$H-NMR was integrated for thirty protons, while $^{13}$C-NMR spectrum demonstrated sixteen signals, DEPT experiment indicated these carbons to be 15 methylenes (fourteen signals were resonating at δ 23~33 and one was observed at δ 62.9) in addition to one quaternary at δ 172.6. Further-more, the presence of a carbonyl of an ester group was deduced from both IR (band at 1744 cm$^{-1}$) and $^{13}$C-NMR at δ 172.6. The $^{1}$H-NMR spectrum revealed the presence of two methylene signals at δ 4.07 ( t, J = 6.7 Hz ) and δ 2.30 ( t, J = 7.5 Hz ) and in the $^{13}$C-spectrum at δ 62.9 and δ 32.9 due to -$CH_2$O- and -$CH_2$-C = O, respectively. COSY experiment indicated that, each of these two $CH_2$ groups is coupled with four proton multiplets centered at δ 1.63 due to the two $CH_2$ groups. All the present data pointed to the presence of an internal ester (at position 3 and 15) of 16-hydroxy palmitic acid. Accordingly, compound (1) is 16-hydroxypalmitic-1, 16-olide. It is of interest to mention that, this compound is isolated for the first time from a natural source.

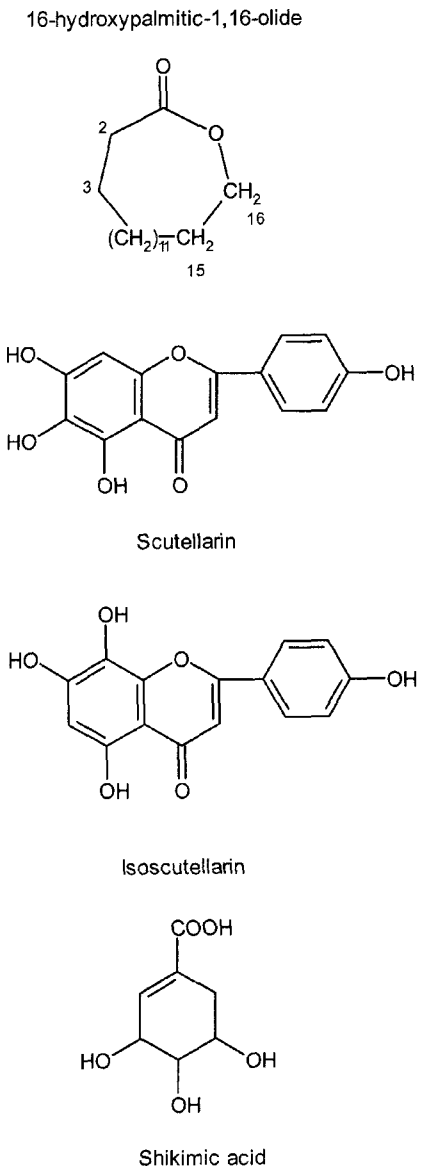


**Fig. 1.** Materials isolated from the berries of *Juniperus phoenicea.*

Physical and chemical properties of Compounds **2** and **3** indicated hydroxylated flavone aglycones. The intense yellow colour produced with alkalis and darken by time indicated high hydroxylation pattern at one of the rings. Compounds **2** and **3** were found to have the same molecular formulae $C_{15}H_{10}O_6$ as observed from mass spectra that revealed the mol. ion peak at *m/z* (286). Furthermore, MS showed mass fragments at *m/z* 168 and 118 indicating trisubstitution on ring A. UV absorption in methanol of both compounds indicated the absence of 3-OH groups. In addition, UV absorption in MeOH and using the shift reagents NaOMe, $AlCl_3$, $AlCl_3$/HCl

ascertained the presence of 4'-OH in both **2** and **3** (NaOMe), the presence of three hydroxyl groups on ring A ($AlCl_3$, $AlCl_3$/HCl) and the absence of 3',4'-O-dihydroxy system on ring B. This was further confirmed from $^1$H-NMR spectra that showed a singlet at δ 6.5 and δ 6.4 for both compounds (**2** and **3**) respectively assigned for H-3. $^1$H NMR spectrum of compound **2** showed the presence of singlet signal at δ 6.79 due to H-8, while that of compound **3** revealed the presence of a signal at δ 6.30 due to H-6. Comparing all the obtained spectral data of 2 and **3** with those previously reported for scutellarin and isoscutellarin, respectively found to be almost identical (Jay and Gonnet, 1973); (Hua and Quing, 2004); and (Iunuma *et al.*, 1980).

The molecular formula of compound **4** was deduced based on different spectral data to be $C_7H_{10}O_5$. $^{13}$C-NMR spectrum revealed the presence of seven resolved carbon signals. DEPT experiment indicated these carbons to be : one $CH_2$, four CH and two quaternary carbons. Addition of Ca $CO_3$ to compound 4 produced effervescence, suggesting the acidic nature of this compound. $^{13}$C-NMR spectrum showed the presence of a carbon signal at δ 174.9 due to the carbonyl of the acid group, most probably in conjugation with a double bond. This was evident by UV spectrum, that revealed the presence of an absorption maximum at λ 213 nm. $^1$H-NMR spectrum showed the presence of one proton singlet at δ 6.60. The spectrum also indicated the presence of two coupled *gem*-protons at δ 2.19 (dd, J = 18.0, 5.3 Hz) and at δ 2.80 (dd, J = 18.0, 7.2 Hz). HMQC experiment indicated their carbon to be at δ 34.1. Moreover, the $^1$H-NMR spectrum revealed the presence of three oxymethine groups appearing at δ 3.58 (dd, J = 8.6, 4.3 Hz), 3.94 (m) and at δ 4.33 (t, J = 4.3 Hz). HMQC experiment indicated their corresponding carbons to be at δ 74.2, 68.1 and 67.0, respectively. Interpretation of the obtained spectral data along with comparison with structurally similar analogues (Haslam and Turner, 1971) have allowed the identification of compound **4** to be shikimic acid, one of the most important precursors in plant biogenesis. It is of interest to mention that this compound is isolated for the first time from *Juniperus species*. Furthermore, quinic acid which is related to shikimic acid reported very recently to be a candidate component for both *in vivo* and *in vitro* biological effects, by inducing cell proliferation arrest and inhibits activation of the transcriptional regulator nuclear factor B (NF-KB). In addition, *in vivo* experiments, it significantly increased number of spleen cells, thus recaptitulating its *in vivo* biological activity. (Aleesson *et al.*, 2005).

**Antihepatotoxic effect** – *Silybum marianum* is a medicinal plant which has been widely used in traditional European medicine. Nowadays, silymarin, the purified flavonlignan mixture of the fruits of *S. marinum* is used in the treatment of liver diseases (Morazzoni and Bombardell 1995). In this work, silymarin was used as a control of the antihepatotoxic effect of the studied plant *Juniperus phoenicea*.

The present work demonstrated significant elevations in serum TNF-α and TGF-β concentrations in rats that received TAA for six weeks compared to control values. These results are in accordance with several studies that point to TNF-α and TGF-β1 as mediators stimulating key events in liver fibrogenesis. TNF-α appears most critical in the early changes of liver fibrosis, such as the loss of vitamin A, matrix degradation, cell migration and proliferation. On the other hand, TGF-β1 is considered a potent mediator for the later phase of HSC activation, characterized by matrix accumulation (Bachem *et al.*, 1993 and Czaja *et al.*, 1989).

Considering the role played by TNF-α in liver fibrogenesis, several data have supported the possible involvement of TNF-α in hepatic fibrogenesis through its ability to stimulate activation of HSC and the synthesis of proteins involved in matrix metabolism. The more HSC are transformed to MFB, the more sensitive the cells become to TNF-α, which may lead to a vicious circle of enhanced fibrogenic and chemotactic mediator production. TNF-α has been also reported to stimulate HSC collagen synthesis and to increase the synthesis of other ECM proteins, particularly of fibronectin and tenascin (Knittel *et al.*, 1997). Additionally, TNF-α may play a role in stimulation of fibroblasts in the liver to proliferate and secrete collagen (He and Liu *et al.*, 1996). In addition TNF-α, as well as TGF-β1 have been reported to activate stress activated protein kinase (SAPK), which is implicated in the transformation process of HSC to MFB (Bahr *et al.*, 1997).

Shifting to the impact of the studied drugs on liver fibrosis, the results of the present work revealed that *Juniperus phoenicea* produced a significant decrease in serum TNF-α and TGF-β1, and a significant improvement in all the estimated parameters of liver function, as well as a significant decrease in hepatic HPO concentration compared to the contnol. The observed antifibrotic effect of *Juniperus phoenicea* can be explained by appreciating its suppressive effect on the cytokines, TNF-α and TGF-β1, thus reducing the deleterious effect of the excess production of both cytokines, which is implicated in the pathogenesis of liver fibrosis. Also, *Juniperus phoenicea*

has pharmacological effects, other than suppressing TNF-α and TGF-β1 that can partially account for its antifibrotic effect.

Concerning the effect of *Juniperus phoenicea* on serum concentrations of TNF-α and TGF-β1, the results of the present work demonstrated a significant decrease in both cytokines in rats that received *Juniperus phoenicea* for six weeks as compared to the control rats. These results are in accordance with those of other studies reporting a decrease in the concentration of TNF-α and TGF-β1 in response to other antioxidants such as vitamin E and N-acetylcysteine (Parola *et al.*, 1992, Victor *et al.*, 1999, Pena *et al.*, 1999).

In the present study, *Juniperus phoenicea* produced a significant improvement in all the estimated parameters of liver function. The results of the present work demonstrated the high antioxidant potential of *Juniperus phoenicea* via prevention of hepatotoxicity-induced hepatic GSH depletion and serum MDA accumulation.

In the present study, the inhibition of histamine and LTs generation from mast cells could contribute to the observed antifibrotic effect of *Juniperus phoenicea*. Mounting evidence indicates that histamine and LTs play an important role in the pathophysiology of the injured liver and an important interrelationship between these mediators and cytokines have been reported (Hiragan *et al.*, 1998). Both mediators have been additionally involved in fibrotic processes. As regards histamine, it has been reported to stimulate collagen synthesis (Kikudin *et al.*, 1995) and to increase its thermal stability (Dabrowski, 1987). Concerning its role in liver fibrogenesis, it has been reported that hepatic histamine content is elevated markedly in $CCl_4$-induced rat liver fibrosis (Suzuki and Nakano 1996). Histamine has been suggested to play an important role in the pathophysiology of liver fibrosis through stimulating the production of proinflammatory cytokines (Umezu *et al.*, 1985). It has been postulated that the hepatotoxin-induced increase in histamine synthesis could be mediated by TNF-α and TGF-β1 (Fernandez *et al.* 1995). Other studies have demonstrated interactions among histamine, TGF-β1 and TNF-α (Endo, 1989).

As regards LTs, their enhanced production in patients with liver fibrosis has been demonstrated (Cincu *et al.*, 1997). Again a role for both TNF-α and TGF-β1 in the overproduction of LTs has been demonstrated. In hepatocytes, LTs are taken up into the cells across the sinusoidal membrane and excreted into the bile across the canalicular membrane by the canalicular isoform of the multidrug resistance-associated protein (cMRP) transporter. It has been reported that during inflammation, this canalicular secretory step is inhibited, but the sinusoidal membrane uptake is unchanged and therefore intracellular concentration of canalicular LTs increases. TNF-α may serve to prime cells for this effect by altering the expression levels, membrane localization, or channel coupling of the cMRP (Meng *et al.*, 1997).

Concerning the role of LTs, it has been shown that cysteinyl LTs affect liver cell viability. It has been also found that the lipooxygenase pathway is involved in HSC transformation and that these cells produced significant quantities of LTs during their activation (Beno *et al.*, 1995). It has been reported that LT production is involved in maintaining the high level of collagen mRNA production by activated HSC and that LTs positively regulate collagen gene expression (Chen *et al.*, 1996). At the same time LTs have been reported to increase the production of proinflammatory cytokines (Alric *et al.*, 1996).

In conclusion, the results of the present study demonstrated that TAA-induced model of rat liver fibrosis is a reliable model for studying liver fibrosis experimentally. Following TAA administration, there was a significant elevation in serum concentration of the cytokines TNF-α and TGF-β1, thus implicating a role for these cytokines in the pathogenesis of liver fibrosis. *J. phonecia* was effective in preventing the fibrogenic process via modulating the action of the cytokines TNF-α and TGF-β1. The antifibrotic potential of *Juniperus phoenicea* can be assessed in other experimental models of fibrosis, in which the cytokines TNF-α and TGF-β1 are also implicated to have a role. Furthermore, the presence of polyhydroxylated flavonoids, quinic acid derivative and polyunsaturated fatty acids in the berries of this plant, might explain its antihepatotoxic effect. The results revealed that there was no significant difference between *J. phoenicea* and silymarin as regard all the investigated parameters. In addition, the two examined extracts of *J. phoenicea* showed almost similar antihepatotoxic effect.

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