A New Flavonoid *C*-Glycoside from *Solanum elaeagnifolium* with Hepatoprotective and Curative Activities against Paracetamol-Induced Liver Injury in Mice

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A new flavonoid *C*-glycoside, kaempferol 8-*C*-*β*-galactoside, along with twelve known glycosidic flavonoids was isolated from the aqueous methanolic extract of *Solanum elaeagnifolium* Cav. (Solanaceae), by conventional chromatographic methods; their structure elucidation was achieved using UV, ESI-MS, and NMR spectral analyses. Groups of six mice were administered *S. elaeagnifolium* extracts at 25, 50, and 75 mg/kg body weight (BW) prior to or post administration of a single dose of paracetamol (500 mg/kg BW). The extract showed significant hepatoprotective and curative effects against histopathological and histochemical damage induced by paracetamol in liver. The extract also ameliorated the elevation in glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP) levels. These findings were accompanied by a nearly normal architecture of the liver in the treated groups, compared to the paracetamol control group. As a positive control, silymarin was used, an established hepatoprotective drug against paracetamol-induced liver injury. This study provides the first validation of the hepatoprotective activity of *S. elaeagnifolium*.

Key words: Solanum elaeagnifolium, Flavonoids, Hepatoprotective

Introduction

The Solanaceae represent a major plant family, with many valuable vegetable crops. They contain several major food plants and provide fruits, spices, and stimulants, as well as chemical compounds which are important in medicine, pharmacology, and drug therapy (Hawkes, 1999).

Solanum is the most representative genus of the Solanaceae, containing about 1,500 species. It is widespread in tropical and subtropical regions of the entire world (Agra, 1999). *Solanum* species are a rich source of steroidal alkaloids and flavonoids, and their glycosides which are known to possess a variety of biological activities (Silva *et al*., 2003). The glycoalkaloids are natural toxins with importance to both ecology and human health, such as being effective against herbivores and pathogenic microorganisms (Fukuhara *et al*., 2004) and as starting material for anabolic, antifertility, anti-inflammatory, and antiallergic drugs, respectively (Mola *et al*., 1997).

Silver-leaf nightshade (*Solanum elaeagnifolium*) is a perennial broadleafed weed with silvery foliage and violet, blue or white flowers (Boyd *et al*., 1984). It is native to the northeast of Mexico and the southwest of the USA (Robinson *et al*., 1978). The steroidal alkaloid solasodine used in the preparation of contraceptive and corticosteroid drugs has been commercially extracted from *S. elaeagnifolium* berries in India (Maiti, 1967) and Argentina, making it the most promising source among the *Solanum* species investigated (Heap and Carter, 1999). Recent studies have identified other potential uses for *S. elaeagnifolium*, as extracts have been found to have moluscicidal and

nematicidal activities, as well as cancer-inhibiting activity. Previous phytochemical studies on *S. elaeagnifolium* have demonstrated the presence of squalene, the saponin 3-deoxy-Δ^3-diosgenin, steroidal glycoalkaloids, solamargine (Guerreiro *et al*., 1971), the flavonoid kaempferol 3-*β*-(6″-*O*-*cis*-cinnamoylglucopyranoside) (Chiale *et al*., 1991), as well as solasurine and solanelagnin (Hanna *et al*., 1996).

In this work, the identification of the new flavonolid *C*-glycoside kaempferol 8-*C*-*β*-galactoside (**1**) along with twelve known flavonoid glycosides, kaempferol 8-*C*-*β*-glucoside (**2**), kaempferol 6-*C*-*β*-glucoside (**3**), kaempferol 7-*O*-*β*-glucoside (**4**), kaempferol 3-*O*-*β*-glucoside (**5**), kaempferol 3-*O*-(6″-*O*-*cis*-*p*-coumaroyl)-*O*-*β*-galactoside (**6**), kaempferol 3-*O*-(6″-*O*-*cis*-*p*-coumaroyl)-*O*-*β*-glucoside (**7**), quercetin 6-*C*-*β*-glucoside (**8**), quercetin 3-*O*-*β*-galactoside (**9**), quercetin 3-*O*-*β*-glucoside (isoquercitrin) (**10**), quercetin 3-*O*-*β*-apiofuranosyl-(1→2)-*O*-*β*-galactoside (**11**), apigenin 6-*C*-*β*-glucoside (vitexin) (**12**), and apigenin 6,8-di-*C*-*β*-glucoside (vicenin II) (**13**), in an aqueous methanolic extract of the aerial parts of *S. elaeagnifolium* is described (Fig. 1). In addition, the hepatoprotective and curative activities of the extract were examined by using paracetamol-induced hepatic injury in mice.

Results and Discussion

Chemical characterization of isolated compounds

The total 70% methanol extract of the air-dried flowering aerial parts of *S. elaeagnifolium* plants was found, by two-dimensional paper chromatography screening, to contain a complicated flavonoid mixture (Fig. 1). Flavonoids **1**–**13** were detected as dark purple spots under UV light, changing to yellow when exposed to ammonia vapour and by spraying with alcoholic aluminum chloride solution. Complete acid hydrolysis of the *O*-glycosides **4**–**7**, **9**–**11**, and ferric chloride degradation of the *C*-glycosides **1**–**3**, **8**, **12**, **13** were carried out, followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties. The structures of the isolated compounds were determined from UV, MS, and NMR spectral data.

Compound **1** was found to be an acid-resistant flavonoid glycoside which identified it as a flavo-

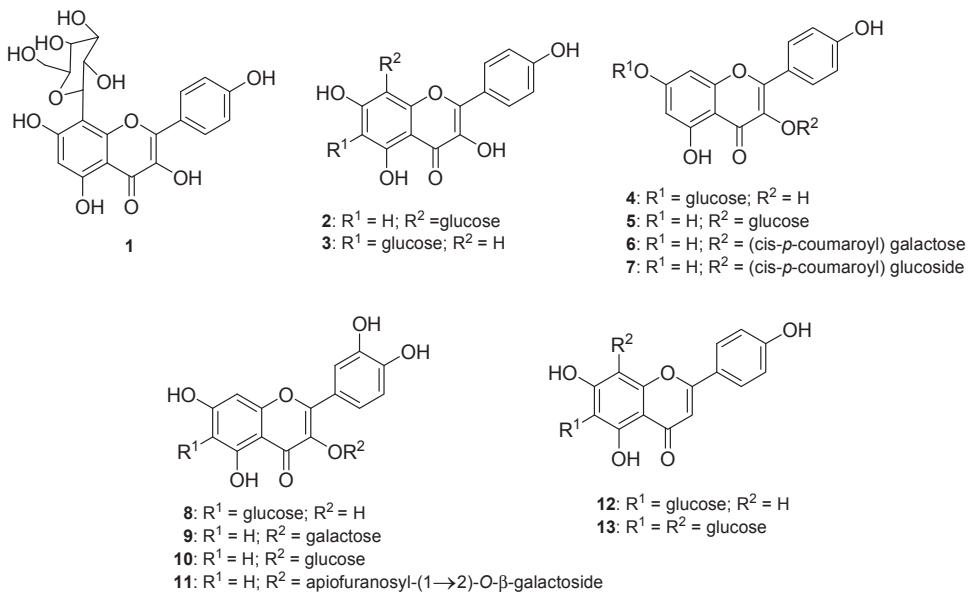

Fig. 1. Chemical structures of the isolated compounds kaempferol 8-*C*-*β*-galactoside (**1**), kaempferol 8-*C*-*β*-glucoside (**2**), kaempferol 6-*C*-*β*-glucoside (**3**), kaempferol 7-*O*-*β*-glucoside (**4**), kaempferol 3-*O*-*β*-glucoside (**5**), kaempferol 3-*O*-(6″-*O*-*cis*-*p*-coumaroyl)-*O*-*β*-galactoside (**6**), kaempferol 3-*O*-(6″-*O*-*cis*-*p*-coumaroyl)-*O*-*β*-glucoside (**7**), quercetin 6-*C*-*β*-glucoside (**8**), quercetin 3-*O*-*β*-galactoside (**9**), quercetin 3-*O*-*β*-glucoside (isoquercitrin) (**10**), quercetin 3-*O*-*β*-apiofuranosyl-(1→2)-*O*-*β*-galactoside (**11**), apigenin 6-*C*-*β*-glucoside (vitexin) (**12**), and apigenin 6,8-di-*C*-*β*-glucoside (vicenin II) (**13**).

noid *C*-glycoside. Furthermore, compound **1** was subjected to ferric chloride degradation (Koeppen and Roux, 1965), and the degradation products were co-chromatographed with authentic sugar samples, whereby galactose was detected.

The UV spectrum of **1** in MeOH gave absorption maxima at 368 (band I) and 268 nm (band II) which are characteristic for flavonols. A bathochromic shift with NaOMe, with reduced intensity of band I (418 nm), is diagnostic for the presence of free 3-OH and 4'-OH groups. The bathochromic shift of band II (278 nm) in the presence of NaOAc, relative to the same band in MeOH, indicated a free 7-hydroxy group (Mabry *et al.*, 1970). Thus, the UV spectral data of compound **1** with the diagnostic shift reagents indicated a flavonol (kaempferol) with free 3,5,7,4'-tetrahydroxy groups.

The ^{1}H NMR spectrum of **1** revealed two distinct patterns of proton resonances, the first being typical for kaempferol with a broad signal at δ_H 13.83 ppm for the exchangeable proton of 5-OH with the 4-keto group. Four aromatic protons were shown as two doublets at δ_H 7.77 and 6.72 ppm, each doublet with J = 8 Hz due to *ortho*-coupling; the protons were assigned to H-2',6' and H-3',5', respectively. A singlet at δ_H 6.26 ppm was assigned to H-8 or H-6. The second signal pattern is characteristic for sugar protons. The anomeric proton of a galactosyl residue appeared at δ_H 4.56 ppm as a doublet (J = 7.8 Hz). Its coupling constant confirmed the β-configuration of the galactose moiety.

As the ^{1}H NMR spectrum of **1** revealed the substituent patterns of kaempferol, displaying however only one singlet proton, the sugar residue must be attached to position 6 or 8. This was fully confirmed by the ^{13}C NMR spectrum of **1** which exhibited close similarity with the carbon resonances of kaempferol and galactose. There was, however, a downfield shift ($\Delta\delta$ = 10.5 ppm) of the C-8 carbon resonance compared to free kaempferol (δ_C 95 ppm), due to glycosylation (Agrawal, 1989), and an accompanying chemical shift of the resonance of the galactose anomeric carbon atom at δ_C 74.3 ppm, indicating that the galactose moiety is attached directly to the benzene nucleus of kaempferol by a carbon-carbon bond. Thus, compound **1** was identified as kaempferol 8-*C*-β-galactoside which has not been reported before to occur in nature.

Paracetamol-induced hepatotoxicity

Acute toxicity

The aqueous methanolic extract of flowering aerial parts of *S. elaeagnifolium* was found to be practically non-toxic when administered orally to mice, and its LD_{50} value was determined to be higher than 500 mg/kg body weight (BW). This allowed us to select safe doses for administration to mice to assess their hepatoprotective effect. The doses used in paracetamol-induced hepatotoxicity tests were 25, 50, and 75 mg/kg BW, respectively.

Biochemical and histopathological results

The present study was conducted to evaluate the protective and curative effects of the *S. elaeagnifolium* aqueous methanolic extract against paracetamol-induced hepatic disorders in mice.

The levels of the three marker enzymes glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and alkaline phosphatase (ALP), in serum increased significantly in the mice after paracetamol administration (P <

Table I. Protective and curative effects, respectively, of the *S. elaeagnifolium* extract and silymarin on serum transaminase activities in paracetamol-treated mice.

Group	ALP [IU/L]	SGPT [IU/L]	SGOT [IU/L]
Control	115.44 ± 1.84	94.12 ± 1.77	127.66 ± 1.58
Paracetamol (500 mg/kg BW)	313.37 ± 1.21*	207.9 ± 9.15*	230.59 ± 3.77*
S. elaeagnifolium extract (25 mg/kg BW) and paracetamol	246.43 ± 1.27**	171.65 ± 1.0**	182.69 ± 3.4**
S. elaeagnifolium extract (50 mg/kg BW) and paracetamol	192.89 ± 1.16**	142.35 ± 0.60**	166.35 ± 0.70**
S. elaeagnifolium extract (75 mg/kg BW) and paracetamol	161.28 ± 0.94**	103.29 ± 1.93**	135.26 ± 0.96**
Silymarin (100 mg/kg BW) and paracetamol	150.16 ± 1.45**	98.32 ± 1.03**	130.02 ± 1.12**
Paracetamol and *S. elaeagnifolium* extract (25 mg/kg BW)	273.12 ± 2.59**	190.22 ± 3.21**	196.20 ± 3.96**
Paracetamol and *S. elaeagnifolium* extract (50 mg/kg BW)	191.81 ± 6.76**	176.13 ± 2.46**	179.3 ± 2.7**
Paracetamol and *S. elaeagnifolium* extract (75 mg/kg BW)	161.61 ± 2.76**	119. 10 ± 2.50**	137.2 ± 8.5**
Paracetamol and silymarin	154.90 ± 2.16**	110.55 ± 3.20**	133.20 ± 5.2**

Data presented as mean ± SE. * Significant (P < 0.05) as compared with the control group; ** significant (P < 0.05) as compared with the paracetamol group.

0.05), compared to the control group (Table I). Examination of liver sections of the control group revealed normal hepatic architecture (Figs. 2A, 3A). Groups treated with 25, 50, and 75 mg/kg BW of the *S. elaeagnifolium* extracts showed normal histology of the liver (data not shown). Changes in liver histology (Figs. 2B, 2C, 3B, 3C), such as hepatocellular necrosis, swollen centrilobular hepatocytes, marked cytoplasmic vacuolation, pyknotic nuclei, obliterated hepatic sinusoids, and moderate

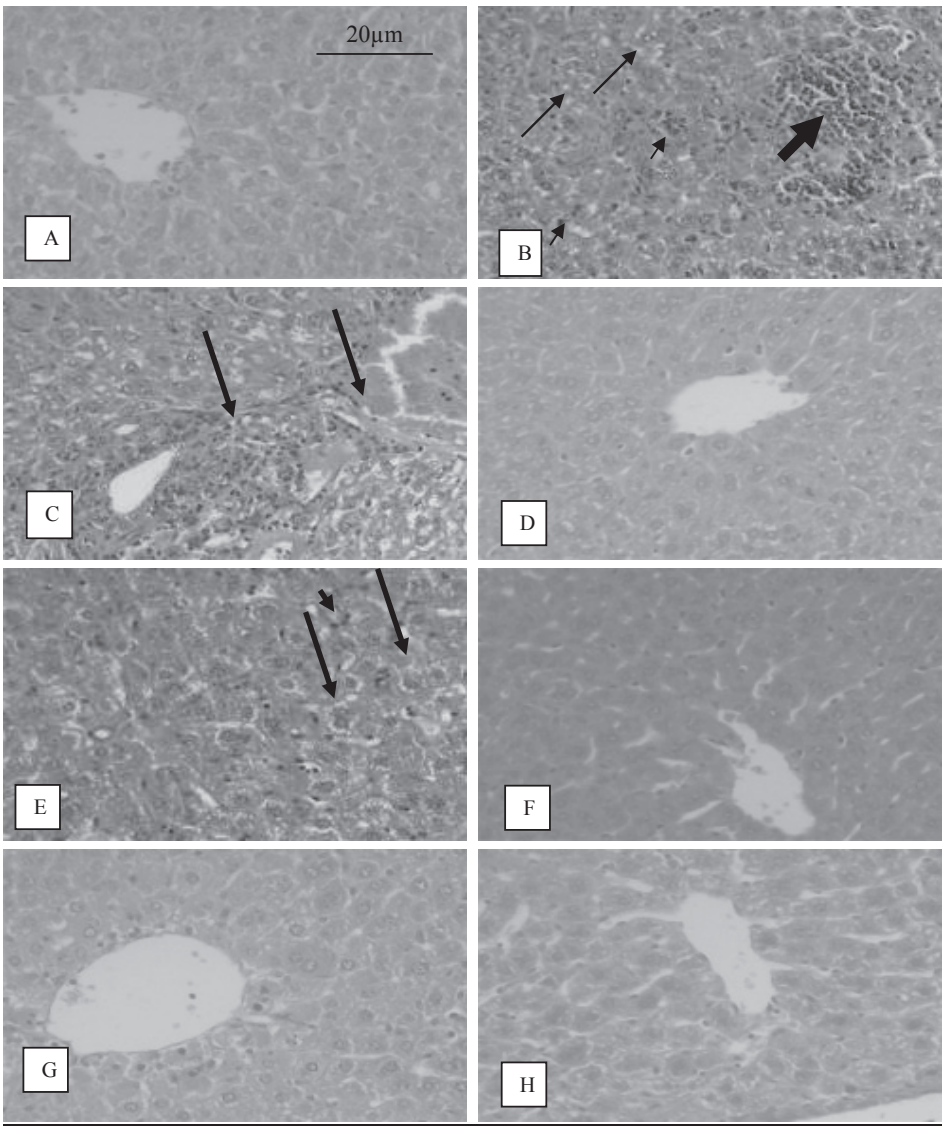

Fig. 2. Protective effects of *S. elaeagnifolium* extract and silymarin on the hepatic architecture of paracetamol-treated mice. (A) Control: normal hepatic architecture. (B) Paracetamol: swollen centrilobular hepatocytes (arrows), highly vacuolated cytoplasm, deeply stained nuclei (arrowheads), focal necrosis of the hepatocytes (large arrow), and obliteration of the intervening sinusoids. (C) as (B) Inflammatory cell infiltration and hemorrhagic area (arrows) in the portal area. (D) Paracetamol + 25 mg/kg BW extract: normal hepatic architecture. (E) as (D), showing many hepatocytes that reveal dark nuclei and vacuolation (arrows). (F, G) Paracetamol + 50 (F) and 75 (G) mg/kg BW extract, respectively, with classical hepatic architecture of nearly normal appearance. Some cells have vacuolated cytoplasm and dark nuclei (arrows). (H) Paracetamol + silymarin: normal hepatic architecture. (H & E, MB: 20 μm)

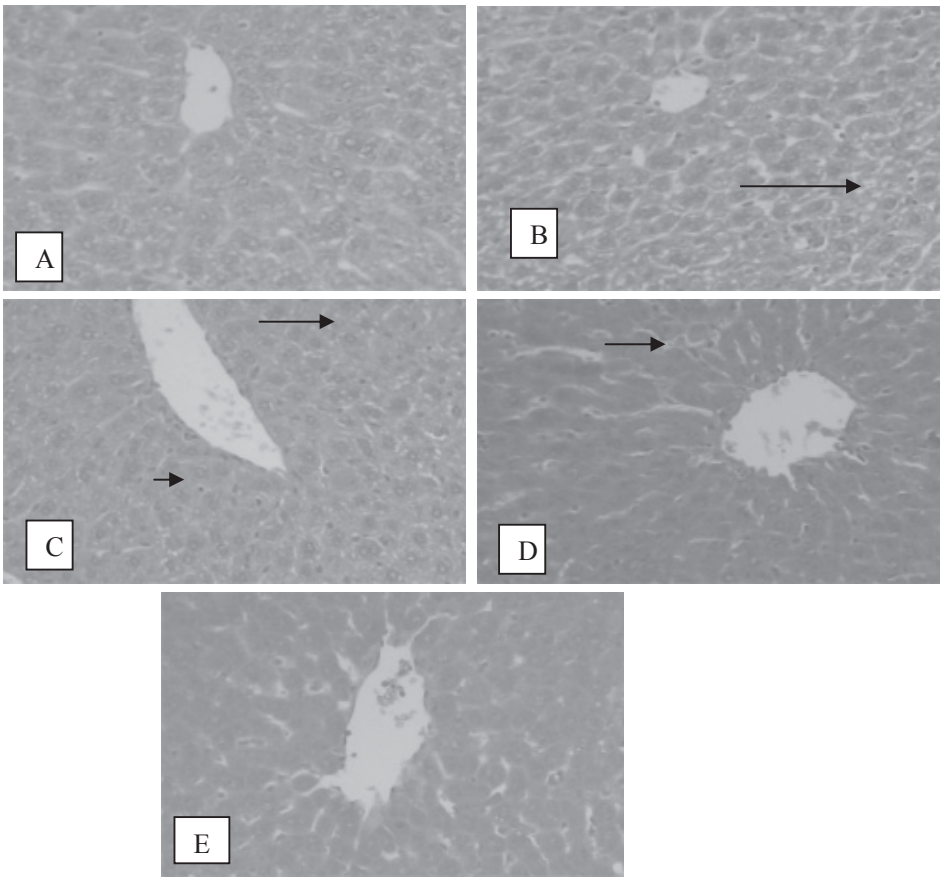

Fig. 3. Curative effects of *S. elaeagnifolium* extract and silymarin on the hepatic architecture of paracetamol-treated mice. (A) Paracetamol + 25 mg/kg BW extract: normal hepatic architecture. (B) as (A) Many of hepatocytes still reveal dark nuclei and vacuolation. (C, D) Paracetamol + 50 (C) and 75 (D) mg/kg BW extract: classical hepatic architecture with nearly normal appearance. Some cells have vacuolated cytoplasm and dark nuclei. (E) Paracetamol + silymarin: normal hepatic architecture. (H & E, MB: 20 μm)

inflammatory cell infiltration in the periportal areas, were observed in paracetamol-treated animals.

It is established that paracetamol administered in an oral therapeutic dose is converted via the cytochrome P-450 pathway to the highly toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Dahlin *et al*., 1984), which is normally conjugated with glutathione, and the conjugates are excreted in the urine. But overdoses of paracetamol deplete the glutathione pool, leading to accumulation of NAPQI, mitochondrial dysfunction (Parmar *et al*., 1995), and the development of acute hepatic necrosis (Janes and Routledge, 1992). Depletion of glutathione also enhances the expression of the tumour necrosis factor alpha (TNF-α) (Agarwal and Piesco, 1994). TNF-α primes phagocytic NADPH oxidase to the enhanced production of free oxygen radicals and thus contributes to liver damage (Gupta *et al*., 1992).

It has been reported that the increased activities of liver marker enzymes, such as GOT, GPT, and ALP, in the serum of paracetamol-treated mice indicate damage to hepatic cells. Damage to the cell integrity of the liver by paracetamol is reflected by an increase in the activity of GOT, which is released into circulation after cellular damage (Rajkapoor *et al*., 2002; Yanpallewar *et al*., 2003).

In animals treated with 25, 50, and 75 mg/kg BW of the *S. elaeagnifolium* extract and with si-

lymarin (positive control), respectively, before or after paracetamol administration, the increased levels of GOT, GPT, and ALP were significantly ($P < 0.05$) reversed compared to animals treated with paracetamol alone (Table I). The histopathological alterations were reduced by treatment with the extract and silymarin, respectively (Figs. 2D, E, F, G, H and 3A, B, C, D, E). At 75 mg/kg BW, the *S. elaeagnifolium* extract provided highest protection against the effects of paracetamol.

These protective effects may be due to the inhibitory effects on cytochrome P-450 and/or promotion of conjugation of NAPQI (Gilman *et al*., 1996), resulting in the inhibition of the formation of hepatotoxic free radicals (Nadeem *et al*., 1997).

Our results are consistent with the general experience that the transaminase activity returns to the normal level in the process of the healing of the hepatic parenchyma and the regeneration of hepatocytes (Ahmed and Khater, 2001). It is well documented that quercetin and kaempferol are strong antioxidants (Chattopadhyay, 1999). Therefore, it is presumed that these compounds which are constituents of the *S. elaeagnifolium* extract may be responsible for the hepatoprotective activity.

Histochemical and image analysis results

DNA

The nuclei of the normal liver exhibited the expected red colour with the Feulgen reagent indicating the presence of DNA, and the cytoplasm was negatively stained. The liver cells of mice treated with paracetamol showed a moderate decrease in the DNA content, and in some nuclei the DNA had disappeared completely. After 7 days of treatment with 25, 50, and 75 mg/kg BW of the *S. elaeagnifolium* extract or silymarin, respectively, *before* paracetamol administration, an increase in the DNA content of the nuclei was visible. The DNA content of the liver cells of mice treated with either 75 mg/kg BW of the *S. elaeagnifolium* extract or silymarin was found to be similar to that of the control group. Likewise, an increase in the DNA content of the liver cells was noticed in the curative groups when the extract or silymarin was given *after* paracetamol administration. The data were quantified by image analysis. The mean values of the levels of the intensity of grey [a grid of pixels each representing the intensity or brightness at that point by a range of numbers, typically from 0 (black) to 255 (white)] indicative of the amount of DNA are presented in Table II. There was a significant ($P < 0.05$) decrease in the DNA content in the liver of the paracetamol-treated group compared to the control group, while the groups treated with the extracts before or after paracetamol administration showed a significant increase in the DNA content compared to the paracetamol-treated group ($P < 0.05$).

Lister and McLean (1997) state that oral overdoses of paracetamol cause inhibition of DNA synthesis in most tissues. In all tissues, with the exception of the liver, DNA synthesis is inhibited within 1 h after administration of an oral dose of 1 g/kg BW paracetamol, which is due to the specific inhibition of the enzyme ribonucleotide reductase, which catalyzes the reduction of ribonucleotides to deoxyribonucleotides. Paracetamol destroys the free tyrosyl radical in the active site

Table II. Protective and curative effects, respectively, of the *S. elaeagnifolium* extract and silymarin on DNA and protein levels in livers of paracetamol-treated mice. Levels are given in intensities of grey.

Group	Protein	DNA
Control	128.30 ±1.80	84.10 ± 2.49
Paracetamol (500 mg/kg BW)	202.84 ± 0.70*	150.38 ± 4.16*
S. elaeagnifolium extract (25 mg/kg BW) and paracetamol	163.66 ± 0.24**	112.36 ± 4.80**
S. elaeagnifolium extract (50 mg/kg BW) and paracetamol	141.70 ± 9.75**	90.17 ± 7.54**
S. elaeagnifolium extract (75 mg/kg BW) and paracetamol	135.17 ± 6.30**	88.26 ± 1.88**
Silymarin (100 mg/kg BW) and paracetamol	131.37 ± 5.36**	85.15 ± 0.99**
Paracetamol and *S. elaeagnifolium* extract (25 mg/kg BW)	179.27 ± 0.38**	138.36 ± 5.69**
Paracetamol and *S. elaeagnifolium* extract (50 mg/kg BW)	168.65 ± 0.70**	108.01 ± 2.10**
Paracetamol and *S. elaeagnifolium* extract (75 mg/kg BW)	160.47 ± 1.66	94.33 ± 2.56**
Paracetamol and silymarin	148.21 ± 1.76	87.23 ± 1.89**

Data presented as mean ± SE. * The means are significantly different as compared with the control group ($P < 0.05$); ** the means are significantly different as compared with the paracetamol-treated group ($P < 0.05$).

located on the small subunit of the enzyme (Uhlin and Eklund, 1994).

Total proteins

Protein in the normal liver showed up as irregular bluish particles in the cytoplasm and nuclei. In the paracetamol-treated group, a decrease in the protein content was observed. The liver of mice treated with 25 mg/kg BW of the *S. elaeagnifolium* extract or silymarin, respectively, for 7 days *before* paracetamol administration had an increased protein content compared to the paracetamol-treated group and the livers of animals treated with 50 and 75 mg/kg BW of the *S. elaeagnifolium* extract or silymarin before paracetamol administration had a protein content similar to that of the control animals. The treatment with extract or silymarin *after* paracetamol administration resulted in an improvement of the total protein content in the liver tissue compared with the paracetamol-treated group. There was a significant ($P < 0.05$) decrease in the protein content in the livers of the paracetamol-treated group compared to the control group, while the groups treated with the extracts before or after paracetamol administration showed a significant increase in the protein content compared to the paracetamol-treated group ($P < 0.05$) (Table II).

The liver is the major source of most of the serum proteins, and its parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors, and most of the α- and β-globulins (Thapa and Walia, 2007). Albumin, being the most abundant plasma protein, accounts for 60% of the total serum protein and is involved in many physiological processes. The observed decrease in the albumin level by paracetamol ingestion could be the result of a decline in the number of cells responsible for albumin synthesis in the liver due to necrosis (Goldwasser and Feldman, 1997). Otherwise, it may be a direct interference with the albumin-synthesizing mechanism in the liver. It has been shown that inflammation exerts a negative influence on albumin synthesis, with albumin RNA decreasing by as much as 90% during inflammation (Rothschild *et al*., 1972). Paracetamol over-dosage is known to be associated with inflammation, marked by an increase in the inflammatory cytokines, TNF-α, interleukin-1α, and interleukin-1β (James *et al*., 2003), as well as up-regulation of the nitrogen oxide (NO) synthesis by macrophages and hepatocytes (Jaeschke *et al*., 2003). Such cytokines produced during inflammation shunt amino acids to increase the synthesis of proteins important in the inflammatory process, thus decreasing the synthesis of albumin which is not essential to inflammation (Rothschild *et al*., 1972).

On the basis of the findings of the present study, it can be concluded that the *S. elaeagnifolium* extract possesses both protective and curative activities against hepatic dysfunctions induced by paracetamol. This action was manifested by the improvement of both histological and histochemical (DNA and protein contents) effects in the liver of mice which had been administered paracetamol and *S. elaeagnifolium* extract. These effects may be related to the antioxidant properties of the flavonoid components of this extract.

Material and Methods

Experimental

UV spectra were recorded on a Shimadzu UV-visible spectrophotometer model-UV 240 (Tokyo, Japan). NMR experiments were performed on a Bruker AMX-400 spectrometer (Bruker Bio Spin, Rheinstetten, Germany). Chemical shifts are given in δ values (ppm) using tetramethylsilane (TMS) as the internal standard; *J* values are given in Hz, with DMSO-d_6 (99.5%; Sigma-Aldrich, St. Louis, MO, USA) as solvent, at room temperature. Column chromatography was performed on silica gel (type 60–230 mesh, 800 g; Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden). Sheets of filter paper Whatman No. 1 & 3 MM (46 x 57 cm) (Maidstone, Kent, England) were used for paper chromatography and developed with solvent mixtures, like BAW (*n*-butanol/acetic acid/water, 4:1:5, v/v/v, upper phase).

Plant material

Solanum elaeagnifolium Cav. was collected from Borg El-Arab city (50 km east of Alexandria), Egypt, in April 2008. The plant was identified by Prof. Ibrahium El-Garf, Botany Department, Cairo University, Cairo, Egypt, and voucher specimens have been deposited at the herbarium of the National Research Center, Dokki, Cairo, Egypt.

Flavonoid isolation and purification

As a preliminary step, the air-dried powdered flowering aerial parts of *S. elaeagnifolium* (430 g) were extracted with 70% aqueous methanol for 3 d at 40–60 °C by maceration. The methanolic extract was filtered and concentrated under vacuum. The total dried crude extract (27.2 g) was subjected to silica gel column chromatography (800 g) and eluted with *n*-hexane/ethyl acetate/methanol in increasing order of polarity. One hundred fractions were obtained and followed by one-dimensional paper chromatography (Whatman No. 1 MM) in order to isolate the flavonoid compounds from the crude extract. The fractions were combined into five fractions (I–V) according to their paper chromatographic characteristics.

Fractions I and II were subjected to paper chromatography (Whatman No. 3 MM), and each chromatogram was eluted twice with 15% acetic acid. Fractions III–V were subjected to paper chromatography (Whatman No. 3 MM), using BAW as eluent. All fractions afforded dark purple spots under UV light, changing to yellow when fumed with ammonia vapour. Each band was cut off and eluted with 70% methanol, followed by successive Sephadex LH-20 column chromatography and elution with MeOH and MeOH/H_2O (8:2 and 1:1, v/v). Fraction I yielded compounds **1** (3 mg), **2** (5.7 mg), **3** (4.1 mg), and **8** (4.6 mg), while fraction II yielded compounds **4** (6.5 mg), **5** (11.2 mg), **9** (3.2 mg), and **10** (12.7 mg). Compounds **6** (13 mg), **7** (9.2 mg), and **12** (4.8 mg) were isolated from fraction III, while compounds **11** (10.6 mg) and **13** (14.5 mg) were isolated from fraction IV.

Kaempferol 8-C-β-galactoside (**1**): Yellow powder. – R_f 0.55 (BAW), 0.28 (15% HOAc). – UV: λ_{max} (MeOH) = 268, 326, 368; (NaOMe) 277, 320, 418 (decomposition); ($AlCl_3$) 271, 304, 348, 426; ($AlCl_3$/HCl) 278, 303, 349, 394; (NaOAc) 275, 376; (NaOAc/H_3BO_3) 273, 349 nm. – (–)-ESI-MS: *m/z* = 447 [M–H]. – (+)-ESI-MS: *m/z* = 449 [M+H], 471 [M+Na]. – ^{1}H NMR (DMSO-d_6): δ = 13.83 (1H, s, 5-OH), 7.77 (2H, d, *J* = 8 Hz, H-2′/6′), 6.72 (2H, d, *J* = 8 Hz, H-3′/5′), 6.26 (1H, s, H-6), 4.56 (1H, d, *J* = 7.8 Hz, H-1″). – ^{13}C NMR (DMSO-d_6): δ = 179.5 (C-4), 167.1 (C-7), 162.5 (C-2), 160.9 (C-9), 159.7 (C-4′), 157.6 (C-5), 135.4 (C-3), 128.2 (C-2′/6′), 121.9 (C-1′), 115.7 (C-3′/5′), 105.4 (C-8/10), 98.5 (C-6), 80.2 (C-5″), 78.9 (C-3″), 74.3 (C-1″), 70.2 (C-2″), 68.9 (C-4″), 59.4 (C-6″).

Animals

Ninety lab-bred adult male Swiss albino mice, strain CD1, weighing 20–25 g, were procured from the animal laboratory house, National Research Center, Cairo, Egypt. The animals were acclimatized to in-house conditions. They were allowed to feed on a commercial pellet diet containing 24% protein, 4% fat, and 4.5% fiber and water *ad libitum*. All experimental protocols were in accordance with the ethical guidelines, and all studies had been approved by the Institutional Animal Ethical Committee.

Chemicals

Paracetamol was purchased from Pharco Pharmaceuticals Company (Alexandria, Egypt) as tablets; each tablet contained 500 mg of paracetamol. Silymarin was purchased from Medical Union Pharmaceuticals Company (Abou Sultan, Ismalia, Egypt) as a gelatinous capsule under the trade name of Hepaticum; each capsule contained 140 mg of silymarin.

Acute toxicity test

Five groups of 6 mice each were administered the aqueous methanolic extract of *S. elaeagnifolium*. One group was used as a control group and given orally the respective volume of the vehicle (distilled water). The extract was suspended in the vehicle immediately before use in the appropriate concentration and orally administered to the groups in increasing doses (100, 200, 300, 400, and 500 mg/kg BW). All groups were kept under observation for 7 d after administration of the extracts to record the mortality. Acute oral toxicity of the extract was evaluated according to OECD 423 guidelines (Ecobichon, 1997).

Paracetamol-induced hepatotoxicity

Mice were divided into ten groups of 6 animals each. Group I served as untreated control group. Group II served as toxicant control group and was treated with paracetamol (500 mg/kg BW). Group III, group IV, group V, and group VI received the extract at the doses of 25, 50, and 75 mg/kg BW, and silymarin at the dose of 100 mg/kg BW, respectively. All animals received these treatments

orally for 7 d (Jafri *et al*., 1999). On the 8th day, all groups, except group I, received a dose of paracetamol (500 mg/kg BW). Groups VII, VIII, IX, and X received a single dose of paracetamol (500 mg/kg BW) and were then treated with 25, 50 and 75 mg/kg BW of the extract or silymarin at the dose of 100 mg/kg BW for 7 d, respectively. Twenty four h after paracetamol administration, animals were anesthetized under light diethyl ether anesthesia. Blood was collected by cardiac puncture in centrifuge tubes, and separated serum was used for the assay of hepatic marker enzymes. After blood samples were taken, the animals were sacrificed under anesthesia. The livers of all animals were excised and preserved in 10% formalin solution for histopathological and histochemical examinations.

Biochemical studies

Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) (Reitman and Frankel, 1957), and alkaline phosphatase (ALP) (Kind and King, 1954) levels were estimated using respective diagnostic kits (Monobind Inc., Costa Mesa, CA, USA).

Histopathological and histochemical studies

Sections, 6 μm in thickness, were prepared and stained with haematoxylin and eosin (H & E) for histopathological examination. For histochemical examination, the Feulgen reaction was used for DNA visualization (Bancroft and Stevens, 1994) and the mercury-bromophenol blue method for protein detection (Mazia *et al*., 1953).

Image analyzer analysis for DNA and total proteins

Data were obtained using a Leica Qwin 500 image analyzer computer system in the Pathology Department, National Research Center, Cairo, Egypt (LEICA Imaging Systems, Cambridge, England). Ten fields were chosen in each specimen, and the intensity of grey of the images was determined. The intensities of the DNA and protein stain, respectively, were measured in nuclei and cytoplasm of liver cells, respectively, at 400x magnification.

Statistical analyses

All statistical analyses were performed using the GraphPad Prism 4 software (GraphPad Software Inc., San Diego, CA, USA). Results were expressed as mean ± standard error (SE). $P < 0.05$ was considered significant.

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