

## Phytochemical and Pharmacological Studies on *Zilla spinosa*

<sup>1</sup>S.A. El-Toumy, <sup>2</sup>F.S. El-Sharabasy, <sup>3</sup>H.Z. Ghanem, <sup>4</sup>M.U. El-Kady, <sup>2</sup>Asmaa F. Kassem

<sup>1</sup>Chemistry of Tannins Department, <sup>2</sup>Chemistry of Natural and Microbial Department, <sup>3</sup>Therapeutic Chemistry Department, National Research Center, 12622 Dokki, Cairo, Egypt, <sup>4</sup>Organic chemistry Department, Faculty of Science, Ain shams university, Cairo Egypt

**Abstract:** The hepatoprotective effect of aqueous ethanol extract of *Zilla spinosa* aerial parts was investigated against carbon tetrachloride-induced acute hepatotoxicity in rats. The hepatoprotective activity of *Zilla spinosa* was evaluated by measuring levels of serum marker enzymes activities: aspartat amino transferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamaglutamyl ( $\gamma$ -GT). The histological studies were also carried out to support the above parameters. Administration of *Zilla spinosa* (100 mg/kg) markedly prevented CCl<sub>4</sub> -induced elevation of serum AST, ALT, ALP and  $\gamma$ -GT activities. A phytochemical study of *Z. spinosa* resulted in the isolation of quercetin 3-*O*-L- rhamnopyranosyl (1-6)- $\beta$ - glucopyranoside, kaempferol 3-*O*-L-rhamnopyranosyl (1-6)- $\beta$ -D-glucopyranoside, quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside, quercetin-3-*O*- $\beta$ - glucopyranoside, kaempferol 3-*O*-  $\beta$ -glucopyranoside, quercetin and kaempferol. These structures of the isolated compounds were established by chromatography, UV and 1D/2D <sup>1</sup>H/<sup>13</sup>C spectroscopy. Hepatoprotective effect of *Zilla spinosa* is probably due to combined action of flavonoids. The aqueous ethanol extract was tested against three human tumor cell lines namely; colon (HCT116), breast (MCF7), and liver (HepG2). The extract showed the highest activity against breast cell line IC<sub>50</sub> 7.5  $\mu$ g/ml.

**Key words:** *Zilla spinosa*, hepatoprotective activity, Flavonoids, cytotoxic

### INTRODUCTION

Despite tremendous advances in modern medicine, hepatic disease remains a worldwide health problem, thus the search for new patents is still ongoing. Numerous formulations of medicinal plants are used to treat liver disorders. Many of this of these treatments act as radical scavengers, whereas others enzyme inhibitors or mitogens (Fadhel and Amran, 2002). Carbon tetrachloride accumulates in hepatic paraenchyma cells and is metabolized to CCl<sub>3</sub>• radicals by liver cytochrome P450-dependent monooxygenases (Recknagel, 1983). Research shows that hepatoprotective effects have been associated with plant extracts that are rich in phenolic compounds (De *et al.*, 1996; Shannmugasundaram and Venkataraman, 2006; Jain *et al.*, 2008; Sabir and Rocha, 2008; Huang *et al.*, 2010; Srivastava and Shivandappa, 2009; Zeashan *et al.*, 2009).

Crucifereae is a large family of 3709 species in 375 genera (Al-Shehbaz *et al.*, 2006), it is also known as the Brassicaceae. It was represented in Egypt by 53 genera and 105 species. It includes vegetable crops; some species were used as food and medicinal plants. They are used as antidiabetic, antibacterial (Radwan *et al.*, 2008), antifungal, anticancer (Vang, 1994; Xiao *et al.*, 2003), antirheumatic (Kirtikau. and Basu, 1984), and show a potent insecticidal effect (Malik *et al.*, 1983). *Z. spinosa* is one of the most common plant species of family Cruciferea, due to its important uses in the folk medicinal; it is used as a drink against kidney stones (Heneidy and Bidak, 2001) or stones of the gall bladder. Previous phytochemical study of *Z. spinosa* led to the isolation of glucosinolates of free sinapine, progotrin, goitrin, flavonioids, triterpenes carbohydrates and sterols (Karawya *et al.*, 1974; El-Menshawy *et al.*, 1980), some of which have a wide range of biological activities including antioxidant, antifungal, hepatoprotective and antiviral activities. However, to the best of our knowledge, the hepatoprotective activity of *Z. spinosa* has not been demonstrated. The present study focused on the evaluating the potential hepatoprotective effects of aqueous ethanol extract from *Z. spinosa* on CCl<sub>4</sub>-induced liver injury in rats, antitumor and flavonoids constituents.

**Corresponding Author:** S.A. El-Toumy, Chemistry of Tannins Department, National Research Center, 12622 Dokki, Cairo, Egypt.  
E-mail: sayedeltomy@yahoo.com.

## MATERIAL AND METHODS

### Plant Material:

Aerial parts of *Z. spinosa* were collected in April 2008 from the road Suez-Cairo desert. Identification of the plants was confirmed by Prof. Dr. Salwa A. Kawashty. National Research Center, Cairo, Egypt and comparison with herbarium specimens. Voucher Specimens were kept in herbarium, National Research Center, Cairo, Egypt

### Extraction and Isolation:

Air-dried ground aerial part of *Z. spinosa* (2kg) were defatted with petroleum ether (40-60 °C), and extracted three times at room temperature with C<sub>2</sub>H<sub>5</sub>OH: H<sub>2</sub>O (7:3). The combined extracts were filtered, evaporated under reduced pressure and lyophilized (180 g). Twenty grams of the dry residue was used for Pharmacological Studies. Weighed samples of *Z. spinosa* extract were used to prepare the solutions, which were diluted with distilled H<sub>2</sub>O to the appropriate concentration for the experiment. The rest of the dry extract was redissolved in 2 L H<sub>2</sub>O and extracted with n-butanol (3 x 2 L). After evaporation of solvents, the n-butanol extract and the remaining H<sub>2</sub>O phase gave dark brown solids 50 and 70 g, respectively. The n-butanol extract was loaded on a polyamide 6S column chromatography (80 x 3 cm). The column was eluted with H<sub>2</sub>O, and then H<sub>2</sub>O-CH<sub>3</sub>OH mixtures of decreasing polarity and 10 fractions (1 L, each) were collected. The major flavonoids fractions obtained were combined into five fractions after chromatographic analysis. Fraction 1 (2.5 g) was fractionated by column chromatography on Sephadex LH-20 with aqueous EtOH (0- 70%) for elution to give compounds **1** (17 mg) and **3** (25 mg). Fraction 2 (1.6 g) was subjected to column chromatography on cellulose and n-BuOH saturated with H<sub>2</sub>O as an eluent to give two major subfractions, then one of them was separately fractionated on a Sephadex LH-20 to yield pure samples **2** (22 mg). Using the same procedure fraction 3 (1.8 g) and fraction 4 (1.4 g) gave chromatographically pure samples **4** (15 mg) and **5** (15 mg). Fraction 5 (1.5 g) was chromatography on Sephadex LH-20 using aqueous acetone (0- 25%) for elution to give pure sample **6** (20 mg) and **7** (25 mg).

### Chemical Characterization of the Isolated Compound:

**Quercetin 3-O- $\alpha$ -L-rhamnopyranosyl(1-6)- $\beta$ -D-glucopyranoside (Rutin) (1):** yellow powder, UV<sub>max</sub> nm (MeOH) 254, 268<sup>sh</sup>, 298<sup>sh</sup>, 354; (NaOMe) 269, 330<sup>sh</sup>, 412; (271, 306<sup>sh</sup>, 431; (AlCl<sub>3</sub>+HCl) 268, 359, 405; (NaOAc) 270, 310<sup>sh</sup>, 398; (NaOAc+H<sub>3</sub>BO<sub>3</sub>) 260, 290, 372. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) 12.55 (s, 1H, 5-OH), 7.51 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.49 (1H, *dd*, *J* = 8.4 and 2.0 Hz, H-6'), 6.81 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.35 (1H, *d*, *J* = 2.0 Hz, H-8), 6.16 (1H, *d*, *J* = 2.0 Hz, H-6), 5.31 (1H, *d*, *J* = 6.8 Hz, H-1''), 4.34 (1H, *d*, *J* = 1.25 Hz, H-1'''), Rha) and 0.95 (3H, *d*, *J* = 6.07 Hz, Rha-6'''). <sup>13</sup>C NMR:  $\delta$  156.94 (C-2), 133.79 (C-3), 177.88 (C-4), 157.14 (C-5), 99.21 (C-6), 164.61 (C-7), 94.13 (C-8), 156.94 (C-9), 104.47 (C-10), 122.11 (C-1'), 115.74 (C-2'), 145.25 (C-3'), 148.92 (C-4'), 116.72 (C-5'), 121.69 (C-6'), 103.22 (C-1''), 774.58 (C-2''), 76.39 (C-3''), 72.35 (C-4''), 76.35 (C-5''), 67.35 (C-6''), 101.25 (C-1'''), 70.88 (C-2'''), 70.50 (C-3'''), 71.06 (C-4'''), 69.76 (C-5'''), 18.24 (C-6''').

**Kaempferol 3-O- $\alpha$ -L-rhamnopyranosyl(1-6)- $\beta$ -D-glucopyranoside (2):** greenish yellow amorphous powder, UV  $\lambda_{max}$  nm: MeOH, 260, 300<sup>sh</sup>, 350; MeOH+ NaOMe, 273, 325<sup>sh</sup>, 405; MeOH + NaOAc, 268, 310, 380; MeOH+NaOA+ H<sub>3</sub>BO<sub>3</sub>, 266, 300, 360; MeOH+AlCl<sub>3</sub>, 278, 360, 398; MeOH+AlCl<sub>3</sub>+HCl: 268, 310, 380: <sup>1</sup>H NMR (DMSO-d<sub>6</sub>):  $\delta$  8.0 (2H, *d*, *J* = 8.5 Hz, H-2', H-6'), 6.85 (2H, *d*, *J* = 8.5 Hz, H-3', H-5'), 6.34 (1H, *d*, *J* = 2 Hz, H-8), 6.15 (1H, *d*, *J* = 2 Hz, H-6), 5.2 ( *d*, *J* = 7.5 Hz, H-1'' of glucose), 4.3 (1H,*d*, *J* = 2.0 Hz, H-1'''' of rhamnose ) 3.28-4.1(m, rest of glucose and rhamnose protons). <sup>13</sup>C NMR:  $\delta$  156.76 (C-2), 133.57 (C-3), 177.82 (C-4), 161.59 (C-5), 99.10 (C-6), 164.63 (C-7), 94.04 (C-8), 156.62 (C-9), 104.34 (C-10), 121.28 (C-1'), 131.24 (C-2', C-6'), 115.47 (C-3', C-5'), 160.31 (C-4'), 101.29 (C-1''), 74.59 (C-2''), 76.80 (C-3''), 70.28 (C-4''), 77.83 (C-5''), 61.28 (C-6''), 101.25 (C-1'''), 70.88 (C-2'''), 70.50 (C-3'''), 71.06 (C-4'''), 69.76 (C-5'''), 18.24 (C-6''').

**Quercetin-3-O- $\alpha$ -L-rhamnopyranoside (3):** yellow powder; UV  $\lambda_{max}$  nm: MeOH, 253, 263<sup>sh</sup>, 344; MeOH+ NaOMe: 272, 322<sup>sh</sup>, 372; MeOH+ NaOAc: 260, 300<sup>sh</sup>, 367; MeOH+ NaOAc + H<sub>3</sub>BO<sub>3</sub>: 272, 382. MeOH+AlCl<sub>3</sub> : 272, 304<sup>sh</sup>, 333<sup>sh</sup>, 430; MeOH+AlCl<sub>3</sub> +HCl: 272, 303<sup>sh</sup>, 353, 401. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 7.33 (1H, *d*, *J* = 2 Hz, H-2' ), 7.28 (1H, *dd*, *J* = 2 Hz, and *J* = 8.5 Hz, H-6'), 6.90 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.42 (1H, *d*, *J* = 2 Hz, H-8), 6.23 (1H, *d*, *J* = 2 Hz, H-6), 5.29 (1H, *d*, *J* = 1.41 Hz, H-1'' of rhamnose), 3.16-3.56 (m, rest of rhamnose protons), 0.85 (*d*, *J* = 6.07 Hz, CH<sub>3</sub> of rhamnose). <sup>13</sup>C NMR:  $\delta$  157.34 (C-2), 134.27 (C-3), 177.79

(C-4), 161.35 (C-5), 98.78 (C-6), 164.34 (C-7), 93.70 (C-8), 156.86 (C-9), 104.16 (C-10), 121.17 (C-1'), 115.52 (C-2'), 145.25 (C-3'), 148.50 (C-4'), 115.72 (C-5'), 120.80 (C-6'), 101.87 (C-1''), 70.43 (C-2''), 70.63 (C-3''), 71.25 (C-4''), 70.11 (C-5''), 17.54 (C-6'').

**Quercetin-3-O-β-glucopyranoside, (4):** yellow amorphous powder; UV  $\lambda_{max}$  nm: MeOH, 253, 263<sup>sh</sup>, 294<sup>sh</sup>, 352; MeOH+ NaOMe, 271, 328<sup>sh</sup>, 410; MeOH + NaOAc, 273, 321, 375; MeOH+NaOA+ H<sub>3</sub>BO<sub>3</sub>, 262, 300<sup>sh</sup>, 377; MeOH+AlCl<sub>3</sub>, 275, 305<sup>sh</sup>, 332<sup>sh</sup>, 435; MeOH+AlCl<sub>3</sub>+HCl: 275, 305<sup>sh</sup>, 361<sup>sh</sup>, 403; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  7.53 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.67 (1H, *dd*, *J* = 2.1 Hz and 8.62 Hz, H-6'), 6.82 (1H, *d*, *J* = 8.6 Hz, H-5'), 6.40 (1H, *d*, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6), 5.37 (1H, *d*, *J* = 7.63 Hz, H-1'' of glucose), 3.28-3.65 (m, rest of glucose protons). <sup>13</sup>C NMR:  $\delta$  156.80 (C-2), 133.60 (C-3), 177.50 (C-4), 161.60 (C-5), 98.90 (C-6), 164.60 (C-7), 93.80 (C-8), 156.60 (C-9), 104.00 (C-10), 121.60 (C-1'), 115.80 (C-2'), 145.80 (C-3'), 148.80 (C-4'), 116.20 (C-5'), 122.00 (C-6'), 101.20 (C-1''), 71.60 (C-2''), 74.40 (C-3''), 70.02 (C-4''), 77.70 (C-5''), 61.50 (C-6'').

**Kaempferol 3-O-β-glucopyranoside (5):** greenish yellow amorphous powder, UV  $\lambda_{max}$  nm: MeOH, 256, 292<sup>sh</sup>, 357; MeOH+ NaOMe, 272, 325<sup>sh</sup>, 415; MeOH + NaOAc, 273, 315, 390; MeOH+NaOA+ H<sub>3</sub>BO<sub>3</sub>, 261, 320<sup>sh</sup>, 377; MeOH+AlCl<sub>3</sub>, 274, 340<sup>sh</sup>, 430; MeOH+AlCl<sub>3</sub>+HCl: 269, 340<sup>sh</sup>, 400; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  7.92 (2H, *d*, *J* = 8.1 Hz, H-2',6'), 6.88 (2H, *d*, *J* = 8.1 Hz, H-3',5'), 6.36 (1H, *d*, *J* = 1.8 Hz, H-8), 6.18 (1H, *d*, *J* = 1.8 Hz, H-6), 5.33 (1H, *d*, *J* = 7.6 Hz, H-1'' of glucose), 3.28-3.65 (m, rest of glucose protons). <sup>13</sup>C NMR:  $\delta$  156.76 (C-2), 133.57 (C-3), 177.82 (C-4), 161.59 (C-5), 99.10 (C-6), 164.63 (C-7), 94.04 (C-8), 156.62 (C-9), 104.34 (C-10), 121.28 (C-1'), 131.24 (C-2', C-6'), 115.47 (C-3', C-5'), 160.31 (C-4'), 101.29 (C-1''), 74.59 (C-2''), 76.80 (C-3''), 70.28 (C-4''), 77.83 (C-5''), 61.28 (C-6'').

**Quercetin, (6):** yellow amorphous powder, UV  $\lambda_{max}$  nm: MeOH, 253, 268<sup>sh</sup>, 297<sup>sh</sup>, 368; MeOH+ NaOMe, 247<sup>sh</sup>, 321. (dec.); MeOH + NaOAc, 257<sup>sh</sup>, 274, 329, 390. (dec.); MeOH+NaOA+ H<sub>3</sub>BO<sub>3</sub>, 261, 303<sup>sh</sup>, 388; MeOH+AlCl<sub>3</sub>, 272, 304<sup>sh</sup>, 333, 458; MeOH+AlCl<sub>3</sub>+HCl: 265, 301<sup>sh</sup>, 359, 428.; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  7.69 (1H, *d*, *J* = 2.1, H-2'), 7.55 (1H, *dd*, *J* = 2.1 Hz, and 8.4 Hz, H-6'), 6.90 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.42 (1H, *d*, *J* = 1.8 Hz, H-8), 6.20 (1H, *d*, *J* = 1.8 Hz, H-6). <sup>13</sup>C NMR:  $\delta$  147.50 (C-2), 136.44 (C-3), 176.55 (C-4), 161.43 (C-5), 98.88 (C-6), 164.59 (C-7), 94.05 (C-8), 156.83 (C-9), 103.71, (C-10), 122.66 (C-1'), 116.31 (C-2'), 145.76 (C-3'), 148.40 (C-4'), 115.76 (C-5'), 120.68 (C-6').

**Kaempferol (7):** greenish yellow powder, UV  $\lambda_{max}$  nm: MeOH, 254<sup>sh</sup>, 268, 322<sup>sh</sup>, 365; MeOH+ NaOMe, 275, 320, 412 (dec.); MeOH + NaOAc, 275, 300, 385; MeOH+NaOA+ H<sub>3</sub>BO<sub>3</sub>, 269, 295<sup>sh</sup>, 370; MeOH+AlCl<sub>3</sub>, 262<sup>sh</sup>, 270, 353, 426; MeOH+AlCl<sub>3</sub>+HCl: 260, 271, 350, 428.; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  7.9 (2H, *d*, *J* = 8.1, H-2', 6'); 6.90 (2H, *d*, *J* = 8.2 Hz, H-3', 5'), 6.42 (*d*, *J* = 2.4 Hz, H-8), 6.20 (1H, *d*, *J* = 2.4 Hz, H-6). <sup>13</sup>C NMR:  $\delta$  146.80 (C-2), 135.84 (C-3), 176.20 (C-4), 161.60, (C-5), 98.60 (C-6), 164.59 (C-7), 93.85 (C-8), 156.40 (C-9), 103.70, (C-10), 121.90 (C-1'), 129.90 (C-2', C-6'), 115.80 (C-3', C-5'), 159.5 (C-4').

#### **Animals:**

Thirty-six male Sprague- Dawley rats weighing (120- 150 g) were purchased from the Animal House, National Research Centre, Dokki, Cairo, Egypt. They were housed at standard environmental condition and were allowed free access to tap water and pellet diet. The study has got the approval from the Local Ethical Committee, in the National Research Centre. Acute toxicity study was performed on rats during 24-72 hrs. Rats were divided into six groups, each of six

**Group A:** Rats served as normal control and were orally administered saline for 30 days.

**Group B:** Rats were administered aqueous ethanol extract of *Z. spinosa* (100 mg/kg b.w., oral) alone for 30 days.

**Group C:** Rats were administered CCl<sub>4</sub> (1.5 mg/kg b.w., oral, 50 % w/w paraffin oil) twice weekly for 30 days.

**Group D:** Rats were administered aqueous ethanolic extract of *Z. spinosa* (100 mg/kg b.w., oral) for 15 days followed by administration of a CCl<sub>4</sub> (1.5 mg/kg b.w., oral) twice weekly until day 30.

**Group E:** Rats were administered CCl<sub>4</sub> (1.5 mg/kg b.w., oral) twice weekly for 15 days followed by administered aqueous ethanolic extract of *Zilla spinosa* (100 mg/kg b.w., oral) until day 30.

**Group F:** Rats were administered aqueous ethanolic extract of *Z. spinosa* (100 mg/kg b.w., oral) concomitant CCl<sub>4</sub> (1.5 mg/kg b.w., oral twice weekly) for 30 days.

**Biochemical Assessment:**

At the end of the experiment, blood samples were obtained from the retro-orbital vein plexuses, under ether anaesthesia. ALT and AST activities in serum were measured according to Reitman and Frankel (1957), determination of ALP activity was done according to the method of Belfield and Goldberg (1971) and  $\gamma$ -glutamyl transferase by Sazasz (1976).

**Histopathological Studies:**

Liver samples were excised, washed with normal saline and processed separately for histopathological observation. Initially the materials were fixed in 10% buffered neutral formalin and paraffin sections were taken at 5  $\mu$ m thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathology changes.

**Cytotoxicity Activity:**

**Cell Lines:**

Human tumor cell lines culture, HCT116 (colon carcinoma human cell line), HepG2 (liver carcinoma human cell line), and MCF-7 (breast carcinoma human cell line). They were obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

**Culture Media:**

HCT116, HepG2 and MCF 7 cells were suspended in RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo, and U.S.A) supplemented with 10 % fetal bovine serum(FBS) (Sigma Chemical Co., St. Louis, Mo, U.S.A), Sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo, U.S.A), and Penicillin/Streptomycin (Sigma Chemical Co., St. Louis, Mo, U.S.A).

**Assay Method for Cytotoxic Activity:**

The sensitivity of the human tumor cell lines to thymoquinone was determined by the Sulphorhodamine-B (SRB) assay. This method was carried out according to that of Skehan *et al.* (1990). HCT116, HepG2 and MCF 7 cell lines were plated in 96-multiwell plates ( $5 \times 10^4$ -  $10^5$  cells/well in a fresh media) for 24 hrs before treatment with the tested sample to allow attachment of cells to the wall of the plate. Then, 200 $\mu$ l aliquot of serial dilution of alcoholic extract (5.0, 12.5, 25, 50  $\mu$ g/ml) was added then plates were incubated for 24, 48 and 72 hrs at 37°C. Control cells were treated with vehicle alone. Following 24, 48 and 72 hrs treatment; the cells were fixed with cold 50% trichloroacetic acid for 1 hr at 4°C. Four wells were prepared for each individual dose. Following 24, 48 and 72 hrs treatment, cells were fixed, washed and stained with Sulforhodamine B stain (sigma, USA). Optical density was measured in an ELISA reader spectrophotometer (Tecan Group Ltd.-Sunrise, Germany). The percentage of cell survival was calculated as follows Survival fraction = O.D. (treated cells)/ O.D. (control cells).

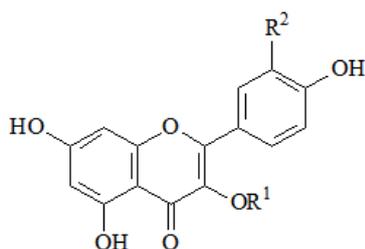
**Statistical Analysis:**

Data were analyzed by student 't' test using Excel 2003 Microsoft Corp (11.5612.5606), Redmond, WA software package. Results were presented as means  $\pm$  standard error of the means ( $X \pm SE$ ). *P-values* < 0.05 were ranged as statistically significant.

**Results:**

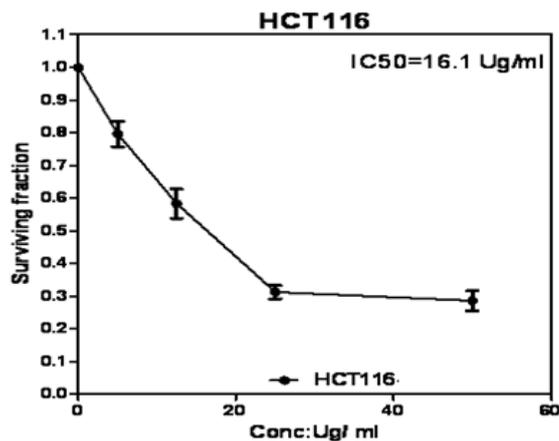
Fractionation of the extract resulted in isolation and identification of seven flavonoids (1-7) (Fig. 1). The structure of the isolated compounds was established through chromatography as well as conventional chemical and spectroscopic methods of analysis (UV, 1/2D NMR). The ethanolic extract was tested for cytotoxicity to three human cancer cell lines, namely, colon (HCT), breast (MCF7), liver (HEPG2) cell lines. The activities were expressed by the IC<sub>50</sub> value and their results are shown in (Fig. 2, 3 and 4). According to the American National Cancer Institute guidelines (Suffness and Pezzuto, 1990) extracts with IC<sub>50</sub> values <30  $\mu$ g/ml were considered active. It was found that the ethanolic extract was active against colon (HCT116), breast (MCF7) and liver (HEPG2) human cancer cell lines with an IC<sub>50</sub>, 16.1; 7.5 and 14.3  $\mu$ g/ml; respectively. The cytotoxicity of ethanolic extract gives a good result in case of breast (MCF7) due to the less IC<sub>50</sub> value.

*Z. spinosa* total alcoholic extract had no toxic signs up to 3 gm/kg b wt oral administration. Pilot experiment was performed to evaluate effective dose to be used in hepatoprotective study. It was shown that

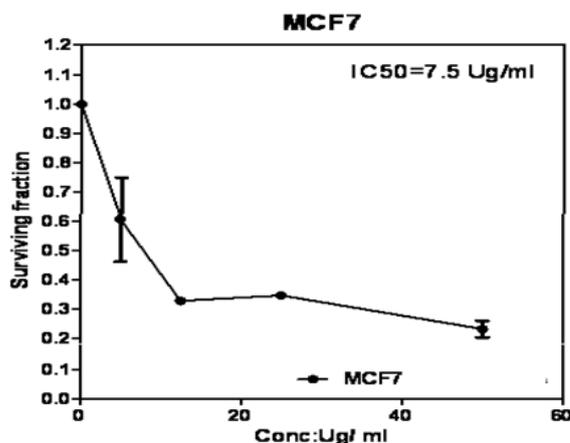


1	R <sup>1</sup> = glucoside(1→6) rhamnoside	R <sup>2</sup> = OH
2	R <sup>1</sup> = glucoside(1→6) rhamnoside	R <sup>2</sup> = H
3	R <sup>1</sup> = rhamnoside	R <sup>2</sup> = OH
4	R <sup>1</sup> = glucoside	R <sup>2</sup> = OH
5	R <sup>1</sup> = glucoside	R <sup>2</sup> = H
6	R <sup>1</sup> = H	R <sup>2</sup> = OH
7	R <sup>1</sup> = R <sup>2</sup> = H	

**Fig. 1:** Chemical structure of the isolated compounds.



**Fig. 2:** Cytotoxicity of ethanolic extract on colon cell line (HCT116).



**Fig. 3:** Cytotoxicity of ethanolic extract on breast cell line (MCF7).

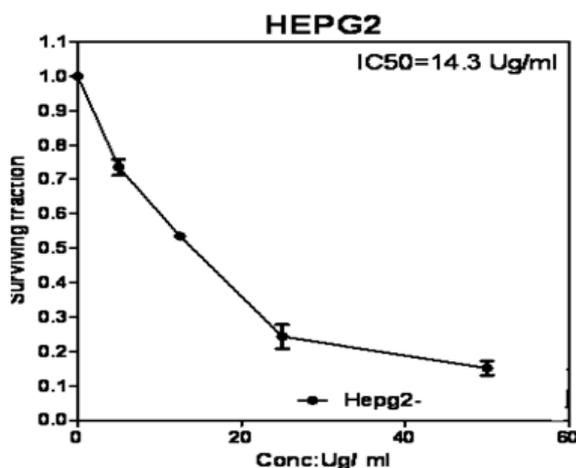


Fig. 4: Cytotoxicity of ethanolic extract on liver cell line (HEPG2).

100 mg/kg the lowest effective. Results in the table 1 showed that ALT, AST, ALP and  $\gamma$ -GT in CCl<sub>4</sub>-injected group significantly increased compared with control. Significant decrease in plasma enzyme activity of AST, ALT, ALP and  $\gamma$ -GT was observed in groups administrated *Z. spinosa* extract. Histopathological study of liver in control group showed a normal hepatic architecture with distinct hepatic cells, sinusoidal spaces, prominent nucleus and a central vein (Fig. 5). The histological architecture of liver sections in rat treated with 100 mg/kg plant extract showed normal lobular pattern with a mild lymphocyte infiltration (Fig 5B). However, CCl<sub>4</sub>-intoxicated group exhibited sever histopathological changes, such as centrilobular hepatic necrosis, fatty change. Degeneration and broad infiltration of the lymphocytes and Kupffer cells around the central vein and portal tract, with the loss of cellular boundaries were also observed (Fig. 5C).

Table 1: Effect of the ethanolic extract of the aerial parts of *Z. spinosa* (100 mg/ Kg) on Serum enzymes of liver (ALT, AST and  $\gamma$ -GT) and (ALP) in normal and carbon tetrachloride- treated rats (n = 6, means $\pm$  SE of the means).

Group	ALT( $\mu$ L)	AST( $\mu$ L)	ALP( $\mu$ L)	$\gamma$ -GT
Group A	47.3 $\pm$ 0.81	126.5 $\pm$ 1.99	215.2 $\pm$ 0.91	6.06 $\pm$ 0.41
Group B	49.1 $\pm$ 0.9**	128.7 $\pm$ 0.2**	216.7 $\pm$ 0.12*	5.05 $\pm$ 0.31
Group C	195.95 $\pm$ 1.11***	193 $\pm$ 2.15**	300.3 $\pm$ 2.19*	9.54 $\pm$ 0.7*
Group D	70.2 $\pm$ 0.19**	135 $\pm$ 0.22**	245 $\pm$ 0.20**	6.8+ 0.51
Group E	150.6 $\pm$ 0.7***	190 $\pm$ 0.1***	290 $\pm$ 0.11**	7.5 $\pm$ 0.44
Group F	140.1 $\pm$ 1.21***	189 $\pm$ 0.99***	250.1 $\pm$ 0.98**	7.2 $\pm$ 0.42

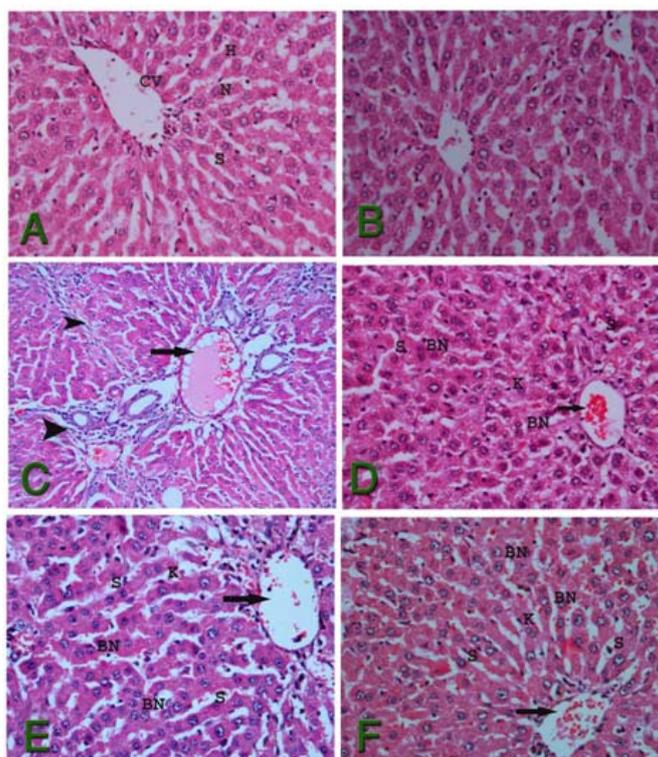
Values statistically significant \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

Pretreatment-rats with *Z. spinosa* showed restoration and apparently normal organ with very few hemorrhage in central vein with binucleated cells were found (Fig. 5D). Thus *Z. spinosa* pretreatment greatly inhibited liver morphological changes and necrosis due to CCl<sub>4</sub> hepatotoxicity. Post-treatment of plant extract after CCl<sub>4</sub> intoxication also attenuated the hepatic damage induced by CCl<sub>4</sub> with dialed of blood sinusoids with the presence of inflammatory cells (Fig. 5E). Fatty change and lymphocyte infiltration were improved in the histological sections of group treated with plant extract concomitant with CCl<sub>4</sub>. Fewer necrotic zones were also observed (Fig.5). The maximum protection was seen in the group pretreatment with *Z. spinosa* extract.

#### Discussion:

Much attention has been focused on the protective biochemical function of naturally occurring antioxidants in biological system and on the mechanisms of their action (Freil and Higdon, 2003; Manach *et al.*, 2005). Liver injury induced by CCl<sub>4</sub> are the best characterized system of xenobiotic-induced hepatotoxicity and commonly used models for the screening of antihepatotoxic and/or hepatoprotective activities of drugs (Lee, et al, 2004).

Carbon tetrachloride, a widely used experimental hepatotoxicant, it is biotransformed by cytochrome P - 450 systems to produce the trichloromethyl free radical (CCl<sub>3</sub>•) that causes lipid peroxidation and, thereby, produce liver damage (Recknagal. 1967). When administered, carbon tetrachloride accumulates in hepatic parenchymal cells, which is metabolized to free radical CCl<sub>3</sub>•. The free radicals react with molecular oxygen



**Fig. 5:** A. Normal photomicrograph of the liver section of a rat showing the architecture of the hepatic lobule, central vein (CV), hepatocytes (H), nucleus and blood sinusoids (S) (H&E. 400). B. Photomicrograph of the liver section of a rat received extract of *Z. spinosa* (100 mg/kg), showing normal architecture of the hepatic lobules (H&E .400). C. Photomicrograph of the liver section of carbon tetrachloride-treated rats showing the distortion of architecture in hepatic cells. Congestion of portal tract (long arrow) and sever cellular infiltration around portal tract (Head arrow). D. Photomicrograph of the liver section of a rat treated with ethanolic extract of *Z. spinosa* (100 mg/kg) for 15 days followed by administration of CCl<sub>4</sub> (1.5 mg/kg) until day 30, showing congestion of central vein with dialated sinusoids and binucleated cells. E. Photomicrograph of the liver section of a rat treated with CCl<sub>4</sub> (1.5 mg/kg) for 15 days followed by administered ethanolic extract of *Z. spinosa* (100 mg/kg) until day 30, showing dialated of central vein with dialated sinusoids and binucleated cells. F. Photomicrograph of the liver section of a rat treated with ethanolic extract of *Z. spinosa* (100 mg/kg) and CCl<sub>4</sub> (1.5 mg/kg) at the same time for 30 days, showing congestion of central vein with dialated sinusoids and binucleated cells.

to produce peroxy radicals (H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub> and <sup>•</sup>OH due to incomplete reduction of molecular oxygen), thereby causing oxidative destruction of polyunsaturated fatty acids (Gebhardt, 2002). These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum, rich in polyunsaturated fatty acids. Lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity (Dhawan *et al.*, 1991). Elevated levels of serum enzymes, ALT, AST and  $\gamma$ -GT are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). Treatment with aqueous ethanol extract of *Z. spinosa* decreased the serum levels of ALT, AST and  $\gamma$ -GT towards their respective normal value; that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue from the damage caused by CCl<sub>4</sub>. On the other hand ALP is an indicator of pathological alteration in biliary flow (Ploa and Hewitt, 1989). CCl<sub>4</sub> induced elevation of serum ALP is in line with high levels of serum bilirubin. Effective control of ALP levels in aqueous ethanol extract of *Z. spinosa* treatment groups points towards an early improvement in the secretory mechanism of hepatocytes. These biochemical findings were further substantiated by histopathological studies.

Histopathology of liver of the normal control rats showed prominent central vein and normal arrangement of hepatic cell (Fig 5A). CCl<sub>4</sub> treatment rats showed massive fatty changes, gross necrosis and broad infiltration

of lymphocytes and Kupffer cells around the central vein and loss of cellular boundaries (Fig 5C). Liver section of rats treated with aqueous ethanol extract of *Z. spinosa* showed significant regeneration against CCl<sub>4</sub> induced liver damage (Fig 5D and 5F). This is in agreement with data report by Rienke *et al.*, (1988), Obi *et al.*, (1998) and Yan-Jun *et al.*, (2004). When hepatocytes are damaged, a variety of enzymes, normally located in the cytosol, are released into the blood and their estimation is a useful quantitative marker of the extent and type of hepatic cell damage (Mitra, *et al.*, 1998). Administration of aqueous ethanol extract of *Z. spinosa* before CCl<sub>4</sub> injection improved liver enzymes. Phytochemical studies revealed that the presence of flavanoids in extract of *Z. spinosa*. Several flavonoids have been shown to have potential as hepatoprotective agents (Middleton and Kandaswami, 1994; Wegner and Fintelmann, 1999; Palanivel *et al.*, 2008).

It is concluded that treatment with aqueous ethanol extract of *Z. spinosa* decreases the CCl<sub>4</sub>-induced elevation in biochemical parameters (liver AST, ALT and ALP). These findings suggest that the aqueous ethanol extract of *Z. spinosa* was effective in bringing about functional improvement of hepatocytes. The healing effect of this extract was also confirmed by histological observations. Our results demonstrated that the possible hepatoprotective mechanisms of aqueous ethanol extract of *Z. spinosa* on CCl<sub>4</sub>-induced liver damage in rats might be due to the following effects: (1) inhibiting the cytochrome P450-dependent oxygenase activity; and (2) stabilizing the hepatocyte membrane. The active compounds of *Z. spinosa* which are responsible for the observed hepatoprotective effects, have been isolated quercetin 3-*O*-L-rhamnopyranosyl (1-6)- $\beta$ -glucopyranoside, kaempferol 3-*O*-L-rhamnopyranosyl (1-6)- $\beta$ -D-glucopyranoside, quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside, quercetin-3-*O*- $\beta$ -glucopyranoside, kaempferol 3-*O*- $\beta$ -glucopyranoside, quercetin and kaempferol.

#### REFERENCES

- Al-Shehbaz, I.A., M.A. Beilstein, E.A. Kellogg, 2006. Systematics and Phytogeography Brassicaceae Phylogeny and trichome evolution. *Am. J. Botany*, 93: 607-619.
- Belfield, A., D. Goldberg, 1971. Colorimetric methods for determination of alkaline Phosphatase. *Enzyme*, 12: 561-574.
- Dhawan, D., A. Goel, K. Karkara, 1991. Effects of carbon tetrachloride and Liv.52 on the clearance rate of 133 I-Rose Bengal in rat liver. *AMPI Med Phys Bul.*, 16: 27-9.
- De, S., V.J. Shukla, B. Ravishankar, G.C. Bhavasar, 1996. A preliminary study on the hepatoprotective activity of methanol extract of *Paederia foetida* leaf. *Fitoterapia*, 67: 106-109.
- Drotman, R.B., G.T. Lawhorn, 1978. Serum enzymes are indicators of chemical induced liver damage. *Drug and Chemical Toxicology*, 1: 163-171.
- El-Menshawly, B., M. Karawya, G. Wassel, J.A. Reish, A. Kjaer, 1980. Glucosinolates in the Genus *Zilla* (Brassicaceae). *J. Nat. Prod.*, 43: 534-536.
- Fadhel, Z.A., S. Amran, 2002. Effects of black tea extract on carbon tetrachloride induced lipid peroxidation in liver, kidneys, and testes of rats. *Phytother. Res.*, 16: 28-32.
- Freil, B., J.V. Higdon, 2003. Antioxidant Activity of Tea Polyphenols in Vivo: Evidence from animal studies. *J. Nut.*, 133: 3275S-3284S.
- Gebhardt, R., 2002. Oxidative stress, plant-derived antioxidants and liver fibrosis. *Planta Med.*, 68: 289-96.
- Heneidy, S.Z., L.M. Bidak, 2001. Multipurpose plant species in bisha, Asir region Southwestern Saudi Arabia. *J.King Saud Univ.*, 13: 11-26.
- Ban, X., J. He, H. Zeng, P. Zhang, Y. Wang, 2010. Hepatoprotective and antioxidant effects of the methanolic extract from *Halenia Elliptica*. *J. Ethnopharmacol.*, 131: 276-281
- Jain, A., M. Soni, L. Deb, A. Jain, S.P. Rout, V.B. Gupta, K.L. Krishna, 2008. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. Leaves. *J. Ethnopharmacol.*, 115: 61-66.
- Karawya, M.S., G.M. Wassel, B.S. El-Menshawly, 1974. Phytochemical study of *Zilla spinosa* (Turra) Prantl. General analysis. Carbohydrates and lipids. *Pharmazie*, 29: 60-61.
- Kirtikau, K.R., L. Basu, 1984. Indian Medicinal plants, vol. 1-V, 2nd ed., Bishen Singh Mahendra pal singh, Dehra Dun, India.
- Lee, K.J., E.R. Woo, C.Y. Choi, D.W. Shin, D.G. Lee, H.J. You, H.G. Jeong, 2004. Protective effect of acteoside on carbon tetrachloride-induced hepatotoxicity. *Life Sci.*, 74: 1051-1064.
- Manach, C., G. Williamson, C. Morand, A. Scalbert, C. Remesy, 2005. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.*, 81: 230-242.
- Malik, R.S., I.J. Anand, S. Srinvasachar, 1983. Effect of glucosinolates in relation to aphid (lipaphis erysimi Kalt.) fecundity in Crucifers. *Ind. J. Trop. Agric.*, 1: 273-278.

Middleton, J.E., C. Kandaswami, 1994. The impact of flavonoids on mammalian biology: implications for immunity, inflammation and cancer, In: Harborne, J.B. (Ed.), *The Flavonoids: Advances in Research Since 1982*. Chapman & Hall, London.

Mitra, S.K., M.V. Venkataraganna, R. Sundaran, S. Gopumadhavan, 1998. Protective effect of HD-03, a herbal formulation against various hepatotoxic agents in rats. *J. Ethnopharmacol.*, 63: 181-6.

Obi, F.O., I.A. Usenu, J.O. Osayande, 1998. Prevention of CCl<sub>4</sub>-induced hepato-toxicity in the rat by *H. rosasinensis* anthocyanin extracts administered in ethanol. *Toxicology*, 131: 93-98.

Palanivel, M.G., B. Pajkapor, R.S. Kumar, J.W. Einstein, E.P. Kumar, M.P. Kumar, K. Kavitha, M.P. Kumar, B. Jayakar, 2008. Hepatoprotective and Antioxidant Effect of *Pisonia aculeata* L. against CCl<sub>4</sub>- Induced Hepatic Damage in Rats. *Sci Pharm.*, 76: 203-215.

Ploa, G.L., Hewitt W.R. (1989). In: Wallace Hyes, A. (Ed.), *Principle and Methods of Toxicology*, vol. II. Raven Press, New York, p. 399.

Recknagal, R.O., 1967. Carbon Tetrachloride Hepatotoxicity. *Pharmacol Rev.*, 19: 145-208.

Recknagal, R.O., 1983. A new direction in the study of carbon tetrachloride hepatotoxicity. *Life Science*, 33: 401-408.

Reink, L.A., E.K. Lai, P.B. McCay, 1988. Ethanol feeding stimulates trichloromethyl radical formation from carbon tetrachloride in liver. *Xenobiotica*, 18: 1311-1318.

Reitman, S., A. Frankel, 1957. Colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *Am J Clin Pathol.*, 28: 56-58.

Sabir, S.M., J.B. Rocha, 2008. Antioxidant and hepatoprotective activity of aqueous extract of *Solanum fastigiatum* (false "Jurubeba") against paracetamol- induced liver damage in mice. *J. Ethnopharmacol.*, 120: 226-232.

Shanmugasundaram, P., S. Venkataraman, 2006. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. *J. Ethnopharmacol.*, 104: 124-128.

Srivastava, A., T. Shivanandappa, 2009. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats *Food Chem.*, 118: 411-417.

Skehan, P., R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, 1990. New colourimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.*, 82: 1107-1112.

Szasz, G., 1976. Reaction-rate method for gamma-glutamyltransferase activity in serum. *Clin Chem.*, 22: 2051-2055.

Vang Ole, 1994. Neue Aspekte Gesund wirkung pflanz. *Nahrungsn vortragstage. Dtsch. Ges. Qualitataetes forsch*, 29th: 74-85.

Wegner, T. and V. Fintelmann, 1999. Flavonoids and bioactivity. *Wien Med Wochenschr.*, 149: 241-247.

Xiao, D., S.K. Srivastava, K.L. Lew, Y. Zeng, P. Hershberger, C.S. Johnson, D.L. Trump, S.V. Singh, 2003. Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing G2/M arrest and inducing apoptosis. *Carcinogenesis*, 24: 891-897.

Yan-Jun, L., Y. Jie-ping, S. Zhao-Hong, L. Wang, 2004. *Gingko biloba* extract reverses CCl<sub>4</sub>- induced liver fibrosis in rats. *World J. of Gastroenterology*, 10: 1037-1042.

Zeashan, H., G. Amresha, S. Singh, C.V. Rao, 2009. Hepatoprotective and antioxidant activity of *Amaranthus spinosus* against CCl<sub>4</sub>-induced toxicity. *J. Ethnopharmacol.*, 125: 364-366.