Phytochemical and pharmacological study of *Ficus cordata* growing in Saudi Arabia

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Abstract: Phytochemical study of the aerial parts of *Ficus cordata* utilizing liquid-liquid fractionation and different chromatographic techniques resulted in the isolation of four furanocoumarins: psoralene (1), hydroxy isoimperatorin (2), oxypeucedanin hydrate (3) and dorsteniol (4), the flavone glycoside rutin (5), *β*-sitosterol and sucrose. Structures of the isolated compounds were established through physical, 1D- and 2D-NMR and MS data. The total extract of the plant was examined *in vivo* for its possible effects as hepatoprotective, nephroprotective, antiulcer and anticoagulant in comparison with standard drugs. Hepatoprotective activitys were accessed via serum biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin. Tissue parameters such as non-protein sulfhydryl groups (NP-SH), malonaldehyde (MDA) and total protein (TP) were also measured. In addition to tissue parameters, nephroprotective effect was evaluated by measuring the serum levels of sodium, potassium, creatinine and urea. Histopathological study for both liver and kidney cells was also conducted. Antiulcer activity was explored by observing stomach lesions after treatment with ethanol. Whole blood clotting time (CT) was taken as measure for the anticoagulant activity of the extract. All the studied parameters indicated that the total extract of *Ficus cordata* at 500mg/kg possess moderate hepatoprotective effect, good protection against ethanol induced ulcer and weak nephroprotective effect. The CT was about one quarter of that of warfarin.

Keywords: *Ficus cordata*; furanocoumarins; hepatoprotective; nephroprotective; antiulcer; anticoagulant.

INTRODUCTION

Members of the genus *Ficus* family Moraceae range from high trees to shrubs (Hutchinson *et al.,* 1958). Plants of the genus are used in folk medicine as tonic and to treat many conditions such as inflammation, epilepsy, bronchitis, influenza whooping cough, tonsillitis, toothache, bacillary dysentery, enteritis and bruises (Kitajima *et al*., 1999; Noumi and Fozi, 2003; Betti, 2004; Fan *et al*., 2005; Lansky *et al*., 2008). Several furanocoumarins such as psoralene, bergapten, 5-O-*β*-D-glucopyranosyl-6-hydroxyangelicin, 6-O-*β*-D-glucopyranosyl-5-hydroxyangelicin, 5,6-O-*β*-D-diglucopyranosylangelicin, 8-O-*β*-D-glucopyranosyl-5-hydroxypsoralen and 5-O-*β*-D-glucopyranosyl-8-hydroxypsoralen were isolated from *Ficus carica* and *Ficus ruficaulis* (Chang *et al*., 2005; Chunyan *et al*., 2009). Triterpenes, fatty alcohol, steroids, coumarin, flavane, 4-hydroxybenzoate, megastigmane [4,5-dihydroblumenol] were isolated from *Ficus microcarpa* (Kuo and Li, 1997). Nine Ursane- and Oleanane-type triterpenes were isolated from the aerial roots of *Ficus microcarpa* (Kuo and Chunyan, 2009). Two isoflavones, ficuisoflavone and isolupinisoflavone E were isolated from the bark of *Ficus microcarpa* (Li and Kuo, 1997). One apocarotenoid, ficusone and two novel lactone derivatives, ficuspirolide and ficusolide as well as 4,5-dihydroblumenol were isolated from the heartwood of *Ficus microcarpa* (Li and Kuo, 2000). Seven flavonoids, 5,6-dihydroxy-2-methylchromone, and *β*-sitosterol-D-glucoside have been isolated from the leaves of *Ficus lyrata* (Basudan *et al*., 2005).

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MATERIALS AND METHODS

General

Melting points were determined in open capillary tubes using *Thermosystem FP800 Mettler FP80* central processor supplied with *FP81 MBC* cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a *Unicum Heyios* α UV-Visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a *Bruker DRX-500* (Central Lab at the College of Pharmacy, King Saud University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard *Bruker* program. MS were obtained using Liquid Chromatography/Mass Spectrometer (*Quattro micro API*) equipped with a Z-spray electrospray ion source (*Micromass®, Quattro*

micro™, WATERS). Centrifugal preparative TLC (CPTLC) was preformed using Chromatotron (Harrison Research Inc. model 7924): 4 mm silica gel P254 disc. Silica gel 60/230-400 mesh (EM Science) and RP C-18 silica gel 40-63/230-400 mesh (Fluka) were used for column chromatography, while silica gel 60 F254 (Merck) was used for TLC.

Plant materials

The aerial parts of *Ficus cordata* ssp. *salicifolia* (Vahl) Berg F. Moraceae was collected from Wadi Lagab, Gazan area south of Saudi Arabia in March 2010. The plants were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Voucher specimen (# 15133) was deposited at the herbarium of this center.

Extraction, Fractionation and Purification

The dried ground aerial parts of *F. cordata* (1300g) were extracted to exhaustion by percolation at room temperature with 90% ethanol (15L), and the extract was evaporated under reduced pressure to leave 145.5g of the total extract. A portion of this residue (90g) was dissolved in 500ml of MeOH and 500 ml water were added to the solution. The resulted solution was defatted with petroleum ether (600ml×3) to yield 20.5g of petroleum ether soluble fraction. Then aqueous methanol fraction was partitioned with EtOAc (600ml×3) to yield 61g of EtOAc soluble fraction and finally with *n*-butanol (400ml×3) to yield 11g of *n*-butanol soluble fraction.

Part of the EtOAc fraction (30g) was chromatographed over silica gel column (700g, 7cm i.d.) using a gradient of EtOAC/MeOH. Fractions 300ml each were collected and screened by TLC and similar fractions were pooled. Fractions 3-7 eluted with EtOAc afforded 135mg of **1** after crystallization from MeOH. Fractions 10-12 eluted with 5% MeOH in EtOAc afforded 50mg of *β*-sitosterol glucoside. Fractions 20-25 eluted with 7% MeOH in EtOAc (2.3g) were further purified by CPTLC (4 mm silica gel GF_{254} disk, solvent: EtOAc/MeOH; 95:5) to give 15 mg of **2,** 17 mg of **3** and 26 mg of **4**.

Fractions 28-29 eluted with 10% MeOH in EtOAc afforded 32mg of **5** after repeated crystallization from MeOH. While fractions 32-35 eluted with 15% MeOH in EtOAc afforded 150mg of sucrose after crystallization from MeOH.

Psoralene (**1**). M.P. 162-163 ^{0}C. UV λ_{max} (MeOH): 240, 245, 292, 327. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 6.30 (d, *J*=9.5 Hz, H-3), 7.32 (s, H-8), 7.40 (s, H-3'), 7.89 (s, H-5), 8.03 (s, H-2'), 8.17 (d, *J*=9.5 Hz, H-4); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 97.52 (C-8), 106.12 (C-3'), 112.69 (C-3), 114.78 (C-10), 120.57 (C-5), 123.86 (C-6), 143.91 (C-4), 146.30 (C-2'), 149.99 (C-9), 158.23 (C-7), 160.59 (C-2). ESIMS: 225 (5, $[M + K]^+$), 209 (33, $[M + Na]^+$), 187 (100, $[M + H]^+$).

5-[4"-hydroxy-3"-methyl-2"-butenyloxy]-6,7-furocoumarin (**2**). M.P. 130-132^{o}C. UV λ_{max} (MeOH): 254, 258, 268, 306. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 1.64 (s, CH_3-5"), 3.86 (d, *J*=4.5 Hz, 2H-4"), 4.96 (t, *J*=5 Hz, OH), 5.07 (d, *J*=6.5 Hz, H-1"), 5.76 (t, *J*= 4.5 Hz, H-2"), 6.32 (d, *J*=9.8 Hz, H-3), 7.35 (s, 2H, H-8+H-3'), 8.04 (s, H-2'). 8.15 (d, *J*=9.8 Hz, H-4); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 13.76 (C-5"), 65.22 (C-4"), 68.94 (C-1"), 93.46 (C-8), 105.55 (C-3'), 106.53 (C-10), 112.36 (C-3), 113.56 (C-6), 117.17 (C-2"), 139.51 (C-4), 142.26 (C-3"), 146.01 (C-2'), 148.54 (C-9), 151.99 (C-5), 157.47 (C-7), 160.12 (C-2). ESIMS: 309 (35, $[M + Na]^+$), 287 (100, $[M + H]^+$).

(+)-Oxypeucedanin hydrate (**3**). M.P. 133-134 ^{0}. $[\alpha]_D$ = + 34.8 (*c*=0.5, MeOH). UV λ_{max} (MeOH): 218, 248, 266 (sh), 313. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 1.12 (s, CH_3-4"), 1.25 (s, CH_3-5"), 3.65 (t, *J*= 7 Hz, H-2"), 4.27 (t, *J*=9 Hz, H-1"), 4.57 (s, 3"-OH) 4.75 (d, *J*=9.7 Hz, H-1"), 5.33 (d, *J*=5.5 Hz, 2"-OH), 6.33 (d, *J*=9.8 Hz, H-3), 7.29 (s, 2H, H-3'), 7.31 (s, H-8), 8.01 (s, H-2'), 8.36 (d, *J*=9.8 Hz, H-4); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 24.11 (C-4"), 27.65 (C- 5"), 70.68 (C-3"), 74.79 (C-1"), 76.21 (C-2"), 93.20 (C-8), 105.49 (C-3'), 106.36 (C-10), 112.01 (C-3), 113.21 (C-6), 140.05 (C-4), 145.85 (C-2'), 149.18 (C-9), 152.01 (C-5), 157.51 (C-7), 160.120 (C-2). ESIMS: 327 (19, $[M + Na]^+$), 305 (100, $[M + H]^+$).

(-)-Dorsteniol (**4**). M.P. 126-127 ^{0}C. $[\alpha]_D$ =- 21.9 (*c*= 0.85, MeOH). UV λ_{max} (MeOH): 225, 251 (sh), 259, 299 (sh), 335. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 1.13 (s, CH_3-5'), 3.22 (m, 2H, H-3'), 3.28 (m, 1H, H-5'), 3.55 (m, 1H, H-3'), 4.64 (s, 4'-OH), 4.84 (bs, 5'-OH), 4.90 (t, *J*=9 Hz, H-2'), 6.21 (d, *J*=10 Hz, H-3), 6.78 (s, H-8), 7.48 (s, H-5), 7.94 (d, *J*= 10 Hz, H-4); ^{13}C-NMR (125 MHz, DMSO-d_6): δ_C 20.78 (C-6'), 28.00 (C- 3'), 65.92 (C-5'), 72.72 (C-4'), 87.51 (C-2'), 96.68 (C-8), 111.92 (C-10), 111.04 (C-3), 123.78 (C-5), 125.72 (C-6), 144.76 (C-4), 155.00 (C-9), 160.57 (C-7), 163.40 (C-2). ESIMS: 285 (26, $[M + Na]^+$), 263 (100, $[M + H]^+$).

Animals

Wistar albino rats (150-200g) roughly the same age (8-10 weeks), obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used. The animals were housed under constant temperature (22±2°C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and free access to drinking water *ad libitum* (Abdel-Kader *et al*., 2010). The experiments and procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, King Saud University.

Chemicals

Silymarin, paracetamol, β-sitosterol, sucrose, rutin and warfarin were obtained from Sigma Chemical Company, USA.

Hepatoprotective & Nephroprotective Activity

Male Wistar rats were divided into five groups five animals each. Group I received normal saline and was kept as a control group. Groups II, III, IV and V received 500mg paracetamol/ 1kg body weight intraperitoneally for two days. Group II received only paracetamol (Pa) treatment. Group III was administered silymarin (Sil) at a dose of 10mg/kg p.o (20.7µmole/kg) Groups IV and V were treated with 250 and 500mg/kg of the total extract. Treatment started 5 days prior to Pa administration and continued till day six. After 24h, following Pa second administration in day 7 the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture and the serum was separated for evaluating the biochemical parameters.

Determination of AST, ALT, GGT, ALP, Bilirubin, Creatinine, Urea, Potassium and Sodium Levels

The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin were estimated by reported methods (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE). Serum creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by the reported method (Varley and Alan, 1984). Potassium level was measured using diagnostic strips (Reflotron®, ROCHE) while photometric determination of sodium level was done using Mg-uranylacetate method (Henry *et al*., 1974).

Determination of Non-protein sulfhydryl groups (NP-SH), Malonaldehyde (MDA) and Total Protein (TP)

The livers and kidneys samples were separately cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02M ethylenediaminetetraacetic acid (EDTA) in a Potter-Elvehjem type C homogenizer. Homogenate equivalent to 100mg tissues were used for the measurements. Non-protein sulfhydryl groups (NP-SH) were quantified by the reported method (Sedlak and Lindsay, 1968).

For the level of MDA Aliquots of homogenate were incubated at 37°C for 3h in a metabolic shaker and then treated as described by Utly *et al*. (Utley *et al*., 1967).

For TP determination parts of the homogenate were treated with 0.7ml of Lowry's solution, mixed and incubated for 20 min in dark at room temperature. Diluted Folin's reagent (01ml) were then added and samples were incubated at room temperature in dark for 30 min. The absorbance of the resulted solutions was then measured at 750 nm (Lowry *et al*., 1951).

Histopathology

The fixed liver and kidney samples were placed in cassettes and loaded into tissue baskets. The samples were paraffinzed and thin sections (3µm) were made using microtome (*Leitz* 1512). Sections were stained with Mayer's hematoxylin solution and counterstained in eosin-phloxine solution (Prophet *et al*., 1994).

Antiulcer activity

Rats were divided into three groups 5 animals each. All animals received 1 ml 80% ethanol. Group I served as positive control. Groups II and III were treated with 250 and 500mg/kg of the total extract 30 min before the administration of ethanol. One hour after the administration of ethanol, rats were scarified and examined for stomach lesions. Patchy lesions of the stomach induced by ethanol were scored according to the method described by Robert *et al*. (Robert *et al*., 1983).

Determination of whole blood Clotting Time (CT)

Clotting time was determined according to the reported method (Dacie and Lewis, 1970). Rats were divided into three groups 5 animals each. Group I received 10 mg/kg of warfarin and served as positive control. Groups II and III were treated with 250 and 500mg/kg of the total extract. Blood samples 0.4ml was collected at 0, 30, 60, 120 min from the retrorbital sinus venous channels. The blood samples were dispensed separately into prewarmed test tubes fixed in a rack previously placed in water bath maintained at 37°C. Tubes were tilted to check for the sign of blood clot every 30 sec. Using stopwatch, the time interval between blood collection and the time the clot appeared in each test tube was recorded in min.

STATISTICAL ANALYSES

For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test were used to determine the significance of the differences. Differences between the control and CCl_4-treated group were compared for significance using Dunnette test for non-paired samples (Woolson and Clarke, 2002). All the values shown are the mean ± S.E.

RESULTS

Phytochemical study of the aerial parts of *F. cordata* resulted in the isolation of four furanocoumarin derivatives, rutin, β-sitosterol and sucrose. Physical and spectral data of the some of the isolated compounds are presented in the experimental section. The total extract was tested for hepatoprotective and nephroprotective effect against paracetamol induced toxicity. The results

are presented in tables 1-3. Antiulcer potential of the extract was also explored against ethanol-induced lesions. Results are presented in table 4. The results of anticoagulant effect in comparison with warfarin based on whole blood clotting time (CT) are presented in table 5.

DISCUSSION

Phytochemical investigation of the aerial parts of *Ficus cordata* ssp. *salicifolia* resulted in the isolation of 7 compounds. *β*-sitosterol (Goad and Akihisa, 1997), sucrose (Popov *et al*., 2006) and the flavone glycoside rutin (5) (Bilia *et al*., 1996) were identified by comparison of their physical and spectral data with literature as well as direct comparison with authentic material.

Four furanocoumarin derivatives were also isolated. Compounds **1** and **3** were identified via comparison of their UV, $[\alpha]_D$, NMR and MS data with literature as psoralene (O'Neil, 2001; Abu-Mustafa and Fayez, 1967) and oxypeucedanin hydrate (Chen *et al*., 1996; Chen *et al*., 2008).

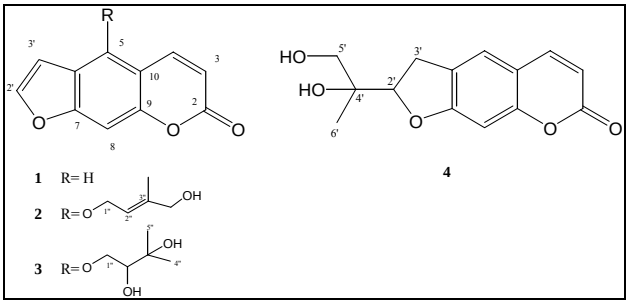


Fig. 1: The structures of compounds **1-4**.

The ESIMS of **2** (see experimental) indicated a molecular formula $C_{16}H_{14}O_5$. All the 14 carbons were clear in the ^{13}C-NMR (table 1). UV, ^{1}H- and ^{13}C-NMR, HMBC were diagnostic for a C-5 substituted furanocoumarin (Murray *et al*., 1982; Miranda *et al*., 1994). ^{1}H- and ^{13}C-NMR indicated the presence of prenyl group composed of an olefin (δ_H 5.76, t, *J*=4.5. 1H-2″, δ_C 117.17), an olefinic quaternary carbon (δ_C 142.26, C-3″) and a methyl group (δ_H 1.64, s,3H-5″, δ_C 13.67). Two oxymethylenes (δ_H 5.07 d, *J*=6.5 Hz, δ_C 68.94 and δ_H 3.86 d, *J*=4.5 Hz, δ_C 65.22). In COSY experiment the OH proton at 4.96 (t, *J*=5 Hz) showed correlation with the oxymethylene at δ_H 3.86 assigned to position 4" and the other oxymethylene at δ_H 5.07 assigned to position 1" as it showed correlation with H-2" at δ_H 5.76. The data of **2** is very close to that of 5-[4″-hydroxy-3″-methyl-2″-butenyloxy]-6,7-furocoumarin (Stavri and Gibbons, 2005). However, physical data and chemical shifts of **2** are apparently different and point out to a possible isomeric structure. In an NOESY experiment (fig. 2) showed NOE correlation between the olefinic proton at δ_H 5.76 and C-4" oxymethylene at δ_H 3.86. The methyl group at δ_H 1.64 and the C-1" oxymethylene at δ_H 5.07. These correlation clearly indicated that **2** is the isomer *(E)*-5-[4″-hydroxy-3″-methyl-2″-butenyloxy]-6,7-furocoumarin.

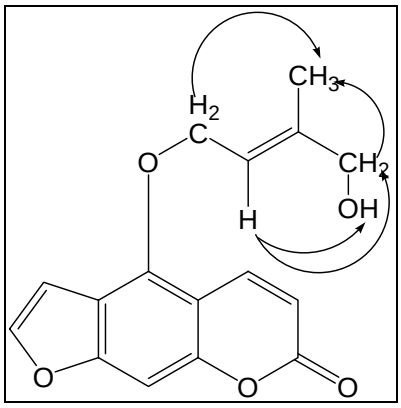


Fig. 2: NOESY correlations of **2**.

^{1}H- and ^{13}C-NMR of **4** (table 1) indicated a 6, 7-disubstituted coumarin derivative where H-3 and H-4 appeared at δ_H 6.21 (d, *J*=10 Hz), 7.94 (d, *J*=10 Hz, H-4) and H-5 and H-8 appeared as to singlets at δ_H 7.48 and 6.78. In addition, 5 more carbons were clear in the ^{13}C-NMR and were sorted by DEPT experiments into CH_3, CH_2, CH_2-O, CH-O and C-O. ESIMS showed ions at m/z 285 (26, $[M+Na]^+$), 263 (100, $[M + H]^+$) for the molecular formula $C_{14}H_{14}O_5$. Literature search revealed that the data of **4** is very similar to that published for dorsteniol (Chen *et al*., 2008; Ricardo *et al*., 1998). However, **4** showed negative sign in optical rotation and consequently was identified as *(-)*-dorsteniol.

Serum Parameters related to Hepatoprotective Activity

Therapeutic doses of Pa eliminated mainly as sulfate and glucoronide (Eriksson *et al*., 1992) and only 5% of the dose is converted into N-acetyl-p-benzoquineimine (NAPQI). However, upon administration of toxic doses of Pa higher percentage of the molecules are oxidized to highly reactive NAPQI by cytochrome p-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI is rapidly conjugated with glutathione (GSH), a sulphydryl donor which results in the depletion of liver GSH pool (Remirez *et al*., 1995). Under conditions of excessive NAPQI formation or reduced of glutathione store, NAPQI covalently binds to vital proteins, the lipid bilayer of hepatocyte membranes and increases the lipid peroxidation (McConnachie *et al*., 2007).

Hepatic toxicity is reflected by increase in the biochemical parameter levels such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin. Treatment of rats with Pa resulted in severe damage of hepatocytes, biliary obstruction and transport inability across the liver as indicated by high levels of AST, ALT, GGT, ALP and bilirubin (table 1) (Edwards and Bouchier, 1991). Pretreatment of rats with Sil, significantly ($P<0.001$)

decreased the raised levels of AST, ALT, GGT, ALP and bilirubin induced by Pa (50.68, 44.00, 47.76, 28.68 and 62.00% respectively) (table 1) indicating a good recovery from the hepatotoxic agent. Treatment with *F. cordata* total extract showed dose dependent reduction in the levels of all the measured parameters. Animal treated with 500 mg/ kg body weight of *F. cordata* showed significant (P<0.01- P<0.001) reduction in the levels of AST, ALT, GGT, ALP and bilirubin (34.87, 35.42, 21.76, 18.39 and 52.94%) indicating good protection against liver damage induced by Pa. Treatment with 250mg/kg body weight resulted in less improvement in the parameters, However, all the results were highly significant (P<0.01- P<0.001) except reduction in ALT (16.49%, P<0.05).

Serum Parameters related to Nephroprotective Activity

Elevations of serum electrolytes, urea and creatinine are considered reliable parameters for investigating drug-induced nephrotoxicity in animals and man (Adelman *et al*., 1981). Pa exhibits a significant rise in the biochemical markers of kidney function like serum urea, serum creatinine, sodium and potassium level. Pretreatment with Sil (10mg/kg p.o) decreased the raised levels of serum urea, serum creatinine, percentage of sodium and potassium (52.0, 54.16, 31.19 and 47.66%) induced by Pa (table 2). Dose dependent reduction in the elevated parameters resulted from the treatment with *F. cordata* total extract. Animal treated with 500mg/kg body weight of *F. cordata* showed highly significant (P<0.001) reduction in the levels of serum urea, serum creatinine, sodium and potassium levels (20.78, 27.23, 22.01 and 38.95%) indicating a moderate protection against Pa induced nephrotoxicity. Treatment with 250mg/kg body weight resulted in less protection and significance ranged from P<0.05- P<0.01 (table 2).

Tissue Parameters related to both Hepatoprotective and Nephroprotective

Treatment of animals with Pa at a dose of 500mg/kg decreased the hepatic and renal NP-SH from 8.34±0.55, 8.96±0.25 to 4.59±0.45, 4.80±0.45 µmol/gm wet weight tissue, respectively (table 3). Pretreatment of the animals with Sil at a dose of 10 mg/kg significantly increased the reduced NP-SH content (p<0.01, p<0.001) to 7.44±0.65 and 7.66±0.56 µmol/gm wet weight tissue in hepatic and renal tissues respectively. Animal received the total extract of *F. cordata* showed significant dose dependent recovery of the NP-SH contents. Pre-treatment with 500 mg/kg *F. cordata* total extract significantly increased the reduced content of NP-SH in the liver and kidney tissues to 6.79±0.47 and 6.21±0.31 µmol/gm wet weight tissue respectively (p<0.05, p<0.01). The 250 mg/kg dose was less effective but statistically significant (p<0.05). The NP-SH contents in the liver and kidney tissues were increased to 5.98±0.38 and 6.31±0.42 µmol/gm wet weight tissue respectively.

The production of malonaldehyde (MDA) is used as a biomarker to measure the level of oxidative stress in an organism (Del Rio *et al*., 2005). Normal control group showed 1.15±0.08 and 1.08±0.05 nmol/l concentrations of MDA in their healthy liver and kidney tissues respectively. The levels of MDA greatly increased after Pa treatment to 6.05±0.52 and 5.022±0.26 nmol/l in liver and kidney tissues respectively. The standard drug Sil was effective in reducing these elevated levels to 2.24±0.21and 1.82±0.10nmol/l in liver and kidney tissues respectively in a highly statistically significant results (p<0.001). *F. cordata* total extract resulted in dose dependent reduction in the MDA levels. Treatment of the animals with 500mg/kg body weight decreased the level of MDA to 3.28±0.19 nmol/l (p<0.001) in liver tissues and 3.60±0.20 nmol/l (p<0.01) in kidney tissues reflecting a moderate level of protection (table 3).

One of the most important liver functions is protein synthesis. Liver damage causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. The TP levels depressed in hepatotoxic conditions due to defective protein biosynthesis. Restoring the normal levels of TP is an important parameter for liver recovery (Navarro and Senior, 2006). Treatment of rats with Pa resulted a approximately 50% reduction in liver and kidney tissue protein contents (table 3). Treatment with Sil increased the levels of TP in both liver and kidney to 89.42±5.04 g/l (84%) and 87.02±1.59 g/l (76%) respectively. The results were highly statistically significant (p<0.01). Highly significant results (p<0.01) also resulted from treatment with 500mg/kg body weight *F. cordata* total extract. The TP levels increased in liver tissue to 75.44±1.60 g/l (71%) and in kidney tissues to 73.45±2.11 g/l (64%) respectively. These results indicated that the extract has more protection on the liver tissues when compared with renal tissues.

Histopathological Study

The histological appearance of the liver and kidney cells reflects their conditions (Prophet *et al*., 1994). The histopathology of the normal hepatic cells is presented in fig.1, L-1. Treatment with 500mg/kg body weight pa resulted in centrilobular necrosis and extensive fatty degeneration of centrolobular hepatocytes (fig. 1, L-2). Liver samples of rats treated with Sil prior the administration of Pa showed significant protection indicated by the absence of necrosis and fatty deposition, however, slight activation of kupffer cells was observed (fig. 1, L-3). Treatment with 500mg/kg body weight *F. cordata* total extract showed good recovery with absence of necrosis and fatty depositions but had slight activation of kupffer cells (fig. 1, L-4). The appearance of hepatocytes treated with 500mg/kg body weight *F. cordata* total extract was comparable with those treated with the standard drug Sil.

Table 1: Effect of *F. cordata* total extract on Serum Biochemical Parameters at 250 and 500 mg/kg

Treatment (n=5)	Biochemical Parameters									
	AST (units/l)		ALT (units/l)		GGT (units/l)		ALP (units/l)		Bilirubin (mg/dl)	
	Mean± S.E.	% Decrease	Mean± S.E.	% Decrease	(Mean± S.E.)	% Decrease	Mean± S.E.	% Decrease	Mean± S.E.	% Decrease
Normal	83.96± 2.32		25.58± 2.68		3.05± 0.13		225.75 ±45.10		0.56± 0.01	
Pa 500 mg	172.66± 7.06***[a]		149.33 ± 5.61***[a]		9.7± 0.27***[a]		493.83 ± 20.00***[a]		2.04 ± 0.06***[a]	
Sil + Pa	96.66± 2.85***[b]	44	78.00 ± 3.63***[b]	47.76	4.78± 0.23***[b]	47.76	352.16 ± 12.53***[b]	28.68	0.77 ± 0.05***[b]	62.00
250 + Pa	142.00± 8.11*[b]	17.76	120.5± 4.78**[b]	19.30	8.1± 0.33**[b]	19.30	418.66 ± 8.23**[b]	15.22	1.03± 0.06***[b]	49.18
500 + Pa	111.5± 4.26***[b]	35.42	116.83± 4.63**[b]	21.76	6.31± 0.17***[b]	21.76	403.00± 3.78**[b]	18.39	0.96± 0.05***[b]	52.94

*p<0.05; ** p<0.01; ***p<0.001,, [a] as compared with the normal saline (control) group; [b] as compared with the CCl_4 only group.

Table 2: Effect of *F. cordata* total extract on kidney function at 250 and 500 mg/kg

Treatment (n=5)	Biochemical Parameters							
	Sodium (mmol/l)		Potassium (mmol/l)		Creatnine (mg/dl)		Urea (mg/dl)	
	Mean ± S.E.	% Change	Mean ± S.E.	% Change	(Mean ± S.E.)	% Change	Mean ± S.E.	% Change
Normal	82.46± 3.37		5.95± 0.18		2.91± 0.14		56.56± 4.23	
Pa 500 mg	163.5± 5.46***[a]		14.3± 0.60***[a]		11.2± 0.28***[a]		178.83± 7.06***[a]	
Sil + Pa	112.5± 3.71***	31.19	7.48± 0.30***[b]	47.66	5.13± 0.19***[b]	54.16	85.83± 6.51***[b]	52.00
250 + Pa	144.66± 2.71*[b]	11.51	11.40± 0.53**[b]	20.27	9.31± 0.34**[b]	16.81	154.00± 6.17*[b]	13.88
500 + Pa	127.5± 6.88**[b]	22.01	8.73± 0.20***[b]	38.92	8.15±***[b]	27.23	141.66± 2.33***[b]	20.78

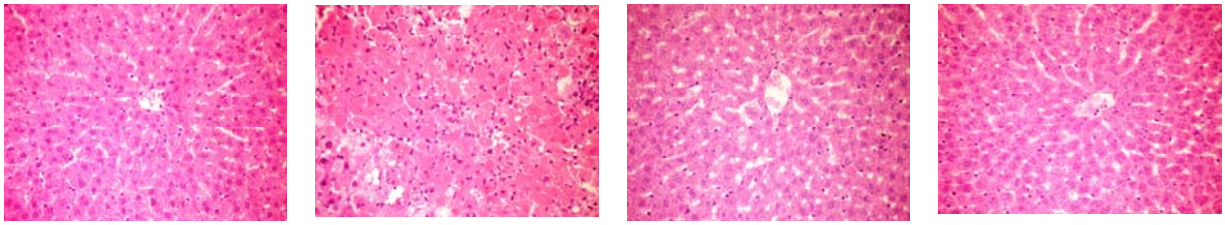
*p<0.05; ** p<0.01; ***p<0.001,, [a]as compared with the normal saline (control) group; [b]as compared with the CCl_4 only group.

Table 3: Effect of *F. cordata* total extract on NP-SH, MDA and TP in rat liver and kidney

Treatment (n=5)	NP-SH (nmol/l)		MDA (nmol/l)		Protein (g/l)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	8.34±0.55	8.96±0.25	1.15±0.08	1.08±0.05	106.58±3.31	114.17±2.11
Pa 500 mg	4.59± 0.45***[a]	4.80± 0.45***[a]	6.05± 0.52***[a]	5.022± 0.26***[a]	51.09± 2.52***[a]	55.48± 2.42***[a]
Sil + Pa	7.44± 0.65***[b]	7.66± 0.56**[b]	2.24± 0.21***[b]	1.82± 0.10***[b]	89.42± 5.04***[b]	87.02± 1.59***[b]
250 + Pa	5.98±0.38*[b]	6.31±0.42*[b]	4.49±0.32*[b]	5.91±0.43[b]	66.66±2.58**[b]	57.48±1.51[b]
500 + Pa	6.79± 0.47**[b]	6.21± 0.31*[b]	3.28± 0.19***[b]	3.60± 0.20**[b]	75.44± 1.60***[b]	73.45± 2.11***[b]

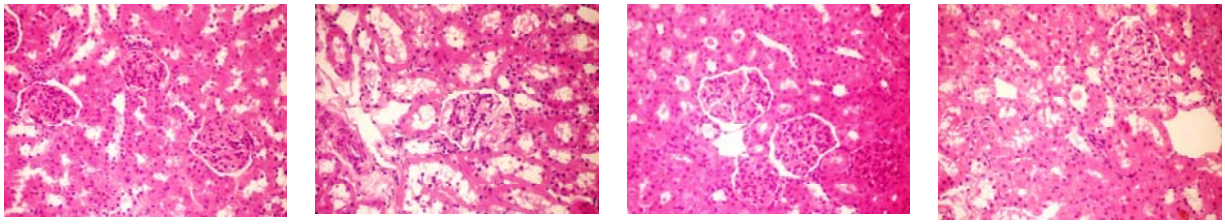
*p<0.05; ** p<0.01; ***p<0.001,, [a]as compared with the normal saline (control) group; [b]as compared with the CCl_4 only group.

Histopathological study revealed the normal renal architecture in control group (fig.2, K-1). Kidney cells of rats treated with Pa showed dramatic histopathological changes appeared as vaculation of epithelial lining renal tubules, pyknosis of their nuclei and endothelial lining glomerular tuft (fig.2, K-2). Pretreatment with Sil resulted in good protection indicated by the absence of any histopathological changes (fig.2, K-3). Renal cells of rats treated with Pa and 500mg/kg body weight *F. cordata* total extract showing vaculation of epithelial lining renal tubules and hypertrophy of glomerular tuft pointing out to poor protection (fig.2, K-4).



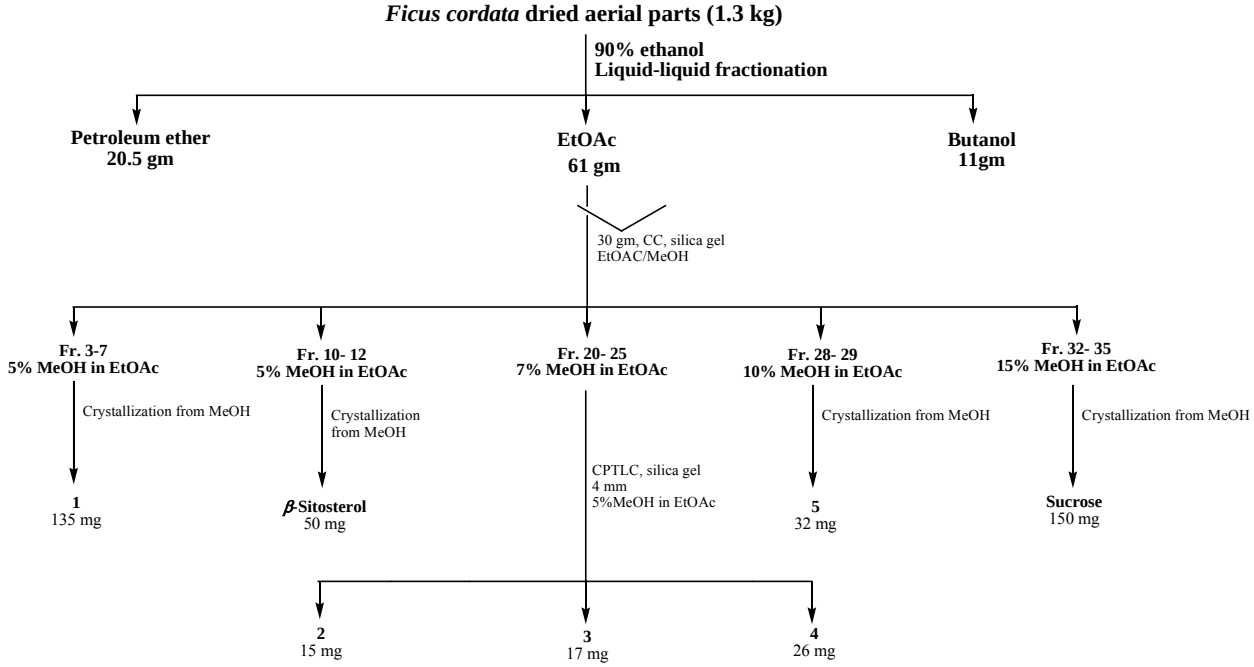
L-1 L-2 L-3 L-4

Fig. 3: Histopathological appearance of liver cells; (L-1) normal cells; (L-2) liver cells of rats treated with Pa showed centrilobular necrosis and extensive fatty degeneration of centrolobular hepatocytes; (L-3) liver cells of rats treated with Pa and Sil showed no necrosis or fatty deposition but had slight activation of kupffer cells; (L-3) liver cells of rats treated with Pa and total extract showing a good recovery with absence of necrosis and fatty depositions but had slight activation of kupffer cells.



K-1 K-2 K-3 K-4

Fig. 4: Histopathological appearance of kidney cells; (K-1) normal cells; (K-2) kidney cells of rats treated with Pa showed vaculation of epithelial lining renal tubules, pyknosis of their nuclei and endothelial lining glomerular tuft; (K-3) kidney cells of rats treated with Pa and Sil showed no histopathological changes; (K-4) kidney cells of rats treated with Pa and total extract showing vaculation of epithelial lining renal tubules and hypertrophy of glomerular tuft.



Scheme 1: Schematic presentation of the extraction and isolation procedures of *F. cordata* compounds.

Antiulcer activity

Damage of mucus membrane that normally protects the esophagus, stomach and duodenum from gastric acid and pepsin causes peptic ulcer (Brenner and Stevens, 2006). Many plants showed good antiulcer activity such as *Jasminum grandiflorum, Anogeissus latifolia, Solanum nigrum, Azadirachta indica* and *Ocimum sanctum* (Sen *et al*., 2009; Kumar *et al*., 2011). *F. cordata* total extract was tested at 250 and 500 mg / kg body weight for possible antiulcer effect against 80% ethanol induced lesions. The ulcer index of the group treated with 80% ethanol was 6.83±0.3 (table 4). Protection against ulcer by *F. cordata*

total extract was dose dependent and statistically significant (P<0.05- P<0.01). Although the protection with the 250mg/kg was weak, the higher dose (500mg/kg) showed good protection with ulcer index =2.5±0.42 (table 4).

Table 4: Effect of *F. cordata* extract at 250 and 500 mg/kg on the gastric lesion induced by 80% ethanol.

Treatment n=5	Ulcer index (Mean ±S.E)
1 ml 80% Ethanol	6.83±0.30
250+1ml 80% Ethanol	5.33±0.42*
500+1 ml 80% Ethanol	2.5±0.42**

*P<0.05,**P<0.001

Table 5: Effect of *F. cordata* extract at 250 and 500 mg/kg on clotting time.

Treatment n=5	0 min	30 min	60 min	120 min
Control	2.16 ±0.04	2.15± 0.03	2.20± 0.02	2.23± 0.06
Warfarin	2.81± 0.15	11.55± 0.33***	16.30± 0.47***	22.93± 0.74***
250	2.16± 0.02	2.30± 0.03	2.31± 0.02*	2.35± 0.01**
500	2.20± 0.03	3.42± 0.02***	4.52± 0.28***	5.70± 0.28***

*p<0.05; ** p<0.01; ***p<0.001

Determination of whole blood Clotting Time (CT)

Coumarins and coumarin derivatives are well known for their anticoagulant effect (Murrey *et al*., 1982; Penning-van Beest *et al*., 2005). As furanocoumarins are the main secondary metabolites present in *F. cordata* the total extract was subjected to CT assay using warfarin as standard. Although the response was dose, time dependent and highly significant (P<0.001) for the 500mg/kg dose of the extract, the effect was about one quarter of that obtained by treatment with warfarin (table 5). The effect of the 250mg/kg dose of the extract was very weak as compared to the warfarin and with the CT at zero time after administration of the extract. The results obtained after 30 minutes was not significant.

CONCLUSION

Phytochemical study of the aerial parts of *Ficus cordata* indicated that furanocoumarins are the major components. *In vivo* evaluation of the hepatoprotective, nephroprotective, antiulcer and anticoagulant in two doses level and in comparison with standard drugs was performed. The higher dose (500 mg/kg) showed moderate hepatoprotective activity as indicated via serum biochemical parameters, tissue parameters and histopathological evaluation. Nephroprotective effect was weak. Antiulcer activity against ethanol induced stomach lesions indicated a good protection power with 2.5±0.42 ulcer index. Whole blood clotting time (CT) was about one quarter of that of warfarin.

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