Research Article

Anti-oxidant and hepatoprotective activities of total flavonoids of *Indocalamus latifolius*

Anti–oxidant and hepatoprotective activities of total flavonoids of *Indocalamus latifolius*

Juan Tan, Shenghua Li, Junying Zeng and Xianjin Wu

The Department of Life Science, Huaihua University, Huaihua 418008, China.

Abstract

The total flavonoids of *Indocalamus latifolius* were evaluated in term of their anti-oxidant and hepatoprotective activities. The results showed that *in vitro* hepatoprotective and anti-oxidant activities of total flavonoids at doses of 200 and 400 mg/kg and 100 mg/kg, respectively, were comparable to those of the known hepatoprotective drug silymarin at 100 mg/kg. These data were supplemented with histopathological studies of rat liver sections. Seven of the main flavonoid compounds purified by column chromatography using silica gel, sephadex LH-20 and develosil ODS, and determined to be vitexin, orientin, isovitexin, homoorientin, tricin, tricin-7-O-β-D-glucopyranoside and quercetin-3-O-glucopyranoside.

Introduction

The *Indocalamus latifolius* (Keng) McClure is extensively cultivated throughout eastern Asia, particularly in China. The young leaves of *I. latifolius* have been used to make tea for many years in China, and have also been used for their anti-oxidant, free radical-scavenging, restraining, antibacterial, preservative and anti-HIV activities (Flora, 1996). The leaves are also used during the annual Dragon Boat Festival in China to parcel polished glutinous rice, which is known as "zongzi" for thousands of years. Several studies have been carried out on the low-molecular-weight compounds present in essential oils (Li et al., 2007) of *I. latifolius*, with particular emphasis on the polysaccharides (Chen et al., 1999), and polyphenols (Luo and Chen, 2003). Based on previous studies, it is well-known that flavonoid compounds exist in a wide variety of different plant species. Several bioactive flavonoid compounds isolated from natural sources have recently attracted considerable attention from researchers working in the fields of biochemistry and pharmacology (Avallone et al., 2000; Xu et al., 2011), and several reports have demonstrated that flavonoid

compounds are the major active constituents in plants belonging to the Bambusoideae family, such as *Indocalamus tessellates* (Xiang et al., 2002) and *Lophatherum gracile* Brongn (Li, 2008). Furthermore, these compounds have been isolated and reported to possess anti-oxidative, antitumor, and immunostimulatory activities. However, studies pertaining to the flavonoid compounds present in *I. latifolius* have been scarce. With this in mind, we became interested in studying the isolation and subsequent characterization of the flavonoid compounds present in the leaves of *I. latifolius*, as well as evaluating the hepatoprotective and anti-oxidant activities of these compounds *in vitro* and *in vivo* models.

Materials and Methods

Plant Material

The *I. latifoliu* were collected locally in July 2011 from their natural habitat in Hunan Province, China, and authenticated by Prof. Xian-jin Wu, Department of Bioscience, Huaihua University, where a voucher specimen was deposited. The plant material was dried at 40–

50°C for 72 hours and then powdered to the extent that all of the material could be passed through a 0.5 mm mesh.

Determination and preparation of total flavonoids

An extract of *I. latifolius* was prepared by the ultrasound-assisted extraction of the powdered material with 70% ethanol for 45 min. The resulting mixture was filtered (paper), and the filtrate was evaporated to dryness under reduced pressure to give a residue (5.0% yield). The extract was then dissolved with hot water, and the resulting solution was applied to a column of D-101 macroreticular resin for adsorption for 12 hours. The column was then washed successively with 20% ethanol to remove any impurities. The column was then eluted with 60% ethanol to allow for the total flavonoids to be washed from the column, and the resulting eluent was collected and evaporated to dryness under reduced pressure to give total flavonoids (1.7% yield).

A standard curve was prepared according to the following procedure. A calibration solution was prepared consisting of 200.8 μg/mL of rutin in 70% ethanol. Seven stock solutions were then prepared by placing 0, 1, 2, 3, 4, 5, and 6 mL of the rutin calibration solution into individual 25-mL volumetric flasks. 6mL of 70% ethanol and 1 mL of 5% $NaNO_2$ were then added to each flask, and the resulting mixture was shaken thoroughly and allowed to stand still for 6 min. Each flask was then treated with 1 mL of a 10% $AlCl_3$ solution, and the resulting mixtures were shaken and allowed to stand for 6 min. Each flask was then treated with 10 mL of a 10% NaOH solution before being diluted to a total volume of 25 mL with 70% ethanol. The resulting mixtures were then mixed thoroughly and allowed to stand for 15 min. The absorbance properties of the different solutions were measured at 500 nm with 70% ethanol being used as a blank control.

A sample (1 mL) of extraction solution described above was transferred to a 25-mL volumetric flask, and its absorbance properties were measured using the procedure described above. In this way, it was possible to calculate the total flavonoid contents of the extract using a regression equation.

Isolation of total flavonoids

The dried powdered leaves of *I. latifolius* powder (5000 g) were extracted with 70% ethanol under ultrasonic irradiation for 45 min, and the resulting mixture was filtrated (paper). The filtrate was evaporated to dryness under reduced pressure to give a residue (1248 g), which was dissolved in hot water (1000 mL). The resulting aqueous solution was applied to a column loaded with D-101 macroreticular resin for adsorption for 12 hours, and then washed successively with 20% ethanol to remove any impurities. The total flavonnoids on the column was then eluted with 60% ethanol, and

the resulting eluent solution was collected and evaporated to dryness under vacuum to give a red solid (1064 g).

A portion (900 g) of the solid was dissolved in water (800 mL), and the resulting solution was washed successively with petroleum ether (800 mL × 5), ethyl acetate (800 mL × 5), and butanol (800 mL × 5) to afford petroleum ether-soluble (28.8 g), ethyl acetate-soluble (171.7 g), and n-butanol-soluble (61.4 g) fractions following the removal of the solvent under vacuum. The petroleum ether-soluble fraction (28.8 g) was subjected to column chromatography over silica gel (762 g) on a standard column (490 × 72 mm inner diameter) eluting with different mixtures of chloroform and methanol [100:0, 95:5, 90:10, 85:15, 80:20, and 70:30 (v/v) with fraction sizes of 8.0, 6.0, 7.0, 12.5, 9.0, and 5.5 L, respectively] to give fractions A1–11. Fraction A8 (2.1 g), which was obtained via the elution of the silica column with an 85:15 (v/v) mixture of chloroform and methanol, was passed through a develosil ODS (4.2 g) pre-column eluting with a 1:1 (v/v) mixture of 80% methanol and water to give a residue (0.29 g). This residue was purified by column chromatography over sephadex LH-20 eluting with methanol to give compounds 1 (13 mg) and 2 (34.5 mg). Fraction A2 (0.5 g) was filtered through a solid-phase extraction cartridge of RP-18 silica to give a solid, which was recrystallized from methanol. The solid material was then purified by RP-HPLC using an XTerra column (10 × 250 mm) eluting with 4:6 (v/v) mixture of methanol and water at a flow rate of 2 mL/min to give compound 3 (18 mg). The ethyl acetate-soluble fraction (171.7 g) was dissolved in water (500 mL), and the resulting solution was loaded onto a HP-20 (280 g) column, which was sequentially eluted with water (100 mL) and methanol (500 mL).

The material from the methanol eluent (90.8 g) was subjected to column chromatography over silica gel (1600 g) on a standard column (590 × 92 mm inner diameter) eluting with different mixtures of chloroform and methanol [90:10, 85:15, 80:20, 70:30 and 60:40 (v/v) at 14 L each] to give fractions B1–8. Fraction B4 (12.6 g), which was obtained from the elution of the silica column with a 85:15 (v/v) mixture of chloroform and methanol, was purified over a develosil ODS (45.0 g) column (360 × 18 mm inner diameter) eluting with different mixtures of methanol and H_2O [1:9, 2:8, 3:7, 4:6, 5:5, 6:4, and 7:3 (v/v) at 450 mL each] to give fractions B4.1–B4.35. Fractions B4.21 (96.6 mg) and B4.22 (178.9 mg) were purified by HPLC eluting with a 1:1 (v/v) mixture of 49% methanol and water at a flow rate of 5 mL/min to give compounds 4 (10.2 mg) and 5 (12.6 mg). Fraction B5 (4.3 g), which was obtained from the elution of the original silica column with 8:2 and 7:3 (v/v) mixtures of chloroform and methanol, was purified over develosil ODS (28.8 g) using a standard

column (230 × 18 mm inner diameter) eluting with different mixtures of methanol and water [1:9, 2:8, 3:7, 4:6, 5:5, and 6:4 (v/v) at 300 mL each] to afford fractions B5.1–B5.10. Fractions B5.8 (76.8 mg) and B5.9 (76.9 mg) were separated by sephadex LH-20 column chromatography eluting with methanol to yield compounds 6 (39 mg) and 7 (14 mg).

In vitro anti-oxidant activity

DPPH radicals scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that shows a maximum absorption at 517 nm, and is widely used to evaluate the free radical scavenging ability of natural compounds. In the DPPH assay, anti-oxidant species reduce the stable DPPH radical to diphenylpicrylhydrazine, which is yellow in color. The anti-oxidant activity of a given sample can, therefore, be expressed in terms of its ability to reduce the DPPH radical. In this study, the anti-oxidant activities of the compounds isolated from *I. latifolius.* were determined by the DPPH scavenging activities of the purified polysaccharides, according to a modified version of the procedure described by elsewhere (Shimada et al., 1992). Briefly, one milliliter of 0.1 mM solution of DPPH in methanol was mixed with different concentrations (i.e., 0.2–1.0 mg/mL) of a purified polysaccharide solution (3.0 mL). The resulting solutions were held at room temperature for 30 min, and their absorbance properties were then measured at 517 nm. The DPPH scavenging effect was calculated as follows:

DPPH inhibition rate (%) = $[(A_s - A_i)/A_s] \times 100$

where As is the absorbance of DPPH alone, and Ai is the absorbance of DPPH in the presence of a specific concentration of the polysaccharide

Solutions of butylated hydroxytoluene (BHT) and ascorbic acid were prepared at the same concentrations and used as reference standards.

ABTS radicals scavenging assay

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity of DDP was identified using a modified version of the method described by elsewhere (Re et al., 1999). ABTS was produced from the reaction of a 7 mM solution of ABTS with a 2.45 mM solution of potassium persulfate. The resulting mixture was held in the dark at room temperature for 16 hours. At the time of use, the ABTS solution was diluted with ethanol to yield an absorbance of 0.7 ± 0.0 at 734 nm. Samples (0.2 mL) of various concentrations (0.2–1.0 mg/mL) were mixed with the ABTS solution (2 mL), and the resulting mixtures were allowed to react at room temperature for 6 min. The absorbance properties of the resulting solutions were then measured at 734 nm. The ABTS scavenging effect was calculated based on the following formula:

ABTS scavenging (%) = $[(A_s - A_i)/A_s] \times 100$

where As is the absorbance of ABTS alone, and Ai is the absorbance of ABTS in the presence of specific concentration of the substrate

Solutions of BHT and ascorbic acid were also prepared at the same concentrations and used as reference standards.

Superoxide radical scavenging assay

The capacity of TFI to scavenge superoxide radicals was examined using a pyrogallol auto-oxidation system (Wang et al., 2012). Reaction mixtures containing test extracts (0.2 mg/mL) in Tris–HCl buffer (4.5 mL, 50 mmol/L, pH 8.2) were incubated for 10 min at 25°C before being treated with 0.15 mL of pyrogallol (3 mmol/L in 10 mmol/L HCl). The absorbance properties of the resulting mixture were immediately measured at 325 nm, and at 30-sec intervals thereafter. The auto-oxidation rate constant (Kb) of pyrogallic acid was calculated from the curve of absorbance at 325 nm versus time. Solutions of BHT and ascorbic acid were prepared at the same concentrations and used as reference standards. The inhibitory activities of the test extracts towards the auto-oxidation rate of pyrogallic acid correlated well with their ability to scavenge superoxide radicals.

Reducing power assay

The reducing powers of the samples were determined according to a modified version of the method described by elsewhere (Oyaizu, 1986). All of the solutions used in the assay were freshly prepared on the same day. Briefly, 6 mL of reaction mixture containing different concentrations of samples (0.2–1.0 mg/mL) in phosphate buffer (0.2 M, pH 6.8) was incubated with a 1% (w/v) solution of potassium ferricyanide in water at 50°C for 20 min. The reaction was terminated by the addition of a 10% (w/v) solution of TCA in water. The resulting solution was then mixed with distilled water and a 0.1% (w/v) solution of $FeCl_3$ in water. The absorbance properties of the resulting solution were then measured at 700 nm, using BHT and ascorbic acid as positive controls.

Increased levels of absorbance from the reaction mixture indicated a greater reducing power.

β-Carotene bleaching assay

The anti-oxidant activity of TFI was evaluated according to a slightly modified version of the β-carotene bleaching method (Shon et al., 2003). Briefly, a solution of β-carotene was prepared by dissolving β-carotene (6 mg) in chloroform (20 mL). A portion (4 mL) of the resulting β-carotene solution was then placed in a 500-mL round-bottomed flask together with purified

linoleic acid (80 mg), and Tween 80 emulsifier (800 mg), and the resulting mixture was thoroughly agitated. The chloroform was then removed under vacuum to give a residue, which was dissolved in aerated distilled water (200 mL) with vigorous shaking. Aliquots (3.0 mL) of the resulting emulsion were transferred into different test tubes containing 0.2 mL of a TFI solution (0.2 mg/mL), and the mixtures were incubated in a water bath at 50°C. The absorbance properties of the mixtures were then recorded at 30-min intervals for 2 hours. BHT was used as standards for comparison. Lipid peroxidation (LPO) inhibition was calculated as follows:

LPO inhibition (%) = [(As − Ai)/As] × 100

where As is the initial absorbance of the assay and Ai is the absorbance of the assay after 2 hours.

Anti-oxidant activity in a linoleic acid system using ferric thiocyanate and thiobarbituric acid

The ferric thiocyanate (FTC) test was conducted as described by Osawa and Namiki (Osawa and Namaki, 1981), with slight modifications. A solution of total flavonoids (0.1 mg) in ethanol (1 mL), was treated with a 2.5% solution of linolenic acid in ethanol (1 mL), a 50 mmol/L solution of phosphate buffer (2 mL, pH 7.0), and distilled water (1 mL) in a clean Eppendorf tube, and the resulting mixture was placed in a dark oven at 40°C for 5 min. A portion (0.1 mL) of the solution was then collected and treated with a 0.02 mol/L solution of ammonium ferrous chloride in 3.5% HCl (0.1 mL), a 30% solution of ammonium thiocyanate, a 75% solution of ethanol (9.7 mL), and a 30% solution of ammonium thiocyanate (0.1 mL). After 3 min, the absorbance properties of the mixture were recorded at 500 nm. A 50% ethanol solution was used as the blank. The absorbance properties of the mixture were determined at 24 hours intervals until a constant maximum was reached. A mixture without total flavonoids was used as a negative control, and BHT and ascorbic acid were used as reference materials.

The thiobarbituric acid (TBA) value of the extract was assayed using the method described by Kikuzaki and Nakatani (Kikuzaki and Nakatani, 1993), with minor modifications. Briefly, a 0.67% solution of TBA (1 mL) and 20% solution of trichloroacetic acid (1 mL) were mixed thoroughly with 0.5 mL of the extract solution, and the resulting mixture was placed in a bath of boiling water for 10 min. The mixture was then allowed to cool to ambient temperature, during which time it was treated with 4 mL of water saturated with the n-butanol extract. The resulting mixture was then centrifuged at 3000 ×g for 10 min, and the absorbance properties of the colored organic phase were measured at 532 nm. The inhibition rate was calculated using the following equation:

Inhibition rate (%) = [(Ac − As)/Ac] × 100%

where Ac is the absorbance of the control and As is the absorbance of the sample

In vivo hepatoprotective and anti-oxidant activities

Experimental animals

Male Sprague-Dawley rats (220 ± 4.5 g) were purchased from the animal house of the Xiangya School of Medicine, Central South University. The rats were kept in departmental animal houses under strict temperature control (25 ± 2°C) with a relative humidity of 50 ± 5% and a 12-hours light/darkness cycle. All the animals were provided with free access to standard laboratory food and tap water prior to the experiment.

Carbon tetrachloride (CCl₄)-induced oxidative toxicity

Sixty rats were divided into six groups of 10 animals. Group I served as a normal group and received distilled water containing 0.3% sodium carboxymethyl cellulose (CMC-Na) (1 mL/kg body weight, p.o.) for 5 days, and olive oil (1 mL/kg body weight, s.c.) on days 2 and 3 (Jain et al., 2008). Group II served as a control group and received 0.3% CMC-Na (1 mL/kg body weight, p.o.) for 5 days, and a 1:1 (v/v) mixture of CCl₄ and olive oil (2 mL/kg body weight, s.c.) on days 2 and 3. Group III was treated with the standard drug silymarin (100 mg/kg body weight, p.o.) daily for 5 days, and also received a 1:1 (v/v) mixture of CCl₄ and olive oil (2 mL/kg body weight, s.c.) on days 2 and 3 at 30 min after the administration of silymarin. Groups IV–VI (test group animals) were given a dose of 100, 200, and 400 mg/kg body weight of total flavonoids (p.o.), respectively, for 5 days. The animals in these groups also received a 1:1 (v/v) mixture of CCl₄ and olive oil (2 mL/kg, s.c.) on days 2 and 3 at 30 min after the administration of total flavonoids. On the sixth day, the animals were anesthetized and their blood was collected and allowed to clot. The serum was then separated for assessment of the biochemical parameters. The rats were then sacrificed by bleeding, and their liver and kidneys were carefully dissected and cleaned of extraneous tissue. Parts of the liver tissue were then immediately transferred into 10% formalin for histopathological investigation.

Serum analysis for liver marker enzymes

Serum samples were assayed for AST, ALT, ALP, and GGT using assay kits, which were obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Serum analysis for biochemical studies

Total bilirubin, cholesterol, triglycerides, protein, and albumin were estimated by the assay kits.

Determinations of anti-oxidant activity in vivo

The superoxide dismutase, catalase, malondialdehyde,

and GSH levels in the liver and kidneys were determined using the assay kits. which were obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Histopathological studies

Liver tissues were fixed in 10% formalin for at least 24 hours, before being embedded in paraffin and cut into 5-µm-thick sections in a rotary microtome. The sections were stained with hematoxylin-eosin dye and observed under a microscope (IX51, Olympus, Japan) to detect histopathological changes in the liver.

Statistical analysis

All of the experiments were performed in triplicate, and the results were reported as the mean values ± standard deviation. The data were analyzed by one-way analysis of variance, and any statistically significant effects were further analyzed. The mean values were compared using the Duncan's multiple range test. Statistical significance was determined at $p < 0.05$.

Results and Discussion

Extraction of total flavonoids

The total flavonoids were extracted from *I. latifolius* with 70% ethanol and purified with D-101 macroreticular resin to give a residue, which was dried under reduced pressured to give a TFI in a yield of 2.3%. Regression equation: $A = 12.36C - 0.086$, $r = 0.9997$, C (concentration), with a better liner correlation between 9.2 and 48.7 µg/mL. Quantitative analysis indicated that the total flavonoids were up to 88.5%. Finally, the main flavonoid compounds were purified by chromatography over silica gel, sephadex LH-20,and develosil ODS. Seven of the compounds detected were vitexin, orientin, isovitexin, homoorientin, tricin, tricin-7-O-β-D-glucopyranoside, and quercetin-3-O-glucopyranoside (Figure 1).

Anti-oxidant activities analysis

DPPH and ABTS radical scavenging activity

DPPH and ABTS are relatively stable free radical compounds that have been widely used to test the free radical-scavenging activities of numerous compounds (Sanchez-Moreno, 2002). The radical scavenging activities of DPPH and ABTS in different concentrations of total flavonoids are shown in Figure 2A and B. In the DPPH scavenging assay, the IC_{50} (the concentration required to scavenge 50% of radical) values of total flavonoids of *I. latifoliu*, BHT, and ascorbic acid were 89.7 ± 3.2, 64.8 ± 1.6, and 29.6 ± 1.1 µg/mL, respectively. In the ABTS scavenging assay, the IC_{50} values of total flavonoids of *I. latifoliu*, BHT, and ascorbic acid were 4.3 ±

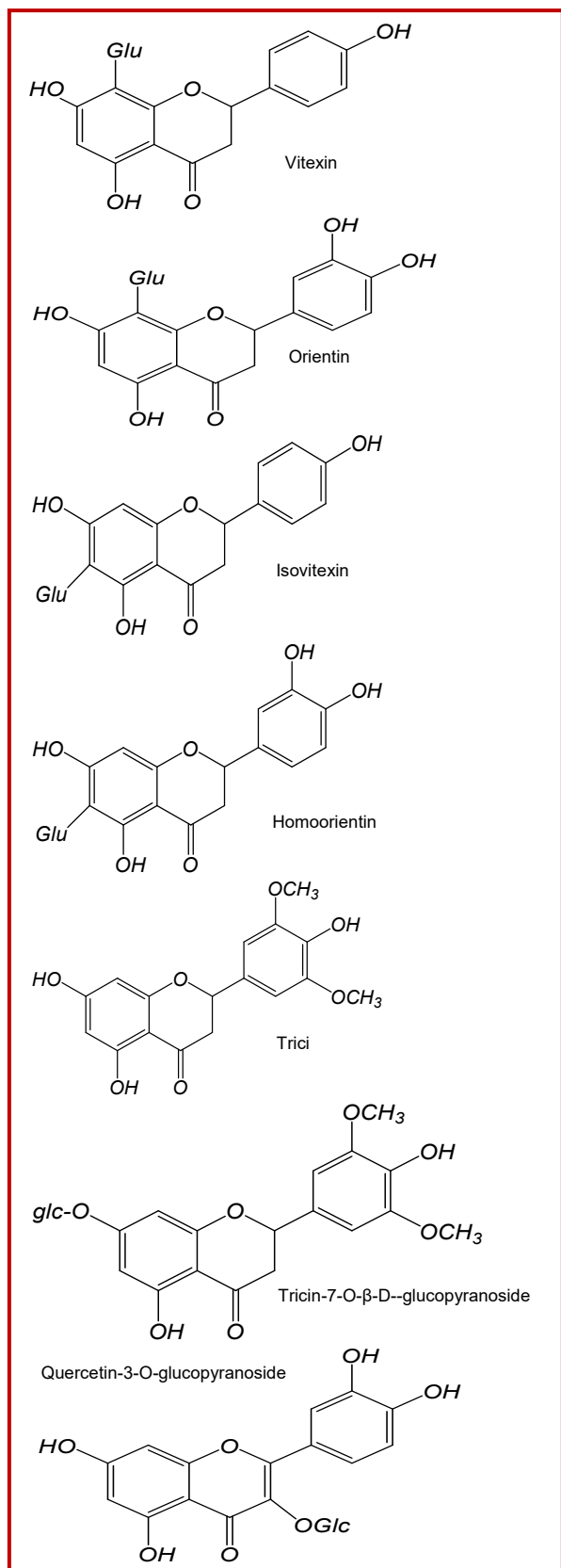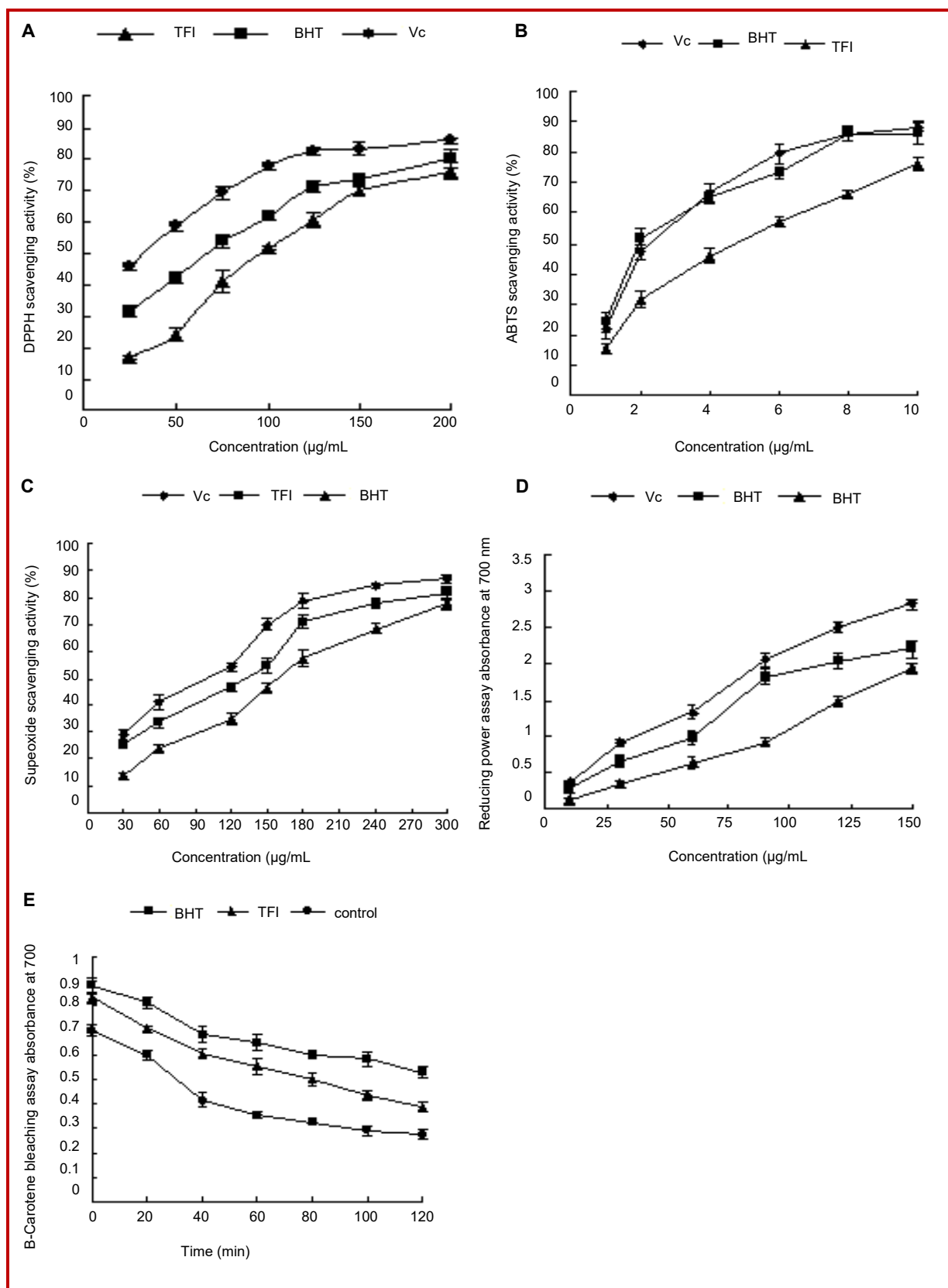Figure 1: Structure of the compounds

Figure 2: Anti-oxidant activities of total flavonoids of *I. latifoliu* as determined by the A) DPPH; B) ABTS; C) $O_2^{\bullet-}$ scavenging activity; D) Reducing power, and E) β-carotene bleaching assay
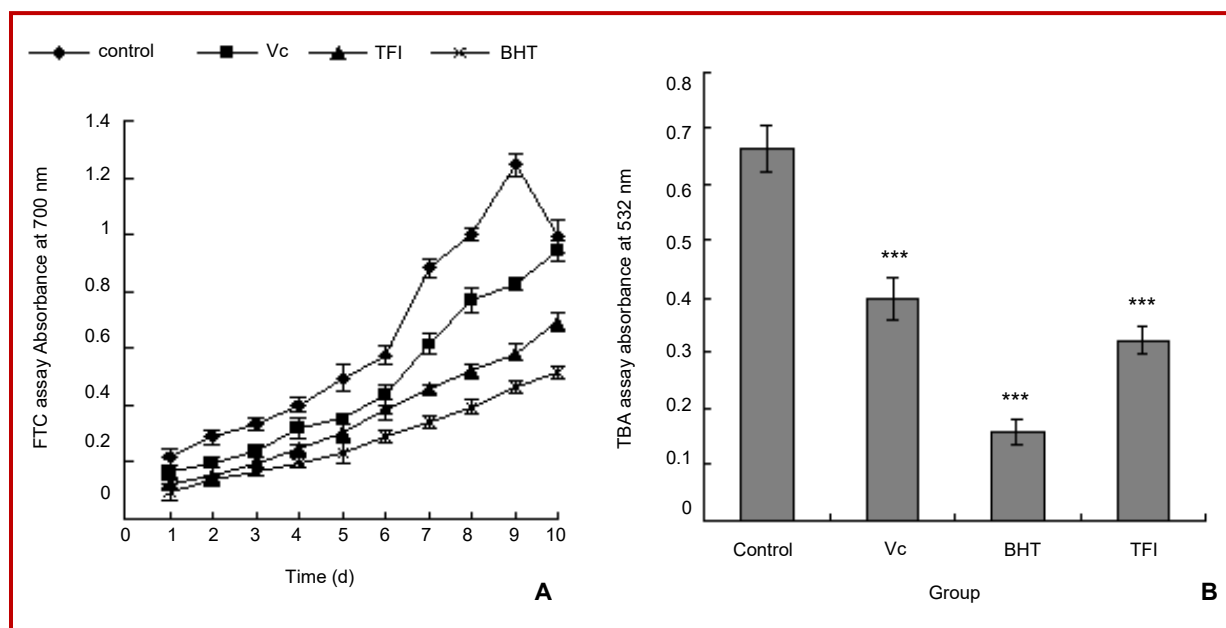
Figure 3: Antioxidant activities of total flavonoids of *I. latifoliu* assayed using the A) FTC and B) TBA methods. Low absorbance values indicate a strong antioxidant activity. All of the samples were measured at the same concentration of 0.1 mg/mL of total flavonoids of *I. latifoliu*. Statistically significant differences have been indicated by asterisks (***p<0.001 compared with the control group)

0.7, 2.0 ± 0.1, and 2.0 ± 0.2 µg/mL, respectively.

Superoxide radical scavenging assay

The superoxide anion radical $(O_2{}^{\bullet-})$ is one of the most common free radical species generated *in vivo*. Pyrogallic acid can auto-oxidize under alkaline conditions to produce $O_2{}^{\bullet-}$ directly, and the rate constant of this auto-oxidation reaction is dependent on the $O_2{}^{\bullet-}$ concentration. The test compound can significantly reduce the rate of the auto-oxidation reaction of pyrogallic acid because of its ability to scavenge $O_2{}^{\bullet-}$ radicals (Xiang and Ning, 2008). The K_b values (×10.4 A/s) of the control, total flavonoids of *I. latifoliu*, BHT, and Vc samples were 6.4 ± 0.6, 2.9 ± 0.4, 5.6 ± 0.3, and 1.2 ± 0.2, respectively (Figure 2C).

Effect of reductive power

The anti-oxidant activity of the plant extract was investigated by measuring the conversion of Fe^{3+} to Fe^{2+} (Meir et al., 1995). The reductive capability was monitored by the formation of Perl's Prussion blue at 700 nm. The resulting data are shown in Figure 2D. The reducing abilities of total flavonoids, ascorbic acid, and rutin increased with increasing concentration. The anti-oxidant activity of ascorbic acid was significantly higher than that of total flavonoids, and slightly stronger than that of rutin. This result was in agreement with that of Afolayan et al. (Afolayan et al., 2008) for their methanol extract of *Malva parviflora*. Based on these results, it could be inferred that the reference

drugs and the total flavonoids were behaving as electron donors, and that this behavior could lead to a reduction in the number of oxidized intermediates resulting from lipid peroxi-dation (Ordonez et al., 2006). This observation could be attributed to the presence of reductones, which are well-known termina-tors of free radical chain reactions that operate through the donation of a hydrogen atom (Apak et al., 2008).

β-Carotene bleaching assay

β-Carotene is a yellow/orange-red fat-soluble pigment that can be bleached by the auto-oxidation products of linoleic acid. Figure 2E shows that BHT has the best activity, and the absorbance of the control group fell sharply with increased time. The LPO inhibition values of BHT and total flavonoids were 76.5 ± 1.7 and 53.2 ± 4.5%, respectively.

Anti-oxidant activity in a linoleic acid system using ferric thiocyanate and thiobarbituric Acid

The auto-oxidation products of linoleic acid can oxidize Fe^{2+} to Fe^{3+}. Fe^{3+} ions can form a thiocyanate complex with SCN^-, which has a maximum absorbance at 500 nm (Liu and Yao, 2007). The final products of linoleic acid oxidation, including thiobarbituric acid reactive species (TBARS), can react with TBA to form a red complex that can be determined at 532 nm. Figure 3A shows that the absorbance of the control increased over time and reached its peak value on day 8. Total flavonoids of *I. latifoliu* and BHT exhibited strong and
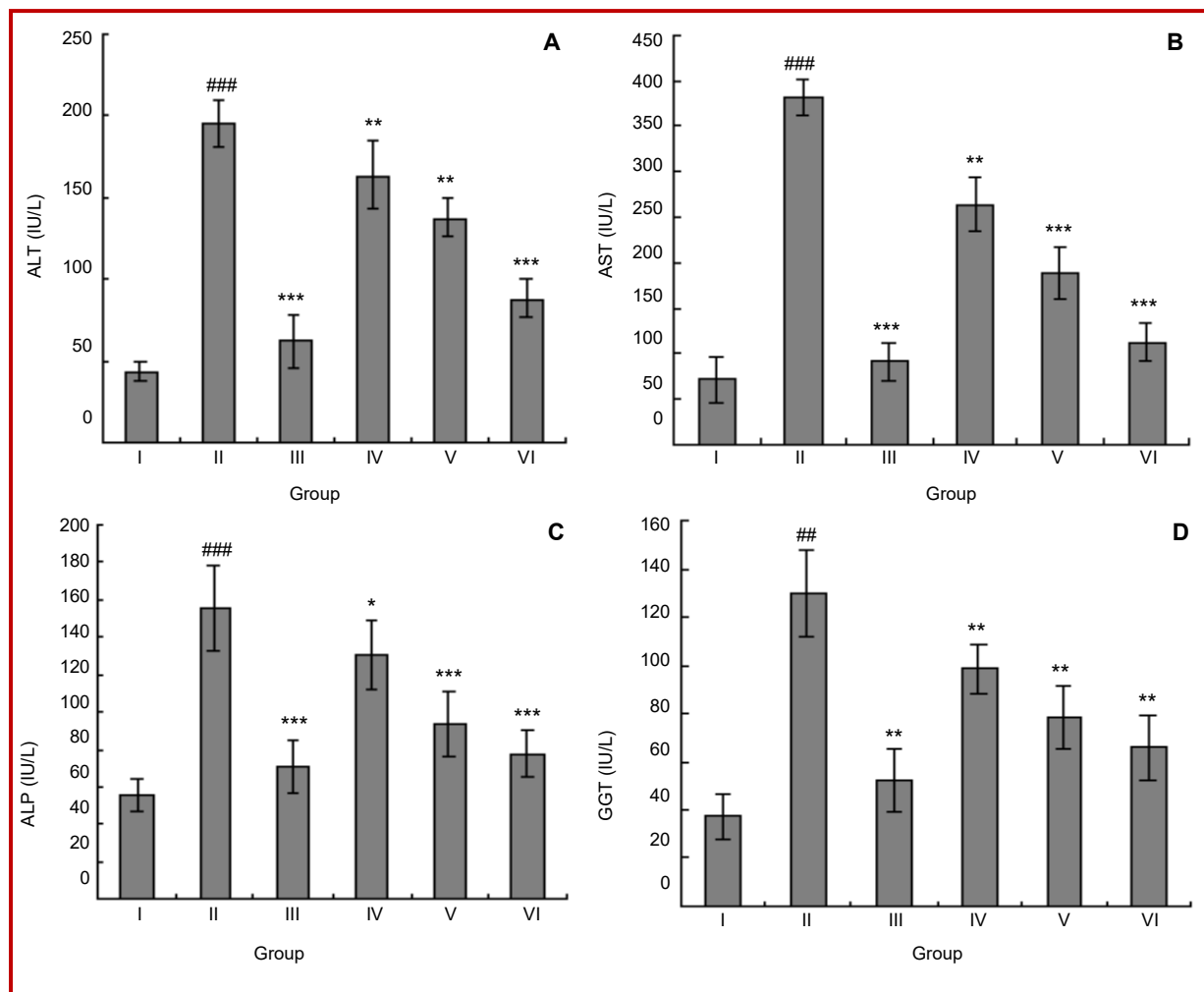
Figure 4: Effect of TFI on the biochemical parameters of CCl_4-damaged livers of rats. (A) AST; (B) ALT; (C) ALP; (D) GGT. The values have been expressed as the mean values ± standard error of the mean, n=10; #p<0.05, ##p<0.01, ###p< 0.001 versus the normal group; *p<0.05, **p<0.01, and ***p<0.001 compared with the CCl_4-intoxicated control group

comparative activities. Figure 3B shows that total flavonoids and BHT also exhibited excellent anti-oxidant activities, and the inhibition rates of ascorbic acid, total flavonoids and BHT were 48.6 ± 3.6, 87.7 ± 1.7, and 93.2 ± 3.6%, respectively.

Effect of total flavonoids on liver marker enzymes

Hepatic injury resulting from CCl_4-induced lipid peroxidation has been used extensively in experimental models to understand the cellular mechanisms behind oxidative damage and evaluate the therapeutic potential of drugs and dietary anti-oxidants (Basu, 2003). In CCl_4-induced toxicity, CCl_4 is metabolized through the cytochrome P_{450} monooxygenase system to produce a trichloromethyl radical, which can react with oxygen to form a trichloromethyl peroxyl radical (Shenoy et al., 2001). Glutamate pyruvate transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) are all important metabolic enzymes in liver

cells, and are released into the blood when the liver cells are damaged. As shown in Figure 4, the enzymatic activities of serum ALT, AST, ALP, and GGT in CCl_4-intoxicated rats sharply increased to 195.1 ± 14.1 (p< 0.001), 382.3 ± 19.7 (p<0.001), 155.7 ± 22.6 (p<0.001), and 129.5 ± 18.2 IU/L (p<0.0 01) compared with values of 42.8 ± 8.3, 71.2 ± 4.5, 55.4 ± 8.7, and 37.9 ± 9.1 in the normal groups, respectively. Administration of total flavonoids (100, 200, and 400 mg/kg) alleviated the toxicity of CCl_4 and the serum levels of AST, ALT, ALP, and GGT reverted back towards those of the normal groups, with significance differences from the control group. Similarly, administration of silymarin (100 mg/kg) alleviated the toxicity of CCl_4 and the serum levels of AST, ALT, ALP, and GGT were observed to be significantly lower than that of the control group.

Total flavonoids on serum biochemical profile

The levels of total cholesterol, triglycerides, total bilirubin, total protein, and albumin in the serum were

Table I				
Protective effect of total flavonoids of *Indocalamus latifolius* on serum biochemical profile				
Group	Total bilirubin (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
I	0.4 ± 0.0	43.6 ± 3.2	28.6 ± 1.1	9.6 ± 1.7	1.1 ± 0.1
II	0.9 ± 0.1[a]	98.7 ± 4.9[a]	72.7 ± 1.7[a]	5.9 ± 0.9[a]	3.2 ± 0.6[a]
III	0.4 ± 0.1[d]	52.8 ± 3.2[d]	35.7 ± 2.14[d]	8.6 ± 0.4[d]	1.4 ± 0.6[d]
IV	0.7 ± 0.1[b]	79.6 ± 3.0[c]	67.4 ± 1.2	8.3 ± 1.3	2.2 ± 0.2[b]
V	0.6 ± 0.1[c]	71.6 ± 1.6[c]	52.6 ± 2.0[c]	7.6 ± 0.7[c]	1.9 ± 0.1[c]
VI	0.5 ± 0.1[d]	59.6 ± 1.3[d]	38.6 ± 1.2[d]	8.4 ± 0.9[d]	1.5 ± 0.1[d]

Values are mean ± standard error of the mean; n=10 animals in each group; symbols represent statistical significance; [a]<0.001 versus the normal group; [b]$p<0.05$; [c]$p<0.01$; and [d]$p<0.001$, compared with the CCl$_4$-intoxicated control group

Table II			
Effects of total flavonoids of *Indocalamus latifolius* on liver superoxide dismutase, catalase, malondialdehyde, and reduced glutathione level in CCl$_4$-intoxicated rats			
Group	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)	Malondialdehyde (nmol/mg protein)	Reduced glutathione (mg/g protein)
I	285.2 ± 5.5	68.5 ± 2.2	1.5 ± 0.04	9.2 ± 0.3
II	179.3 ± 10.9[d]	32.5 ± 3.2[d]	3.5 ± 0.2[d]	3.8 ± 0.2[d]
III	241.5 ± 5.9[a]	61.3 ± 2.34[c]	1.6 ± 0.1[c]	8.7 ± 0.3[c]
IV	208.1 ± 9.23[b]	42.1 ± 1.1[a]	2.9 ± 0.1[a]	4.2 ± 0.2[a]
V	234.0 ± 6.3[b]	51.4 ± 2.1[b]	2.2 ± 0.1[b]	6.7 ± 0.3[b]
VI	258.5 ± 8.2[c]	61.3 ± 3.1[c]	1.8 ± 0.04[c]	7.9 ± 0.2[c]

Values are mean ± standard error of the mean, n = 6 animals in each group; symbols represent statistical significance: [d]$p<0.001$ compared with the normal control group. [c]$p<0.001$, [b]$p<0.01$, and [a]$p<0.05$ compared with the CCl$_4$-intoxicated control group

estimated and the results are shown in Table I. Administration of CCl$_4$ in rats caused a significant increase ($p<0.01$) in levels of total bilirubin, total cholesterol, triglyceride, and albumin in the serum from 0.4 ± 0.04, 43.6 ± 3.2, 28.6 ± 1.1 mg/dL and 1.1 ± 0.1 g/dL for the normal control rats to 0.9 ± 0.1 mg/dL, 98.7 ± 4.9 g/dL, 72.6 ± 1.7 mg/dL, and 3.2 ± 0.6 g/dL in the rats treated with CCl$_4$, respectively. In contrast, there was a significant decrease ($p<0.01$) in the level of TP in the serum from 9.6 ± 1.7 to 5.9 ± 0.9 g/dL following treatment with CCl$_4$. However, the protective adminis-tration of different doses of total flavonoids led to a decrease in the CCl$_4$-elevated levels of serum total cholesterol, triglyceride, total bilirubin, and albumin ($p<0.05$). The effect of total flavonoids in this regard was similar to that of the positive control, silymarin. In contrast, the administration of total flavonoids led to an increase in the CCl$_4$-elevated levels of serum total protein.

Effect of total flavonoids on liver anti-oxidant enzymes

The CCl$_4$-induced hepatotoxicity model has been used extensively to evaluate the anti-oxidant effects of drugs and plant extracts (Awaad et al., 2006). Both superoxide dismutase and catalase play important roles in human defense mechanisms against the harmful effects of reactive oxygen species and free radicals. Reduced glutathione content is another important parameter that can be used to reveal oxidative damage in liver. Malondialdehyde is a cytotoxic product that is used as a hallmark of lipid peroxidation (Huang et al., 2010). The results of our study of the *in vivo* anti-oxidant activities of total flavonoids in liver are shown in Table II. Compared with the normal group, the CCl$_4$-intoxicated rats exhibited a significant decrease in total superoxide dismutase, catalase, and reduced glutathi-one in their liver ($p<0.001$), as well as a significant increase in the level of malondialdehyde ($p<0.001$). These changes were significantly reversed after treat-ment with total flavonoids and silymarin.

Effect of total flavonoids on histopathology of liver

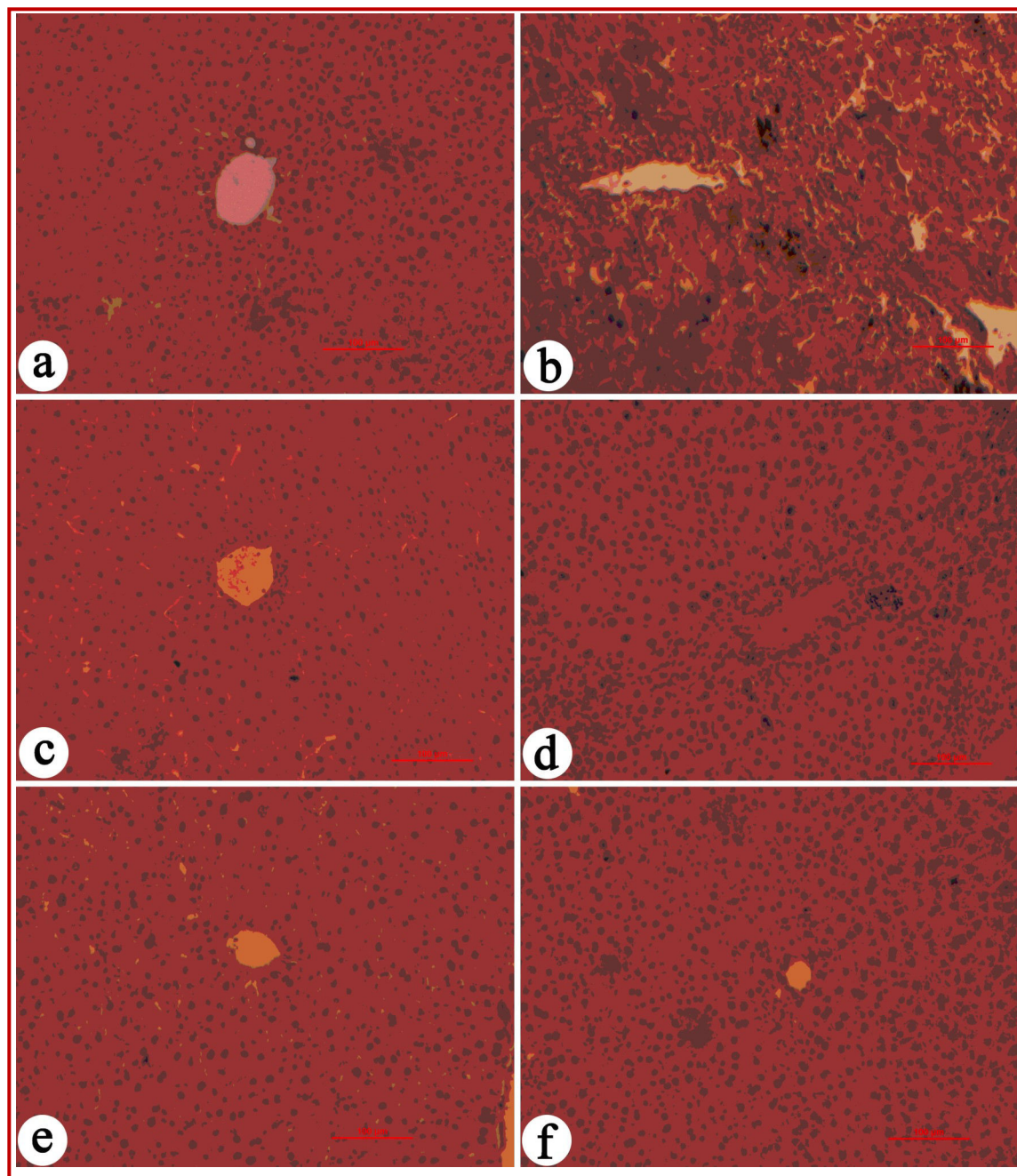Histopathological examination of liver sections from the control group showed normal cellular architecture

Figure 5: Sections of the livers of CCl$_4$-treated rats showing the ventral vein (CV) and hepatic cells (hematoxylin-eosin staining, 200×)

(a) Normal group, (b) control group, (c) CCl$_4$ + silymarin (100 mg/kg), (d) CCl$_4$ + TFI (100 mg/kg), (e) CCl$_4$ + TFI (200 mg/kg), and (f) CCl$_4$ + TFI (400 mg/kg)

with distinct hepatic cells, sinusoidal spaces, and a central vein (Figure 5A). In contrast, the CCl$_4$ group revealed severe damage to the tissue, with the liver sections showing massive fatty changes, necrosis, ballooning degeneration, broad infiltration of the lymphocytes, and loss of the cellular boundaries (Figure 5B). The liver sections of the rats treated with TFI (Figure 5E–F) showed a relatively normal lobular pattern with mild degrees of fatty change, necrosis, and lymphocyte infiltration, that were almost comparable to those of the control (Figure 5A) and the silymarin-treated (Figure 5C) groups.

Conclusion

An ethanol extract of *I. latifolius* exhibited strong hepatoprotective and anti-oxidant activities in a rat model of CCl$_4$-induced hepatotoxicity. The hepatopro-

tective activity of total flavonoids could be attributed to its free radical-scavenging and anti-oxidant activities, resulting from the presence of some flavonoids in the extracts.

Ethical Issue

All of the studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee.

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Author Info

Xianjin Wu (Principal contact); e-mail: hhuxianjin@163.com

First two authors contributed equally