Protective effects of *Forsythia suspensa* extract against oxidative stress induced by diquat in rats

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**Abstract**

Forsythia suspensa extract has been proved as a potential antioxidant in the recent years. The present study was undertaken to obtain the optimal antioxidant fraction in vitro and examine its antioxidative potential against diquat-induced oxidative stress in male Sprague Dawley rats in vivo. In vitro, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging experiment indicated that the CH\(_2\)Cl\(_2\) fraction of *F. suspensa* (FSC) exerted the strongest scavenging activities; forsythoside A, forsythoside A and philogenin from it might be the major antioxidant constituents. In vivo, pretreatment of rats with different doses of FSC (25, 50 and 100 mg/kg bw) and vitamin C (100 mg/kg bw, positive control) for 15 days significantly lowered the tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1(\(\beta\)) (IL-1), and interleukin-6 (IL-6) in plasma compared to the negative control group. Also, FSC significantly increased the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and the levels of glutathione (GSH) in plasma, liver and kidney whereas it decreased the levels of malondialdehyde (MDA) in plasma and kidney. Moreover, the protective effect of FSC (100 mg/kg bw) was better than vitamin C. These results revealed that FSC exerted a protective effect against diquat-induced oxidative stress and is worthy of becoming a potential dietary antioxidant.

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1. Introduction

Oxidative stress are caused by excessive oxidative radicals including reactive oxygen species (ROS) or reactive nitrogen species (RNS), which damage DNA, bio-membrane lipids, proteins, and other macromolecules. Further, oxidative stress is related to a number of aging-dependent pathogenic processes including cancers, arteriosclerosis, arthritis, neurodegenerative disorders and other diseases (Valko et al., 2006). However, excessive oxidative radicals can be eliminated by the use of an antioxidant system including non-enzymatic components and a series of antioxidant enzymes. Non-enzymatic components include glutathione (GSH), Se and some vitamins such as vitamin C and vitamin E; the antioxidant enzymes include superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which are the major antioxidant enzymes that are capable to minimize oxidative stress in the organelle (Cadenas and Davies, 2000). The degree of lipid peroxidation is often used as an indicator of ROS mediated damages (Kühn and Borchert, 2002) and the concentrations of malondialdehyde (MDA) in blood and tissues are generally used as biomarkers of radical-induced damage and the endogenous lipid peroxidation (Sehirli et al., 2007; Yousef et al., 2008). Some antioxidants are reported to have protective effects of diquat-induced oxidative stress, such as 1,3-bis(2-chloroethyl)-N-nitrosourea (BCNU) (Smith, 1987). In the present study, a proper oxidative stress inducer diquat (1,1-ethylene-2,2'-dipyridylum dibromide) was used and natural antioxidant *Forsythia suspensa* is supposed to help to maintain a proper oxidative status.

Diquat is a commonly used bipyridyl herbicide and potent photoproduct, which has been used as a model chemical for in vivo studies of oxidative stress (Gallagher et al., 1995). Diquat utilizes molecular oxygen to produce superoxide anion radical, and subsequently hydrogen peroxide. Further, it induces oxidative stress in animals to a greater extent compared to oxidized fish oil (Yuan et al., 2007). Some antioxidants are reported to have protective effects of diquat-induced oxidative stress, such as 1,3-bis(2-chloroethyl)-N-nitrosourea (BCNU) (Smith, 1987). In the present study, natural products of herbal origin are supposed to be use as dietary source in food and feed to reduce the severity of diquat-induced oxidative stress.

*Forsythia suspensa* Vahl (Oleaceae) is such an antioxidant source which is a climbing plant widely distributed in China, Japan and Korea. The extracts of the dried fruits have been used for a long time and the plant is considered as a natural medicine for various diseases (Cadenas and Davies, 2000). The extracts of the dried fruits have been used for a long time and the plant is considered as a natural medicine for various diseases (Cadenas and Davies, 2000). Forsythoside A, forsythoside A and philogenin from it might be the major antioxidant constituents. In vivo, pretreatment of rats with different doses of *Forsythia suspensa* extract (25, 50 and 100 mg/kg bw) and vitamin C (100 mg/kg bw, positive control) for 15 days significantly lowered the tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), interleukin-1(\(\beta\)) (IL-1), and interleukin-6 (IL-6) in plasma compared to the negative control group. Also, *Forsythia suspensa* significantly increased the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and the levels of glutathione (GSH) in plasma, liver and kidney whereas it decreased the levels of malondialdehyde (MDA) in plasma and kidney. Moreover, the protective effect of *Forsythia suspensa* extract against diquat-induced oxidative stress is worthy of becoming a potential dietary antioxidant.

**Abbreviations:** FSC, CH\(_2\)Cl\(_2\) fraction of *Forsythia suspensa* extract; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); IL-1, interleukin-1(\(\beta\)); IL-6, interleukin-6; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; i.p., intraperitoneal; bw, body weight.

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time as traditional Asian medicines to treat gonorrhea, erysipelas, inflammation and pharyngitis (Piao et al., 2008a). The major active components of *F. suspensa* are phenethyl alcohol glycoside, lignan, pentacyclic triterpenoids and volatile oil (Zhang, 2000). It was reported to exhibit potential antibacterial (Li et al., 2007; Liu et al., 2007; Niu et al., 2002), antiviral (Liu et al., 2004), and anti-inflammatory (Hu et al., 2007). In the recent years, numerous studies for its antioxidant activity were intensively conducted (Qu et al., 2008; Schinella et al., 2002; Piao et al., 2009, 2008b; Wang et al., 2008). Previous studies on this plant proved that phyllin and forsythoside, two of the major components, are responsible for the antioxidant activities of the herb (Qu et al., 2008). New monooxoxygenangens forsythialan A, forsythialan B and two known components, phylgenin and 8-hydroxyxypinosel, extracted from *F. suspensa* fruit, also showed their protective effects against peroxynitrite-induced oxidative stress in LLC-PK1 cells (Piao et al., 2008a). In addition, *F. suspensa* extract was able to reduce oxidative stress of broiler chickens under high ambient temperatures (Wang et al., 2008). The aim of this study was to obtain the optimal antioxidant fraction of *F. suspensa*, investigate its protective effect against diquat-induced oxidative stress in Sprague-Dawley rats, and provide theoretical basis for the development of *F. suspensa* extract as a dietary antioxidant in food and feed.

2. Materials and methods

2.1. Materials

Dried fruits of *F. suspensa* were purchased from Tong Ren Tang (Beijing, China; collected from Shanxi province, August). Diquat dibromide monohydrate was purchased from Chem Service (West Chester, PA) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other all chemicals were of analytical grade (SinoPharm Chemical Reagent Beijing Co., Ltd., Beijing, China).

2.2. Extraction and fractionation from *F. suspensa*

*F. suspensa* extract (FSE) was obtained as described in Li and Chen (2005). Briefly, ground, dried fruits of *F. suspensa* (500 g) were extracted with 2500 mL of 80% ethanol and sonicated for 1 h three times. Extracts were combined and concentrated under reduced temperature (45 °C and pressure), and freeze dried to give a powdered mass (62.2 g). The crude extract was then re-dissolved in water and further fractioned with CH2Cl2 three times and BuOH four times in succession. Fractions were dried by rotary vaporization (Buchi, Rotavapor R-124, Flawil, Switzerland; Piao et al., 2008b) and yielded CH2Cl2 fraction 26.4 g and BuOH fraction 21.8 g. The fractions were stored in a vacuum desiccator.

2.3. Activity-guided fractionation and isolation

An aliquot (20 g) of the CH2Cl2 fraction was divided into six fractions on silica gel column chromatography (Qingdao Chem, 200–400 mesh, 600 g) with using CHCl3–MeOH mixtures of increasing polarity (100:1 to 2:1). Repeated silica gel column chromatography of the fraction yielded four compounds. Based on their mass spectral, 1H NMR, and 13C NMR data, the chemical structures of the compounds were identified.

2.4. DPPH radical-scavenging activity

The level of oxidation was determined by spectrophotometry as described previously with minor modifications (Hatano et al., 1989). Solutions (100 μL) of the total extract of ethanol and fractions of BuOH, CH2Cl2, CH3OH, H2O, phylgenin, phylrin, forsythialan A and forsythoside A at different concentrations were prepared and then 100 μL of DPPH 120 μM in ethanol was added to each sample. Ethanin (100 μL) was used as control. After gentle mixing and standing 30 min at room temperature, the DPPH radical level was measured at 517 nm by a microplate reader (SPECTRAmax 340PC, Molecular Devices, Sunny Vale, CA, USA). The antioxidant activity was expressed as the inhibition rate of DPPH radicals or IC50 concentration in μg/mL required to inhibit DPPH radical formation by 50%.

The inhibition rate (%) was calculated as follows: \( \% = \left( \frac{A_0 - A_i}{A_0} \right) \times 100 \)

where \( A_0 \) is the absorbance of the control reaction and \( A_i \) is the absorbance of the test sample. IC50 of different components was determined from the log-dose inhibition curve.

2.5. Animals

Male Sprague Dawley rats (3–4 weeks, 72±1.5 g) obtained from Beijing Administration Office of Laboratory Animals (Beijing, China) were used for the study. Rats were divided into six equal groups of six and caged together at 23 °C with a 12 h light then 12 h dark schedule and provided standardized pellet fed (Beijing Laboratory Animal Research Center, Beijing) and clean drinking water *ad libitum*. All rats used in this study were humanely managed according to the China Department of Agriculture guidelines and the experimental protocol was approved by the China Agricultural University Animal Care and Use Committee.

2.6. Behavioral and toxic effects

Different groups of mice were orally treated with graded doses of the FSC (25, 50, 100, 200 and 400 mg/kg bw). One group was maintained as control, and was given 0.1% CMC (w/v) in distilled water. The level of oxidation was determined by spectrophotometry as described previously with minor modifications (Hatano et al., 1989). Solutions (100 μL) of the to-

2.7. Animal experimental design

After a week of acclimatization, male Sprague Dawley rats were divided to six in groups of six animals each. (1) CT: orally treated with equal volume saline, received saline, i.p. on d 15, serving as a normal control; (2) NC: orally treated with equal volume saline, received diquat, i.p. on d 15, serving as a negative control; (3) CL: orally treated with FSC at 25 mg/kg bw; (4) CM: orally treated with FSC at 50 mg/kg bw; (5) CH: orally treated with FSC at 100 mg/kg bw; and (6) PC: orally treated with vitamin C at 100 mg/kg bw. On d 15, all treatment groups, except CT, received 0.1 mmol/kg bw of diquat (i.p. (Smith, 1987) dissolved in normal saline. CT group received an equal dose of normal saline i.p. The different doses of CH2Cl2 fraction of *F. suspensa* were determined according to the Chinese Pharmacopoeia (2005): low dose (CL) was half the recommended dose, middle dose (CM) was the recommended dose, and high dose (CH) was two times the recommended dose. Blood was drawn from the posterior vena orbitalis 3 h after i.p., and the liver, kidney and ileum were immediately removed.

2.8. Plasma collection and tissue preparation

Blood samples were placed on ice immediately after collection. Plasma samples were obtained by centrifugation at 3000g for 10 min and stored at −20 °C until analysis. The same parts of liver, kidney and ileum of each rat were washed using chilled saline solution, packed with surgical pledget, and then frozen by immersion in liquid N2 and stored at −80 °C until analysis. Tissues were minced and homogenized (10% w/v) in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 0.86% NaCl. The homogenate was centrifuged at 3000g for 10 min at 4 °C; the resultant supernatant was used for the determination of enzyme activities.

2.9. Assay of TNF-α, IL-1β, and IL-6 biological activity in plasma

Plasma levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) were quantified according to the manufacturer’s instructions using enzyme-linked immunosorbent assay (ELISA) kits specific for the previously mentioned rat cytokines (R&D Systems China Co. Ltd., Shanghai, China).

2.10. Assay of antioxidant indices in plasma and tissue samples

Assay kits for the antioxidant index and protein were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Prepared sample supernatants and plasma were analyzed for the activities of antioxidant enzymes including SOD, GSH-Px, GSH and the indicators of lipid peroxidation including MDA, SOD, GSH-Px, MDA and GSH levels were assayed by a spectrophotometer (Leng Guang SFZ1606017568, Shanghai, China) using the following methods: SOD activity was measured by the xanthine oxidase method, which monitors the inhibition of nitro blue tetrazolium reduction by the sample (Sun et al., 1988); GSH-Px activity was detected with 5,5′-dithiobis-p-nitrobenzoic acid and the change in absorbance at 412 nm was measured (Hafeman et al., 1974); MDA level was analyzed with 2-thiobarbituric acid and the change in absorbance was read at 532 nm (Ohkawa et al., 1979); GSH can react with DTNB to produce oxidized glutathione GSSG and chromophore TNB which can be detected at 412 nm (Tietze, 1969). Quantitative estimation of cellular protein was made by the method of Lowry et al. (1951). Data were expressed as U/mg of protein for tissues and U/mL for plasma.

2.11. Statistics

Data were analyzed by ANOVA using the GLM procedures of SAS (v 8.2, SAS Inst., Inc., Cary, NC), according to Mao et al. (2005) and Yousef et al. (2009). Significant differences between treatment means were determined by using Duncan’s multiple-range tests. Linear and quadratic effects of FSC on all indices were deter-
mined according to the REG procedure of SAS. The initial statistical model included the level of FSC (0, 25, 50, or 100 mg/kg bw). All the values were represented as mean ± SE (n = 6) and those at p < 0.05 were considered significant.

3. Results

3.1. DPPH radical-scavenging activity

Effects of the total extract of ethanol and fractions of CH2Cl2, BuOH and H2O from F. suspensa on DPPH radical inhibition were shown in Fig. 1. The scavenging effect of total extract of ethanol, fractions of CH2Cl2, BuOH and H2O on the DPPH radical linearly increased (p < 0.05) in a dose-dependent manner. In particular, the CH2Cl2 fraction of F. suspensa (FSC) exerted the strongest inhibition effect on DPPH radical generation, showing an inhibition 46.7% at 64 µg/mL and 76% at 256 µg/mL.

3.2. Isolation and identification of the compounds and toxicity studies

The activity-guided fractionation and isolation method yielded four compounds. These compounds were identified as phillygenin (16.7 mg), phillyrin (81.7 mg), forsythialan A (41.3 mg) and forsythoside A (16.5 mg) (Fig. 2) based on their mass spectral, 1H NMR, and 13C NMR data (Rahman et al., 1990; Piao et al., 2008a; Ming et al., 1999). In acute toxicity, no mortality was observed up to a dose level of 400 mg/kg body weight.

3.3. Antioxidative effects of compounds isolated from FSC

Of all the compounds tested, forsythoside A, forsythialan A and phillygenin showed high scavenging properties with IC50 values of 10.43, 29.85 and 53.64 µg/mL, respectively (Table 1), whereas phillyrin’s IC50 value was over 300 µg/mL.

3.4. Assay of TNF-α, IL-1β and IL-6 biological activity of the plasma

The levels of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, were represented in Figs. 3–5. The level of TNF-α was increased (p < 0.01) in NC compared to CT and was reduced linearly (p < 0.01) in a dose-dependent manner in the FSC pre-treated groups. The level of TNF-α in CM was comparable to that of PC. Similarly, IL-1β and IL-6 were also increased (p < 0.01) in NC; however, when rats were treated with FSC, these cytokines were linearly decreased (p < 0.05) back to normal control level.
compared to CT. SOD increased \((p < 0.01)\) when different doses of FSC were pretreated compared to NC. Effects of the antioxidant activity in CM, as measured by SOD, GSH-Px, GSH and MDA, were comparable to those of PC.

Lower activities \((p < 0.05)\) of GSH-Px were observed in liver and kidney of rats in NC compared with CT and the levels of GSH-Px increased \((p < 0.05)\) in a linear dose-dependent manner. The levels of SOD did not decrease \((p > 0.05)\) in NC compared with CT, however, the levels linearly increased \((p < 0.05)\) when different doses of FSC were pretreated for 15 days. Similarly, the levels of GSH in both liver and kidney decreased \((p < 0.05)\) in NC and linearly increased \((p < 0.05)\) to normal levels in the kidney and a little higher in the liver \((p < 0.05)\). The MDA levels in liver did not differ among treatments whereas NC had greater \((p < 0.05)\) levels of MDA than CH and CM in kidney. Overall, the protective effects of 100 mg/kg bw of vitamin C were between the 100 mg/kg bw and 50 mg/kg bw of FSC in SOD, GSH-Px, GSH and MDA. The data of antioxidant indices in the ileum were not different (data not shown).

### 4. Discussion

The current data indicated that acute diquat exposure yielded oxidative status alterations in the liver and kidney with a concomitant increase in pro-inflammatory cytokines in the plasma. On the other hand, the optimal antioxidant fraction FSC reduced the severity of injury, depressed the concentration of these cytokines and increased the antioxidant capacity of the biological organism through both enzymatic system and non-enzymatic system.

First of all, determining disappearance of free radicals, such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH), is a rapid and stable method to assess the antioxidant activity of plant extracts \((\text{Samarth et al., 2007})\). In the study, the total extract and all the fractions showed their remarkable inhibitions of DPPH, while FSC exerted the strongest antioxidant activity (see Fig. 1). Several studies conducted using DPPH experimentation also suggested that the extract of \textit{F. suspensa} exhibited strong antioxidant activity \((\text{Wang et al., 2008; Qu et al., 2008})\).

Phenylethanoid glycosides like forsythoside A, forsythoside B, and lignans such as phillyrin, forsythialan A, forsythialan B, phillygenin, and 8-hydroxypinoresinol were reported as antioxidant components of \textit{F. suspensa} \((\text{Qu et al., 2008; Piao et al., 2008a})\). Qu et al. \((2008)\) utilized the DPPH radical to evaluate the antioxidant activity of the two monomers, phillyrin and forsythoside, and reported that forsythoside contributed 50% of the antioxidant activities of \textit{F. suspensa}. Piao et al. \((2008a)\) suggested that new monoepoxylignans forsythialan A, forsythialan B and two known components, phillygenin and 8-hydroxypinoresinol, which were isolated from the FSC, also showed a noticeable protective effect against peroxynitrite-induced oxidative stress in LLC-PK1 cells. In this study, we isolated phillygenin, forsythoside A, forsythialan A and phillyrin, three of which exerted a remarkable DPPH scavenging ability (Table 1). So the optimal antioxidant fraction FSC, which had the best radical scavenging capability, might have some correlation with the isolated constituents above. Also, they might contribute a lot to the protection of FSC.

In the \textit{in vivo} study, the bipyrindyl herbicide diquat was used as an oxidative inducer. Toxicity of diquat is a potent redox cyclers and is readily converted to a free radical, which when combined with molecular oxygen, generates superoxide anion and subsequently other redox products. These products can induce lipid peroxidation in cell membranes and potentially cause cell death \((\text{Jones and Vale, 2000})\). Previous studies have reported its toxicity in the liver, \((\text{Reif et al., 1988; Smith et al., 1985; Tsokos-Kuhn et al., 1988})\) kidney \((\text{Xu et al., 2007})\) and small intestine \((\text{Rawlings et al., 1994})\). Thus, in this study, these major targets of diquat were chosen to analyze...

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**Table 2** showed that SOD and GSH-Px, the two main antioxidant enzymes, decreased \((p < 0.05)\) in NC, whereas the MDA, a maker of free radical mediated lipid peroxidation injury, increased \((p < 0.05)\) compared to CT. SOD increased \((p < 0.01)\) and MDA decreased linearly \((p < 0.05)\) when different doses of FSC were pretreated compared to NC. Effects of the antioxidant activity in CM, as measured by SOD, GSH-Px, GSH and MDA, were comparable to those of PC.

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the body oxidative status. In addition, it was shown that mRNA expression for several drug-metabolizing and antioxidant enzymes in the liver, such as SOD, was altered after a single dose of diquat (0.1 mmol/kg bw, i.p.) to Sprague Dawley rats (Gallagher et al., 1995). In another study, hepatic activity of GSH was slightly increased 3 h after administrating a single dose of diquat to Sprague Dawley rats (Smith et al., 1985). In the present study, a single dose of diquat at 0.1 mmol/kg bw was chosen as a challenging oxidative inducer and the cytokines and the antioxidant indices were assayed 3 h after diquat treated. The significant changed data showed in plasma and organs indicated that a successful oxidative model was established.

When oxidative stress causes damage in organs, a variety of cytokines and enzymes located in the cytosol are released into the blood, thereby causing changes in cytokine and enzyme levels in the plasma. The assays of cytokines and enzymes in the plasma are useful quantitative indicators for the extent and types of organ damage. In the present study, diquat activated inflammatory cells, leading to the synthesis and release of certain pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 (Figs. 3–5). By analyzing the level of those inflammatory mediators in plasma, a significant increase was found which verified that diquat toxicity was closely related with inflammatory mechanisms when oxidative damage occurred. However, when animals were treated with FSC, these cytokine levels were comparatively lower than the NT group in a dose-dependent manner, which is likely to relate to the anti-inflammatory activity of F. suspensa previously reported (Hu et al., 2007; Ozaki et al., 1997). FSC suppressed the production of TNF-α, IL-1β, and IL-6 inflammatory cytokines, which suggested FSC had protective capabilities due to its anti-inflammatory activity.

### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD’ (U/mg)</th>
<th>GSH-Px’ (U/mg)</th>
<th>GSH (mg/L)</th>
<th>MDA’ (nmol/mL)</th>
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</thead>
<tbody>
<tr>
<td>CT</td>
<td>360.7 ± 6.7b</td>
<td>1461.9 ± 94.0a</td>
<td>33.4 ± 2.6m</td>
<td>5.19 ± 0.41b</td>
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<tr>
<td>NC</td>
<td>326.3 ± 11.1h</td>
<td>1461.9 ± 95.1a</td>
<td>33.7 ± 4.3h</td>
<td>5.17 ± 0.13a</td>
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<tr>
<td>CL</td>
<td>314.9 ± 8.3a</td>
<td>1300.0 ± 105.8a</td>
<td>28.4 ± 3.4a</td>
<td>5.30 ± 0.10ab</td>
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<tr>
<td>CM</td>
<td>361.2 ± 25.6</td>
<td>1347.6 ± 132.4a</td>
<td>33.9 ± 1.4a</td>
<td>5.20 ± 0.55b</td>
</tr>
<tr>
<td>CH</td>
<td>361.2 ± 9.9a</td>
<td>1240.4 ± 99.8a</td>
<td>32.3 ± 3.4a</td>
<td>4.37 ± 0.93a</td>
</tr>
<tr>
<td>PC</td>
<td>357.4 ± 15.5b</td>
<td>12350.0 ± 193.3b</td>
<td>33.1 ± 1.9b</td>
<td>5.39 ± 0.50ab</td>
</tr>
</tbody>
</table>

Mean values within a row lacking common superscript letters (a–d) differed (p < 0.05).

### Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD’ (U/mg protein)</th>
<th>GSH-Px’ (U/mg protein)</th>
<th>GSH (mg/g protein)</th>
<th>MDA’ (nmol/g protein)</th>
</tr>
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<tbody>
<tr>
<td>CT</td>
<td>62.3 ± 4.3a</td>
<td>824.6 ± 53.8a</td>
<td>3.2 ± 0.8a</td>
<td>1.28 ± 0.20</td>
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<tr>
<td>NC</td>
<td>53.4 ± 6.9a</td>
<td>651.8 ± 117.9a</td>
<td>1.6 ± 0.1a</td>
<td>1.54 ± 0.31</td>
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<tr>
<td>CL</td>
<td>50.6 ± 4.5b</td>
<td>607.2 ± 22.1b</td>
<td>2.1 ± 0.2c</td>
<td>1.49 ± 0.27</td>
</tr>
<tr>
<td>CM</td>
<td>62.2 ± 8.9a</td>
<td>787.3 ± 203.3a</td>
<td>4.3 ± 0.6b</td>
<td>1.40 ± 0.28</td>
</tr>
<tr>
<td>CH</td>
<td>63.5 ± 13.4a</td>
<td>837.8 ± 49.3a</td>
<td>5.8 ± 2.2a</td>
<td>1.19 ± 0.14</td>
</tr>
<tr>
<td>PC</td>
<td>60.7 ± 8.5b</td>
<td>811.2 ± 105.3a</td>
<td>2.2 ± 1.4d</td>
<td>1.44 ± 0.18</td>
</tr>
</tbody>
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CT: normal control; NC: negative control; CL: pretreatment with FSC at 25 mg/kg bw; CM: pretreatment with FSC at 50 mg/kg bw; CH: pretreatment with FSC at 100 mg/kg bw; PC: pretreatment with vitamin C at 100 mg/kg bw.

Values are expressed as means ± SE; n = 6 for each treatment group.

### Table 4

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Values are expressed as means ± SE; n = 6 for each treatment group.

Mean values within a row lacking common superscript letters (a–d) differed (p < 0.05).
depletion of GSH resulted in enhanced lipid peroxidation, and excessive lipid peroxidation caused increased GSH consumption (Yousef et al., 2009). The level of GSH reflected the non-enzymatic antioxidant defense system, so it suggested that FSC also maintained the redox balance of ROS through the enzymatic system as well as non-enzymatic antioxidant defense system.

The level of MDA, which is a major product of lipid peroxidation, approached the normal control in high dose of FSC pretreated animals exposed to diquat. Restoration to nearly normal levels of MDA by these extracts may be due to an enhancement of antioxidant enzymes, such as SOD and GSH-Px. In addition, effects of FSC on SOD and MDA were consistent with studies conducted by Hou and Yang (2006), which found that F. suspensa extract increased the heart SOD level and decreased the heart MDA level in mice after exhaustive exercise. Also, the result agreed with Wang et al. (2008), which stated that group treated with the extract of F. suspensa had greater muscle and hepatic SOD activity and lower MDA than the negative control group in broiler chickens under heat stress.

Generally, the protective effects of PC were not as good as CH, though vitamin C was reported to be a potential antioxidant in many studies. Likely, the dose of the vitamin was insufficient, since Xavier et al. (2007) mentioned a dose of 250 mg/kg bw that altered pilocarpine-induced oxidative stress in the brain of rats. Another reason might be that FSC has a multi-function activity to protect rats from oxidative stress, like anti-inflammatory as well as antioxidant activities.

All the antioxidant indices trends in the liver and kidney were almost the same as in the plasma, while no significant change occurred in the ileum. This suggested that the liver and kidney were more sensitive than the ileum in the model of diquat-induced oxidative stress.

In conclusion, FSC had the optimal antioxidant activities in a dose-dependent manner when measured by DPPH scavenging experiment and reduced diquat-induced oxidative stress towards normalization. Contents of the extract not only had anti-inflammatory activity but also activated the antioxidant enzyme system and enhanced the non-enzymatic system to restore the impaired balance of ROS induced by diquat. The beneficial effect of FSC might be due to the presence of glycoside forsythoside A, which exhibited remarkable antioxidant activities. These results suggested that the compounds present in the plant extract efficiently worked on the liver and kidney to keep them functioning normally and could be developed as a potential natural antioxidant in food and feed.

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