Hepatoprotective Effect of the Aqueous Extract of *Flemingia macrophylla* on Carbon Tetrachloride-Induced Acute Hepatotoxicity in Rats Through Anti-Oxidative Activities

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Abstract: This study investigated the protective effect of the aqueous extract of *Flemingia macrophylla* (AFM) against hepatic injury induced by CCl₄. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected as biomarkers in the blood to indicate hepatic injury. Product of lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and reduced glutathione (GSH) contents were evaluated for oxidative stress in hepatic injury. Moreover, histopathological observation
was assayed for the degree of hepatic injury. After oral administration of AFM, 0.5 g/kg and 1.0 g/kg doses significantly decreased ALT and AST, attenuated the histopathology of hepatic injury, ameliorated oxidative stress in hepatic tissue, and increased the activities of CAT, SOD and GSH-Px. The hepatoprotective effect of daidzein and genistein were consistent to that of AFM. This study demonstrated for the first time that AFM has hepatoprotective effect on acute liver injuries induced by CCl$_4$, and the results suggested that the effect of AFM against CCl$_4$-induced liver damage was related to antioxidant properties.

**Keywords:** Flemingia macrophylla; Carbon Tetrachloride; Hepatotoxicity; Nitric Oxide; Tumor Necrosis Factor-α.

**Introduction**

Reactive oxygen species (ROS) including oxygen free radicals are causative factors of degenerative diseases, including some hepatopathies (Poli, 1993). The enhanced production of free radicals and oxidative stress can be induced by a variety of factors, such as ionizing radiation, and exposure to drugs or xenobiotics (e.g., carbon tetrachloride). CCl$_4$, an analog of human hepatotoxin, has been used extensively in animal models to induce liver damage. Liver damage caused by CCl$_4$ is characterized by inflammation in the early stage. In damaged hepatocytes, CCl$_4$ is reductively bioactivated by cytochrome P450 2E1 into a trichloromethyl radical (-CCl$_3$), which is subsequently converted into a peroxyl radical (-OCCl$_3$) in the presence of oxygen. These reactive free radical metabolites can covalently bind to macromolecules and initiate lipid peroxidation (Recknagel et al., 1989; Goeptar et al., 1995). Antioxidant action plays an important role by which various natural products protect against CCl$_4$-induced liver damage (Halim et al., 1997).

CCl$_4$ toxicity is thought to be mediated by at least two sequential processes. The first involves cytochrome P450-mediated metabolism of CCl$_4$ to a highly reactive trichloromethyl radical, which initiates lipid peroxidation and leads to hepatocellular membrane damage (Sipes et al., 1974). It is followed by the release of inflammatory mediators from activated hepatic macrophages, which are thought to potentiate CCl$_4$-induced hepatic damage (Badger et al., 1996). Macrophages release a number of inflammatory mediators with cytotoxic potentials. Two mediators of interest are tumor necrosis factor-α (TNF-α) and nitric oxide (NO). TNF-α is unique among cytokines in that it can also induce cytotoxicity directly and has been implicated in apoptosis (Wang and Liu, 1995). NO is a highly reactive oxidant, produced by parenchymal and nonparenchymal liver cells from L-arginine via the action of inducible nitric oxide synthase (NOS II). When released by macrophages against infectious agents, NO has been shown to inhibit mitochondrial respiration and DNA synthesis (Nathan, 1992). Macrophages and inflammatory mediators, including TNF-α and nitric oxide, have been implicated in liver damage induced by a number of different toxicants (Laskin and Pendino, 1995).

The Flemingia genus, known as ‘I-Tiao-Gung’ in Chinese, is distributed in tropical areas. The traditional usages of the roots of Flemingia species have been for the treatment of rheumatism, arthropathy, leucorrhrea, menalgia, menopausal syndrome, chronic nephritis,
and improvement of bone mineral density (Li et al., 2008). Only few studies have confirmed the pharmacological activity of members in the Flemingia genus. For example, it was reported that the root extract of *F. philippinensis* (*F. prostrata* (FP)) exhibited antioxidative, anti-inflammatory, estrogenic, and anti-estrogenic activities (Li et al., 2008). The stem of *F. macrophylla* has been used in traditional medicine as an antirheumatic and anti-inflammatory agent and for improving blood circulation. Furthermore, its flavonoids have inhibitory effects on Aβ-induced neurotoxicity (Shiao et al., 2005). In our previous studies, FM had both antioxidant and antidiabetic activities (Hsieh et al., 2010). The objective of this study was to better understand the hepatoprotective effect of FM on CCl₄-induced rat liver damage.

**Materials and Methods**

**Chemicals**

CCl₄, silymarin, olive oil, and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutathione (GSH) were purchased from Randox Laboratory Ltd. TNF-α and IL-1β concentrations were quantified using a commercial ELISA kit (Biosource International Inc., Camarillo, CA, USA). Daidzin, daidzein, genistin, and genistein were purchased from Sigma Chemicals Co. (USA).

**Plant Material**

Plant materials were collected from Taichung County, Taiwan. They were identified and authenticated by Dr. Chao-Lin Kuo, Associate Professor and Chairman, Department of Chinese Medicine Resources, China Medical University, Taichung, Taiwan.

**Preparing the Aqueous Extract**

Dried herb roots were boiled with one liter distilled water for one hour. Filtrate and collection of the extracts were done three times. The filtrate was concentrated to powder by a freeze dryer (Christ Alpha, Germany) and stored at −20°C.

**Compositional Analysis of AFM by HPLC**

HPLC was performed with a Hitachi Liquid Chromatography (Hitachi Ltd., Tokyo, Japan), consisting of two L-7100 model pumps, and one L-7455 model photodiode array detector (254 nm). AFM extracts were filtered through No. 1 filter-paper. The filtrate was diluted to 100 μl with 70% methanol. This solution was then passed through a 0.45 μm PVDF-filter and the filtrate was injected into an HPLC (10 μl) equipped with a Mightysil RP-18 GP column (5 μm, 250 × 4.6 mm I.D.). The method involved the use of a binary gradient with mobile phases containing: (A) phosphoric acid in water (0.1%, v/v), and (B) H₂O/CH₃CN: 20/80 (v/v). The solvent gradient elution program was as follows: 0–15 min, 100–95% A,
0–5% B; 15–20 min, 95–85% A, 5–15% B; 20–30 min, 85–45% A, 15–55% B; 30–45 min, 45–25% A, 55–75% B; 45–50 min, 25–0% A, 75–100% B; and finally 50–60 min, 0% A, 100% B. The flow-rate was kept constant at 0.8 ml/min. A precolumn of \( \mu \)-Bondapak\textsuperscript{TM} C\textsubscript{18} (Millipore, Milford, MA, USA) was attached to protect the analytical column.

**Animals**

Male SD rats, aged six to eight weeks and weighing 180–200 g, were selected for the study. They were maintained at a controlled temperature of 25–28°C with 12 h light/dark cycles and fed a standard diet and water ad libitum. Animal studies were conducted according to the regulations of the Institute Animal Ethics Committee and the protocol was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Rats were divided into six groups of eight animals each. Rats in the normal control and negative control were orally administered with distilled water. The positive control was orally administered with silymarin (25 mg/kg in 1% carboxymethyl cellulose) once daily for seven days. In the three experimental groups, the rats were pretreated orally with AFM (0.1 g/kg, 0.5 g/kg, and 1.0 g/kg) once daily for seven consecutive days. One hour after the last treatment, all the rats, except those in the normal control, were treated with CCl\textsubscript{4} (1.5 ml/kg in olive oil, 20%, ip). 24 h after the CCl\textsubscript{4} treatment, animals were anesthetized with ethyl ether, and blood samples were collected through their carotid arteries. The mortality rate and body weight were recorded daily.

**Histopathology**

Small pieces of liver, fixed in 10% buffered formalin were processed for embedment in paraffin. Sections of 5–6 \( \mu \)m were cut and stained with hematoxylin and eosin before they were examined for histopathological changes under the microscope (Nikon, ECLIPSE, TS100, Japan). Images were taken with a digital camera (NIS-Elements D 2.30, SP4, Build 387) at original magnification of \( \times 200 \).

**Antioxidant Enzyme Activities**

The following biochemical parameters were analyzed to detect the hepatoprotective activity of AFM. SOD activity was determined by monitoring the inhibition of cytochrome \( c \) reduction at 550 nm using xanthine and a xanthine oxidase system. One SOD unit was defined as the amount of enzyme required to inhibit cytochrome \( c \) reduction by 50% (Lee and Yu, 1990).

Catalase activity was measured by the method of Aebi (1984). A reaction mixture (2 ml), which contained 0.01 ml of liver homogenate, 0.09 ml of 1% Triton X-100 and 1.9 ml of 20 mM phosphate buffer, was added to a crystal cuvette containing 1 ml of 0.03 M H\textsubscript{2}O\textsubscript{2}. The change in the absorbance after 1 min was read at 240 nm. Using the reaction time interval (\( \Delta t \)) of absorbance (\( A_1 \) and \( A_2 \)), the rate constant (K) was calculated using
the equation $K = (2.3/\Delta t) \log (A1/A2)$. The specific activity of the enzyme was expressed as K/mg protein.

GSH-Px activity was measured according to the method of Paglia and Valentine (1967). The liver supernatant was added to the reaction mixture comprised of 1 mM EDTA, 1 unit of glutathione reductase, 1 mM glutathione, 0.25 mM H$_2$O$_2$ and 1 mM sodium azide in 50 mM phosphate buffer (pH 7.0). The reaction was initiated by the addition of 0.2 mM NADPH, and GSH-Px activity was defined as the amount required to oxidize 1 $\mu$M of NADPH in one min. Resulting values of GSH-Px activity were expressed as U/mg protein in each supernatant.

**Determination of GSH**

Hepatic GSH level was determined as described previously (Ellman, 1959) with slight modifications. Briefly, 720 $\mu$l of liver homogenate in 200 mM Tris buffer (pH 7.2) was diluted to 1440 $\mu$l with the same buffer. Five percent TCA (160 $\mu$l) was added and mixed thoroughly. The samples were then centrifuged at 10,000 $\times$ g for 5 min at 4°C. Ellman’s reagent (DTNB solution) (660 $\mu$l) was added to the supernatant (330 $\mu$l). Finally the absorbance was taken at 405 nm.

**Determination of Hepatic Lipid Peroxidation**

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reactive substances (TBARS) as previously described (Uchiyama and Mihara, 1978). Briefly, 1 g of liver was homogenized in 10 ml of KCl 1.15 % (w/v), and the homogenate was filtered through four folded-gauze. 0.5 ml of liver homogenate was mixed with 3 ml of H$_3$PO$_4$ 1% (v/v) and 1 ml of TBA 0.6% (w/v), and then heated to and maintained at 100°C for 45 min. The samples were allowed to cool down to room temperature and 3 ml of $n$-butanol was added. After shaking vigorously with the vortex, the butanolic phase was obtained by centrifugation at 4000 $\times$ g for 10 min to determine the absorbance at 535 nm. The standard was 1, 1, 1, 3-tetraethoxypropane.

**Determination of Nitric Oxide (NO)**

The production of NO was assessed indirectly by measuring the nitrite levels in the plasma by a calorimetric method based on the Griess reaction (Green et al., 1982). Plasma samples were diluted four times with distilled water and deproteinized by adding 1/20 volume of zinc sulfate (300 g/L) to a final concentration of 15 g/L. After centrifugation at 10,000 $\times$ g for 5 min at room temperature, 100 $\mu$l supernatant was added to a microliter plate well, followed by 100 $\mu$l of Griess reagent (1% sulfanilamide and 0.1% N-1-naphthylethlenediamine dihydro-chloride in 2.5% polyphosphoric acid). After 10 min of color development at room temperature, the absorbance was measured at 540 nm with a Micro Reader (Hyperion, Inc., FL, USA). Nitrite was quantified by using sodium nitrate as the standard curve.
Measurement of Serum TNF-α and IL-1β

The serum level of TNF-α and IL-1β were determined using a commercially available enzyme linked immunosorbent assay (ELISA) kit (Biosource International Inc., Camarillo, CA, USA) according to the manufacturer’s instructions. TNF-α and IL-1β were determined from a standard curve. The concentrations were expressed in pg/ml.

Statistical Analysis

Results were expressed as mean ± S.E.M. and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey post-hoc analysis. A p value less than or equal to 0.05 was considered significant.

Results

Compositional Analysis of AFM by HPLC

Four marker compounds were selected for HPLC chromatographic fingerprint analysis of AFM as shown in Fig. 1, with the markers identified as daidzin (retention time, 27.3 min), daidzein (43.5 min), genistin (35.8 min) and genistein (48.7 min) (Butyl p-hydroxybenzoate is internal standard (IS)). Based on the plots of the peak-area (y) vs. concentration (x, μg/ml), the regression equations of the three phenolic constituents and their correlation coefficients ($r^2$) were as follows: daidzin, $y = 0.0509x + 0.2852$ ($r^2 = 0.9981$); daidzein,
Activities of ALT and AST in Serum

The serum activities of ALT and AST were used as biochemical markers for the early acute hepatic damage. The effect of oral administration of AFM on serum AST and ALT levels is as shown in Fig. 2. AFM at the dose of 1.0 g/kg significantly reduced serum AST and ALT levels in liver damaged rats (p < 0.001). The standard drug, silymarin at dose of 10 mg/kg, also had similar effects. It was confirmed that AFM could ameliorate hepatic function in CCl₄ induced liver injury.

Effect of AFM on Liver Histology

The histological features of the livers from the control and experimental groups are as shown in Fig. 3. Figure 3A shows the hepatic cell structure of the control and is a representation of normal liver lobular architecture, which has no pathological changes and with central vein and radiating hepatic cords. Figure 3B shows multiple and extensive areas of portal inflammation and hepatocellular necrosis randomly distributed throughout the parenchyma, as well as a moderate increase in inflammatory cell infiltration. Changes were improved in AFM and silymarin pretreated rats, which exhibited areas of normal liver architecture and patches of necrotic hepatocytes (Figs. 3C–3F).

Effect on CAT, SOD, and GSH-Px Activities in CCl₄-Induced Hepatic Injury

CAT is a key component of the antioxidant defense system. Inhibition of this protective system results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. CAT activities in total liver homogenates were shown in Fig. 4A. CAT activity in liver homogenate of the CCl₄ group (3.46 ± 0.16 U/mg protein) was conspicuously lower than that of the control group (5.18 ± 0.21 U/mg protein). CAT activities of liver homogenates from the 0.5 g/kg (3.70 ± 0.17 U/mg protein, p < 0.05) and 1.0 g/kg (4.36 ± 0.29 U/mg protein, p < 0.01) AFM groups were significantly higher than that of the CCl₄ group. In this study, CAT was increased by the administration of AFM, suggesting that it could restore CAT enzyme activity.

SOD plays an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in hepatic tissues. SOD activity of the liver homogenate in the CCl₄ group (9.97 ± 0.46 U/mg protein) was lower than that of the control group (14.83 ± 0.63 U/mg protein). The SOD activities in liver homogenates of the groups treated with 0.1 g/kg (11.40 ± 0.51 U/mg protein, p < 0.05), 0.5 g/kg (12.48 ± 0.83 U/mg protein, p < 0.01), and 1.0 g/kg (13.47 ± 0.87 U/mg protein, p < 0.001) of AFM were significantly higher than that
Figure 2. Effect of AFM on the activities of serum AST (A) and ALT (B) in CCl₄-treated rats after 24 h of treatment. The rats were pre-treated with AFM (0.1, 0.5, and 1.0 g/kg) once daily for seven consecutive days. Three hours after the final treatment, the rats were treated with CCl₄ (1.5 ml/kg, ip) and then killed 24 h later. Hepatotoxicity was determined 24 h later by quantifying the serum activities of AST and ALT. Each value is the mean ± S.E.M. *p < 0.05, compared to the control group. **p < 0.05, ***p < 0.01, and ****p < 0.001 compared to the CCl₄ group.
Figure 3. The effect of *Flemingia macrophylla* on CCl₄-induced liver damage. The rats were pretreated with AFM (0.1, 0.5, and 1.0 g/kg) once daily for seven consecutive days. Three hours after the final treatment, the rats were treated with CCl₄ (0.5 ml/kg, ip) and then killed 24 h later. In turn, their livers were removed, fixed and embedded in paraffin. Sections were stained with hematoxylin-eosin (×100). (A) normal control; (B) received CCl₄ (1.5 ml/kg); (C) silymarin (200 mg/kg) + CCl₄ (1.5 ml/kg); (D) AFM (0.1 g/kg) + CCl₄ (1.5 ml/kg); (E) AFM (0.5 g/kg) + CCl₄ (1.5 ml/kg); and (F) AFM (1 g/kg) + CCl₄ (1.5 ml/kg) (200×).
Figure 4. Effect of AFM on antioxidant enzyme activities of CCl$_4$-treated rats. Activities of SOD, superoxide dismutase, CAT, catalase and GPx, glutathione peroxidase are shown in the figure. The values are mean ± S.E.M. done in triplicates. *p < 0.05, compared to the control group. **p < 0.01 and ***p < 0.001 compared to the CCl$_4$ group.
of the CCl₄ group (Fig. 4B). The observed increase in SOD activity suggested that AFM had an efficient protective mechanism in response to ROS.

Figure 4C shows the GSH-Px activity in the liver homogenate of CCl₄-treated rats after continual administration with different doses of AFM and 10 mg/kg silymarin. GSH-Px activity in the liver homogenate from the CCl₄ group (3.02 ± 0.20 U/mg protein) was lower than that of the control group (4.78 ± 0.31 U/mg protein). GSH-Px activities in liver homogenates from the experimental groups pretreated with 0.1 g/kg (3.86 ± 0.17 U/mg protein, p < 0.01), 0.5 g/kg (4.43 ± 0.21 U/mg protein, p < 0.001), and 1.0 g/kg (4.76 ± 0.29 U/mg protein, p < 0.001) AFM were significantly higher than that of the CCl₄ group.

**Effect on the GSH Levels in CCl₄-Treated Rats**

GSH is an intracellular reductant and plays major roles in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. Significant depletion of GSH was detected in CCl₄-treated rats (10.85 ± 0.63 U/mg protein in the control vs. 5.60 ± 0.21 U/mg protein in the CCl₄ group). Furthermore, 0.5 g/kg and 1.0 g/kg of AFM significantly ameliorated CCl₄-induced depletion of GSH in the hepatic tissue (7.52 ± 0.49 U/mg protein in the 0.5 g/kg AFM group and 8.31 ± 0.91 U/mg protein in the 1.0 g/kg AFM group, with p < 0.05 and p < 0.01 respectively when compared to the CCl₄ group, Fig. 5).
Effect on Hepatic TBARS Levels

CCl₄ caused a marked lipid peroxidation in hepatic tissue. The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver homogenate, is as shown in Fig. 6. MDA content in the liver total homogenate was dramatically increased in the CCl₄ group (2.33 ± 0.16) as compared to the control group (0.69 ± 0.08). MDA level was significantly inhibited in 0.1 g/kg (0.44 ± 0.11, p < 0.05), 0.5 g/kg (1.34 ± 0.18, p < 0.01) and 1.0 g/kg (0.85 ± 0.07, p < 0.001) of AFM-treated groups.

Effect on Serum NO, TNF-α, and IL-1β Levels

CCl₄ induced hepatotoxicity was associated with marked increase in the level of NO, TNF-α and IL-1β. As shown in Fig. 7A, the production of NO in the plasma was significantly increased in CCl₄-treated rats as compared to the normal control group (4.04 ± 0.35 μM vs. 2.45 ± 0.14 μM). However, pre-treatment of silymarin and AFM decreased NO production in CCl₄-treated rats. NO level was significantly inhibited in the groups pretreated with 0.5 g/kg (3.27 ± 0.23 μM, p < 0.01) and 1.0 g/kg (2.64 ± 0.33 μM, p < 0.001) of AFM.

The production of TNF-α and IL-1β in the serum was significantly increased in CCl₄-treated rats (132.47 ± 1.46 pg/ml and 377.79 ± 34.80 pg/ml) as compared to the normal control group (82.23 ± 11.20 pg/ml and 145.02 ± 18.93 pg/ml). At the dose of 0.1 g/kg, AFM produced significant decreases in TNF-α and IL-1β levels, as shown in Figs. 7B and 7C. Furthermore, treatment with AFM (0.5 and 1.0 g/kg) over seven days, similar to

Figure 5. Effect of AFM on glutathione contents in CCl₄-treated rats. The values are mean ± S.E.M. done in triplicates. *p < 0.05, compared to the control group. **p < 0.01 and ***p < 0.001 compared to the CCl₄ group.
Figure 6. Effect of AFM on TBARS formation in CCl₄-treated rats. The values are mean ± S.E.M. done in triplicates. *p < 0.05, compared to the normal control group. **p < 0.01, and ***p < 0.001 compared to the negative group.

Figure 7. Effect of AFM on (A) NO production; (B) TNF-α; and (C) IL-1β concentrations in CCl₄-treated rats. The values are mean ± S.E.M. in triplicates. #p < 0.05, compared with the normal control group. *p < 0.05, **p < 0.01, and ***p < 0.001 compared with the negative group.
silymarin, produced more pronounced ($p < 0.001$) dose-dependent decreases in the levels of TNF-α and IL-1β.

**Discussion**

Liver injury induced by CCl₄ is a classical system of xenobiotic-induced hepatotoxicity and has been used extensively for decades for the screening of antihepatotoxic/hepatoprotective
activities of different drugs (Alqasoumi, 2010). Oxidative stress has been postulated as a major molecular mechanism involved in experimental animal models. In the present study, we have evaluated the hepatoprotective effect of AFM against CCl₄ induced acute hepatotoxicity in rats. The consistency of chemical composition in AFM is important in safe guarding the reliability of the research results. The chemical profile of AFM was recorded by HPLC analysis. The HPLC chemical profile could be delineated by the measurement of relative retention times of major characteristic peaks using genistein and daidzein as markers. The resulting chromatogram was used as the standard for the assessment of all extracts used in the present study. Genistein has anti-inflammatory effects on experimental liver damage caused by CCl₄. Genistein reduces liver damage by preventing lipid peroxidation and strengthening antioxidant systems (Kuzu et al., 2007).

It is well known that CCl₄ is activated by the cytochrome P450 system. The initial metabolite is the trichloromethyl free radical, which is believed to stimulate the biochemical events that ultimately culminate in liver cell necrosis (Lin et al., 2000). In response to hepatocellular injury initiated by the biotransformation of CCl₄ into reactive radicals, “activated” Kupffer cells respond by releasing increased amount of active oxygen species and other bioactive agents (Yam et al., 2007). CCl₄-induced generation of peroxyl and superoxide radicals result in the inactivation of CAT and SOD. Our results also showed that CCl₄ significantly decreased the activities of CAT, SOD and GSH-Px in the liver. Cells have a number of self-protecting mechanisms against the toxic effects of ROS, including free radical scavengers and chain reaction terminators such as SOD, CAT, and GPx systems. SOD removes superoxide radicals by converting them into H₂O₂, which in turn, can be rapidly converted into water by CAT and GPx. However, such protective effect of AFM against NO has not been elucidated and may be related to its antioxidant properties. Cellular injury occurs when ROS generation exceeds the cellular capacity of removal (Tang et al., 2010).

Oxidative stress causes depletion of intracellular GSH, leading to serious consequences (Ha et al., 2005). AFM administration inhibited lipid peroxidation at higher levels after CCl₄ treatment. Interestingly, 0.5 g/kg and 1.0 g/kg of AFM were capable of increasing the activity of endogenous antioxidant enzymes (SOD, CAT, and GSH-Px) and the level of GSH in hepatic tissue. AFM pretreatment was demonstrated to inhibit MDA from producing reactive oxygen radicals. Anti-inflammatory effect has also been shown in the hepatoprotective agent, silymarin.

The pro-inflammatory cytokine, TNF-α, has been reported to play a key role in the pathogenesis of various liver diseases. Following its release from activated Kupffer cells, TNF-α aggravates both oxidative stress and inflammatory responses in the liver (Nagata et al., 2007). The key role of TNF-α in CCl₄ induced liver damage has also been substantiated in an earlier study where treatment with soluble TNF-α receptors prevented liver injury and decreased mortality in rats. TNF-α has also been shown to increase the release of reactive oxygen intermediates and to augment lipid peroxidation in cultured rat hepatocytes (Roome et al., 2008). AFM not only inhibited the release of inflammatory mediators NO, TNF-α, and IL-1, based on the findings of our study, the hepatoprotective effect of AFM could also be attributed to its anti-inflammatory properties.
These results have provided evidence for the pharmacological effect of AFM on CCl₄-induced hepatotoxicity. Overall, AFM not only provided maximum conjugation with injurious free radicals and diminished their toxic properties, but also suppressed the inflammatory responses in CCl₄-induced liver injury. Further studies will be required to fully understand the association between CCl₄ induced oxidative stress and inflammatory responses in the liver with the hepatoprotective effect of AFM.

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