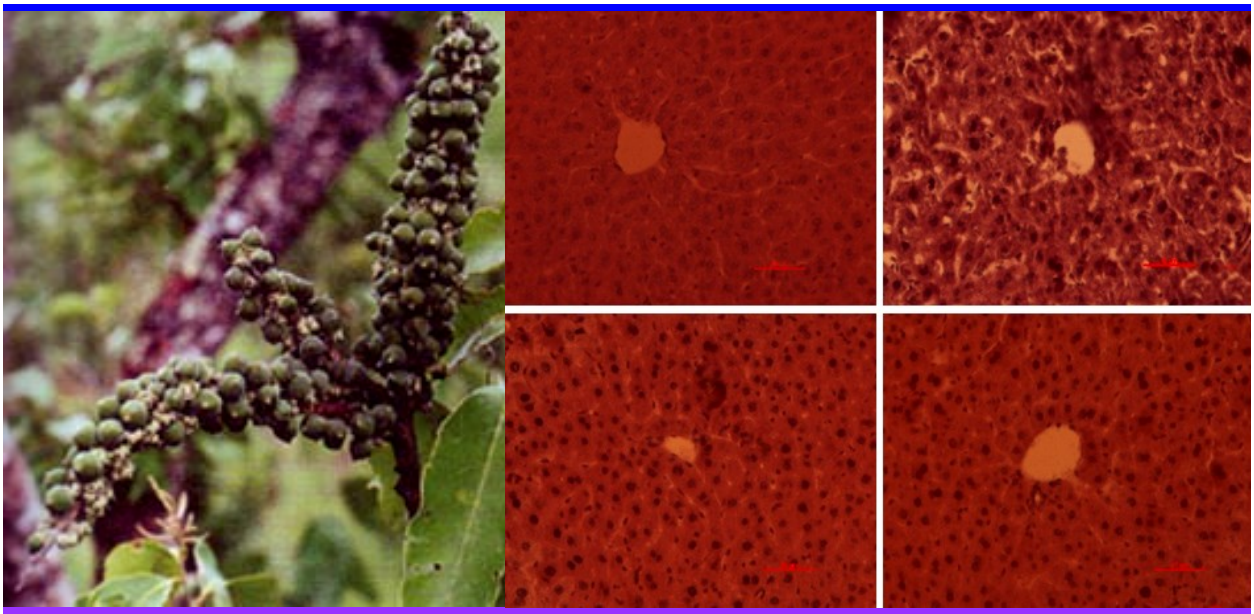

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Anti-oxidant and hepatoprotective effects of *Lithocarpus polystachyus* against carbon tetrachloride-induced injuries in rat

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Abstract

The present study aims to investigate the hepatoprotective and anti-oxidant effects of the total flavonoid of *Lithocarpus polystachyus* Rehd.(LP-F) *in vitro* and *in vivo*. The *in vitro* anti-oxidant property of total flavonoids was investigated by employing various established systems. Rats with carbon tetrachloride-induced liver injury were used to assess the hepatoprotective and anti-oxidant effect of total flavonoids *in vivo*. The level of activity of glutamate pyruvate transaminase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase, total bilirubin, total cholesterol, triglycerides total protein and albumin contents in the serum and malondialdehyde, superoxide dismutase, catalase, and glutathione in the liver and kidney of the rats were assayed using standard procedures. The results showed the total flavonoids of *L. polystachyus* has strong hepatoprotective and anti-oxidant activity *in vitro* and *in vivo*. These data were supplemented with histopathological studies of rat liver sections. This suggests that the hepatoprotective activity of formulation is possibly attributed to its free radical scavenging properties.

Introduction

The liver is a vital organ and has many important functions, including metabolism and detoxification of hepatotoxicants (Altasey al., 2011). In most cases, liver damage is a widespread pathology which involve oxidative stress and a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Srivastava and Shivanandappa, 2010). Many people die every year due to liver anomalies. Among them, most common are cirrhosis, cholestasis, hepatitis, portal hypertension, hepatic encephalopathy, hepatic failure and certain tumors like hepatoma.

As one of the aspects of the body's natural ecosystem, it is being realized that the majority of the diseases/ disorders are mainly due to the hyper physiological burden of free radicals, causes imbalance in homeostatic phenomena between oxidants and anti-oxidants in the body. CCl_4 is a classical hepatotoxin leading to hepatic diseases, and is generally used in animal models for inducing acute liver injury (Weber, Boll and Stampfl, 2003). Reductive dehalogenation of CCl_4, by the P_{450} enzyme system in the endoplasmic reticulum, into highly reactive trichloromethyl-free radicals ($^{\cdot}CCl_3$ or $CCl_3OO^{\cdot}$) initiates the process of lipid peroxidation. This is considered to be the most important mechanism in the pathogenesis of liver damage induced by CCl_4 (Altas et al., 2011). CCl_4-induced damage is also able to change the anti-oxidant status of the tissues, which is

manifested by abnormal histopathological alteration with steatosis, centrilobular necrosis and cirrhosis in the liver (Xu et al., 2010).

Hepatoprotective and anti-oxidant effects have been investigated recently in different plant extracts due to their potent antioxidant activities, such as *Carum copticum* (Gilani et al., 2005), *Trianthema decandra* (Balamurugan and Muthusamy, 2008), *Anogeissus leiocarpus* (Atawodi et al., 2011), *Impatiens bicolor* (Nisar et al., 2012); *Meconopsis integrifolia* (Maxim.) Franch (Zhou et al., 2013), Huangshan Maofeng (Lu et al., 2013) and *Litchi chinensis* (Basu et al., 2012). However, many other plants, including *Lithocarpus polystachyus* have not been studied for such effects.

L. polystachyus of family Fagaceae, is a shrub distributed widely in the mountainous area in southern China (Institute of Botany, 1972). Its tender leaves have been a traditional Chinese herb and been taken as a sweet tonic drink for several hundreds of years (China Pharmacopoeia Committee, 1999). In the course of seeking the sweet components in the leaves, the sweet compounds were identified phlorizin, phloretin, quercetin, dihydrochalcone-2'-β-D-glucopyranoside, luteolin, and quercitrin (Li et al., 2010), and their content in the leaves was up to around 10.6% (Li et al., 2008), *L. polystachyus* is claimed to possess multiple bioactivities, including hepatoprotective, anti-hypertensive, antioxidant, vasodilative, cerebrovascular, cardiovascular, hypoglycemic, and antiobesity activities (Dong et al., 2012; Hou et al., 2012; Liao et al., 1994; Yang et al., 2007). According to the Dictionary of Chinese Traditional Drugs, hepatoprotective activity is the major pharmacological effect of *L. polystachyus* but no scientific data has been reported for the hepatoprotective effect of this medicinal plant so far.

The present study was carried out to investigate the hepatoprotective effect and a possible underlying antioxidant activity of indifferent extracts of the leaves of *L. polystachyus* by assaying various marker enzymes, antioxidant enzymes in carbon tetrachloride-induced rats.

Materials and Methods

Plant materials

L. polystachyus was collected from the Huaihua county of the western region of Huna province, China in March, 2010 and authenticated by Prof. Xianjin Wu, Department of Bioscience, Huaihua University, where a voucher specimen has been deposited. The plant material was air dried indoor at room temperature before extraction.

Experimental animal

Male Wistar rats (220 ± 4.5 g) purchased from the animal house of Xiangya School of Medicine of Central South University. They were kept in departmental animal house in an environment-tally control room (25 ± 2°C), relative humidity 50 ± 5% RH and with 12 hours light and 12 hours darkness cycle. All the animals were free to standard laboratory feed and tap water before experiment. All 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.

Determination and preparation of total flavonoids of L. polystachyus

The extract from *L. polystachyus* was obtain-ed by ultrasound-assisted extraction with 70%ethanol for 45 min (Li et al., 2008), and then filtrated through filter paper The filtrate was evaporated till dryness under reduced pressure weighed, the yield of the extract 15.2%. The extract was solved with hot water, added into column loading-treated AB-8 macroreticular resin for adsorption for 12 hours, and then washed with 20% ethanol respectively for getting rid of impurity. LP-F in the column was eluted with 60%ethanol, eluting solution was evaporated till dryness under reduced pressure and weighed, the yield of total flavonoids of *L. polystachyus* 8.7%.

Preparation of standard curve: A calibration rutin solution containing 200.8 μg/mL rutin was prepared with 70%ethanol. In all 0, 1, 2, 3, 4, 5 and 6 mL of calibration rutin solution was respectively added to seven 25 mL volumetric flasks; to each was added 6 mL of 70%ethanol and 1 mL 5%$NaNO_2$. Each flask was shaken fully, mixed and then stand still for 6 min; each flask was added 1 mL 10%$AlCl_3$ solution, shake them fully again, mixed and stand still 6 min subsequently, 10 mL 10%NaOH solution was added into each volumetric flask and dilution to the mark with 70% ethanol, shaken fully, mixed and then let it rest for 15 min, Its absorbency (A) was measured at 500 nm, 70% ethanol as a blank control.

One mL of above the extraction was transferred to a 25 mL volumetric flask, to measure its absorbance (A). Using the methodology described above, we finally calculated its total flavones content according to a regression equation

In vitro anti-oxidant activity

Scavenging activity on DPPH radical

The radical scavenging activity of total flavonoids of *L. polystachyus* was determined according to our previous procedure (He et al., 2012). In brief, 3.0 mL of 0.1 mM DPPH in methanol solution was added to 1.0 mL of total flavonoids of *L. polystachyus* preparative solution at different concentrations. The mixture was stood for 20 min in the dark before the absorbance was measured with the visible spectrometer at 517 nm. The DPPH scavenging effect was calculated as follows: DPPH scavenging activity (%) = [1 − (Absorbance of sample− Absorbance of background)/Absorbance of blank] ×

100.

Scavenging activity on ABTS radical

The ABTS radical cation scavenging activity of DDP was identified using a method (Re et al., 1999) with some modifications. ABTS was produced from the reaction of 7 mM of ABTS solution with 2.45 mM of potassium persulfate. The mixture was then kept 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 0.70 ± 0.02 at 734 nm. Samples (0.2 mL) of various concentrations (0.2 to 1.0 mg/mL) were mixed with ABTS solutions (2 mL). After reaction at room temperature for 6 min, the absorbance at 734 nm was recorded. The ABTS scavenging effect was calculated based on the following formula: ABTS scavenging activity (%) = [1 − (Absorbance of sample− Absorbance of background)/ Absorbance of blank] × 100.

Scavenging activity on hydroxyl radical

The HO^--scavenging activity of LP-F was measured by an impro-ved Fenton-type reaction (Wang et al., 2012). In brief, 1.0 mL of LP-F, at different concentrations, was spiked with 1.0 mL of 6 mM $FeSO_4$, 1.0 mL of 6 mM salicylic acid–ethanol and 1.0 mL of 6 mM H_2O_2 and incubated at 37°C for 1 hour. BHT was used as positive control. The absorbance at 510 nm was measured with a visible spectrometer. The percentage of HO^--scavenging activi ty of total flavonoids of *L. polystachyus* was calculated according to the following formula: Scavenging activity against HO^- (%) = [1-(Absorbance of sample—Absorbance of blank)/Absorbance of control] × 100.

Scavenging activity on superoxide radical

The scavenging activity on $O_2^{\bullet -}$ of total flavonoids of *L. polystachyus* was determined as described in our previous study (Wang et al., 2012). 1.0 mL of Mm nitroblue tetrazolium, 1.0 mL of mM nicotinamide adenine diuncleotide hydrogen, 1.0 mL of different concentration sample solution and 0.4 mL of 3 mM phenazine methosulfate were successively added into test tube, the reaction mixture was incubated at ambient temperature for 5 min and the absorbance of the mixture solution was determined at 560 nm with a visible spectrometer. $O_2^{\bullet -}$ scavenging capability of total flavonoids of *L. polystachyus* was calculated according to the following formula: Suppression rate (%) = $O_2^{\bullet -}$(%) = [1-(Absorbance of sample−Absorbance of blank)/Absorbance of control] × 100.

In vivo studies

Carbon tetrachloride-induced hepatotoxicity

Rats (n = 60) were divided into six groups consisting of 10 animals in each group. Group I served as normal group and received distilled water with 0.3%sodium carboxy-methylcellulose (CMC-Na)(1 mL/kg body weight, p.o.) for 5 days, and olive oil (1 mL/kg bodyweight, s.c.) on days 2 and 3 (Jain et al., 2008). Group II served as control group and received 0.3% CMC-Na (1 mL/kg body weight, p.o.) for 5 days, and CCl_4–olive oil (1:1, 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 CCl_4–olive oil (1:1, 2 mL/kg body weight, s.c.) on days 2 and 3, 30 min after administration of silymarin. Groups IV–VI (test group animals) were administered orally a dose of 100, 200, and 400 mg/kg body weight of total flavonoids of *L. polystachyus* (p.o.), respectively, for 5 days; in addition, they received CCl_4–olive oil (1:1, 2 mL/kg, s.c.) on days 2 and 3, 30 min after administration of total flavonoids of *L. polystachyus*. On day 6, animals were anesthetized, blood was collected and allowed to clot, and serum was separated for assessment of biochemical parameters. The rats were then sacrificed by bleeding, the liver and kidney were carefully dissected and cleaned of extraneous tissue, and part of the liver tissue was immediately transferred into 10%formalin for histopathological investigation.

Serum analysis for liver marker enzymes

Serum samples were assayed for alkaline phosphatase, glutamate pyru-vate transaminase, aspartate aminotransferase, gamma-glutamyl transpeptidase by assay kits which were obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Serum analysis for biochemical studies

Total bilirubin, total cholesterol, triglycerides total protein and albumin were estimated by assay kits which were obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China).

Determinations of antioxidant activity in vivo

The liver and kidney of superoxide dismutase, catalase, malondi-aldehyde and glutathion were determined by 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, 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

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- or Two-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 5%level of significance ($p<0.05$).

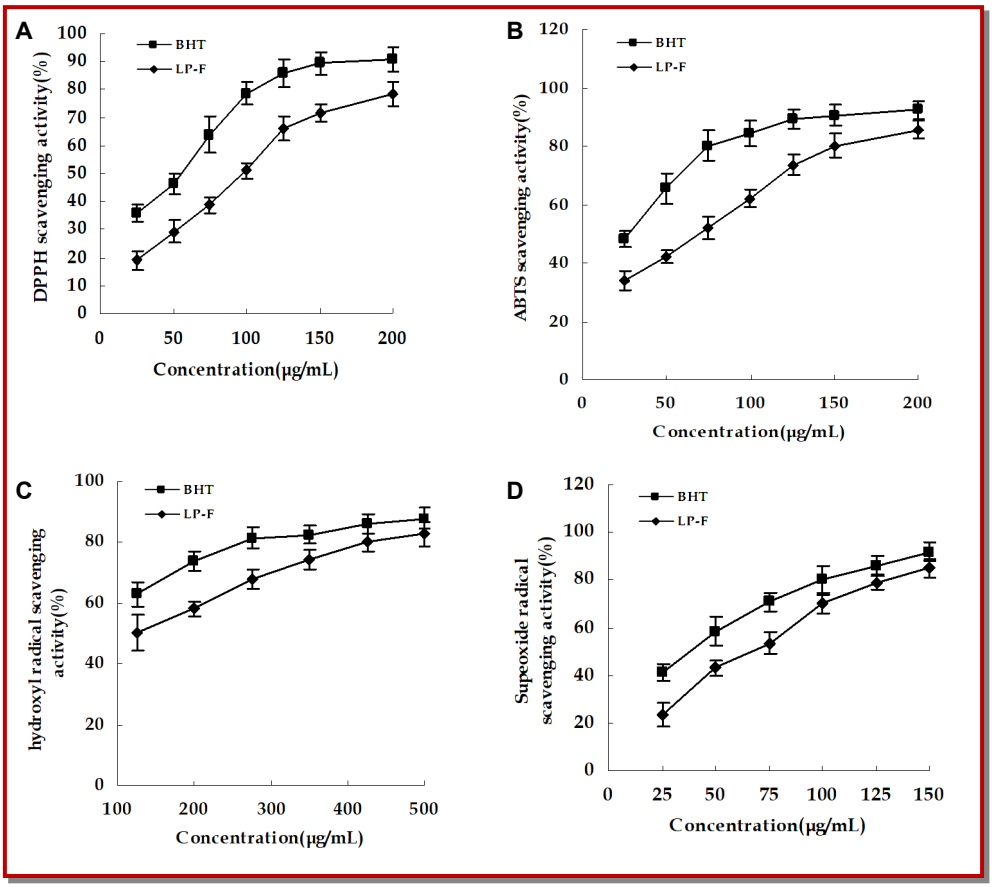

Figure 1: *In vitro* free radical scavenging activity and antioxidant effects of LP-F. (A) DPPH- scavenging activity of various concentrations of total flavonoid of *L. polystachyus* and reference BHT. (B) ABTS-scavenging effect of LP-F and BHT. (C) HO^--scavenging activity of LP-F and BHT. (D) $O_2^{\bullet -}$-scaven ging activity of total flavonoid of *L. polystachyus* and BHT, Data are presented as means ± SD (n = 3)

Results

Total flavonoids of *L. polystachyus* was extracted from *L. polystachyus* with 70%ethanol and purified with AB-8 macroreticular resin, dried under reduced pressure and weighed, the yield of 8.7%. Regression equation: A= 10.014C−0.007, r=0.9998, *C* (concentration), with a better linear correlation between 7.9 and 54.4 μg/mL. Quantitative analysis indicated that the content of total flavonoids of *L. polystachyus* was up to 76.8%.

In this study, the *in vitro* antioxidant capacity of total flavonoids of *L. polystachyus* was estimated with DPPH, ABTS, hydroxyl radical, and reducing power systems. As shown in Figure 1A, total flavonoids of *L. polystachyus* concentration-dependently displayed DPPH-scavenging effects of 27.9, 39.9, 5 0.1, 62.4, 71.5, 79.2 and 84.7% at the tested concentrations of 25, 50, 75, 100, 125, 150 and 200 μg/mL, respectively. Similarly, total flavonoids of *L. polystachyus* at the concentrations of 25–200 μg/mL also exhibited obvious scavenging activity (31.9, 36.9, 53.6, 67.2, 76.2, 81.3 and 86.6%) against ABTS, respectively (Figure 1B). Total flavonoids of *L. polystachyus* also showed the significant scavenging effects (49.5, 59.3, 70.3, 77.6, 82.5 and 86.6%) against HO^- in a concentration-dependent manner (125–500 μg/mL, Figure 1C). As shown in Figure 1D, total flavonoids of *L. polystachyus* also showed the significant scavenging effects (24.6, 4 5.8, 55.5, 69.5, 79.6 and 85.6%) against $O_2^{\bullet -}$ in the broad range of 25–150 μg/mL, The data presented here indicates that total flavonoids of *L. polystachyus* has potential to be explored as a strong anti -oxidant, although it is inferior to the reference BHT in antioxidant capacity (Figure 1).

As depicted in Table I, the enzymatic activities of serum glutamate pyruvate transaminase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in CCl_4-intoxicated rats sharply increased to 135.2 ± 11.3 ($p<0.001$), 223.5 ± 15.6 ($p<0.001$), 197.5 ± 16.3 ($p<0.001$) and 132.6 ± 5.8 IU/L ($p<0.001$) from 32.6 ± 2.9, 65.3 ± 1.7, 48.3 ± 1.7 and 31.3 ± 2.2 IU/L of the normal group, respectively. Administration of LP-F (100, 200 and 400 mg/kg) alleviated the toxicity of CCl_4 and the changed serum level of glutamate pyruvate transaminase, aspartate aminotrans -ferase, alkaline phosphatase and gamma-glutamyl

Table I

Protective effect of total flavonoid of *L. polystachyus* on liver marker enzymes

Group	Glutamate pyruvate transaminase (IU/L)	Aspartate ami-notransferase (IU/L)	Alkaline phosphatase (IU/L)	Gamma-glutamyl transpeptidase (IU/L)
Normal	32.6 ± 2.9	65.3 ± 1.7	48.3 ± 1.7	31.3 ± 2.2
Control	135.2 ± 11.3[a]	223.5 ± 15.6[a]	197.5 ± 16.3[a]	132.7 ± 5.8[a]
Silymarin 100 (mg/kg)	48.7 ± 5.6[b]	72.8 ± 4.9[b]	61.4 ± 5.3 [b]	41.1 ± 4.2[b]
Extract 100 (mg/kg)	105.7 ± 8.5[c]	136.5 ± 19.6[c]	135.4 ± 8.5[c]	102.9 ± 7.6[c]
Extract 200 (mg/kg)	68.5 ± 2.7[b]	98.7 ± 8.5[b]	109.4 ± 7.6[c]	76.6 ± 4.4[b]
Extract 400 (mg/kg)	54.3 ± 4.6[b]	76.5 ± 6.3[b]	76.2 ± 5.0[b]	52.4 ± 6.5[b]

Values are expressed as mean ± SEM, n = 10; [a]p<0.001 considered statistically significant as compared to normal control group; [b]p<0.001, [c]p<0.05 considered statistically significant as compared to carbon tetrachloride treated group

Table II

Protective effect of LP-F on serum biochemical profile

Group	Total bilirubin (mg/dL)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Normal	0.6 ± 0.9	68.6 ± 5.6	32.7 ± 1.0	8.2 ± 0.7	1.0 ± 0.1
Control	1.9 ± 0.2[a]	113.6 ± 6.6[a]	79.4 ± 2.2[a]	4.9 ± 0.1[a]	2.7 ± 0.0[a]
Silymarin 100 (mg/kg)	0.8 ± 0.1[b]	73.7 ± 3.3[b]	38.6 ± 4.2[b]	7.6 ± 0.4[b]	1.1 ± 0.1[b]
Extract 100 (mg/kg)	1.4 ± 0.1[c]	98.7 ± 4.7[c]	71.6 ± 1.2[c]	5.6 ± 0.3[c]	2.1 ± 0.1
Extract 200 (mg/kg)	1.2 ± 0.1[c]	87.7 ± 2.9[c]	52.3 ± 1.0[c]	7.0 ± 0.3[c]	1.8 ± 0.2[c]
Extract 400 (mg/kg)	0.8 ± 0.1[b]	76.4 ± 7.7[b]	40.6 ± 1.1[b]	7.4 ± 0.9[b]	1.2 ± 0.1[b]

Values are expressed as mean ± SEM, n = 10; [a]p<0.001 considered statistically significant as compared to normal control group; [b]p<0.001, [c]p<0.05 considered statistically significant as compared to carbon tetrachloride treated group

transpeptidase, reverted towards the normal group with significance difference from the control group. Similarly, administration of silymarin (100 mg/kg) alleviated the toxicity of CCl_4 and serum level of glutamate pyruvate transaminase, aspartate amino-transferase, alkaline phosphatase and gamma-glutamyl transpeptidase was observed significantly less than that of control group.

The levels of serum total bilirubin, total cholesterol, triglycerides, total protein and albumin were estimated and the results were given in Table II. Administration of CCl_4 in rats caused a significant increase ($p<0.05$) in serum total bilirubin, total cholesterol, triglycerides and albumin levels, ranging from 0.6 ± 0.9, 68.6 ± 5.6, 32.6 ± 1.0 mg/dL, 8.2 ± 0.7 and 1.0 ± 0.1 g/dL for the normal control rats to 1.9 ± 0.2, 113.6 ± 6.6, 79.4 ± 2.2 mg/dL, 4.9 ± 0.1 and 2.7 ± 0.0 g/dL, while caused a significant decrease ($p<0.05$) in serum total protein ranging from 8.2 ± 0.7 g/dL for the normal rats to 4.9 ± 0.1 g/dL respectively. However, the protective administration of indifferent dose of LP-F decreased the CCl_4-elevated levels of serum total bilirubin, total cholesterol, triglycerides and albumin ($p<0.05$), and silymarin as positive control also exhibited a similar effect. While increased the CCl_4-elevated levels of serum total protein.

Determinations of the *in vivo* anti-oxidant activities of total flavonoids of *L. polystachyus* in liver and kidney are presented in Figure 2. Compared with the normal group, the CCl4-intoxicated rats exhibited a significant decrease in total superoxide dismutase, catalase and glutathione (liver and kidney) ($p<0.001$), significant increase in the level of malondialdehyde (in liver and kidney) ($p<0.001$). These changes were significantly reversed after treatment with LP-F and silymarin.

Histopathological observations of liver sections from the control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Figure 3A). In contrast, the CCl_4 group revealed the most severe damage of any of the groups; the liver sections showed massive fatty changes, necrosis, ballooning degeneration, broad infiltration of lymphocytes, and the loss of cellular boundaries (Figure 3B). The liver sections of the rats treated with total flavonoids of *L. polystachyus* (Figure 3E–F) showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration almost comparable to the control (Figure 3A) and the silymarin-treated group (Figure 3C).

Discussion

The assays for scavenging DPPH, ABTS, $HO^-$and $O_2^{\bullet -}$ showed that total flavonoids of *L. polystachyus* had a

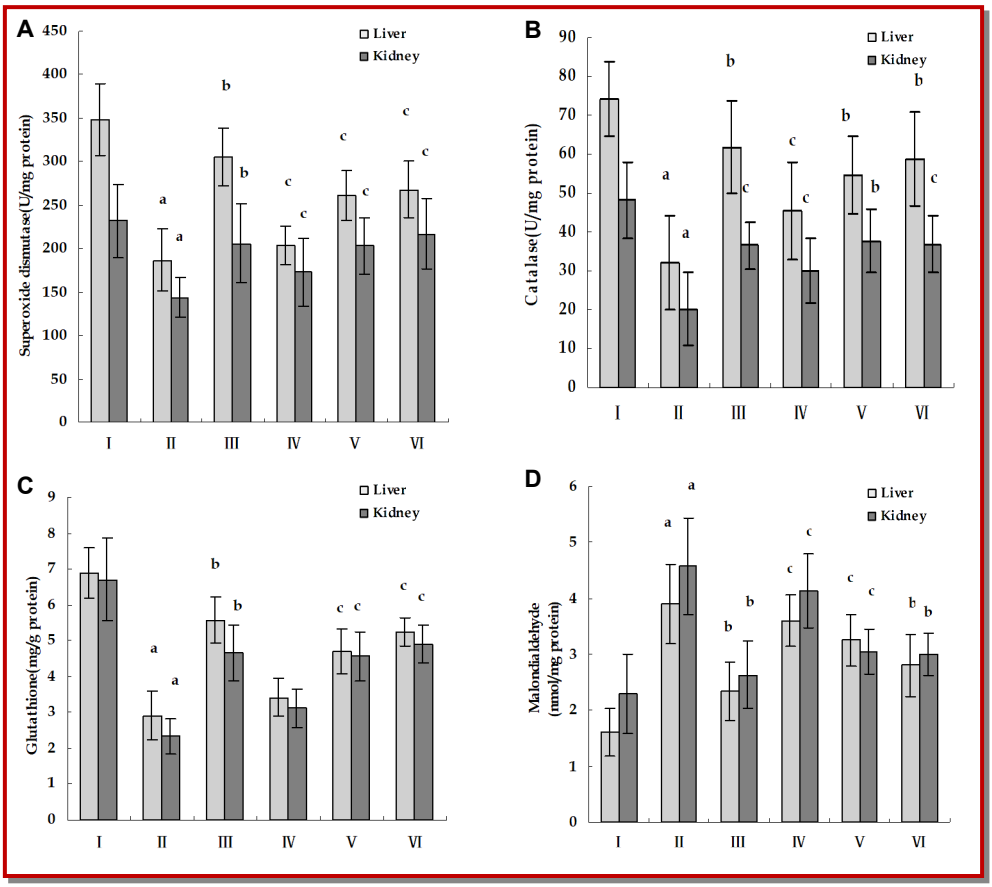

Figure 2: Total flavonoid of *L. polystachyus* on liver and kidney superoxide dismutase, catalase, glutathione and malondialdehyde in CCl_4-intoxicated mice

Values are expressed as mean ± SEM; n = 10; [a]p<0.001 considered statistically significant as compared to normal control group; [b]p<0.001, [c]p<0.05 considered statistically significant as compared to carbon tetrachloride treated group; Group I: Normal, Group II: control, Group III: silymarin 100 (mg/kg), Group IV: total flavonoid of *L. polystachyus* 100 (mg/kg), Group V: total flavonoid of *L. polystachyus* 200 (mg/kg), Group VI: total flavonoid of *L. polystachyus* 400 (mg/kg)

concentration-dependent anti-oxidant effect *in vitro*, and this effect was further verified by testing the anti-oxidant potential of total flavonoids of *L. polystachyus* in an animal model with experimental CCl_4-induced oxidative stress.

In this study, elevated levels of liver marker enzymes such as glutamate pyruvate transaminase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase in serum are obtained with CCl_4 treatment indicating the pathophysiology of liver. Generally, CCl_4 is metabolized by the liver in to highly reactive metabolites which either directly or indirectly cause lipid peroxidation of the hepatocytes. Different cytosolic liver marker enzymes would then leaked out from these swollen and necrotic hepatocytes in to blood circulation and evidently elevated levels are obtained that is associated with the massive centrilobular necrosis, ballooning, degeneration and cellular infiltration of the liver (Singh et al., 2008). Treatment of rats by both CCl_4 and the LP-F/silymarin reversed the increase in liver marker enzymes towards the control group and hepatic lesions are also minimized (Table I).

It is well known that the liver play a pivotal role in the regulation of various chemicals. Administration of CCl_4 causes hepathopathy as indicated by elevation in serum level of total bilirubin, total cholesterol, triglycerides and albumin whereas decreases total protein in rat. These pathological changes signify the potential damage in hepatic induced with CCl_4 treatment. Treatment of rat with CCl_4 and with either LP-F or silymarin ameliorated the toxic effects of CCl_4 and restored the level of above biochemicals towards the normal group in accordance with other findings (Lin et al., 2008).

Carbon tetrachloride is accumulated in hepatic parenchyma cells and metabolized to the $CCl_3^{\bullet}$ (Recknagel, 1983). $CCl_3^{\bullet}$ radical reacts very rapidly with oxygen to yield a highly reactive $CCl_3OO^{\bullet}$. These radicals react with proteins and lipids. They remove hydrogen atoms from unsaturated lipids thus initiating lipid peroxidation, which causes loss of integrity of cell membranes and damage to hepatic tissue (Zhou et al., 2010). The enzymes superoxide dismutase, catalase and glutathione are key anti-oxidant enzymes that protect

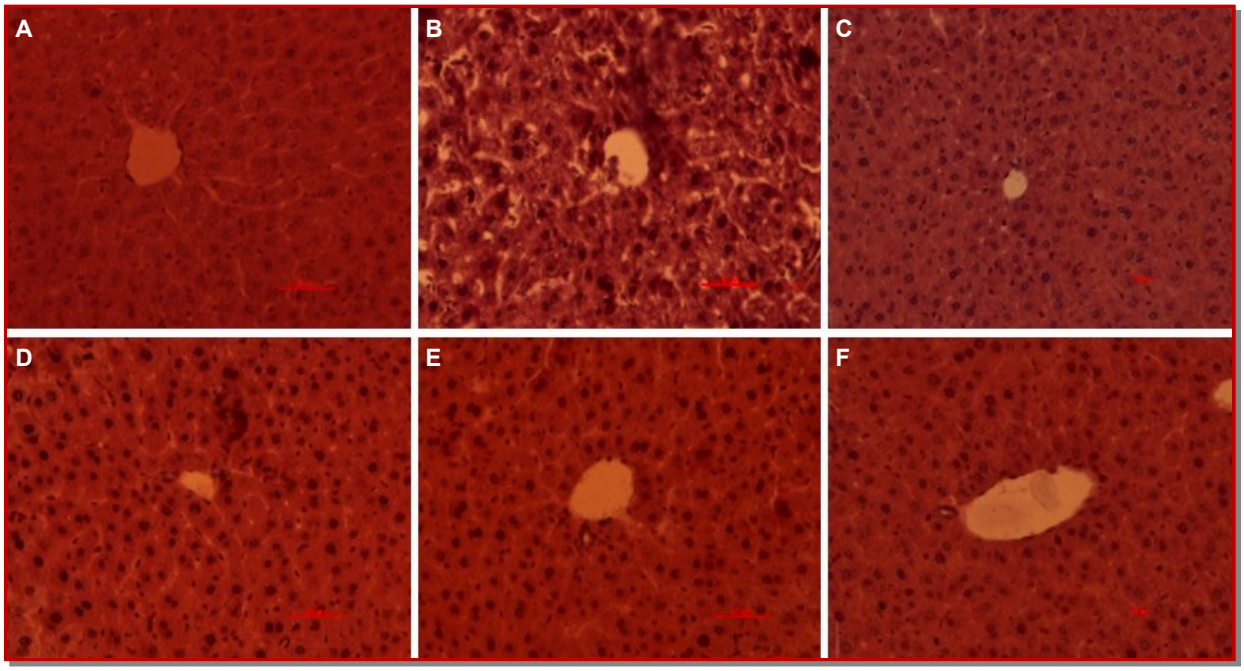

Figure 3: Sections of the livers of CCl_4-treated rats showing the ventral vein (CV) and hepatic cells (hematoxylin–eosin staining, 200x) (A) Normal group, (B) Control group, (C) CCl_4 + silymarin (100 mg/kg), (D) CCl_4 + total flavonoid of *L. polystachyus* (100 mg/kg), (E) CCl_4 + total flavonoid of *L. polystachyus* (200 mg/kg), and (F) CCl_4 + total flavonoid of *L. polystachyus* (400 mg/kg).

against oxidative stress and tissue damage (Halliwell and Gutteridge, 1990).

These enzymes are critical for defence mechanisms against the harmful effects of reactive oxygen species (ROS) and free radicals in biological systems. The superoxide dismutase converts superoxide radicals into H_2O_2 and O_2, thus participating with other anti-oxidant enzymes, in the enzymatic defense against oxygen toxicity. Catalase is a key component of the anti-oxidant defense system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage. The decrease of catalase may result in a lot of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Srinivasan et al., 2007). glutathione content was another important parameter that revealed oxidative damage in both liver and kidney. glutathione constitutes the first line of defense against free radicals. Reduction in liver glutathione activity in carbon tetrachloride-treated rats as observed in this study indicates the damage to the hepatic cells. Malondialdehyde is a cytotoxic product that is a hallmark of lipid peroxidation. The fact that LP -F treatment reduced elevated malondialdehyde and increased levels of superoxide dismutase, catalase and glutathione, indicated that it prevent the peroxidation of lipids by carbon tetrachloride.

Conclusion

L. polystachyus possessed strong hepato-protective and anti-oxidant activity in a rat model of CCl_4-induced. The hepatoprotective activity may be due to its free radical-scavenging and anti-oxidant activity, resulting from the presence of some flavonoids in the extracts.

Contribution

First two authors contributed equally in this study

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Conflict of Interest

Authors declare no conflict of interest

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