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Original Article

Antinociceptive, anti-inflammatory, and antioxidant properties of flavonoids extracted from *Cinnamomum longepaniculatum*

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Abstract: *Cinnamomum longepaniculatum* is an endemic plant species in China; it belongs to the family Lauraceae and is rich in flavonoids. Flavonoids have various biological activities, including antinociceptive, anti-inflammatory, and antioxidant activities. However, limited information is available about the biological activities of flavonoids extracted from *C. longepaniculatum* (FCL). *Therefore,* the aim of the current study is to assess the understanding of the antinociceptive, anti-inflammatory, and antioxidant properties of FCL. The results of hot plate and acetic acid-induced writhing tests revealed that 250 and 500 mg/kg FCL possessed obvious antinociceptive effects. Moreover, the anti-inflammatory activity of FCL was reflected by the repair of liver injury, the decrease in serum white blood cells and pro-inflammatory mediators, both of which are factors inducing the adverse effects of lipopolysaccharide challenge. FCL also had antioxidant properties through increasing antioxidant enzyme activities and decreasing the MDA levels, and consequently attenuated the adverse effects of 50% ethanol exposure in mice. In conclusion, FCL had obvious antinociceptive, anti-inflammatory, and antioxidant properties. The antinociceptive properties of 250 and 500 mg/kg FCL were close to those of 250 mg/kg *Ligusticum chuanxiong* Hort. Moreover, the anti-inflammatory properties of 500 mg/kg FCL were close to those of 40 μg/kg dexamethasone.

Keywords: Antinociceptive, anti-inflammatory, antioxidant, flavonoids, *Cinnamomum longepaniculatum*

Introduction

Cinnamomum longepaniculatum is an endemic plant species in China; it belongs to the family Lauraceae and is mainly distributed throughout Yibin, Sichuan Province, China. *C. longepaniculatum* leaves are rich in flavonoids, with the flavonoid content in the leaves reaching up to 39.68 mg/g [1]. An *in vitro* study in our laboratory demonstrated that flavonoids extracted from *C. longepaniculatum* could scavenge free radicals and inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* (Unpublished data).

Flavonoid compounds are secondary metabolites that are widely accumulated in vascular plants and, to a lesser extent, in mosses. More than 6,000 kinds of flavonoids have been detected and purified from nature [2]. Flavonoids possess a wide array of biological properties [3]. First, flavonoids can inhibit autoxidation reactions and scavenge free radicals, which depend on their structure. A previous study revealed that only flavonoids with a free hydroxyl group at the C-3 position of the flavonoid skeleton could show high inhibitory activity against β-carotene oxidation [4]. Second, flavonoids are potential anti-inflammatory agents [5]. Nuclear factor-kappa B (NF-κB) is a key modulator that regulates the inflammatory response [6]. The activation of NF-κB can increase the levels of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α), which induce cell apoptosis [7]. Jie et al. (2011) [8] reported that flavonoids

could inhibit the lipopolysaccharide (LPS)-induced activation of NF-κB in RAW-Blue cells and suppress inflammation. Third, flavonoids possess antinociceptive properties. The acetic acid-induced writhing test is a visceral pain model that is widely employed. It has frequently been used to evaluate peripheral antinociceptive activity [9]. Previous studies have revealed that flavonoids extracted from *Carthamus tinctorius* [10], *Cistus laurifolius* [11], and *Morus nigra L.* [12] could significantly decrease acetic acid-induced writhing episodes. However, limited information is available about the biological activities of flavonoids extracted from *C. longepaniculatum* (FCL). We hypothesized that FCL might exert antioxidant, anti-inflammatory, and antinociceptive effects in mice.

In the present study, we assessed the antioxidant, anti-inflammatory, and anti-nociceptive activities of FCL in mice.

Materials and methods

C. longepaniculatum sample preparation

C. longepaniculatum leaves were collected from Yibin, Sichuan Province, China. After 3 h of steam distillation, the *C. longepaniculatum* leaves were dried at 60°C to a constant mass and then pulverized. The powders were sieved to pass through a 60-80-mesh sieve, degreased by Soxhlet extraction, dried at 60°C again, and flavonoids were extracted by using ultrasonic method.

Assessment of in vivo antinociceptive activity

Hot plate test: All the experimental protocols used in the study were approved by the Animal Care and Use Committee of the Sichuan Agricultural University, China. The hot plate test was performed according to the method of [13], with slight modifications. A transparent glass cylinder (16 cm in height, 16 cm in diameter) was used to keep the mice on the heated surface of the plate. The temperature of the hot plate was maintained at 55 ± 0.5°C. Five groups consisting of 10 mice each were treated with normal saline (control, 250 mg/kg body weight, orally), FCL (125, 250, and 500 mg/kg body weight, orally), and *Ligusticum chuanxiong* Hort (positive control, 250 mg/kg body weight, orally). Before the hot plate test, all the groups were orally administered the different agents for 5 days. Following this, 30, 60, and 90 min after the last administration, the period between the zero point and the time when the animal licked its back paw or jumped off to avoid thermal pain were recorded; antinociceptive activity was expressed as the time duration.

Acetic acid-induced writhing test: The acetic acid-induced writhing test was performed according to the method of Wang et al. [10], with slight modifications. Five groups consisting of 10 mice each were treated with normal saline (250 mg/kg body weight, orally), FCL (125, 250, and 500 mg/kg body weight, orally), and *L. chuanxiong* Hort (positive control, 250 mg/kg body weight, orally). Before the acetic acid-induced writhing test, all the groups were orally administered the different agents for 5 days. Following this, 1 h after the last administration, 0.6% acetic acid (8.75 ml/kg body weight) was intraperitoneally injected into the mice. The number of abdominal constrictions and stretches was recorded in a period of 20 min. The nociceptive thresholds for the acetic acid-induced writhing test were converted to the percentage of maximum possible effect according to the following formula [14]:

$$\% \text{ of maximum possible effect} = \frac{a - b}{a} \times 100\%$$

where a = the number of writhing episodes in the control group; b = the number of writhing episodes in the treatment group.

Assessment of in vivo anti-inflammatory activity

In total, 60 SD mice were assigned to one of six treatment groups, with 10 mice being present in each group: control (250 mg/kg saline, orally), LPS (100 μg/kg LPS, intraperitoneally), positive control (LPS + 40 μg/kg dexamethasone, intraperitoneally), and FCL (125, 250, and 500 mg/kg body weight, orally). Before the test, each FCL group was orally administered FCL for 4 days, while the other groups were orally administered 250 mg/kg saline. On day 4, 30 min after the last administration, LPS was intraperitoneally injected into the mice in the LPS and FCL groups, while dexamethasone was

Table 1. Effects of FCL on antinociceptive activities of mice in hot plate test[1,2]

Treatment	The length of time (s)		
	30 min	60 min	90 min
Control	12.67 ± 8.62[a]	24.00 ± 10.44[a]	31.33 ± 10.60[a]
Positive control	58.33 ± 2.89[b,c,d]	60.00 ± 0.00[b]	56.33 ± 6.35[b]
FCL (mg/kg)			
125	39.33 ± 12.06[b]	37.67 ± 7.57[a]	36.67 ± 17.62[a]
250	54.00 ± 10.39[b,c]	53.00 ± 6.08[b]	50.33 ± 10.60[a,b]
500	46.33 ± 12.10[b]	55.33 ± 4.16[b]	56.67 ± 5.77[b]

[1]Each value represents the mean values of 10 replicates (n = 10). [2]Means without a common letter are different, $P < 0.05$; Control = mice were treated with normal saline (250 mg/kg, body weight, oral); Positive control = mice were treated with normal *Ligusticum chuanxiong* hort (250 mg/kg, body weight, oral); FCL = flavonoids extracted from *Cinnamomum longepaniculatum;* Antinociceptive activities was expressed as the time period between the zero point when the animal licked its back paw or jumped off to avoid thermal pain. [a,b,c,d]Means without a common letter are different.

intraperitoneally injected into the mice in the positive control group. After another 8 h, all the mice were weighed, sacrificed by cervical dislocation, and their blood was collected. The weights of their heart, liver, spleen, lung, and kidney were determined, and the organ-to-body weight ratios were calculated. Following this, the liver samples were fixed in 4% paraformaldehyde for standard histological analysis.

Histological assay: In brief, the 4% paraformaldehyde-fixed liver sections were embedded in paraffin. Following this, 4-μm-thick sections were cut and mounted on microscope slides. The sections were deparaffinized in xylene, rehydrated using graded ethanol solutions, and then stained with hematoxylin and eosin, as described by Picut et al. [15].

Routine blood test: White blood cell (WBC), red blood cell (RBC), lymphocyte (LYM), granulocyte (GRA) neutrophil, and monocyte (MON) counts, RBC volume (hematocrit, HCT), mean corpuscular volume (MCV), mean platelet volume (MPV), and mean corpuscular hemoglobin (MCH) level were measured using an automated hematology analyzer (Yellow Springs Instrument, Yellow Spring, OH).

Assessment of blood inflammatory cytokines: TNF-α, cyclooxygenase-2 (COX-2), IL-1, IL-6, IL-8, and IL-10 levels were determined using enzyme-linked immunosorbent assay (Nanjing Jiancheng Bioengineering Institutes, Nanjing, China).

Assessment of in vivo antioxidant activity

In total, 50 SD mice were assigned to one of five treatment groups, with 10 mice being present in each group: control, ethanol, and three FCL groups (125, 250, and 500 mg/kg body weight, orally). During days 1-30 of the trial, each FCL group was orally administered FCL, while the other groups were orally administered 250 mg/kg saline daily. On day 30 of the trial, 30 min after the last administration, the FCL and ethanol groups were orally administered 50% ethanol (1 mg/kg). After another 16 h, all the mice were sacrificed by cervical dislocation, their blood was collected, and serum was isolated for antioxidant index analysis.

Antioxidant activity analysis: The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), total antioxidant capacity (T-AOC), and the malonaldehyde (MDA) level were determined using specific detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA; SPSS 17, IBM Corp., Chicago, IL). Differences between treatment groups were detected using Duncan's multiple range test, and significant differences were considered at $P < 0.05$. The results were expressed as the means and standard deviations.

Results

Antinociceptive activity

Hot plate test: As shown in **Table 1**, compared with the control group, the three FCL groups showed significantly increased latency to the first pain reaction 30 min after the last administration ($P < 0.05$). The 250 and 500 mg/kg FCL groups showed significantly increased latency to the first pain reaction 60 and 90 min after the last administration ($P < 0.05$). However, this antinociceptive effect was lesser than that observed in the positive control group.

Table 2. Effects of FCL on antinociceptive activities of mice in acetic acid induced writhing test[1,2]

Treatment	Number of writhing episodes	%Maximum possible effect
Control	16.20 ± 3.27[a]	-
Positive control	9.40 ± 2.30[b]	41.98 ± 14.21
FCL (mg/kg)		
125	8.80 ± 2.05[b]	45.68 ± 12.65
250	5.20 ± 2.59[c]	67.90 ± 15.97
500	6.40 ± 2.51[b,c]	60.49 ± 15.49

[1]Each value represents the mean values of 10 replicates (n = 10). [2]Means without a common letter are different, $P < 0.05$; Control = mice were treated with normal saline (250 mg/kg, body weight, oral); Positive control = mice were treated with normal *Ligusticum chuanxiong* hort (250 mg/kg, body weight, oral); FCL = flavonoids extracted from *Cinnamomum longepaniculatum;* Antinociceptive activities was expressed as the number of abdominal constrictions and stretching in a period of 20 min and the percent maximum possible effect. [a,b,c]Means without a common letter are different.

Acetic acid-induced writhing test: As shown in **Table 2**, compared with the control group, the three FCL groups showed significantly decreased numbers of acetic acid-induced writhing episodes ($P < 0.05$). The 250 mg/kg FCL group showed significantly lower numbers of acetic acid-induced writhing episodes than the *L. chuanxiong* Hort group ($P < 0.05$). In the 125, 250, and 500 mg/kg FCL groups, the nociceptive thresholds decreased by 45.68%, 67.90%, and 60.49%, respectively ($P < 0.05$). Thus, 250 and 500 mg/kg FCL possessed obvious antinociceptive activity.

Anti-inflammatory activity

Visceral index assessment: As shown in **Table 3**, LPS group showed significantly higher spleen, lung, and kidney weights than the control group. FCL could counteract the adverse effects in a dose-dependent manner ($P < 0.05$).

Histological assay: As shown in **Figure 1**, the liver samples of mice in the control group showed no evidence of inflammation. The liver samples of mice in the LPS group showed signs typical of granulomatous inflammatory reactions, namely capillary congestion, and inflammatory cell infiltration. The positive control group showed capillary congestion and slight inflammatory cell infiltration. The 125 mg/kg FCL group showed erythrocyte hemolysis and enormous inflammatory cell infiltration, while the 250 mg/kg FCL group showed capillary congestion and slight inflammatory cell infiltration. The 500 mg/kg FCL group showed congestion of the central vein and slight inflammatory cell infiltration.

Routine blood test: As shown in **Table 4**, when compared to control mice, the LPS group showed significantly higher serum WBC, RBC, GRA, and MON counts, HGB, HCT, and MPV levels ($P < 0.05$). The positive control group showed significantly higher serum WBC counts, RBC counts, HGB levels, and HCT levels than the control group ($P < 0.05$). Moreover, the three FCL groups showed significantly higher serum WBC counts, RBC counts, HGB levels, and HCT levels than the control group ($P < 0.05$). The serum WBC, GRA, and MON counts significantly decreased in the three FCL groups. FCL could partially counteract the adverse effects in the mice.

Blood inflammatory cytokines: As shown in **Table 5**, the LPS group showed significantly higher serum TNF-α, COX-2, IL-1, IL-6, IL-8, and IL-10 levels than the control group ($P < 0.05$). The positive control group showed significantly lower serum TNF-α, COX-2, IL-1, IL-6, IL-8, and IL-10 levels ($P < 0.05$) than the LPS group. Meanwhile, the three FCL groups showed lower serum TNF-α, COX-2, IL-1, IL-6, IL-8, and IL-10 levels ($P < 0.05$) than the LPS group. The higher the dosage of FCL, the better was the effect. The beneficial effects of 500 mg/kg FCL were close to those of 40 μg/kg dexamethasone.

Antioxidant activity

As shown in **Figure 2**, the exposure of mice to 50% ethanol significantly ($P < 0.05$) decreased their serum SOD, GSH-Px, and CAT activities and T-AOC and increased their serum MDA levels. The administration of 125 mg/kg FCL could counteract the adverse effects. The 250 and 500 mg/kg FCL groups showed significantly ($P < 0.05$) higher SOD activity, CAT activity, and T-AOC and lower MDA levels than the control group.

Table 3. Effects of FCL on viscera index of mice in anti-inflammatory activity studies[1,2]

Treatment	Heart	Liver	Spleen	Lung	Kidney
Control	0.52 ± 0.04	3.57 ± 0.11[a]	0.26 ± 0.02[a]	0.55 ± 0.03[a]	0.80 ± 0.05[a]
LPS	0.49 ± 0.04	4.61 ± 0.59[a,b]	0.40 ± 0.09[b]	0.82 ± 0.05[c]	0.96 ± 0.05[b]
Positive control	0.48 ± 0.04	4.45 ± 0.27[a,b]	0.34 ± 0.05[a,b]	0.71 ± 0.09[a,b]	0.91 ± 0.02[a,b]
FCL (mg/kg)					
125	0.47 ± 0.03	4.78 ± 0.40[b]	0.36 ± 0.09[a,b]	0.82 ± 0.03[c]	0.95 ± 0.06[b]
250	0.49 ± 0.06	4.33 ± 0.68[a,b]	0.34 ± 0.08[a,b]	0.70 ± 0.07[a,b]	0.90 ± 0.11[a,b]
500	0.47 ± 0.02	4.10 ± 0.24[a,b]	0.32 ± 0.04[a,b]	0.64 ± 0.04[a]	0.91 ± 0.10[a,b]

[1]Each value represents the mean values of 10 replicates (n = 10). [2]Means without a common letter are different, $P < 0.05$; Control = mice were treated with normal saline (250 mg/kg, body weight, oral); LPS = mice were treated with normal Lipopolysaccharide (100 μg/kg, intraperitoneal); Positive control = 100 μg/kg Lipopolysaccharide + 40 μg/kg dexamethasone; FCL = flavonoids extracted from *Cinnamomum longepaniculatum*. [a,b,c]Means without a common letter are different.

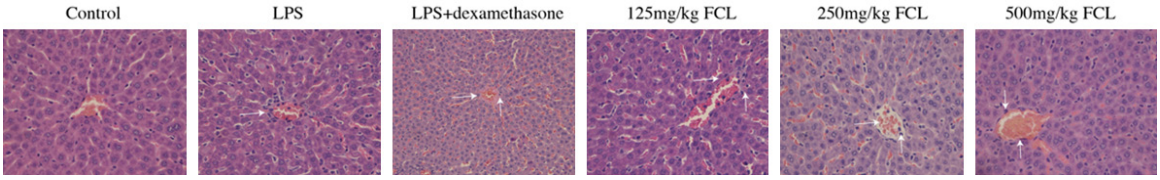

Figure 1. Effects of FCL on liver histology of mice in anti-inflammatory activity studies (×400). White arrows indicated the capillaries were congested and inflammatory cells infiltrated. Control = mice were treated with normal saline (250 mg/kg, body weight, oral); LPS = mice were treated with normal Lipopolysaccharide (100 μg/kg, intraperitoneal); Positive control = 100 μg/kg Lipopolysaccharide + 40 μg/kg dexamethasone; FCL = flavonoids extracted from *Cinnamomum longepaniculatum.*

Table 4. Effects of FCL on routine blood test of mice in anti-inflammatory activity studies[1,2]

Treatment	Control	LPS	Positive control	FCL (mg/kg)		
				125	250	500
WBC (10^9/L)	8.72 ± 3.80[a]	21.14 ± 4.75[d]	9.18 ± 2.35[a,b]	15.24 ± 3.24[c]	12.12 ± 1.47[b,c]	13.50 ± 2.83[b,c]
RBC (10^{12}/L)	4.91 ± 1.82[a]	7.58 ± 0.61[b,c]	6.208 ± 0.55[b]	7.190 ± 0.32[b,c]	8.24 ± 0.88[c]	6.79 ± 0.92[b]
LYM (g/L)	4.16 ± 1.89[a]	10.32 ± 2.51[a,b]	3.78 ± 0.96[a]	7.98 ± 1.38[a,b]	6.80 ± 2.04[a,b]	14.02 ± 14.01[b]
GRA (10^9/L)	3.24 ± 1.97[a]	10.16 ± 3.31[c]	5.06 ± 1.45[a,b]	6.76 ± 2.06[b]	4.86 ± 2.05[a,b]	6.08 ± 2.49[a,b]
MON (10^9/L)	0.32 ± 0.23[a]	0.66 ± 0.15[b]	0.34±0.207[a]	0.50 ± 0.19[a,b]	0.46 ± 0.15[a,b]	0.40 ± 0.12[a]
HCT (%)	30.82 ± 12.72[a]	47.00 ± 3.23[b,c]	39.66±4.50[b]	45.70 ± 2.30[b,c]	51.58 ± 4.57[c]	42.76 ± 4.61[b,c]
MCV (fL)	62.08 ± 3.18[a]	62.12 ± 1.49[a]	63.9±2.69[a]	63.62 ± 0.86[a]	62.82 ± 3.01[a]	63.28 ± 2.50[a]
MPV (fL)	5.76 ± 0.59[a,b]	6.26 ± 0.71[b]	5.32±0.278[a]	6.14 ± 0.34[b]	6.18 ± 0.67[b]	6.08 ± 0.52[b]
MCH (pg)	19.32 ± 3.77[a]	21.36 ±0.85[a,b]	22.00 ± 1.48[a,b]	22.40 ± 0.38[b]	21.10 ± 2.56[a,b]	22.64 ± 0.85[b]

[1]Each value represents the mean values of 10 replicates (n = 10). [2]Means without a common letter are different, $P < 0.05$; Control = mice were treated with normal saline (250 mg/kg, body weight, oral); LPS = mice were treated with normal Lipopolysaccharide (100 μg/kg, intraperitoneal); Positive control = 100 μg/kg Lipopolysaccharide + 40 μg/kg dexamethasone; FCL = flavonoids extracted from *Cinnamomum longepaniculatum*; WBC = white blood cell; RBC = red blood cell; LYM = lymphocyte; GRA = neutrophil granulocyte; MON = monocytes; HCT = Red blood cell volume; MCV = mean corpuscular volume; MPV = mean platelet volume; MCH = Mean corpuscular hemoglobin. [a,b,c,d]Means without a common letter are different.

Discussion

C. longepaniculatum has been widely cultivated as a major economic crop in the Yibin region (Sichuan, China). A previous study on *C. longepaniculatum* mainly focused on the extraction such as essential oils and proanthocyanidins from *C. longepaniculatum* leaves [16-18]. However, little is known about the biological activity of flavonoids extracted from *C. longepaniculatum*. In the present study, the results of hot plate and acetic acid-induced writhing tests revealed that 250 and 500 mg/kg FCL had obvious antinociceptive effects. Wang et al. [10] found that flavonoids extracted from safflower could significantly inhibit nociception in-

Table 5. Effects of FCL on inflammatory cytokines of mice in anti-inflammatory activity studies[1,2]

Treatment	Control	LPS	Positive control	FCL (mg/kg)		
				125	250	500
TNF-α (pg/ml)	104.83 ± 5.71^{a,b}	239.37 ± 14.81^{f}	120.57 ± 7.80^{b,c}	211.52 ± 8.05^{e}	131.75±16.469^{c}	151.49 ± 2.24^{d}
COX-2 (ng/ml)	21.80 ± 4.43^{a}	47.83 ± 1.14^{d}	29.41 ± 1.14^{b}	39.34 ± 3.08^{c}	32.85 ± 0.37^{b,c}	32.72 ± 4.32^{b,c}
IL-1 (pg/ml)	73.67 ± 6.36^{a}	154.55 ± 25.04^{c}	76.70 ± 4.21^{a}	108.67 ± 5.88^{b}	74.66 ± 5.24^{a}	72.92 ± 6.01^{a}
IL-6 (pg/ml)	142.31 ± 4.71^{a}	170.59 ± 15.53^{b}	135.66 ± 1.09^{a}	171.72 ± 4.30^{b}	140.45 ± 4.91^{a}	146.24 ± 8.41^{a}
IL-8 (pg/ml)	35.75 ± 1.63^{a,b}	92.23 ± 0.36^{d}	29.68 ± 4.60^{a}	59.71 ± 7.62^{c}	48.75 ± 14.43^{b,c}	40.78 ± 6.84^{a,b}
IL-10 (pg/ml)	25.34 ± 3.34^{b,c}	17.74 ± 1.82^{a}	36.98 ± 2.68^{e}	27.95 ± 2.52^{c,d}	35.79 ± 4.44^{d,e}	30.17 ± 3.92^{c,d}

[1]Each value represents the mean values of 10 replicates (n = 10). [2]Control = mice were treated with normal saline (250 mg/kg, body weight, oral); LPS = mice were treated with normal Lipopolysaccharide (100 μg/kg, intraperitoneal); Positive control = 100 μg/kg Lipopolysaccharide + 40 μg/kg dexamethasone; FCL = flavonoids extracted from *Cinnamomum longepaniculatum*; TNF-α = Tumor necrosis factor-α; COX = cyclooxygenase-2; IL = interleukin. [a,b,c,d,e,f]Means without a common letter are different.

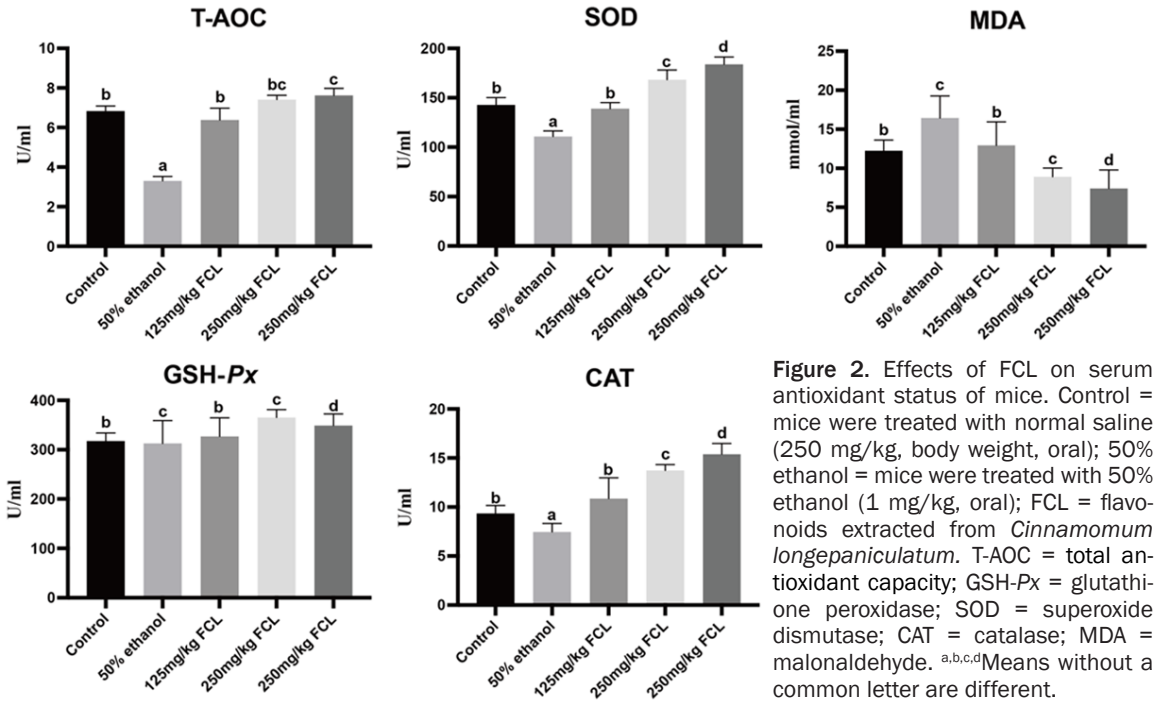

Figure 2. Effects of FCL on serum antioxidant status of mice. Control = mice were treated with normal saline (250 mg/kg, body weight, oral); 50% ethanol = mice were treated with 50% ethanol (1 mg/kg, oral); FCL = flavonoids extracted from *Cinnamomum longepaniculatum*. T-AOC = total antioxidant capacity; GSH-*Px* = glutathione peroxidase; SOD = superoxide dismutase; CAT = catalase; MDA = malonaldehyde. [a,b,c,d]Means without a common letter are different.

duced by acetic acid and formalin and that kaempferol 3-o-rutinoside and kaempferol 3-o-glucoside were the major active components involved in this antinociceptive activity. Chen et al. [12] reported that flavonoids extracted from black mulberry exhibited antinociceptive effects in the two nociceptive phases of the formalin test. Our results supported these finding. *L. chuanxiong* Hort is a well-known traditional medicinal herb that can inhibit the LPS-induced proinflammatory response and alleviate pain. We found that the antinociceptive effects of 250 and 500 mg/kg FCL were similar to those of *L. chuanxiong* Hort [19]. FCL can be used as a potential antinociceptive drug.

LPS can induce a systemic inflammatory response and activate the immune system [20]. LPS significantly increased the visceral index, which was not consistent with the previous study by Rui et al. [21]. This may be due to the compensatory growth of visceral caused by LPS. Meanwhile, the exposure of mice to LPS could significantly increase the aspartate aminotransferase and alanine aminotransferase levels in their blood, cause apoptotic and necrotic changes in their hepatocytes, and result in a high degree of lethality [22]. The main function of WBCs is defense, and inflammation can cause an increase in the WBC count. TNF-α, COX-2, IL-1, IL-6, IL-8, and IL-10 are the typical

pro-inflammatory mediators, and LPS can induce an increase in the levels of these cytokines [23]. Yeilada et al. [24] reported that flavonoids extracted from Cistaceae could decrease serum IL-1α and prostaglandin levels. Moreover, Küpeli et al. [11] reported that flavonoids extracted from cistaceae had potent inhibitory activity against carrageenan-induced hind paw edema. Chen et al. [12] found that flavonoids extracted from black mulberry significantly decreased the serum IL-1β, TNF-α, interferon-γ, and NO levels and alleviated the adverse effects. Our results were in accordance with these findings. The results of the visceral histopathology, WBC counts, and blood inflammatory cytokine levels indicated that FCL had anti-inflammatory properties and that the beneficial effects of 500 mg/kg FCL were close to those of 40 μg/kg dexamethasone. Collectively, FCL can be used as a potential anti-inflammatory drug.

Furthermore, we found that FCL could increase the antioxidant activity of mice. The administration of FCL to mice significantly increased their serum SOD, GSH-Px, and CAT activities and T-AOC and decreased their serum MDA levels. SODs can prevent the accumulation by converting superoxide into H_2O in the cell [25]. CAT plays a critical role in protecting cells against the toxic effects of hydrogen peroxide (H_2O_2) [26]. GSH-Px is an important antioxidant enzyme that can convert H_2O_2 into H_2O. MDA is the most important lipid peroxidation marker [25, 27]. A previous study reported that flavonoids extracted from flowers of *Ablemoschus Manihot* (L.) with a medical dose dependently decreased the MDA level and increased the GSH level in the liver, in addition to increasing the SOD activity, CAT activity, and T-AOC [28]. Our results were in accordance with these findings, and FCL could be used as a potential antioxidant.

Conclusion

Taken together, in the present study, the results of hot plate and acetic acid-induced writhing tests revealed that 250 and 500 mg/kg FCL possessed obvious antinociceptive properties. Moreover, FCLs exhibited anti-inflammatory effects, as reflected by the repair of liver injury, the decrease in WBCs and pro-inflammatory mediators, which are factors inducing the adverse effects of LPS challenge. The anti-inflammatory effects of 500 mg/kg FCL were close to those of 40 μg/kg dexamethasone. Furthermore, FCLs exhibited antioxidant effects, evidenced by the increased antioxidant enzyme activities and the decreased MDA levels, which were induced by the exposure of mice to 50% ethanol.

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Disclosure of conflict of interest

None.

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