

## Original Research Article

# Hepatoprotective and antioxidant effects of *Cymbopogon citratus* Stapf (Lemon grass) extract in paracetamol-induced hepatotoxicity in rats

Suphaket Saenthaweesuk<sup>1</sup>, Narongsuk Munkong<sup>2</sup>, Wason Parklak<sup>1</sup>, Atcharaporn Thaeomor<sup>3</sup>, Janeyuth Chaisakul<sup>4</sup> and Nuntiya Somparn<sup>1\*</sup>

<sup>1</sup>Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Pathum Thani, <sup>2</sup>Department of Medicine, School of Medicine, University of Phayao, Phayao, <sup>3</sup>School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, <sup>4</sup>Department of Pharmacology, Phramongkutkiao College of Medicine, Bangkok, Thailand

\*For correspondence: **Email:** [nuntiya\\_tom@hotmail.com](mailto:nuntiya_tom@hotmail.com); **Tel/Fax:** +66-2926-9710

Received: 4 July 2016

Revised accepted: 5 December 2016

## Abstract

**Purpose:** To investigate the protective effect of *Cymbopogon citratus* Stapf. (CS, lemongrass) extract on paracetamol (PCM)-induced hepatotoxicity in rats.

**Methods:** The rats were orally administered CS extract (1000 mg/kg/day) for 30 days prior to induction of hepatotoxicity by a single oral administration of PCM (3 g/kg). Hepatoprotection was assessed by measuring the level of hepatic markers including aspartate transaminase (AST), alanine transaminase (ALT) and oxidant/antioxidant markers including Malondialdehyde (MDA), protein carbonyl, and glutathione (GSH) in liver homogenate and serum. Phytochemical screening of the CS extract was also performed.

**Results:** Phytochemical screening of the extract indicate the presence of tannins, flavonoids, and phenolic compounds. Elevation of serum AST, ALT, and MDA levels along with depletion GSH in the liver were observed in rats treated with PCM alone compared with control ( $p < 0.05$ ). Pre-treatment of the animal with CS extract reduced the levels of hepatic markers (AST and ALT). Pre-treatment with CS extract also significantly reduced oxidative stress induced by PCM as shown by an increase in GSH level and reduction of MDA compared to rats treated with PCM alone ( $p < 0.05$ ).

**Conclusion:** The results indicate that CS possesses antioxidant activity and it exerts its effect by reducing lipid peroxidation and restoring GSH. Pre-treatment with CS extract reduces oxidative stress and ameliorates hepatic injury induced by PCM.

**Keywords:** *Cymbopogon citratus*, Antioxidant, Oxidative stress, Hepatotoxicity

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Paracetamol (PCM) is an over-the-counter drug commonly used for its analgesic and antipyretic activities. At therapeutic doses, PCM is considered safe. However, PCM overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by liver enzymes to N-acetyl-p-benzoquinoneimine (NAPQI), a

toxic metabolite that can be detoxified by glutathione (GSH), which results in extensive hepatic GSH depletion and oxidative stress [1]. GSH depletion leads to lipid peroxidation, which further leads to the initiation of liver damage. Several studies have demonstrated that the antioxidant properties of certain medicinal plants, such as ginseng, soybean, and pomegranates,

can help provide substantial protective and health benefits against oxidative stress [2,3].

*Cymbopogon citrates* Stapf. (CS), commonly known as lemongrass, is a widely used herb in Southeast Asia. Studies have reported the pharmacological properties, including free radical scavengers, anti-oxidant, anti-inflammatory and anti-mutagenicity properties, of specific parts of CS [4]. Moreover, the hypoglycemic and hypolipidemic effects of fresh CS leaf extracts have been demonstrated [5]. Therefore, the present study investigates the antioxidant potential of CS against PCM-induced hepatotoxicity in rats.

## EXPERIMENTAL

### Chemicals and drugs

Paracetamol, 2, 2-dipheneyl-1-picrylhydrazil (DPPH), thiobarbituric acid (TBA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

### Plant material and identification

Fresh CS was procured from a local agricultural field in Khon Kaen Province, Thailand in November 2014. The plant was authenticated by Ms. Dujhathai Anekchai, a taxonomist from the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla, Thailand. A voucher specimen (no. SKP 081030301) was deposited in the herbarium of the same institution.

### Extraction and phytochemical screening

Whole plants were washed thoroughly in water, cut into small pieces, soaked in 50 % ethanol (1 part of plant material: 2 parts of 50 % ethanol) for 72 h, and then filtered. The filtrate was concentrated using rotary vacuum evaporation and then lyophilized with a freeze dryer. The yield of the extract was 1.86 %. The extract was kept at  $-80^{\circ}\text{C}$  before its screening for the presence of tannins, flavonoids, and phenolic compounds [6].

### Total phenolic content assay

The CS extract was assayed for total phenolic content using the Folin-Ciocalteu reagent according to a previously described method [6]. Gallic acid was used as a standard, and the results were expressed as gallic acid equivalent per gram CS extract.

### 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical-scavenging activity was determined as per the previously described method [7]. Briefly, 0.08 mM DPPH was prepared in 100 % ethanol. DPPH solution, Tris buffer, and 80 % ethanol were mixed in order to obtain a 1:1:1 ratio for 1.8 mL. Then the plant extract (0.6 mL) was added and incubated for 30 min in the dark. The absorbance was read at 525 nm.

### Animals and experimental design

The animals were divided into 6 groups, each of which consisted of 6 rats that were treated as follows:

- Group I: (Control) distilled water (DI)
- Group II: (PCM only) DI + PCM
- Group III: (positive control) DI + PCM followed by N-acetylcysteine (NAC).
- Group IV: DI + PCM followed by CS extract
- Group V : CS extract
- Group VI: CS extract followed by PCM

DI (1 mL/kg) and CS (1,000 mg/kg) were administered orally once daily for 30 consecutive days, and then, the animals were administered with a single oral dose of PCM (3 g/kg) on the 31<sup>st</sup> day. Groups III and IV received NAC (1 g/kg) or CS (1,000 mg/kg) orally after the single oral dose of PCM.

At 24 h after PCM administration, all the animals were anesthetized and euthanized. Blood was collected by cardiac puncture, and the liver tissue was dissected for biological and histological evaluation. The protocol for the present study was approved by the Animal Research Committee of Thammasat University, Thailand (license no. AE 005/2015). All institutional and national guidelines for the care and use of laboratory animals were followed in this study [8].

A single dose of PCM was selected according to the previous reports in the hepatotoxicity-induced rat model [9,10]. The dose of extract (1000 mg/kg) used in the present study was selected based on our preliminary study on serum total antioxidant capacity (TAC). Serum TAC, which is considered as the cumulative action of all the antioxidants, was demonstrated to be significantly elevated only in 1000 mg/kg CS treated rat ( $1.74 \pm 0.45$  mM trolox equivalent) compared to the control group ( $1.05 \pm 0.75$  mM trolox equivalent). However, the doses of 250 mg/kg and 500 mg/kg ( $1.15 \pm 0.77$  and  $1.10 \pm 0.87$  mM trolox equivalent, respectively) did not

demonstrate the significant changes in the serum TAC.

### Acute toxicity studies

Thirty-six male Sprague-Dawley rats, weighing 200 - 250 g, were used for the acute toxicity study. They were allowed to acclimatize for seven days to the laboratory condition before the experiment. The procedure was performed according to OECD 425 guidelines [11], with respect to the guidelines that the volume of administration should not exceed 1 mL/100 g body weight. The maximum concentration of CS dissolved in aqueous solution is 1000 mg/mL. Therefore, our limit test dose is 2000 mg. The rats received 2,000 mg/kg CS extract orally. On testing the first dose with one animal, we found that the animal survived. Thus, the remaining animals ( $n = 4$ ) were administered with 2,000 mg/kg CS extract. They were continuously observed for 48 h to detect the changes in autonomic or behavioral responses. Any mortality or behavioral changes during the experimentation period of 15 days were also recorded. All the animals survived during the study period, and we could not increase the test dose due to the limits of the dissolution. We, thus, anesthetized and euthanized the animals. Blood was collected by cardiac puncture, and liver tissue was dissected out for biological and histological evaluation. The  $LD_{50} > 2000$  mg/kg body weight may be assumed.

### Evaluation of antioxidant activity

Malondialdehyde (MDA), a marker of lipid peroxidation, was assayed in plasma as thiobarbituric acid reactive products (TBAR) [12]. In brief, 150  $\mu$ L of plasma was reacted with 125  $\mu$ L of 10 % TCA, 125  $\mu$ L of 5 mM EDTA, 125  $\mu$ L of 8 % SDS, and 10  $\mu$ L of 0.5  $\mu$ g/mL of an antioxidant, butylhydroxytoluene (BHT), to prevent auto-oxidation during the assay. The mixture was left for 10 min, then 435  $\mu$ L of 0.6 % thiobarbituric acid (TBA) was added, and the mixture was heated for 30 min in a boiling water bath. After cooling to room temperature, 10,000 g of the mixture was centrifuged at 25  $^{\circ}$ C for 10 min. The absorbance of the supernatant was measured at 532 nm. A standard curve of MDA was generated by using appropriate concentrations of standard 1,1,3,3-tetraethoxypropane (TEP) (0.3 - 10  $\mu$ mol/L).

Serum protein carbonyl was assessed with protein carbonyl content assay kit (MAK094, Sigma Chemical Company (St. Louis, MO, USA)) while the GSH content in liver was assessed with

glutathione assay kit (CS0260, Sigma Chemical Company (St Louis, MO, USA)).

### Measurement of liver and kidney function markers

The blood of animals from both acute toxicity test and hepatoprotective study was collected by cardiac puncture. 10 mL of whole blood was allowed to clot in a conical tube at room temperature. After coagulation, the blood was centrifuged at 2,000 rpm 4  $^{\circ}$ C for 10 min to separate the serum. The levels of liver function markers, including kinetic assay of enzyme aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and kidney function markers, including blood urea nitrogen (BUN) and serum creatinine (Cr), were measured using automated machine analyzer at The Clinical Pathology Laboratory Unit, Thammasat University Hospital.

### Histopathological examination of rat liver

All animals from the acute toxicity test and the hepatoprotective study were anesthetized and euthanized. The liver tissue was dissected out, washed in ice cool PBS pH 7.4, fixed in 4 % paraformaldehyde, and embedded in paraffin. The serial sections were cut 5  $\mu$ m thick and stained with hematoxylin-eosin (H&E) and examined under a photomicroscope.

### Statistical analysis

Data are presented as mean  $\pm$  SEM. Analysis of variance (ANOVA) with Duncan post-hoc test was used to determine significant differences between each experimental group. An ANOVA on rank test was also performed for the non-parametric test. The level of significance was set at  $p < 0.05$  using SigmaStat software version 4 (Systat Software, Inc. California, USA).

## RESULTS

### Antioxidant properties

The CS extract contain several chemicals with antioxidant properties including polyphenolic compounds. The *extract* contained phenolic compounds 1,400.10  $\pm$  0.47 mg of gallic acid equivalents per gram CS extract. The free radical scavenging activity assessed by DPPH assay gave  $IC_{50}$  of 168.77  $\pm$  3.32  $\mu$ g/mL, which is relatively lower than that of BHT with  $IC_{50}$  of 12.34  $\pm$  1.14  $\mu$ g/mL.

### Acute toxicity of the CS extract

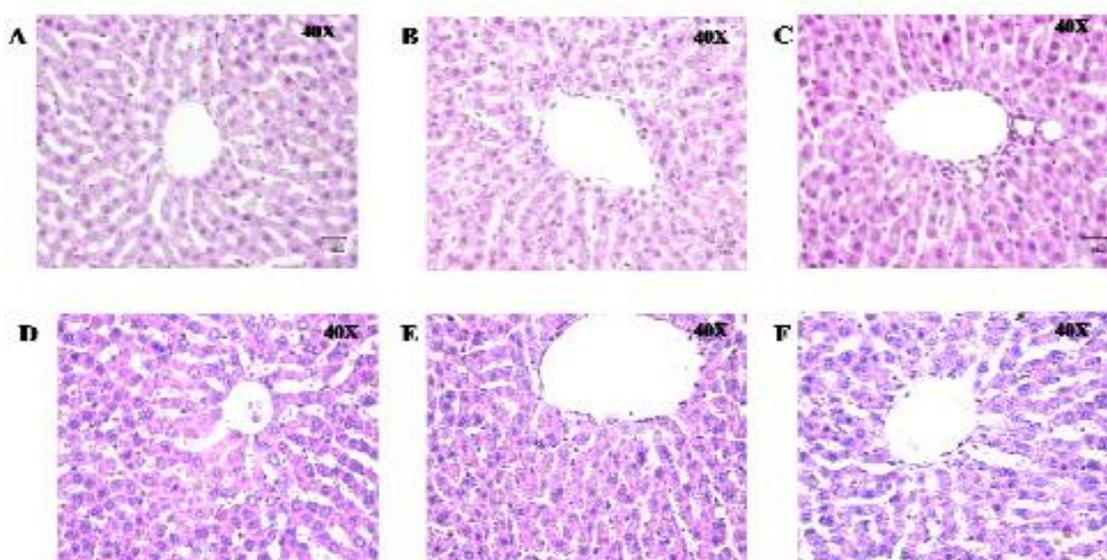
After receiving a single dose of 2,000 mg/kg, the rats showed no mortality or any clinical signs during the experimental period. In addition, treatment with a single dose of CS extract did not

affect the serum markers of liver and kidney function. The serum AST, ALT, BUN, and Cr levels were normal (Table 1), and no abnormality of the liver tissue was observed (Figure 1). It may be assumed that the LD<sub>50</sub> of CS is > 2000 mg/kg.

**Table 1:** Effect of CS extract on serum markers of PCM-induced toxicity rats

Treatment	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Cr (mg/dL)
Control	37.6±4.2	87.5±10.2	23.6±4.3	0.24±0.01
PCM alone	42.2±1.6	159±9.8 <sup>#</sup>	23.4±5.4	0.32±0.01
PCM + NAC	36.3±4.8*	95±4.6*	20.01±4.6	0.22±0.02*
PCM + CS extract (single dose)	38.5±9.5	156.7±12.6 <sup>#</sup>	21.6±6.6	0.22±0.01*
CS extract (30 days)	36.4±3.3	91±6.5	31.8±5.4	0.32±0.11
CS extract (30 days) + PCM	38.5±4.8	119.5±9.4*	20.8±2.1	0.24±0.02*

Values are mean ± SEM (n = 6); <sup>#</sup>significant difference when compared with control (p<0.05); \*significant difference when compared with PCM alone (p <0.05)



**Figure 1:** Histopathological examination of the rat liver. Representative slides from corresponding groups are shown. (A) Normal control; (B) liver section from acute toxicity test using a single dose CS extract of 2000 mg/kg; (C) liver from rat treated with CS extract 1,000 mg/kg/day, for 30 consecutive days. There were no abnormalities of the liver tissue in any rat treated with CS extract compared with normal control; (D) Liver tissue from PCM (3 g/kg) alone reveals cellular infiltration and necrosis (black arrow) ; (E) PCM (3 g/kg) + NAC (1 g/kg); and (F) Pre-treatment with CS extract 1,000 mg/kg/day, for 30 days + PCM (3 g/kg) showed healing of the liver and less degree of cellular damage

### Effect of CS extract on the serum markers of liver and kidney function

Administration of the CS extract alone for 30 days did not affect liver and kidney function as shown by the normal serum AST, ALT, BUN and Cr levels in rats group V (Table 1). No abnormality of the liver tissue was observed in any rat treated with CS extract for 30 days. These findings indicated that the CS water extract administration in rats was generally safe

even with at a dose up to 1,000 mg/kg/day for 30 consecutive days.

Treatment of rats with PCM alone showed a significant increase in serum ALT and AST levels compared with normal control rats, indicating that the hepatotoxicity was induced by PCM. Pre-treatment with 1,000 mg/kg/day of the CS extract for 30 days or treatment with NAC significantly reduced the elevated serum AST and ALT levels (p < 0.05) (Table1). The results indicated a

**Table 2:** Levels of glutathione (GSH), protein carbonyl and malondialdehyde (MDA) in rats treated with CS and/or PCM

Parameter	Protein carbonyl (mmole/mg protein)	MDA (mmole/mg protein)	GSH (mmole/mg protein)
Control	0.72±0.01	15.39±1.98	262.58±48.93
PCM alone	0.92±0.06 <sup>#</sup>	34.01±2.65 <sup>#</sup>	135.80±24.56 <sup>#</sup>
PCM + NAC	0.64±0.04 <sup>*</sup>	12.27±1.96 <sup>*</sup>	223.84±22.09 <sup>*</sup>
PCM + CS extract (single dose)	0.66±0.03 <sup>*</sup>	16.72±4.03 <sup>*</sup>	197.10±33.12 <sup>*</sup>
CS extract (30 days)	0.71±0.02	18.19±2.23	244.95±18.67
CS extract (30 days) + PCM	0.70±0.08 <sup>*</sup>	16.46±3.27 <sup>*</sup>	187.14±23.98 <sup>*</sup>

Values are mean ± SEM (n = 6); <sup>#</sup>significant difference when compared with control (p < 0.05); <sup>\*</sup>significant difference when compared with PCM alone (p < 0.05)

hepatoprotection of CS extract against the hepatotoxic effect of PCM.

### Effect of CS extract on oxidant and antioxidant status

The serum MDA level was measured as a marker of lipid peroxidation, and protein carbonyl was measured as a marker of protein oxidation. It was found that MDA and protein carbonyl levels were significantly elevated in animals treated with PCM compared to control animals (p < 0.05) (Table 2). Lipid peroxidation and protein oxidation levels were reduced by pre-treatment with the CS extract. In addition, depletion of liver GSH was found in rats treated with PCM compared with control (p < 0.05). Pre-treatment with CS extract showed an elevation of liver GSH content compared to the PCM treated alone (Table 2).

### Effect of CS extract on liver histology

When compared with control rats, the liver section of PCM-treated rats exhibited cellular infiltration and necrosis. Pre-treatment with CS extract showed healing of the liver and less degree of cellular damage, whereas the treatment with NAC can prevent the cellular damage (Figure 1).

## DISCUSSION

PCM is the most common drug used for pain and fever. Generally, it is safe at the recommended dose; however, its long-term usage or abuse by taking high dose can induce hepatocellular damages or necrosis [13]. Hepatotoxicity of PCM has been attributed to the formation of toxic metabolites called NAPQI, which is activated by hepatic cytochrome p450. Initially, NAPQI is detoxified with reduced glutathione (GSH).

However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or

sulfhydryl group of proteins. GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from polyunsaturated fatty acid, which ultimately damages liver tissue [13,14].

One of the end-products of lipid peroxidation is MDA. The increase in MDA level in liver, which can be induced by PCM intake, suggests enhanced lipid peroxidation and is an indicator of hepatic tissue damage [14,15]. Beside the depletion of glutathione, NAPQI can exert initial cell stress through a wide range of mechanisms including binding to enzymes, lipids, nucleic acids, and other cell structures [16]. After cellular damages, hepatic enzymes including AST and ALT are released into circulation. A significant elevation of AST and ALT levels is, thus, often found in PCM intoxication with hepatocellular injury [17,18].

In the present study, treating rats with high-dose PCM alone resulted in the significant increases in AST, MDA, and protein carbonyl, and a significant decrease in GSH level. It is interesting to discover that rats treated with CS extract (both PCM followed by single dose of CS and 30-day CS followed by PCM) had a significant reduction of lipid peroxidation and resumption of GSH level compared to rats treated with PCM alone. The degree of liver tissue damage as measured by the level of AST enzyme was also significantly improved among rats treated with CS extract compared to rats treated with PCM alone. These results are consistent with the histopathological findings of the liver tissue from the tested rats.

CS is an edible herb commonly used worldwide, and its safe consumption is historically appreciated. Consumption of CS water extract for 30 days in our study showed its safety profile in the experimental animals at the dose up to 1000 mg/kg/day. In line with other studies, we found that lemongrass contains alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids, and tannins [19]. We also demonstrated the antioxidant activity of the CS

extract. This radical scavenging activity and antioxidant effect could be due to the presence of one or more active principles in the plant.

Several studies showed that plants extracts containing antioxidants exhibit the hepatoprotective activity through the regulatory action on cellular permeability, stability, and suppressing oxidative stress [20-22]. Certain flavonoids have been demonstrated to have a protective effect on liver attributable to their antioxidant properties [20-22]. Tannins, which have potent antioxidants and anti-inflammatory activities, have been reported to reduce the levels of lipid peroxide in liver cells and thus, prevent hepatocytes from the destructive effects [23]. Phenolic compounds were also reported as the important active ingredients of natural products that are responsible for the antioxidant effect [24-27]. Findings from our phytochemical analysis showed that the water extracts of CS contained phenolic compounds and tannins. It is likely that these compounds contribute to the hepatoprotective effects of the extract. However, the complete phytochemical constituents of the CS extract, and their mechanism of action needs further investigated.

## CONCLUSION

The results suggest that treatment with CS water extract exhibits antioxidant effect by reduction of lipid peroxidation and induction of GSH levels, thereby, ameliorating the liver injury induced by PCM.

## DECLARATIONS

### Acknowledgement

This work was supported by a grant (no. TP 2/31/2015) from Thammasat University, Thailand. The authors would like to acknowledge Dr Pholawat Tingpej and Mr Sebastien Maury for their help in proofing and editing the manuscript.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

## Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

## REFERENCES

1. Reid AB, Kurten RC, McCullough SS, Brock RW, Hinson JA. Mechanisms of acetaminophen-induced hepatotoxicity: role of oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. *J Pharmacol Exp Ther* 2005; (312): 509-516.
2. Greenwald P, Milner JA, Anderson DE, McDonald SS. Micronutrients in cancer chemoprevention. *Cancer Metastasis Rev* 2002; (21): 217-230.
3. Surh YJ, Ferguson LR. Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential-highlights of a symposium. *Mutat Res* 2003; (523-524): 1-8.
4. Suaeayun R, Kinouchi T, Arimochi H, Vinitketkumnuen U, Ohnishi Y. Inhibitory effects of lemon grass (*Cymbopogon citratus* Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. *Carcinogenesis* 1997; (18): 949-955.
5. Adeneye AA, Agbaje EO. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus* Stapf. in rats. *J Ethnopharmacol* 2007; (112): 440-444.
6. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agric Food Chem* 2003; (51): 609-14.
7. Okawa M, Kinjo J, Nohara T, Ono M. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biol Pharm Bull* 2001; (24): 1202-1205.
8. Kunjara P, Chatikavanij P. Research animals for scientific purposes in Thailand: Ethics & policies. *Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences. AATEX Special Issue* 2007; (14): 189-191.
9. Handa SS, Sharma A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. *Indian J Med Res* 1990; (92): 284-292.
10. Mayuren C, Reddy VV, Priya SV, Devi VA. Protective effect of Livactine against CCl<sub>4</sub> and paracetamol induced hepatotoxicity in adult Wistar rats. *N Am J Med Sci* 2010; (2): 491-495.

11. OECD. *Guidelines for testing chemicals, Guidelines 425, Acute Oral Toxicity: Acute Toxic Class Method* Paris, 2008.
12. Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S, Hadley M. A comparative evaluation of thiobarbituric acid methods for the determination of malondialdehyde in biological materials. *Free Radic Biol Med* 1993; (15): 353-363.
13. Vermeulen NP, Bessems JG, Van de Straat R. Molecular aspects of paracetamol-induced hepatotoxicity and its mechanism-based prevention. *Drug Metab Rev* 1992; (24): 367-407.
14. Savides MC, Oehme FW. Acetaminophen and its toxicity. *J Appl Toxicol* 1983; (3): 96-111.
15. Jaeschke H, Williams CD, McGill MR, Xie YC, Ramachandran A. Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. *Food Chem Toxicol* 2013; (55): 279-289.
16. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther* 1973; (187): 211-217.
17. Kuvandik G, Duru M, Nacar A, Yonden Z, Helvacı R, Koc A, Kozlu T, Kaya H, Sogut S. Effects of erdosteine on acetaminophen-induced hepatotoxicity in rats. *Toxicol Pathol* 2008; (36): 714-719.
18. Madhavan V, Pandey AS, Murali A, Yoganarasimhan SN. Protective effects of *Capparis sepiaria* root extracts against acetaminophen-induced hepatotoxicity in Wistar rats. *J Complement Integr Med* 2012; (9): Article 1.
19. Avoseh O, Oyedeji O, Rungqu P, Nkeh-Chungag B, Oyedeji A. *Cymbopogon* species; ethnopharmacology, phytochemistry and the pharmacological importance. *Molecules* 2015; (20): 7438-7453.
20. Liu DD, Chen C, Li RW. Protective effect of flavonoids from *pericarpium citri reticulatae* (chenpi) against oxidative stress induced by exhaustive exercise. *Afr J Microbiol Res* 2011; (5): 50-56.
21. Zhong MM, Chen FH, Yuan LP, Wang XH, Wu FR, Yuan FL, Cheng WM. Protective effect of total flavonoids from *Bidens bipinnata* L. against carbon tetrachloride-induced liver injury in mice. *J Pharm Pharmacol* 2007; (59): 1017-1025.
22. Sasaki N, Toda T, Matsuo M. Protective effect of flavonoids on the cytotoxicity of linoleic acid hydroperoxide toward rat phenochromocytoma PC12 cells. *Free Radical Bio Med* 2002; (33): S268-S268.
23. Gali H, Perchellet E, Makkar H, Perchellet J. Ability of tannins extracted from the leaves of various trees and shrubs to inhibit the biomarkers of tumor promotion in mouse skin in vivo. *Int J Oncol* 1996; (9): 801-809.
24. Diaz P, Jeong SC, Lee S, Khoo C, Koyyalamudi SR. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. *Chin Med* 2012; (7): 26.
25. Sieniawska E, Baj T, Los R, Skalicka-Wozniak K, Malm A, Glowniak K. Phenolic acids content, antioxidant and antimicrobial activity of *Ligusticum mutellina* L. *Nat Prod Res* 2013; (27): 1108-1110.
26. Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB. Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. *Int J Mol Sci* 2010; (11): 2362-2372.
27. Adedapo AA, Jimoh FO, Afolayan AJ, Masika PJ. Antioxidant activities and phenolic contents of the methanol extracts of the stems of *Acokanthera oppositifolia* and *Adenia gummifera*. *BMC Complement Altern Med* 2008; (8): 54.