Effect of Aqueous Extract from *Cleistocalyx nervosum* on Oxidative Status in Rat Liver

**Taya S, Punvittayagul C, Chewonarin T, Wongpoomchai R.**

Department of Biochemistry, Faculty of Medicine, Chiang Mai University.

**ABSTRACT**

*Cleistocalyx nervosum* var. *paniala*, Ma-kiang, is a local plant in northern region of Thailand presenting high antioxidant activity in vitro. Our previous study found that 5 g/kg bw of *C. nervosum* aqueous extract had no acute toxic effect on rat. The present study was designed to determine effect of aqueous extract of *C. nervosum* on oxidative status in rat liver. Male Wistar rats were divided into 3 groups. Rats in group 1 were received water as a vehicle control, while group 2 and 3 were received 100 and 500 mg/kg bw of aqueous extract via intragastrium 5 times a week for 4 weeks. At the indicated time, the effect of *C. nervosum* on oxidative stress and antioxidant system were evaluated. Aqueous extract of *C. nervosum* did not affect the level of total glutathione, glutathione peroxidase and catalase activities. Low dose of *C. nervosum* (100 mg/kg bw) significantly increased malondialdehyde formation but high dose (500 mg/kg bw) did not. However, 500 mg/kg bw of *C. nervosum* extract significantly enhanced heme oxygenase-1 activity. Although the aqueous extract of *C. nervosum* at low dose exhibited the pro-oxidant effect but at high dose, it reduced oxidative stress in rat liver. In conclusion, the aqueous extract of *C. nervosum* might show biphasic effect on oxidative status of rat liver.

**Keywords:** Antioxidant, *Cleistocalyx nervosum*, liver, Ma-kiang and oxidative stress
INTRODUCTION

Epidemiological studies suggest that the consumption of fruits and vegetables reduces the risk of cardiovascular diseases, diabetes, arthritis and cancer due, at least in part, to their antioxidant and anti-inflammatory activities.\(^1\)\(^2\) Recent studies have shown flavonoids and related polyphenols in fruits and vegetables contribute significantly antioxidant activity.\(^3\) Anthocyanins are flavonoids containing antioxidant properties in certain colorful fruits, such as grapes and cranberries.\(^4\) Many studies have shown that anthocyanins may have potential effects in reducing the risk of cardiovascular diseases and cancer by antioxidant, anti-inflammatory and chemoprotective properties.\(^5\)\(^6\)

*Cleistocalyx nervosum* var. *paniala*, Ma-kiang, is found growing in scatter locations in some villages of the northern provinces of Thailand. The rich purplish red color of *C. nervosum* was characterized by an anthocyanin profile and a major compound was cyanidin 3-glucoside.\(^7\) Our previous study found that *C. nervosum* had no acute toxic effect on rat.\(^8\) There is no report on biological activity of *C. nervosum*. Then, the present study was designed to determine effect of aqueous extract of *C. nervosum* on antioxidant enzymes in rat liver.

![Figure 1 Fruit of Cleistocalyx nervosum var. paniala (Ma-kiang)](image)

MATERIALS AND METHODS

**Extraction of *C. nervosum***

The fruit of *C. nervosum* (Figure 1) was collected from Tambol Choeng Doi, Amphur Doi Saket Chiang Mai, Thailand, in July-August, 2008. Fresh ripe fruit of *C. nervosum* was extracted with distilled water and then dried by lyophilizer. The aqueous extract was kept in dark at -20 °C until used to determine antioxidant activity *in vivo*.

**Animals**

Male Wistar rats, weight ranged 150 - 180 g were obtained from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. They were housed under standard environmental conditions of temperature at 24 °C under a 12 hr dark-light cycle, and allowed free excess to drinking water and pelleted diet. An experimental protocol was approved by The Animal Ethics Committee of Faculty of Medicine, Chiang Mai University.

The male Wistar rats were divided into 3 groups of five animals each with an initial body weight of 199±0.58 g. Rats in group 1 were received water as a vehicle control, while group 2 and 3 were received 100 and 500 mg/kg bw of aqueous extract via intragastrium 5 times a week for 4 weeks. Body weight and food intake were recorded weekly. At the end of the experiment, rats were food deprived for 12 h, and then were anesthetized by diethyl ether. Liver tissue was immediately excised, rinsed with 0.9% NaCl solution, and frozen in liquid nitrogen. The frozen pieces of liver tissue were kept at -80 °C until the determination of oxidative status and antioxidant system.

**Liver tissue preparation**

The liver was homogenized with potassium phosphate buffer containing potassium chloride, EDTA and PMSF as a protein inhibitor. Liver homogenate was centrifuged at 14,000 rpm at 4 °C for 20
The supernatant was transferred into a fresh cleaned tube and centrifuged at 100,000 g at 4 °C for 1 h. The supernatant and pellet were separately collected which were represented the cytosolic and microsomal fractions, respectively.

**Assay of lipid peroxidation products in liver tissues**

The quantitative estimation of lipid peroxidation was performed by determining the concentration of malondialdehyde (MDA) in the liver using TBARs assay which described by the method of Ohkawa et al. The amount of TBARs was quantified and used as an index of lipid peroxidation. The results were expressed as pmol of MDA per milligram protein.

**Determination of glutathione content**

Total glutathione was determined by using glutathione recycling system. The conjugation of total glutathione and DTNB which generated yellow color of 2-nitro-5-thiobenzoic acid was measured the absorbance at 405 nm.

**Measurement of some antioxidant enzyme activities**

Catalase (CAT) activity was measured according to Aebi H. The enzyme reaction assay contained 30 mM H$_2$O$_2$, 50mM phosphate buffer (pH 7.0) and sample in a total volume of 750 μl. CAT activity was estimated by the decrease in absorbance of H$_2$O$_2$ at 240 nm and was expressed as nmol of H$_2$O$_2$ decomposed per min per mg protein.

Glutathione peroxidase (GPx) plays important role in protecting of organisms from oxidative damage. GPx reduces hydroperoxide coupling with oxidizes glutathione (GSH) to oxidized glutathione (GSSG). Then, GSSG was further reduces to GSH by glutathione reductase using NADPH as an electron donor. The decrease of NADPH is relative to GPx activity. The decrease of NADPH can be measured by absorbance at 340 nm.

Heme oxygenase (HO) is a potent antioxidant enzyme and is important in the cellular response to oxidative injury. HO is an antioxidant enzyme catalyzing degradation of heme. The end products of HO are carbon monoxide, biliverdin, and free iron. Biliverdin can be subsequently converted to bililubin by biliverdin reductase. Bilirubin levels are then measured by a spectrophotometric method using the difference in absorption at 460 and 530 nm.

**Statistical analysis**

Results were expressed as mean ± standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test.

**RESULTS**

Body weight and consumption of diet and water did not show any significant differences in treated groups when compared to control group (Data not shown). The effect of *C. nervosum* aqueous extract on antioxidant and biochemical parameters was summarized in Table 1. The MDA formation resulting in lipid peroxidation was measured in terms of the TBARs formation. The level of MDA in the liver homogenate was significantly increased in the rats treated with 100 mg/kg bw of *C. nervosum* extract when compared to the control group. However, high dose of *C. nervosum* extract (500 mg/kg bw) significantly increased heme oxygenase (HO-1) activity. The aqueous extract did not modulate on total glutathione level, glutathione peroxidase and catalase activities.

**DISCUSSION AND CONCLUSION**

In the previous study showed that the aqueous extract of *C. nervosum* contained total phenolic
The 2nd National Conference in Toxicology 17-18 December 2009

Table 1 Effect of *C. nervosum* aqueous extract on the level of malondialdehyde, total glutathione, glutathione peroxidase, catalase and heme oxygenase-1 in rat liver

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aqueous extract of <em>C. nervosum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 mg/kg bw</td>
</tr>
<tr>
<td>MDA formation (pmol/mg protein)</td>
<td>12.36 ± 4.03</td>
<td>20.60 ± 5.37*</td>
</tr>
<tr>
<td>Total glutathione (nmol/mg protein)</td>
<td>17.61 ± 3.72</td>
<td>15.94 ± 5.92</td>
</tr>
<tr>
<td>Glutathione peroxidase activity (pmol/min/mg protein)</td>
<td>25.22 ± 2.62</td>
<td>27.11 ± 4.94</td>
</tr>
<tr>
<td>Catalase activity (nmol/min/mg protein)</td>
<td>19.08 ± 2.72</td>
<td>14.04 ± 1.53</td>
</tr>
<tr>
<td>Heme oxygenase-1 activity (umol/min/mg protein)</td>
<td>820.83 ± 81.38</td>
<td>982.64 ± 164.92</td>
</tr>
</tbody>
</table>

Values are mean±SEM from 5 rats in each group. *Significantly different from control group; p<0.05.

Compounds 181.16 ± 0.59 mg gallic acid equivalents per 100 gram fresh weight and total flavonoids 54.86 ± 3.45 mg catechin equivalents per 100 gram fresh weight. The phenolic compounds found in aqueous extract of *C. nervosum* might take a part of its antioxidant capacity. The mechanism of antioxidant activity of *C. nervosum* might be involved in scavenging free radical and/or chelating iron generating Fenton reaction. Lipid peroxidation and antioxidant systems were used as important biomarkers for detection of antioxidant property of aqueous extract. Four antioxidant systems (GSH, GPx, CAT and HO-1) and MDA formation were evaluated for oxidative status. The results in the present study have demonstrated that aqueous extract of *C. nervosum* could affect both of antioxidant system and MDA formation in rat liver. It showed that oxidative stress significantly increased in rats treated with aqueous extract at a low dose (100 mg/kg bw). The increasing of oxidative stress in liver was observed in only rat received low dose of *C. nervosum* extract which might be a dose of prooxidant. Phenolic phytochemicals are not only recognized as antioxidants, but they can also exert prooxidant activities. In fact, most free-radical scavengers act in oxidation-reduction reactions that are reversible, and some, such as phenolic phytochemicals, can act both as antioxidants and prooxidants depending on their structure and the conditions. The prooxidant activity is thought to be directly proportional to the total number of hydroxyl groups. So, *C. nervosum* might exhibit an antioxidant activity at high dosage while act as a prooxidant at low dosage.

Glutathione is one of the most abundant non-enzymatic biological antioxidants present in the liver. Its functions include removal of free radicals such as hydrogen peroxide, superoxide anions and alkoxy radicals, maintenance of membrane protein thiols and acting as a substrate for glutathione peroxidase (GPx). GPx catalyses the reduction of H$_2$O$_2$ and hydroperoxides to nontoxic products. CAT catalyses the reduction of H$_2$O$_2$ and protects the tissue from highly reactive oxygen free radicals and hydroxyl radicals. The result showed that aqueous extract of *C. nervosum* did not affect on total glutathione, glutathione peroxidase and catalase activities. Other antioxidant enzyme which determined in the study is
heme oxygenase (HO-1). It mediated cytoprotection which is important for tissues that are vulnerable to oxidative stress. Aqueous extract at dosage of 500 mg/kg bw significantly increased heme oxygenase activity and also decreased MDA formation. Although at low dosage of C. nervosum extract enhance oxidative stress in rat liver, on the other hand at high dosage of C. nervosum extract might be able to reduce oxidative stress by enhancement of non-glutathione pathway such as heme oxygenase. In conclusion, the aqueous extract from C. nervosum might show biphasic effect on oxidative stress in rat liver.

ACKNOWLEDGEMENTS

This work was supported by the Endowment Fund for Medical Research (2009), Faculty of Medicine, Chiang Mai University. The authors would like to thank Ms. Pimwalun Pinthuprapa for providing C. nervosum in this study.

REFERENCES

8. Taya S, Punvittayagul C, Wongpoomchai R. Acute and subacute toxicity studies of antioxidative compounds extracted from Ma-kiang (Cleistocalyx nervosum var. paniala) in wistar rat. In The 1st CMU Graduate Research Conference; 2009 Nov 27-28; Chiang Mai, Thailand.