ABSTRACT

Cleistocalyx nervosum var. paniala, Ma-kiang, is a native plant in northern region of Thailand. So far, there is no study on the in vivo biological activities of its fruit. The present study was designed to investigate toxicity, antioxidant activity and chemopreventive effect on hepatocarcinogenesis of an aqueous extract of the pulp of C. nervosum. Some major chemical constituents and in vitro antioxidant activity of an aqueous extract of C. nervosum were performed. One hundred grams of the fresh fruit contained 181.16±0.59 mg GAE of total phenolic compounds and 54.86±3.45 mg CE of total flavonoids. The major anthocyanins in this extract were cyanidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-5-glucoside. The C. nervosum extract presented antioxidant activity in a dose-dependent manner using in vitro DPPH radical scavenging and deoxyribose assays. Its inhibitory mechanism might be involved with either scavenging free radicals or chelating iron generating Fenton reaction.

The toxicity tests in animal model were performed. In acute toxicity test, oral administration of 5000 mg/kg bw of C. nervosum extract produced neither mortality nor significant changes in behavior and gross appearance of the internal organs of male and female rats. In subacute toxicity study, no mortality was observed when the two doses of 100 and 500 mg/kg/day of C. nervosum extract were administered orally for a period of 4 weeks. There were no significantly differences in hematological analysis and clinical blood chemistry between the controls and the treated animals of both sexes. It was suggested the aqueous extract of C. nervosum containing...
antioxidant activity had no acute and subacute toxic effects on wistar rat. Furthermore, antioxidant activity of *C. nervosum* extract was confirmed in male rats. The results showed the activity of heme oxygenase in rats administrating *C. nervosum* extract for 4 weeks was significantly enhanced but the other antioxidant markers were similar among treatment group.

To study effect of *C. nervosum* extract containing antioxidant activity on oxidative stress induced early stage of hepatocarcinogenesis in rats, the protocol using the combination of diethylnitrosamine (DEN), a genotoxic carcinogen and phenobarbital (PB), a nongenotoxic carcinogen were performed. Male wistar rats were divided into 5 groups. Group 1 was a negative control receiving 0.9% NSS injection once a week for 3 weeks and tap water as drinking water throughout the experiment. Groups 2 to 5 were intraperitoneally injected with 100 mg/kg bw of DEN once a week for 3 weeks and received 500 ppm of PB in drinking water for 4 weeks. Group 2 was a positive control group receiving distilled water via gavage feeding throughout 8 weeks of the experimental period. Two weeks before the first injection, group 3 was intragastrically fed with 500 mg/kg bw of *C. nervosum* extract for 8 weeks. Group 4 and 5 were fed with 500 mg/kg bw of *C. nervosum* extract and 100 mg/kg bw of silymarin (a known antioxidant), respectively, at the same time of PB administration. The treatments of *C. nervosum* extract both prior and after initiation tended to decrease the number of GST-P positive foci in liver when compared to the positive control. Interestingly, the administration of silymarin, a known antioxidant, significantly increased the number of GST-P positive foci when compared to a positive control. The *C. nervosum* extract did not alter the number of preneoplastic lesions in the liver of DEN-, PB- induced carcinogenesis; this result might be partly due to either strong carcinogenic potency of chemicals in this model or low antioxidant capacity of the *C.nervosum* extract.

The further protocol was designed by reducing potency of carcinogens and increased concentration of the extract. Male wistar rats were divided into 4 groups. Group 1, a negative control, was intraperitoneally injected with 0.9% NSS once a week for 2 weeks and received tap water as drinking water throughout the experiment. Groups 2 to 4 were intraperitoneally injected with 100 mg/kg bw of DEN once a week
for 2 weeks and received 500 ppm of PB in drinking water for 4 weeks after the last injection. Group 2, a positive control, was intragastically fed with distilled water throughout 8 weeks of the experimental period. Two weeks before the first injection, groups 3 and 4 were intragastically fed with 500 and 1000 mg/kg bw of *C. nervosum* extracts, respectively, for 8 weeks until the end of experiment. The results showed that the number of GST-P positive foci was decreased in the liver of rats treated with 1000 mg/kg bw of the aqueous extract. The *C. nervosum* extract reduced malondialdehyde in serum and liver of rats receiving DEN and PB. It also modulated glutathione level and the activities of glutathione peroxidase, catalase and heme oxygenase.

In conclusion, the aqueous extract of *C. nervous* containing anthocyanins demonstrated cancer chemopreventive effect on chemicals induced early stages of rat hepatocarcinogenesis. The chemopreventive mechanism might be partly due to either enhancement of the antioxidant status or reduction of oxidative stress in the liver of carcinogens induced rats.
Cleistocalyx nervosum var. paniala

\[ \text{cyanidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-5-glucoside} \]

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\text{DPPH radical scavenging and deoxyribose assays} \\
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จากการวิจัยสามารถสรุปได้ว่า สารต่อเนื่องย่อยส่วนน้ำที่ประกอบด้วยสารกลุ่มแอนไฮดริช อนินนิวคลีอิทตันการเกิดเรื่องดับในระยะเริ่มต้นนั้นโดยกลไกการป้องกันที่เป็นไปได้อาจเกิดขึ้นกับการเหนื่อยง่ายระบบด้านอนุมูลอิสระและการลดภาวะเครียดออกซิเดชันในเด็กหนุ่มที่ได้รับสารก่อนเรื่อง