

Effects of curcuminoid on ethanol-induced toxicity in hepatic cells and ratsRuttiya Thongrung^{1*}, Sakonwun Praputbut¹¹Department of pharmaceutical sciences, Naresuan University, Phitsanulok 65000, Thailand

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Abstract

Alcoholic liver disease (ALD) is caused by excessive consumption of alcohol. The pathological progress of ALD involves in increasing reactive oxygen species (ROS) and nitric oxide (NO) production, cytokines secretions, and inflammatory reactions. Curcuminoid, a mixture of active substance derived from turmeric compound, has exhibited an antioxidant and anti-inflammatory properties. This study aimed to examine effect of curcuminoid on NO production in ethanol-stimulated hepatic cells and on hepatoprotective effect in ethanol-induced toxicity rats. We found that curcuminoid at lower concentration (0.313 and 0.625 µg/ml) tended to reduce NO production in the ethanol-stimulated cells. In addition, curcuminoid at concentration 500 and 750 µg/ml decreased the liver function enzymes significantly in the ethanol-induced toxicity rats. The results suggested that curcuminoid had a potential property to be use as a hepatoprotective agent in ethanol-induced hepatic toxicity.

Keywords: alcoholic liver disease, ethanol, nitric oxide, hepatoprotective agent, curcuminoid

Introduction

The excessive consumption of alcohol has been identified as the leading cause of ALD. The pathological changes of ALD range from fatty liver (steatosis), hepatic inflammation and cell injury (hepatitis), cell fibrosis (cirrhosis) and finally, hepatocellular carcinoma. (1) On the early stages, alcohol increases fatty molecule in the hepatic cells, lipid peroxidation and ROS that resulting in initiation of inflammatory process. (2) On the other hand, the direct alcohol toxicity is contributed from its metabolism pathway to ROS production. (3) Several studies have shown that NO, an interesting ROS generated from hepatic cells involve in both physiological and pathological roles in the liver. (4, 5) NO, an endogenous gas with short half-life (< 10 seconds) is synthesized from L-arginine by enzyme nitric oxide synthase (NOS). (6) Under normal condition, the hepatic cells produce low level NO to regulate vascular perfusion, however, in ALD, the large amounts of NO are produced. (7) The mechanisms of alcohol induced hepatic toxicity are complex with diverse consequences in different cell types and tissues. In addition, there is no pharmacological agent for ALD treatment. Now a day, one idea of developing hepatoprotective agent from herbal plants to reduce production of ROS is remarkably under investigation. Curcuminoid, a yellow pigment in turmeric compound is derived from *Curcuma Longa Linn* in ginger family. (8) Curcuminoid has been shown variety of pharmacological actions such as anti-inflammatory, antimicrobial, antioxidant and anticarcinogenic properties. (9, 10) Curcuminoid interacts with numerous target molecules; enzymes, transcription factors, growth factors, receptors or metals, that supports the notion of its influence of many biological cascades. (10) We are interested in the effect of curcuminoid as a hepatoprotective agent against alcohol-induced toxicity. The aim of this study was to investigate effects of curcuminoid on NO production in ethanol-stimulated cells and on aminotransferase enzymes in rats.

Methods

Cell culture: The human liver cell line, Hep G2 cells were obtained from American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM)/F12 containing 10% fetal bovine serum and 1% penicillin-streptomycin to 90% confluence.

Effect of curcuminoid on nitric oxide production: All cells were plated into 96-black well plates at density 3×10^4 cell/well for 24 hours. Then, the medium was removed and cells were pre-treated with various concentrations of curcuminoid in serum free medium for 2 hours. After that cells were added with 10% (V/V) of ethanol for 22 hours. The diaminofluorescein-2 diacetate (DAF-2 DA) was added to the wells and incubated for 30 minutes in dark. Fluorescence was read at excitation 485 nm, emission 535 nm. The cells were lysed and measured protein content by BCAkits®. (Bio-rad, Philadelphia)

Animals: Sprague-Dawley rats (weight 180-220 g) were obtained from National laboratory animal center, Mahidol University, Nakornpathom. All rats were rested 7 days before experiments. The rats were fed with regular diet and water ad libitum.

Effect of curcuminoid on ethano- induced toxicity rats: Rats were divided into 6 groups of six rats in each group. Group I was the control animal. Group II was the rats received vehicle. Group III was the rat received isocaloric 60% glucose. Group IV, V, and VI, VII, the rats were received ethanol (6 g/kg /day p.o.) for 14 weeks and on week 8th the rats were received sylimarin ((Legalon®) 100 mg/kg/day or curcuminoid 250,500 and 750 mg/kg/day respectively. Serums from the animals were analyzed for alnine aminotransferase (ALT) and aspartate aminotransferase (AST) by LFEkits® (S.E. supply, Bangkok)

Results

Effect of curcuminoid on NO production in ethanol stimulated Hep G2 cells

Hep G2 cells, stimulated with various concentrations of ethanol for 24 hours, increased NO production as a dose-dependent manger. The ethanol concentration at 7.5% and 10% v/v could induce the cells to generate amounts of NO significantly, comparing with the control cells (figure 1A.) When 10% ethanol-stimulated Hep G2 cells were pre-incubated with curcuminoid, we found that curcuminoid at lower concentration (0.313 and 0.625 µg/ml) trends to decrease NO productions. However, curcuminoid at higher concentration could enhance NO productions in the stimulated cells. (figure 1B.)

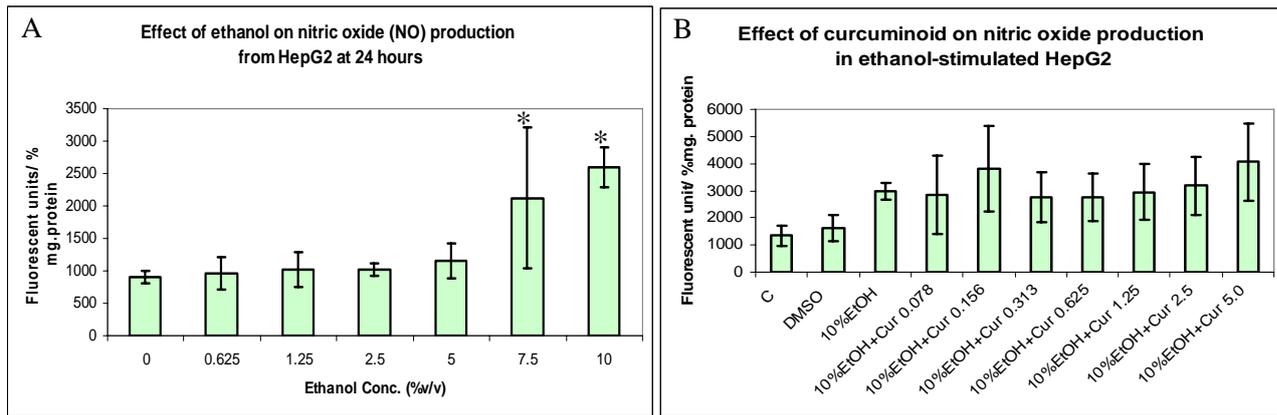


Figure 1. Nitric oxide (NO) production in ethanol stimulated Hep G2 cells. Cells were stimulated with ethanol with various concentration (0.625, 1.25, 2.5, 5.0, 7.5, and 10 % v/v) of ethanol for 24 hours (A.) The ethanol stimulated cells were pre-incubated with curcuminoid (B.) NO productions were measured by DAF-2DA reagent. Data were from 3 separated experiments (n=3) and shown as mean ± SD of fluorescent unit/%mg. protein. Data were analyzed statistic significantly by ANOVA, comparing to the control (p ≤ 0.05).

Effect of curcuminoid on aminotransferases in ethanol-induced toxicity rats

Serum ALT and AST levels were increased significantly from all groups received ethanol, comparing to the control group ($p \leq 0.05$). (figure 2A, 2B.) After that the ethanol-induced toxicity rats were received a vehicle, sylimarin (hepatoprotective agent) or various doses of curcuminoid for another 6 weeks. The results showed that curcuminoid concentration at 750 and 1000 $\mu\text{g/ml}$ decrease the serum ALT and AST levels significantly ($p \leq 0.05$) and this effect of curcuminoid seemed to be similar to sylimarin. (figure 2A, 2B.) The serum level of ALT and AST showed fewer changes in the rats received only the vehicle.

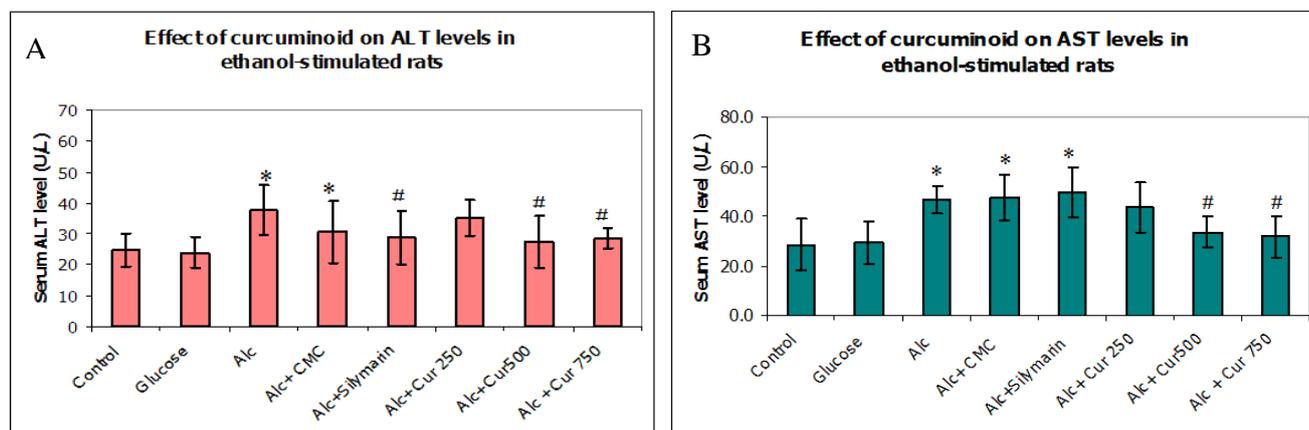


Figure 2. Effects of curcuminoid on serum ALT and AST in ethanol-induced hepatic toxicity rats. Rats were received ethanol (6 g/kg/day) for 8 weeks and then were given carboxymethylcellulose (CMC), sylimarin or curcuminoid (250, 500, 750 $\mu\text{g/ml}$) for another 6 weeks. Serum ALT and AST levels were measured with the liver function enzyme kits. Data were from 6 animals ($n=6$) and shown as mean \pm SD. Data were analyzed statistic significantly by ANOVA ($p \leq 0.05$), comparing to the control group(*) or comparing to the ethanol induced hepatic toxicity baseline (#).

Discussions

It has been demonstrated that enzyme NOS are upregulated in cirrhosis livers and they involve in pathological process. (11, 12) NO modulates different inflammatory cells and prolong cytokines secretions such as $\text{TNF-}\alpha$, resulting in hepatitis. (5) In this present study the ethanol-stimulated hepatic cells enhance NO production. When we tested the effects of curcuminoid on ethanol-stimulated cells, the results showed that curcuminoid at lower concentration trends to reduce NO production. However, curcuminoid at high concentration seemed to accelerate NO production in the cells. We also studied the effect of curcuminoid in long term ethanol-induced rats. Curcuminoid could significantly reduce the enzyme ALT and AST which are the markers for chronic hepatitis. Many studies before have been demonstrated that curcuminoid contains anti-inflammatory and antioxidant effects. (10) For example, curcuminoid has protective effect on iron-induce hepatotoxicity (13) and ethanol induced pancreatitis. (14) Our studies confimed that curcuminoid decrease ethanol-induce hepatic toxicity in rats as well as reduce NO production in ethanol-stimulated hepatic cells

Conclusion

In this study, we showed that curcuminoid in lower concentration reduce NO production in the ethanol-stimulated hepatic cells. In chronic ethanol-induced rats, curcuminoid also decreased the liver function enzymes, ALT and AST significantly. These results suggest that curcuminoid might delay the inflammatory progression in chronic hepatitis and be use as hepatoprotective agents. However, further investigation in mechanism of actions and toxicity of curcuminoid on ethanol-stimulated in vitro and in vivo are need to be done.

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