RESEARCH ARTICLES

Effect of Barakol on Cytochrome P450, UDP-Glucuronyl-transferase and Glutathione S-Transferase in Isolated Rat Hepatocytes

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Abstract

Effect of barakol at various concentrations (0.025, 0.05, 0.075, 0.10 and 0.15 mM) was studied directly in isolated rat hepatocytes by determining the activities of phase I enzyme, aminopyrine N-demethylase (CYP2B, CYP2C), and phase II enzymes including UDP-glucuronyltransferase and glutathione S-transferase. The release of cellular transaminase (ALT,AST), the reduced glutathione (GSH) contents and the extent of lipid peroxidation (as indicated by malondialdehyde (MDA) formation) were also measured and regarded as the cytotoxic criteria. Results indicated that barakol at all concentrations studied, increased the activities of aminopyrine N-demethylase and glutathione S-transferase with the reduction in UDP-glucuronyltransferase activity. Increase in the release of ALT, AST and GSH contents were found only with high concentrations of barakol (0.10 and 0.15 mM). There was no change in MDA formation. In conclusion, cytotoxicity induced by high concentrations of barakol may involve the activities of certain phase I and phase II enzymes but not the lipid peroxidation.

Key words : barakol, cytochrome P450, UDP-glucuronyltransferase, glutathione S-transferase

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บทคัดย่อ

ทำการศึกษาผลของบาราคอลในความเข้มข้นต่างๆ (0.025, 0.05, 0.075, 0.10 และ 0.15 mM) ที่มีโดยตรงต่อเซลล์ตับอิสระที่แยกจากหนูขาว โดยตรวจวัดการทำงานของเอนไซม์ใน phase I คือ aminopyrine N-demethylase (CYP2B, CYP2C) และเอนไซม์ใน phase II คือ UDP-glucuronyltransferase และ glutathione S-transferase รวมทั้งศึกษาผลที่อาจเกิดพิษต่อเซลล์ตับในขนาดที่ใช้ โดยวิเคราะห์หาปริมาณของเอนไซม์ transaminase (ALT, AST) ที่ถูกปล่อยออกจากเซลล์ ปริมาณของ reduced glutathione ที่อยู่ในเซลล์และการเกิด lipid peroxidation (ปริมาณของ malondialdehyde (MDA) ที่เกิดขึ้น) ผลการทดลองพบว่าบาราคอลในทุกความเข้มข้นมีผลเพิ่มการทำงานของเอนไซม์ aminopyrine N-demethylase และ glutathione S-transferase พร้อมกับมีผลลดการทำงานของ UDP-glucuronyltransferase ปริมาณของเอนไซม์ ALT และ AST รวมทั้ง GSH เพิ่มขึ้นเมื่อได้รับบาราคอลในความเข้มข้นสูงเท่านั้น (0.10 และ 0.15 mM) แต่ไม่พบความแตกต่างของ MDA ที่เกิดขึ้น จากผลการทดลองสรุปได้ว่าบาราคอลทำให้เกิดพิษต่อตับได้ในขนาดสูง โดยไม่สัมพันธ์กับการเกิด lipid peroxidation และอาจเกี่ยวข้องกับการทำงานของเอนไซม์ใน phase I และ phase II

คำสำคัญ: บาราคอล, ไซโตโครมพี 450, ยูดีพี-กลูคูโรนิลทรานสเฟอเรส, กลูตาไทโอน เอส-ทรานสเฟอเรส

Introduction

Barakol is an active constituent found in young leaves and flowers of *Cassia siamea* Lamk. (known as Kheelek in Thai) (Figure 1). This plant has been used in traditional medicines treating many aliments such as insomnia, asthma, hypertension, diabetes and constipation. The biological activity studies of *C. siamea* indicated a wide variety of effects on different systems including central nervous system, cardiovascular system and hepatic drug metabolism[1-5]. Acute hepatitis was reported in patients at Phramongkutklao and Chulalongkorn Memorial Hospitals in 1997 when taking 1-4 capsules/day of *C. siamea* (400 mg dried leaves/capsule) for 7-60 days[6]. From previous studies, rats feeding with 5% dietary *C. siamea* leaves for 14 days demonstrated a markedly increase in activity of phase II detoxifying enzymes, UDP-glucuronyltransferase (UGT) and glutathione S-transferase (GST) with a decrease in phase I bioactivating enzymes such as aniline hydroxylase (ANH) and aminopyrine N-demethylase (AMD)[7]. Subchronic toxicity of barakol in rats (30 mg/kg/day, po, for 90 days) showed a decrease in CYP1A2 activity in both normal and high cholesterol conditions and a decrease in CYP1A1 activity in high cholesterol diet group with no changes in CYP2B1/2B2, CYP2E1 and GST activities[8].

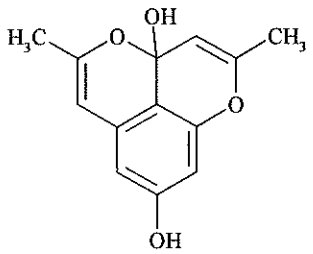

Figure 1 Chemical structure of barakol (3α,4-dihydro-3α,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene)

Since the effect of barakol on hepatic drug-metabolizing enzymes was studied mostly *in vivo*. The present investigation was aimed to examine the direct effect of barakol on phase I enzyme (AMD) and phase II enzymes (UGT and GST) using isolated rat hepatocytes as the *in vitro* model. The release of cellular transaminase (ALT, AST), the reduced glutathione (GSH) contents and the extent of lipid peroxidation (as indicated by MDA formation) were also measured and regarded as the cytotoxic criteria. This study was ethically approved by the Ethical Committee on Animal and Human Research Studies, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Materials and Methods

Test compound

A stable salt of barakol, anhydrobarakol hydrochloride was provided by Assoc. Prof. Chaiyo Chaichantipyuth, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Anhydrobarakol hydrochloride solution was freshly prepared by dissolving anhydrobarakol hydrochloride in distilled water and wrapped with aluminium foil.

Animals

Male Wistar rats (200-250 g.) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom, Thailand. Rats were kept for 3 days under standard laboratory conditions in 12 h light/dark cycle at 25 $\pm$ 2 °C before the experiment for acclimatization with free access to rodent pellet and water.

Preparation of isolated rat hepatocytes

Isolated rat hepatocytes were prepared using the method of collagenase perfusion[9,10]. Liver cell viability greater than 90% of trypan blue exclusion test was used throughout the experiment. Aliquot of cell suspension was incubated at a

density of 3 ml (5-6 x 10^6 cells/cm^3) in 25 ml Erlenmeyer flask with the desired concentrations of barakol (0.025, 0.05, 0.075, 0.10 and 0.15 mM) and distilled water for control group. The incubation was performed at 37 ^{0} C for 1 h on metabolic shaker bath and gassed with carbogen (O_2 95%, CO_2 5%).

Enzyme assays

AMD was determined by measuring formaldehyde production[11,12]. Activity of cytosolic GST was measured spectrophotometrically according to the method of Habig using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate[13]. UGT activity was assayed fluorometrically by method of Bock[14]. 1-Naphthol was used as substrate and α-naphthyl β-D-glucuronide as standard.

Cytotoxicity assays

ALT and AST activities were determined by measuring pyruvate formation using ALT & AST kits[15]. GSH content was determined by the method of Ellman and Jollow[16,17]

Lipid peroxidation in isolated rat hepatocytes was monitored by MDA formation using the thiobarbituric acid assay[18]

Statistical analysis

Data were presented as Mean ± SEM. and statistically assessed by one-way analysis of variance (ANOVA) using LSD test. Probability levels of less than 0.05 were considered significant.

Results

Exposure of isolated rat hepatocytes to 0.025-0.15 mM of barakol significantly increased AMD and GST activities (Figures 2 and 3), while UGT activity was markedly reduced (Figure 4).

From cytotoxic studies, barakol at 0.10 and 0.15 mM significantly increased ALT activity (Figure 5) and GSH content (Figure 7). AST activity was dramatically increased when incubated isolated rat hepatocytes with 0.0075, 0.10 and 0.15 mM of barakol (Figure 6). There was no change in MDA formation (Figure 8).

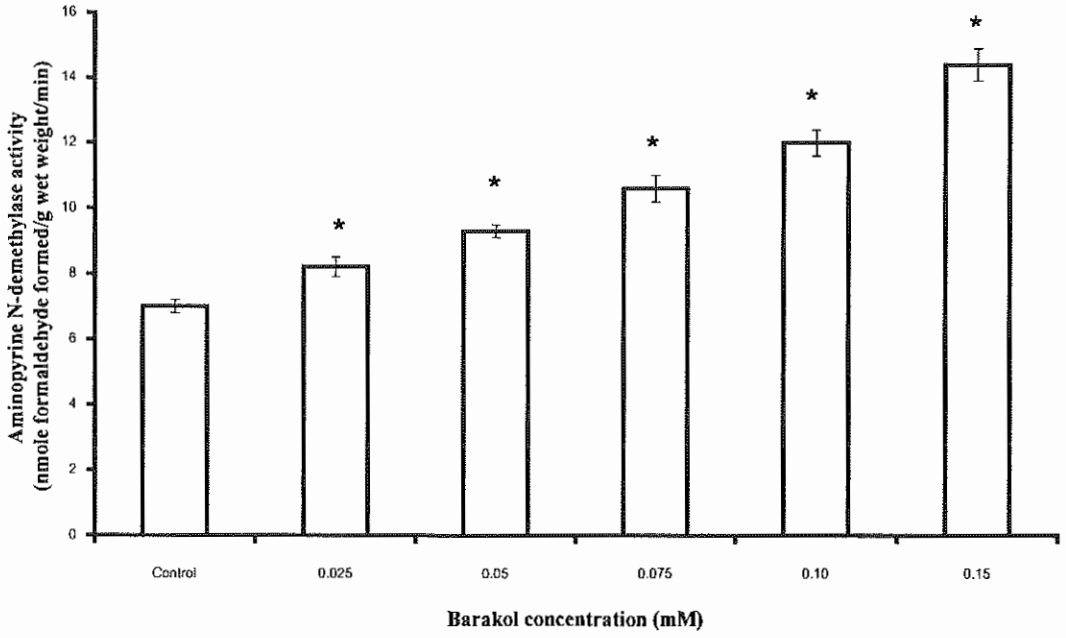

Figure 2 Effect of barakol on aminopyrine N-demethylase activity in isolated rat hepatocytes. (N=9 , *P<0.05 significant differences from the control)

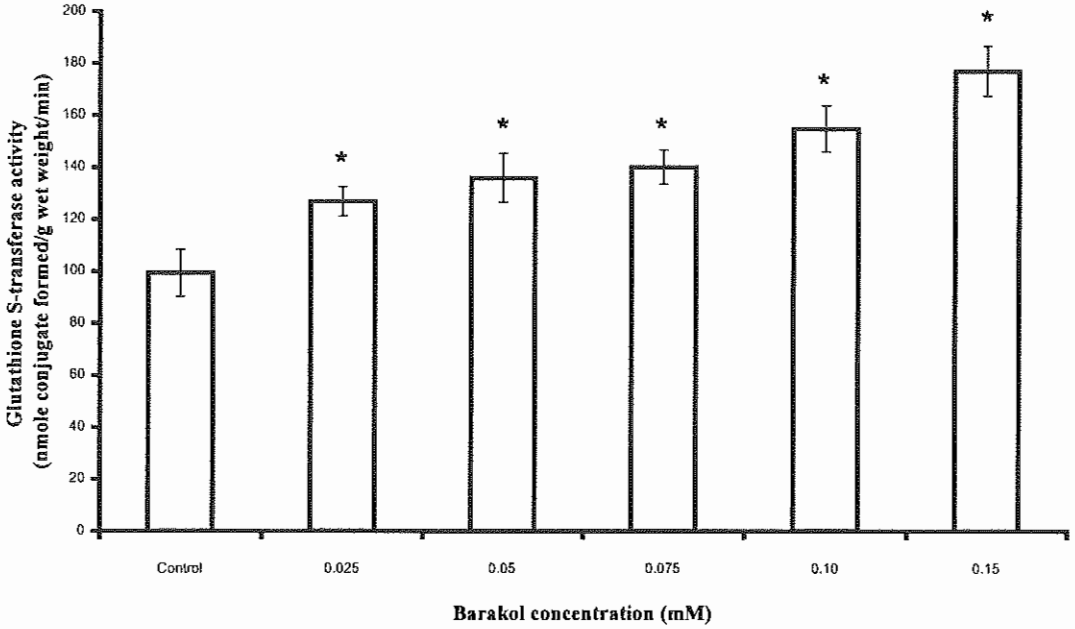

Figure 3 Effect of barakol on glutathione S-transferase activity in isolated rat hepatocytes. (N=9 , * P<0.05 significant differences from the control)

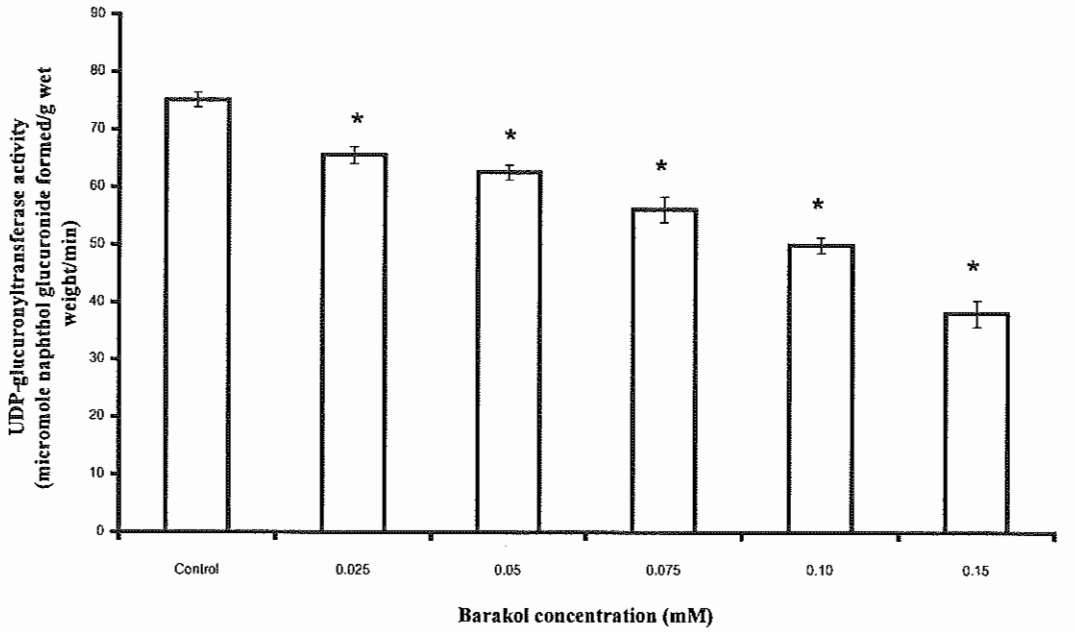

Figure 4 Effect of barakol on UDP-glucuronyltransferase activity in isolated rat hepatocytes. (N=9, * P<0.05 significant differences from the control)

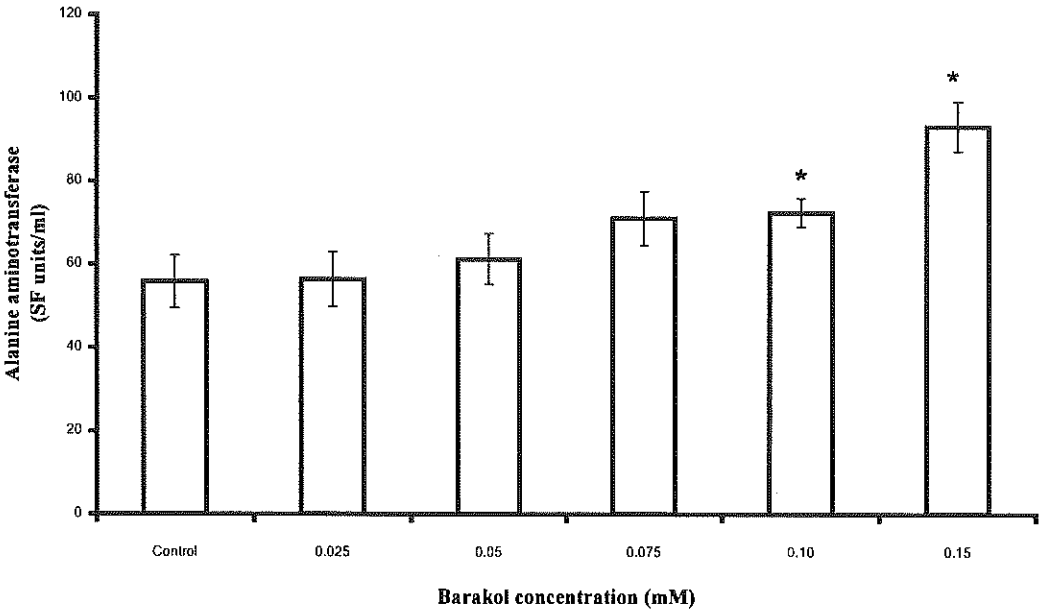

Figure 5 Effect of barakol on alanine aminotransferase activity in isolated rat hepatocytes. (N=9 , * P<0.05 significant differences from the control)

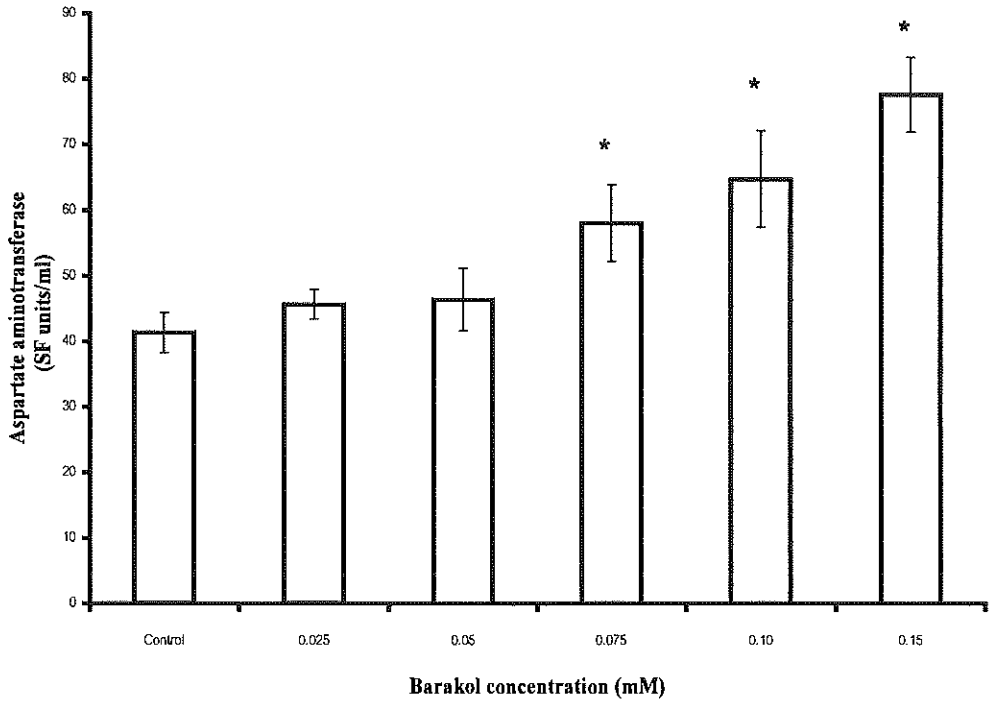

Figure 6 Effect of barakol on aspartate aminotransferase activity in isolated rat hepatocytes. (N=9 , * P<0.05 significant differences from the control)

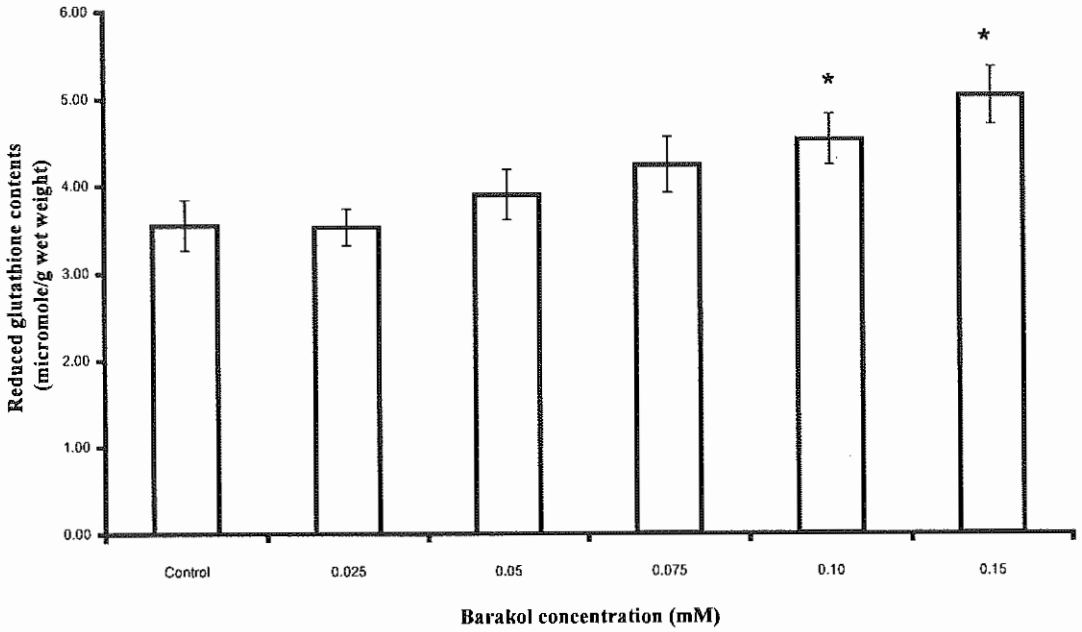

Figure 7 Effect of barakol on reduced glutathione contents in isolated rat hepatocytes. (N=10 , * P<0.05 significant differences from the control)

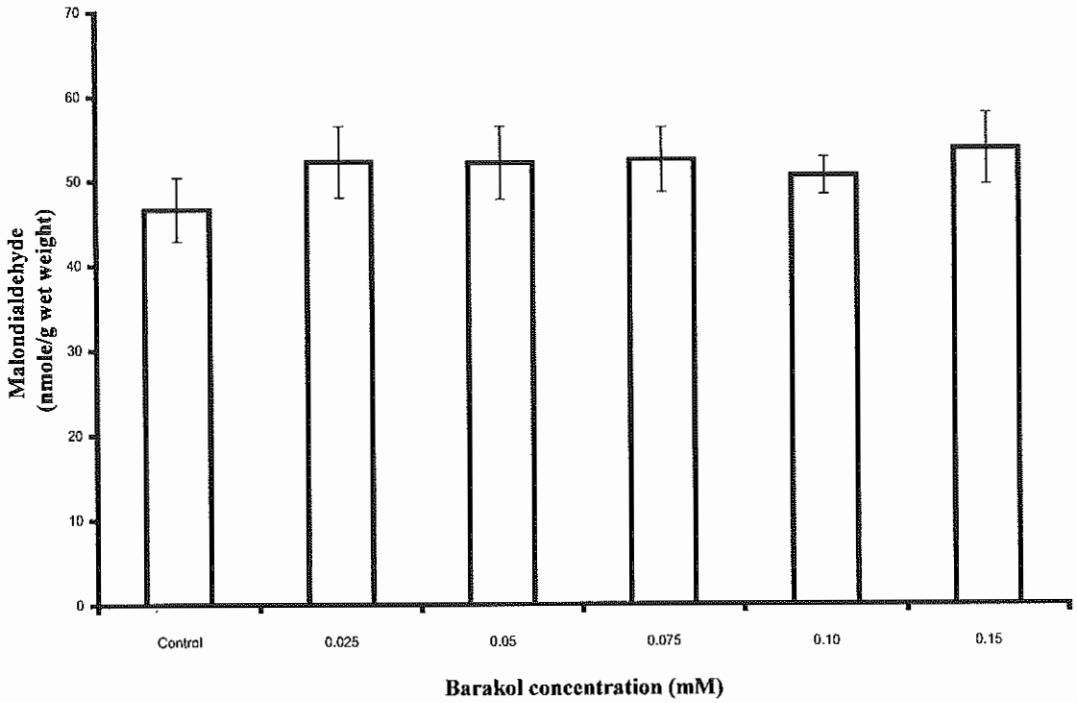

Figure 8 Effect of barakol on lipid peroxidation (as malondialdehyde formation) in isolated rat hepatocytes.(N=13)

Discussion and conclusion

In vitro experimental system, isolated rat hepatocytes have been used to investigate the direct effects of various concentrations of barakol on the activities of phase I and phase II enzymes. Moreover cytotoxic effect were observed.

From the result, all concentrations of barakol induced both AMD (as markers of CYP2B , CYP2C) and GST activities while UGT activity was reduced. Suggesting that barakol may be metabolized by CYP2B and CYP2C isoforms. The classical inducer of CYP2B and CYP2C is phenobarbital[19] which is known to accelerate its own biotransformation. So it is possible that barakol may be a substrate of CYP2B and CYP2C.

UGT activity was measured using 1-naphthol as substrate of UGT1A6 and UGT1A7 isoforms. In rats, these isozymes are induced by 3-methylcholanthrene which is also an inducer of CYP1A. Moreover, glucuronidation is substrate specific reaction and its rate is substrate-dependent [20]. From this point of view, 1-naphthol may not be a specific substrate of UGT isozymes which metabolized barakol.

All GST activities were significantly increased. Conversely, GSH, the conjugating agent of glutathione conjugation was increased at high concentrations of barakol. This result suggested that barakol may not be a substrate of GST. The induction of GST activity by barakol needs further clarification.

In cytotoxicity study, the release of ALT and AST, and GSH contents were increased only with high concentrations of barakol. Meanwhile there was no change in MDA formation. This indicated that cytotoxicity induced by barakol is dose-dependent and may not involve the lipid peroxidation reaction.

In conclusion, cytotoxicity induced by high concentrations of barakol may involve the activities of certain phase I and phase II enzymes but not the lipid peroxidation reaction.

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