RESEARCH ARTICLES

Subchronic Effects of Barakol on Blood Clinical Biochemistry Parameters in Rats Fed with Normal and High Cholesterol Diets

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Abstract

Subchronic effects of barakol, a major constituent in flowers and young leaves of *Cassia siamea* Lam., on blood clinical biochemistry parameters were investigated in this study. Thirty-two male Wistar rats were randomly assigned into 4 groups. The first two groups were fed with normal or high cholesterol diets serving as control groups. The other two groups were fed with normal or high cholesterol diet along with 30 mg/kg/day barakol given orally. After 90 days of treatment period, serum sample and whole blood of each rat were assessed for blood clinical biochemistry parameters and hematology. Normal or high cholesterol diet-fed rats treated with barakol demonstrated a significant decrease in TG but increases in total and direct bilirubins. A significant decrease in ALP was found in high cholesterol diet-barakol treated group, but there was no change in normal diet-barakol treated group. There were no significant effects of barakol on the following parameters: SGOT, SGPT, BUN, SCr, total cholesterol, LDL-C, HDL-C, LDL-C/HDL-C ratio, serum glucose, Hb, Hct, platelet count, WBC count and % differential WBCs in both dietary conditions. In addition, rats fed with high cholesterol diet showed significant increases in SGOT, SGPT, ALP, total cholesterol, LDL-C, and LDL-C/HDL-C ratio as compared to the normal diet group. The alterations of these parameters might indicate the different types of liver injury caused by barakol treatment and hypercholesterolemia condition. Further studies on the effects of various doses of barakol on blood clinical biochemistry parameters as well as the mechanisms of which barakol-induced liver injury are in progress.

Key words: barakol, *Cassia siamea* Lam., high cholesterol diet, blood clinical biochemistry parameters

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ผลระยะกึ่งเรื้อรังของบาราคอลต่อค่าชีวเคมีคลินิกในเลือดของหนูขาวที่ได้รับอาหารปกติและอาหารคอเลสเตอรอลสูง

รวิวรรณ มณีรัตนโชติ[1], พรพิมล กิจสนาโยธิน[1], ลัดดาวัลย์ ผิวทองงาม[2], วัชรีวรรณ ทองสะอาด[2], นวลศรี นิวัติศัยวงศ์[1], และสมทรง ลาวัณย์ประเสริฐ[1,3]

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บทคัดย่อ

งานวิจัยนี้ได้ทำการศึกษาถึงผลระยะกึ่งเรื้อรังของบาราคอล ซึ่งเป็นสารสำคัญในดอกและใบอ่อนของขี้เหล็ก (*Cassia siamea* Lam.) ต่อค่าชีวเคมีคลินิกในเลือด หนูขาวเพศผู้พันธุ์วิสตาร์จำนวน 32 ตัวถูกแบ่งโดยการสุ่มออกเป็น 4 กลุ่ม สองกลุ่มแรกเป็นกลุ่มควบคุมที่ได้รับอาหารปกติ และอาหารคอเลสเตอรอลสูง ส่วนอีกสองกลุ่มที่เหลือได้รับอาหารปกติ และอาหารคอเลสเตอรอลสูงร่วมกับบาราคอลโดยการป้อนทางปากในขนาด 30 มก/กก/วัน หลังจากครบระยะเวลา 90 วัน นำตัวอย่างซีรัมและเลือดของหนูขาวแต่ละตัวมาวัดค่าชีวเคมีคลินิก และค่าโลหิตวิทยา ตามลำดับ ผลการทดลองพบว่าหนูขาวที่ได้รับอาหารปกติ และอาหารคอเลสเตอรอลสูงร่วมกับบาราคอลมีค่า TG ลดลงอย่างมีนัยสำคัญ แต่พบว่าค่า total และ direct bilirubin เพิ่มสูงขึ้น พบการลดลงอย่างมีนัยสำคัญของค่า ALP ในกลุ่มที่ได้รับอาหารคอเลสเตอรอลสูงร่วมกับบาราคอล ไม่พบผลของบาราคอลต่อค่าพารามิเตอร์ต่างๆ ดังต่อไปนี้: SGOT, SGPT, BUN, SCr, total cholesterol, LDL-C, HDL-C, อัตราส่วนของ LDL-C ต่อ HDL-C, serum glucose, Hb, Hct, platelet count, WBC count และ % differential WBCs ในหนูขาวกลุ่มที่ได้รับอาหารทั้งสองชนิด นอกจากนี้พบว่าหนูขาวที่ได้รับอาหารคอเลสเตอรอลสูงมีค่า SGOT, SGPT, ALP, total cholesterol, LDL-C, อัตราส่วนของ LDL-C ต่อ HDL-C สูงขึ้นอย่างมีนัยสำคัญ เมื่อเทียบกับหนูขาวกลุ่มที่ได้รับอาหารปกติ การเปลี่ยนแปลงของค่าพารามิเตอร์เหล่านี้อาจชี้ให้เห็นถึงลักษณะที่แตกต่างกันของการบาดเจ็บของตับอันเนื่องมาจากการได้รับบาราคอล และภาวะคอเลสเตอรอลสูง ควรมีการศึกษาเพิ่มเติมถึงผลในขนาดต่างๆ ของบาราคอลต่อค่าชีวเคมีคลินิกในเลือด รวมถึงกลไกการเกิดพิษต่อตับที่เหนี่ยวนำโดยบาราคอลต่อไป

กุญแจคำ: บาราคอล, ขี้เหล็ก, อาหารคอเลสเตอรอลสูง, ค่าชีวเคมีคลินิกในเลือด

Introduction

Cassia siamea Lam. is a plant in family Leguminosae[1] that is generally grown in tropical countries. In Thailand, flowers and young leaves of this plant have been used as a sleeping aid, increase appetite as well as an ingredient in Thai food recipe. In 1949, Arunlaksana found that crude ethanol extract of *C. siamea* leaves decreased movement and activity in animals but not sleep despite being given at high doses[2]. The major constituent in flowers and young leaves of *C. siamea*, barakol, was first extracted by Hassanali *et al* in 1969[3]. Barakol ($C_{13}H_{12}O_4$) has a chemical name of 3α,4-dihydro-3α,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene or 2,5-dimethyl-3αH-pyrano-[2,3,4-de]-1-benzopyran-3α,8-diol (Figure 1)[4]. The physical characteristics of this compound are pale yellow needle crystals with boiling point of 165°C [3]. A proposed synthesis of barakol was described by Bycroft et al. in 1970[5]. Subsequently, pharmacological and physiological properties as well as toxicity of barakol were studied continuously, including the effects on central nervous system, cardiovascular system and antimicrobial effect, as summarized by Thongsaard [6].

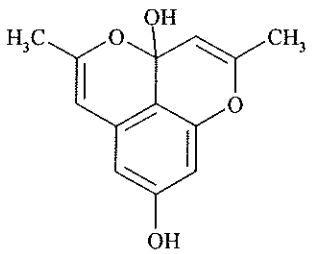


Figure 1 Chemical structure of barakol (3α,4-dihydro-3α,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene)

Following intraperitoneal administration of barakol to mice in an acute toxicological study, it was found that barakol possessed a median convulsant dose, CD_{50}, of 296.71 mg/kg and a median lethal dose, LD_{50}, of 324.09 mg/kg[7]. Acute hepatitis was reported in patients at Phramongkutklao and Chulalongkorn Memorial Hospitals in 1999 when 20-40 mg/day of *C. siamea* capsules were taken for 7-60 days[8].

Recent pharmacological study has focused on the anticarcinogenic and anti-mutagenic effects of *C. siamea* leaves. Interestingly, it was found that hexane, chloroform and methanol extracts of flowers and young leaves of *C. siamea* protected rats against aflatoxin B_1-induced mutagenesis[9]. Moreover, the chloroform and methanol extracts prevented mutagenesis induced by benzo(a)pyrene[9]. This group of researchers also studied the effects of *C. siamea* leaves on hepatic drug-metabolizing enzymes in rats. Feeding rats with 5% dietary *C. siamea* leaves for 14 days resulted in a significant increase in phase II detoxifying enzyme activities including GST and uridine 5′-diphosphoglucuronosyltransferase (UDPGT), along with a decrease of some bioactivting enzyme activities in the CYP systems such as aniline 4-hydroxylase and aminopyrine N-demethylase[10].

Since subchronic toxicity of barakol has not yet been reported, blood clinical biochemistry parameters in rats were investigated in this study. In addition, barakol was shown to possess an advantage on the cardiovascular system such as hypotensive effect in both systolic and diastolic blood pressure in rats and cats, reduction in the contraction of isolated rat thoracic aorta induced by phenylephrine[11], and reduction of heart rate in anesthesized rats[12]. Hypercholesterolemia is a risk factor of atherosclerosis, which is generally associated with almost cases of cardiovascular disease[13]. Therefore, this experiment was performed in rats fed with normal or high cholesterol diet to study the lipid-lowering effects of this compound as well.

Materials and Methods

Barakol solution

Barakol was extracted by Assist. Prof. Dr. Watchareewan Thongsaard, Department of Physiology, Faculty of Medicine, Srinakharinwirot University,

Thailand. Briefly, fresh young leaves of *C. siamea* were cut into small pieces and boiled with 0.5% sulfuric acid. The water extract was alkalinized with concentrated sodium hydrogen carbonate solution, further extracted with chloroform, shaked with 5% aqueous acetic acid until colorless, neutralized with concentrated ammonia solution, and cooled. Crude crystallized barakol (greenish yellow) was added with concentrated hydrochloric acid and dried immediately with filtrated vacuum. The obtained crystallized-yellow needles was anhydrobarakol hydrochloride (MW 251.4)[4]. The administrated solution was freshly prepared by dissolving anhydrobarakol hydrochloride in distilled water to make a concentration of 30 mg/ml. The solution appeared greenish yellow in color and was reported to be stable for about 1 hour after preparation[4].

Animals

Thirty-two male Wistar rats weighing between 200-250 g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom, Thailand. Animals were kept in animal care facility at Srinakharinwirot University, Bangkok, Thailand and acclimatized for at least 1 week before the experiment. All animals were allowed free access to food (S.W.T. Ltd., Thailand) and drinking water. Light/dark period and temperature were controlled at 12/12 hour cycle and 25°C, respectively. During the time of experimentation, body weight of each rat was recorded every 2 weeks.

Animal treatment

Rats were randomly assigned into 4 treatment groups fed orally with 1) normal diet, 2) high cholesterol diet, 3) normal diet with 30 mg/kg/day barakol, and 4) high cholesterol diet with 30 mg/kg/day barakol for 90 days. High cholesterol diet composed of 1% cholesterol diet mixed with 2% sodium choleate.

At the end of the treatment period, the animals were fasted for 10 hours before anesthesized intraperitoneally with 0.6 - 1 ml of pentobarbital sodium (Nembutal®). Blood was drawn from the left ventricle for an approximate volume of 5 ml. Five hundred microlitre of whole blood was transferred to a microtube containing a few grains of EDTA sodium and mixed thoroughly. The remaining blood was transferred to another tube, allowed to stand in a slope posture in order to collect the highest amount of serum and kept at – 4°C until the time of analysis. Whole blood and serum were investigated for the hematology and blood clinical biochemistry parameters, respectively. Meanwhile, the liver was also removed and weighed.

Determination of blood clinical biochemistry parameters

Serum samples of individual rat were measured for the following parameters using specific test kits: serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) (Enzyline®, bioMé rieux sa), total and direct bilirubin (Bili-T/D®, Mérieux Vitek Inc.), blood urea nitrogen (BUN) (Urée cinétique UV®, bioMérieux sa), serum creatinine (SCr) (Créatinine cinétique®, bioMérieux sa), total cholesterol (Cholestérol RTU®, bioMérieux sa), triglyceride (TG) (Triglycérides Enzymatique PAP 150®, bioMérieux sa), glucose (Glucose RTU®, bioMérieux sa), low density lipoprotein cholesterol (LDL-C) (LDL-C Plus®, Rocke) and high density lipoprotein cholesterol (HDL-C) (HDL-C Plus®, Roche). Whole blood samples were measured for hemoglobin (Hb), hematocrit (Hct), platelet count, white blood cell (WBC) count and % differential WBCs.

Statistics

All numeric data were presented as mean ± SE. Statistical analysis was run on SPSS for Window version 7.5. Independent-samples *t* test was used for comparisons between the two groups at a significant level of $p < 0.05$.

Results

Barakol had no effects on body weight gain (Figure 2), terminal body weight, liver weight and relative liver weight in both normal diet and high cholesterol diet rats (Table 1). However, high cholesterol diet rats comparing with normal diet control rats showed a significant increase of liver weight (16.32 ±1.02 vs. 10.36 ± 0.23; $p<0.05$) and relative liver weight (3.55 ± 0.24 vs. 2.34 ± 0.08; $p < 0.05$).

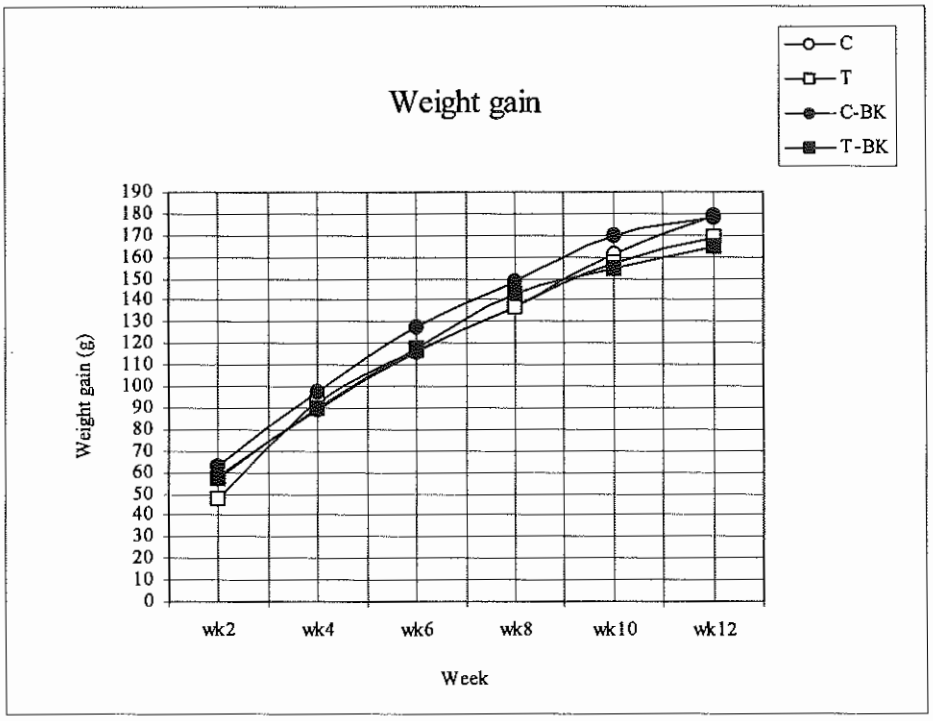
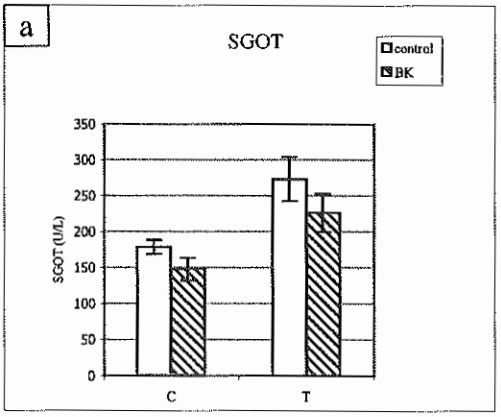


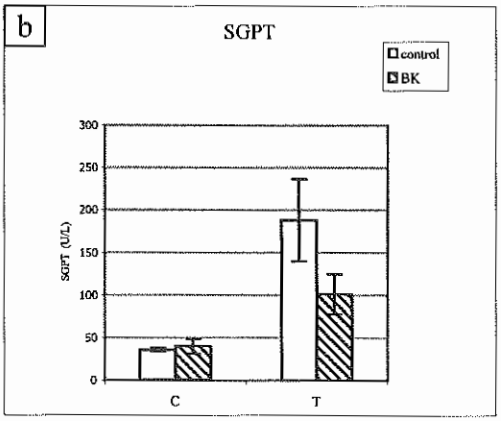
Figure 2 Subchronic effects of barakol on body weight gain
Rats were given normal diet (C), high cholesterol diet (T), normal diet with oral barakol 30 mg/kg/day (C-BK) and high cholesterol diet with oral barakol 30 mg/kg/day (T-BK) for 90 days. Body weight gain of each rat was recorded every two weeks. The statistical analysis was performed using independent-samples *t* test for comparisons between the two groups (C-BK vs. C and T-BK vs. T) at a significant level of $p < 0.05$.

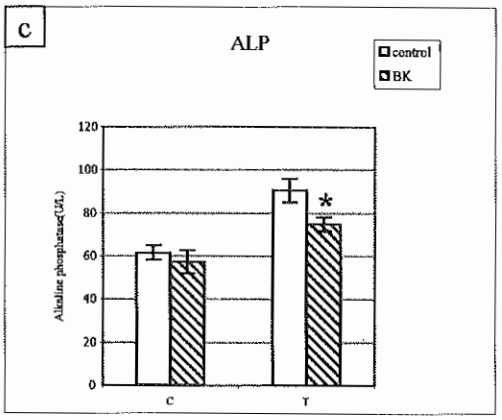
Table 1 Subchronic effects of barakol on terminal body weight, liver weight and relative liver weight

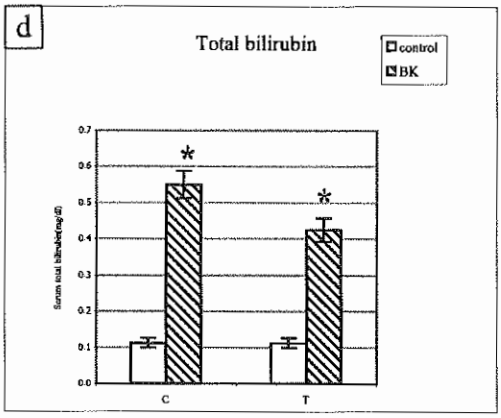
	Group			
	C	C-BK	T	T-BK
Terminal body weight (g)	444 ± 11	426 ± 14	463 ± 16	422 ± 22
Liver weight (g)	10.36 ± 0.23	10.29 ± 0.60	16.32 ± 1.02	16.29 ± 1.25
Relative liver weight (% of body weight)	2.34 ± 0.08	2.41 ± 0.11	3.55 ± 0.24	3.82 ± 0.15

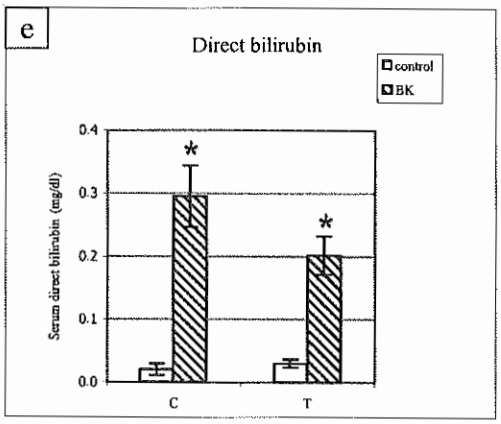
Rats were given normal diet (C), high cholesterol diet (T), normal diet with oral barakol 30 mg/kg/day (C-BK) and high cholesterol diet with oral barakol 30 mg/kg/day (T-BK) for 90 days. The statistical analysis was performed using independent-samples *t* test for comparisons between the two groups (C-BK vs. C and T-BK vs. T) at a significant level of $p < 0.05$.

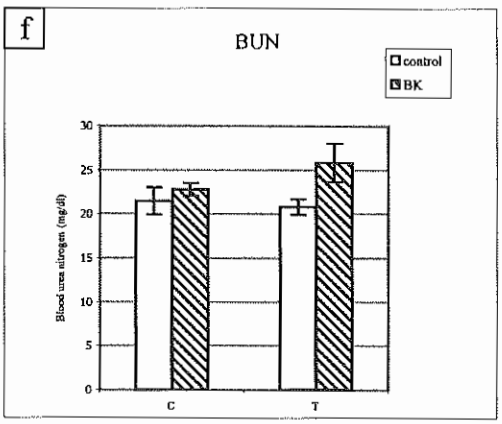

a
SGOT
control
BK
SGOT (U/L)
350
300
250
200
150
100
50
0
C
T

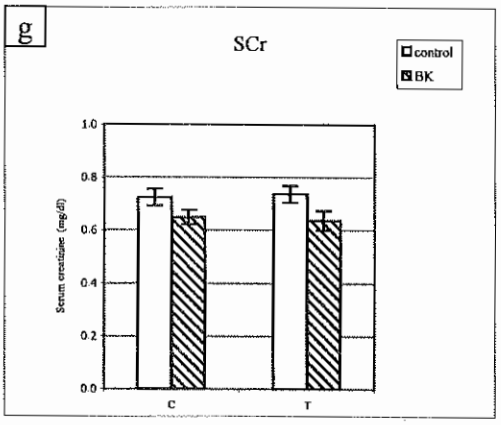

b
SGPT
control
BK
SGPT (U/L)
300
250
200
150
100
50
0
C
T

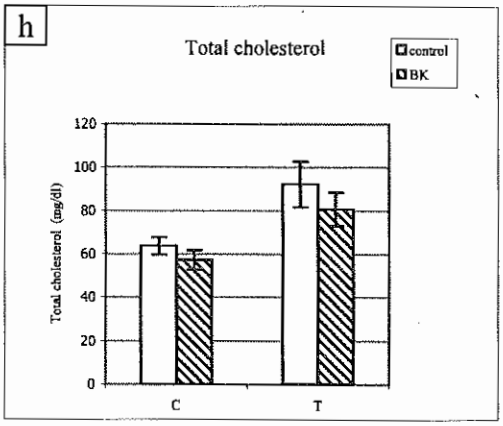

c
ALP
control
BK
120
100
80
60
40
20
0
C
T
*
*


d
Total bilirubin
control
BK
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C
T
*
*


e
Direct bilirubin
control
BK
Serum direct bilirubin (mg/dl)
0.4
0.3
0.2
0.1
0.0
C
T
*
*


f
BUN
control
BK
Blood urea nitrogen (mg/dl)
30
25
20
15
10
5
0
C
T


g
SCr
control
BK
Serum creatinine (mg/dl)
1.0
0.8
0.6
0.4
0.2
0.0
C
T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Total cholesterol
control
BK
Total cholesterol (mg/dl)
120
100
80
60
40
20
0
C
T

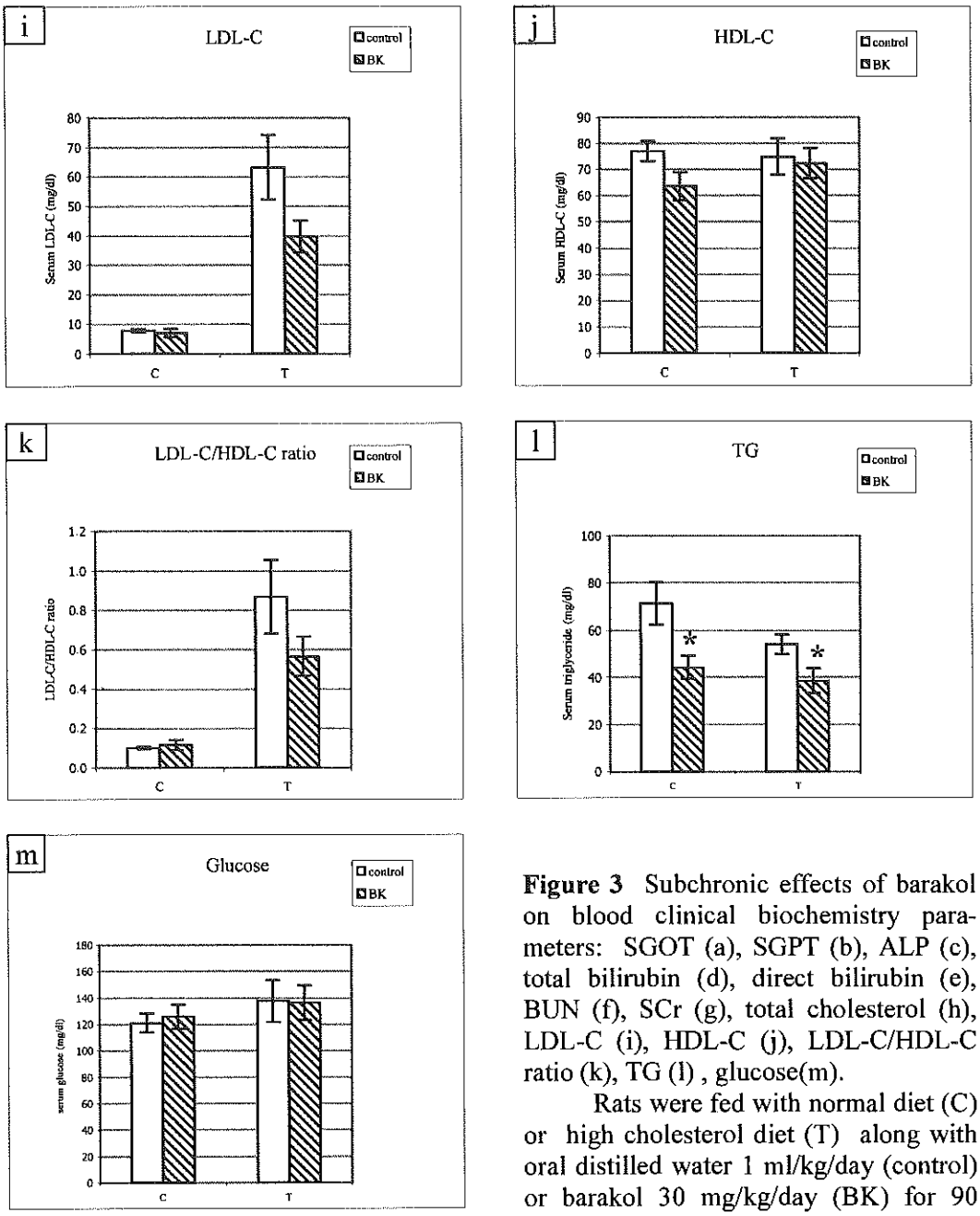

Figure 3 Subchronic effects of barakol on blood clinical biochemistry parameters: SGOT (a), SGPT (b), ALP (c), total bilirubin (d), direct bilirubin (e), BUN (f), SCr (g), total cholesterol (h), LDL-C (i), HDL-C (j), LDL-C/HDL-C ratio (k), TG (l) , glucose(m).

Rats were fed with normal diet (C) or high cholesterol diet (T) along with oral distilled water 1 ml/kg/day (control) or barakol 30 mg/kg/day (BK) for 90 days. The statistical analysis was done by using independent-samples t test for comparisons between the two groups at a significant level of $p < 0.05$.

* significantly different from the corresponding diet control groups.

Table 2 Subchronic effects of barakol on hematology

Hematology	Group			
	C	C-BK	T	T-BK
Hb	14.53 ± 0.40	14.86 ± 0.42	13.88 ± 0.25	14.32 ± 0.28
Hct	44 ± 1	44 ± 1	42 ±1	43 ± 1
Platelet count	329 ± 51	314 ± 26	346 ± 18	331 ± 23
WBC count	1.53 ± 0.27	1.91 ± 0.19	1.94 ± 0.34	1.90 ± 0.44
% differential WBCs				
- Neutrophil	29 ± 2	23 ± 2	24 ± 5	24 ± 4
- Leucocyte	69 ± 2	72 ± 2	72 ± 4	73 ± 3
- Monocyte	2 ± 0	4 ± 2	2 ± 1	2 ± 0
- Eosinophil	1 ± 0	1 ± 0	1 ± 0	1 ± 0

Rats were given normal diet (C), high cholesterol diet (T), normal diet with oral barakol 30 mg/kg/day (C-BK) and high cholesterol diet with oral barakol 30 mg/kg/day (T-BK) for 90 days. Values perform as mean ± SE. The statistical analysis was performed using independent-samples *t* test for comparisons between the two groups (C-BK vs. C and T-BK vs. T) at a significant level of $p < 0.05$.

Serum sample and whole blood of individual rat were measured for blood clinical biochemistry parameters and hematology, respectively. Subchronic exposure (90 days) of oral barakol 30 mg/kg/day in rats fed with normal and high cholesterol diets demonstrated a significant increase of both serum total and direct bilirubins (Figure 3d,3e) but a significant decrease of TG comparing with their corresponding control rats (Figure 3l). Barakol showed an advantageous effect on liver function of high cholesterol diet-fed rats as demonstrated by a decrease of ALP comparing with the corresponding control group (Figure 3c). There were no significant effects of barakol on the following parameters: SGOT, SGPT, BUN, SCr, total cholesterol, LDL-C, HDL-C, LDL-C/HDL-C ratio, glucose, Hb, Hct, platelet count, WBC count and % differential WBCs in both dietary conditions (Figure 3a-3b,3f-3k,3m) (Table 2).

Compared with normal diet rats, high cholesterol diet rats demonstrated significant increases of SGOT (274 ± 30 vs 179 ± 10; $p<0.05$), SGPT (188 ± 48 vs. 36 ± 2; $p<0.05$), ALP (91 ± 5 vs. 62 ± 3; $p<0.05$), total cholesterol (92 ± 10 vs. 64 ± 4; $p<0.05$), LDL-C (63 ± 11 vs. 8 ± 0.5; $p<0.05$) and LDL-C/HDL-C ratio (0.868 ± 0.186 vs. 0.101 ± 0.006; $p<0.05$). Markedly increases of SGPT and LDL-C/HDL-C ratio in high cholesterol diet rats accounted for about 5 and 9 fold, respectively, of those in the normal diet rats.

Discussion

Barakol, a major constituent in flowers and young leaves of *C. siamea*, was investigated for subchronic effects (90 days) on blood clinical biochemistry in rats fed with normal and high cholesterol diets. A study by Thongsaard and collaborators in 1996 indicated that barakol given intraperitoneally to rats at a dose of 10 mg/kg demonstrated an anxiolytic profile on the elevated plus-maze similar to that induced by diazepam. An increase in exploratory and locomotor behavior, which was not produced by diazepam, was also found at this dose of barakol[14]. This was an interesting advantage of barakol over benzodiazepines regarding its anxiolytic effect without sedation. However, at the higher doses (25 and 50 mg/kg, i.p.), barakol produced less effects on anxiolytic property and the decreased in exploratory and locomotor behavior were observed.

At very high dose (75 mg/kg, i.p.), lost an anxiolytic property but the sedative effect was found[14]. Sedation and antianxiety were indications of *C. siamea* capsules in clinical use. It may be traumatic for the animals to receive the drug i.p. for 3 months to investigate subchronic effect, oral route was used to study instead. Because of the effects of absorption and bioavailability of drugs, oral administration given lower plasma concentration than i.p. administration[15]. And due to no previous report on pharmacokinetic of barakol, the oral dose used in this study (30 mg/kg/day) was approximately expected to give the same degree of plasma concentration of i.p. dose with effectively anxiolytic effect (10 mg/kg).

Subchronic treatment of oral 30 mg/kg/day barakol demonstrated a marked increase of total and direct bilirubin in both normal diet- and high cholesterol diet-fed rats. These findings were consistent with those studied in rats receiving dry *C. siamea* leaves at dosages of 200 and 2,000 mg/kg/day for 6 months[16] as well as those reported in patients with history of taking 20-40 mg/day *C. siamea* capsules for 7-60 day at Phramongkutklao and Chulalongkorn Memorial Hospitals[8]. The elevation of serum levels of endogenous compounds that are normally concentrated in bile such as bile acid and billirubin, is a useful biochemical marker indicating liver injury from biliary secretion defect defined as canalicular cholestasis[17]. Barakol did not cause an increase of SGOT, SGPT and ALP in both normal and high cholesterol diet-fed rats. This might be an implication that biliary secretion defect should be responsible for the liver injury induced by barakol at the dosages used in this study rather than an effect of barakol on hepatic parenchymal cells.

High cholesterol diet-fed rats demonstrated an increase of total cholesterol, LDL-C, LDL-C/HDL-C ratio along with an increase of SGOT, SGPT, ALP as well as the relative liver weight. There are two broad categories of liver injury commonly found, cholestasis and hepatocellular damage. Cholestasis is characterized by a predominant elevation of ALP and gamma-glutamyltranspep-tidase (γ-GT) whereas hepatocellular damage is detected by an elevation of hepatic parenchymal enzymes such as SGOT and SGPT[18]. It is likely that high cholesterol diet feeding is responsible for a mixed pattern of these abnormalities. Accompanying the increase of relative liver weight that indicates enlargement of the liver, an accumulation of fat in the liver might be involved in lipid induced liver injury in this group of animals.

Barakol seemed to possess an advantage on liver function as demonstrated by a decrease of ALP in high cholesterol diet group. In general, no single test such as aminotransferases, ALP and so on is satisfactory for the assessment of all forms of liver injury. The 4 major test categories recommended for evaluation of experimental hepatic injury in laboratory animals include serum hepatic enzyme tests, hepatic excretory tests, alterations in the chemical constituents of the liver and histologic analysis of liver injury[19]. Therefore, a decrease of serum ALP but not serum aminotransferases and bilirubin caused by barakol treatment in high cholesterol diet rats was not a clear indication regarding significant improvement of hepatic function.

According to the Frederickson/ WHO calssification of hyperlipoproteinemia, the pattern of elevations of LDL-C and total cholesterol (but not necessarily an increase of TG) is classified as Type IIa hyperlipoproteinemia in human that indicates high atherosclerosis risk[20]. The high cholesterol diet rats in this study possessed all of those characteristics (elevation of total cholesterol, LDL-C, LDL-C/HDL-C ratio, but not TG) as mentioned in Type IIa hyperlipoproteinemia. Barakol (30 mg/kg/day) administration orally along with normal or high cholesterol diet caused only a significant decrease in TG, but showed no significant effects on total cholesterol, LDL-C, HDL-C and LDL-C/HDL-C ratio. This characteristic effect of barakol at the dose used in this study seemed to offer no advantage for this model of hyper-

cholesterolemia. This result was inconsistent with a previous study in which *C. siamea* leaves were given to rats at the dosage of 2,000 mg/kg/day for 6 months. They found that *C. siamea* leaves caused a significant decrease in serum cholesterol and triglyceride[16]. The differences of doses, duration of treatment or other constituents in *C. siamea* leaves may contribute to the lowering effects of the lipid profiles in serum.

In this study, barakol exhibited no significant effects on these following parameters: BUN, SCr, serum glucose, Hb, Hct, platelet count, WBC count and % differential WBCs. These findings provided a preliminary information that barakol given subchronically at the dose used in this study exhibited no toxic effects on renal function, carbohydrate metabolism and hemopoietic system. Further studies on the mechanisms of which barakol induced liver injury as well as effects of various doses of this compound on blood clinical biochemistry parameters should be performed.

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