Note

Effect of the water extract of *Macrosolen cochinchinensis* (Lour.) Tiegh. leaves on 7,12-dimethylbenz [*a*] antracene induced female mice liver carcinogensis

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Abstract: Liver cancer is the third most common cause of death from cancer worldwide. Recently, natural products were used widely as an alternative therapy for liver cancer. Previous study reported *Macrosolen cochnichinensis* (Lour.) Tiegh. that grows in the host star fruit inhibited breast cancer cells growth in vitro. This study aims to observe the effect of water extract of *M. cochinchinensis* leaves (MCE) on Balb/c mice hepatocyte after initiation of 7,12-dimethylbenz [*a*] anthracene (DMBA) as a liver cancer model inducer. The experiment consisted of four mice groups, corn oil solvent control group, the DMBA dose 20 mg/kgBW p.o. ten times twice a week, DMBA+MCE dose 250 mg/kgBW, and DMBA+MCE 750 mg/kgBW. Extract which was dissolved into 0.5% CMC-Na was administered daily by the oral route 1 week before, during and terminated 1 week after the DMBA induction. At the end of the study, rat livers were collected and stained with Haematoxyllene and Eosin (H&E) method. Administration of MCE could not inhibit hepatic carcinogenesis in DMBA-induced female mice. There was no difference in liver tissue histopathology profile between the extract treatment group and DMBA control group.

Keywords: *Macrosolen cochincinensis*; Carcinogenesis; Liver cancer; DMBA

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1. Introduction

Liver cancer is the third of causes of cancer death in the world[1]. The number of deaths in the world caused by liver cancer showed more than one million deaths per year. Several methods for liver cancer therapy such as surgery, chemotherapy, hormonal therapy and radiotherapy had been established. Surgery is no longer effective for cells that have metastatic disease[2]. Effectivity of chemotherapeutic agents were limited because of drug resistance problem and its toxicity in normal cells[3]. Different research findings were developed to overcome this problem. Strategies to solve the problem include the use of an agent that is called chemopreventive agent.

Chemoprevention is defined as the use of substances of natural origin, phytochemical agents, synthetic, or chemical compounds to prevent or suppress cancer progression, reverse to normal physiological functions and perform early detection of pathological cancer conditions[4]. Phytochemicals are defined as bioactive non-nutrient components of plant parts, such as seeds, leaves, and rhizomes[5]. Indonesia, as a megabiodiversity country in the world, provides many herbal and traditional medicines for cancer therapy.

One of Indonesian plants that are potentially efficacious as cancer chemopreventive agent is mistletoe (*Macrosolen cochinchinensis*). Previous study in the water extract of *M. cochinchinensis* leaves that grows in the host star fruit (*Averrhoa carambola*) (MCE) was able to inhibit the growth of MCF-7 breast cancer cells with IC_{50} 0.63 ppm[6]. In vitro anti-cancer tests of MCE have also been performed on L1210 leukemia cancer cells with IC_{50} 41.0 ppm, HCT116 colon cancer cells with IC_{50} 20 ppm, and A431 cancer cells with IC_{50} 20 ppm[6]. Study of MCE on B16 cancer cells showed that MCE at concentration of 100 ppm had no cytoxicity (cell viability 93%), but at the concentrations of 200 and 400 ppm indicated toxicity (cell viability 26% and 9%)[6]. There is no previous study on effect of MCE on liver cancer cells. A study of MCE mentioned that MCE inhibit carcinogenesis on rat liver induced by benzidin[7].

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We would like to explore the effect of MCE on female mice liver carcinogenesis induced by DMBA because *Macrosolen cochinchinensis* is traditionally used to treat many kinds of cancers including liver cancer in Indonesia. In our study, we extrapolated the daily dose of MCE for mice from human treatment.

2. Materials and methods

2.1. Materials

The water extract of *M. cochinchinensis* leaves was obtained from Ir. Nina Artanti (Research Centre for Chemistry-Indonesiaan Institute of Sciences (RCChem-LIPI), Serpong). DMBA (7,12-dimetilbenz [*a*] anthracene) was obtained from Sigma (Saint Louis, MO).

2.2. Animals

Balb/c female mice (*Mus musculus*) (40 days old) weighed from 18 to 25 g were obtained from the Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia. The mice were kept for at least one week before use, and were given standard pellet diet and water ad libitum, and kept on a 12:12 h light/dark cycle.

Forty mice were devided into four groups (10 mice per group). The experimental design is summarized in Figure 1. Beginning at 5 week of age, solvent control (group 1) was administered with corn oil, groups of treatment (groups 2, 3, and 4) intended for carcinogen were treated with single oral dose of DMBA (Sigma) (20 mg/kgBW), twice a week for 5 weeks. DMBA was dissolved in corn oil and MCE was dissolved in 0.5% CMC-Na as vehicle. Group 3 and group 4 were administered orally once a day by 250 and 750 mg/kgBW, respectively for 7 weeks, started 1 week before DMBA initiation until 1 week after DMBA administration. Body weight was recorded weekly throughout the study.

2.3. H&E staining

After 16 weeks of experiment, all animals were sacrificed by ether as scheduled. At autopsy, liver organ were removed and fixed in 10% buffered formalin. After 12–24 h of fixation, 3–5 mm tissue slices were embedded in paraffin, and stained with H&E for microscopy.

2.4. Statistical analysis

A statistically significant difference in body weight was evaluated by ANOVA continued with LSD. $P<0.05$ between groups was considered statistically significant using SPSS (SPSS release 17.0; SPSS Inc.).

3. Results

There was no direct evidence of toxicity due to MCE treatment. Body weight change of the animals treated with corn oil, DMBA and DMBA+MCE was compared (Fig. 2). In the beginning of study, there was no difference in body weight change. DMBA group was significantly different from corn oil in

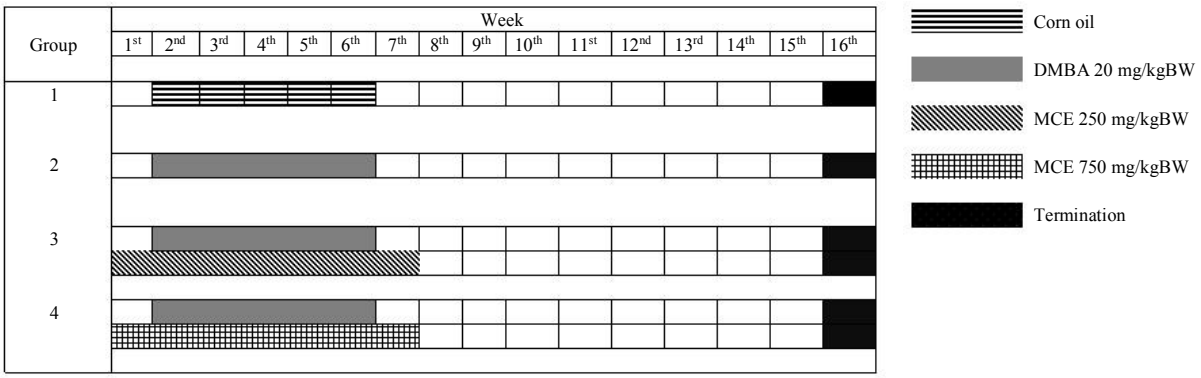

Figure 1. Experimental time line.

second week, and DMBA+MCE 750 mg/kgBW in the third week (P<0.05). Final body weight change showed that DMBA was no significant different from other groups (P>0.05). The body weight from animals treated with DMBA+MCE 250 mg/kgBW was significantly different from those treated with corn oil and DMBA+MCE 750 mg/kgBW (P<0.05) (Table 1 and Fig. 2). The survival curves for the DMBA and DMBA+MCE were practically identical before the 13rd week of experiment, but after that the survival curve of the DMBA started to shift towards a higher life-span as compared to the MCE treatment group (Fig. 3). Observation was terminated at 8 week after the last DMBA initiation because of the bad condition of the animals characterized by clinical symptoms of the breath failure and convulsions. Morphologically, there was macroscopic difference in liver organ (Fig. 4). The livers from DMBA treatment group showed swelling and were more pale than corn oil group, while the morphology of livers from MCE treatment group was similar to that from DMBA group.

The histopathological appearance of liver tumors in DMBA and DMBA+MCE treated mice was depicted in Figure 5. H&E staining showed two types of cancers in liver, hepatocellular carcinoma or primary liver cancer and lymphoblastic cells in the liver or lymphosarcoma. Treatment of corn oil did not induce cancer in mice liver. The incidence of liver cancer in the DMBA and DMBA+MCE did not differ significantly (Table 1). Incidence of lymphosarcoma (Table 1) was higher than primary liver cancer. In general, treatment of MCE did not repaire liver morphology induced by DMBA. The higher dose of MCE was related to the higher incidence of lymphosarcoma. In addition, target organ site of DMBA in female mice strain Balb/c was the lymphoid system.

Table 1. Effect of MCE on DMBA-induced liver carcinogenesis

Treatment group	Final body weight change (g)[1]	Final liver tumor incidence (%)	Final liver lymphosarcoma incidence (%)
Corn oil	1.00±0.47	0.0	0.0
DMBA	–0.40±0.32	20.0	90.0
DMBA+MCE 250 mg/kgBW	–3.00±1.35[a]	14.3	85.7
DMBA+MCE 750 mg/kgBW	0.56±0.59[b]	16.7	100.0

[1]Values represent means±SE. Means among treatment groups with different superscripts are statistically significant (P<0.05) by analysis of variance continued by benferroni test. a, significant difference with corn oil group (P<0.05); b, significant difference with DMBA+MCE 750 mg/kgBW (P<0.05).

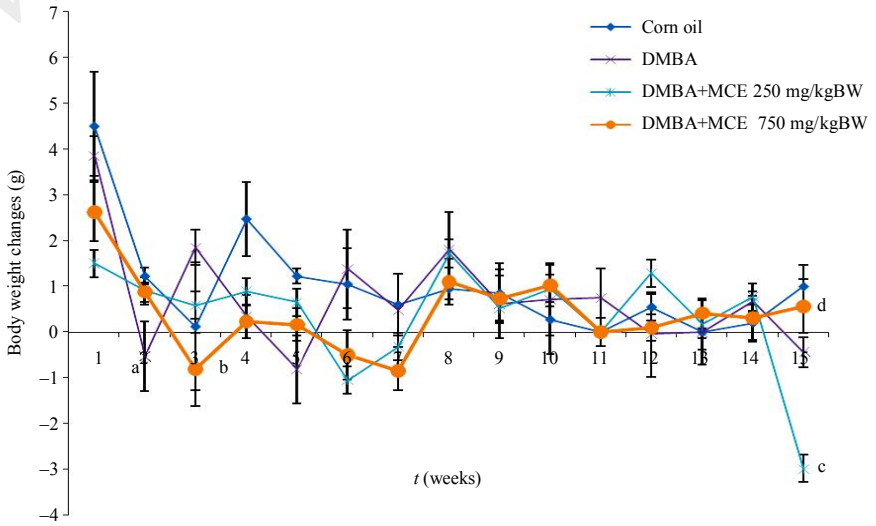

Figure 2. Body weight change of animals. a, Significant difference between corn oil group and DMBA group (P<0.05) in the second week; b, Significant difference between DMBA group and DMBA+MCE 750 mg/kgBW group (P<0.05) in the third week; c, Significant difference between corn oil group and DMBA+MCE 250 mg/kgBW group (P<0.05) in the fifteen week; d, Significant difference between DMBA+MCE 250 mg/kgBW group and DMBA+MCE 750 mg/kgBW group (P<0.05) in the fifteen week.

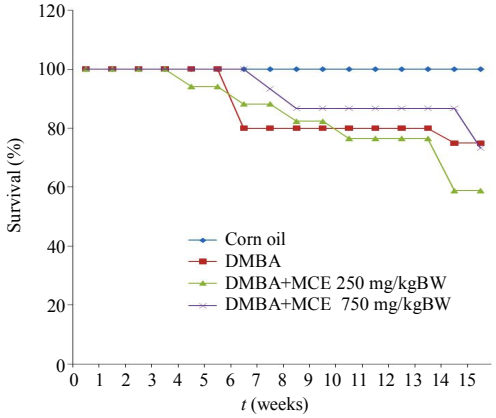

Figure 3. Survival curve of animals. The Y-axis represents the ratio of live mice (%) and the X-axis is the time from the start of the experiment (week).

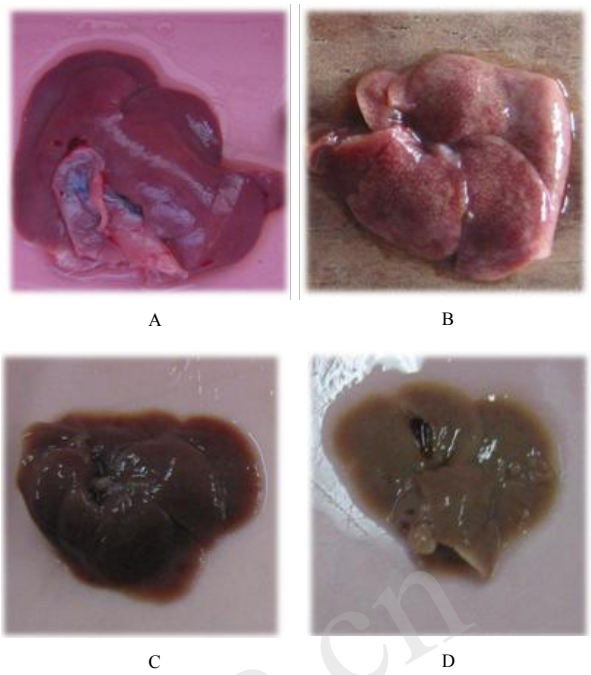

Figure 4. Morphological difference of liver treated with MCE in DMBA-induced female mice liver cancer. Rats were divided into 4 groups. (A) Corn oil; (B) DMBA control group: administered 20 mg/kgBW in corn oil; (C) DMBA+250 mg/kgBW MCE; (D) DMBA+750 mg/kgBW MCE. At autopsy, liver organs were removed and fixed in 10% buffered formalin.

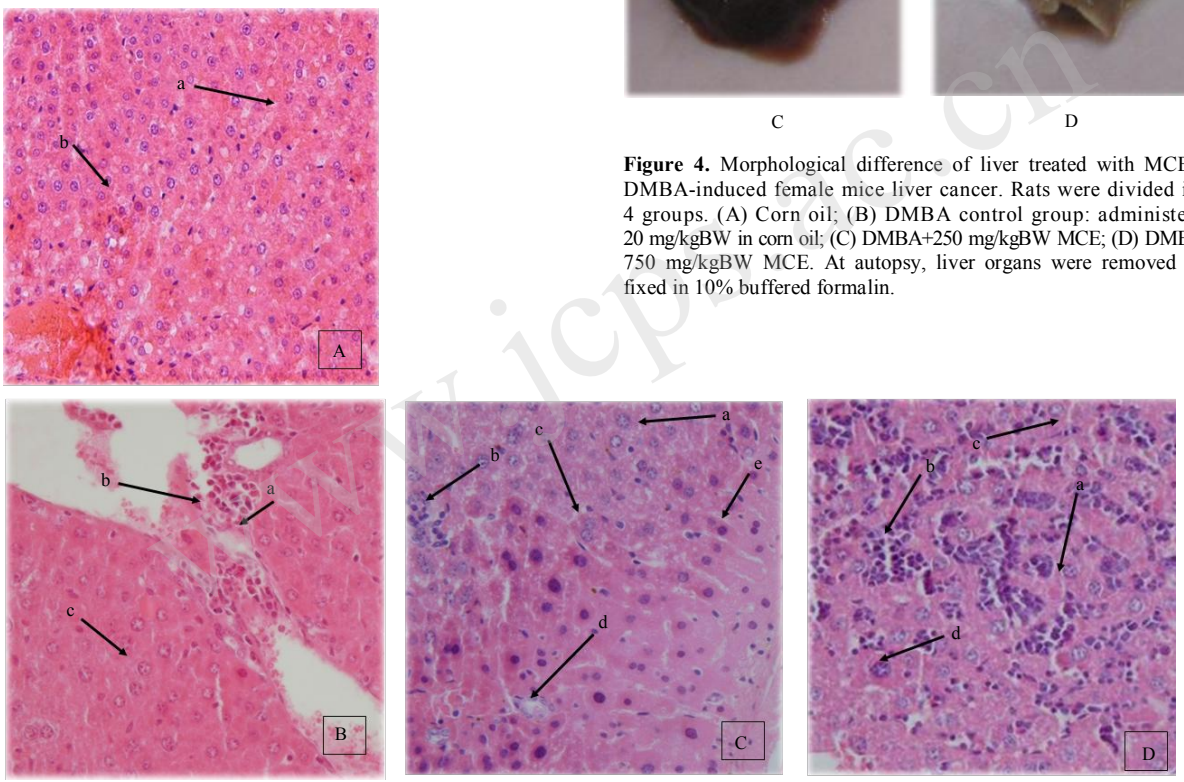

Figure 5. The histological evaluation of liver tissues in all groups. Rats were divided into 4 groups. (A) Corn oil; (B) DMBA control group: administered 20 mg/kgBW in corn oil; (C) DMBA+250 mg/kgBW MCE; (D) DMBA+750 mg/kgBW MCE. At autopsy, liver organs were removed and fixed in 10% buffered formalin. (a, hepatocyte; b, sinusoid; c, lymphoblastic cell; d, focus of liver tumor; e, mitotic cell).

4. Discussion

This study explored the effect of *M. cochinchinensis* leaves water extract on DMBA induced female mice liver. In this study, MCE was given a week before, during and after induction of DMBA. MCE treatment was designated to prevent metabolic activation of DMBA and suppress liver cancer progression. Our study showed that DMBA induced lymphosarcoma was more intensive than primary liver cancer. Lymphosarcoma is a common neoplasm, characterized by enlargement of lymph nodes in peripheral areas of neoplastic lymphocytes and eventually it would enter the blood circulation. Thymus gland and bone marrow are primary lymphoid organ, while the secondary lymphoid organs include the spleen and lymph node[8].

DMBA, a polysiclic aromatic hydrocarbon (PAH), is a procarcinogen, therefore it needs to be metabolized by cytochrome P450 (CYP) to become a reactive metabolites that triggers DNA damage[9]. Individual PAH may affect their own metabolism catalyzed by CYP 1A1, 1A2, and 1B1[10]. PAH also acts as autoinducer of CYP[11].

Arylhydrocarbon receptor (AhR) is a transcription factor of numerous CYP expression in the organs such as liver, mammae, and bone marrow. Bond between the AhR with PAH compounds such as DMBA will induce expression of CYP like CYP 1A1. DMBA caused damage of hematopoiesis in bone marrow and affected the development of B cells[9], then caused lymphosarcoma.

Glutathione-*S*-transferase (GST), a phase II metabolic enzyme, detoxifies carcinogens and facilitates their excretion by promoting the conjugation of electrophilic compounds with glutathione[12]. GST deactivates and protects the surrounding tissues from mutagenesis and carcinogenesis. Most of GST inducers affect the activity of GST gene transcription via the Antioxidant-Responsive Element (ARE), Xenobiotik-Responsive Element (XRE), GST-P Enhancer 1 (GPE) or Glucocorticoid-Responsive Element (GRE)[13].

Previous study mentioned that major constituent of MCE is flavonoids[6]. Flavonoids might enhance activation of carcinogens via induction of specific CYP[13]. This mechanism is associated with the enhanced activity of CYP1 family enzymes including CYP 1A1 and 1B1[15] which are responsible to the activation of carcinogens such as benzo[*a*]pyrene, DMBA and aflatoxin B1[10]. On the other hand, various flavonoids inhibit CYP activity involved in carcinogen activation and scavenging of reactive species formed by CYP mediated carcinogens[16]. Several CYPs are also involved in flavonoids metabolism[16]. Previous in vitro study using mouse and human microsomes mentioned that CYP 1A2 was capable to metabolize all five investigated flavonoids such as hesperetin, naringenin, apigenin, kaempferol and tamarixetin[17]. These results may decrease the effect of flavonoid as a cancer chemopreventive agent. Flavonoids from MCE might be dominated by flavonoid which induces the CYP expression rather than inhibits the CYP activity, but this proposed mechanism needs to be explored with more details.

Many studies have shown that flavonoids can stimulate GST, a promising strategy for the prevention[18]. Kaempferol, luteolin, quercetin and galangin inhibited GST activity in MCF-7 breast cancer cells[12]. Another study showed that plant phenolic compounds ellagic acid and curcumin inactivate human GSTs[19]. Flavonoids from MCE probably induced GST expression, while some of them played as inhibitors of GST activity, but this proposed mechanism needs to be further clarified.

Indonesia, a mega biodiversity country, provides many herbs for cancer treatment, but there is no evidence of their safety and efficacy. In the previous study, MCE inhibited some cell line, but did not inhibit liver cancer growth in animal model. Cancer is complex disease that involves many regulatory proteins those differ from one to another kind of cancer tissue. This study contribute to giving scientific information that MCE did not inhibit liver cancer growth, but it might has effect on other cancer tissues such as breast cancer or lung cancer.

In the present study, MCE did not inhibit tumor incidency based on histopathological profile. We conclude that MCE did not inhibit carcinogenesis of DMBA on female mice liver. However, the proposed molecular mechanism involving CYP and GST by the extract needs to be further explored.

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References

[1] Jemal, A.; Siegel, R.; Xu, J.; Ward, E. *CA Cancer J. Clin.* **2010**, *60*, 277–300.

[2] King, R.J.B. *Can*cer *Biology*, 2 nd Ed. London: Pearson Eduation Limited. **2000**, 1–7.

[3] Hornsby, P.J. *J. Clin. Oncol.* **2007**, *25*, 1852–1857.

[4] Greenwald, P. *BMJ.* **2002**, *324*, 714–718.

[5] Ikai, T.; Akao, Y.; Nakagawa, Y.; Ohguchi, K.; Sakai, Y.; Nozawa, Y. *Biol. Pharm. Bull*. **2006**, *29*, 2498–2501.

[6] Artanti, N.; Jamilah, A.H.; Meiyanto, E.; dan Darmawan, A. *Laporan Teknis Sub Tolok Ukur Pengembangan Senyawa Potensial antikanker dari Taxus sumatrana dan Benalu.* Serpong: Puslit Kimia LIPI. **2004**. (Technical Report Development of Potential Anticancer Agent form Taxus sumatrana and Mistletoe, RCChem-LIPI, Serpong, **2004** (In Indonesian)).

[7] Hudayato, P.A. Pengaruh Infus Benalu Teh terhadap Karsinogenisitas benzidin pada Tikus Putih Jantan, *Skripsi (Bachelor Thesis)*, Fakultas Farmasi UGM, Yogyakarta. **1988** (In Indonesian).

[8] Baratawidjaya, K.G. *Imunologi Dasar*. Jakarta: Balai Penerbit Fakultas Kedokteran Univrsitas Indonesia. **2006**, 20–25 (Basic Immunology, Faculty of Medicine University of Indonesia Press, Jakarta, 20–25 (In Indonesian)).

[9] Weimer, T.L.; Ashok, P.R.; Ulrich, H.; David, A.S.; Craig, S.; Michael, R.M.; William, B.; Jerry, H.; Bailey, G. *Toxicol. Sci.* **2000**, *57*, 217–228.

[10] Shimada, T.; Guengerich, F.P. *Chem. Res. Toxicol*. **2006**, *19*, 288–294.

[11] Gregus, Z.; Klaasen, C.D. *Mechanisms of Toxicity*. In Klaasen, C.D. *Casarett & Doull's Toxicology*: *The Basic Science of Poisons*, 6th ed. New York: Mc. Graw-Hill. **2001**, 40–41.

[12] van Zanden, E.; Geraets, L.; Wortelboer, H.M.; van Bladeren, P.J.; Rietjens, I.M.C.M.; Cnubben, N.H.P. *Biochem. Pharmacol.* **2004**, *67*, 1607–1617.

[13] Hayes, J.D.; Pulford, D.J. *Chem. Mol. Biol*. **1995**, *30*, 445–600.

[14] Deng, Y.; Bi, H.C.; Zhao, L.Z.; He, F.; Liu, Y.Q.; Yu, J.J.; Ou, Z., M.; Ding, L.; Chen, X.; Huang, Z.Y.; Huang, M.; Zhou, S.F. *Xenobiotika*. **2008**, *38*, 465–481.

[15] Shimada, T.; Fuji-Kuriyama, Y. *Cancer Sci*. **2004**, *95*, 1–6.

[16] Hodek, P.; Trefil, P.; Stiborova, M. *Chem. Biol. Interact*. **2002**, *139*, 1–21.

[17] Breinholt, V.M.; Offord, E.A.; Brouwer, C.; Nielsen, S.E.; Brosend, K.; Friedberg, T. *Food Chem. Toxicol*. **2002**, *40*, 609–616.

[18] Ren, W.; Zhenhua, Q.; Hongwei, W.; Lei, Z.; Li, Z. *Med. Res. Rev*. **2003**, *23*, 519–534.

[19] Hayeshi, R.; Mutingwende, I.; Mavengere, W.; Masiyanise, V.; Mukanganyama, S. *Food Chem. Toxicol.* **2007**, *45*, 286–295.