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Protective effect of *Brassica oleracea* var. *botrytis* L. against theophylline-induced hepatocellular abnormalities in rats

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ABSTRACT

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BACKGROUND
Indole and its derivatives, such as indole acetic acid are active constituents of cauliflower (*Brassica oleracea var. botrytis* L.) with are known to function as plant growth hormones. Drug metabolism mainly occurs in the liver, so that the possibility of damage to this organ is considerable. The purpose of this study was to evaluate the effect of cauliflower extract on alanine transaminase (ALT) levels and on the hepatocyte morphology induced by theophylline in Wistar rats**.**

METHODS
Twenty Wistar rats were divided randomly into four groups. Group I was given only theophylline 20 mg/kg BW. Groups II, III, and IV were given cauliflower extract 200 g/kg BW, for 5, 10, 15 days, and on the last day were given theophylline 20 mg/kg BW. On days 5, 10, and 15 the animals were terminated, their livers taken and observed for microscopic appearance, while blood samples were taken for measuring ALT levels.

RESULTS
Cauliflower affected a reduction in ALT levels, mean serum ALT concentration in rats given cauliflower for 5 days (109.86 ± 2.20 IU/L was significant higher compared to rats given cauliflower for 15 days (71.58 ± 3.17 IU/L) (p=0.000). Mean values of histopathologic liver cells in rats given cauliflower for 15 days (1.17 ± 0.01) significant lower compared to rats given cauliflower for 5 days (1.45 ± 0.06) (p=0.000).

CONCLUSIONS
Cauliflower extract reduced the level of ALT and improved the microscopic appearance of hepatic cells in the rats. Cauliflower extract could lead to the development of potent anti-tumor agents for hepatic cancer treatment.

Keywords: Cauliflower extract, theophylline, liver microscopic appearance, Wistar rats

Efek protektif dari Brassica oleracea var. botrytis L. terhadap kelainan hepatoseliler pada tikus diinduksi teofilin

ABSTRAK

LATAR BELAKANG

Kubis bunga (Brassica oleracea L. var. botrytis L.) mengandung senyawa aktif indole acetic acid (IAA) memiliki fungsi sebagai hormon pertumbuhan pada tumbuh-tumbuhan. Interaksi cauliflower dengan teofilin metabolismenya terjadi di hepar, kemungkinan terjadinya perubahan komposisi sel hepar menjadi sangat besar. Penelitian ini bertujuan untuk menilai pengaruh pemberian ekstrak cauliflower terhadap perubahan kadar alanine transaminase (ALT) dan gambaran mikroskopis sel hepar pada tikus Wistar yang diberi teofilin.

METODE

Duapuluh ekor tikus Wistar dibagi secara acak menjadi 4 kelompok. Kelompok I berupa kelompok kontrol hanya diberi teofilin 20 mg/kgBB/1x pemberian. Kelompok II, III, dan IV adalah kelompok perlakuan diberi ekstrak cauliflower dengan dosis 200g/kgBB selama 5, 10, dan 15 hari, pada hari terakhir perlakuan diberi teofilin 20 mg/kgBB/1x pemberian. Pada hari ke-5, 10, 15 dilakukan terminasi, organ hepar diamati gambaran mikroskopis dan sampel darah untuk pengukuran kadar ALT.

HASIL

Hasil penelitian menunjukkan bahwa rata-rata kadar enzim ALT pada pemberian kubis bunga selama 5 hari (109,86 ± 2,20 IU/L) lebih tinggi secara bermakna dibandingkan pemberian ekstrak cauliflower selama 15 hari (71,58 ± 3,17 IU/L) (p=0,000). Rata-rata nilai histopatologi sel hepar pada tikus yang diberi ekstrak kubis bunga selama 15 hari (1,17 ± 0,01) lebih rendah secara bermakna dibandingkan pemberian ekstrak kubis bunga selama 15 hari (1,45 ± 0,06) (p=0,000).

KESIMPULAN

Pemberian ekstrak kubis bunga mampu menurunkan kadar enzym ALT dan memperbaiki sel hepar menuju normal pada tikur yng diinduksi dengan teofiline. Ekstrak kubis bunga cauliflower perlu dikembangkan lebih lanjut sebagai agen anti kanker yang bermanfaat untuk pengobatan kanker hati.

Kata kunci: *Ekstrak bunga kubis, teofilin, mikroskopik hepar, tikus Wistar*

INTRODUCTION

Cauliflower is a common vegetable in the diet of many communities. This plant belongs to the family Brassicaceae and its scientific name is *Brassica oleracea L.*, comprising a large number of varieties, including broccoli. The regular consumption of cauliflower results in a high probability that this vegetable is taken concurrently with drugs. Cauliflower is known to contain compounds capable of accelerating the biotransformation of concurrently ingested drugs,[1] as it contains the active compounds indole and sulforaphane. Indole has been recognized as an inducer of enzymes for the biotransformation of drugs. Both compounds are capable of inducing cytochrome P-450 and several enzymes catalyzing conjugation reactions.[2,3] In addition, both components have definite anticancer effects.[4] Cauliflower also has antioxidant properties, since it contains vitamin A, vitamin C, and several minerals. Cauliflower is capable of repairing the cytoplasmic membrane of hepatocytes and one of the mechanisms involved is through its antioxidant effect. One study reported that progoitrin was one of the

predominant glucosinolates in *Brassica oleracea* (cabbage). Glucosinolates and their derived products have been reported to have health beneficial effects by reducing the risk of certain cancers in humans.[5]

Theophylline is one of the antiasthma drugs that is still frequently utilized. In addition to its relatively low cost, this drug is also much prescribed by doctors. Furthermore, the side effects of theophylline are known, and may therefore be anticipated by doctors, while it is clinically beneficial as a bronchodilator.[6] Theophylline has even been recommended by the Ministry of Health of the Republic of Indonesia, and in (2007/2008) year it was classified as a drug for respiratory tract complaints obtainable without prescription in the "One thousand drugs program" *("Program obat seribu")*. However, because theophylline is metabolized in the liver and has a narrow therapeutic range, it may become relatively dangerous if not consumed according to need. Drugs that are metabolized in the liver may have potential hepatotoxic effects. Xenobiotics or drugs that are given in high doses and/or for long periods result in an intimate and longer contact with the hepatocytes.[6] Drug interactions are considered to be clinically important if they increase the toxicity or decrease the effectiveness of the interacting drugs. In the hepatocellular environment the effect of these interactions may result in inflammation, degeneration, intracellular deposits, necrosis, and fibrosis. These effects depend on the xenobiotics in question, but are also affected by their dose and duration of contact.[6]

One simple and routine test for determining liver functions is the measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). ALT is bound to the cytoplasm of hepatocytes, while AST is bound to their organelles. Hepatocytes undergoing necrosis will cause a rise in the serum concentrations of both enzymes. Although ALT and AST are frequently considered to be liver enzymes, because of the high concentration of both enzymes in hepatocytes, only ALT is specific to the liver. In contrast, AST is also found in cardiac muscle, skeletal muscle, the kidneys, and the pancreas.[7] The purpose of this study was to evaluate the effect of administration of cauliflower extract on ALT concentrations and hepatocyte morphology in Wistar rats given theophylline.

METHODS

Study design

This was an experimental laboratory study using a post test only with control group design to evaluate the effect of cauliflower extract on ALT concentrations and hepatocyte morphology in Wistar rats given theophylline. The study was conducted from January until June 2011.

Preparation of cauliflower extract

The cauliflower sample (*Brassica oleracea* var. *botrytis* L.) came from the area of Bandungan in Central Java. To prepare the extract, the vegetable was macerated in water at low temperature, to obtain a concentration and volume corresponding to the desired dose.

Experimental animals

The experimental animals used were male Wistar albino rats, 2-3 months old and weighing 190-250 grams, obtained from the Integrated Research and Testing Institute (*Lembaga Penelitian dan Pengujian Terpadu*, LPPT), Gajah Mada University, Yogyakarta. The study used a total of twenty animals, based on a sample size of 5 animals per group. The inclusion criteria were: Wistar rats, male, age 2-3 months, weight 190-250 grams, in healthy condition. Rats with anatomical deformity were excluded from the study.

The twenty male Wistar rats were assigned to 4 groups by simple randomization, resulting in 5 animals in each group. Group I was given a single oral theophylline dose of 20 mg/kgBW,[5] while groups II-IV received cauliflower extract orally for 5 days at a concentration of 200g/

kgBW, for 5, 10, and 15 days, respectively, followed by a single oral theophyilline dose of 20mg/kgBW on the last day of intervention. On days 5, 10, and 15 the animals were terminated and the livers taken and observed for abnormal hepatocyte morphology, which was scored by the method of Maretnowati, [8] Blood samples was also taken for determination of ALT concentrations. The use of a single oral theophylline dose of 20 mg/kgBB was based on the therapeutic dose generally used in medical practice, and the cauliflower extract was given for 5, 10 and 15 days to protect the hepatocytes against theophylline-induced abnormalities.

Measurement of ALT

Blood samples were collected from the lateral ocular vein for determination of post test ALT concentrations and analyzed by means of a 4010 photometer and GPT ready-made reagents. The enzyme concentration was measured by putting 100 µl serum into a cuvette, adding 1000 µl of the enzyme reagent R1, letting the mixture stand for 5 minutes, then adding 200 µl of the substrate reagent R2, mixing, and reading the result in a spectrophotometer at 340 nm wavelength.

Preparation of histopathology slides

Liver tissue was fixed in formalin 10%, made into blocks and sectioned with a microtome at a thickness of ± 3 mm, then stained with hematoxylin-eosin, and observed under the microscope at a magnification of 400x. The degree of hepatocyte abnormality was scored according to the method used by Maretnowati (Table 1).[8]

Table 1. Scoring of hepatocyte abnormality [8]

Degree of abnormality	Score
Normal cellular morphology	1
Parenchymatous degeneration	2
Hydropic degeneration	3
Necrosis	4

The scoring results were obtained from observation of 20 hepatocytes in five different fields of view, thus obtaining a total of 100 cells, at a magnification of 400x, then the scores were used to calculate the histopathologic value of the hepatocytes, expressed as the mean (± standard deviation) of 100 observations for each group.

Ethical clearance

Ethical clearance was obtained from the Committee for Animal Research Ethics at the Faculty of Public Health, Diponegoro University.

Data analysis

The obtained results in the form of data on serum ALT activity and histopathologic value of hepatocytes were tested by one way Anova at confidence level of 95%, using SPSS version 13.

RESULTS

Administration of cauliflower extract for 5, 10 and 15 days resulted in measurable changes in ALT concentrations, determined on the last day of intervention. The resulting serum ALT concentrations are shown in Table 2. These demonstrate the occurrence of interactions between theophylline and cauliflower, with

Table 2. Mean serum ALT* concentrations by intervention group

	Intervention				
	Theophylline (n=5)	Cauliflower extract for 5 days + theophylline (n=5)	Cauliflower extract for 10 days + theophylline (n=5)	Cauliflower extract for 10 days + theophylline (n=5)	p
ALT level (IU/L)	176.26 ± 5.48	109.86 ± 2.20	85.10 ± 3.89	71.58 ± 3.17	0.000

*ALT=Alanine aminotransferase

Table 3. Mean values of histopathologic of hepatocytes, by intervention group

	Intervention				p
	Theophylline (n=5)	Cauliflower extract for 5 days + theophylline (n=5)	Cauliflower extract for 10 days + theophylline (n=5)	Cauliflower extract for 15 days + theophylline (n=5)	
Hepatocyte histopathologic value	2.77 ±0.04	1.45 ± 0.06	1.26 ±0.04	1.17 ± 0.01	0.000

increasing length of administration of cauliflower, leading to a reduction in serum ALT concentrations in the rats. Mean serum ALT concentration in rats given cauliflower for 5 days (109.86 ± 2.20 IU/L was significant higher compared to rats given cauliflower for 15 days (71.58 ± 3.17 IU/L) (p=0.000).

The results of Tukey multiple comparison test indicated significant differences in ALT concentrations between theophylline and cauliflower administration for 5, 10 and 15 days followed by theophylline (p=0.000).

Histopathologic analysis of the hepatocytes was performed qualitatively and quantitatively to determine the hepatocyte morphologic abnormalities induced by interactions between theophylline and cauliflower, with increasing length of administration of cauliflower. The data on microscopic observation of the hepatocytes representative of each intervention group, may be seen in Table 3 and Figures 1-4.

Table 3 shows that the histopathologic value of the hepatocyte after administration of cauliflower extract for 5, 10, and 15 days, decreased with increasing length of administration. Mean values of histopathologic liver cells in rats given cauliflower for 15 days (1.17 ± 0.01) significant lower compared to rats given cauliflower for 5 days (1.45 ± 0.06) (p=0.000) This may be explained by the presence in cauliflower of sulforaphane and indole, which are capable of restoring the cytoplasmic membrane of hepatocytes, to accelerate their regeneration. The histopathologic morphology representing the various histopathologic values obtained in this study may be seen in Figures 1 – 4.

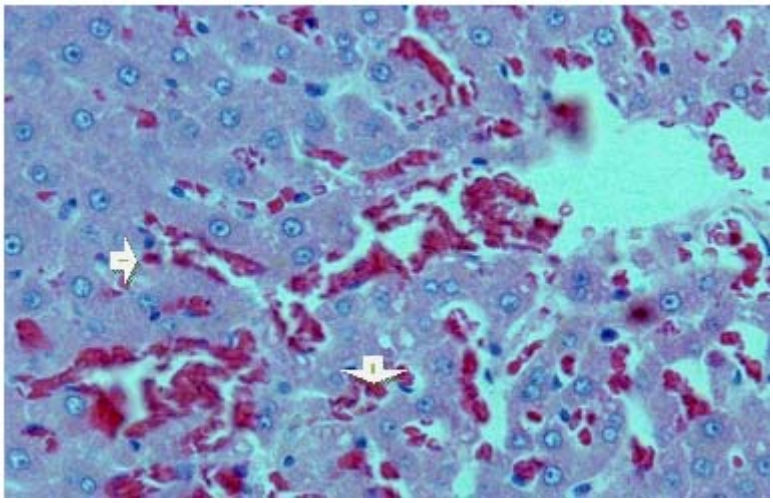

Figure 1. Hepatocyte morphology, magnification 400x , HE stain. (→) : congested cells (2.778)

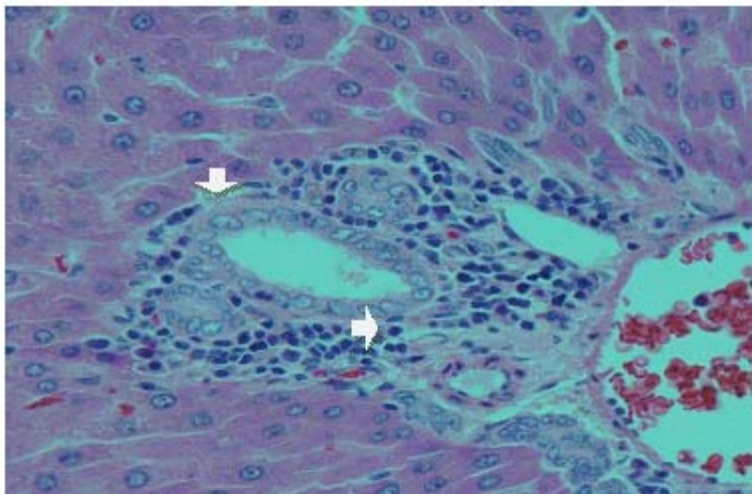

Figure 2 Hepatocyte morphology, magnification 400x , HE stain.(→) : cells with parenchymatous degeneration (1.456)

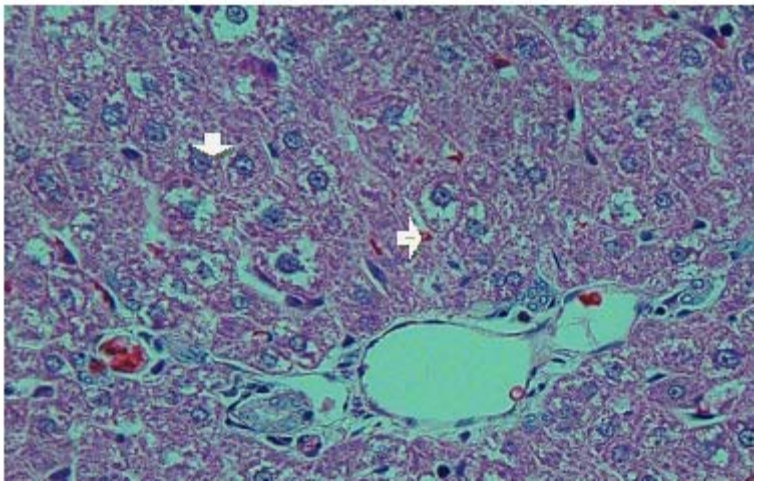

Figure 3. Hepatocyte morphology, magnification 400x, HE stain.(→) : cells with parenchymatous degeneration. (1.266)

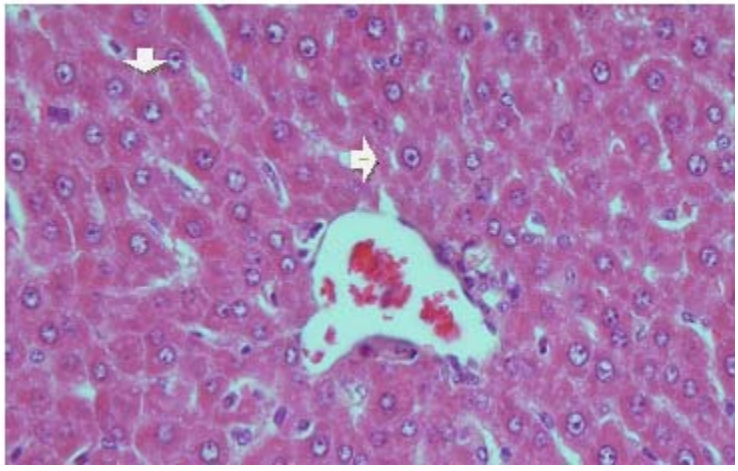

Figure 4. Hepatocyte morphology, magnification 400x , HE stain.(→) : normal cells (1.176)

DISCUSSION

The present study demonstrates that administration of cauliflower extract for 5, 10, and 15 days was capable of reducing serum ALT concentrations in rats given a single dose of theophylline. The results of this study are consistent with those of a study on the administration of *Brassica oleracea var. botrytis* L. for 30 days to female rats followed by intraperitoneal diethylnitrosamine (DEN) for 7 days.[9,10]

Essentially similar results were obtained in another study, in which oral administration of broccoli caused a significant decrease in serum ALT levels in rats with hepatotoxic lesions induced by N-nitrosodiethylamine (NDEA) and carbon tetrachloride (CCl4).[11]

The biomarkers used in this study provide the measures of carcinogen exposure in rats as an area of high risk for development of hepatocellular carcinoma. This study revealed that oral administration of cauliflower extracts caused a significant decrease in serum levels of ALT. Broccoli is a plant of the family *Brassicaceae*. The observed enhancement level by *Brassica* vegetables was due to their content of glucosinolates, flavonoids and other phenolics.[12]

The anticarcinogenic activity is related to the presence of biologically active components that modulate the activity of phase I and II detoxification enzymes and other mechanisms triggered by glucosinolates, which are formed as a result of hydrolysis and catalyzed by the enzyme myrosinase. Hepatotoxic abnormalities may be evaluated from the aspects of both cellular structure and function. Evaluation of hepatocellular structure was performed by light microscopy. Cauliflower with its indole and sulforaphane content is capable of inducing conjugases through phase II drug metabolism, as demonstrated by its ability to reduce ALT concentrations.[9] In addition, its high antioxidant content also plays a role in improving liver functions. Hepatotoxic effects may take the form of inflammation, degeneration, necrosis, and fibrosis.

The observed prophylactic effect of cauliflower extract on hepatocyte morphology of the experimental animals, indicate the occurrence of significant between-group differences in histopathologic picture. The hepatocyte abnormalities may take the from of parenchymatous degeneration, hydropic degeneration, as well as necrosis. On average, histopathologic picture of the hepatocytes tended to decrease in proportion to the length of administration of the cauliflower extract. These hepatocellular changes may be due to decreased ATP levels caused by covalent binding of theophylline to intracellular proteins, resulting in abnormalities of action. Failure of actin fibril assembly on the hepatocyte surface causes cytoplasmic membrane rupture.[13]

The reduction in ALT concentrations and improvement in hepatocyte morphology by cauliflower extract in this study are due to the central role of the liver in the detoxification of drugs such as theophylline. The high content of antioxidants in cauliflower, such as vitamin A, vitamin C, and several minerals, which may act synergistically, is capable of restoring cellular functions and repairing hepatocyte menbrane damage. In addition, cauliflower contains indole and sulforaphane, which are capable of restoring hepatocellular functions to their original status.[4,12,13] This is also supported by previous studies demonstrating that cauliflower (*Brassica oleracea* var. *botrytis* L.) is also capable of reducing LDL and increasing HDL cholesterol in hyperlipidemic rats, as well as decreasing total cholesterol and MDA (malondialdehyde).[14] A Phase I clinical study have characterized the metabolism and excretion of glucosinolates and isothiocyanates of broccoli sprouts in humans to assist in the design of dosing regimens for studies of efficacy.[15] Studies of cultured cancer cells and animal models of cancer provide the most compelling evidence that the nonnutrient phytochemical composition of Brassica reduces tumor incidence.[16,17]

CONCLUSIONS

Administration of cauliflower (*Brassica oleracea* var. *botrytis* L.) has a protective effect on hepatocyte abnormalities and reduced the ALT concentrations induced by theophylline in rats. Therefore this study recommends increasing the dietary intake of cauliflower (*Brassica oleracea* var. *botrytis* L), as a complementary medicines for patients with liver cancer.

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