Biomedical Research 2017; 28 (9): 4196-4201

ISSN 0970-938X
www.biomedres.info

Tolerability and safety of black cumin seed oil (Bcso) administration for 20 days in healthy subjects.

Akrom Akrom[1,2*], Endang Darmawan[1]

[1]Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta, DIY, Indonesia

[2]Ahmad Dahlan Drug information and Crisis Center, Yogyakarta, DIY, Indonesia

Abstract

The objective of this research was to determine the effect of black cumin seed oil (BCSO) on hematology, level of aspartat aminotransferase (AST)/alanin aminotransferase (ALT), level of ureum and creatinine, and also immune response in healthy subjects. This study applied a phase 1 of clinical trial. The 36 healthy subjects were divided into three groups, 12 subjects each group. Group I, subjects received 1 × 3 soft capsules of BCSO. Group II, subject received 2 × 3 soft capsules of BCSO and in groups III subject received 3 × 3 soft capsules of BCSO. Each soft capsule contained of 0.5 ml standardized BCSO. On the 21st day, the blood cell, level of ALT and AST and level of ureum and creatinine were measured. The average number of blood cells, levels of ALT/AST and level of ureum and creatinine then were been analyzed with one way ANOVA test. Statistical analysis was performed on 95% confidence level. The results showed that the administration of three doses of BCSO soft capsule did not increase number of blood cells, level of ALT/AST and level of ureum and creatinine (p>0.05). BCSO also did not affect number of CD4+ Th and CD4+CD25+ Treg cells and also expression of IFN-γ (p>0.05). The consumption of BCSO at three doses 1.5, 3, and 4.5 ml/d for 21 days in healthy subjects were tolerable and safety.

Keywords: Black cumin seed oil, Phase 1 clinical trial, Safety, Soft capsule immunax.

Accepted on February 28, 2017

Introduction

Black cumin seed oil (BCSO) has often been used by people as an alternative and complementary medicine. BCSO contains a lot of unsaturated fatty acids such as linoleic acid and linolenic acid. It also contains of essential oil with active substances such as *thymoquinone*, and nigellone (dithymoquinone) [1-3]. Unsaturated fatty acids and *thymoquinone* are powerful antioxidants and immunomodulatory agents [4-7]. The mechanism actions of antioxidant and anti-inflammatory effects of BCSO have been investigated both in preclinical [8-10] and clinical studies [11-13].

Unfortunately, the administration of a combination of BCSO and other herbal preparations has been reported cause hepatic dysfunction in Indonesia. Furthermore, the administration of a combination between BCS and Phyllantus niruri increases the activity of ALT and AST in hospitalized-patients. But, other clinical trials showed that 300-500 mg/d of BCS powders supplied with standard drugs in patients improve the profile blood cholesterol, lowering the percentage of HbA1C and blood pressure in patients menopause and non-menopause and is not accompanied by increase in SGPT and SGOT or their harmful side effects [14-16].

This study was aimed to determine the effect of BCSO at dose of 1.5, 3 and 4.5 ml/d for 20 days on peripheral blood, liver and kidney function in healthy subjects to study the safety of BCSO. The influence of BCSO on immune responses was observed by measuring the expression of IFN-γ in T lymphocytes and the number of CD4+ Th and CD4+CD25+ Treg cells. In this study, BCSO was prepared in soft capsules to be more acceptable by the subjects.

Methods

Materials

BCSO was standardized for the concentration of thymoquinone and then was formed in soft capsules immunax (SCI). Specification of BCSO is presented in Table 1. The capsules were given to the subjects orally daily at 1.5 ml (1 × 3 SCI), 3 ml (2 × 3 SCI) and 4.5 ml (3 × 3 SCI).

Table 1. *The content of BCSO in the soft capsules immunax (SCI).*

Content of BCSO	Relative concentration (%)
Thymoquinone	2.72
Caprilic acid	0.21

Capric acid	0.15
Lauric acid	0.1
Myristic acid	0.18
Palmitic acid	12.27
Palmitoleic acid	0.28
Heptadecanoic acid	0.1
Oleic acid	0.07
Linoleic acid	2.85
Linolenic acid	0.1
Eicosanoic acid	3.15
Eicosenic acid	0.15
Eicosadienoic acid	0.25
Arachidonic acid	0.03
Eicopentanoic acid	0.03
Behenic acid	0.06
Docoheksanoic acid	0.04
Teracosanoic acid	0.02

Subjects

The inform consent and the ethical clearance for this study was reviewed and approved by Research Ethics Committee, Ahmad Dahlan University (No: 011503029). Respondents for the study were selected based on the inclusion and exclusion criteria. The inclusion criteria were men/women aged 18-60, be healthy evidenced by a health certificate, and willing to be the respondent (by filling the informed consent). The exclusion criteria were woman pregnancy and hypersensitive history to ingredient of Nigella sativa oil. Physical examination and clinical examination of prospective subjects are conducted to ascertain the health status.

Recruitment of subjects

The brochures/leaflets, the recruitment procedure, the informed consent, the research procedures as well as case report form have been reviewed and approved by the Ethics Committee of Ahmad Dahlan University. Researchers distributed the brochures and the announcement about the recruitment of volunteers of BCSO safety testing in the campus of Ahmad Dahlan University and Gadjah Mada University as well as in some public facilities in Yogyakarta. Interested people should call on the phone number listed in the brochure or leaflet. For prospective volunteers who have signed up are then invited for an explanation of the purpose, benefits, and procedures as well as the consequences and the rights of research volunteers. After signing the informed consent, the subjects performed a physical and clinical examination to ascertain their health status. A total of 36 volunteers received health certificates and signed the informed consents.

Experimental design

A total of 36 healthy subjects were randomly divided into three experimental groups. Group 1 was given 1 × 3 BCSO soft capsules (SC) (1.5 ml/d), group 2 was given 2 × 3 BCSO SC (3 ml/d) and group 3 was given 3 × 3 BCSO SC (4.5 ml/d). BCSO SC were orally administered every day for 20 days. In day 21, the peripheral blood, the levels of AST/ALT, BUN and creatinine level, number of CD4+ Th and CD4+ CD25+ Treg cells and expression of IFN-γ were determined.

Blood pressure and heart rate measurement

Measurement of blood pressure and heart rate is performed three times during the study by professionals. Blood pressure was measured in the left arm using a mercury sphygmomanometer, supine sleeping position, wide cuff adapted to the size of the arm, as recommended by WHO. Each blood pressure measurement was repeated 2 times with 30 minutes interval time between measurements. The heart rate was calculated on the radial artery and repeated 3 times. The value of blood pressure and heart rate were presented as average value.

Peripheral blood, renal and liver function analysis

Peripheral blood was taken and collected from the cubital vein by authorized analyst. The blood was then examined for the peripheral blood and kidney function by measuring level of urea and creatinine using hematoanalyzer as performed in a previous study [17].

Expression of IFN-γ and number of CD3+CD4+ Th and CD3+CD4+CD25+ treg cells analysis

Immune response was observed by measuring the expression of IFN-γin T lymphocytes and the number of CD4+ Th and CD4+CD25+ Treg cells. IFN-γ expression and the number of CD4+ Th and CD4+CD25+ Treg cells been measured using flow cytometry.

Data analysis

The significant difference between groups was tested by one way Anova analysis. The difference intern group before and after treatment were tested by two-way ANOVA. The statistical test was performed at 95% confidence level. The average difference in blood pressure and heart rate between measurements in one group was analyzed with repeated ANOVA.

Results

Demographic characteristic of respondents

Respondents who participated in this study are 36 subjects consisting of 10 (22.92%) men and 26 (77.08%) women. Characteristics of the respondents are presented in Table 2. Respondents in this study aged 18-60 years. Based on body mass index (BMI), there were 15 (40.83%) people having BMI

<25 (non-obese) and 21 (59.17%) subjects having BMI ≥ 25 (obese). Based on a history of hypertension owned respondents, there were 2 (5.17%) people with history of hypertension and 34 (94.83%) had no history of hypertension. Based on the level of education, respondents who had finished high school were 9 (25.00%) people and 27 (75%) respondent have passed university. While based on marital status, 5 (14.58%) respondents are categorized as married and 31 (85.42%) people are unmarried. Statistically the sex, age, BMI, history of hypertension, education, occupation and marital status of respondents were not different (p>0.05).

Table 2: *Characteristic of respondents consummated BCSO 1.5; 3; 4.5 ml/d.*

Characteristic of the respondents	n	%	p
Sex			
Men	10	27.8	0.35
Women	26	72.2	
Age			
≤ 25 years	30	83.33	0.947
>25 years	6	16.67	
Body mass index (BMI)			
<25 (kg/cm^2)	15	41.7	0.918
≥ 25 (kg/cm^2)	21	58.3	
History of Hypertension			
Yes	2	4.17	0.837
No	34	95.83	
Education			
High School	9	25.00	0.056
Graduate program	27	75	
Occupation			
Student	28	79.17	0.734
Non-government	6	14.58	
entrepreneur	2	6.25	
Marital status			
Married	5	14.58	0.673
Not married	31	85.42	
Health assurance			
Private health assurance	3	8.3	0.056
Public heath assurance	10	27.8	
Not health assurance	23	63.9	

Clinical characteristic of respondents

The clinical condition of the volunteers before treatment are presented in Table 3. As shown in Table 3, the clinical condition of the subject prior to the administration BSCO under similar conditions. Levels of hemoglobin, number of erythrocytes, leukocytes and platelets within the normal range and comparable between treatment groups (p>0.05). The results of the number of leukocytes to monocytes, neutrophils, eosinophils and basophils in all three groups were comparable (p>0.05).

Table 3: *Clinical characteristics of the healthy subject before BSCO administration.*

Clinical characteristic	Treatment Groups			p
	Group I (n=12)	Group II (n=12)	Group III (n=12)	
Age (years)	25.83 ± 6.58	24.08 ± 4.48	23.83 ± 4.45	0.6
Body Weight (Kg)	57.42 ± 11.33	58.00 ± 16.18	61.50 ± 17.66	0.8
Pulse (frex/menutes)	73.00 ± 4.39	76.16 ± 7.79	75.50 ± 5.60	0.6
Systolic blood pressure (mmHg)	116.25 ± 16.80	117.00 ± 14.19	114.35 ± 17.89	0.8
Diastolic Blood Pressure (mmHg)	73.75 ± 10.02	73.33 ± 13.70	73.75 ± 12.45	0.7
Hb (pg)	13.28 ± 13.28	13.04 ± 1.17	14.19 ± 0.74	0.6
Erithrocytes (×10^6/mL)	4.885 ± 0.37	5.02 ± 0.43	5.15 ± 0.51	0.2
Leucocytes (×10^3/mL)	8.22 ± 2.05	7.42 ± 1.68	9.26 ± 4.45	0.5
Thrombocytes (×10^3/mL)	311.17 ± 73.73	302.17 ± 85.96	299.90 ± 56.35	0.6
Limphocytes (%)	30.58 ± 4.85	36.41 ± 8.48	31.67 ± 9.61	0.6
Monocytes (%)	7.67 ± 1.30	7.00 ± 1.28	6.17 ± 1.11	0.3
Neutrophil (%)	57.92 ± 6.68	53.83 ± 9.67	59.42 ± 10.48	0.8
Eosinophil (%)	3.83 ± 2.62	2.75 ± 1.76	2.75 ± 1.54	0.9
ESR hour 1 (mm/h)	14.92 ± 11.06	16.33 ± 12.87	15.67 ± 12.18	0.8
ESR hour 2 (mm/h)	34.67 ± 24.42	32.75 ± 22.17	32.25 ± 20.33	0.9
MCV (fl)	81.94 ± 7.02	81.49 ± 7.82	83.02 ± 5.67	0.5
MCH (pg)	27.41 ± 2.63	26.81 ± 3.48	27.75 ± 1.90	0.5
MCHC (%)	33.44 ± 0.72	32.82 ± 1.41	33.45 ± 0.66	0.4
RDW (%)	13.77 ± 1.86	13.66 ± 1.75	13.05 ± 0.78	0.6
Glucose (mg/dl)	89.17 ± 13.23	94.33 ± 14.67	93.33 ± 16.52	0.7
Total cholesterole (mg/dl)	177.00 ± 23.33	197.25 ± 24.38	183.67 ± 31.26	0.2
Triglyceride (mg/dl)	97.08 ± 34.91	149.33 ± 95.34	99.42 ± 53.89	0.1
ALT (mg/dl)	23.58 ± 29.26	18.03 ± 16.80	20.06 ± 17.69	0.9
AST (mg/dl)	22.47 ± 21.51	20.76 ± 11.62	19.19 ± 9.18	0.9

BUN (mg/dl)	7.96 ± 1.80	8.43 ± 2.31	7.55 ± 2.31	0.4
Creatinine (mg/dl)	0.71 ± 0.11	077 ± 0.16	0.76 ± 0.16	0.5
IFN-γ (%)	3.06 ± 1.95	2.19 ± 1.51	3.44 ± 2.86	0.6
CD4+ Th Cells (%)	46.19 ± 7.55	41.49 ± 8.61	39.30 ± 9.17	0.6
CD4+CD25+ Treg Cells (%)	24.30 ± 6.71	28.30 ± 9.50	25.20 ± 6.17	0.5

Analysis of blood pressure and heart rate

In this research, monitoring clinical conditions of the respondents included blood pressure and heart rate. Clinical examination includes blood pressure, temperature; rhythm and heart rate were performed on day 0, 10 and 20. The average of blood pressure of the respondents is presented in Table 4.

Table 4: *The average of blood pressure between control and treatment groups treated by BCSO.*

Groups	Parameters	Blood pressure (mmHg) and pulse (frex/men) (mean ± SD)			p
		Control	Day 10	Day 20	
Group I (n=12)	Systolic BP (mmHg)	116.25 ± 16.80	109.50 ± 11.29	110.83 ± 11.64	>0.05
	Diastolic BP (mmHg)	73.75 ± 10.02	70.08 ± 7.26	69.58 ± 9.40	>0.05
	Pulse (frex/menut)	73.00 ± 4.39	75.00 ± 5.42	76.33 ± 8.97	>0.05
Group II (n=12)	Systolic BP (mmHg)	117.00 ± 14.19	116.67 ± 14.03	112.50 ± 12.88	>0.05
	Diastolic BP (mmHg)	73.33 ± 13.70	70.00 ± 9.04	65.83 ± 11.83	>0.05
	Pulse (frex/menit)	76.16 ± 7.79	78.33 ± 8.97	74.16 ± 7.00	>0.05
Group III (n=12)	Systolic BP (mmHg)	114.35 ± 17.89	112.08 ± 14.37	107.92 ± 16.98	>0.05
	Diastolic BP (mmHg)	73.75 ± 12.45	72.91 ± 9.40	70.41 ± 12.14	>0.05
	Pulse (frex/menit)	75.50 ± 5.60	74.83 ± 5.74	77.33 ± 7.92	>0.05

Overall, the average of the systole and diastole blood pressure both on day 0 and day 10 was not significant ($p>0.05$). While there was a significant difference in the average of blood pressure on day 0 and day 10 compared to day 20 both systole and diastole ($p<0.05$). However, there are no significant differences in the average blood pressure in both systole and diastole between the groups treated with control ($p>0.05$). BCSO did not significantly affect to the blood pressure and heart rate than the control group. Thus, the consumption of BCSO for 20 days does not significantly affect blood pressure in healthy subjects.

Blood composition and liver & renal function analysis

The administration of 1 × 3, 2 × 3 and 3 × 3 SC BCSO for 20 days in healthy respondents did not affect the blood components. The number of blood cells such as leukocytes, erythrocytes and platelets within normal limits and there was no significant difference between treatment groups ($p>0.05$). The normal value of hemoglobin is >13 ml/dl in men or >12 mg/dl in women. Hb value of all groups was >13 mg/dl. The number of erythrocytes for normal condition is $3^{-7} \times 10^3$ cell/dl. The number of erythrocytes in all groups was $>4 \times 10^6$ cells/dl. The results showed that administration of a 1 × 3, 2 × 3 and 3 × 3 BCSO SC in healthy respondents did not affect blood cell number.

Table 5. *The clinical characteristic of healthy subject after administration 1.5, 3 and 4.5 ml/day BCSO for 20 days (each group n=12 subject).*

Blood components	Treatment groups			p
	Group1 treated 1.5 ml BCSO	Group2 treated 3 ml BCSO	Group3 treated 4.5 ml BCSO	
Hemoglobin (pg)	13.33 ± 1.47	13.62 ± 1.60	14.12 ± 0.57	>0.05
Erythrocyte ($\times 10^6$/ml)	4.87 ± 0.31	5.09 ± 0.41	5.06 ± 0.46	>0.05
Hematocrit (%)	39.53 ± 3.79	4.0 ± 3.87	41.69 ± 1.3	>0.05
Leucocyte ($\times 10^3$/ml)	8.34 ± 1.64	8.44 ± 1.85	76.1 ± 1.63	>0.05
MCV (fl)	81.30 ± 7.3	80.98 ± 8.0	82.84 ± 5.57	>0.05
MCH (pg)	27.38 ± 2.75	26.9 ± 3.45	28.04 ± 1.98	>0.05
MCHC (%)	33.66 ± 0.81	33.13 ± 1.32	33.86 ± 0.56	>0.05
RDW (%)	13.97 ± 2.29	13.75 ± 1.80	13.14 ± 0.80	>0.05
Thrombocyte ($\times 10^3$/ml)	302 ± 70.56	312 ± 80.71	305 ± 52.66	>0.05
Lymphocyte (%)	31.75 ± 4.98	33.33 ± 7.86	33.58 ± 8.24	>0.05

Monocyte (%)	7.00 ± 1.54	6.67 ± 1.23	8.42 ± 3.90	>0.05
Neutrophil (%)	57.83 ± 6.28	57.67 ± 8.82	55.42 ± 10.76	>0.05
Eosinophil (%)	3.42 ± 2.81	2.33 ± 1.44	2.82 ± 2.02	>0.05
ESR hour 1 (mm/h)	14.58 ± 11.0	14.00 ± 11.0	16.08 ± 11.07	>0.05
ESR hour 2 (mm/h)	34.25 ± 20.67	30.75 ± 22.12	32.75 ± 20.63	>0.05
Glucose (mg/dl)	89.17 ± 13.23	94.33 ± 14.67	93.33 ± 16.52	>0.05
Total cholesterol (mg/dl)	177.00 ± 23.33	197.25 ± 24.38	183.67 ± 31.26	>0.05
Triglyceride (mg/dl)	97.08 ± 34.91	149.33 ± 95.34	99.42 ± 53.89	>0.05
AST (mg/dl)	22.81 ± 21.70	19.18 ± 7.80	18.52 ± 3.38	>0.05
ALT (mg/dl)	19.57 ± 25.59	16.13 ± 14.53	16.82 ± 11.96	>0.05
BUN (mg/dl)	8.17 ± 1.68	9.13 ± 3.05	7.83 ± 2.27	>0.05
Creatinine (mg/dl)	0.72 ± 0.0.07	0.78 ± 0.15	0.77 ± 0.14	>0.05
IFN-γ expression (%)	4.52 ± 2.84	3.65 ± 1.54	5.02 ± 2.60	>0.05
CD4+Th (%)	53.49 ± 7.76	49.94 ± 6.94	46.24 ± 5.33	>0.05
CD4+CD25+Treg (%)	13.23 ± 5.99	12.11 ± 2.18	12.44 ± 3.19	>0.05

Table 5 revealed that the administration of 1 × 3, 2 × 3 and 3 × 3 BCSO SC for 20 days did not affect the levels of AST, ALT, BUN, and creatinine. The levels of BUN, creatinine, and ALT/AST of subjects within the normal range. The levels of BUN, creatinine and AST/ALT after the treatment did not differ between the experimental groups ($p>0.05$). ALT and AST are useful biomarkers of liver function. ALT and AST values on all treatment groups were in the normal range. In recent years it was reported that the administration of BCSO combined with other herbal preparations caused hepatic dysfunction, but our study indicate that administration for 20 days BCSO does not affect liver function and kidney function.

Expression of IFN-γ and Number of CD4+ Th and CD4+CD25+ Treg cells analysis

The administration of 1 × 3, 2 × 3 and 3 × 3 BCSO SC for 20 days in healthy subjects did not affect the number of CD4+ Th cells, CD4+CD25+ Treg cells and expression of IFN-γ in lymphocytes. Table 5 shows that the BCSO administration for 20 days in healthy subject did not affect the number of CD4+ Th and CD4+CD25+ Treg cells and also expression of IFN-γ ($p> 0.05$). BCSO may increase the immune response. Our preclinical study demonstrated that administration of BCSO increases macrophage activity and T lymphocytes in rats exposed by dimethylbenzantracene (DMBA) [17].

Acknowledgements

We acknowledge to all volunteers who are willing to become study subjects. Thanks are also expressed to the Ministry of Higher Education, Research and Technology, Indonesian Government for funding this research.

References

1. Akrom A. Chemopreventive mechanisms hexane extract of Nigella sativa seeds on DMBA-induced SD rats: study of the antioxidant and immunomodulatory. Dissertation, Doctoral Program of the Faculty of Medicine and Health, Gadjah Mada University, Yogyakarta.
2. Farrah KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immunopotentiating effects of Nigella sativa L., oil in streptozotocin-induced diabetic hamsters. Res Vet Sci 2004; 77: 123-129.
3. Nickavar B, Mojab F, Javidnia K, Amoli MA. Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran. Z Naturforsch C 2003; 58: 629-631.
4. Iddalmadeniya SS, Thabrew ML, Wickramasinghe SMDN, Ratnatunge N, Thammitiyagodage MG. A long-term investigation of the anti-hepatocarcinogenic potential of an indigenous medicine comprised of Nigella sativa, Hemidesmus indicus and Smilax gabra. J Carcinog 2006; 8: 6.
5. El Sayed I, Fukushima S. Chemopreventive potential of volatile oil from black cumin (Nigella sativa L) seeds against rat colon carcinogenesis. Cancer 2003; 45: 195-202.
6. Mousa D, Dilsiz N, Gumushan H. Antitumor activity of an ethanol extract of Nigella sativa seeds. Biologia-Bratislava 2004; 59: 735-740.
7. Randhawa MA, Al-Ghamdi MS. A review of the pharmaco-therapeutic effects of Nigella sativa. Pakistan J Med Res 2002; 41: 2.
8. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000; 14: 323-328.

9. El Aziz MA, Hassan HA, Mohammed MH, Meki AR. The biochemical and morphological alterations following administration of melatonin, retinoic acid and Nigella sativa in mammary carcinoma: an animal model. Int J Exp Pathol 2005; 86: 383-396.
10. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol 2001; 110: 239-251.
11. Fallah AM, Mohtashami R, Ghamarchehre ME, Sadeqhi Z, Kianbakht S, Huseini FA. Blood pressure lowering effect of Nigella sativa L. seed oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. Phytother Res 2013; 27:1849-1853.
12. Dehkordi FR, Kamkhah AF. Antihypertensive effect of Nigella sativa seed extract in patients with mild hypertension. Fundam Clin Pharmacol 2008; 22: 447-452.
13. Ibrahim RM, Hamdan NS, Ismail M, Saini SM, Abd Rashid SN. Protective Effects of Nigella sativa on Metabolic Syndrome in Menopausal Women. Adv Pharm Bull 2014; 4: 29-33.
14. Najmi A, Nasiruddin M, Khan RA, Haque SF. Therapeutic effect of NS (Nigella sativa) in patients of poor glycemic control. Asian J Pharm Clin Res 2012; 5: 224-228.
15. Najmi A, Nasiruddin M, Khan RA, Haque SF. Indigenous herbal product Nigella Sativa proved effective as an antihypertensive in metabolic syndrome. Asian J Pharm Clin Res 2013; 6: 61-64.
16. Shah A, Khan GM, Badshah A, Shah SU, Shah KU, Mirza SA. Nigella sativa provides protection against metabolic syndrome. Afr J Biotech 2012; 10919-10925.
17. Akrom A, Nurani LN, Hidayati T. Study of the immunomodulatory activity of the active isolate agent chemopreventive N. sativa extract on breast cancer caused by exposure to DMBA in the rat. Research Reports LPP UAD Yogyakarta 2008.

*Correspondence to

Akrom Akrom

Head of Ahmad Dahlan Drug Information and Crisis Center

Universitas Ahmad Dahlan

Yogyakarta

Indonesia