

Effect of Steamed Broccoli Juice (*Brassica oleraceae* L. var. *italica*) to the Serum Interleukin 8 Level in Colitis Murine Model

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ABSTRACT

Background: Ulcerative colitis (UC) is a bowel inflammation that happens in the colon and rectum. In UC there is an increased level of inflammatory mediators, one of which is interleukin-8 (IL-8), the main chemoattractant for polymorphonuclear (PMN) inflammatory cells causing inflammation to worsen. Broccoli (*Brassica oleraceae* L. var. *italica*) contains sulforaphane which can inhibit nuclear factor kappa B (NF- κ B), resulting in the decrease of IL-8. This study was performed to identify the effect of steamed broccoli juice to the serum interleukin-8 level in murine model of colitis.

Method: This study was a true experimental laboratory study using complete randomized design. Experimental animals being used were 28 murines with Balb/c strain and were divided into 4 intervention groups. Serum IL-8 level was measured by using enzyme-linked immunosorbent assay (ELISA) method. Data analysis was performed using analysis of variance (ANOVA) test, followed by multiple comparisons Tukey honest significant difference (HSD) test ($\alpha = 0.05$) and $p \leq 0.05$.

Results: The highest mean of IL-8 level was found in the positive control group. This study also showed highly significant difference ($p < 0.001$) of IL-8 level between positive control group and treatment group.

Conclusion: Steamed broccoli juice could decrease serum interleukin-8 level in murine model of colitis.

Keywords: ulcerative colitis, sulforaphane, interleukin-8

ABSTRAK

Latar belakang: Kolitis ulseratif (KU) adalah peradangan usus yang terjadi pada kolon dan rektum. Pada KU terjadi peningkatan kadar mediator inflamasi, salah satunya adalah interleukin-8 (IL-8) yang merupakan kemoatraktan utama untuk sel radang polymorphonuclear (PMN), menyebabkan peradangan semakin parah. Brokoli (*Brassica oleraceae* L. var. *italica*) mengandung sulphoraphane yang dapat menghambat nuclear factor kappa B (NF- κ B) sehingga terjadi penurunan IL-8. Penelitian ini dilakukan untuk mengetahui pengaruh sari kukusan brokoli terhadap kadar interleukin-8 serum pada mencit model kolitis.

Metode: Penelitian ini adalah penelitian eksperimental laboratorium sungguhan dengan rancangan acak lengkap (RAL). Hewan percobaan yang digunakan adalah mencit galur Balb/c sebanyak 28 ekor dan dibagi menjadi 4 kelompok perlakuan. Kadar IL-8 serum diukur dengan menggunakan metode enzyme-linked immunosorbent assay

(ELISA). Analisis data dilakukan dengan menggunakan analisis varian (ANOVA) satu arah, dilanjutkan dengan uji multiple comparisons Tukey honest significant difference (HSD) ($\alpha = 0,05$) dan $p \leq 0,05$.

Hasil: Rerata kadar IL-8 yang paling tinggi terdapat pada kelompok kontrol positif. Penelitian ini juga menunjukkan perbedaan yang sangat signifikan ($p < 0,001$) kadar IL-8 antara kelompok kontrol positif dan kelompok terapi.

Simpulan: Sari kukusan brokoli dapat menurunkan kadar interleukin-8 serum pada mencit model kolitis.

Kata kunci: kolitis ulserativa, sulforaphane, interleukin-8

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammation of the bowel, which consists of Chron's disease (CD) and ulcerative colitis (UC). Pathogenesis of these two diseases has not been fully understood. Genetic and environmental factors hold important role in the dysregulation of intestinal immunity which further causes trauma to the digestive tract.¹ UC is the most frequent form found in IBD patients. The incidence of UC worldwide reaches 1.2-20.3 cases per 100,000 individual per year. The highest incidence and prevalence of IBD were found in America and North Europe, while the lowest incidence and prevalence were found in Asia. However, based on the available data, there was increased incidence of IBD in Asia. This is associated with the environmental condition and life style in Western Countries, which may cause the incidence of IBD, such as: smoking, high fat diet, sugar, stress, particular drugs, and the high socio-economic status.^{2,3} Data from endoscopy unit in several hospitals in Jakarta revealed IBD cases with chronic diarrhea 12.2%, IBD with haematochezia 3.9%, IBD with stomachache 2.8%, and IBD with chronic diarrhea, haematochezia, and stomachache 25.9%.⁴

Pathogenesis of UC is often associated with the presence of increased inflammatory mediators and oxidative stress.⁵ Nuclear factor kappa B (NF- κ B) is a cytokine regulator and pro-inflammatory chemokine. One of the main chemokines is interleukin 8 (IL-8).⁶ The level of IL-8 in the intestinal mucosa of patients with UC is higher compared to patients with CD. The increase of IL-8 is in accordance with the severity of disease histopathologically.^{7,8} IL-8 is the main chemoattractant in CD and UC.^{9,10,11}

Broccoli (*Brassica oleracea* L. var. *italica*) belongs to the family Brassicaceae. Broccoli contains sulforaphane (SFN) substance which has protection benefit towards bacteria, cancer, and diabetes.¹² Previous studies showed that administration of broccoli to murine might give preventive effect towards

colitis induction by dextran sulphate sodium (DSS) in the form of amelioration in diarrheal degree and clinical score.¹³ SFN which is contained in broccoli can decrease the concentration of proinflammatory cytokine produced by macrophage in bacteria infected murine model.¹⁴ SFN has been studied to have anti-inflammatory effect. SFN can bind to Nrf-2 which further cause the release of phase II enzyme and is produced by non-toxic agent to inhibit inflammatory process. Anti-inflammatory mechanism of SFN is also associated with the inhibition of pro-inflammatory molecule signal from NF- κ B. In vitro study showed that SFN decreased the release of interleukin, and TNF- α through direct inhibition of NF- κ B pathway which function in regulation of inflammatory cytokines and inflammatory chemokines.^{13,15,16} Until recently, there is no study of steamed broccoli juice as a treatment of UC. Based on the aforementioned things, this study was performed to know the role of steamed broccoli juice in decreasing serum IL-8 level in colitis murine model. The novelty of this study was to identify how steamed broccoli juice colitis could play role as a curative agent in colitis murine model by suppressing IL-8 pathway as a chemotaxis pathway.

METHOD

This study was a true experimental laboratory study with complete randomized design. The serum IL-8 level was measured by using enzyme-linked immunosorbent assay (ELISA) and was read using ELISA plate reader with 450 nm wavelength. Experimental animals being used were 28 male murines BALB/c strain aged 8 weeks, with weight ranged around 25 grams, divided into 4 groups of intervention ($n = 6$) based on the formula $(t-1) (n-1) \geq 15$ ¹⁷ in which t is the total intervention group and n is the number of murine being used. Research groups consisted of negative control (NC), broccoli control (BC), positive control (PC), and broccoli treatment (B) groups. From day-1 to day-7 the negative control and broccoli control groups were given aquadest, while the

colitis group consisted of positive control and broccoli treatment groups were given DSS 2.5% (w/v) as much as 0.5 mL through oral route using nasogastric tube. On day-15 all murines' serum blood were taken for serum IL-8 level examination. Data was obtained in pg/mL, analysed using one way analysis of variance (ANOVA) test continued with multiple comparisons test Tukey honest significant difference (HSD) with $\alpha = 0.05$ using SPSS version 17 software, in which a difference were marked to be significant if $p \leq 0.05$.

RESULTS

Serological examination by using ELISA method to all serum murine groups which had been given steamed broccoli juice intervention for 7 days showed the mean of IL-8 level as shown in Table 1.

Table 1. Mean of IL-8 level after 7 days of treatment

	Mean IL-8 level (pg/mL)			
	NC	BC	PC	B
1	0.184	3.870	16.339	7.848
2	0.000	1.435	15.996	6.528
3	0.000	3.576	12.765	7.848
4	0.259	1.078	18.335	6.142
5	0.430	0.110	17.971	7.220
6	0.259	0.120	18.551	11.653
Mean	0.188	1.698	16.660	7.873
STDEV	0.167	1.655	2.182	1.975

NC: negative control (administration of aquadest ad libitum); BC: broccoli control (administration of steamed broccoli juice 1.5 mL); PC: positive control (administration of DSS 2.5% (w/v)); B: broccoli treatment (administration of DSS 2.5% (w/v) followed by steamed broccoli juice 1.5 mL)

Mean IL-8 data (Table 1) in every treatment showed that lowest serum IL-8 level was found in negative control (NC) group, while highest IL-8 level was found in positive control group, which was induced by DSS without administration of steamed broccoli juice.

Table 2. ANOVA effect of steamed broccoli juice to IL-8 level

	Sum of squares	df	Mean square	F	p
Between groups	1007.707	3	335.902	117.483	0.000
Within groups	57.183	20	2.859		
Total	1064.890	23			

$F_{count} = 117.483 > F_{table 0.05 (3,20)} = 3.10$ with $p = 0.00 < \alpha$, therefore H_0 was rejected, and H_1 was accepted, meaning that there was difference of serum IL-8 level between at least 1 pair of intervention group. Further, to determine which of it between different groups, Post Hoc test was performed using Tukey HSD method. Results of Tukey HSD analysis could be seen in Table 3 and Figure 1.

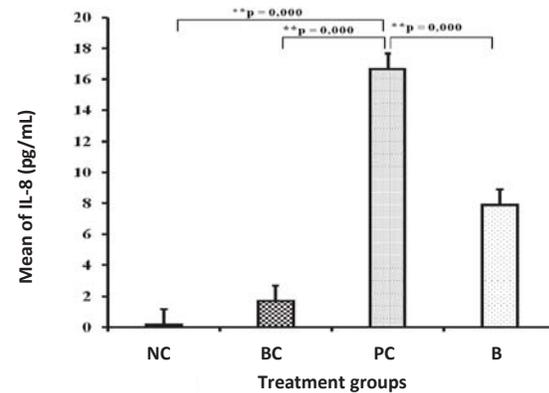
Table 3. Level of IL-8 based on mean differences test Tukey-HSD method

Treatment Groups	NC	BC	PC	B
NC		0.430 ^{ns}	0.000 ^{**}	0.000 ^{**}
BC			0.000 ^{**}	0.000 ^{**}
PC				0.000 ^{**}
B				

NC: negative control (administration of aquades ad libitum); BC: broccoli control (administration of steamed broccoli juice 1.5 mL); PC: positive control (administration of DSS 2.5% (w/v)); B: broccoli treatment (administration of DSS 2.5% (w/v) followed with steamed broccoli juice 1.5 mL); ns: not significant; **: highly significant

Based on the results of Tukey HSD test (Table 3), it could be seen that there was highly significant difference of IL-8 level ($p < 0.01$) between negative control (NC) and positive control (PC) and broccoli treatment (B) groups. Further, there was also not significant difference in NC and broccoli control (BC) groups. IL-8 level had highly significant difference ($p = 0.00$) in DSS group compared to the broccoli control (BC) and broccoli treatment (B) groups with p value < 0.01 in each groups.

Comparison of each experimental group can be seen in Figure 1 below.



NC: negative control (administration of aquades ad libitum); BC: broccoli control, administration of steamed broccoli juice 1.5 mL; PC: positive control (administration of DSS 2.5% (w/v)); B: broccoli treatment (administration of DSS 2.5% (w/v) followed with steamed broccoli juice 1.5 mL); NS: not significant; **: Highly Significant

Figure 1. Diagram comparing mean of IL-8 level on day 8

DISCUSSION

Results of this study showed that DSS might induce the occurrence of colitis in murine. This was supported with the presence of increased IL-8 level. Table 1 revealed the mean of IL-8 in positive control group, which was given DSS 2.5% markedly increased compared to those with negative control group. Table 2 also showed the presence of difference of IL-8 level that was highly significant between negative control and positive control groups. In broccoli control group,

there was also increased IL-8 serum level. This was caused by the murines being used were not sterile murine and the room in which the murines' cages were put were also not sterile, thus there could be a possibility of exposures to microbes or other substances which could cause increased of IL-8.

This study also revealed that steamed broccoli juice might give therapeutic benefits. Administration of steamed broccoli juice to colitis murine model can decrease the level of IL-8. In Table 1, it was shown that the lowest mean of IL-8 level was found in negative control group, while the highest was found in positive control groups. However, in groups receiving intervention, IL-8 level was found to be lower compared to the positive control groups. Table 3 also showed highly significant difference of IL-8 level ($p = 0.000$) between negative control groups and positive control group and broccoli treatment group. Additionally, in Table 3, it was shown that there was highly significant difference of IL-8 level ($p = 0.000$) between positive control and broccoli treatment groups. This showed that steamed broccoli juice could lower IL-8 level. Difference of IL-8 level in each groups was also shown in Figure 1.

UC is marked by the mucosal damage and ulceration involving the rectum and spreading to the colon. In UC, there was involvement of mediators, such as the release of TNF- α , Interleukin-1 and Interleukin-6. These mediators cause the occurrence of clinical inflammatory symptoms, particularly diarrhoea and weight loss.¹⁶ Other mediators which cause inflammation are chemokines. One of the chemokines playing important role was IL-8. IL-8 was a potent chemoattractant to the inflammatory cells, particularly neutrophils. The presence of neutrophil infiltration in the inflamed tissue, such as intestinal crypts and mucosa will cause the inflammatory reaction to be more severe which is shown in clinical or even histopathological appearance.¹¹

Broccoli contains sulforaphane that is a derivate of isothiocyanate and has chemopreventive effect towards inflammation and cancer. One of the mechanisms of sulforaphane is by inhibiting NF- κ B which is the main regulator of inflammatory cytokine and chemokine. SFN selectively inhibit DNA binding to NF- κ B, either direct or indirectly. Directly, SFN inhibit degradation from I κ B- α which was regulator of NF- κ B, while indirectly through the interaction with redox regulator including thioredoxin and Ref-1 which was associated with NF- κ B activity.^{18,19} SFN which is associated with Nrf-2 will activate phase II enzymes which further can

inhibit NF- κ B, thus decrease the expression of IL-8.²⁰ Based on the explanation above, the administration of steamed broccoli juice for 7 days might decrease the IL-8 serum level in colitis murine models which were induced by DSS. This study explained how was the curative effect of broccoli towards IL-8 in colitis murine model. Application of this research in the future was that broccoli could be used as a complementary treatment in patients with ulcerative colitis. Researches on the effect of broccoli towards clinical score and pathway which activate proinflammatory cytokine such as TNF- α are needed to further explain the molecular mechanism underlying the inhibitory process of broccoli towards inflammatory reaction.

CONCLUSION

Steamed broccoli juice decreased serum IL-8 serum level in colitis murine model.

REFERENCES

- Bernstain C.N, Fried M, Cohen H. Inflammatory Bowel Disease: A Global Perspective. World Gastroenterology Organisation Global Guidelines, 2009.p.112-24.
- Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med 2011;365:1713-25.
- Silva BC, Lyra AC, Rocha R, Santana GO. Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis. W J Gastroenterol 2014;20:9458-67.
- Djojoningrat D. Perdarahan saluran cerna bagian bawah (hematokezia). In: Sudoyo AW, Setyohadi B, Alwi I, Simadibrata M, Setiati S, eds. Buku Ajar Gastroenterologi. 1st ed. Jakarta: Interna Publ 2011.
- Head KA, Jurenka JS. Inflammatory bowel disease part I: ulcerative colitis– pathophysiology and conventional and alternative treatment options. Alternative Medicine Review 2003;8:247-83.
- Roebuck KA. Regulation of interleukin-8 gene expression. J Interferon Cytokine Res 1999;19:429-38.
- Mahida YR, Ceska M, Effenberger F, Kurlak L, Lindley I, Hawkey CJ. Enhanced synthesis of neutrophil-activating peptide-1/interleukin-8 in active ulcerative colitis. Clin Sci 1992;82:273-5.
- Mazzucchelli L, Hauser C, Zraggen K, Wagner H, Hess M, Laissue JA, et al. Expression of interleukin-8 gene in inflammatory bowel disease is related to the histological grade of active inflammation. Am J Pathol 1994;144:997–1007.
- Mitsuyama K, Toyunaga A, Sasaki E, Watanabe K. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. Clin Exp Immunol 1994;96:432–6.
- Banks C, Bateman A, Payne R, Johnson P, Sheron N. Chemokine expression in IBD: mucosal chemokine expression is unselectively increased in both ulcerative colitis and Crohn's disease. J Pathol 2003;199:28–35.
- Atreya I, Atreya R, Neurath MF. NF- κ B in inflammatory bowel disease. J Intern Med 2008;263:591-6.

12. Jahan IA, Mostafa M, Nimmi I, Hossain MH, Ahsan M, Chowdhury JU. Chemical and antioxidant properties of broccoli growing in Bangladesh. *J Pharm Sci* 2010;9:31-7.
13. Khiong K, Darsono L, Yolanda R. Sulforaphane prevents colitis-associated cancer by inducing phase II enzymes activity of liver detoxification. *Jurnal Kedokteran Maranatha* 2011;11:64-9.
14. Mueller K, Blum NM, Mueller AS. Examination of the anti-inflammatory, antioxidant, and xenobiotic-inducing potential of broccoli extract and various essential oils during a mild DSS-induced colitis in rats. *ISRN Gastroenterology* 2013;2013:1-14.
15. Kong JS, Yoo SA, Kim HS. Inhibition of synovial hyperplasia, rheumatoid T cell activation, and experimental arthritis in mice by sulforaphane, a naturally occurring isothiocyanate. *Arthritis Rheum* 2010;62:159-70.
16. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417-29.
17. Hanafiah KA. Rancangan percobaan: teori dan aplikasi. Jakarta: Raja Grafindo Persada 2000.p. 6-7.
18. Surh YJ. NF- κ B and Nrf-2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities. *Asia Pac J Clin Nutr* 2008;17:269-72.
19. Geisel J, Bruck J, Glovoca I. Sulforaphane protects from T cell-mediated autoimmune disease by inhibition of IL-23 and IL-12 in dendritic cells. *J Immunol* 2014;192:3530-9.
20. Hung CN, Huang HP, Wang CJ, Liu KL, Lii CK. Sulforaphane inhibits TNF- α -induced adhesion molecule expression through the Rho A/ROCK/NF- κ B signaling pathway. *J Med Food* 2014;17:1095-102.