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Effects of scopoletin from noni fruit (*Morinda citrifolia* L.) to IL-10 levels in male white mice with hypersensitivity type I.

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ABSTRACT

A study about scopoletin activity on IL-10 levels of hypersensitivity type I male white mice has been conducted. The scopoletin was isolated from noni fruit (*Morinda citrifolia* L.) with soxhletation method using dichloromethane and purified by column chromatography method. Scopoletin was administered orally to hypersensitivity type I mice with dose variation 1, 3 and 10 mg/kg BW. After 24 hours of scopoletin administration, mice's blood was taken and amount of IL-10 was determined by Elisa method. The results of this study showed that administration scopoletin at doses of 1, 3 and 10 mg/kgBW reduce the amount of IL-10 in mice with hypersensitivity type I ($p < 0.01$). Administration 3 mg/kgBW and 10 mg/kgBW of scopoletin can decrease IL-10 levels in mice with type 1 hypersensitivity to the normal range ($p < 0.01$).

Keywords: *Morinda citrifolia* L, scopoletin, hypersensitivity type I, IL-10.

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INTRODUCTION

The sources of hypersensitivity type I reactions are protein, pollen, food, cold and dry air, dust, smoke, animal, medicine, fungi, viruses, chemicals and industrial products, and stress. Allergens cause a wide variety of hypersensitivity type I reactions, such as bronchial asthma, allergic rhinitis, atopic dermatitis, anaphylactic shock and any others [1,2].

Allergens that enter the body caused an immune response with the formation of immunoglobulin-E (IgE) and subsequently the IgE bonded to surface of mast cells and basophil cells [1,3]. Allergen exposure process begins with phagocytosis by macrophages. Macrophage cells will break the allergen into peptide fragments and then the fragments were ligated by mayor histocompatibility complex (MHC) class II and carried to macrophages cell surface and subsequently presented to Th₀ cells (helper naive). Macrophage cells will release several cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). T helper cell (Th) which receive signals from macrophages go through differentiation and proliferation into Th1 and Th2 cells. Allergens also phagocytosed by mastcell and basophils, then these cells will release IL-4. High levels of IL-4 caused the proliferation and differentiation of Th₀ cells toward Th2 cells. Th2 cells will release several cytokines such as IL-4, IL-5, IL-10 and IL-13 [4]. IL-4 has a direct effect on lymphocytes B and these cells differentiate and proliferate into plasma cells, which in turn produces IgE [5,6].

IL-10 is a cytokine produced by Th2 cells, CD8 T cells, lymphocytes B and macrophages. IL-10 has ability to inhibit inflammation, inhibit antigen recognition process by macrophages and cells dendrit with suppression of MHC class II expression and inhibit the production of Th1 cells cytokines. IL-10 also inhibit TCD4 cell proliferation into Th1 cells, so that proliferation process of Th2 cells will increase [4].

Two main functions of IL-10 are inhibit the production of several cytokines (TNF, IL-1, chemokines and IL-12) and inhibit the function of macrophages in T cell activation. Macrophage dysfunction occurs because IL-10 suppress the expression of MHC class II on macrophages and reduces the expression of co-stimulatory. The final impact of the IL-10 activity is inhibit the non-specific and specific inflammatory reaction mediated by T cells, so that IL-10 also known as cytokine synthesis inhibitor factor and anti-inflammatory cytokines [4,7].

Suppressing synthesis of IL-10 caused Th1 cells will increase INF production then proliferation and differentiation of Th1 and Th2 cells will be balance and amount of IL-4 will decrease. INF also can inhibit the binding of IL-4 to its receptor on the cell plasma so IgE formation can be inhibited [4].

Previous research has been conducted against hypersensitivity type I. In this Study presented scopoletin that isolated from noni fruit may inhibit active cutaneous anaphylaxis reaction [8] and decrease IgE levels [9]. Scopoletin was able to reduce levels of IL-4 from the male white mice who had hypersensitivity type I [10]. In addition, it was also reported that scopoletin can inhibit the production of PGE₂ (prostaglandin E₂), TNF- α , IL-1 β , IL-6 and suppresses COX-2 [11,12] and hepatoprotective [13].

Based on this background, we tried to prove the effect of scopoletin from noni fruit (*Morinda citrifolia L.*) on IL-10 levels in male white mice with hypersensitivity reaction type I. This study is an advanced of previous research about scopoletin effects on IgE levels [9], and the IL-4 levels of mice with hypersensitivity type I [10].

MATERIAL AND METHODS

Tools and materials

The tools used were a measuring cup, animal scales, oral needles, analytic balance, mortar and pestle, surgical scissors, a set of centrifuges, HPLC (Detector: Prominence DAD SPD-M20A, Coloumn: RP C18-Shimadzu) and a spectrophotometer IR (Perkin Elmer). Materials used were scopoletin [10], distilled water, physiological saline, Na CMC, ovalbumin (Brand No. Lot.20HO763 A-5253) and Platinum Mouse IL-10 ELISA kit (eBioscience, BMS 614/2, No. 887 904).

Preparation of hypersensitivity type I animals technique

Healthy mice weight 20-25 g injected intraperitoneally with 250 mg/kg BW ovalbumin. On the third day, ovalbumin was administrated again with the same dose given subcutaneously. Animal sensitivity declared if on the seventh day administration 250 mg/kg BW ovalbumin subcutaneously raised reddish color in place of injection [10].

Determination of scopoletin purity level

Extrasynthese (France) as standard compound and scopoletin isolated from noni fruit was diluted with methanol. To create a calibration curve, standard compound (Extrasynthese France) was made in different concentrations those are 3 mg/mL, 6 mg/mL, 12 mg/mL, 24 mg/mL, 48 mg/mL. Each sample was filtered before injected into HPLC, then 20 mL of the sample injected to HPLC. Eluent used were methanol: aquabidest (9:1) with a flow rate of 0.5 mL/min at room temperature, using a detector at 345 nm wavelength.

Administration of test compound

The treatment group consisted of five groups, namely: Group I was a normal animal, group II was mice with hypersensitivity type I (positive control) given only normal saline, group III was given 1 mg/kg BW scopoletin, group IV was given 3 mg/kg BW scopoletin and Group V was given 10 mg/kg BW scopoletin. Scopoletin was given at the visible signs of redness place of injection.

Determination of the IL-10 levels

IL-10 levels was determined by Enzyme Linked Immunosorbent Assay (ELISA) method using a Platinum Mouse IL-10 ELISA kit.

RESULTS AND DISCUSSION

Scopoletin used in this study was isolated based on previous studies [10] and gained as much as 0.01% scopoletin from noni fruit. According to Indonesian Herbal Pharmacopoeia first edition (2008) scopoletin in noni fruit simplesia (powder) not less than 0.02%. This may be due to differences place and processes to grow plants. the scopoletin obtained are still relatively small.

Scopoletin used in this study has same Rf value with scopoletin comparator (Exrtasynthese France) with n-hexane: ethyl acetate (1.5: 3.5) as eluent, that is 0.56 (Fig. 1).

Spectrum of ultraviolet data of scopoletin in Fig.2. Scopoletin spectrum in this study equal to Scopoletin comparator spectrum (Exrtasynthese France).

IR spectrum Examination showed scopoletin isolated from noni fruit has same spectrum with scopoletin from Exrtasynthese France and the spectrum can be seen in Fig. 3 and Fig. 4.

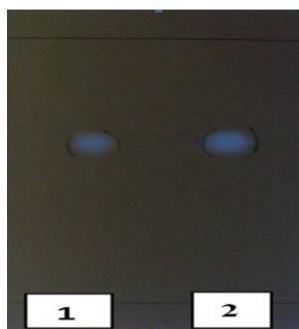


Fig. 1. Thin-layer chromatograms of scopoletin under uv light 365 nm wavelength (1. Scopoletin isolated from noni fruit; 2. Scopoletin comparator compound (Exrtasynthese French).

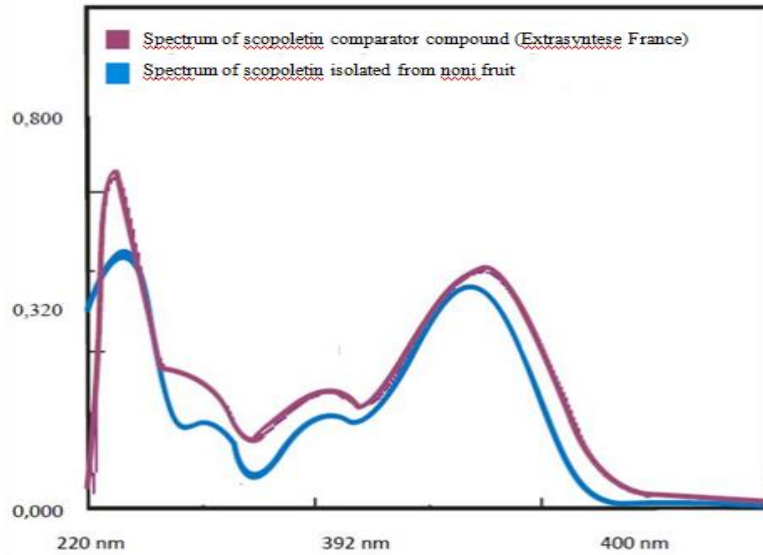


Fig. 2. UV spectrum of scopoletin isolated from noni fruit and scopoletin comparator compound (Extrasynthese France) [10].

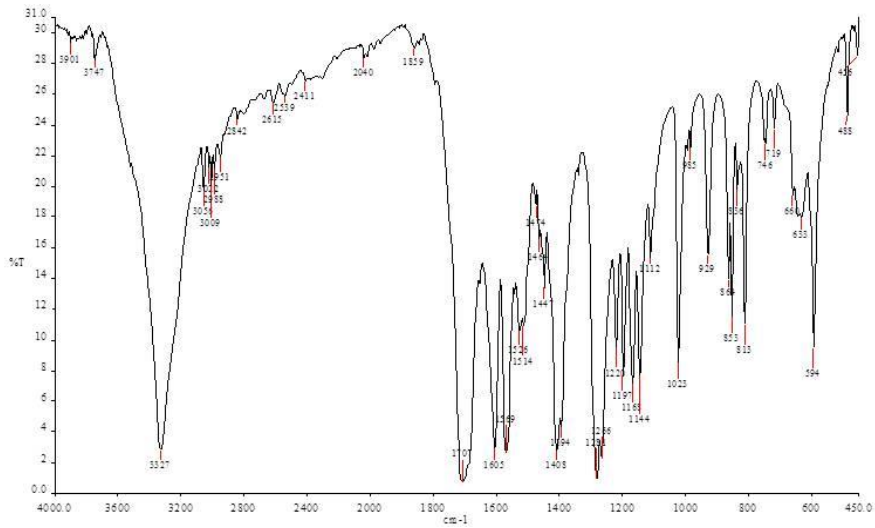


Fig. 3. IR spectrum of scopoletin isolation from noni fruit [10].

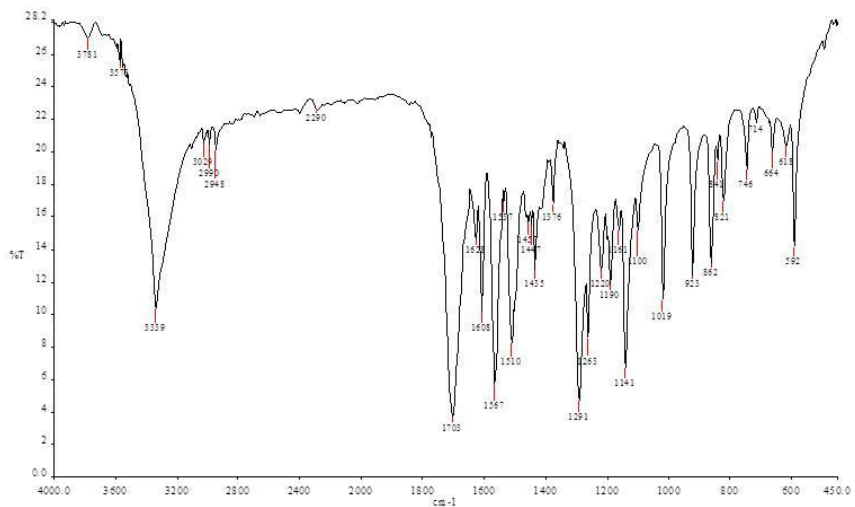


Fig. 4. IR spectrum of scopoletin comparator compound (Extrasynthese France) [10].

In this study, purity of scopoletin was determined by HPLC, by measuring area under curve of scopoletin. Scopoletin was detected at retention time (Rt) about 6.05 minutes. HPLC chromatogram of scopoletin is showing in Fig. 5. area measurement of a standard scopoletin (Extrasynthese, France) at several concentrations obtained calibration curve as shown in Fig. 6.

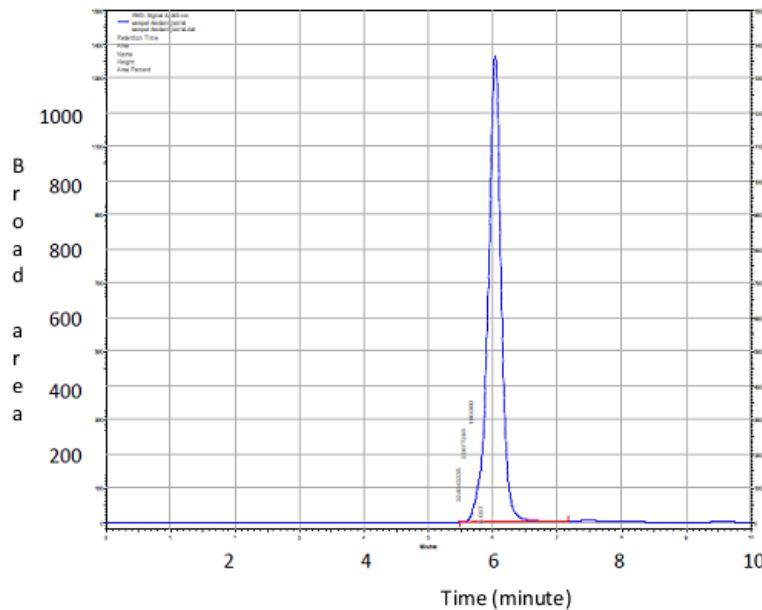


Fig. 5. The curve of scopoletin injected as much as 20 mL with methanol : aquabidest eluent (9:1) flow rate 0.5 mL/min at room temperature, using a detector on 345 nm wavelength.

Linear equation of the standard calibration curve of scopoletin is $Y = 675227,333 + 2511296,498 X$. Using this equation, obtained the purity of scopoletin isolated from noni fruit, that is 104.22%

IL-10 in mice hypersensitivity type I serum measured using Platinum Mouse IL-10 ELISA kit (eBioscience, BMS 614/2, No. 887 904). To determine levels of IL-10, standard curve of IL-10 was made used a standard compound in the kit at a wavelength of 450 nm. The standard curve of IL-10 in the blood serum of mice can be seen in Fig. 7.

IL-10 Levels in the serum of hypersensitivity type I mice after administration of scopoletin was determined using a calibration curve (Fig. 7). The results of IL-10 serum levels on five treated groups can be seen in Table 1.

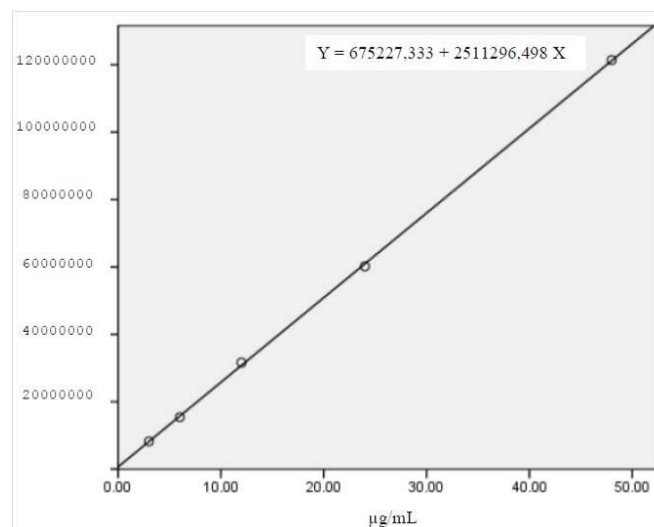


Fig. 6. The calibration curve of scopoletin standard compound (Extrasynthese, France).

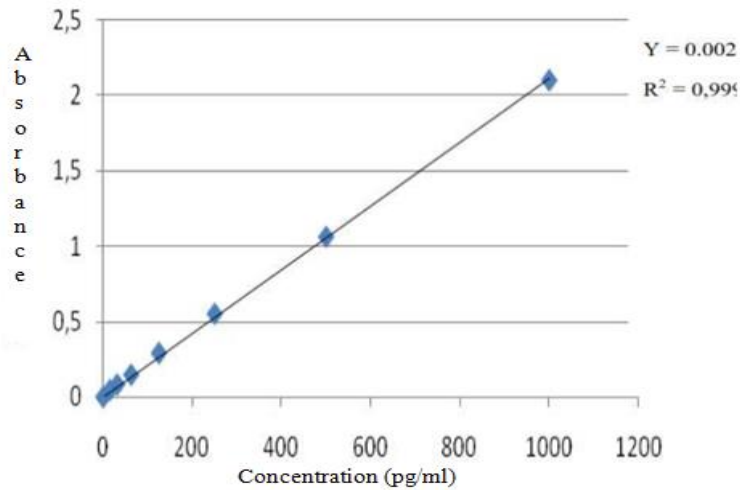


Fig. 7. The standard curve of IL-10 in the blood serum of mice on 450 nm wavelength.

Administration of scopoletin at doses of 1, 3 and 10 mg / kg bw in mice with hypersensitivity type I reaction intended to decreased levels of IL-10. After analyzed statistically by one direction analysis of variance method, Scopoletin was able to reduce the levels of IL-10 very significantly ($p <$

No.	Group	IL-10 levels (pg/ml)					Rate of IL-10 levels (pg/ml)
		1	2	3	4	5	
1	I	229,00	228,00	189,00	220,00	275,00	228,20 ± 30,80
2	II	364,00	381,00	427,00	413,00	427,00	402,40 ± 28,53
3	III	338,00	279,00	364,00	346,00	306,00	326,60 ± 33,90
	IV	313,00	236,00	267,00	323,00	309,00	289,60 ± 36,82
5	V	267,00	232,00	263,00	379,00	291,00	286,40 ± 55,86

Table 1. IL-10 serum levels on male white mice with hypersensitivity type I after administration of scopoletin from noni fruit. I = normal animals, II = positive control animals (NaCMC solution), III = hypersensitivity type I animals with 1 mg/kg BW dose, IV = hypersensitivity type I animals with 3 mg/kg BW dose, V = hypersensitivity type I animals with 10 mg/kg BW dose.

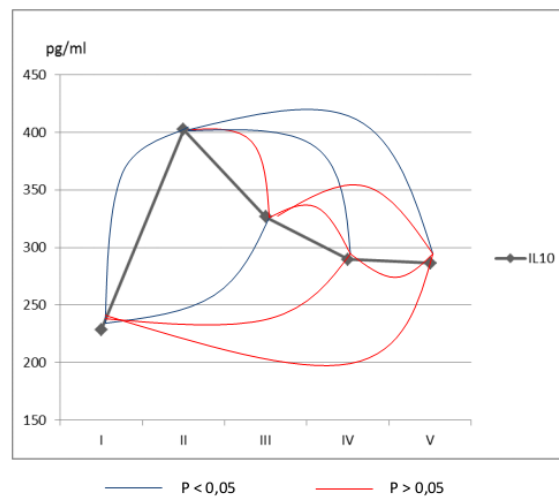


Fig. 8. The relationship between the dose of scopoletin compound given to male white mice with hypersensitivity type I to IL-10 levels. I = normal animals, II = positive control animals (NaCMC solution), III = hypersensitivity type I animals with 1 mg/kg BW dose, IV = hypersensitivity type I animals with 3 mg/kg BW dose, V = hypersensitivity type I animals with 10 mg/kg BW dose.

Statistical analysis followed by Bonferroni test in order to determine differences ability of each dose in decreasing IL-10 levels. Results of Benferroni statistic test can be seen in Fig. 8. Administration 1 mg/kg BW

scopoletin to hypersensitivity type I mice has not decreased IL-10 levels significantly ($p > 0.05$) when compared to IL-10 levels of the positive control group. After administration 3 mg/kg BW and 10 mg/kg BW scopoletin, IL-10 levels was decreasing significantly ($p < 0.05$). This means, 1 mg / kg BW scopoletin had no effect and the effect seen at dose of 3 mg / kg and 10 mg / kg BW. However, scopoletin ability to decrease IL-10 levels provided by 1 mg/kg BW was same with 3 mg/kg BW dose and 10 mg/kg BW dose statistically. Meanwhile, the ability of 3 mg/kg BW scopolatin can reduce IL-10 levels become lower and reach animals normal levels of IL-10 in mice with hipersensitivity type I ($p > 0.05$).

IL-10 levels in normal group of mice was 228.20 ± 30.80 pg/ml. if mice had hypersensitivity type I reacted with unannounced test compound (positive control), the IL-10 levels increase up to 176% of normal levels. Increasing of IL-10 levels in hypersensitivity type I mice was not as high when compared with the levels of IL-4 [10].

Increasing of IL-10 levels in mice with hypersensitivity type I also affects activity of CD4 T and IL-4. it will go through cell proliferation and differentiation to Th2 cells [13]. Moreover, IL-10 also has capability to inhibit the production of cytokines by Th1 cells and inhibit the function of monocytes or macrophages [14]. Decreasing of IL-10 levels in mice with hypersensitivity type I will improve the balance of proliferation and differentiation of CD4 T cells and then IL-4 production by Th2 cells will be decreased.

The increase of IL-10 levels in mice with hypersensitivity was not as high as increasing of IL-4 levels [10] and scopoletin ability to decrease levels of IL-10 is weak. Scopoletin ability with dose 1 mg/kg BW in decreasing IL-10 levels in mice with hypersensitivity type I was not significant ($p > 0.05$) compared to mice with hypersensitivity type I which were not given the test compounds.

CONCLUSION

These results indicated that administration of scopoletin with dose of 1, 3, and 10 mg / kg of body weight can decrease serum IL-10 mice with type I hypersensitivity. Administration 3 mg / kg BW scopoletin can decrease IL-10 levels in mice with type 1 hypersensitivity to the normal range.

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