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THE EFFECT OF CROCATIN AND DEACETYL CROCATIN ISOLATED FROM RED BETEL (*Piper cronatum*, RUIZ & PAV.) LEAVE ON MICE ANTIBODY TITER

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Abstracts

The aim of this research was to investigate antibody titer effect in mice treated with crocatin and deacetyl crocatin isolated from red betel (*Piper crocatum* Ruiz & pav.). The Balb/c mice immune response were induced with Listeria monocytogenes. Antibody titer effect was tested using mouse IgG elisa kit. The effect of both crocatin and deacetyl crocatin IgG titers, at the dose of 2,5 and 10 mg/kg BW, occurred at 10th days after *L. monocytogenes* infection. Both compound showed no significant difference compared to the control group on day 21th after *L. monocytogenes* infection.

Keywords : *Piper crocatum* Ruiz & Pav., crocatin and deacetyl crocatin, IgG titer

INTRODUCTION

The activity of the compounds in the extract of red betel leaf (*Piper crocatum* Ruiz & Pav) was reported (Wicaksono *et al*., 2009; Rachmawaty *et al*., 2013). Its imunommodulatory activity was also reported (Hartini *et al.*, 2013a). In general, plants that have imunommodulatory activity has a stimulating activity of specific and non-specific immunity (Wagner & Proskh, 1985). Some of these plants stimulate the humoral and cellular immunity, while others simply activate the cellular components of the immune system, such as phagocytosis function without effect on humoral and cellular immunity (Bafna & Misrha, 2004). The two compound isolated from red betel leaf (crocatin and deacetyl crocatin) activate the phagocytic function (Hartini, *et al*., 2013b). This research aim to know the effect of crocatin and deacetyl crocatin on humoral immunity.

MATERIALS AND METHODS

Preparation of methanol extract of red betel leaves was done by maceration. The extract was further fractionated by the method of Vacuum Liquid Chromatography, successively using n-hexane, chloroform, ethyl acetate, and methanol. Crocatin and deacetyl crocatin are in the 3rd and 4th of 5 methanolic extracts fractions. Isolation of the two compounds was conducted by preparative Thin Layer Chromatography.

Male Balb/c mice 8 weeks old weighing about 20-25 g and *Listeria monocytogenes* were used for the experiments. All procedures were approved by The Ethical Clearance Commision for pra-clinically research of Laboratorium Penelitian dan Pengujian Terpadu Gadjah Mada

University, Yogyakarta, Indonesia. In the preliminary study, Balb/c mice were divided into treatment group and control group. The treathment group, received 10 mg/kg BW deacetyl crocatin while the control group received 0.7 ml of 1% sodium carboxy methyl cellulose as solvent control, per oral for 14 days. On 15th day (=day 0), 0.2 ml *L. monocytogenes* containing 5x10^{3} cfu/ml are injected intraperioneally to all mice. On day 0, day 3, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the infra-*orbital* plexus of mice.

In the main study, Balb/c mice were divided into nine groups. Group A, received 2.5 mg/kg BW crocatin, Group B, received 5 mg/kg BW crocatin, Group C, received 10 mg/kg BW crocatin, Group D, received 2.5 mg/kg BW deacetyl crocatin, Group E, received 5 mg/kg BW deacetyl crocatin, Group F, received 10 mg/kg BW deacetyl crocatin, per oral for 14 days. Group G, didn't received drugs, as normal control, Group H, received 0.7 ml of 1% sodium carboxy methyl cellulose per oral as solvent control, and Group I, received 100 mg/kgBW product-X® (contain echinacea extract) per oral as positive control. On 15th day (= day 0) and 25th day 0.2 ml *L. monocytogenes* containing 5x10^{3} cfu/ml are injected intraperioneally to all mice. On day 0, day 10 and the twenty-one days after *L. monocytogenes* infection, 0.5 ml of blood was taken from the infra-*orbital* plexus of mice.

The humoral immune response determined by measuring the titer of immunoglobulin G (IgG). Measurement of IgG titers using mouse IgG elisa kit. The data were analyzed by one-way ANOVA followed by Tukey test.

RESULT AND DISCUSSION

The compounds isolated from red betel are neolignan. The scientific name of red betel is *Piper crocatum* Ruiz & Pav., so that isolate 1 was named crocatin while isolate 2 was named deacetyl crocatin. The existence of an acetyl group (OCH_3) at $C_{1'}$ to distinguish crocatin of deacetyl crocatin having hydroxyl groups (OH). The chemical structure differences crocatin and deacetyl crocatin are shown in Figure 1. Croatin is *2-allyl-4-(1'-hydroxy-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa-3, 5-dienone and deacetyl crocatin is 2-allyl-4-(1'-acetyl-1'-(3 ", 4", 5 "-trimethoxyphenyl) propan-2'-yl) -3,5-dimethoxycyclohexa- 3,5-dienone* (Kustiawan, 2012). Aside from the relatively high rendemen, size crocatin and deacetyl crocatin spotting on TLC chromatogram is relatively large and the color intensity of damping patches on UV detection at 254 nm is very strong. Processes, equipment, and means of detection croctin and deacetyl crocatin fairly simple, allowing the two compounds used as chemical markers for leaves of *Piper crocatum*. Crocatin and deacetyl crocatin can be used as a marker compound, which is a therapeutic components for *Piper crocatum*.

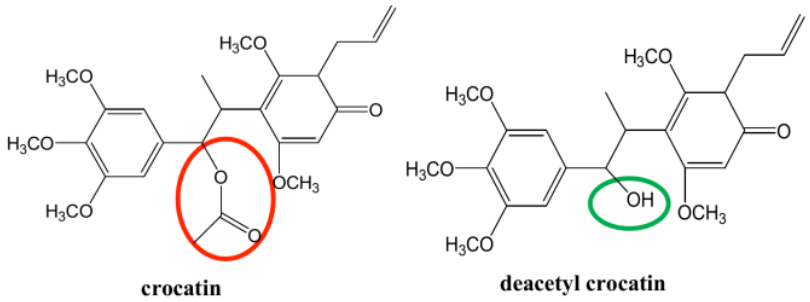

Figure 1. The chemical structure differences between crocatin and deacetyl crocatin.

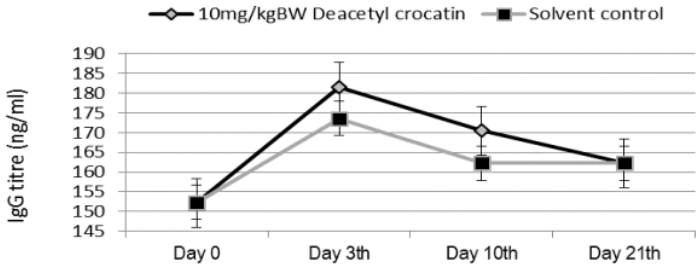

Figure 2. IgG titer levels after the mice were infected by *L. monocytogenes*.

Figure 1 shows the result of preliminary study. In this study, IgG titers of mice treated with 10 mg/kgBW deacetyl crocatin showed increase on day 3th, then decreased on day 10th and it was as same as the control group on day 21th. Although any differences IgG titers on day 3th and 10th, but statistically analysis showed no significant difference between treatment group and control group. It indicates that treatment with 10 mg/kgBW deacetyl crocatin have no IgG titers differences compare to control group. Probably due to on the day-10, it need to boost the mice immune responses, so that in the main study we use twice *L. monocytogenes* infection. In the preliminary study the dose of 10 mg/kgBW deacetyl crocatin showed increasing IgG titer, in order to know the potential level of the compound, we use 2 lower doses in the main study. The main study tested 3 range doses of crocatin and deacetyl crocatin ie : 2,5; 5; and 10 mg/kgBW. The result of main study can see on Figure 3.

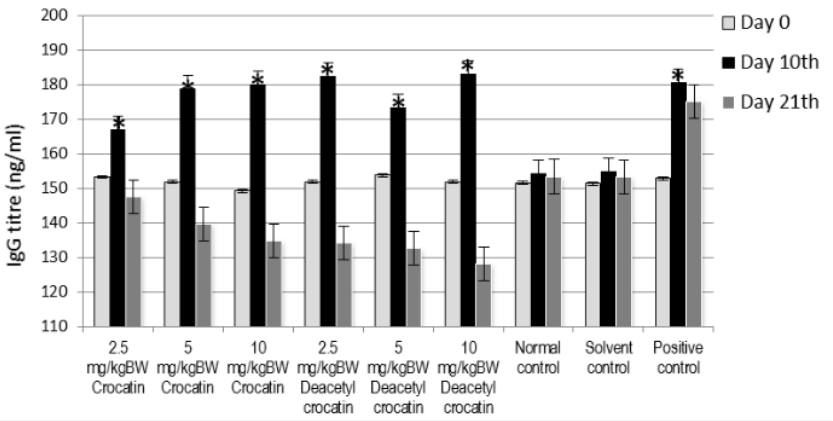

Figure 3. The effect of crocatin and deacetyl crocatin against IgG titers in mice after twice infection with *L. monocytogenes*. Values are mean ± SD of 3 replicate, *denotes significant difference ($P < 0.05$) to the normal control and the solvent control.

The normal control and solvent control showed the same level of IgG titers, the solvent did not give unexpected effect, 1% sodium carboxy methyl cellulose is an appropriate solvent for this study. In the day 0 (before infection with *L. monocytogenes*) there are no differences effect on all of groups. There are no differences IgG titers of mice before *L. monocytogenes* infection, It indicates that treatment with crocatin, deacetyl crocatin (at dose of 2.5; 5; 10 mg/kgBW) and product-X® (contain echinacea extract, at dose of 100 mg/kgBW) per oral for 14 days, didn't effect on IgG titers. At day 10 after infection of *L. monocytogenes*, the treatment group showed significantly different IgG titers, but on day 21th IgG titers decline, in contrast to the control group but the difference was not significant. Possibly because of the amount of microbial increased on day 10, and then on day 21 had a decline. According Unanue (1997), curve number of *L. monocytogenes* were alive after 0-14 days in mice infected with *L. monocytogenes* showed a slight decrease and then rose on the third day until day 10 reached a peak, and then decreased on day 14 reached zero.

Echinacea is reported to have no effect on the stimulation of IgG immune response, one week following the secondary sheep RBC's subcutaneously infection (Dennis, 1999). Our study using *L. monocytogenes*, an intracellulair bacteria, for antigen. Although the test result showed similarity on day 21 after infection antigen, but on day 10. These differences may lead to differences in test result. *L. monocytogenes* induce the cellulair immune responses, maybe the humoral immune response wasn't stimulated therefore no effect on the IgG titer.

There are no differences effect of crocatin and deacetyl crocatin on the IgG titers of mice infected with *L. monocytogenes*. Both of the neolignans didn't show significantly effect on the mice IgG titer on the 21th day after *L. monocytogenes* infection, compare to control group.

Probably due to *L. monocytogenes* is an intracellular microorganisms, so that it effect on the cellular immune response but humoral response. As it has reported, the differences of both neolignan are crocatin not cause toxic effects on the kidneys and liver either, but deacetyl crocatin that have OH at $C_{1'}$ cause liver damage even though safe for the kidneys (Hartini et al., 2013b).

CONCLUSION

There are no differences effect of crocatin and deacetyl crocatin on the IgG titers of mice infected with *L. monocytogenes*. Both of the neolignans didn't show significantly effect on the mice IgG titer, compare to control group.

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