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## Research Article

# Immunomodulatory Effect of Orally Red Fruit (*Pandanus conoideus*) Extract on the Expression of CC Chemokine Receptor 5 mRNA in HIV Patients with Antiretroviral Therapy

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## Abstract

**Background and Objective:** The C-C chemokine receptor 5 (CCR5) is a coreceptor of human immunodeficiency virus (HIV) and is related to immune cells. Therefore, it was investigated immunostimulant effect of red fruit (*Pandanus conoideus*) oil extract on the level of CCR5 mRNA in HIV infected patients with taking antiretroviral therapy. **Materials and Methods:** Seventy of HIV infected patients (age of 17-45 years) were into the antiretroviral (ARV) control (n = 35) and the antiretroviral+red fruit capsule (ARV+RFC)-treated (n = 35) groups. In ARV+RFC group, they were given oral RFC (1 g daily for two months). Monitoring evaluations of the laboratory were performed at baseline (0 months) and two months during the study. The quantification of CCR5 messenger ribonucleic acid (mRNA) in human blood immune cells was determined using real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay. **Results:** In this study, it was found that ARV+RFC exhibited a significantly increased level of CCR5 mRNA of HIV-infected patients compared with ARV alone (p<0.001). **Conclusion:** From this, it was concluded that the red fruit oil extract may have an excellent immunostimulant effect and has potential as an adjuvant in the management of HIV-infected patients.

**Key words:** Antiretroviral therapy, CCR5, HIV/AIDS, immune response, *Pandanus conoideus*

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Human immunodeficiency virus (HIV) caused a problem in immune systems and can develop become an infectious disease namely the acquired immune deficiency syndrome (AIDS)<sup>1-3</sup>. In 2015, WHO reported that 36.7 million people were infected with HIV and causing 1.1 million deaths<sup>3</sup>, while in Indonesia estimated 690,000 people living with HIV<sup>4</sup>. Papua Province is one of Indonesia province with a higher prevalence of HIV infection and AIDS; Merauke is a regency in Papua Province with a significant prevalence of HIV infection and AIDS<sup>5</sup>. A total of 2,502 cases were reported for HIV infection (1,063 cases), AIDS (902 cases) and AIDS-related death (537 cases) in Merauke Regency from 1992 to January, 2017<sup>5</sup>. Therefore, this study focused on management HIV-infected patients with antiretroviral therapy and combined with red fruit extract in Merauke District of Papua Province, Indonesia.

To date, one successful alternative management in HIV-infected patients was used antiretroviral therapy (ARV)<sup>6,7</sup>. However, there were limited to ARV treatment in HIV-infected patients include drug resistance<sup>8-10</sup>, toxicity<sup>11</sup>, drug-drug interaction<sup>12</sup>, drug-food interaction<sup>13</sup>, required lifelong use, failed treatment response, the optimal time to start treatment and switching regimens<sup>14</sup>. Therefore, new strategies are required that can reduce the negative impact and improve the efficacy of ARV therapy for the management of HIV-infected patients. One of the alternatives that can be used is medicinal plants; because it contains metabolite compounds that are responsible for some pharmacological properties<sup>15-21</sup>. Some reasons use the medicinal plant for a treat of people with HIV infection include to enhance their immune function, to treat symptoms, to improve their quality of life and to reduce side effects related to medications<sup>22</sup>. Recent clinical research reported that there was a change in the concentration of antiretroviral drugs in the body and it had increased efficacy when HIV-infected patients were treated with combination medicinal plant extracts from Papua New Guinea with ARV<sup>23</sup>. Another literature showed that combination treatment of Chinese herbal compound and antiretroviral agents increased antiviral benefit compared with antiretrovirals alone<sup>24</sup>.

The immune system has a significant damage problem in infection of HIV on human<sup>25</sup>. The C-C chemokine receptor 5 (CCR5), a  $\beta$ -chemokine receptor, expressed on immune cells such as monocytes<sup>26</sup>, T cells<sup>27,28</sup>, dendritic cells<sup>29</sup> and macrophages<sup>30</sup>. A number of studies have reported that CCR5 is the significant coreceptors of HIV required for successful viral entry to the host cell<sup>31-33</sup>. Another function showed that CCR5 is involved in signaling and coordination of immune

responses<sup>34</sup>. Therefore, the improvement level of CCR5 may indicate an increase immune response in HIV-infected patients.

The red fruits or *Pandanus conoideus*, called "BuahMerah" in Indonesia, is family *Pandanaceae* that most known and widely distributed in the Papua Province of Indonesia<sup>35</sup>. This plant has a high value because in traditional of Papua community can be used to treat cancer, rheumatoid arthritis, stroke and HIV/AIDS<sup>36</sup>. Previous works have shown that red fruit has some pharmacological activities such as anticancer, anti-inflammation, antioxidant, antibacterial activities<sup>37-40</sup>. Another research showed that red fruit oil could decrease the level of creatine kinase enzyme at maximum physical activity<sup>41</sup>. Red fruit contains various nutrient including fat, carbohydrate, vitamin C, phosphorus, calcium, carotenoids and tocopherols<sup>35</sup>. Another literature showed that red fruit contains various phytochemicals such as phenolic, flavonoid<sup>40</sup>, oleic acid, linoleic acid<sup>42</sup>, triolein, palmito-diolein, linoleo-diolein and palmito-linoleo-olein<sup>35</sup>.

This study aimed to the evaluation of the level of CCR5 mRNA effect of red fruit (*Pandanus conoideus*) oil extract on HIV-infected patients with taking antiretroviral therapy.

## MATERIALS AND METHODS

**Ethical considerations:** The Medicine Faculty Research Ethics Committee of the Hasanuddin University, Indonesia, approved the study protocol (532/H4.8.4.5.31/PP36-KOMETIK/2017). The study was conducted in randomized controlled groups. Patients qualified for inclusion in the study were ambulatory, with the base CD4 line was between 200-349 cells  $\mu\text{L}^{-1}$ , the body weight of 45-70 kg and treated with ARV. Excluded were pregnant women and kidney failure, body weight under than 45 kg and no treated ARV in three months.

**Study location and design:** Recruitment and drug administration took place in Merauke Hospital, Merauke Regency of Papua Province, Indonesia. Laboratory tests were performed at a Molecular Biology and Immunology Laboratory for Infectious Diseases, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Seventy HIV-infected patients of either gender aged between 17 and 45 years were selected in this study. The patients were divided into the ARV (antiretroviral) control ( $n = 35$ ) and the antiretroviral+red fruit capsule (ARV+RFC)-treated ( $n = 35$ ) groups. RFC gelatin (1000 mg red fruit extract per capsule) was administered to the patients. The dosage was 1000 mg (one capsule) daily for two months. Monitoring evaluations of the laboratory were performed at baseline (0 months) and two months during the study.

**Determination level of CCR5 mRNA:** The determination of CCR5 mRNA used human blood immune cells and it is determined using RT-PCR assay<sup>43</sup>. The CCR5 gene was amplified by RT-PCR using forward primer 5'-GCTGTGTTTTCGCTCTCTCCAGGA-3' and reverse primer 5'-CTCACAGCCCTGTGCCTCTTCTTC-3'<sup>43</sup>. The level of CCR5 copies of samples was determined based on the standard curve of known CCR5 gene copies of a plasmid used as the RNA standard for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) real-time RT-PCR. Thus, the CCR5 mRNA levels are expressed as the mean copy number of CCR5 mRNA per milliliter of total RNA.

**Statistical analysis:** One-way analysis of variance (ANOVA) was employed to assess significant differences in CCR5 mRNA expressions at  $p < 0.001$ . Statistical analysis was performed using software of SPSS 16.00 for Windows (SPSS Inc., Chicago II, USA).

## RESULTS AND DISCUSSION

Figure 1 showed the level of CCR5 mRNA of ARV and ARV+RFC-treated groups at baseline (0 months), two months and mean change. The result shows that the level of CCR5 mRNA in the ARV+RFC-treated group was a gradual increase in the mean CCR5 mRNA from  $7.25 \pm 1.86$  copies  $\text{mL}^{-1}$  at baseline (0 months) to  $9.25 \pm 1.87$  copies  $\text{mL}^{-1}$  after two months treatment of ARV+RFC-treated (Fig. 1). While in ARV group showed that a lower increase in the mean level of CCR5 mRNA from  $7.84 \pm 1.55$  copies  $\text{mL}^{-1}$  at baseline (0 months) to  $8.12 \pm 2.13$  copies  $\text{mL}^{-1}$  after two months therapy. One-way ANOVA with LSD test showed that ARV+RFC-treated group has a significant difference of the CCR5 mRNA copies with ARV group with  $p < 0.001$ ; mean change level of CCR5 mRNA in the ARV+RFC-treated group was  $2.27 \pm 1.93$  copies  $\text{mL}^{-1}$  higher than ARV group with value mean change of  $0.29 \pm 1.56$  copies  $\text{mL}^{-1}$ . The percentage of CCR5 mRNA was increased by 13.54% in the ARV+RFC-treated group while only 1.82% in ARV group. The CCR5 was known as a protein on the surface of white blood cells and its involvement in the immune system with a function as a receptor for chemokines<sup>44</sup>. Therefore, these results indicated that the application of ARV+RFC could be an enhancement of the immune status of the patients compared with ARV only; red fruit oil extract has potential as an adjuvant in the management of HIV patients.

Effect of red fruit oil extract on the expression of CCR5 was probably derived from its metabolite compounds, one of

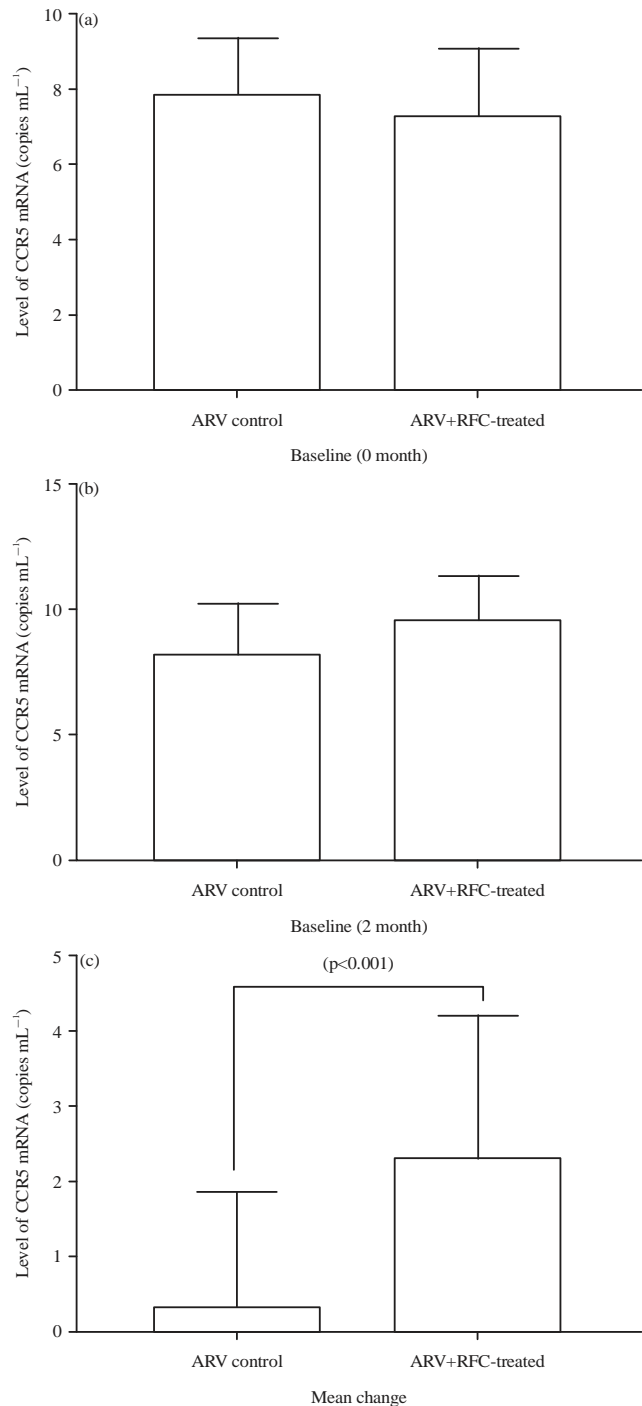


Fig. 1(a-c): Level of C-C chemokine receptor 5 (CCR5) mRNA of ARV (antiretroviral) control and ARV+RFC (red fruit capsule)- treated in the, (a) Baseline, (b) Two months and (c) Mean change

which is carotenoid<sup>35</sup>. Carotenoid has been widely reported and has been shown to possess antioxidant<sup>45,46</sup>, anticancer<sup>47</sup> and immunomodulatory capacity<sup>48</sup>. As an immunomodulator,

carotenoid has been shown to enhance lymphocyte blastogenesis, increase the population of specific lymphocyte subsets, increase lymphocyte cytotoxic activity and stimulate the production of various cytokines<sup>49</sup>. Another mechanism reported that the carotenoid has immunomodulatory effects by increasing INF- $\gamma$  and IL-2 production without inducing cytotoxicity<sup>50</sup>. In HIV-infected patients, the role of carotenoid was as antioxidant appears to be related to both direct immune modulation and inhibition of cytokine and NF- $\kappa$ B activation and inhibiting HIV replication<sup>51</sup>.

In the context of an immune system in HIV-infected patients, the CCR5 changes after the administration of red fruit oil extract have a definite meaning and benefit. The natural target of HIV infection was CD4<sup>+</sup> T lymphocytes<sup>52</sup>. Some studies have reported that CCR5 to be associated with disease progression and level of CD4 in HIV-infected patients<sup>53-55</sup>. Regulatory CCR5 expression affects many factors, including genetic mutation<sup>56</sup>, activation, signaling and trafficking and environmental<sup>57</sup>. Increasing effect of CCR5 mRNA levels is expected to improve the effectiveness of the immune response in HIV-infected patients.

Results showed that level of CCR5 mRNA in ARV+RCV treatment was significantly increased in HIV-infected patients as compared to ARV alone (Fig. 1). Results indicated that red fruits extract oils functional as immunostimulants; because the CCR5 as a coreceptor of the CD4<sup>+</sup> cell and CD4<sup>+</sup> cell counts reflect the immunologic status of HIV-infected patients<sup>58-60</sup>. Thus it is possible that the functional interaction of red fruit extract oils with antiretroviral therapy as an immunostimulant in HIV-infected patients occur at the level of metabolism through enzyme induction or inhibition<sup>23</sup>. Another possible mechanism that metabolite constituents of red fruits extract oils remedies may affect ARV metabolism as a result of their efflux drug transporter systems<sup>61,62</sup>.

This work shows the first report of immunostimulants effect on HIV-infected patients with ARV therapy that intervened with RFC. Thus, the combination of RFC and ARV can be alternative in the management of HIV-infected patients. The small of a sample size of current research is limited, so further research needs to be done by using a large number of samples.

## CONCLUSION

In conclusion, this study shows that the increased level of CCR5 mRNA expression by the ARV+RFC-treated group provided positive benefits in HIV/AIDS therapy that its combination can increase the mechanism of the immune system.

## SIGNIFICANCE STATEMENT

This study discovered the immuno stimulant effect of red fruit (*Pandanus conoideus*) oil extract in HIV infected patients with taking antiretroviral (ARV) therapy. This study will help the researcher to uncover the critical areas of immunological abilities from red fruit oil extract in combination with ARV therapy on the HIV-infected patients that many researchers were not able to explore. Thus, the red fruit oil extract could be a combination with ARV therapy that has potential as an adjuvant in the management of HIV-infected patients.

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