

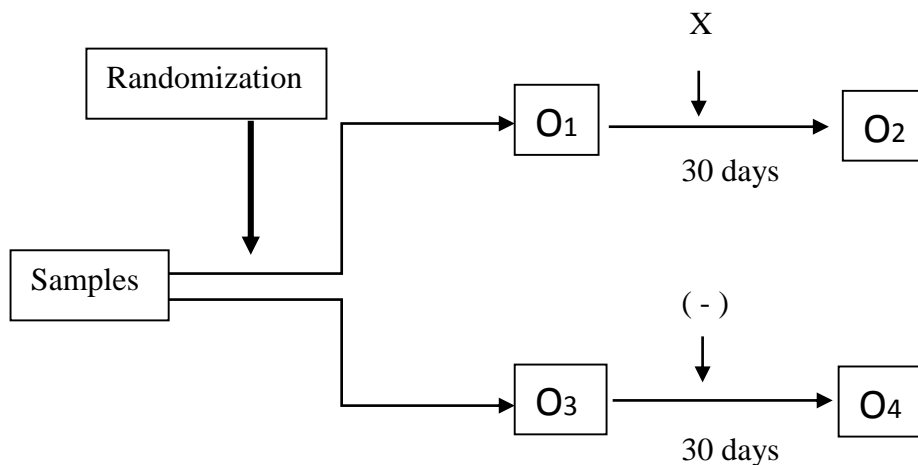
CHAPTER IV
RESEARCH METHODE

4.1 Research Scope

Scope of study focuses on immunological aspect, infectious diseases and phytopharmacology.

4.2 Research Design

Design of this research is the experimental study with *Double Blind Randomized Control Group Pre test – Post test Design*. Samples were randomly with permuted randomization, allocated into 2 groups : treatment group and control group. The scheme of research as follow :



Note :

X = Treatment group (antiretroviral therapy and mangosteen peel extract)

(-) = Control group with (antiretroviral therapy and placebo)

O1 = Pre test examination of CD8+ T cell count and CD38 expression on the treatment group

O2 = Post test examination of CD8+ T cell count and CD38 expression on the treatment group

O3 = Pre test examination of C8+ T cell count and CD38 expression on the control group

O4 = Post test examination of CD8+ T cell count and CD38 expression on the control group

4.3 Place and Time of the Research

4.3.1 Research Place

- The study was observed in Klinik Seroja Gunung Jati Hospital Cirebon.
- The laboratory examination was conduct in the Laboratory of Clinical Pathology Faculty of Medicine, Gajah Mada University.

4.3.1 Research Time

This research was conducted at Klinik Seroja Gunung Jati Hospital Cirebon for 30 days, from 3 November to 3 December 2014.

4.4 Population and Sample

4.4.1 Target Population

HIV/AIDS patient with antiretroviral therapy in Gunung Jati Hospital

4.4.2 Accessible Population

Population of study are HIV/AIDS patient in Klinik Seroja Gunung Jati Hospital who come to Klinik Seroja to take an antiretroviral therapy.

Inclusion Criteria :

- i. Age of 17 – 40
- ii. Agree to enroll in this study by signed informed consent letter
- iii. Clinical status : do not have an opportunistic infection and co-infection.
- iv. Get antiretroviral therapy for six months minimum with good adherence (>90%)
- v. Not in immunostimulatory and antioxidant therapy and agree not to take another immunostimulatory and antioxidant therapy during the study.

Exclusion criteria :

- i. Resigned before study completion

4.4.3 Sample size

Sample size was calculated by the formula :

$$n = \frac{2(Z_{1-\alpha} + Z_{1-\beta})^2 \sigma^2}{d^2}$$

n = sample size

$Z_{1-\alpha}$ = mean deviation alpha significance level (95%=1,645)

$Z_{1-\beta}$ = mean deviation beta significance level (power 80%=0,842)

σ = estimated population standard deviation (30)

d = significant difference (20)

from the calculation, the number of samples per group of 13. Drop out Risk : 50%

so we added 7 patients for each group so each group is 20

4.5 Research Variables

4.5.1 Independent variable

Given of mangosteen (*Garcinia mangostana*) peel extract dose 2400mg/day
divide 3 times for 30 days

4.5.2 Dependent variables

- a. The number of CD8⁺ T lymphocytes
- b. The level of CD38 expression

4.6 Operational Definition

4.6.1 Mangosteen (*Garcinia mangostana*) peel extract

Mangosteen (*Garcinia mangostana*) peel extract is the standardized mangosteen extract from sidomuncul that was given orally in dose 2400 mg/day divided in three time for two weeks.

Scale : nominal

4.6.2 The number of CD8⁺ T lymphocytes

Number that identified the absolute and presentation number of CD8⁺T cells count in, whole blood taken for measured the number of CD8⁺T cell count by flow cytometry.

Scale : rasio

4.6.3 The level of CD38 expression

The level that identified the expression of CD38 from CD8⁺ T cell. Peripheral whole blood taken for measured the frequency and density of the expression of CD38 from CD8⁺ T cell by flow cytometry.

Scale : rasio

4.7 Material and Method

4.7.1 Research tool and material

a. Research tool

- Research data collect by questioner anamnesis and collecting blood samples.
- Questioner anamnesis include : identity, subject characteristic (age, gender, sexual, risk of infection, depression, etc)
- Blood sample collect use vacutainer with EDTA for 5 cc
- Measurement of the number of CD8⁺ T cell and the level of CD38 expression using flow cytometry in the Laboratory of Clinical Pathology Faculty of Medicine, Gajah Mada University.

b. Material

1. Mangosteen (*Garcinia mangostana*) peel extract in capsul standardized, by Sidomuncul @400mg
2. Vacutainer with EDTA
3. Falcon disposable 12 x 75-mm capped polystyrene test tubes
4. Micropipet and tip
5. BD fluorochrome-conjugated monoclonal antibodies to human cell surface antigens
6. Vortex mixer
7. FACS Lysing Solution (10x)
8. FACS flowcytometry

4.7.2 Method

a. Research method

1. All HIV/AIDS patients in Klinik Seroja Gunung Jati Hospital assess for clinical status, antiretroviral treatment, fulfill inclusion criteria, ready to join for initial procedure, and signed informed consent.
2. Randomization use permuted random sampling, divide into two groups, treatment group and control group.
3. Drug coding do by researcher, doctor and nurse that give treatment to patients not known about group divide (*double blind*).
4. Mangosteen (*Garcinia mangostana*) peel extract given in dose 3x400 mg for 30 days as same as placebo and serial monitoring use phone call, short message and medical report book patients.
5. Collect blood aseptically by venipuncture into a sterile K₃ EDTA Vacutainer blood collection tube. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20⁰ to 25⁰C) until ready for staining and lysing.
6. Collecting peripheral whole blood done twice, pretest examination and post test examination. Peripheral whole blood collect and measured for the number of CD8⁺ T cell and the level of CD38 expression (post test).

b. Laboratory Method

Lysing and Staining

1. For each specimen, add a precise volume of EDTA anticoagulated whole blood of 50 uL in to a 12 x 75 mm tube
2. Add 10 uL of Tritest CD8/ CD38/ CD45 perCP or Tritest CD3 FITC/ CD8 PE/ CD45 per CP
3. Vortex gently and incubate for 15 minutes in the dark at room temperature (20⁰ – 25⁰ C)
4. Dilute 50 uL 10x FACS Lysing Solution with 450 uL distilled water
5. Add 450 uL of 1x FACS Lysing Solution
6. Vortex gently and incubate for 15 minutes in the dark at room temperature (20⁰ – 25⁰ C)
7. Analyze in a FACS Calibur flow cytometer with multiset software. Mix samples thoroughly before acquisition.

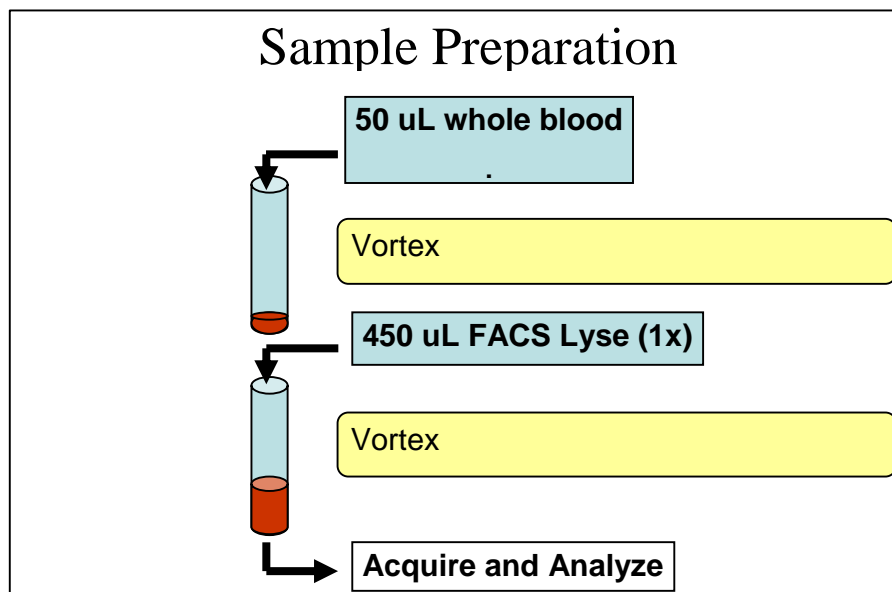
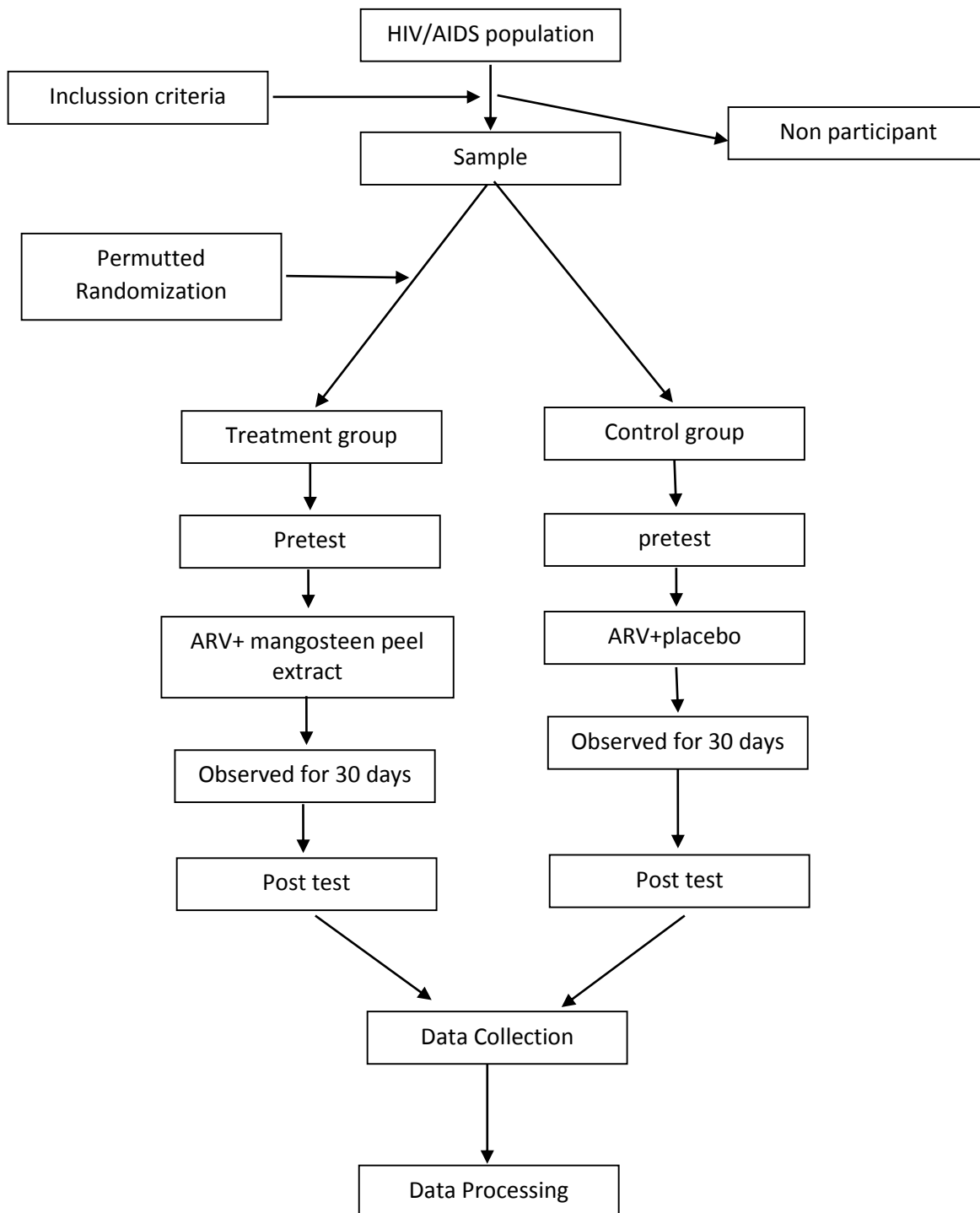


Fig. 4.1 Sample Preparation steps

4.7.3 Flow Chart Schematic Research



4.8 Data Processing and Analysis

Nominal P values are presented $p < 0.05$ is considered a statistically significant difference. Normal distribution was assessed by *Saphiro Wilks* normality test. All analyses were performed using SPSS software version 22.0 program for windows. Continuous variables are represented as mean \pm SD values or median \pm minimal-maximal values. Differences between the two groups were compared by the Independent Sample t test at baseline and at the end of the study period. The changes of the indices from baseline were calculated and compared between the two groups by the same method. If the variance not equal, the *non-parametric Mann-Whitney* test was used for comparisons. Differences between before and after consumption of mangosteen peel extract and placebo were analyzed by the Paired Sample t test. If the variance not equal, the non-parametric *Wilcoxon Smith* test was used for comparison. The χ^2 test was used to analyzed the data obtained from the subjects self-report questionnaire.

4.9 Research Ethics

The ethical clearance was approved by the Ethical Committee for Medical Research of Faculty of Medicine, Diponegoro University, No. 581/EC/FK-RSDK/2014.