

## ANTI INFLAMMATORY ACTIVITIES OF UNRIPE, RIPE *Muntingia Calabura* L. FRUITS AND *Muntingia Calabura* L LEAVES IN WISTAR WHITE RAT

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### Abstrak

Inflammation is a local protective response resulting from trauma or damage in the tissue functioning to destroy or to reduce injury agent or injured antigen. The objective of study was to compare the anti-inflammatory effect of unripe *Muntingia calabura* L, ripe *Muntingia calabura* L fruits and *Muntingia calabura* L leaves in Wistar white rat on anti-inflammation. The anti-inflammatory effect testing was carried out using 8 rats per group, each of which was treated with carrageenan induction. Each group was given treatment: the first group was given 1% CMCNa, the second group was given Na-diclophenac, the third unripe *Muntingia calabura* L was given fruit extract 50 mg/kg BW, the fourth group was given unripe *Muntingia calabura* L fruit extract 100 mg/kg BW, the fifth group was given ripe *Muntingia calabura* L fruit extract 50 mg/kg BW, the sixth group was given ripe *Muntingia calabura* L fruit extract 100 mg/kg BW, the seventh group was given *Muntingia calabura* L leaves 50 mg/kg BW, and the eighth group was given *Muntingia calabura* L leaves 100 mg/kg BW. The research to know anti-inflammatory activity of *Muntingia calabura* L. unripe, ripe fruits and leaves ethanol extract of carrageenan induced rat paw edema, percentage (%) of anti-inflammatory of ethanol extract of *Muntingia calabura* L. unripe, ripe fruits and leaves, and effect of *Muntingia calabura* L. unripe, ripe fruits and leaves on carrageenan induced inflammation change of inflammatory inhibition percentage (%). The result showed that unripe *Muntingia calabura* L, ripe *Muntingia calabura* L fruits, and *Muntingia calabura* L leaves groups had a significantly different anti-inflammatory effect compared to Na-diclophenac in inhibiting edema volume in male white rat.

**Keywords:** Na-diclophenac, Unripe *Muntingia calabura* L fruit extract, ripe *Muntingia calabura* L fruit, *Muntingia calabura* L leaves, anti-inflammation, combination.

### 1. INTRODUCTION

Some scientific studies have been conducted on the *talok* (cherry or Japanese kerst in English) plant providing scientific data on the content of plant. For example, a study by a Malaysian herbal author finding that the boiled *talok* leaves can be used to kill *C. diphtheria*, *S. aureus*, *P. vulgaris*, *S. epidemidis* and *K. rizophil* through in vitro experiment. The boiled *talok* leaves also has anti-inflammatory activity or reduces inflammation and lowers body temperature; such the data was obtained from the studies with rat as research object.

Inflammation is a local protective response generated by tissue injury or damage, functioning to destroy, to reduce or

to sequester injurious agent or the injured antigen (Dorland 2002). The cellular damage related to inflammation affects the cellular membrane leading the leukocyte to release lysosomal enzymes, arachidonic acid and then various eucosanoids were released from previous compounds. *Cyclooxygenase* (COX) path of arachidonic metabolism provides prostaglandin exerting an effect on blood vessel, nerve ends and cells involved in inflammation. *Cyclooxygenase-2* is induced during inflammation process and used to facilitate the inflammation response (Katzung and Trevor 2002).

## 2. RESEARCH METHOD

### Material

The materials used in study were *Muntingia calabura* L. leaves derived from Baluwarti, Surakarta; 95% ethanol as extraction solution; the tested animal consisted of 2-3 months aged male rats with 150-200 gram body weight; 1% carageenan was used as inflammation inducer; Na-Diclophenac was used as reference drug and CMC-Na was used as negative control.

### Tested Animal

Healthy wistar male rat with 150-200 gram weight.

### The Preparation of Unripe, Ripe *Muntingia calabura* L Fruits and *Muntingia calabura* L Leaves Extracts

50 gram *talok* leaves powder, and then put into reflux tool added with 95% ethanol until one and a half circulation occurred and connected to cooler or condenser, and then heating was conducted until the extraction proceeded. The extraction process was carried out until clear filtrate was obtained. The extract obtained was concentrated using *rotary evaporator* vacuum to get thick (concentrate) extract.

### Anti-inflammatory activity Test on Karagenan-Induced Foot

Each animal group was administered suspended tested compound in 0.5% CMC

per oral. A half of hour later, the animal was induced for inflammation with 0.05 mL karagenan 1% intraplantar. The foot volume was measured before injection (t0) and every hour after injection up to 7 hours (t1-t7). The increased means of edema volume for each tested animal were compared with the foot volume before induction (t0). The percentage inhibition was estimated by comparing the mean edema volume of tested group and that of control group (Jain et al., 2004).

### Data Analysis

The effect of unripe, ripe *Muntingia calabura* L fruits and *Muntingia calabura* L leaves extracts administration resulting from fractionation on the anti-inflammatory effect of Na-dichlophenac was estimated by measuring edema volume. Edema volume was the difference of rat's feet before and after being inflamed using caraagenan 1% sub plantar injection.

#### Group

1. CMC Na 1%
2. Na-Dichlophenac
3. E.unripe talok fruit 50 mg/kg bw
4. E.unripe talok fruit 100 mg/kg bw
5. E.ripe talok fruit 50 mg/kg bw
6. E.ripe talok fruit 100 mg/kg bw
7. Talok leaf 50 mg/kg bw
8. Talok leaf 100 mg/kg bw

Table 1. Anti Inflammatory activity of *Muntingia calabura* L. Unripe, Ripe Fruits and leaves ethanol extract of Carrageenan induced rat paw edema

| Group | Mean edema volume (ml) |       |       |       |       |       |       |       |
|-------|------------------------|-------|-------|-------|-------|-------|-------|-------|
|       | t0                     | t1    | t2    | t3    | t4    | t5    | t6    | t7    |
| 1     | 0.028                  | 0.06  | 0.066 | 0.07  | 0.076 | 0.072 | 0.072 | 0.072 |
| 2     | 0.026                  | 0.054 | 0.058 | 0.058 | 0.048 | 0.04  | 0.03  | 0.03  |
| 3     | 0.028                  | 0.056 | 0.058 | 0.062 | 0.06  | 0.038 | 0.028 | 0.028 |
| 4     | 0.028                  | 0.062 | 0.064 | 0.064 | 0.054 | 0.04  | 0.032 | 0.032 |
| 5     | 0.024                  | 0.06  | 0.062 | 0.062 | 0.054 | 0.04  | 0.03  | 0.03  |
| 6     | 0.028                  | 0.064 | 0.062 | 0.058 | 0.048 | 0.038 | 0.028 | 0.028 |
| 7     | 0.028                  | 0.062 | 0.064 | 0.064 | 0.054 | 0.04  | 0.03  | 0.03  |
| 8     | 0.03                   | 0.054 | 0.064 | 0.068 | 0.06  | 0.044 | 0.034 | 0.034 |

Tabel 2. Percentage (%) of Anti Inflammatory of ethanol extract of *Muntingia calabura* L. Unripe, Ripe Fruits and leaves

| Groups | Percentage (%) of Anti Inflammatory |        |        |        |        |        |        |
|--------|-------------------------------------|--------|--------|--------|--------|--------|--------|
|        | t1                                  | t2     | t3     | t4     | t5     | t6     | t7     |
| 1      | 120                                 | 140    | 153.33 | 176.66 | 163.33 | 163.33 | 163.33 |
| 2      | 120                                 | 133.33 | 133.33 | 93.33  | 60*    | 20*    | 66.66* |
| 3      | 106.66                              | 113.33 | 130    | 120    | 36.66* | 6.67*  | 13.33* |
| 4      | 130                                 | 136.67 | 136.67 | 100    | 50*    | 20*    | 20*    |
| 5      | 156.67                              | 166.67 | 170    | 116.66 | 70*    | 26.67* | 26.67* |
| 6      | 133.33                              | 126.67 | 106.66 | 80     | 36.66* | 0*     | 0*     |
| 7      | 130                                 | 136.67 | 136.67 | 100    | 50*    | 26.67* | 20*    |
| 8      | 80                                  | 113.33 | 126.66 | 100    | 46.66* | 13.33* | 13.33* |

\* = P < 0.001 statistically significant compared to positive control statistically significant compared to positive control (n=6)

Tabel 3. Effect of *Muntingia calabura* L. Unripe, Ripe Fruits and leaves on Carrageenan induced osteoarthritis change of Inflammatory Inhibition Percentage (%)

| Groups | Percentage (%) of Inflammatory Inhibition |       |       |       |        |        |        |
|--------|---|-------|-------|-------|--------|--------|--------|
|        | t1  | t2    | t3    | t4    | t5     | t6     | t7     |
| 2      | 10  | 12.12 | 17.14 | 36.84 | 44.44* | 58.33* | 72.22* |
| 3      | 6.66                                      | 12.12 | 11.43 | 92.11 | 47.22* | 61.11* | 61.11* |
| 4      | 3.33                                      | 3.03  | 8.57  | 28.95 | 44.44* | 55.55* | 55.55* |
| 5      | 0   | 6.45  | 12.9  | 40.74 | 80*    | 140*   | 140*   |
| 6      | 6.67                                      | 6.06  | 17.14 | 36.84 | 47.22* | 61.11* | 61.11* |
| 7      | 3.33                                      | 3.33  | 8.57  | 28.95 | 44.44* | 58.33* | 58.33* |
| 8      | 10  | 3.03  | 2.86  | 21.05 | 38.89* | 52.78* | 52.78* |

\* = P < 0.001 statistically significant compared to positive control statistically significant compared to positive control (n=6)

### 3. RESULT AND DISCUSSION

Unripe *Muntingia calabura* L fruit extract, ripe *Muntingia calabura* L fruit extract, *Muntingia calabura* L leaves at 50 mg/kg bw dose, unripe *Muntingia calabura* L fruit extract, ripe *Muntingia calabura* L fruit extract, *Muntingia calabura* L leaves at 100 mg/kg bw dose, positive control Na-Dichlophenac, respectively.

The statistic test carried out using Kolmogorov Smirnov test. The result showed  $p > 0.05$ , meaning that the data was distributed normally. The result of ANOVA was 0.000 ( $p < 0.05$ ) meaning  $P < 0.001$  that there was a significant difference between treatments. To find out whether or not there was a significant difference between treatments, the Post Hoc test was conducted.

Effect of *Muntingia calabura* L on paw oedema volume in Wistar white rats. Paw edema was measured every hour after

the administration of carrageenan. \* =  $P < 0.001$  statistically significant compared to positive control (n = 6).

At t5, the groups 2-8 obtained P (probability) values  $P < 0.001$ , meaning that there was a significant difference of anti-inflammatory activity compared with CMC as the positive control. At t6, the groups 2-8 obtained P (probability) values  $P < 0.001$ , meaning that there was a significant difference of anti-inflammatory activity compared with CMC as the positive control. At t7, the groups 2-8 obtained P (probability) values  $P < 0.001$ , meaning that there was a significant difference of anti-inflammatory activity compared with CMC as the positive control.

Na-dichlophenac had a working mechanism in the same path in inhibiting the prostaglandin synthesis with cyclooxygenase enzyme. Based on the data of percentage

anti-inflammatory activity, it could be found that unripe talok fruit, ripe talok, and talok leaves have anti-inflammatory effect. In addition to the same working mechanism, the inhibition of prostaglandin synthesis through cyclooxygenase enzyme work, in the

previous studies it was presumed that the extracts of unripe *Muntingia calabura* L fruit, ripe *Muntingia calabura* L, and *Muntingia calabura* L leaf contain flavonoid having anti-inflammatory effect.

Figure 1: Result of identification on flavonoid content in talok *Muntingia calabura* L, ripe fruits, and leaves

| Test                 | Extract of Leaves              | Extract of Unripe Fruit | Extract of Ripe Fruit |
|----------------------|--------------------------------|-------------------------|-----------------------|
| Saponin              | -                              | -                       | -                     |
| Flavonoid            | +                              | +                       | +                     |
| Kuinon               | +                              | +                       | +                     |
| Gelatin              | -                              | +                       | -                     |
| TaninKatekat         | -                              | +                       | -                     |
| TaninGalat           | -                              | -                       | -                     |
| Steroid/Triterpenoid | + (steroid, because it is red) | +                       | -                     |
| Alkaloid             | -                              | -                       | -                     |

Notes:

+ = contains

- = no contains

Previously, Preethi (2012) had conducted a study finding that the extract of *Muntingia calabura* L fruit at doses 100 mg/kg bw, 200 mg/kg bw, and 300 mg/kg could act as anti-inflammatory agent. This current study, as the expansion of Preethi's study, employed unripe, ripe talok fruits and talok leaves and found that all of extracts had anti-inflammatory effect.

#### 4. CONCLUSION

Both doses of ripe *Muntingia calabura* L fruits at the dose of 50 and 100 mg/kg showed higher anti-inflammatory effect on wistar white rat compared to unripe fruits and leaves. There was a significant difference of anti-inflammatory effect in the groups 2-6 at t6.

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