Ethanolic extracts of pleurotus ostreatus in the prevention of raised body temperature and C-Reactive Protein

S B Rahimah¹*, R K Putri², V Hendrayani², W Maharani³, J Hartati³, D D Djunaedi⁴, A Y Soeroto⁵ and T Bisri⁶

¹ Department of Pharmacology, Medical faculty, Universitas Islam Bandung, Bandung, Indonesia
² Clinical program, Medical faculty, Universitas Islam Bandung, Bandung, Indonesia
³ Department of Microbiology, Medical faculty, Universitas Islam Bandung, Bandung, Indonesia
⁴ Department of Biochemistry, Medical faculty, Universitas Padjadjaran, Bandung, Indonesia
⁵ Department of Internal Medicine, Medical faculty, Universitas Padjadjaran, Bandung, Indonesia
⁶ Department of Anesthesia, Medical faculty, Universitas Padjadjaran, Bandung, Indonesia

* santun@unisba.ac.id

Abstract. Anti-inflammatory effect of ethanolic extractions of Pleurotus ostreatus would measure on body temperature and the C-Reactive protein (CRP) in rats induced by carrageenan. In this study, 30 rats were divided; group I (normal control) group II (negative control- carrageenan), group III (positive control- ibuprofen 400 mg / time), group IV and V (treatment I and II: ethanolic extract at 250 and 500 mg / kg BB). The Rats’ body temperature was measured at 0, 2, 4, 6 hours and at the 6th hour the qualitative and semiquantitative CRP was examined. The results showed that the temperature was reduced in the 1st to 6th hour, with different patterns. The Kruskal Wallis test shows significant P-values in the 2nd hour (0.02) and 6th (0.02). The qualitative CRP was analyzed using the Fisher Test and showed a significance at 1:2 dilutions (p=0), while the semiquantitative CRP is only at 1: 1 a dilution (p= 0.0). The ethanol extraction of Pleurotus ostreatus at the dose of 250 mg and 500 mg / Kg BB showed a decrease in temperature and CRP, which was quite good when compared with ibuprofen. This result confirms about anti-inflammatory effect of the mushroom.

1. Introduction
Mushrooms have been used in the world as medicine since a long time ago and are useful in preventing several diseases such as hypertension, hypercholesterolemia and carcinoma. The white oyster mushroom (Pleurotus ostreatus Jacq: Fr Kumm) is the most popular commercial mushroom, contains high nutrients and various secondary metabolites that have pharmacological effects [1,2]. One of the potential pharmacological effects of white oyster mushrooms is its anti-inflammatory effect. The secondary metabolites of white oyster mushroom which act as an anti-inflammatory such as phenolic, flavonoids,
terpenoids, polysaccharides, lectins, steroids, glycoproteins, some lipid components, ergothioene (ET) and Beta glucan (β-glucan) [3-6].

Inflammation mostly underlies the pathogenesis of various diseases. Acute inflammation can become a chronic inflammation and can eventually develop into malignancy [5,6]. Excessive inflammatory response will be dangerous and cause various diseases. An increase in body temperature is a parameter that can be assessed to see the inflammatory response that occurs, due to the stimulation of interleukin-1 (IL-1) to the central nervous system. At each stage of inflammation there are roles of various inflammatory cells and mediators of inflammation [7-9]. The measurement of inflammatory parameters can be a clue as to how inflammatory conditions are happening, as well as providing a prognosis of the disease. One important inflammatory parameter is the C-reactive protein (CRP). C-reactive protein is an acute phase protein that describes the initial response to an inflammation or infection [10-11].

The most commonly used drugs as anti-inflammatory drugs are non-steroidal anti-inflammatory groups (NSAIDs) such as ibuprofen. Long-term use of NSAIDs can increase the risk of gastrointestinal bleeding [12]. Another alternative to treating inflammation, is to use herbal remedies such as the White Oyster Mushroom [3,4]. The antiinflammatory mechanism of fungal metabolites varies widely and has not been clearly disclosed, but several in vitro and in vivo studies have proven the anti-inflammatory potential of white oyster mushrooms [3,4,13-15].

Based on the data above, the researchers were interested in examining the inflammatory effects of white oyster mushrooms on inflammatory model rat induced with carrageenan, and assessing the inflammatory parameters that occurred by assessing changes in body temperature and CRP levels in rats.

2. Research methods

2.1. The design of the research
In vivo experimental using the Post Test-Only Control Group. Subjects of this study were male rats with Wistar strain, weighing 200-300 grams, aged 2-3 months and adapting for 7 days. The number of samples per group was calculated by the Ferderer's formula and obtained 30 total samples. The dependent variable in this study was the rat body temperature and C-reactive protein, while the independent variable was the dosage of ethanolic extraction of the White Oyster Mushroom.

2.2. Preparation of ethanolic extraction of white oyster mushrooms
The Ethanol extraction of the white oyster mushroom is an herbal preparation made by extracting white oyster mushrooms using 70% alcohol, and the dosages used are 250 and 500 mg / kg BB. Comparative drugs use 200 mg of Ibuprofen 1,2 Extractions make from fresh white oyster mushrooms, which are sliced and dried in an oven. The dried powder is then macerated with 70% alcohol and let stand for 24 hours. The aqueous extraction obtained from the macerator is then concentrated using a rotary evaporator [16].

2.3. Induction of carrageenan
The carrageenan used is a carrageenan solution in 0.9% NaCl with a concentration of 1-3%. This solution is them injected into the intraplantar at a dose of 50-150 μl. Inflammatory effects on the hind legs or thighs of the rat can be seen within 30 minutes after injection [17,18].

2.4. Measurement of rat body temperature
The rat body temperature is measured per rectal at 0, 2, 4 hours, and 6 hours after the carrageenan injection. The temperature is measured using a digital thermometer, because the tools are simple, have a short time detection, easy to read and accurate.
2.5. Measurement of C-reactive protein
The C-reactive Protein (CRP) is an acute phase of protein that plays a role in the inflammatory process. The amount can be detected in the blood in 6-10 hours, with a peak level of around 2 hours. The CRP examination was carried out qualitatively and semi-quantitatively by an agglutination method at 6 hour intervals. CRP titers are the highest dilution that still occurs in agglutination [10,11].

2.6. Data analysis
The test for normality and homogeneity of data distribution uses the Saphiro Wilk Test. Because the data is not normally distributed, the analysis is then continued using the cruscal Wallis nonparametric test and the Fisher Test.

2.7. Place, time and ethical aspect of the Research
The study was conducted in the laboratory of the Bandung Islamic University Faculty of Pharmacy Research Laboratory. The Research Time is from March to October 2018. The Research that uses experimental animals must apply the 3R principles, namely replacement, refinement, and reduction.

3. Results and discussion

3.1. Results of rat body temperature

| Group    | Average Rat Body Temperature (°C) |
|----------|-----------------------------------|
|          | 0      | 2nd    | 4th    | 6th    |
| I        | 33.2   | 34.1   | 34.7   | 35.6   |
| II       | 33.3   | 34.9   | 35.1   | 36.3   |
| III      | 33.6   | 32.6   | 34.6   | 34.8   |
| IV       | 33.9   | 34.5   | 34.4   | 35.4   |
| V        | 34.2   | 35.1   | 34.5   | 35.5   |

Information:
Group I (normal group) : normal food and drink
Group II (negative control) : carrageenan, CMC 2 cc / 200gram
Group III (positive control) : carrageenan, ibuprofen 400 mg / time
Group IV (treatment I) : carrageenan, ethanolic extraction at 250 mg / KgBB
Group V (treatment II) : carrageenan, ethanolic extraction at 500 mg / KgBB

The table 1 shows that at 0 hours, the lowest temperature is 33.2 °C and the highest temperature is 33.9°C. Two hours after induction, the whole group experienced a rise in temperature, except for group III who received ibuprofen. At the 4th and 6th hours, the second group experienced a progressive temperature increase, while the treatment group which received the white oyster mushroom ethanol extraction has decreased in temperature compared to the second hour, but the decline was unstable. At the 6th hour, almost all groups begin to increase again.

Analysis of the data starts with the normality test using the Saphiro Wilk test. The results of the normality test indicate that the data is not normally distributed. Therefore, the data analysis is then carried out by Kruskall-Wallis test. The results of the Kruskal Wallis test showed that the p value at 0 hours (0.20) and the 4th hour (0.38) was not significant, meaning that there was no significant difference in body temperature between treatment groups, while in the 2nd hour (0.02) and 6th (0.02) there are significant differences, which means that all groups at that hour has a significant temperature difference.
3.2. C-Reactive Protein (CRP) test results

The levels of CRP in the rat experimental animals is not as large as in humans, so that at the time of examination it is often doubtful whether the results are positive or negative. Positive results illustrate the existence of coagulation that is very clear in accordance with existing standards, while the "positive-negative" examination shows a coagulant but it is not as clear as the standard. "Positive-negative" results are categorized as "positive" on statistical checks.

Table 2. Results of CRP examination of Rat Inflammatory models.

| Groups | Result | Qualitative | Semi Quantitative |
|--------|--------|-------------|-------------------|
|        |        |             | Dilution 1:1       | Dilution 1:2       | Dilution 1:4       | Dilution 1:8       |
| I      | Positive | 0           | 0                  | 0                  | 0                  | 0                  |
|        | Negative | 6           | 6                  | 6                  | 6                  | 6                  |
| II     | Positive | 6           | 6                  | 4                  | 0                  | 0                  |
|        | Negative | 0           | 0                  | 2                  | 6                  | 6                  |
| III    | Positive | 3           | 3                  | 2                  | 0                  | 0                  |
|        | Negative | 3           | 3                  | 4                  | 0                  | 0                  |
| IV     | Positive | 3           | 1                  | 0                  | 0                  | 0                  |
|        | Negative | 3           | 5                  | 6                  | 6                  | 6                  |
| V      | Positive | 2           | 2                  | 2                  | 6                  | 6                  |
|        | Negative | 4           | 4                  | 4                  | 6                  | 6                  |

Information:
- Group I (normal group): normal food and drink
- Group II (negative control): induction of carrageenan, CMC 2 cc / 200gram
- Group III (positive control): induction of carrageenan, ibuprofen 400 mg / time
- Group IV (treatment I): Ethanolic extraction of white oyster mushroom 250 mg / KgBB
- Group V (treatment II): Ethanolic extract of white oyster mushroom 500 mg / KgBB

The table 2 shows that the measurement of qualitative CRP levels in group 1 is entirely negative, in the two groups which are entirely positive-negative and groups of 3.4 and 5 vary. While semiquantitative measurement results show that positive results are only seen at the 1:1 dilution.

Analysis of CRP level measurement data using Fisher's test and the P value was said to be significant if p <0.05. The Fisher Test Results of Qualitative CRP obtained p = 0, meaning that there are differences between each group. Fisher Test Results of Semi Quantitative CRP obtained p = 0 at the 1:1 dilution showed significance, but the subsequent dilution did not show any significant differences.

4. Discussion

In group 2 (negative control), there was an increase in CRP levels and consistent temperature that began to be seen starting at the 2nd after induction. Increases in body temperature and CRP levels of rats showed an inflammatory response that emerged, after induction, of intraplantar carrageenan resulting in inflammation and edema of the rat’s paw. Carrageenan is a polysaccharide obtained from red seaweed, forms a gel and can thicken, which is obtained from the extraction of a rhodophyceae species which is a red seaweed species. The effect of carrageenan is biphasic: the initial phase (1-2 hours) will induce the release of histamine, serotonin and bradykinin. In the final phase (3-5 hours) prostaglandin and other inflammatory mediators are released [17-18].

In this study as a comparison, the usual dose of Ibuprofen of 400 mg was used as an anti-inflammatory, which caused a decrease in temperature and changes in CRP levels. Ibuprofen showed a significant decrease in body temperature at 2 o'clock, in the following hours it appeared that there was an increase in the temperature again but when compared with the negative controls it showed a good temperature reduction effect. The half-life of ibuprofen is only 2 hours and will be excreted immediately.
through the kidneys and metabolism in the liver, so that at 4 o'clock the temperature seems to begin to rise again. The anti-inflammatory mechanism of ibuprofen is due to non-selective inhibition of COX1 and COX2 [12].

The ethanol extraction of white oyster mushroom at a dose of 250 mg / Kg BW and 500 mg / Kg BB showed a decrease in temperature and a decrease in CRP levels which was quite good when compared with ibuprofen. This is likely related to the active metabolites of white oyster mushrooms. Active metabolites in fungi contain polysaccharides, glycosphingolipids, and glycoprotein proteins which can inhibit acute inflammatory processes. Other studies have shown that both white oyster mushroom extracts and beta glucan compounds can reduce edema in the limbs of mice arthritis with examples induced with formalin and methotrexate [13-14]. Phenolic or polyphenol compounds are thought to inhibit a TNF-α gene expression and inhibit its production. This fungus is also known in vitro to inhibit NF-kB activation through the inhibition of protein kB (IkBα) inhibitory phosphorylation [15]. The difference is in the pattern of temperature reduction in both doses, probably due to differences in the concentration of active substances that contained therein. The pattern of decreasing body temperature at a dose of 500 mg / kgBB, seems more stable compared to a dose of 250 mg / KgBB, although the onset of action is faster at a dose of 250 mg / Kg BB. The effect of the white oyster mushroom ethanol extraction also appears to prevent an increase in CRP levels, although the effect cannot be distinguished on both extraction doses. The higher CRP level indicates that the inflammatory process is ongoing.

5. Conclusion
Ethanol extractions of white oyster mushrooms at a dose of 250 mg / Kg BB and 500 mg / kg BB can prevent an increase in body temperature in wistar strain rats that received carrageenan induction and can prevent an increase in C-Reactive protein levels in wistar strain male rats that received carrageenan induction.

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