Effect of ethanolic extract of cashew leaf (Anacardium occidentale L) on edema, hyperalgesia and malondialdehyde levels of the rat foot of an inflammatory model compared to diclofenac

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ABSTRACT

Inflammation is a body tissue reaction to the damage caused by foreign object. One of the plants that the community uses to eliminate inflammation is cashew leaf (Anacardium occidentale L). This research aimed to find out if the guava extract has an anti-inflammatory effects compared with diclofenac. This was an experimental study using 30-tails Wistar strain, which is divided into 6 groups that every 1 hour later induced lambda-carrageenan. Group I and IV are given ethanolic extract of cashew leaf 300 mg/kg BW. Group II and V were given cellulose (CMC) 1% as a control. Group III and VI were given diclofenac 4.5 mg/kg of BW. Edema volume from hour 1 to 6 and MDA levels of foot tissue in the hour 6 were measured in group I, II, III. Hyperalgesia was measured in group IV, V and VI. All research results were statistically tested with ANOVA test followed by the Newman Keuls test. Ethanolic extract of cashew leaf 300 mg/kg BW inhibited the formation of edema volume, preventing hyperalgesia significantly (P < 0.05) compared to a group of control and diclofenac. Measurement of MDA levels between groups with ethanolic extract of cashew leaf, control, and diclofenac was not significantly different (P > 0.05). Ethanolic extract of cashew leaf has an anti-inflammatory effect based on inhibition of edema volume, hyperalgesia.

INTRODUCTION

Inflammation is caused by various things such as trauma, temperature, radiation, chemical substances, bacteria-induced infections, viruses, fungi or parasites and can also be caused by an immune reaction. Inflammation can be acute or chronic. Acute inflammation runs fast and in a short time, starting from a few days. Chronic inflammatory can run longer form a few days to years (Mitchell and Cotran, 2003).

One of the signs of inflammation is pain. Pain is a tissue response to tissue-damaging stimuli and then causes activation of pain receptors. Mediators that are released in the event of tissue damage will increase pain sensitivity such as prostaglandin (Guyton and Hall, 2006).

Anti-inflammatory drugs are groups of drugs that have pressing or reducing inflammatory activity. Anti-inflammatory drugs are two types of nonsteroidal and steroid classes. Nonsteroidal anti-inflammatory drugs work by inhibiting prostaglandin synthese. Non-steroidal anti-inflammatory drugs are widely used in the field of dentistry as both anti-inflammatory and analgesic among other diclofenac. The use of non-steroidal
anti-inflammatory drugs and steroids gives a benefit, but non-steroidal anti-inflammatory drugs also do little to provide harm due to side effects (Roberts and Morrow, 2001; Wagner et al., 2004).

Indonesian people have long known and used medicinal plants as one of the efforts in tackling health problems in the hereditary. The use of natural ingredient drugs, in general, is assessed safer than the use of modern medicine. This is because natural medicinal ingredients have relatively fewer side effects than modern drugs (Ekor, 2014).

Cashew (Anacardium occidentale L) is two-LEED plants (dicot). Cashew nuts are one of the crops that can be utilized as medicinal plants. The parts used are bark, bark, leaves, and fruit. Cashew crops grow a lot in the Wonogiri area. Cashew leaf have been used by society in treating inflammation by boiling 5 sheets of cashew leaf. Cashew leaf are used because of the leafy cashew trees throughout the year. Cashew leaf contain chemical compounds such as tannins, flavonols, anacardol acid, phenol com- pounds, cardol which are beneficial as antibacterial and antiseptic. Flavonol as an anti-inflammatory by inhibiting cyclooxygenase and lipoxygenase. Anacardol acid is antioxidant so that the acid Anacardol has an anti-inflammatory effect. Besides, the young Pink leaf also has a chemical content composition such as vitamin A and vitamin C (Agedah et al, 2010; Sheny et al., 2011). Lambda-carrageenan is a complexity that can cause inflammation of the foot of the rat injected into intraplantar 0.1 ml (Morris, 2003; Romano et al., 1997).

MATERIALS AND METHODS

Research subject
In this study, the subject used was a 30-tailed white rat Wistar strain that was split into 6 groups. Each group consisted of 5 rats. The rats must meet the inclusion criteria: male, healthy and active, weight approximately 180-200 grams and about 12 weeks old. Changes in the exclusion of weight changes during adaptation of more than 10%.

Research materials
Cashew leaf were obtained from Subang, West Java. The ethanolic extracts wered manufactured in the chemistry laboratory of Universitas Padjadjaran, Bandung. Diclofenac sodium tablets 50 mg was used as a comparative drug. Lambda-carrageenan in a solution of NaCl 0.9% which was used as an infusion of inflammation, breast milk on the soles of rats. The control groups were given Carboxyl Methyl Cellulose (CMC) 1% solution.

Research tools
Simple surgical apparatus, a syringe with oral needle, mortar and stamper, Plethysmometer (edema volume gauge), spectrophotometer to check MDA levels, and water bath.

Research methods
The definition of a variable concept is divided into two variables (the dose of cashew-leaf ethanol extract, diclofenac dose, dose lambda-carrageenan. Variable bound (volume of rat foot edema, hyperalgesia, and MDA levels).

The definition of operation and consists of the volume of the rat foot edema measured with a plethysmometer, Hyperalgesia measured from the foot inserted into the water bath until the foot is pulled spontaneously, MDA levels are examined by assessing the absorbancy of Thiobarbituric acid Reac- ting substances (TBARS) using the spectrophotometer.

The number of samples per group is determined according to the formula Federer (R-1) (T-1) > 15; (R-1) (6-1) > 15; 5 (R-1) > 15; 5r > 20; R > 4. Based on the formula above the minimum number of each group is 4 tails. In this study, each group used 5 rats.

Medicinal materials manufacture
Cashew leaf were washed clean and then dried indoors so the resulting simplisia. Simplisia is then made into powder by smoothing with grinding tools, then in maceration with ethanol 99% in Macer- ator. The maceration process was done several times until not colored and then performed with evaporator until the ethanol solvent evaporates and obtained cashew leaf extract.

Research procedure
Rats were first adapted for seven days in the laboratory. The rat were restored a day before the experiment. The allocation of rats in six groups was done randomly block permutation. The first group received ethanolic extract of cashew leaf of 300mg/kg BW to be measured the volume of swelling of the rat foot and the MDA tissue level of the rat paw. The second group received a carboxy methylcellulose (CMC) 1% solution as much as 2 ML mouths as the control to be measured the volume of swelling of the rat foot and the MDA tissue level of the rat Kaiki. The third group received diclofenac 4.5 mg/kg BW mouth as a comparative drug that will be measured volumes of edema of rat feet and MDA tissue levels of rat feet. The fourth group received the ethanolic extract of cashew leaf as much as 300 mg/kg BW mouth which will be tested the condition of its hyperequipment. The fifth group received a 1% Carboxyl methyl cellulose (CMC) solution of 2 mL peroral as a control to which the condition will
Table 1: The average of edema volume of each group

| Group | 1 hour (SD) | 2 hours (SD) | 3 hours (SD) | 4 hours (SD) | 5 hours (SD) | 6 hours (SD) |
|-------|-------------|--------------|--------------|--------------|--------------|--------------|
| I     | 9.00 (0.71) | 11.20 (1.30) | 10.80 (0.84) | 10.60 (0.55) | 11.00 (1.00) | 11.20 (0.84) |
| II    | 9.40 (0.55) | 12.00 (0.61) | 11.80 (0.45) | 12.40 (0.55) | 13.40 (0.55) | 14.00 (0.71) |
| III   | 7.00 (1.00) | 8.00 (0.71)  | 7.00 (0.71)  | 7.80 (0.84)  | 8.00 (0.71)  | 8.40 (1.52)  |

Description: I. Test group Ethanol extract of cashew leaf 300 mg/kgBW
II. Negative control (CMC 1%)
III. Positive control (Diclofenac 4.5 mg/kgBW).

Table 2: The Anava advanced test for the edema volume in the sixth hour

| Diclofenac | Ekstrak daun cashew leaf | CMC |
|------------|--------------------------|-----|
| 8.40       | 11.20                    | 14.00 |

Table 3: Average foot pull-off time to measure the state of hyperaglesia

| Group | 1 hours (SD) | 2 hours (SD) | 3 hours (SD) | 4 hours (SD) | 5 hours (SD) | 6 hours (SD) |
|-------|--------------|--------------|--------------|--------------|--------------|--------------|
| IV    | 3.60 (2.13)  | 3.12 (1.03)  | 5.12 (1.80)  | 3.12 (1.33)  | 4.02 (1.95)  | 4.16 (1.42)  |
| V     | 3.38 (0.97)  | 2.06 (0.39)  | 2.22 (0.27)  | 2.40 (0.62)  | 1.88 (0.62)  | 1.84 (0.92)  |
| VI    | 2.30 (0.99)  | 1.90 (1.24)  | 2.10 (0.50)  | 2.70 (1.85)  | 1.32 (0.16)  | 2.14 (0.78)  |

Description: IV. The test group of ethanol extract Daunjambu cashew 300 mg/kgBW
V. Negative control (CMC 1%)
VI. Positive control (Diclofenac 4.5 mg/kgBW).

Table 4: The Anava advanced test for hyperaglesia in the sixth hour

| CMC | Diclofenac | Ekstrak daun cashew leaf | Daun cashew leaf |
|-----|------------|--------------------------|------------------|
| 1.84| 2.14       | 4.16                     |                  |

| CMC  | Diclofenac | Ethanol extract of cashew leaf |
|------|------------|-------------------------------|
| 1.84 | 2.14       | 4.16 0.0155* 0.0238*          |

Table 5: Average levels of MDA (nmol/ml) of each group

| Groups                       | Average levels of MDA (nmol/ml) |
|------------------------------|---------------------------------|
| Ethanol extract of cashew leaf| 21.883 (3.2857)                 |
| CMC                          | 25.566 (8.6867)                 |
| Diclofenac                   | 32.282 (11.803)                 |
Table 6: The Anava advanced test for MDA levels in the sixth hour

|                          | Ethanolic extract of cashew leaf | CMC       | Diclofenac |
|--------------------------|---------------------------------|-----------|------------|
| Ethanolic extract of     | 21.883                          | 25.566    | 32.282     |
| cashew leaf              |                                 |           |            |
| CMC                      | 25.566                          | 0.4011    | 0.0924     |
| Diclofenac               | 32.282                          | 0.0924    | 0.3354     |

The sixth group receives 4, 5mg/KGBW mouth diclofenac which will be tested in the condition of the hyperfittings.

Measurement of edema volume

Measuring edema volume by using a tool called a plentisthermometer. The stage is that every foot of the rat is marked as the dyeing boundary in the plentimeter vessel containing mercury. The first group was given a cashew-leaf ethanol extract at a dose of 300 mg/kg of BW, the second group was given carboxyl methylcellulose (CMC) 1% as much as 2 mL of Mouth and a third group was given diclofenac at a dose of 4.5 mg/kg BW mouth. An hour later, all rats in each group were lambd-carrageenan + 1% of 0, 1mL on the left foot of the Intraplantar rat. On the first hour until the sixth hour after induced measurements of the volume of left foot edema with Pletisometer.

Measurement of hyperalgesia state

In forth, fifth and sixth groups, the value of foot pull-off time of each rat was measured by dipping the left foot that has been marked with a waterproof ink as the dyeing boundary into a water bath containing hot water at 50 °C and seen in how many seconds the rat would pulling his feet. Once the lambda-carrageenan was induced, it was measured again in one to the sixth hour.

Measurement of MDA levels

The MDA levels was inspected after the six-hour measurement of the edema volume. Soft tissue samples were taken from the left foot of the rat. Rats were previously in anesthesia with Pentotal. Then taken the left foot tissue, then crushed in a mortar that has been cooled before, then made homogenate 20% with NaCl 0.9%. The process continues with the TBARS method. The stages of TBARS method were homogenate 700 ml, solution of BHT 50 ml, acetic acid 1500 ml. After the ingredients are mixed, heat the water bath 100 °c for 60 minutes. The solution is then cooled immediately. The solution is then disentriguated with a speed of 2325 rpm for 10 minutes. The Supernatan was then measured in absorption at a wavelength of 532 nm with a spectrophotometer. MDA levels (nmol/mL) are then calculated with the sample absorption formula divided by standard absorbance of standard concentrations.

Data analysis

The Data was analyzed statistic using the Analysis of Variance (ANOVA) followed by the Newman Keuls test.

Place and time of research

The research was conducted at the SMF Pharmacology Laboratory and UPK Faculty of Medicine Universitas Padjadjaran Bandung from December 2010 to January 2011.

Animal ethics implications

This research on animals carries the impact of ethical implications on animals that are in the form of animal discomfort. 3R approach (refinement, reduction, and replacement) was used to reduce the possible discomfort of the animals. The refinement principle involves the use of methods that reduce or relieve pain to ensure the welfare of experimental animals. The principle of reduction by using fewer experimental animals. The accuracy of a study depends on the size of the sample and the error variance and not on the weight of the experimental animals. The principle of replacement includes methods that allow achieving research objectives without the use of experimental animals. The experimental animals were the lowest number on the phylogenetic scale and the least taste. At the end of the study, the animals were sacrificed with deep anesthesia techniques and then buried accordingly.

RESULTS AND DISCUSSION

The research was conducted on 30 rats that were divided into 6 groups, each consisting of 5 rats. The first and forth group received the ethanolic extract of cashew leaf, the second and fifth group to receive 1% CMC and the third and sixth group receiving diclofenac. Edema volume and MDA levels were measured in the first to third group. Hyperalgesia was measured in the forth to sixth group.
Measurement of edema volume

Edema volume was measured in the left foot of rats at 1, 2, 3, 4, 5, and 6 hours after induction of lambda-carrageenan. Measurements were performed with a plethysmometer apparatus and results expressed in milliliters. The average result of edema volume of the rat foot of the inflammatory model from 1 to 3 hours can be seen in the Table 1.

Table 1 shows the average volume of edema from one to six hour with the result: Group I with ethanolic extract of cashew leaf 300mg/KGBW in the sixth hour increased compared to the hour to one but lower than the group II with the administration of CMC 1%. Group II with the granting of CMC solution 1% from hour to one to the sixth hour experienced an increase. Group III with the administration of Diclofenac 4.5 mg/kg BW from hours to one hour to six also experienced increased although lower than Ethanolic extract of cashew leaf 300 mg/kg BW and CMC 1%.

In the sixth hour test results with Anava followed by a Newman Keuls test (Table 2) obtained significant results with Fcount = 33.60 and P-value < 0.05. The average volume of edema CMC 1% (14.00) is higher than the introduction of ethanolic extract of cashew leaf 300mg/KGBW or diclofenac 4, 5mg/KGBW. On average the volume of ethanolic extract of cashew leaf is 300mg/kg BW with CMC 1% indicating a significant difference of 11.20 ml and 14.00 ml (p = 0.0004). The average volume of edema of cashew leaf extract is 300mg/kg BW compared with diclofenac indicating a significant difference of 11.20 ml and 8.4 ml (P = 0.0068).

Measurement of hyperalgesia state

The measurement of hyperalgesia performed on the left foot every hour after induced with lambda-carrageenan up to six hour. The state of the hyperalgesia is rated from the foot pulled in second. The average measurement of hyperalgesia from one to six hour is shown in Table 3. In group IV which is a test with ethanolic extract of cashew leaf 300mg/KGBW, there is a difference where the sixth hour is higher than the hour to one. In group V which is a test with the CMC 1%, the average value in the sixth hour shows the smallest average value compared to the average value at the hour to one. In a group of VI which is a test with a diclofenac 4.5mg/KGBW, the average value of the sixth hour is not much different from the hour to one, which is 2.14 and 2.30.

Test results with Anava followed by advanced Test Neuman Keuls (Table 4) obtained significant results with fcoun = 6.85 or P-value = 0.0103 which is smaller than 0.05. It can be interpreted in the sixth hour, the average Hiperalgesia cashew leaf extract 300mg/kgBW with CMC 1% shows significant differences of 4.16 seconds and 1.84 seconds (P = 0.0155). Average Hiperalgesia Cashew leaf extract 300mg/kgBW with diclofenac 4, 5mg/KGBW showed significant differences of 4, 16 seconds and 2, 14 seconds (P = 0.0238).

Measurement of MDA levels

Inspection of MDA levels of rat foot tissue was performed 6 hours after the induction of lambda-carrageenan. The examination is done with the TBARS method, then its measurements use spectrophotometry at 532 nmol/ml wavelengths.

Referring to Table 5, the average of MDA level in diclofenac 4.5mg/KGBW group was the highest but the advanced test result showed in Table 6 indicates no significant difference between three groups. It means that all three treatments had the same effect on MDA levels.

Edema

The inflammatory process marked edema caused by the occurrence of capillary vasodilation and an increase in blood flow in inflammatory areas. Capillaries vasodilation and increased blood flow will increase the intravascular hydrostatic pressure resulting in the movement of fluid out of the blood vessels. This fluid is called transudate. The transudation process will be followed by increased vascular permeability which causes fluid and cell displacement to the interstitium called an exudate. The accumulation of fluid occurring in the extracellular tissue is called edema. This inflammatory process is mediated by mediators such as histamine, prostaglandin, leukotriene, and Bradikine (Reid and Roberts, 2005).

The inflammation of the rat foot due to lambda-carrageenan injection, due to its mechanism that stimulates the release of inflammatory mediators, especially histamine, bradykinin, and prostaglandin. Diclofenac can inhibit the formation of edema through inhibition of the cyclooxygenase enzyme. This enzyme plays a role in the biosynthesis of prostaglandins from arachidonate acid (Reid and Roberts, 2005).

Ethanolic extract of cashew leaf can inhibit the formation of edema formed due to inflammation formed due to the induction of lambda-carrageenan due to the presence of active substances in cashew leaf that are flavonol and anacardol acid. The effect of flavonol as an anti-inflammatory through inhibitory mechanisms against cyclooxygenase and lipoxygenase pathways thus lowering the...
metabolism of arachidonate acid and prostaglandin synthesis. Patil (2003) Ahwa Ethanolic extract of cashew leaf 300 mg/kg BW could inhibit edema formation in the inflammatory caused by lambda-carrageenan injection (Patil et al., 2003). In this study, the suppression of edema volume in the administration of ethanolic extract of cashew leaf although not as strong as diclofenac, but showed a significant difference when compared to the administration of CMC.

**Hyperalgesia**

In an inflated state, it will be a state of increased pain sensitivity called hyperalgesia. Some mediator substances that play a role in pain processes such as histamine, Bradykine, leukotriene, and prostaglandins are involved in this inflammatory process. Prostaglandins cause increased nociceptor sensitization by lowering the activation threshold for sodium canals.

The ability of diclofenac to inhibit the reduction of pain thresholds or hyperalgesia on the feet of lambda-carrageenan-induced rats due to its ability to inhibit prostaglandin Biosintesias through inhibition of cyclooxygenase enzyme.

Ethanolic extract of cashew leaf has a preventative effect of hyperalgesia through its active substances that are flavonol and tannins that inhibit the pathway of cyclooxygenase and lipoxygenase to cause the prevention of prostaglandin biosynthesis and leukotriene is responsible for the pathophysiology of pain and hyperalgesia.

In this research extract of the ethanol jambu leaves of the cashew effect of hyperalgesia is higher than the diclofenac extract caused by cashew leaf ethanol shall more than one active substance.

**MDA levels**

A lambda-carrageenan injection at the foot of the rat caused inflammation. In inflammatory processes occur vascular changes that are vasodilation of local blood, and increased capillary permeability and cellular change i.e. migration of leukocytes from capillaries to surrounding tissues. Leukocytes that migrate to tissues will do phagocytosis. During phagocytosis will occur free radical liberation known as Reactive Oxygen Species (ROS) by macrophages, neutrophils. ROS on numerous amounts will cause cell damage. Lipid peroxidation is a sign of cell damage. Lipid peroxidation will increase MDA levels (Morris, 2003; Whiteley and Dalrymple, 2001).

MDA is an organic compound mainly produced by the fat peroxidation process and is a biomarker of oxidative stress and cell damage. Malondialdehyde can also be produced from the metabolic process of arachidonate acid into Prostaglandins. MDA comes from solving PGH2, an unstable compound in PG biosynthesis. The breakdown of PGH2 to MDA can occur spontaneously or catalyze by Ferro Ion or heme (Roberts and Morrow, 2001; Wagner et al., 2004).

Diclofenac may inhibit elevated MDA levels in inflammatory tissues due to its ability to inhibit prostaglandin biosynthesis. This inhibition will reduce the MDA levels formed through the prostaglandin biosynthesis pathways. Reduced prostaglandins will reduce the number of leukocytes that migrate to the inflammatory regions, thereby reducing the process of phagocytosis and the release of free radicals resulting in increased MDA levels are inhibited (Gan, 2010).

Ethanolic extract of cashew leaf can inhibit the increase of MDA levels because it contains the flavonol and anode acid that are antioxidant, thereby inhibiting the release of free radicals that can cause fat peroxidation.

MDA levels can be enforced quite sensitive but not specific. This is due to the TBARS method having some disadvantages. Some aldehyde compounds in addition to MDA can also react and at the same measured spectrophotometry inspection as well as MDA.

**CONCLUSIONS**

Ethanolic extract of cashew leaf has an anti-inflammatory effect based on inhibition of edema volume and hyperalgesia. The effectiveness of cashew leaf ethanol in inhibiting edema and MDA level is comparable to diclofenac and higher in inhibiting hyperalgesia.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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