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Anti-inflammatory activity extract of Katola (Arcangelisia flava (L.) Merr.) on the Expression of Cyclooxygenase-2 enzyme in Wistar rats Induced Complete Freund,s Adjuvant (CFA)

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ABSTRACT— The aim of this research were to determine the anti-inflammatory activity infusa extract of katola (Arcangelisia flava (L.). Merr.) on cyclooxygenase-2 enzyme expression in Wistar rats induced Complete Freund,s Adjuvant (CFA). The examination was using immunohistochemical methods. Immunohistochemical preparations were made to treat the experimental animals as inflamed with Complete Freund,s Adjuvant (CFA) induction. The conclusion was the infusa of katola stem bark (A.flava (L.) Merr.), which consisted of Berberine HCl 5.69±0.18 (rerata±SD) at the dose 450 mg/kg BW significantly decreased the expression of COX-2 different from negative control, the level at significantly by 95%, $p<0.05$.

KEYWORDS: Katola, Arcangelisia flava, immunohistochemical, Cox-2

1. INTRODUCTION

Metabolic disease and degenerative diseases are increasing because of the people's behavior themself and the side effect of the synthetic drug. Scientific research to find an alternative using local wisdom or local knowledge that has been used by the local community in Indonesia could be used a primary strategy to find the right choice for metabolic disease. At a price that can be affordable, sufficient raw material with minimal side effects continues to be done to overcome the problems faced. One of the plants used in traditional medicine at "MUNA" district was katola stem bark (Arcangelisia flava (L.) Merr.). The conventional recipe was used for the treatment of loose stools with blood. Phytochemical studies on the plant species Arcangelisia flava (L.) Merr. Lately, much increased. This is because it is driven by the knowledge that the plant consists of isoquinoline alkaloid e.g.Jatrorridzine, palmatine, columbamine, and berberine with diverse useful as antifungal bioactivity, antiasthma, antibacterial, antimalaria, antitumor and anti-inflammatory (Anonymous, 2010; Wongbutdee, 2009). Research has also conducted on the extraction and isolation of compounds content (Meistiani, 2001). The antimicrobial activity and in vitro and in vivo was done (Larisu, 2010). Hot water-soluble extract of katola was used as a medicinal remedy, abdominal pain, jaundice, and sore eyes by the Dayak people in Borneo (Sitepu and Sutikno, 2001). The research on anti-inflammatory activity in the timber katola (Arcangelisia flava (L.) Merr.) has not already made. It was as an effort to utilization diversify findings of this research. This study was also to develop a timber katola (Arcangelisia flava (L.) Merr.) as an Indonesian herbal remedy in anti-inflammatory medicine in the future.

2. METHODOLOGY

2.1 Materials

Katola (Arcangelisia flava L.Merr) obtained from Muna, Southeast Celebes Province, Complete Freund's Adjuvant as an inflammatory inducer (CFA) 0.1% (Sigma Chemical Co.., MO., USA), Berberine HCl (Sigma-Aldrich) and pure diclofenac sodium (Global Chemindo Megatrading), male Wistar rats aged 6-7 weeks obtained from the Animal Experiment Unit Development (UPHP) UGM, Sodium CMC 0.5%, NaCl 0,9%, Methanol, Isopropanol, glacial acetic acid, Dragendroff and Cerium (IV) sulfate.

2.2 Sample Preparation

The sample in the form of logs katola (Arcangelisia flava L.Merr.) Obtained from protected forests Laende Muna Southeast. Extraction was done by hot water 100 oC. The water-soluble extract was dried by using a freeze dryer.

2.3 Animal

Normal Wistar male rats 110-150 g body weight, the age of 5-7 weeks from the Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta. The Rats was acclimatized for seven days for adaptation to the conditions of the study, then Complete Freund's Adjuvant (CFA) rats induced by subplantar (0,1 ml) ((Sigma Chemical Co.., MO., USA). Thirty minutes after drug administration, rats induced with 0.1 ml of CFA 0.1%. Rats were divided into 6 groups each of 5 animals, and consist of a group of untreated, one group is Negative control: CFA-induced 0.1% without any medication, two groups were given diclofenac sodium 9 mg/kg body weight (Positive Controls: CFA-induced 0.1%), 3 group was extract dose of 114 mg/kg, a dose of 225 mg/kg, doses of 450 mg/kg body weight (Group E1, E2, and E3: CFA-induced 0.1%), 6 groups prepared Na. CMC 0.5% is administered orally.

2.4 Mode of feeding

The suspension extracts a dose of 114 mg/kg BW, 225 mg/kg body weight, and 450 mg/kg BW and Sodium diclofenac dose of 9 mg/kg BW administered once daily for 21 days as peroral with a maximum volume of 2 ml/200 g.

2.5 Immunohistochemistry assay

Joint torsocrural blocks prepared slide with a thickness of 4 μm slices. Slides made with xylene and hydrated deparafinasi with alcohol. The technique used is the indirect method; the slides were washed PBS, poured Trekkie Universal Link, tracks HRP, DAB, Cardassian chromogen antibody, and cyclooxygenase-2 (COX-2). Slide covered with a coverslip and observed using light microscopy, and then calculated the number of inhibition of cyclooxygenase-2 (COX-2) (Sofian and Kampono., 2006).

3. RESULTS AND DISCUSSION

Infusa katola was 35.165 g (the extractable was 19.536%). The infusa extract was brownish-yellow powder, bitter taste, odorless, and hygroscopic. The statistical analyzes of Inhibition of COX-2 expression performed by one-way ANOVA followed by post hoc testing with the LSD method. The analysis showed that the extract by the dose of 450 mg/kg body weight decreased expression of COX-2 inhibition was better. This was according to the content of the active constituent of Berberine chloride extract greater doses of 450 mg/kg. Berberine chloride was an alkaloid isoquinoline that could be found in the roots and stems of some of the plants in the family of Menispermaceae and have spare pharmacology activity as an anti-inflammatory (Singh et al., 2011). Inhibition of COX-2 expression to look at the molecular mechanisms of the inflammatory score was calculated based on the decreasing expression of COX-2 inhibition, as shown in fig.1 and fig. 2. The analysis for hot water extract katola (A.flava (L.) Merr.) inhibition values obtained were

not significantly different from the positive control (diclofenac sodium) 9 mg/kg BW, was the dose extract of 450 mg/kg BW at significant at the level of 95% ($p < 0.05$).

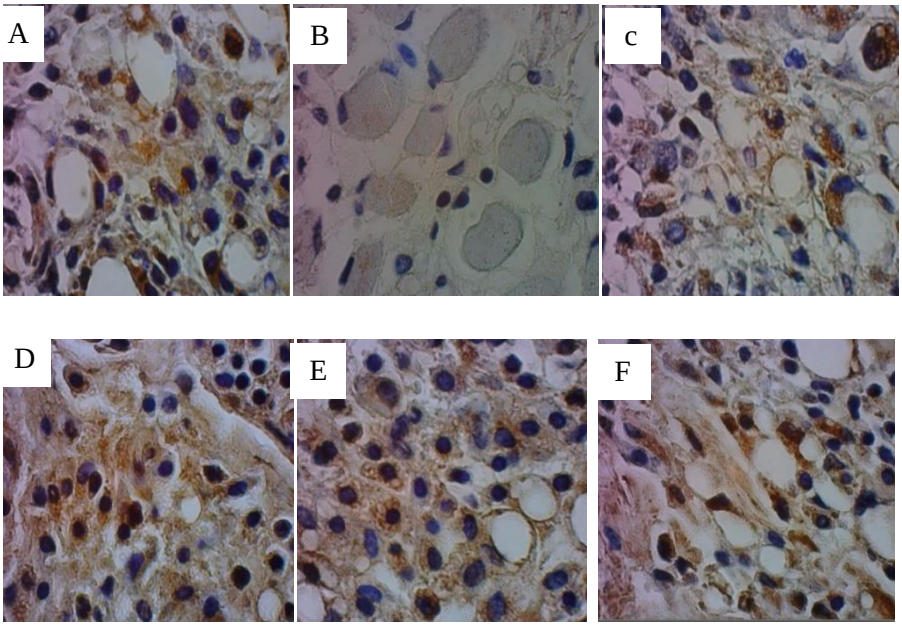

Figure 1. Immunohistochemistry torsocrural rats induced Complete Freund's Adjuvant ((CFA), A. Negative control. B. Normal control. C. Positive control (Diclofenac sodium 9 mg/kg BB). D. Extract doses 114 mg/kg BW E. Extract doses 225 mg/kg BW. F Extract doses of 450 mg/kg BW

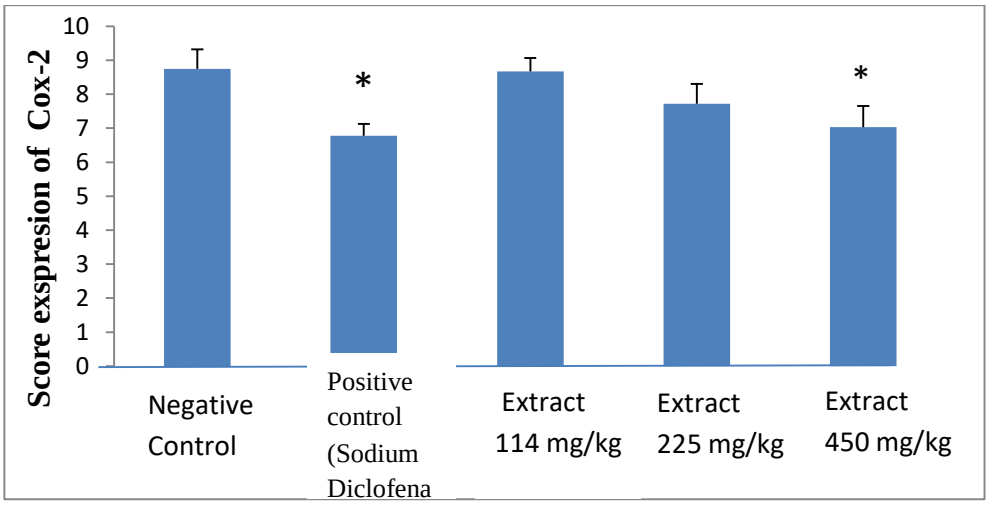

Figure 2. Chart bar Score Expression of COX-2 *p<0,05, values represent mean ± SD of 5 animals.

4. CONCLUSION

Infusa extract katola (Arcangelisia flava (L.) Merr.) showed anti-inflammatory activity through the decreased expression of COX-2 in Wistar male rats induced Complete Freund's Adjuvant (CFA).

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