
STUDY ON ANTIINFLAMMATORY EFFECT OF n-HEXANE AND ETHYL ACETATE EXTRACT OF AVOCADO SEED (*Persea americana* MILL.) ON WHITE MICE (*Mus musculus*)

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ABSTRACT

Background : Avocado leaves have been reported to have antitussive effects, antidiabetic and anti-inflammatory. Fraction of n-hexane and ethyl acetate avocado leaves shown to have anti-inflammatory effects at a dose of 100 mg / kg, 200 mg / kg and 400 mg / kg. In addition avocado leaves, avocado seed is also used traditionally to tackle anti-inflammatory and analgesic.

Objective: Study of antiinflammatory effect from extract of *n*-hexane and ethyl acetate seeds of *Persea americana* Mill on male white mice with paw edema methods.

Methods: Avocado seed that has been chopped and dried extracted by maceration stratified using a solvent n-hexane and ethyl acetate followed by maceration, in order to get the avocado seed extract thick. The subjects in this research were 40 mice of which divided into 8 groups; negative control (Na CMC), positive control (Na diclofenac dose 6.5 mg/kgBW) and extract group of *n*-hexane with doses 100, 200 and 400 mg/kgBW; ethyl acetate with the doses 100, 200 and 400 mg/kgBW. Na CMC, Na diclofenac and extract supplementation were each one done per oral. Thirty minutes after administration of the control solution or test solution, left foot all mice were injected by intra-plantar with 1% carrageenan suspension as much as 0.05 mL. Edema volume measurement was performed with interval one hour for 5 hours assessing with pletysmometer. Then be calculated parameter of the inhibition percent of edema volume.

Outcome measured: inhibition percent of edema volume.

Results : The result of this study showed there was significant difference of inhibition percent from extract of the dose 100, 200 and 400 mg/kgBW with negative control ($p < 0,05$) but wasn't significant difference of inhibition percent of positive control ($p > 0,05$).

Conclusion: The seeds of *Persea americana* Mill were active as anti-inflammatory agents the dose 100, 200 and 400 mg/kgBW.

Keywords: *Persea americana* Mill, anti-inflammatory, avocado seed, carrageenan

INTRODUCTION

Avocado leaves have been reported have antitussive effects, antidiabetic and anti-inflammatory (Adeyemi *et al*, 2002), vasorelaksation in rat aorta (Owolabi *et al*, 2005), may decrease blood pressure and heart rate (Yuslinda, 2007) and antidiabetic activity (Antia *et al*, 2005). The study on antimicrobial effect of ethanol extract of its seeds on the growth of *Staphylococcus aureus* in vitro data obtained Minimum Inhibitory Concentration (MIC) at 0.5% (Rahma *et al*, 2012). In-vitro study avocado seed inhibit α -



glucosidase enzyme activity (Nasution *et al.*, 2014). In effect hipolipemic research, avocado seed extract has decrease blood cholesterol levels in rat (Asaolu *et al.*, 2010). The content of chemical coumpound in the avocado seed that reported containing alcaloids, tannins, flavonoids, terpenoids and saponins (Marlinda *et al.*, 2012). Beside avocado leaves, avocado seed is also used traditionally to treat toothache, chronic gastritis, hypertension and diabetes.

METHODS

Materials

Adult white mice strain DYY 2-3 months old, weighing between 20-30 grams were used for the study. The materials used: *Persea americana* Mill seeds, NaCMC, natrium diclofenac, carrageenan, n-hexane and ethyl acetate.

Preparation of extracts

Avocado seeds were collected from Lintau, West Sumatra Province, Indonesia. One kg of dried samples were extracted by stratified maceration method using n-hexane and ethyl acetate, successively. The sample was soaked for 5 days at place protected from light while stirring repeatedly. The macerate was filtered and residue of sample was re-macerated in the same way until macerate produced translucent color. Furthermore the filtrate was concentrated using rotary evaporator. The yield of extract of n-hexane and ethyl acetate were 5.11 and 4.93 g, respectively based on dry weight. Then n-hexane and ethyl acetate extracts obtained were then subjected to anti inflammatory test.

Anti inflammatory activity

Anti inflammatory effect of n-hexane and ethyl acetate extracts of seeds of *Persea americana* Mill has tested on male white mice using paw edema method. The subjects in this research were 40 mice which divided into 8 groups, including negative control (Na CMC), positive control (Na diclofenac dose 6.5 mg/kgBW), n-hexane extract groups with doses of 100, 200 and 400 mg/kgBW and ethyl acetate extract groups with doses of 100, 200 and 400 mg/kgBW. Na CMC, Na diclofenac and extracts were administered per orally. Thirty minutes after administration of control or test solution, left foot all mice were injected intra-plantar with 1% carrageenan suspension as much as 0.05 mL. Measurement of edema volume was performed with one hour interval for 5 hours assessing using Pletysmometer (Ugo Basile). The percent of inhibition was then calculated from data of edema volume.

Data analysis

The percent of inhibition from data of edema volume were analyzed using Two Way Analisis Of Varian SPSS program, followed by Tukey HSD Pos Hoc Test. The data were compared with control data and mean difference is significant at $p < 0.05$ level.

RESULTS AND DISCUSSION

The effect of n-hexane and ethyl acetate extracts on carrageenan (1%, w/v)-induced inflammation was investigated following the method of Winter *et al.*, (1962) with minor modifications using mice as experimental animal. The course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar *et al.*, 1969). The first phase occurs within an hour of carrageenan

injection and is partly due to the trauma of injection and also due to release of histamine and serotonin (Crunkhon and Meacock, 1971). Prostaglandins (PGs) play a major role in the development of the second phase of reaction that is measured around 3-hour time (Crunkhon and Meacock, 1971; Vinegar *et al.*, 1969). The presence of PGs in the inflammatory exudates from the injected foot can be demonstrated at 3-hour and period thereafter (Vinegar *et al.*, 1969). Results of testing performed on the anti-inflammatory activity of white mice to extract n-hexane with Tukey test to variable doses, it is known that the group of n-hexane extract dose of 100, 200 and 400 mg/kgBW had a percent of inhibition of data that differ significantly from the negative control group (Na CMC) and did not differ significantly with the positive control group (diclofenac sodium) at $p < 0.05$ (Figure 1). Diclofenac sodium as a positive control has strong anti-inflammatory power. Diclofenac sodium is a phenylacetic acid derivative. Its mechanism works by inhibiting the enzyme cyclooxygenase nonselective so inhibited prostaglandin formation (Katzung, 2002). This indicates that n-hexane extract of avocado seeds afforded anti-inflammatory activity. Based on screening of chemical constituents, it was shown that n-hexane extract contains secondary metabolites class of steroid compounds. The ability to inhibit edema suspected generated from steroid content of the extract. Further tests of statistical analysis with Tukey test between dose groups, found that among treatment groups given n-hexane extract doses of 100, 200 and 400 mg/kgBW did not differ significantly. This means that increasing in dose of n-hexane extract gave no significant increase in percent of inhibition.

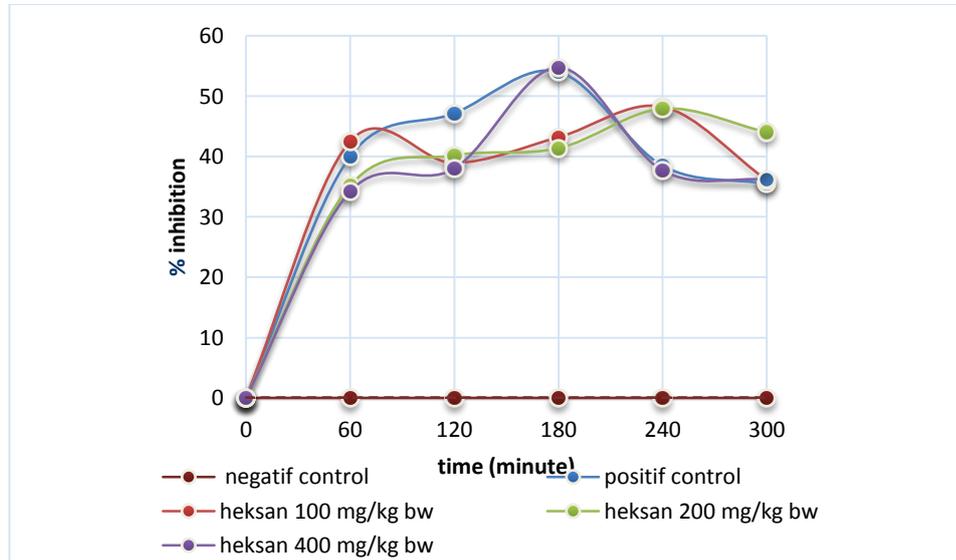


Figure 1. The percent of inhibition of administration of n-hexane extract of *Persea americana* Mill seeds with various doses on carragenan-induced white mice.

Results of testing performed on the anti inflammatory activity of white mice to ethyl acetate extract with Tukey test to variable doses, it was known that group of ethyl acetate



extract doses of 100, 200 and 400 mg/kgBW had percent of inhibition of data that differ significantly from negative control group (Na CMC) and did not differ significantly with positive control group (diclofenac sodium) at $P < 0.05$ (Figure 2). This indicates that ethyl acetate extract of avocado seeds have anti-inflammatory activity. Flavonoids, phenolics as well as terpenoids were indicated in its ethyl acetate extract. The ability of edema inhibition suspected that flavonoid content in the extract. Further tests of statistical analysis with Tukey test between dose groups, found that between the treatment groups given ethyl acetate extract doses of 100, 200 and 400 mg/kgBW did not differ significantly. This means that with increased dose of ethyl acetate extract exhibited no increase in percent inhibition.

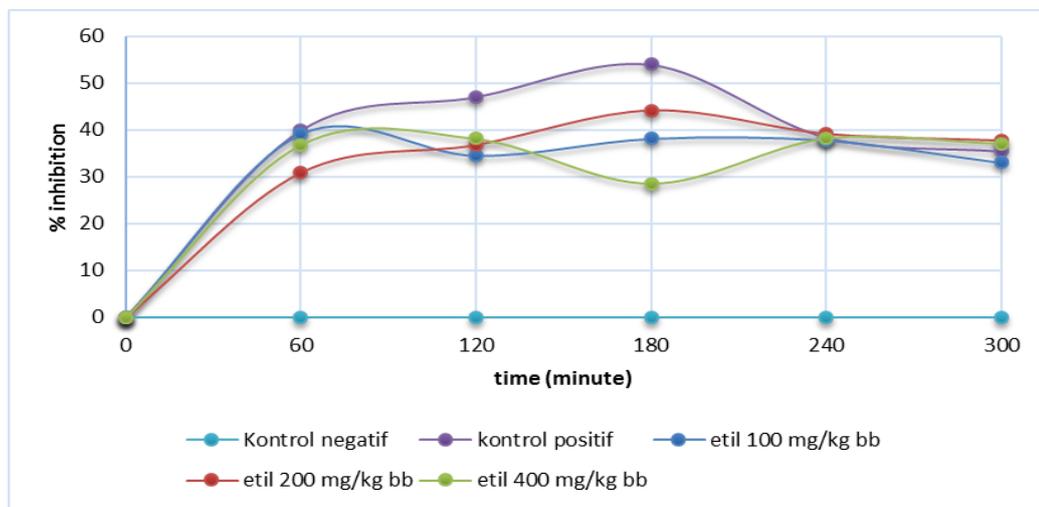


Figure 2. The percent of inhibition of administration of ethyl acetate extract of *Persea americana* Mill seed with various doses on carrageenan-induced white mice.

CONCLUSION

The n-hexane and ethyl acetate extracts of seeds of *Persea americana* Mill were active as anti-inflammatory agents with doses of 100, 200 and 400 mg/kgBW ($p < 0.05$) on white mice (*Mus musculus*)

DISCLOSURE

There is no conflict of interest between the writers.

ACKNOWLEDGEMENT :-

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