
Anti-inflammatory effects of avocado peels against inflammation induced by carrageenan in mice

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ABSTRACT

The aim of this research is to investigate the anti-inflammatory activity of avocado peel against carrageenan-induced inflammation in mice. The group of mice that was used as a negative control group to test the anti-inflammatory activity of infusion and decoction (group I) were given aquadest, while the group for testing the activity of extract (group II) was given CMC-Na. The positive control group (group III) was given potassium diclofenac. Groups IV-VI were given avocado peel infusion with the following doses 667.5; 1335; and 2670 mg/kgBW respectively. Groups VII-IX were given avocado peel decoction with the same doses as the previous groups. Groups X-XII were given 830; 1670; and 3330 mg/kgBW of avocado peel extract respectively. The paw edema were measured using a digital caliper for 6 hours afterwards after carrageenan injection. There were a significant ($p < 0.05$) reduction in paw edema at all doses of infusion, decoction, and extract of avocado peel. Based on the research, it can be concluded that the avocado peels have anti-inflammatory activities.

Keywords: avocado, peel, anti-inflammatory, carrageenan

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INTRODUCTION

Inflammation is the body's response to eliminate either foreign substances, pathogens, or irritants and manage tissue repairment (Karch, 2003). In inflammation, there is a process where free radicals are produced (Ardhie, 2011). Free radicals can cause tissue damage which triggers the biosynthesis of arachidonic acid, which were converted to prostaglandin as a mediator of inflammation. Common signs of inflammation are swelling, pain, redness, heat, and loss of function. These signs often cause discomfort for people so they need to be treated quickly (Supriyatna *et al.*, 2015).

The bioactive contents of avocado showed higher antioxidant activities towards DPPH method (Malangngi *et al.*, 2012). Antioxidants binds free radicals and inhibit the cyclooxygenase enzyme as the mechanism of inflammatory inhibition to prevent the production of prostaglandin. This will impact in the inhibition of inflammatory mediators. According to this thing, another research shows the existence of analgesic activity from avocado seed (Kyakulaga *et al.*, 2012; Josephine and Ngozi, 2013). However, the research of anti-inflammation activity of avocado peel is never reported, although avocado peels have higher antioxidant activities and more bioactive contents such as phenolic compounds compared to avocado seeds. Based on those things, the research objective is to find out the anti-inflammation activity in infusion, decoction and extract of avocado peels on mice.

MATERIAL AND METHODS

Plant material and chemicals

Avocado peels were collected from Yogyakarta which were determined by the Department of Biology at the Faculty of Pharmacy of Gadjah Mada University, Yogyakarta. Aquadest, NaCl 0.9% solution, CMC-Na, Potassium diclofenac (Cataflam Fast®) and Carrageenan (Sigma Chemical Company) were obtained from Pharmacology and Toxicology Laboratory of the Faculty of Pharmacy of Sanata Dharma University.

Animal

Adult male Swiss mice weighing 20-30 g were used for this experiment. All of mice were obtained from Imono Laboratory of the Faculty of Pharmacy of Sanata Dharma University.

Preparation of Avocado Peel Extracts

Each avocado peel infusion and avocado peel decoction were obtained by measuring dry powder of avocado peel \pm 8 g, then putting it in the infusion pan and mixing with 16 mL of aquadest. The next step, the infusion pan was heated with a hotplate with temperature of 90 °C within 15 minutes for the avocado peel infusion and 30 minutes for the avocado peel decoction. Each mixture was filtered using flannel cloth to obtain infusion and decoction of avocado peel respectively.

The dry powder of avocado peel (50 g) was extracted with methanol (90% v/v) for 24 hours at room temperature. The mixture was filtered using Buchner funnel, then concentrated by using rotary evaporator to yield a semi solid extract.

Study Design

This research has been approved by the Medical and Health Research Ethics Committee (MHREC) of Faculty of Medicine of Gadjah Mada University with approval number ref: KE/FK/0873/EC/2017. A total of 60 mice were divided randomly into 12 groups. Acute edema was induced by injection of carrageenan 1% into the sub-plantar region of hind-paw of mice (Chamundeeswari *et al.*, 2004; Tjandrawinata *et al.*, 2015). The mice of group I, as a negative control group for avocado peel infusion and decoction, were given aquadest. Group II, as negative control group for avocado peel extract, were given CMC-Na, and group III, as positive control, were given Cataflam Fast® (Pottasium Diclofenac) 4.48 mg /kgBW. Groups IV – VI were given avocado peel infusion with 3 levels of doses, 667.5; 1335; and 2670 mg/kgBW (Kristanti *et al.*, 2017). Groups VII – IX were given avocado peel decoction with 3 levels of doses which were 667.5; 1335; and 2670

mg/kgBW. Groups X – XII were given avocado peel extract with 3 levels of doses which were 830; 1670; and 3330 mg/kgBW (Kristanti *et al.*, 2017).

Edema thickness in mice's paw was measured using digital caliper at 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and 360 minutes after carrageenan injection (Tjandrawinata *et al.*, 2015; Hendra *et al.*, 2017; Kristanti *et al.*, 2017) using the equation as followed:

$$Tu = Tt - To \quad (1)$$

Tu: Thickness of mice foot edema in particular time

Tt: Thickness of mice foot after 1% carrageenan induction

To: Thickness of mice foot before 1% carrageenan induction

The calculation of the edema volume was conducted using formula area under curve (AUC) and percentage of inhibition of inflammation (Boaky-Gyasi *et al.*, 2008; Nguemfo *et al.*, 2007; Chaulya *et al.*, 2012). Area Under Curve (AUC) was calculated for each minute within 0-6 hours using trapezoid method formula. These are detailed as below:

$$AUC_{t_{n-1}} = \frac{T_{t_{n-1}} - T_{t_n}}{(t_n - t_{n-1})} \quad (2)$$

$T_{t_{n-1}}$: Average edema volume on t_{n-1}

T_{t_n} : Average edema volume on t_n

$$\text{Inhibition of inflammation (\%)} = \frac{(AUC_{0-x})_0 - (AUC_{0-x})_n}{(AUC_{0-x})_0} \times 100\%$$

$(AUC_{0-x})_0$ = average AUC_{0-x} for negative control group,

$(AUC_{0-x})_n$ = AUC_{0-x} for each animal given test drug at dose of n

Statistical Analysis

Total AUC value are expressed as mean \pm standard deviation (SD). Data were analyzed using one-way analysis of variance followed by *post-hoc* tests using SPSS 22. A *p*-value <0.05 was considered statistically significant. Statistical differences were determined using the Student's *t*-test, with *p*-values being indicated for each figure.

RESULTS AND DISCUSSION

The present study determines the anti-inflammatory activity of avocado peel against carrageenan-induced inflammation in mice. Carrageenan-induced paw edema has been commonly used for acute inflammation animal model. Inflammation induced by carrageenan was observed to have two phases, early phase (up to 2 h) and late phase (1-6h) as shown at graph of negative control (Figure 1-3). The avocado peel showed a reduction in paw edema at all time interval.

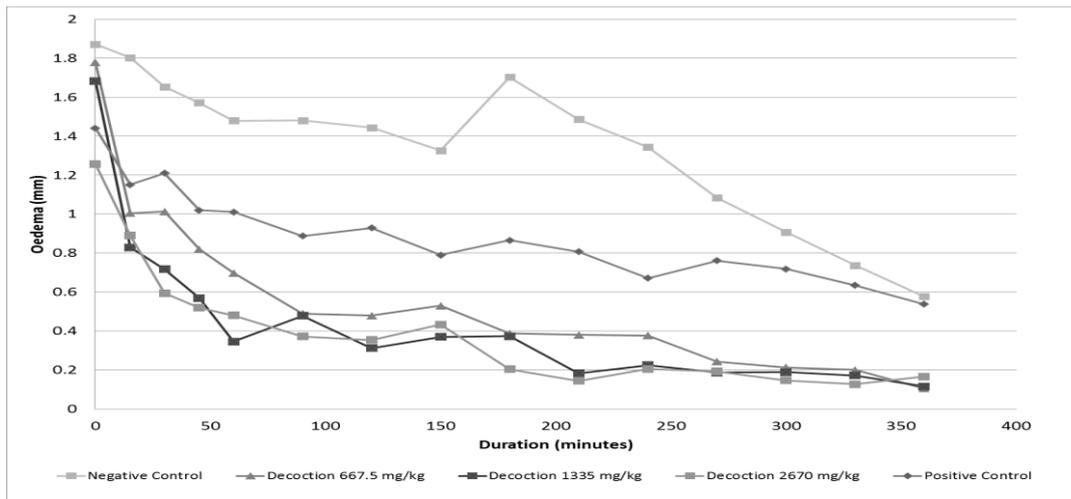


Figure 1. Graph of duration vs oedema thickness of avocado peel decoction

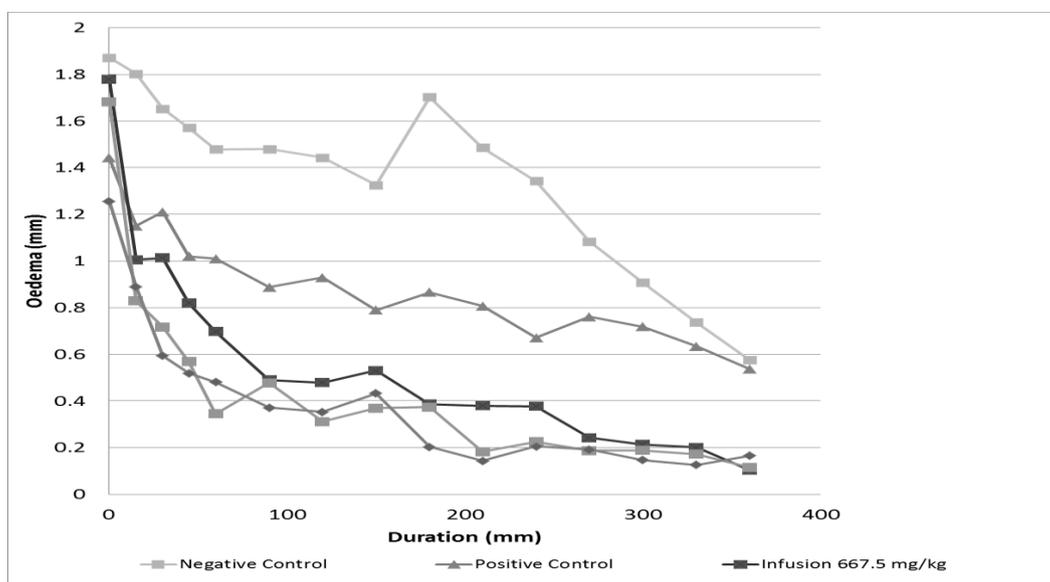


Figure 2. Graph of duration vs oedema thickness of avocado peel infusion

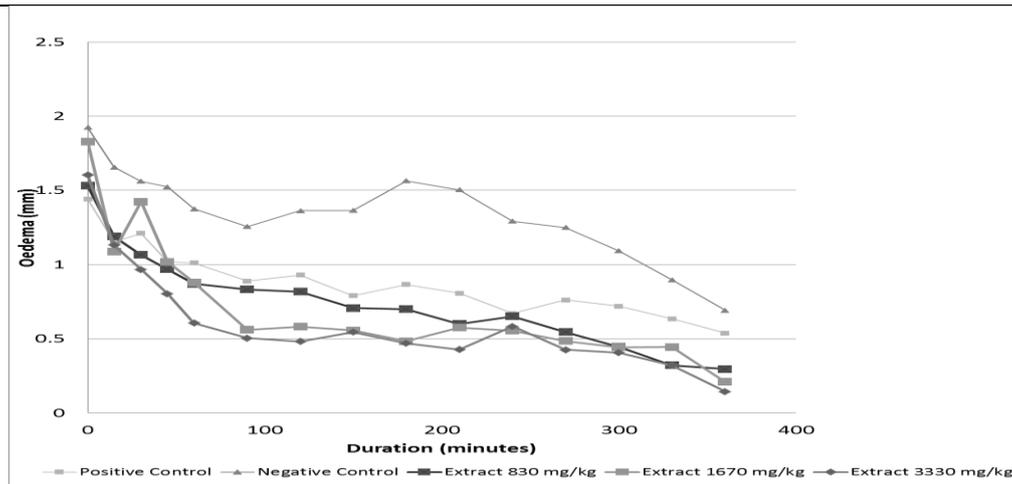


Figure 3. Graph of duration vs oedema thickness of avocado peel extract

In carrageenan-induced paw edema model, it was found that both avocado peel infusion and avocado peel decoction exhibited a significant inhibition ($p < 0.05$) compared negative control aquadest on paw edema formation. Percentage inhibition were 65.6, 72.0, and 75.2% at the doses 667.5; 1335; and 2670 mg/kgBW of avocado peel infusion, while positive control potassium diclofenac was 47.7% (Table I). Avocado peel decoction also reduced paw edema compared negative control aquadest by 36.8, 39.9, 45.5% at the dose 667.5; 1335; and 2670 mg/kgBW respectively. There were a reduction in paw edema significantly ($p < 0.05$) at all doses of the extract in comparison to negative control CMC-Na by 49.3, 50.5, and 58.6% at the doses 833; 1670; and 3330 mg/kgBW respectively. All of the effects were non-dose dependent manner.

The results of this study showed that all doses levels of infusion, decoction and extract of avocado peels had a significant anti-inflammatory activity. The shorter extraction time by infusion (15min) showed the best activity compared decoction (30 min). This evidence strongly suggests that different types of extraction method and solvent had great influence on the anti-inflammatory property of avocado peels. However, it is not yet known exactly which class of compounds contained in avocado peels that are responsible for anti-inflammatory activity.

Table I. Effect of avocado peels on carrageenan-induced mice

Group	AUC (mm.minute)	% Inhibition
Negative control (aquadest)	476.59 ± 7.25 ^b	0 ± 1.5
Negative control (CMC-Na)	474.34 ± 7.99 ^b	0 ± 1.7
Positive control (potassium diclofenac 4,48 mg/kg BW)	249.11 ± 7.80 ^{a,c}	47.7 ± 1.6
Avocado Peel Infusion 667.5 mg/kg BW	163.76 ± 12.21 ^{a,b}	65.6 ± 2.6
Avocado Peel Infusion 1335 mg/kg BW	133.42 ± 7.56 ^{a,b}	72.0 ± 1.6
Avocado Peel Infusion 2670 mg/kg BW	118.25 ± 8.37 ^{a,b}	75.2 ± 1.8
Avocado Peel Decoction 667.5 mg/kg BW	301.24 ± 5.97 ^{a,b}	36.8 ± 1.3
Avocado Peel Decoction 1335 mg/kg BW	286.69 ± 7.10 ^{a,b}	39.9 ± 1.5
Avocado Peel Decoction 2670 mg/kg BW	259.96 ± 2.29 ^a	45.5 ± 0.5
Avocado Peel Extract 833 mg/kg BW	240.34 ± 6.04 ^c	49.3 ± 1.3
Avocado Peel Extract 1670 mg/kg BW	234.72 ± 9.83 ^c	50.5 ± 2.1
Avocado Peel Extract 3330 mg/kg BW	196.32 ± 17.98 ^{b,c}	58.6 ± 3.8

Values are expressed as mean ± SD of five animals in each group; a: $p < 0.05$ vs aquadest negative control; b: $p < 0.05$ vs positive control; c: $p < 0.05$ vs CMC-Na negative control

The results obtained in the rat paw edema show that the active principles present in the avocado peel may act analogously in the inflammatory response produced by carrageenan. These results are similar with a previous report in which the same doses of avocado seed infusion and extract had anti-inflammatory activities (Kristanti *et al.*, 2017). Avocado leaves also possess anti-inflammatory activity (Adeyemi *et al.*, 2002).

The phytochemical screening of avocado peel showed the presence of flavonoid (Vinha *et al.*, 2013) and these antioxidants might contribute as anti-inflammatory that is able to capture free radicals that cause the emergence of inflammatory responses (Rodriguez-Carpena *et al.*, 2011; Kosinska *et al.*, 2012; Arukwe *et al.*, 2012). The flavonoid mechanism of stabilizing reactive oxygen species (ROS) is reacting with the reactive compound of the radicals so that the radicals become inactive (Pourmourad *et al.*, 2006). Flavonoids have been determined to produce anti-inflammation effect by suppressing the lipoxygenase and/or cyclo-oxygenase activities. The above results on anti-inflammatory of avocado peels corresponds to these findings. However, the specific of flavonoid of avocado peel has not been determined.

CONCLUSION

Avocado peel can represent a useful source of anti-inflammatory activity in dose dependent manner. Further studies involving the isolation of anti-inflammatory compounds is needed.

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