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RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF CATECHINS ISOLATE OF *UNCARIA GAMBIR ROXB* ON CARRAGEENAN INDUCED PAW EDEMA IN WISTAR MALE RATS

*Muhammad Yanis Musdja, Nelly Suryani and Pipit Pitriyah

Department of Pharmacy, Faculty of Health Sciences, State Islamic University, Jakarta, Indonesia

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ABSTRACT

A mixture *Uncariagambir Roxb* (gambir) is often used by some communities in Indonesia and other countries in Southeast Asia and South Asia to treat inflammation and other treatments. Catechins as the main content of the gambir are allegedly powerful that have anti-inflammatory properties. The objective of this study was to determine the anti-inflammatory activity of catechins isolate of gambir on carrageenan induced paw edema in wistar male rats. Catechins of gambir were isolated by using partition method with ethyl acetate solvent. A total of 30 wistar male rats were divided into 5 groups. Tests of anti-inflammatory effects was done based on Winter's modified paw edema method. Rats were made to suffer inflammation by the injected with carrageenan 0.1 ml of 1 % (b/v). The effect of paw edema in male rats was done 1 hour after administration of test preparation for 6 hours by using plethysmometer. A total of 3 groups were given catechins isolate per oral with a dose of 1 mg/kg.b.w, 10 mg/kg b.w and 100 mg/kg b.w respectively. Test preparations were suspended in Na-CMC 0,5 %. As positive control was used Na-diclofenac 5,14 mg/kg b.w and as negative control was used Na-CMC 0,5 %. Based on the results of statistical tests was obtained a dose of 100 mg / kg b.w gives the greatest anti-inflammatory effect, ie 59.19%. while for doses of 1 mg / kg, b.w and 10 mg / kg b.w also significantly different compared to negative controls ($p \leq 0.05$). The dose of 10 mg / kg b.w have same effect with dose 1% Na-diclofenac For anti-inflammatory.

INTRODUCTION

In traditional medicine, *Uncariagambir Roxb* (gambir) is often used to cure inflammation, disorders mouth, diarrhea, gastric disease, burns, acne, as antioxidants, cancer and gambir also is used to as one of the ingredients for betel quid chewing (Hamda, 2014; Lee *et al.*, 2016; Hanny, 2017; Pa *et al.*, 2017) Catechins are polyphenolic phytochemicals compound as main content of gambir. Other plants with their main content of catechins are *Camellia sinensis* (tea) and *Acacia catechu*. Gambir is a more potential plant for the source of catechins than green tea (*Camellia sinensis*), black tea and *Acacia catechu*, because gambir contains higher levels of catechins than these plants. Based on research of Amos (2010), total catechins content of the extract Gambir in Indonesia 40-80%. (Amos, 2010). Research of Hilal and Engelhardt's (2007) of grefrom the German market were from green tea 8.5 - 20.6%, black tea 0.74 - 10% (Hilal *et al.*, 2007) and content of catechins of *Acacia catechu* 9-12%. (Rangari, 2007). The content of catechins between gambir and green tea is different. Based on research of Taniguchi *et al.* (2008) was obtained 9 types of catechins in gambir namely (+)-catechin, (-)-epicatechin Gambiriin, A1, Gambiriin A2, Gambiriin B1, Gambiriin B2, Catechin-(4 α -8)-ent-epicatechin, Gambirflavan D1 and Gambirflavan D2. Main content of gambir is (+)-catechin (Biasi *et al.*, 2011.). The content of catechins of green tea are -epicatechin (EC) 35%, -epigallocatechin (EGC) 15%, -epicatechin-gallate (ECG) 4%, and -epigallocatechin-3-gallate

(EGCG) 38-40%. (Taniguchi *et al.*, 2008; Atomssa *et al.*, 2008; Fei *et al.*, 2017.)

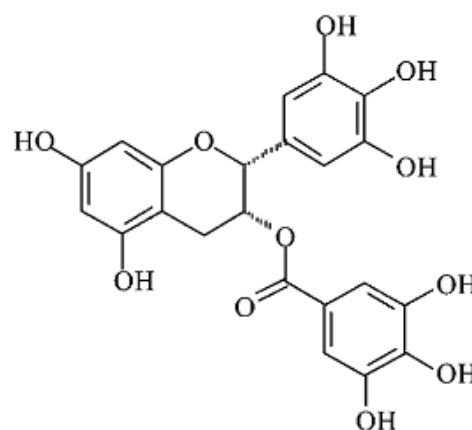


Figure 1. Epigallocatechin-3-gallate (EGCG) (Main content of green tea)

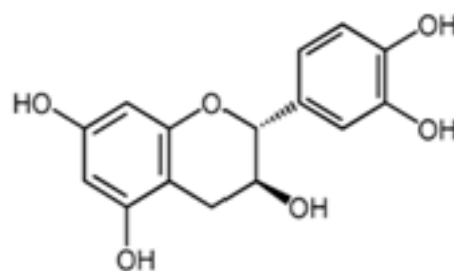


Figure 2. (+)-Catechin, (Main content of gambir)

*Corresponding author: Muhammad YanisMurdja,
Department of Pharmacy, State Islamic University, Jakarta, Indonesia.

Green tea with its main content EGCC, according to in-vivo studies have shown efficacious as anti-inflammatory, (Fei-Yan *et al.*, 2017; Biasi *et al.*, 2011; Vasconcelos *et al.*, 2012), but gambir with its main content (+)-catechin has not been investigated as an in-vivo anti-inflammatory. Carrageen induced inflammation has been used as the most widely used in vivo model for assessing anti-inflammatory agents. The inflammation formed can be measured thoroughly using a plethysmometer (Fei-Yan *et al.*, 2017).

MATERIALS AND METHODS

Gambir was obtained from Limapuluhkota districts, Indonesia, These districts are the largest gambir producer in the world, then plant authentication was conducted at the Biology research center, Indonesian Institute of Sciences, Bogor, Indonesia. Screening of chemical compound group of gambir extract was done based on Harbone (1998) methods. Catechins isolation was done based on National Standard Indonesia: SNI 01-3391-2000, "A total of 250 g of gambir powder was extracted with a water solvent at temperature of 90 - 96 °C for 15 minutes while stirring. Then the infusion was filtered in hot conditions using a funnel coated with filter paper. The extract obtained was partitioned with ethyl acetate, the ratio of extract with ethyl acetate (1 : ½). Ethyl acetate phase was taken and the water phase was partitioned repeatedly with ethyl acetate until a clear solution was obtained. The ethyl acetate phase was condensed with an evaporator, then washed with cold water and filtered. Catechins that obtained were dried in an oven at temperature 70 °C". The yield of crude catechins obtained from the gambir isolate was determined as (+)-catechin (Biomarker) by comparing with standard (+)-catechin and measured by a spectrophotometer UV-VIS at wavelength 279 nm. Quality of gambir was determined based on National Standard Indonesia: SNI 01-3391-2000.

The anti-inflammatory effect test was investigated based on a modification methods of Winter *et al.* (1962). "Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay. The rats were acclimated for 15 days, The rats qualified for the experiment were divided into 5 groups. Each group consists of 6 rats, before the experiment, rats were fasted for 12 hours. A total of 3 groups were given catechins isolate of gambir with different doses, administered intraperitoneally, ie: 1 mg/kg bw, 10 mg/kg b.w and 100 mg / kg b.w. As positive control was used Na-diclofenac 5,14 mg/kg b.w. To make pharmaceutical preparations of catechins suspension extract was used 0.5% Na-CMC (neutral substance) as an emulsifying solution. therefore for negative control group, only 0.5% Na-CMC/kg b.w was given. After 1 hour, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The anti-inflammatory effect was measured by comparing the volume of rat paw between before and after administering test preparation. Measurement of inflammatory volume was carried out by inserting the rat paw into the plethysmometer until the volume limit mark. It were measured every 1 hour interval for 6 hours (Winter *et al.*, 1962)

RESULTS AND DISCUSSION

The results of the authentication of plants was carried out by the Center for Biological Research of Indonesian Institute of

Sciences, Bogor, Indonesia. Plant used for this research was gambir (*Uncaria gambir Roxb*). Screening of chemical compound group of gambir extract based on Harbone methods was founded, that gambir extract containing flavonoids, phenolic and saponin. The Catechins in this study, there were about 9 types of catechins, this is based on research of Taniguchi *et al.*, (2008) was obtained 9 types of catechins in gambir namely (+)-catechin, (-) – epicatechin Gambiriin A1, Gambiriin A2, Gambiriin B1, Gambiriin B2, Catechin-(4 α -8)-ent-epicatechin, Gambirflavan D1 and Gambirflavan D2. Main content of gambir is (+)-catechin (Taniguchi *et al.*, 2008) However (+)-Catechin is the highest level among 9 types of catechins. which is about 45.66% of the total catechins (Musdja). Therefore (+)-catechin can be used as a biomaker to determine the quality of gambir. Therefore in this study only (+)-catechin (biomarkers) was measured by a spectrophotometer UV-VIS at wavelength 279 nm. Then based on our study before, about this study (Musdja, *et al.*, 2017), where we was done isolation catechin (Figure 2) from gambir with methods : "Isolation of (+)-catechin was done by using a column chromatography, silica gel was used as stationary phase and for mobile phase was used as the mixture of chloroform and methanol (4 : 1). Gambir dry extract was dissolved in eluent. Droplets of liquid that come out of the column are collected and add eluent until was obtained liquid 30 mL for each fraction. The (+)-catechin analysis for each fraction was carried out using Thin Layer Chromatography. Chloroform and methanol with a ratio (3: 1) are used as a mobile phase. To find spots used a solution of 10% vanillin in H2SO4 concentration. Then, the spots formed are compared to (+)-catechin standards". With this methods, we were obtained the yield of (+)-catechin about about 45.66% of the total catechins. (Musdja *et al.*, 2017). Gambir quality requirements based on the National Standards of Indonesia: (Standar Nasional Indonesia: SNI 01-3391-2000) were compared with quality of gambir for this experiment, as shown in Table 1.

The quality of the gambir that was used in this experiment, when compared to the requirements written on the National Standards of Indonesia: (Standard National of Indonesian: SNI 01-3391-2000) was a type of gambir with the quality of number 1 or excellent. This was indicated by Physical condition, Water content w/w, Ash content w/w, Catechin concentration w/w of dry weight and Insoluble content in water and alcohol are eligible, as shown in Table 1. The results of anti-inflammatory activity of catechin isolate of gambir was done by comparing the percentage of rat paw edema and the percentage of inhibition of rat paw edema in each group. As shown in Table 2 and Figure 3. In the negative control group, the percentage of edema was much greater than the low dose group (1 mg / kg b.w), medium dose (10 mg / kg b.w), high dose (100 mg / kg b.w) and positive control. On the test preparation dose group 10 mg / kg b.w, the percentage of edema was smaller in T1 (1st hour) to T3 (3r hour), compared with the dose group 100 mg / kg b.w, but the percentage of edema in T4 (4th hour) to T6 (6th hour) was greater than the dose group 100 mg / kg b.w. Effect of Carrageenan Induced Paw Edema in Wistar Male Rats, as shown on Table 2 and Figure 3. At a dose group of 1 mg / kg b.w the percentage of edema is greater than the group of 10 mg / kg b.w, 100 mg / kg b.w and positive control. While the positive control group percentage of edema smaller than the dose test group 1 mg, 10 mg / kg b.w and dose 100 mg / kg b.w, these were due to the effect of the test preparation. This showed that edema caused by carrageenan induction in rat's paw was reduced compared

Table 1. Comparison of gambir quality that was used for experiment with gambir quality requirements written on the National Standards of Indonesia

Type of test	Quality number 1	Quality number 2	Gambir for experiment
a. Physical condition	Intact	Intact	Intact
• shape	Yellow to brownish yellow	Yellow brown to yellow-black	Yellow to brownish yellow
• color smell	Specific	Specific	Specific
b. Water content w/w	≤ 14%	≤ 16%	0.9%
c. Ash content w/w	≤ 5%	≤ 5%	2.5%
d. Catechin concentration w/w of dry weight	≥ 60%	≥ 50%	88.65%
e. Insoluble material content of :			
Water w/w of dry weight	≤ 7%	≤ 10%	3.7%
Alcohol w/w of dry weight	≤ 12%	≤ 15%	7.5%

(Standar Nasional Indonesia : SNI 01-3391-2000)

Table 2. Average percentage of rat paw edema at various time intervals, after were induced with carrageenan (n = 6)

Hour/T (Time)	% Average value of edema in rat paw				
	NC	PC	ICG1	ICG10	ICG100
0	0	0	0	0	0
1	42.66	38.5	31.30	27.87	32.81
2	56.17	42.44	51	57.81	74.57
3	69.09	44.13	63.06	59.65	62.83
4	70.14	37.76	80.98	61.08	50.36
5	57.38	39.18	6.51	48.96	47.90
6	67.54	39.23	60.84	41.54	28.18

Note: NC= Negative Control (Na CMC); PC = Positive Control (Na diclofenac); ICG1= Isolat Catechins of gambir 1mg; ICG 10= Isolat Catechims of gambir 10 mg

Table 3. Average percentage value of inhibition edema in rat paw

Hours	% average value of inhibition edema				
	NC	PC	ICG1	ICG10	ICG100
0	0	0	0	0	0
1	0	23.47	36.80	28.87	25.78
2	0	25.05	14.26	17,32	2.85
3	0	34.04	14.46	17,35	12.86
4	0	49.9	12.24	14.99	24.18
5	0	35.51	5.96	28.63	26.53
6	0	33.33	13.98	33.57	59.19

Note: NC = Negative Control (Na-CMC); PC = Positifve Control (Na-diclofenac); ICG1 = IsolatcatechihnsGambir 1 mg; ICG10 = IsolatcatechihnsGambir 10 mg; ICG100 = IsolatcatechihnsGambir 100mg

with negative controls that was not given medications or extracts. Percent of edema in all test groups gradually decreased while in the negative control group percent edema persisted until 6 hours, ie about 67.54%. Time can affect the process of healing on edema, which can be seen in the presence of a maximum percentage that slowly decreases at a certain time even if no drugs or extracts are given. The results of this study showed that all doses of the test preparation group have anti-inflammatory effect, namely: the mean percent edema value of each test preparation group was smaller than negative control group. The percent value of edema continues to increase from the first hour until 4 hour and then decreases at 5th and 6th hours. In Figure 4 showed that negative control have no effect in inhibiting edema due to carrageenan induction, The average percentage of inhibition of rat paw edema after induced with carrageenan, as shown in Table 3. In Table 2 showed that the largest percentage of edema occurred in the negative control group that was equal to 67.54% at 6th hours. While the dose group 1 mg/kg b.w, 10 mg/kg b.w, 100 mg/kg b.w and positive control had percent edema were 60.84%, 41.54%, 28.18% and 39.23% respectively. These were due to the effects of catechins and positive controls. Group dose of 100 mg/kg b.w has a high potential for anti-inflammatory, i.e. 59.19%. While the dose group of 1 mg/kg b.w and dose of 10 mg/kg b.w had a smaller anti-inflammatory effect, ie: 13.98% and 33.57%, respectively.

Group dose of 100 mg/kg b.w has a high potential for anti-inflammatory, i.e. 59.19%. While the dose group of 1 mg/kg b.w and dose of 10 mg/kg b.w had a smaller anti-inflammatory effect, ie: 13.98% and 33.57%, respectively. However, the best optimal dose in inhibiting inflammation in rat paw edema was the dose of catechins isolate 10 mg/kg b.w because its inhibitory power was same with the positive control (Na diclofenac) with dose 5,14 mg/kg b.w. The results of statistical tests with ANOVA and Kolmogorov Smirnov test to see the differences of each group and followed by homogeneity test using Levene method. Statistical data indicate that all test groups are distributed homogeneously. Then proceed with the test Analysis of Variance (ANOVA) with 95% confidence level. From the results of statistic test was obtained significantly different from each treatment group so followed by Least Significant Difference (LSD) test to see the difference between treatment groups. The LSD test results showed that at the 2nd, 3rd, 4th, 5th and 6th hours. dose 1 mg / kg b.w, dose 10 mg / kg b.w and dose 100 mg / kg b.w has significant difference with negative control ($p \leq 0,05$) but dose 1 mg/kg b.w, dose 10 mg / kg b.w, dose 100 mg/kg b.w did not differ significantly with positive control ($p \geq 0,05$). This showed that dose 1 mg / kg b.w, dose 10 mg / kg bw, dose 100 mg/kg b.w were potentially reduces the volume of edema. The dose 1 mg/kg b.w showed a significant difference ($p \leq 0,005$) with dose of 100 mg/kg b.w at 6th hours, while the dose 10 mg / kg b.w

and dose 100 mg/kg b.w did not show significant difference with the positive control ($p \geq 0,05$).

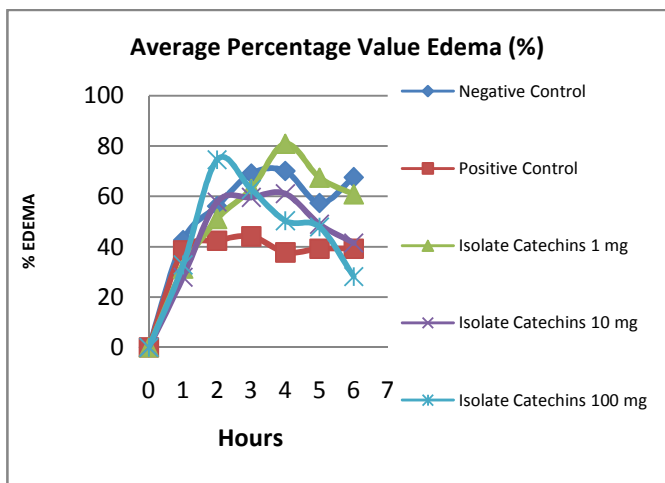


Figure 3. Average Percentage Value of Edema in Rat Paw

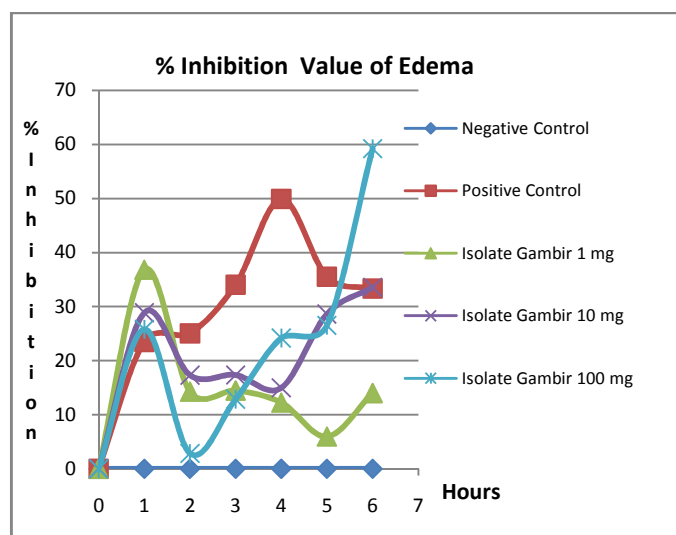


Figure 4. Average Percentage Inhibition Value of Edema in Rat Paw

This indicates that catechins isolates dose 10 mg / kg b.w and dose of 100 mg / kg b.w have better edema inhibiting ability than dose of 1 mg/kg b.w. However, when observed from the percentage of inflammatory inhibition dose 100 mg/kg b.w has a higher inhibitory power with 59.19%. And the dose 10 mg/kg b.w has the same ability with positive control in inhibition of rat paw edema

Conclusion

1. Catechin isolate of gambir with dose 1 mg/kg b.w has anti-inflammatory effect on carrageenan induced paw edema in wistar male rats
2. The dose 10 mg/kg b.w catechin isolate of gambir was the best dose in reducing edema because it has an edema almost the same with the Na-diclofenac.
3. Effect of anti-inflammatory dose 100 mg/kg b.w was not difference significant compared with dose of 10 mg/kg b.w.

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