Edisi Cetak Dentika Dental Journal, Juli 2011 (ISSN: 1693-671X)

EFFECT OF LATEX AND EXTRACT OF JATROPHA CURCAS LINN ON EXPRESSION OF SUBSTANCE P (SP) AND CYCLOOXYGENASE-2 OF DENTAL PULP

(EFEK LATEKS DAN EKSTRAK JATROPHA CURCA LINN TERHADAP EKSPRESI SUBSTANSI P DAN COX2 PULPA GIGI)

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Abstract

Inflammatory pulpal pain may arise due to the increased pressure inside the pulp or the release of prostaglandin E_2 (PGE_2). In the inflamed pulp, levels of PGE_2 and substance P (SP) is higher than those of normal pulp. PGE_2 sensitizes all nociceptor while SP can induce the activity of cyclooxygenase-2 (COX-2) and is an exitatory neurotransmitter. *Jatropha curcas* Linn latex is widely used for pulpal pain relief. The aim of this study was to evaluate the effect of the latex and extract of *J. curcas* on the dental pulp expression of COX-2 and SP. Thirty-six pulpitis-induced dental pulp of *Macaca fascicularis*, were divided into 3 groups: Group 1 served as controls, Group 2 was given latex, and Group 3 was given extracts. The ELISA assay was used to determine the levels of SP and COX-2. SP data was analyzed with ANOVA ($p < 0.05$) while the COX-2 data was analyzed with *Mann Whitney*. The results showed that the levels of SP (pg/mL) of the control, latex, and extract group were 28.94; 26.22; 28.89 respectively, while levels of COX-2 (ng/ml) of control, latex, and extract group were 0.04; 0.08; 0.10 respectively. In conclusion, *J. curcas* can reduce the levels of SP, *J. curcas* latex has lower levels of SP than extract, but does not provide clear results in decreased levels of COX-2. Further study requires the mechanism of SP, and the concentration of COX-2 needs to be further investigated using different methods.

Key words: Jatropha curcas, substance P, cyclooxygenase-2

INTRODUCTION

Pains of dental origin are common, according to a National Health Survey, odontalgia is one of the prevalent diseases of Indonesian population. To overcome the disease, instead of visiting the dentist, patients often try to treat themselves, due to several factors such as fear, the distance to the health services, or financial factors.

Self medicated treatment includes the use of medicinal plants; the popular one is the latex of *Jatropha curcas* L.[1,2] (Greek: *Jatros* means doctors, *trophe* means food or nutrition). Based on these facts, it is necessary to investigate the scientific basis of this plant in reducing dental pains. The scientific data of medicinal plant is one of the several requirements required as a phytopharmacologycal drug.

According to Hargreaves, pulpal pain is caused by the release of mediators and the increasing of intrapulpal pressure. Mediators involved in the processing of pain is prostaglandin E_2 (PGE_2), which sensitizes the nociceptor. [3] Cyclooxygenase (COX) is an enzyme responsible for converting arachidonic acid to PGE_2.[4] There are three isoforms of this enzyme namely the COX-1 and COX-2 and COX-3. The COX-1 is a constitutive enzyme while the COX-2 is stimulated by inflammation. COX-3 is an enzyme released in the brain and spinal cord, but the mechanism is not known.[4,5] Inhibition of COX-1 is

reported to cause adverse effects on the kidneys, stomach, or platelet,[5] and the selective COX-2 drug, has also reported to cause adverse effects on the cardiovascular system and cause hemorrhage.[5-7] Another mediator involved in pain processing is substance P (SP) which is reported to induce the release of COX-2 and acts as excitatory neurotransmitter in medullary dorsal horn. [8] Considering the side effect of COX inhibitor and COX-2 selective inhibitor, as mentioned above, this study will investigate the effect of *J. curcas* on the expression of SP as well as on COX-2.

MATERIALS AND METHODS

This study was conducted in adult 6 years old males of *Macaca fascicularis*, at the Animals Primate Center for Studies, Research & Community Service, Bogor Agricultural Institute, and has received ethical approval from the Commission on Ethics and Animal Welfare (ACUC Number: P.02-09 - IR)

The sample size was calculated by the formula of Frederer. $(t - 1)(r - 1) \geq 15$

t= number of treatment groups and r= number of samples of tooth .

There three treatment groups (group of latex, extract, and control), according to this equation, the samples (r) of each group were 10 teeth (minimal sample). In this experiment, the sums of samples were 12.

The latex was taken directly from the trunk of the *J. curcas* tree and stored in temperature of -20º C. The amount used in this study was 0.5 ml drops of 0.025 ml. The extracts were obtained by extracting 450 ml of latex with 8 l methylen chloride resulting in 2.42 g of extracts. It means that 1ml latex is equivalent with 2,42/450 or 5,37 mg/ml.

The procedures included the animal quarantine for one month, pulpitis induction, and measurement the level of SP and COX-2 by ELISA. Occlusal cavity was prepared in three teeth in one quadrant (second premolar, first molar, dan third molar) until the pulp was perforated, using No.1 round bur. Anesthesia was obtained by administration of intramuscular ketamine hydrochloride 20mg/kg. During the cavity preparation, the cavities were always dripped with drops of NaCl solution to keep it fresh. The cavities were left open for 60 minutes.[9]

After putting the latex or extract to the experimental group, the cavities were covered by temporary filling for 3 hours.[9] The control groups were given sterile cotton pellet without experiment materials and covered with temporary filling.

On day 1, the teeth on one side of the mandible were extracted, and those on the other side of the mandible were extracted on day 8. To collect the pulp tissue, the teeth were split and subsequently the pulp was stored in microcentrifuge tubes containing 0.2 ml phosphate-buffered saline solution (PBS) at – 70º C.[10]

The expression of SP was calculated through competitive ELISA assay (substance P Assay KGE 007 (R&D Systems, USA) while the expression of COX-2 was calculated with Direct Sandwich ELISA assay (human *COX-2* Assay Kit-IBL–Immuno Biological Laboratories C, Ltd.).

Pulp tissues were homogenized in 1 ml of methanol 15% in 0.1 M sodium phosphate buffer pH 7.5 (100 mg in 1 ml of methanol-buffer) and stored in microtubes. The next step was the microcentrifugation for 5 minutes.

Precondition was performed with Sep-Pak C18 column (Waters Corporation); the column was washed with 2 ml of methanol followed by 2 ml water. Insert the sample into the column and adjust the flow rate to 1 ml per minutes. The column was then washed with 2 ml 15% methanol in water followed by 2 ml petroleum ether. Elution was performed with 2 ml methyl formate, and the eluate was evaporated with nitrogen gas flow and after added with PBS the eluate was stored in Eppendorf at-80° C.

SP data was analyzed with ANOVA ($p < 0.05$) while COX-2 data was analyzed with Mann Whitney test.

RESULTS

The control group has the highest level of SP while the latex group has the lowest level of SP. It is also demonstrated that there are only little differences in the three groups. According to ANOVA there were no significant differences ($p = 0,147$) in the three groups, or the concentration of SP in the three treatment groups were similar, (Table 1).

Table 1. Statistical analysis of substance P concentration

Group	n	Mean	Standard of Deviation	p
Control	12	28,94	4,65	
Latex	12	26,22	2,20	0,147
Extract	12	28,89	4,05	

Furthermore, multiple comparison test was used to see the difference between pairs of treatments.

The average difference in SP concentration on treatment groups can be seen in the error bar graphic, that illustrates the average with 95% confidence interval, (Figure 1).

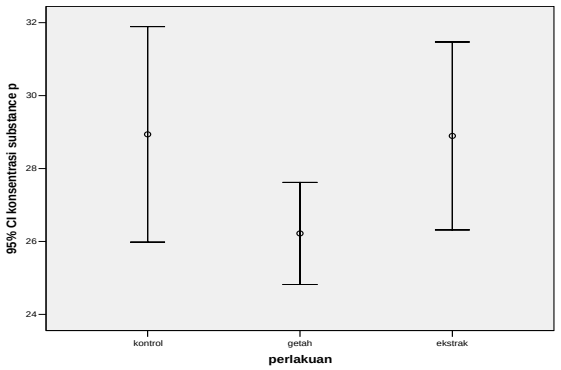

Figure 1. Distribution estimated graphic mean of *substance P* concentration

The average SP in latex group was lower than the control group. However, the average difference was not significant because most of the values of SP on the estimated 95% of the latex was the host from the control group. Extract group had an average value equal to the control group because the estimation was the estimated value of the control group. (Figure 1).

The highest level of COX-2 is 0.10 pg/mL (extract group) and is about three times compare to control group. In the latex group, the level of *COX-2* is two times higher (0.08) than the control group (0.04). There are a little difference between the extract group and the latex group. (Table 3)

Table 3. Concentration of *COX-2* according to treatment group (pg/mL)

Group	n	Mean	Standard of Deviation
Control	12	0,04	0,05
Latex	12	0,08	0,13
Extract	12	0,10	0,12

COX-2 levels on average between the three treatment groups showed that there was no significant difference. Estimated levels of COX-2 on average according to treatment group are shown in Figure 2.

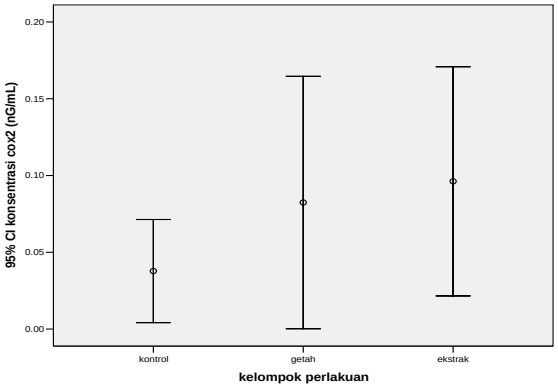

Figure 2. Graphic of distribution of estmated *COX-2* concentration on treatment group

Based on the graphic above, the level of COX-2 was smaller than the control and extract groups. Estimated levels of COX-2 on latex and extract groups were almost similar. Estimated levels of COX-2 in the control group was narrow and its estimated value was in the range of the latex groups. In other words, the estimated levels of COX-2 among the three treatment groups were similar.

Due to the concentration of COX-2 is very slightly below the standard value, it is necessasry to perform statistical data conversion so the COX-2 values obtained is in accordance with the standard curve in the ELISA kit used. Based on Mann-Whitney test, the average concentration of COX-2, the three treatments showed no significant difference. This means that the concentration of COX-2 in the latex, extract and control was not significantly different.

DISCUSSION

The latex of *J curcas* is widely used as dental pain reliever tooth in many places in Indonesia.[1,2] Usually, two or three drops of the latex were dripped into the cavity. The latex of *J. curcas* is a herbal medicine. To be a phytopharmacologycal drug, the scientific mechanism of its pharmacologycal properties should be elucidated. To date, the analgesic effect of the latex of *J. curcas* was demonstrated confirmed further by Irmaleny through chemically through the hotplate method writhing test,[9]

Table 4. Distribution of COX-2 Concentration using Mann-Whitney analysis

Group	Mean Concentration of COX-2 (nG/mL)	SE	95% CI		p		
					K–G	K–E	G–E
Control	0,04	0,02	0,0042	0,0714			
Latex	0,08	0,04	0,0002	0,1646	0,052	0,052	0,755
Extract	0,10	0,03	0,0216	0,1709			

and wasinduced writhing test, [10] the last test is considered more sensitive than the first one.[11] The latex of *J. curcas* was shown to have capacity to lower the synthesis of PGE_2, an inflammatory mediator that sensitizes the nociceptors, in primate *(Macaca nemestrina)*.[9] To fullfil the requirement as a phytopharmacologycal drug, we futher investigated not only the effect of the latex, but also the extract, on the expression of cyclooxygenase-2 (COX-2). The extract is considered worthy to be investigated because it is more practical and the efficacy of the extract is 2-6 times compare to the latex. Nevertheless, the results were not consistent with this statement because the latex, although it was not statistically significant, was revealed to be stronger than the extract.

Prostaglandin E2 (PGE_2) is an inflamatory mediator that sensitizes the nociceptors, mediated by COX. At time, there are three isoforms of COX. COX-1 is a constitutive enzyme and plays role in physiological processes. COX-2 expression is induced by inflammation. COX-3 is issued on brain. Non selective non-steroidal antiinflammatory drugs inhibit the activity either COX-2 or COX-1, resulting in many side effects due to interference to physiological process. Selective COX-2, however, is reported to cause side effects on the cardiovascular system and cause hemorrhage.[4,5,6] Therefore, the effect of the latex and the extract of *J. curcas* on SP (substance P) was included in the present study. Substance P is a mediator that activates the COX-2, induces and degranulates mass cell to release histamin (a pain mediator). Substance P is also an exitatory neurotransmitter in the medullary dorsal horn.[7,8] Considering these capacities of SP and the disadvantages of COX inhibitors, it is worthy to investigate the effect of this herbal on the expression of SP and the results may give another alternative in the management of pain.

There were no significant differences of the expression of COX-2 in all groups. The expression of COX-2 in the control group was smaller than the expression of COX-2 in other groups. These results were not consistent with previous research, conducted in *Macaca nemestrina*,[9] which showed that the latex could reduce the levels of PGE_2, pain mediator mediated by COX-2 enzyme. PGE_2 is a highly conserved mediators, i.e. it plays the equal role in large different species.[12]

These results might be caused by several factors; there was conversion in the standard curve, the position of teeth used for the experiment was too closed, producing immunological response bias, and the exposure time (3 hours) was not enough. Therefore, further research would be a very valuable thing to do.

In the present study, the level of SP was decreased in both groups, either extract or latex. Although non statistically significant, the ability of latex to reduce the level of SP was greater than the extract. This signifies that the experiment material (*J curcas*), does have the ability to reduce the level of SP.

The present study was performed in *Macaca fascicularis*. This nonhuman primate has the similarity with human in the type, the sum and the order of eruption of the teeth, and that it is a good model for dental research.

This study was approved by Ethical Committee and the samples of this study was restricted in relation to animal welfare. The experiments on animals should follow the three R principles: reduction, refining and replacing.[13,14] Further study, therefore, either in nonhuman primate or other animals would be very valuable to confirm the present study.

In conclusion, the latex of *J. curcas* can reduce the concentration of SP, more than do the extract, while the effect of latex and extract of *J. curcas* do not give a clear result in decreasing the concentration of COX-2. The mechanism of SP, and the concentration of COX-2 needs to be further investigated using different methods.

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Edisi Cetak Dentika Dental Journal, Juli 2011 (ISSN: 1693-671X)