

## Research Report

# Analgesic effect of coconut shell (*Cocos nucifera* L) liquid smoke on mice

Meircurius Dwi C.S, Tantiana and Ira Arundina

Department of Oral Biology

Faculty of Dentistry, Universitas Airlangga

Surabaya – Indonesia

### ABSTRACT

**Background:** Drugs can be used to eliminate pain by inhibiting the activity of converting arachidonic acid into prostaglandin. The chemical compositions of coconut shell are cellulose, pentosan, lignin, solvent extraction, uronate anhydrous, nitrogen, and water. One active ingredient in coconut shell is phenyl propanoid (consisting in lignin structure) and guaicol. Phenyl propanoid and guaicol are phenolic compounds that can be used as antioxidant, antiseptic, anti-inflammatory, anesthetic and analgesic. Liquid smoke of coconut shell (*Cocos nucifera* L) contains phenolic compound is believed able to bind a component converting arachidonic acid into prostaglandin. **Purpose:** The study was aimed to examine the analgesic effect of liquid smoke of coconut shell (*Cocos nucifera* L). **Methods:** The study was a laboratory experimental research, conducted on 2-3 months old male mice (*Mus musculus*) with 20-30 grams of weight. There were control group and treatment groups each of which had seven mice. Control group was orally given 0.01 ml/weight (ml/gr) of distilled water, after 30 minutes 0.01 ml/weight (ml/gr) of acetic acid 0.6% was delivered via intraperitoneal injection. The treatment groups were given liquid smoke of coconut shell (*Cocos nucifera* L) with the concentrations of 25%, 50%, and 100% respectively. The analgesic effect was then determined by decreasing of writhing reflex on mice recorded every 5 minutes for 30 minutes. **Results:** There were significant differences of writhing reflexes in the treatment groups given liquid smoke of coconut shell with the concentrations of 25%, 50%, and 100%. The higher concentration of liquid smoke the higher its analgesic effect. **Conclusion:** Liquid smoke of coconut shell (*Cocos nucifera* L) has analgesic effect.

**Key words:** Analgesic effect, liquid smoke of coconut shell, acetic acid

### ABSTRAK

**Latar belakang:** Salah satu mekanisme obat yang digunakan untuk menghilangkan rasa nyeri adalah menghambat aktivitas konversi asam arakhidonat menjadi prostaglandin. Komposisi kimia tempurung kelapa terdiri dari selulosa, petosan, lignin, solvent, uronat unhidrat, nitrogen dan air. Salah satu bahan aktif dalam tempurung kelapa adalah phenyl propanoid (terdapat dalam struktur lignin) dan guaiakol. Phenyl propanoid dan guaiakol adalah suatu senyawa fenol yang mempunyai sifat sebagai antioksidan, antiseptik, anti-inflamasi, anastesi dan analgesik. Liquid smoke tempurung kelapa (*Cocos nucifera* L) mengandung senyawa fenol yang dapat mengikat komponen dalam konversi asam arakhidonat menjadi prostaglandin. **Tujuan:** Studi ini bertujuan untuk meneliti efek analgesik dari liquid smoke tempurung kelapa (*Cocos nucifera* L). **Metode:** Penelitian ini adalah eksperimental laboratoris dengan jenis post test only control group design pada mencit (*Mus musculus*) jantan usia 2-3 bulan dengan berat badan 20-30gram. Kelompok kontrol dan perlakuan terdiri dari 7 hewan coba. Kelompok kontrol diberi aquades 0.01ml/bb (ml/gr) (po) dan setelah 30 menit diberi asam asetat 0.6% 0.01ml/bb (ml/gr) (ip). Kelompok perlakuan diberi liquid smoke tempurung kelapa (*Cocos nucifera* L) dengan konsentrasi 25%, 50% and 100%. Efek analgesik ditentukan dengan melihat penurunan writhing reflex (liukan atau geliat tubuh hewan coba) yang dihitung setiap 5 menit selama 30 menit. **Hasil:** Terdapat perbedaan yang bermakna jumlah writhing reflex pada pemberian

liquid smoke tempurung kelapa konsentrasi 25%, 50% dan 100%. Semakin tinggi konsentrasi liquid smoke, semakin tinggi pula efek analgesic yang ditimbulkan. **Kesimpulan:** Liquid smoke tempurung kelapa mempunyai efek analgesik.

**Kata kunci:** Efek analgesik, liquid smoke tempurung kelapa, asam asetat

*Correspondences:* Ira Arundina, c/o: Departemen Biologi Oral, Fakultas Kedokteran Gigi Universitas Airlangga. Jln. Prof. Dr. Moestopo No. 47 Surabaya 60132, Indonesia. Email: arundinafg@yahoo.com.

## INTRODUCTION

Nociception is a neural tissue response towards unpleasant stimuli while pain is a perception that arises as a result of nociception.<sup>1</sup> Inflammation is a complex biological response which include vascular tissue response to both damaged tissue and irritation.<sup>2</sup> Tissue damage from excessive stimulus are associated with unpleasant and uncomfortable sensory called pain.<sup>1,3</sup> Pain management that can be conducted involves reducing variety of factors both peripheral and central. There are three classes of drugs that can be used to relieve pain, namely non-opioid analgesics, opioid analgesics, and local anesthetics.<sup>4</sup>

Coconuts in Indonesia is produced about 15.5 billion/year.<sup>5</sup> Unfortunately, the processing industry of coconut in general is still focused on the processing of it as fruit, as the primary outcome, while the by-products processing industry such as coconut husk and coconut shell is still be conducted traditionally.<sup>6</sup> Coconut shell is one part of the agricultural products which has high economic value and high functional value.<sup>7</sup> Chemical composition of coconut shell consists of cellulose, pentosan, lignin, ash, solvent extraction, uronat anhydrous, nitrogen, and water.<sup>8</sup> One of active components in the coconut shell is phenylpropanoid compound contained in lignin. Phenylpropanoid compound is a phenol compound that can be used as an antiseptic, antioxidant, anti-inflammatory, anesthetics, and analgesics.<sup>9,10</sup>

Liquid smoke is available from condensation of coconut shell through pyrolysis process at 400 °C. Liquid smoke contains many chemical components, such as *phenols*, aldehydes, ketones, organic acids, alcohols, and ester.<sup>11</sup> Those various chemical components may act as an antioxidant and antimicrobial effect, as well as give color and distinctive flavor to food products.<sup>12</sup> Currently, liquid smoke of coconut shell has been widely used by food industry to give flavor and texture of food products, such as meat, fish, and cheese.<sup>13</sup>

In Indonesia, liquid smoke is also used for making smoked fish,<sup>14</sup> as well as for preservatives of tuna, eel, and fresh noodles.<sup>15</sup> In addition, liquid smoke can also be used as a substance that can reduce pain in wound, and can remove the scar. Knowledge about medicinal plants, based on experience and skills, has been passing from generation to another. However, the role of medicinal plants still needs to be justified medically through analysis and scientific experiments. Efforts towards a rational scientific evidence must be done through the analysis of substances

contained as well as their therapeutic effects.<sup>16</sup> Therefore, it is necessary to examine the analgesic effect of liquid smoke of coconut shell (*Cocos nucifera L*) as the by-products.

## MATERIALS AND METHODS

This research was laboratory experimental with post test only control group design. Materials used in this research were shells of 6–8 months old coconut (*Cocos nucifera L*) obtained and identified in Plant Conservation and Botanical Garden, Purwodadi, Pasuruan. Experimental animals used were healthy male mice (*Mus musculus*) in the age of 2–3 months with the weight of 20–30 grams. Those animals were obtained from the Unit of Experimental Animals in Biochemistry Laboratory of Medical Faculty, Airlangga University.

Liquid smoke of coconut shells (*Cocos nucifera L*) was obtained through pyrolysis process. Pyrolysis was a thermochemical decomposition of organic materials at above 430 °C without oxygen.<sup>17</sup> The raw materials needed were 5 pounds of coconut shells producing 20 ml of liquid smoke through pyrolysis process. The making process of liquid smoke of those coconut shells (*Cocos nucifera L*) was then conducted in Research Laboratory and Industry Consultant, Surabaya.

This research was considered as a preliminary study on the analgesic effect of liquid smoke of coconut shells (*Cocos nucifera L*). Liquid smoke used in this research was at concentration of 100% obtained through pyrolysis. To obtain liquid smoke of coconut shells with lower concentrations (50% and 25%), dilution was conducted by distilled water. The use of the distilled water, as a result, could change the concentration and enlarge the volume, but still stable.<sup>18</sup>

Afterwards, analgesic test on liquid smoke of coconut shells (*Cocos nucifera L*) was determined by *acetic acid*-induced writhing reflex. This method is used for screening peripheral acting analgesics, local peritoneal cell response, and prostaglandin pathway.<sup>19</sup> Mice used as experiment were divided into 4 groups, each group consisted of 7 mice (n = 7) and treated as follows: Control Group was given orally 0.01 ml/gr of distilled water (bb.po); Group I was given 0.01 ml/g of 100% liquid smoke of coconut shells (*Cocos nucifera L*) (bb.po), Group II was given 0.01 ml/g of 50% liquid smoke of coconut shells (*Cocos nucifera L*) (bb.po); and group III was given 0.01 ml/g of 25% liquid smoke of coconut shells (*Cocos nucifera L*) (bb.po). After

thirty minutes, those mice were given 0.6% acetic acid at a dose of 0.01 ml/g intra-peritoneal (bb.ip) to induce pain. After 5 minutes, writhing reflex (abdominal constriction) of those experimental animals was measured every 5 minutes for 30 minutes.<sup>20,21</sup> The effect of analgesics was determined by comparing the percentage of analgesic power to writhing reflex (abdominal constriction) between the control group and the treatment groups given with liquid smoke of coconut shells (*Cocos nucifera L*)<sup>22</sup> with formula:<sup>23</sup>

$$\text{Percentage of inhibition} = \frac{\text{Control group mean} - \text{test group mean}}{\text{Control group mean}} \times 100\%$$

The number of writhing reflex (canting or stretching) was then analyzed by using one-way ANOVA with 95% level of significance. If the results showed no difference, then Least Significant Difference test (LSD test) would be conducted.

## RESULTS

Injection of acetic acid through intraperitoneal in experimental animals can cause pain response described as writhing reflex (stretching or canting of experimental animals' body). But, with liquid smoke of coconut shell (*Cocos nucifera L*) at three different concentrations of 100%, 50% and 25% could decrease the writhing reflex. The data obtained were analyzed by Kolmogorov Smirnov test. The result showed that all groups had probability value greater than 0.05 ( $p > 0.05$ ), indicating that the data were normally distributed. Afterwards, Levene test was conducted to indicate whether the data are homogeneous or not. The result then showed that the significance value was greater than 0.05,  $p = 0.608$  ( $p > 0.05$ ) which indicates the data were homogenous. Since the data were normally distributed and homogenous, one-way ANOVA was conducted to analyze the data.

Concentration of 100% liquid smoke had 40.29% inhibition. It is also known that 50% liquid smoke of coconut shells had 25.28% inhibition. Meanwhile, 25% liquid smoke had 19.70% inhibition. Data analysis using One-Way ANOVA showed the greater level of significance ( $p < 0.05$ ), which means that there was significant difference of stretching or canting of experimental animals' body among the groups. Thus, LSD test was then conducted to know the difference among experimental groups (Table 2).

There were significant differences between the control group given with distilled water and the treatment group given with 100% liquid smoke, between the treatment group given with 50% liquid smoke and the treatment group given with 25% liquid smoke, between the treatment group given with 50% liquid smoke and the control group as well as the treatment group given with 100% liquid smoke, and between the treatment group given with 25% liquid smoke and the control group as well as the treatment group given with liquid smoke 100% ( $p < 0.05$ ), which means that there was a reduction of stretching or canting of experimental animals' body.

## DISCUSSION

Liquid smoke (liquid of evaporation result) is a result of condensation of vapor derived from burning process of materials containing a lot of lignin, cellulose, hemicellulose, and carbon compounds.<sup>24</sup> Moreover, liquid smoke can also be considered as a complex system consisted of both dispersed liquid phase and gas phase as dispersant. Smoke is actually produced by incomplete combustion involving constituent decomposition reaction of polymers into organic compounds with low molecular mass because of heat including oxidation reaction, polymerization reaction, and condensation reaction. The nature of liquid smoke is affected by main materials, namely cellulose, hemicellulose,

**Table 1.** Mean of canting and stretching in animals' body, standard deviations and the percentage of inhibition (power analgesics)

Group	Treatments	$\bar{X} \pm SD$	Percentage of inhibition
Control	Distilled water	48.57 ± 4.79086	–
1	25% liquid smoke of coconut shell	39.00 ± 2.70801	19.70%
2	50% liquid smoke of coconut shell	36.29 ± 3.30224	25.28%
3	100% liquid smoke of coconut shell	29.00 ± 4.54606	40.29%

**Table 2.** LSD test between the control group and the treatment groups

	A	B	C	D
Control group (A)		*	*	*
100% liquid smoke of Coconut Shell (B)	*		*	*
50% liquid smoke of Coconut Shell (C)	*	*		*
25% liquid smoke of Coconut Shell (D)	*	*	*	

\* : There was significant difference among the groups

and lignin with various proportions depended on types of the materials in pyrolysis.<sup>25</sup>

Furthermore, one of materials used in the process of making liquid smoke is coconut shell which is part of the coconut fruit. Coconut shell is widely used to produce liquid smoke because it consists of lignin, cellulose, and metoksil causing good organoleptic nature.<sup>13</sup> There are actually twelve identified components in liquid smoke of coconut shell, mainly from thermal degradation of wood carbohydrates, such as ketones about 6.53%, carbonyl and acid about 2.98%, as well as furan and pyran derivatives about 3.02%. In addition, liquid smoke of coconut shell also contains 28 components derived from degradation of lignin thermal, such as phenol about 24.11%, guaiacol and its derivatives about 36.58%, and syringol and its derivatives about 18.26 %, and alkyl aryl ether about 8.5%.<sup>15</sup>

A compound that can cause irritation on tissue (irritant substance), such as acetic acid, can stimulate the release of prostaglandins through nociceptive neurons that are sensitive to non-steroidal anti-inflammatory drug. In other words, irritant substance can cause the release of endogenous substances, such as prostaglandins, which can stimulate peripheral nociceptor and neurons that are sensitive to non-steroidal anti-inflammatory drug.<sup>26</sup> Writhing reflex induced significantly decreased due to the provision of liquid smoke of coconut shell (*Cocos nucifera L*). It means that the provision of liquid smoke of coconut shell (*Cocos nucifera L*) could reduce response to pain. This is because active ingredient contained in the liquid smoke of coconut shell is phenolic compound that can inhibit pain. Phenolic compound contained in the liquid smoke is a compound of phenol and guaiacol, which is a potent radical trapping compound. This compound is also considered as an antioxidant compound that has the ability as a redox compound, which serve as a reductant, hydrogen supplier, and inhibitor of the initiation stage of the lipid oxidation reaction.<sup>27,28</sup> Antioxidant is actually considered as an anti-inflammatory agent that works through the capture of free radical oxygen released by peroxide. Phenol can inhibit prostaglandin production and reduce oxidized cyclooxygenase enzyme that plays a role in the metabolism of arachidonic acid altered into prostaglandin  $H_2$  ( $PGH_2$ ), an unstable molecule that can turn into a variety of pro-inflammatory compounds.<sup>29</sup>

The mechanism of liquid smoke of coconut shell to inhibit pain response was due to the role of phenolic compound that inhibits cyclooxygenase of tissue by reducing the synthesis of prostaglandin  $E_2$  ( $PGE_2$ ).<sup>30</sup> The decreasing of prostaglandin  $E_2$  ( $PGE_2$ ) is caused by the binding of prostaglandin compound,  $G_2$  ( $PGG_2$ ), and prostaglandin compound,  $H_2$  ( $PGH_2$ ), when arachidonic acid is converted into prostaglandin compound,  $E_2$  ( $PGE_2$ ), by phenolic compound. Both compounds can actually be considered as endoperoxide, a compound produced during the conversion of arachidonic acid to prostaglandins. As a consequence, the provision of liquid smoke of coconut shell (*Cocos nucifera L*) is related to the mechanism of

cyclooxygenase inhibiting in peripheral tissues, so the synthesis of prostaglandins can be reduced, and the main transduction mechanisms of afferent nociceptor can be disrupted.<sup>31</sup> The mechanism of analgesic effect caused by liquid smoke of coconut shell then works by inhibiting the production and function of prostaglandins. Therefore, it can be said that the liquid smoke of coconut shell is a non-steroidal anti-inflammatory like drug.

The percentage of inhibition is the ability of liquid smoke of coconut shell (in %) in reducing the number of writhing reflex (stretching or canting of experimental animals' body) due to acetic acid. In other words, percentage of inhibition will increase as the concentration of liquid smoke of coconut shell increases. There was a significant difference between the percentage of inhibition of liquid smoke of coconut shell with concentration of 100% and that with concentrations of 50% and 25%. The difference is caused by the fact that the amount of phenolic compound contained in liquid smoke of coconut shell with concentration of 100% is considered as a minimum amount that can inhibit the production of prostaglandins. In other words, the concentration of phenolic compound in each liquid smoke concentration is proportional to the concentration of liquid smoke itself. The study showed that liquid smoke of coconut shell (*Cocos nucifera L*) has analgesic effect.

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