


# Potential of Distilled Liquid Smoke Derived from Coconut (*Cocos nucifera* L) Shell for Traumatic Ulcer Healing in Diabetic Rats

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## Abstract

**Objective** Distilled liquid smoke (DLS) is a result of coconut processing by-product that not only serves as a natural food preservative but also has a promising therapeutic effect. The healing potential of DLS derived from coconut (*Cocos nucifera* L) shell was investigated on a traumatic ulcer with the diabetic rat.

**Materials and Methods** DLS was analyzed the component by gas chromatograph mass spectrometry. Diabetic condition was induced by alloxan in 55 male Wistar rats. Ten mm of traumatic ulcer was made along the labial fornix incisive inferior after the diabetic condition was confirmed. Then DLS coconut shell, benzydamine hydrochloride, and sterile distilled water were applied topically for 3, 5, and 7 days. The potential healing was evaluated based on the expression of nuclear factor kappa beta (NFκB) and tumor necrosis factor alpha (TNF-α) on macrophages using immunohistochemical staining and the amount of collagen using Masson Trichome staining. The difference between each group was analyzed using one-way analysis of variance. The least significant difference test is used to determine the significant difference ( $p < 0.05$ ).

**Results** The major compounds found were phenol (36.6%), 2-methoxyphenol (guaiacol) (25.2%), furfural (17.8%), and 4-ethyl-2-methoxyphenol (3.5%) with 28 other minor constituents. The lowest NFκB and TNF-α expression on macrophage was observed by topical application of DLS derived from coconut shell for 3, 5, and 7 days of treatment. The amount of collagen was increased and indicated by the highest result of DLS compared to others.

**Conclusion** The DLS derived from coconut (*Cocos nucifera* L) shell was able to improve traumatic ulcer healing in a person with diabetes.

### ► Keywords

- coconut shell
- diabetic
- distilled liquid smoke
- traumatic ulcer healing

## Introduction

Liquid smoke is a liquid product of wood smoke condensation that is burned indirectly at high temperatures. Liquid smoke contains many compounds formed from the pyrolysis process of wood components, such as cellulose, hemicellulose, and lignin.<sup>1</sup> Liquid smoke is produced from biomass such as coconut shell,<sup>2</sup> palm shell,<sup>3</sup> walnuts,<sup>4</sup> and rice hull.<sup>5</sup> Pyrolysis process produces heavy tar, light tar (liquid smoke), charcoal, and uncondensed gases.<sup>6</sup> Indonesian uses liquid smoke not only as a natural preservative for various processed meat products<sup>3,7,8</sup> but also apply topically for the skin burns.<sup>9</sup>

Liquid smoke contains polyaromatic hydrocarbon (PAH) such as benzo (a)pyrene that is carcinogenic and causes damage to the amino acid and vitamins.<sup>10</sup> Purification is the process to separate and eliminate an undesirable component.<sup>11</sup> One of the purification techniques for liquid smoke is distillation. The purpose of distillation is to remove the undesirable compound like PAH.<sup>6</sup>

The significant component of liquid smoke derived from coconut shell is phenolic compounds.<sup>12</sup> This compound is a potent antioxidant that is able to bind free radicals such as reactive oxygen species (ROS) formation, inhibit nitric oxide (NO) production, production of proinflammatory cytokines tumor such as tumor necrosis factor alpha (TNF- $\alpha$ ),<sup>13</sup> and the activation of nuclear factor kappa beta (NF $\kappa$ B).<sup>14</sup> The phenolic compound may be responsible for therapeutic effect in liquid smoke derived from coconut shell. Our previous study showed per-oral administration of DLS derived from coconut (*Cocos nucifera* L) shell owned an analgesic effect,<sup>15</sup> and topical application can accelerate the contraction and increase the number of fibroblasts in skin burn wound healing.<sup>9</sup>

Oral ulcers in diabetic patients have the potential to delay the healing process, although the diabetic condition is controlled.<sup>16</sup> Patients with controlled type 2 diabetes mellitus and uncontrolled type 2 diabetes mellitus have an oral mucosal abnormality in the form of the oral ulcer with prevalence of 22%<sup>17</sup> and 6.7%, respectively.<sup>18</sup> Several factors influence the process of delayed healing oral ulcers in diabetes mellitus; one of them is the TNF- $\alpha$ .<sup>19</sup> Individuals with recurrent aphthous stomatitis without systemic abnormalities increased by two to five times.<sup>20</sup> Also, free radical levels such as malondialdehyde, NO, and oxidative stress index were higher compared to individuals without recurrent aphthous stomatitis.<sup>21</sup> Increasing TNF- $\alpha$  expression, not only occurs systemically but also increases in tissue, so oral ulcer in diabetes mellitus has the potential to delay the healing.<sup>22</sup> Topical treatment, such as benzydamine hydrochloride (BHCl), is unable to improve ulcer healing. It is possible that the mechanism of BHCl inhibits prostaglandin production and cannot inhibit the production of proinflammatory cytokines.<sup>23</sup> Phenolic compound on liquid smoke derived from coconut shell<sup>12</sup> is a potent antioxidant that is able to bind free radicals such as ROS formation and inhibit NO production, production of proinflammatory cytokines tumor such as TNF- $\alpha$ ,<sup>13</sup> and the activation of NF $\kappa$ B.<sup>14</sup>

Despite the exciting findings across data literature, the importance to explore the in vivo activity of DLS derived from coconut (*Cocos nucifera* L) shell and the composition should be conducted. The results bring evidence about the effect of DLS derived from coconut (*Cocos nucifera* L) shell for topical treatments and the healing potential in traumatic ulcer under diabetic condition. The therapeutic effect combined with safe doses of DLS derived from coconut shell (*Cocos nucifera* L) can give value in therapies or pharmacy.

## Materials and Methods

### Raw Materials

The coconut (*Cocos nucifera* L) shell was collected from the local market in Surabaya, Indonesia, November 2016. The identification of coconut shell was conducted in the Plant Conservation Centre, Institute of Science, Purwodadi, Indonesia.

### Pyrolysis and Distillation of Liquid Smoke

The pyrolysis process was conducted in the Research and Development of Forest Products Laboratory, Bogor, Indonesia (ISO number 17025:2008). The coconut shell dried at room temperature. The pyrolysis furnace was equipped with a kerosene pump stove as the heater and an encircling reactor with a diameter and height of 30 cm and 40 cm, respectively. The furnace could be charged with 5000 g of material. The furnace was connected by a pipeline to the cooling tubes to condense the fumes and generate the liquid smoke. Pyrolysis was carried out at a temperature of 400°C<sup>6</sup> with a heating rate of 3.33°C for 4.5 hours.

The yield of the liquid smoke (%) was calculated using the following formula:

$$\text{yield}(\%) = \frac{\text{liquid smoke (g)}}{\text{coconut shell (g)}} \times 100$$

The liquid smoke produced in this process was then settled for 48 hours before filtering with Whatman 52 (Whatman 52, 110 mm circle, Cat No 1452, GE Healthcare Life Science, Singapore). Then, the purification process was conducted through distillation at 120 to 150°C.<sup>8</sup> The yield of DLS derived from coconut shell (%) was calculated using the following formula:

$$\text{DLS}(\%) = \frac{\text{condensed liquid smoke (mL)}}{\text{liquid smoke (mL)}} \times 100$$

### Chemical Analysis of Distilled Liquid Smoke Derived from Coconut Shell

The 1,000  $\mu$ L DLS was shaken with 1,500  $\mu$ L dichloromethane (Merck, pure-analysis or p.a.). The solvent layer was then separated. The sample was re-extracted twice with the 1,000  $\mu$ L dichloromethane, and the extract layer of solvent was collected.

The components were then determined using gas chromatograph mass spectrometry (GC-MS) model 6890N

(Agilent Technologies, Inc., Santa Clara, California, United States), equipped with a mass spectrometer detector 5975B and DB-5MS UI column (Agilent Technologies, stationary phase; polyethylene glycol, 30 m × 0.25 mm; i.d. 0.25 μm), the temperature of the injector was 250°C. The carrier gas was helium at a constant flow rate of 1 mL/min. The initial oven temperature of the column was raised from 40° to 300°C at 10 C/min and then maintained for 4 minutes at 300°C. The mass spectrometer detector conditions were a capillary direct interface temperature of 270°C, MS Source temperature 230°C, and MS Quad temperature 150°C. Ionization energy was 70 eV, mass range was *m/z* 30-600 a.m.u., and scan rate was 1.4 scan/s. The total flow was 104 mL/minute, column flow was 1 mL/minute, and the linear velocity was 36.262 cm/second—the identification of the individual constituents was based on the comparison between mass spectra and retention time index of authentic reference compounds stored in the NIST14 mass spectral data library.

### Animals

Ethics approval was obtained from the Ethical Clearance of Health Experiment Committee, Faculty of Dental Medicine, Airlangga University, Surabaya with registered number 236/HRECC.FODM/X/2016.

Male Wistar rat, 2-month-old, weighing 120 to 160 g was used in this experiment. The treatment and experiment of animals were conducted in Animal Testing Laboratory, Faculty of Veterinary, Airlangga University, Surabaya. The animal sample was housed in collective cages with controlled-temperature (27°C) and artificially lighted rooms on 12 hours light/12 hours dark cycle with free access to water and standard diet.

### Diabetic Rat Induction

This research was an experimental laboratory using a posttest-only of control group design. Fifty-four Wistar rats were intraperitoneally induced with alloxan monohydrate (Alloxan monohydrate A7413, Sigma Aldrich., St. Louis, Missouri, United States) at a dose of 150 mg/kg to stimulate a diabetic condition.<sup>24</sup> Preparation of alloxan was performed by dissolving 0.9 g of alloxan monohydrate into 6 mL of phosphate-buffered saline to produce a concentrate of 150 mg/mL.<sup>25</sup> The rats did not receive food or water for more than 12 hours overnight before the induction of alloxan occurred. The development of diabetes mellitus in these animals was confirmed 72 hours after the alloxan induction and confirmed by the level of fasting glucose that was more than 200 mg/dL using GlucoDR (AGM-2100, All Medicus, Korea).<sup>24</sup>

### Traumatic Ulcer Induced in Diabetic Rat

After the animals confirmed as presenting the condition of diabetes mellitus (fasting glucose >200mg/dL), a traumatic ulcer sized 10 mm was created along the labial fornix incisive inferior,<sup>26</sup> using a round stainless steel blade.<sup>27</sup> Before this traumatic ulcer being made, the animals were anesthetized using a ketamine/xylazine cocktail.<sup>28</sup> The traumatic ulcer confirmed after 24 hours with the clinical appearance of a yellowish-white ulcer with a reddish edge.

At this point, the therapeutic topical applications of sterile distilled water, BHCl (1.25%, Tantum Verde, Soho, Jakarta, Indonesia) and DLS derived from coconut shell (*Cocos nucifera* L) were performed on the traumatic ulcers of all animals with the following distribution (► **Table 1**).

### NFκB, TNF-α Expression, and Amount of Collagen on Traumatic Ulcer

After treatment for 3, 5, and 7 days in each group, the rats had terminated, their labial fornix incisive inferior tissue was subjected to biopsy before an immunohistochemistry staining to determine the NFκB and TNF-α expression (*NFκB anti-antibody monoclonal, e381, Abcam*) (*TNF-α anti-antibody monoclonal, ab220210, Abcam*). The histological examination was performed using Masson Trichome staining to determine the amount of collagen. NFκB and TNF-α expression were counted directly from macrophages cells that expressed the NFκB and TNF-α.<sup>29</sup> The density of collagen expressed as a percentage (%) was calculated by dividing the area of collagens observed (μm<sup>2</sup>) by the total area of measurement. All measurements using a light microscope (Nikon H600L microscope; Nikon, Japan) at a magnification of 200× (DS Fi2 300MP digital camera; Nikon, Japan, digital software imaging by Nikkon Image System, Nikon, Japan).

### Statistical Analysis

Data were expressed as the mean standard values deviation for each measurement. The data were then analyzed using analysis of variance (one-way analysis of variance). The least significant difference test was used to determine the significant difference (*p* < 0.05). The SPSS 24.0 for Windows was used for the analysis.

## Results

### Pyrolysis and Distillation of Liquid Smoke Derived from Coconut Shell

The pyrolysis process of 5,000 g coconut shell was carried out with the final temperature 400°C (heating rate 3.33°C/min)

**Table 1** Animal distribution in the experiment

Group	Treatment	Duration
A3	Aqua Dest sterile	Once a day for 3 days at a dose of 20 μL/20g weight
B3	Benzydamine hydrochloride	
C3	Distilled liquid smoke	
A5	Aqua Dest sterile	Once a day for 5 days at a dose of 20 μL/20g weight
B5	Benzydamine hydrochloride	
C5	Distilled liquid smoke	
A7	Aqua Dest sterile	Once a day for 7 days at a dose of 20 μL/20g weight
B7	Benzydamine hydrochloride	
C7	Distilled liquid smoke	

for 4.5 hours. The pyrolysis produced 51% of liquid smoke, charcoal 33.87%, and heavy tar 7.28% (►Table 2). Then the liquid smoke from the pyrolysis was distilled at 120 to 150°C, and the final yield was 84%.

### Component of Distilled Liquid Smoke Derived from Coconut Shell

Around 32 components were identified in DLS derived from coconut shell (*Cocos nucifera* L) (►Table 3) with seven groups

**Table 2** The product of pyrolysis process of coconut shell

No	Product	Weight (g)	(%)
1	Liquid smoke	2,240	51
2	Heavy tar	320	7.28
3	Charcoal	1,488	

of the identified component using GC-MS (►Table 4). The primary group was phenol, guaiacol, furan, and pyran derivative. The minor group was a ketone, carbonyl, acid, syringol, and alkyl aryl ether.

Phenol was the most significant components of 36.6%, then followed by furfural of 17.8%, guaiacol of 14.4%, 2-methoxy-5-methylphenol of 5.2%, 4-ethyl-2-methoxyphenol (EMP) of 3.5%, and 2-methylphenol of 3% (►Table 3).

### NFκB, TNF-α Expression, and Amount of Collagen on Traumatic Ulcer

The NFκB and TNF-α expression were counted directly from some macrophages that expressed the NFκB and TNF-α (►Fig. 1A-C and ►Fig. 2A-C, respectively),<sup>30,31</sup>

The NFκB expression was increasing for 3 (79.5 ± 34.63), 5 days (64.5 ± 16.79) and 7 days (92.67±23.6) on topical

**Table 3** Identified components in distilled liquid smoke derived from coconut shell (*Cocos nucifera* L) using GC-MS

Components	RT	Area (%)
Phenol	73.555	36.6
2-Methylphenol	83.889	3.0
2,6-Dimethylphenol	92.585	0.4
2-Ethyl-phenol	96.240	0.4
2,3-Dimethylphenol	98.256	1.1
2,4,6-Trimethylphenol	107.079	0.1
p-Cresol	87.796	2.0
2-Methoxyphenol	90.191	14.4
2-Methoxy-3-methylphenol	96.618	0.1
2-Methoxy-3-methylphenol	102.920	0.8
2-Methoxy-5-methylphenol	104.306	0.5
2-Methoxy-5-methylphenol	105.314	5.2
4-Ethyl-2-methoxyphenol	117.413	3.5
4-Ethyl-2-methoxyphenol	117.917	0.1
Trans-Isoeugenol	134.805	0.1
Trans-Isoeugenol	140.477	0.1
2-Methoxy-4-propylphenol	129.260	0.4
Furfural	49.987	17.8
2-Furanmethanol, acetate	75.193	0.1
3,4-Dimethyl-2-cyclopentene-1-one	75.823	0.2
2,3-Dimethyl-2-cyclopentene-1-one	82.503	0.3
2-Methyl-2-cyclopentene-1-one	65.867	0.7
3-Methylcyclopentane-1,2-dione	80.738	0.7
3-Ethyl-2-hydroxy-2-cyclopentene-1-one	93.971	0.2
4-Methoxy benzoic acid methyl ester	131.024	0.2
4-Oxo-pentanoic acid methyl ester	74.689	0.2
1H-Inden-1-one, 2,3-dihydro-2-methyl	122.202	0.1
Acetic acid, phenyl ester	85.023	0.6
2,6-Dimethoxyphenol	127.118	0.6
2-Methoxy-3-(2-propenyl)-phenol	128.00	0.3
3,4-Dimethoxytoluene	111.238	0.3
3,5-Dimethoxy-4-hydroxytoluene	139.343	0.1

**Table 4** Classes of components in distilled liquid smoke derived from coconut shell (*Cocos nucifera* L)

Classes	Identified compound	Percentage (%)
Phenol and its derivative	7	43.6
Guaiacol and its derivative	10	25.2
Furan and pyran derivative	2	17.9
Ketone	5	2.1
Carbonyl and acid	4	1.1
Syringol and its derivative	2	0.9
Alkyl aryl ether	2	0.4
Total identified		91.8

application of DLS. The topical application of distilled liquid smoke on traumatic ulcer for 3, 5, and 7 days showed the lower NF $\kappa$ B expression compared to BHCl and sterile distilled water. NF $\kappa$ B expression of DLS ( $37.83 \pm 8.19$ ) for 3 days was significantly lower than BHCl ( $112.17 \pm 16.29$ ) and sterile distilled water ( $88.5 \pm 16.84$ ). While the NF $\kappa$ B expression was significantly lower than BHCl ( $80.17 \pm 18.06$ ) when compared to DLS ( $37.83 \pm 8.19$ ) for 5 days ( $\rightarrow$  Fig. 1D).

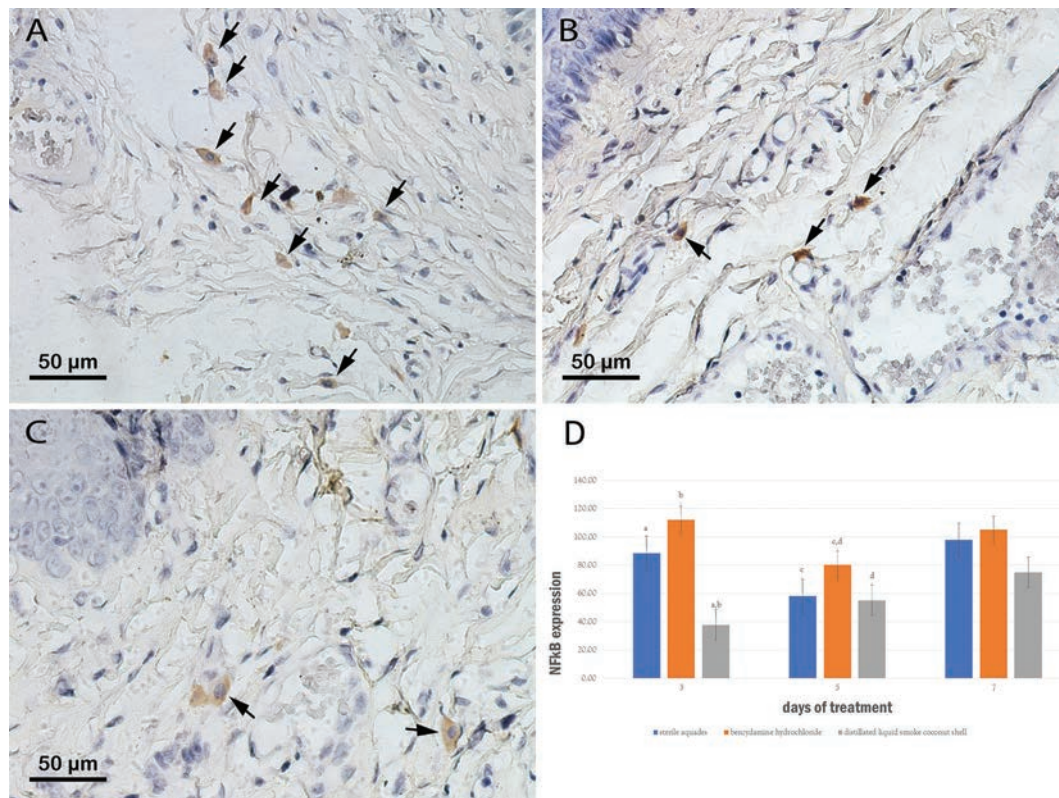
The TNF- $\alpha$  expression showed the same pattern after topical application of DLS, BHCl, and sterile distilled water increased

for 3, 5, and 7 days of application. Only topical application for 3 days showed the different TNF- $\alpha$  expression. Topical application of DLS ( $34.5 \pm 3.27$ ) for 3 days was significantly lower than BHCl ( $38 \pm 6.84$ ) and topical application of BHCl ( $38 \pm 6.84$ ) for 3 days was significantly lower than sterile distilled water ( $48.5 \pm 6.38$ ) ( $\rightarrow$  Fig. 2D).

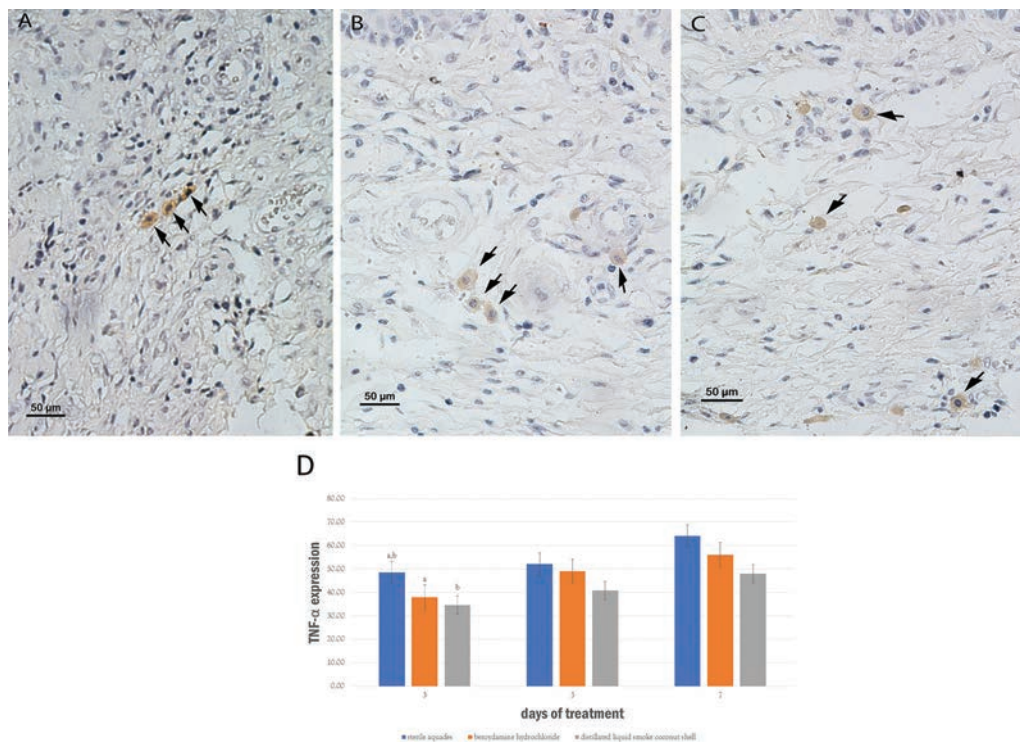
The amount of collagen was counted microscopely as a density of collagen ( $\rightarrow$  Fig. 3A–C). The topical application of DLS derived from coconut shell (*Cocos nucifera* L) on traumatic ulcer of the diabetic patient for 3, 5, and 7 days showed the highest amount of collagen compared to BHCl and sterile distilled water. The amount of collagen was significantly different between 3 and 5 days of topical application. The topical application of DLS ( $67.15 \pm 6.09$ ) for 3 days was significantly higher than BHCl ( $56.14 \pm 8.07$ ) and sterile distilled water ( $39.45 \pm 10.82$ ), while topical application of BHCl ( $56.14 \pm 8.07$ ) was significantly higher than sterile distilled water ( $39.45 \pm 10.82$ ). The 5 days topical application of DLS ( $84.27 \pm 6.76$ ) was significantly higher than BHCl ( $68.24 \pm 9.23$ ), while topical application of BHCl ( $68.24 \pm 9.23$ ) was higher than sterile distilled water ( $77.53 \pm 3.69$ ) ( $\rightarrow$  Fig. 3D).

## Discussion

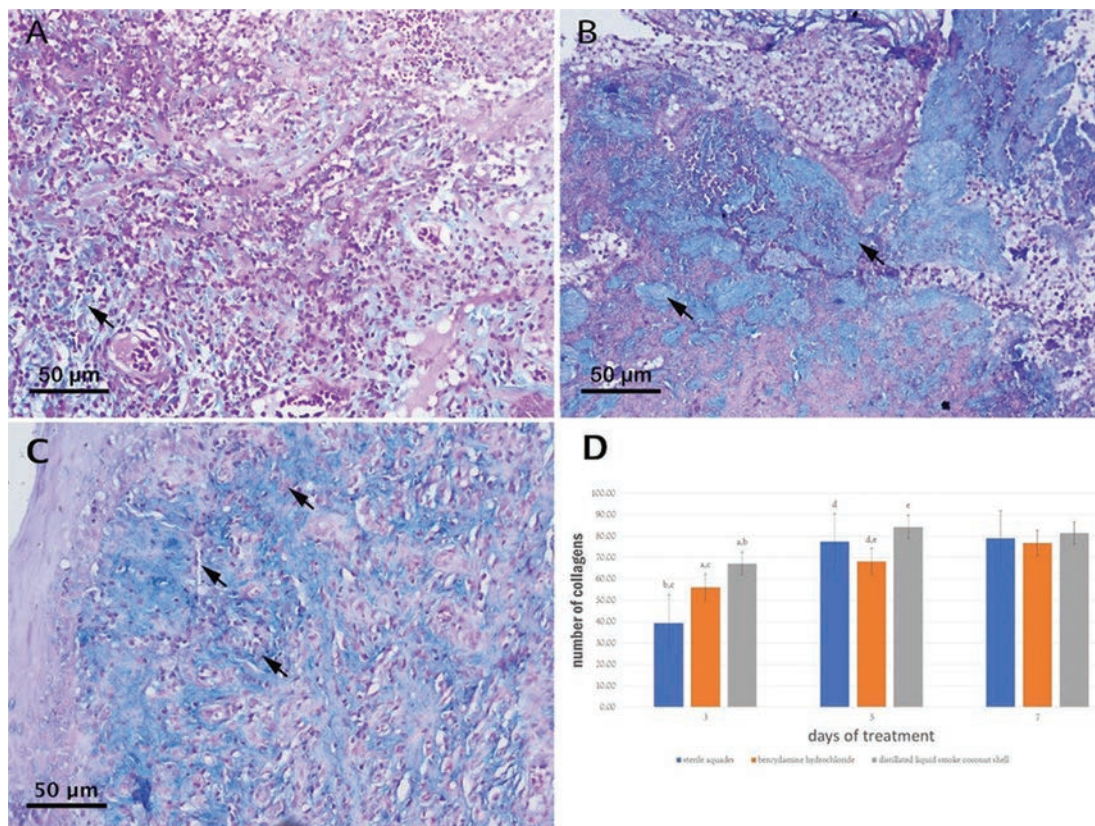
Pyrolysis process of coconut shells was carried out at 400°C with a heating rate of 3.3°C/min. The process resulted in 51%



**Fig. 1** The macrophage that expresses nuclear factor Kappa B (NF $\kappa$ B) on traumatic ulcer with diabetic rat (arrow). (A) The traumatic ulcer treated with sterile distilled water. (B) Traumatic ulcer treated with benzylamine hydrochloride (BHCl). (C) Traumatic ulcer treated with liquid smoke coconut shell (*Cocos nucifera* L). (D) NF $\kappa$ B expression on the traumatic ulcer. The same character means a significant difference between each group ( $p < 0.05$ ).



**Fig. 2** The macrophage that expresses tumor necrosis factor-alpha (TNF-α) on traumatic ulcer with diabetic rat (arrow). (A) The traumatic ulcer treated with sterile distilled water. (B) Traumatic ulcer treated with benzylamine hydrochloride (BHCl). (C) Traumatic ulcer treated with liquid smoke coconut shell (*Cocos nucifera* L). (D) TNF-α expression on the traumatic ulcer. The same character means a significant difference between each group ( $p < 0.05$ ).



**Fig. 3** The amount of collagen on traumatic ulcer with diabetic rat (arrow). (A) The traumatic ulcer treated with sterile distilled water. (B) Traumatic ulcer treated with benzylamine hydrochloride (BHCl). (C) Traumatic ulcer treated with liquid smoke coconut shell (*Cocos nucifera* L). (D) Amount of collagen on the traumatic ulcer. The same character means a significant difference between each group ( $p < 0.05$ ).

of liquid smoke, 7.28% of heavy tar, and 33.87% of charcoal. Ali et al produced  $68.8 \pm 1.5\%$  of liquid smoke and heavy tar and  $19.7 \pm 1.3\%$  of charcoal with the final temperature of  $995^\circ\text{C}$ ,<sup>32</sup> while Lombok et al produced 14.71% of liquid smoke, 2.29% of heavy tar, and 28.75% of charcoal with the final temperature of  $400^\circ\text{C}$ .<sup>6</sup> The differences in these results were due to the different quality of coconut shell, the design of pyrolysis reactor,<sup>33</sup> the final temperature,<sup>33</sup> and the heating rate.<sup>34</sup> The final temperature of  $400^\circ\text{C}$  was chosen for the pyrolysis process due to the formation of liquid smoke derived from coconut shell that was optimal at this temperature. The principle of the pyrolysis process is the decomposition of biomass that contains cellulose, hemicellulose, and lignin at high temperatures.<sup>33</sup> Liquid smoke as the pyrolysis product was obtained at the maximum temperature of 400 to  $550^\circ\text{C}$ .<sup>35</sup> Temperature below  $300^\circ\text{C}$  produced a dominant tar. Temperature above  $450^\circ\text{C}$  will decrease the decomposition process because of the decline in volatile condensable product.<sup>34</sup> The temperature more than  $700^\circ\text{C}$  will increase the carbon content in the form of PAH, such as pyrene and phenanthrene, due to decarboxylation and dehydration reactions.<sup>36</sup> Lignin is the dominant component in a coconut shell,<sup>37</sup> which decomposes at 300 to  $550^\circ\text{C}$ ,<sup>38</sup> and produces major compounds in liquid smoke, that is, phenolic compounds (phenol and guaiacol).<sup>38,39</sup> The final temperature of pyrolysis determines the content of phenol in the liquid smoke coconut shell. The optimal temperature of phenol formation is  $400^\circ\text{C}$ . Temperature of  $350^\circ\text{C}$  produced 2.92% of phenol, temperature of  $400^\circ\text{C}$  produced 4.63% of phenol, and temperature of  $450^\circ\text{C}$  produced 3.67% of phenol.<sup>40</sup> Budaraga et al also showed similar results, the final temperature of  $400^\circ\text{C}$  produced 23.45% phenolic compound, while the final temperature lower than  $400^\circ\text{C}$  resulted in 22.26% of phenolic compound.<sup>37</sup> This happened because liquid smoke and phenol reached the maximum concentration at temperatures between 400 and  $550^\circ\text{C}$ , and then declined. At temperatures higher than  $600^\circ\text{C}$ , the product will convert into gas and cannot be condensed.<sup>36</sup>

Liquid smoke contains PAH such as benzo (a)pyrene that is carcinogenic and causes damage to amino acids and vitamins.<sup>10</sup> Purification is the process of separating and eliminating an undesirable component like benzo (a)pyrene.<sup>11</sup> One of the purification methods is distillation. The distillation process in liquid smoke is carried out to remove that compound.<sup>6</sup>

The main compound of DLS derived from coconut shell (*Cocos nucifera* L) is phenol (36.6%), guaiacol (14.4%), and EMP (3.5%). This proportion was different in the liquid smoke derived from coconut shell that pyrolyzed at  $400^\circ\text{C}$  and not distilled. Another study showed that the proportion of guaiacol and EMP was 3.08 and 8.34%, respectively, in liquid smoke derived from coconut shell with pyrolysis at temperature of 350 to  $420^\circ\text{C}$ .<sup>41</sup> Therefore, phenol, guaiacol, and EMP compounds were found in liquid smoke coconut shells even with or without distillation process.

The topical application of coconut shell distilled liquid smoke to the traumatic ulcer with diabetes showed the expression of NF $\kappa$ B and TNF- $\alpha$  in macrophage cells that were lower than BHCl and sterile distilled water. Significant differences were observed after topical application for 3 days.

Mechanisms that might be involved in this process were the content of phenolic compounds such as EMP and guaiacol in distilled coconut shell liquid smoke. The guaiacol is a phenolic compound with two functional groups in the form of hydroxyl groups (-OH) and methoxy groups (-OCH<sub>3</sub>).<sup>42</sup> This compound has high reactivity to free radicals.<sup>43</sup> The guaiacol reactivity is related to the ability of -OH to bind free radicals,<sup>44</sup> because of its high ionization potential, which is capable of forming strong intermolecular and intramolecular hydrogen bonds.<sup>45</sup> Guaiacol can inhibit superoxide radicals (O<sub>2</sub><sup>-</sup>) by giving hydrogen atoms (H<sup>+</sup>) to -OH to form H<sub>2</sub>O.<sup>46</sup> While EMP plays a role in binding free radicals derived from nitrogen. Free radicals from nitrogen such as NO are produced by L-arginine and nicotinamide adenine dinucleotide phosphate through nitric oxide synthase (NOS).<sup>47</sup> NO will react with O<sub>2</sub><sup>-</sup> and produce peroxy nitrite (ONOO<sup>-</sup>). EMP is a guaiacol compound that has stronger antioxidant properties than guaiacol itself. EMP as a potent NO scavenger can protect cells from these free radicals by binding ONOO<sup>-</sup>.<sup>48</sup> Binding O<sub>2</sub><sup>-</sup> by guaiacol and ONOO<sup>-</sup> by EMP will inhibit phosphorylation and activation of NF $\kappa$ B inhibitor kinase (IKK), I $\kappa$ B degradation or inhibit phosphorylation and translocation of p65 to the cell nucleus so that NF $\kappa$ B decreases.<sup>14</sup> The decrease in NF $\kappa$ B causes a decrease in TNF- $\alpha$ .

The reduction in NF $\kappa$ B expression was also significantly observed after 5 days application of distilled coconut shell liquid smoke but did not reduce the TNF- $\alpha$  expression. The activation of NF $\kappa$ B during the inflammation process not only produces pro-inflammatory cytokines TNF- $\alpha$  but also can activate gene expression to produce other proinflammatory cytokines involved in inflammation, such as interleukin 1 $\beta$  (IL-1 $\beta$ ) and interleukin 6 (IL-6).<sup>49</sup> It is possible that distilled coconut shell liquid smoke also has a mechanism for the production of IL-1 $\beta$  and IL-6 through activation of NF $\kappa$ B. Thus, TNF- $\alpha$  expression was not significantly different when given 5 days of DLS.

Coconut shell liquid smoke not only affects the inflammatory phase through the changed of NF $\kappa$ B expression that becomes lower but also played a role in the proliferation phase by increasing collagen formation in traumatic ulcers with diabetes mellitus. The topical application of coconut shell liquid smoke showed a higher amount of collagen compared to topical application of BHCl and sterile distilled water. The amount of collagen observed increased with the duration of topical application in traumatic ulcers. This difference in the amount of collagen was significantly observed after topical application for 3 and 5 days. The mechanism that can be explained was the topical application of coconut shell liquid smoke to the traumatic ulcer due to the increasing fibroblast cells.<sup>9</sup> Fibroblasts synthesize different amounts of collagen because fibroblasts also synthesize the degradation factor, collagenase. The granulation tissue formation at the proliferation stage of the wound healing process is causing fibroblasts to reduce collagenase resulting in a decrease in the degradation process. Also, at this stage, fibroblasts undergo the apoptosis process so that the granulation tissue will be replaced by collagen.<sup>50</sup> In diabetic conditions, the fibroblast apoptosis process occurs through a

mechanism that involves activation of the FOXO1 transcription factor by increasing caspase-3 activation by advanced glycation end-products (AGEs).<sup>51,52</sup> AGEs can stimulate the apoptosis process through a mechanism involving the ROS formation in fibroblasts.<sup>53</sup> The previous study by Yang et al that the topical application of liquid smoke can reduce the ROS formation<sup>13</sup> and increase the number of fibroblast cells.<sup>9</sup> The results of this study that showed a change in the NFκB expression to be lower and an increase in the amount of collagen, it might explain the mechanism of the traumatic ulcer healing process with diabetes mellitus after the application of coconut shell liquid smoke. The topical application of coconut shell liquid smoke to the traumatic ulcer causes a decrease in the ROS formation, resulting in a decrease in the NFκB expression to produce TNF-α. The decrease in production of pro-inflammatory cytokine resulted in a decrease in activation of the FOXO1 transcription factor, so the caspase-3 activation that caused fibroblast apoptosis also decreased. Decreasing fibroblast apoptosis will increase collagen synthesis, and the proliferation phase in the traumatic ulcer healing process can be achieved so that the healing process will be faster.

This study was the first study to analyze the therapeutic potential of coconut shell DLS in traumatic ulcers with diabetes mellitus. This study showed that topical application of DLS coconut (*Cocos nucifera* L) shell could affect or increase the traumatic ulcer healing process. The limitations of this study only analyzed the mechanism on the inflammatory phase but also analyzed the further mechanism in the proliferation phase. In this context, the mechanism of coconut shell DLS to increase the amount of collagen due to the TNF-α expression reduction still had not been fully explained. Therefore, it needs further analysis.

## Conclusion

The DLS coconut shell (*Cocos nucifera* L) examined in the current study showed the inhibition of NFκB, TNF-α expression and increased the amount of collagen when given topically. Those results indicate the promising pharmaceutical potential of DLS coconut shell (*Cocos nucifera* L) to promote a traumatic ulcer healing. Accordingly, the pharmacological actions of distilled liquid smoke coconut shell (*Cocos nucifera* L) may be related to the presence of phenol, guaiacol, and EMP as the significant component in the distilled liquid smoke coconut shell (*Cocos nucifera* L).

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### Conflict of Interest

None declared.

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