Research Article

Comparison of the Antioxidant Effect of Robusta and Arabica Coffee Ethanol Extracts on Oxidative Stress in Diabetic Wounds

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Abstract.
Impaired wound healing in diabetes mellitus is caused by metabolic disorders due to hyperglycemia. Hyperglycemia causes an auto-oxidation reaction of glucose, which synthesizes the formation of reactive oxygen species (ROS). Robusta and Arabica coffee contain chlorogenic acid, caffeine, and trigonelline, which can prevent oxidative stress. This study aimed to analyze the antioxidant effect of the ethanol extract ointment of robusta coffee and arabica coffee on oxidative stress in diabetic wounds by measuring the decrease in plasma malondialdehyde (MDA) and leukocytes. This study used 24 experimental animals consisting of a positive control group, a negative control group, a group of rats with diabetic wounds who were given a 15% concentration of Robusta coffee ethanol extract ointment, and a group of rats with diabetic wounds who were given a 15% concentration of Arabica coffee ethanol extract ointment. After eight days of treatment, plasma MDA and leukocyte counts were examined. The results showed that plasma MDA levels and leukocytes did not differ between the diabetic wound groups that were given a 15% concentration of Robusta coffee extract ointment and a 15% concentration of Arabica coffee extract ointment. Robusta coffee and Arabica coffee ethanol extract ointments have the same effect on reducing plasma MDA levels and leukocytes.

Keywords: arabica, diabetic wound, malondialdehyde, robusta

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1. Introduction

Hyperglycemia can reduce the function of the immune system in defending itself from being infected with microorganisms that attack people with diabetes mellitus (DM). In hyperglycemia, autonomic neuropathy can occur, which causes a loss of ability to moisturize the surface of the skin due to decreased secretory function of the sebaceous glands and sweat glands, so that the skin of the feet becomes dry and facilitates the spread of infection [1–4]. Hyperglycemia conditions can also cause a decrease in the number of basal cells and impaired proliferation. Keratinocytes and skin barrier function, thus interfering with the wound healing process [1,4,5]. Hyperglycemia causes an auto-oxidation reaction of glucose, which can trigger free radicals, namely reactive oxygen

species (ROS) [1,6]. Oxidant compounds can affect the level of production of ROS and inflammatory mediators, so the higher the production of ROS due to decreased antioxidants, the higher the free radicals, resulting in oxidative stress [6].

Wounds that occur in conditions of hyperglycemia can cause oxidative stress, thereby increasing plasma MDA levels. The increase in MDA is caused by several proinflammatory mediators resulting from skin injury. As is well known, hyperglycemia will also induce an inflammatory response, oxidative stress, and an increase in the number of free radicals, which in turn will increase plasma MDA levels and reduce antioxidants [3,7].

Indonesian people believe that coffee beans are an ethnomedicine because they have many benefits and are easily available to the public. Coffee beans contain polyphenolic compounds such as chlorogenic acid, ferulic acid, caffeine, and trigonelline, which have antioxidant properties to neutralize the effects of oxidative damage to the skin and also have antibacterial, antiviral, and anti-inflammatory properties [8–10]. The administration of antioxidants can suppress the effects of free radicals that arise from the inflammatory process due to diabetic wounds [10,11]. Administration of a 15% concentration of robusta coffee ethanol extract ointment has been known to increase fibroblasts, resulting in diabetic wound healing [11]. Other studies also state that a 30% concentration of robusta coffee extract ointment is capable of repairing diabetic wounds [12]. However, there is still not much data explaining the effectiveness comparison between Robusta and Arabica coffee ethanol extract ointments for healing diabetic wounds. This study aimed to analyze the antioxidant effect of the ethanol extract ointment of robusta coffee and arabica coffee on oxidative stress due to diabetic wounds by measuring the decrease in plasma malondialdehyde (MDA) and leukocytes in hyperglycemic rats.

2. Methods

This research is a laboratory experimental study with a post-test-only control group design. The research subjects were white rats (Rattus norvegicus) of the Wistar strain, which had been acclimatized for seven days. The rats were divided into four groups, consisting of a positive control group, a negative control group, a group of hyperglycemic rats that experienced incisions and were given a 15% concentration of robusta coffee ethanol extract ointment, and a group of hyperglycemic rats that experienced incisions and were given arabica coffee ethanol extract ointment. concentration 15%.

2.1. Diabetic wounds

Induction of hyperglycemia in experimental animals by giving alloxan 200 mg/kg BW intraperitoneally. Diabetic wounds in rats are made by making an incision in the skin. Before the incision, the rats were given intraperitoneal general anesthesia. The rat skin was disinfected with 10% betadine before the incision. Making an incision 2 cm long using a scalpel with a depth of 0.2 cm to the hypodermis layer, the incision is then sutured and covered with sterile gauze.

2.2. Preparation of robusta coffee and arabica coffee ethanol extract ointment

In this study, an ointment made from the ethanol extract of Robusta coffee and Arabica coffee was used. The ointment used is fat-based, using Vaseline. The standard ointment base formula used is 100 grams of Vaseline. The ointment used in this study has a concentration of 15%.

2.3. Examination of plasma malondialdehyde levels with the TBARs method

Animal blood was taken from the retroorbital vein in as much as 3 mL. Then the blood was centrifuged, and then the plasma was added with Na2EDTA, 300 µl of aqua dest, 200 µl of SDS solution, 50 µl of BHT solution, 50 µl of EDTA solution, 1500 µl of acetic acid solution, and 1500 µl of TBA solution. The mixture was heated for 45 minutes in boiling water (100^{o} C), and then immersed in a bath filled with ice water. The sample was then centrifuged, and then read using a spectrophotometer at a wavelength of 532 nm.

3. Analysis

Data analysis to determine the comparative effect of Robusta Coffee and Arabica Coffee ethanol extract ointment on blood sugar levels used the Kruskall-Wallis test and continued with the Mann-Whitney post-hoc test, while for the comparative effect on MDA levels and leukocyte counts used the one-way ANOVA and Tukey post-hoc test.

3.1. Aspects of research ethics

This research has obtained permission from the Health Research Ethics Commission, Faculty of Medicine, Universitas Jenderal Achmad Yani No: 019/UH.1.11/2021.

4. Results

After the rats were induced with alloxan, there was an increase in blood glucose levels in the positive control (KP), whereas in K1 (ethanol extract of Robusta coffee concentration 15%) and K2 (ethanol extract of Arabica coffee concentration 15%), blood glucose levels were not higher when compared to KP. Blood glucose examination results can be seen in Table 1.

The results of the analysis with Kruskal-Wallis revealed that the blood glucose levels between the treatment groups differed significantly with a p-value <0.05. Furthermore, the Mann-Whitney test was carried out between groups. The results of the analysis showed that blood glucose levels were significantly different between the KP groups compared to the KN, KI, and K2 groups.

Table 1: Blood glucose levels, MDA plasma, leukocytes.

Groups	Mean ± SD		
	Glucose	MDA	Leukocytes
KN	77.6±16.3	0.83±0.3	9.7±1.7
KP	299±53.8	17.0±17.6	14.8±3.0
K1	90.8± 13.1	1.5±1.3	7.1±2.7
K2	108±29.8	1.7±0.6	8.6±1.6
p-value	0.000	0.000	0.000

Note: Kruskal Wallis p value <0.05 for blood glucose. One-way ANOVA ; *)p <0.05 for MDA and leukocyte count. KN = negative control, KP = positive control, K1 = 15% concentration of Robusta coffee ethanol extract, K2 = 15% concentration of Arabica coffee ethanol extract.

The plasma MDA levels of rats from each group are presented in Table 1. The highest mean plasma MDA levels were shown in the KP group, and the lowest average plasma MDA levels were shown in the KN group. Increased plasma MDA levels in KP could be due to incisions and hyperglycemia in rats, resulting in the production of several free radicals and ultimately an increase in plasma MDA levels.

Based on these data, it is known that the highest leukocyte count was in the KP group compared to the other groups. Incision wounds that occur in hyperglycemic rats cause an inflammatory reaction, increasing the number of leukocytes. In Table 1, it is known that there are significant differences in plasma MDA levels between groups with a p-value <0.05. Furthermore, from these data, a post-hoc Tukey test was carried out, with the result that there was a significant difference between KP and KN, K1, and K2. While the KN group, compared to K1 and K2, showed no significant difference in plasma MDA levels. Robusta coffee and arabica coffee ethanol extract ointments have the same effectiveness in reducing plasma MDA in hyperglycemic rats that have had incisions.

5. Discussion

Hyperglycemia affects keratinocyte and fibroblast activity, which induces protein synthesis, proliferation, and migration and increases oxidative stress [3].

Antioxidants such as chlorogenic acid, caffeine, and trigonelline, which are found in robusta and arabica coffee, can suppress the appearance of free radicals resulting from the inflammatory process. A decrease in plasma MDA levels may reflect the ability of antioxidants to suppress oxidative stress. Antioxidants in coffee can accelerate wound healing, increase hydroxyproline, reduce malondialdehyde and nitric oxide, and increase glutathione reductase [3,5].

Malondialdehyde is a compound produced from plasma lipid peroxidation by several free radicals such as ROS. Reactive oxygen species (ROS) and nitric oxide appear due to an inflammatory process due to incision wounds and hyperglycemia conditions. Increased antioxidant defense against free radicals causes a decrease in plasma malondialdehyde levels and accelerates wound healing [12–14].

Another indicator to determine the effect of the ethanol extract ointment on Robusta coffee and Arabica coffee is by measuring leukocyte levels. The number of leukocytes between the treatment groups was shown to be different based on the data in Table The results of Tukey's post hoc test analysis showed that the number of KP leukocytes was significantly different from KN, K1, and K2, with a p-value <0.05. Impaired wound healing in diabetes is associated with reduced levels of nitric oxide [6]. Nitric oxide (NO) plays an important role in the inflammatory process because it has a vasodilator, an antimicrobial effect, prevents platelet aggregation, and increases vascular permeability.

NO is responsible for upregulation and downregulation in the inflammatory phase of wound healing [6,15].

Hyperglycemia stimulates leukocytes to release a number of proinflammatory mediators in response to increased glucose. In diabetes, there is an imbalance between pro- and anti-inflammatory cytokines. Neutrophils as the first line of defense against inflammation show decreased functional activity and cause susceptibility and severity of infection.[2] Chlorogenic acid, caffeine, and trigonelline can accelerate wound healing because they provide anti-inflammatory and antibacterial effects [2,4,11].

6. Conclusion

The antioxidants of robusta and arabica coffee ethanol extracts have the same effect on oxidative stress in diabetic wounds, reducing plasma MDA (malondialdehyde) levels and leukocyte count.

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