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HEMATOLOGICAL PROFILE AND SPLEEN HISTOLOGY IMPROVEMENT IN DIABETIC RATS TREATED WITH PLGA NANOPARTICLES-ETHANOL EXTRACT OF JENGKOL (*Archidendron pauciflorum*) FRUIT PEEL

PERBAIKAN PROFIL HEMATOLOGIS DAN HISTOLOGIS LIMPA PADA TIKUS DIABETES YANG DIOBATI NANOPARTIKEL PLGA-EKSTRAK ETANOL KULIT BUAH JENGKOL (Archidendron pauciflorum)

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ABSTRACT

Jengkol (Archidendron pauciflorum) fruit peel-ethanol extract (JFP) contains several chemical compounds that act as antioxidant agents and are considered to have the potential to treat chronic disorders such as diabetes mellitus. Plant extract is generally applied in polylactic-co-glycolic acid (PLGA) nanoparticles due to its efficiency in entering the target organs. This study aims to investigate the effect of PLGA nanoparticles-ethanol extract of jengkol fruit peel (PLGA nanoparticle-JFP) on improving the hematological profile and the spleen histology of streptozotocin-induced diabetic rats. The treatment group was divided into six groups: (1) control group, (2) streptozotocin control (STZ); (3) 10 mg/kg BW of glibenclamide (GLB); (4) 770 mg/kg BW of jengkol fruit peel-ethanol extract (JFP1); (5) 110 mg/kg BW of JFP (JFP2); and (6) 770 mg/kg BW PLGA nanoparticle-JFP (PLGA-JFP). PLGA-JFP group showed a significant increase in erythrocytes and leukocytes counts ($7.73x10^6 \pm 0.02$ cell/mm^3 and $9.68x10^3 \pm 3.0$ cell/mm^3, respectively), a decrease in lymphocytes and neutrophils percentage (66.5±0.5% and 28±1.4% respectively) compared to the STZ group, and no significant difference in monocyte, eosinophils, and basophils percentage within groups. On the spleen histology, the white pulp diameter and the red pulp area showed significantly smaller (168.31±10.69 μm and 80130.28± 480.33 μm^2, respectively) compared to the STZ group. Almost all parameters showed no significant difference compared to the GLB group but are significantly different from the control group. The administration of PLGA nanoparticle-JFP was proven to reverse hematological parameters and improve the spleen histology but has not yet reversed the diabetic rats' condition back to normal.

Keywords: *diabetes mellitus, jengkol fruit peel, spleen, PLGA nanoparticle, hematological profile*

ABSTRAK

Ekstrak etanol kulit buah jengkol (Archidendron pauciflorum) (JFP) mengandung beberapa senyawa kimia yang berperan sebagai antioksidan dan dianggap berpotensi untuk mengobati gangguan kronis seperti diabetes mellitus. Ekstrak tumbuhan umumnya diaplikasikan dalam nanopartikel polylactic-co-glycolic acid (PLGA) karena efisiensinya dalam memasuki organ target. Penelitian ini bertujuan untuk mengetahui pengaruh nanopartikel PLGA -ekstrak etanol kulit buah jengkol (PLGA-JFP) terhadap peningkatan profil hematologis dan histologis limpa tikus diabetes yang diinduksi streptozotocin. Kelompok perlakuan dibagi menjadi enam kelompok: (1) kelompok kontrol, (2) kontrol streptozotocin (STZ); (3) 10 mg/kg BB glibenklamid (GLB); (4) 770 mg/kg BB ekstrak etanol kulit buah jengkol (JFP1); (5) 110 mg/kg BB dari JFP (JFP2); dan (6) 770 mg/kg BB PLGA nanopartikel-JFP (PLGA-JFP). Kelompok PLGA-JFP menunjukkan peningkatan jumlah eritrosit dan leukosit yang signifikan (masing-masing 7,73x106 ± 0,02 sel/mm^3 dan 9,68x103 ± 3,0 sel/mm^3), penurunan persentase limfosit dan neutrofil (66,5 ± 0,5% dan 28 ± 1,4% masing-masing) dibandingkan dengan kelompok STZ, dan tidak ada perbedaan yang signifikan dalam persentase monosit, eosinofil, dan basofil antar kelompok. Pada histologis limpa, diameter pulpa putih dan area pulpa merah menunjukkan secara signifikan lebih kecil (168,31 ± 10,69 μm dan 80130,28 ± 480,33 μm^2) dibandingkan dengan kelompok STZ. Hampir semua parameter tidak menunjukkan perbedaan yang signifikan dibandingkan dengan kelompok GLB tetapi berbeda nyata dengan kelompok kontrol. Pemberian nanopartikel PLGA-JFP terbukti dapat membalikkan parameter hematologis dan memperbaiki histologis limpa namun belum mengembalikan kondisi tikus diabetes menjadi normal.

Kata kunci: *diabetes mellitus, kulit jengkol, limpa, nanopartikel PLGA, profil hematologis*

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder affecting 19,5 million people in Indonesia by 2021 and is expected to rise to 28,6 million by 2045 [1]. Its prominent characteristics are known to have higher blood glucose levels, otherwise known as hyperglycemia. Prolonged hyperglycemia condition later promotes excessive production of reactive oxygen species and chronic inflammation by upregulating some inflammatory markers. Later on, oxidative stress condition takes part to decrease insulin secretion and chronic inflammation in DM can lead to the development of cardiovascular disease [2].

Hyperglycemia condition in DM can also be reflected in the hematological profile. Overwhelming levels of reactive oxygen species produced during oxidative stress can directly impact cell membrane integration and erythrocytes, being the most vulnerable to lipid peroxidation, endure a significant impact of oxidative stress, later affecting the blood circulation system [3, 4]. Moreover, diabetes mellitus condition is also known to affect the immune system, indicated by the increase of leukocyte levels, as well as lymphocytes and neutrophils levels in response to chronic inflammation [5, 6]. As a consequence of the disturbed body's immune system and erythrocytes membrane integration, it later affects the histology of the spleen, being one of the organs that takes a huge role in the immune system. The change in the red pulp area and white pulp diameter of the spleen indicates increased activity in erythrocyte remodeling and leukocyte production [7].

The prevalence of diabetic people happens to increase particularly in lower-middle-income countries and most of them remain inadequately treated [8]. Herbal treatment has been ubiquitously developed due to fewer side effects and is much more affordable. It is also known as one of the alternative treatments used in diabetic people. Indigenous people in Karangwangi village, Cianjur, West Java, have been known to treat diabetes with jengkol fruit peel [9]. Jengkol fruit peel (*Archidendron pauciflorum*) contains various phytochemical compounds, including alkaloids, flavonoids, tannins, quinones, polyphenols, saponins, glycosides, steroids (triterpenoid) [10] which have proven to have an impact on lowering blood glucose levels [11]. Flavonoids, alkaloids, and polyphenols are known to function as antioxidants which can hinder free radical molecules from interacting with lipid molecules in the cell membranes.

In general, the herbal extract is not easy to formulate effectively in oral dosage forms due to the low level of stability and its permeability once it enters the digestive tracts. One of the best ways to overcome such issues is by using the nanoparticulate system in which the herbal extract is encapsulated in a nanoparticle scale range in advance to protect the extracts from degradation and effectively enter the target organs [12]. One of the common polymers used to make nanoparticles is polylactide-co-glycolide (PLGA). PLGA is a biodegradable synthetic polymer as it can be degraded to endogenic lactate acid and glycolate acid. PLGA application for humans is approved by Food and Drug Administration (FDA) due to its low level of systemic toxicity [13].

This experimental research is aimed to acknowledge the effect of PLGA Nanoparticles-ethanol extract of jengkol (*Archidendron pauciflorum*) fruit peel on the hematological profile and the spleen histology through the observation of the white pulp diameter and the red pulp area of diabetic rats (*Rattus norvegicus*).

METHODS

Animals, treatments, and experimental approach. Twenty-four female Wistar rats (*Rattus norvegicus*) 8-12 weeks with an average weight of 182.6 grams were used in this study. The rats were maintained in an animal house under controlled temperature (22-30°C), 12 hours light-dark cycle, and fed with standard pellet CP 511 (5 gr/100 gr BW/day) and water ad-libitum. A diabetic model of rats was induced intravenously by streptozotocin (STZ) of dose 65 mg/kg BW in a 0.1 M citrate buffer solution (pH 4.5). The STZ-induced rats with blood glucose ≥250 mg/dl in 72 hours were indicated as having diabetes [14]. The rats were randomly divided into six treatment groups with four repetitions: (1) control group (0.5% of Carboxymethyl Cellulose (CMC); (2) STZ control (streptozotocin 65 mg/kg BW (STZ)); (3) GLB group (STZ+10 mg/kg BW of glibenclamide as commercial drug comparison); (4) JFP1 group (STZ+770 mg/kg BW of jengkol fruit peel-ethanol extract (JFP)); (5) JFP2 group (STZ+110 mg/kg BW of JFP); and (6) PLGA-JFP (STZ+770 mg/kg BW of PLGA nanoparticles-ethanol extract of jengkol fruit peel). All treatments were given with gavage for 14 days consecutively. On the 15th day, all the rats were sacrificed with cervical dislocation and blood collection was immediately performed and later spleen isolation.

Preparation of jengkol peel-ethanol extract and PLGA nanoparticles. Jengkol fruit peel collected from the market waste products is cleaned, dried, and grounded into simplicia. The simplicia was soaked in 70% ethanol for 3x24 hours and concentrated into a paste with a rotary evaporator. The resulting extract of jengkol fruit

peel (5 kg) consists of 650 grams of simplicia powder and 37.88 grams of jengkol fruit peel-ethanol extract paste, thus resulting in 5.83% of ethanol extract yield. Ethanol extract obtained by the maceration method was prepared into PLGA nanoparticles using the nanoprecipitation technique [15]. Jengkol fruit peel-ethanol extract (10 mg) and PLGA (50 mg) are first dissolved in acetone (3 ml). Eventually, the solution is added dropwise for 0.5 ml/minute into the solution of stabilizer (20 ml) and polyoxyethylene polyoxypropylene (1%) (F68; b/v), then stirred for 400 rpm at room temperature until the evaporation of organic solvent reached the perfect point then followed by centrifugation (25.000 rpm) at 4°C for 30 minutes. The resulting pellet is then suspended in a flask of distilled water and rinsed three times. Finally, the suspension with solid particles is heated at 40°C for 24 hours.

Spleen isolation. Isolated spleens were made into longitudinally sectioned histology slide preparation with the paraffin method and stained with hematoxylin-eosin. The spleen histology slides were observed under 400x magnification with a light microscope and the parameters observed were the white pulp diameter and the red pulp area (field of view = 5). The parameters were calculated and analyzed using ImageJ software.

Statistical analysis. All data were presented as mean ± standard deviation (SD). Data were analyzed with a one-way analysis of variance (ANOVA) followed by a Duncan's Multiple Range test using SPSS Statistics version 21. The value was considered statistically significant when Fobtained > Ftable with a confidence level of 95%.

RESULTS AND DISCUSSION

The observed parameters include complete counts of erythrocytes, leukocytes, and leukocyte differentiation. Leukocytes' differentiation cells from blood smears can be seen below in Figure 1. One-way ANOVA analysis results showed (Table 1) that there was a significant effect of the treatments given on the erythrocytes, leukocytes, lymphocytes, and neutrophils counts ($p < 0.05$).

The smears of rats' spleen histology in Figure 2 showed that every treatment altered the white pulp diameter and the red pulp area of the spleen. The white pulp diameter and the red pulp area of the control group were in the normal size whilst the white pulp diameter and the red pulp area of the STZ group were increased. The white pulp diameter and the red pulp area of GLB, JFP1, and PLGA-JFP groups were quite similar to the control group whilst the diameter of white pulp and area of red pulp of the JFP2 group were similar to the STZ group (Figure 2). Moreover, the one-way ANOVA analysis results showed (Table 2) that there was a significant effect of the treatments given on spleen white pulp diameter and red pulp area ($p < 0.05$).

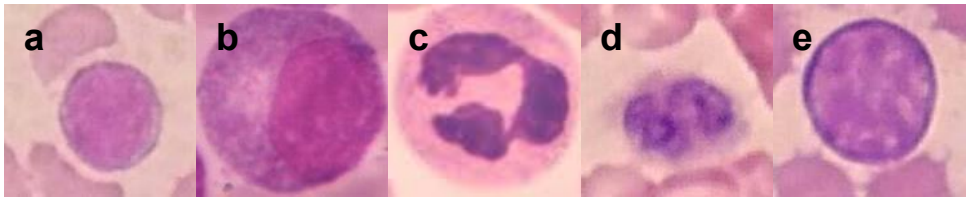

Figure 1. Leukocytes' differentiation cells. a) lymphocyte, b) monocyte, c) neutrophil, d) eosinophil, e) basophil

Table 1. The hematological profile of post-treatment rats

Treatment	Parameters			
	Erythrocytes ($x10^6$) cell/mm^3	Leukocytes ($x10^3$) cell/mm^3	Lymphocytes (%)	Neutrophiles (%)
Control	8.57 ± 0.7^{b}	4.94 ± 0.6^{a}	65 ± 0.82^{a}	28.8 ± 0.9^{b}
STZ	5.72 ± 2.4^{a}	14.6 ± 3.4^{d}	72 ± 0.82^{b}	22.3 ± 0.9^{a}
GLB	8.40 ± 0.6^{b}	7.38 ± 0.4^{ab}	66.3 ± 1.5^{ab}	28.5 ± 1.3^{b}
JFP1	7.13 ± 0.1^{ab}	10.6 ± 2.6^{bc}	66.8 ± 0.9^{ab}	27.8 ± 2.2^{b}
JFP2	7.04 ± 1.0^{ab}	12.0 ± 0.8^{cd}	$67\pm1.8^{a.b}$	26.5 ± 1.3^{b}
PLGA-JFP	7.73 ± 0.02^{b}	9.68 ± 3.0^{bc}	66.5 ± 0.5^{ab}	28 ± 1.4^{b}

Note: Data are shown in Mean±SD and analyzed with a one-way ANOVA test with a confidence level of 95% (α=0.05) which was subsequently tested with the Duncan test. Different letters in one column indicate a significant difference. Control group (CMC 0.5%); STZ group (STZ 65 mg/kg BW+CMC 0.5%); GLB (10 mg/kg BW of glibenclamide); JFP1 (770 mg/kg BW of JFP); JFP2 (110 mg/kg BW of JFP); PLGA-JFP (110 mg/kg BW of PLGA-nanoparticles of JFP ethanol extract).

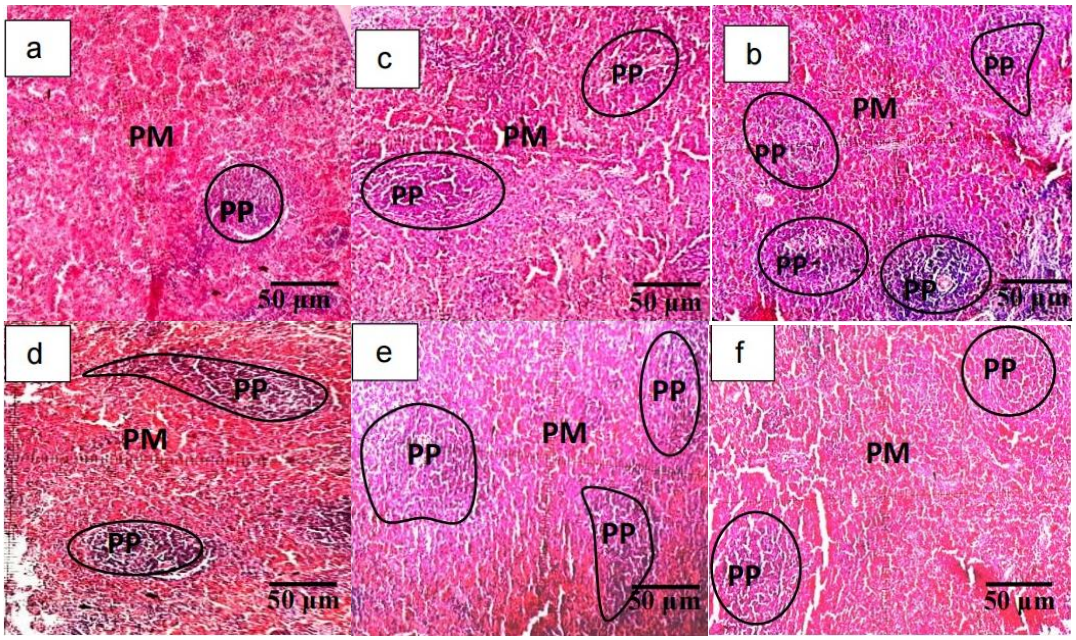

Figure 2. Histological evaluation of rats' spleen. The sagittal sections of spleens were stained with Hematoxylins-Eosin and in magnification of 100X showed the white pulp diameter and red pulp area in each treatment. A) Control group (CMC 0.5%); B) STZ group (STZ 65 mg/kg BW+CMC 0.5%); C) GLB (10 mg/kg BW of glibenclamide); D) JFP1 (770 mg/kg BW of JFP); E) JFP2 (110 mg/kg BW of JFP); F) PLGA-JFP (110 mg/kg BW of PLGA-nanoparticles of JFP ethanol extract). PP: White Pulp Diameter, PM: Red Pulp Area.

Table 2. The spleen histology of post-treatment rats

Treatment	Parameters	
	White Pulp Diameter (µm)	Red Pulp Area (µm^2)
Control	145.73 ± 4.77^a	78766.88 ± 483.17^a
STZ	221.84 ± 18.38^d	91793.50± 522.74^d
GLB	167.95 ± 9.46^b	80025.50 ± 749.57^b
JFP1	177.55 ± 4.54^b	80739.90 ± 216.28^b
JFP2	199.18 ± 5.96^c	85004.78 ± 93.98^c
PLGA-JFP	168.31 ± 10.69^b	80130.28± 480.33^b

Note: Data are shown in Mean±SD and analyzed with a one-way ANOVA test with a confidence level of 95% (α=0.05) which was subsequently tested with the Duncan test. Different letters in one column indicate a significant difference. Control group (CMC 0.5%); STZ group (STZ 65 mg/kg BW+CMC 0.5%); GLB (10 mg/kg BW of glibenclamide); JFP1 (770 mg/kg BW of JFP); JFP2 (110 mg/kg BW of JFP); PLGA-JFP (110 mg/kg BW of PLGA-nanoparticles of JFP ethanol extract).

Discussion. This present study showed that streptozotocin (STZ)-induced rats had blood glucose increased 72 hours after the injection. Increased blood glucose can cause hyperglycemia [16] which then leads to increased free radical molecule production and eventually disturbs the integration of membrane cell molecules and cell death. Reactive Oxygen Species (ROS) are free radical molecules and known to be highly reactive because the presence of one or more unpaired electrons in their valence shell causes the tendency to interact with other molecules around them, such as interacting with erythrocytes membrane cells and resulting in cell damage in erythrocyte [17, 18].

The results of erythrocytes, leukocytes, and leukocytes differentiation cell counts (Table 1), also the white pulp diameter and the red pulp area (Table 2) showed that the effect of treatment with PLGA nanoparticle-JFP (PLGA-JFP) gave a similar effect with the glibenclamide treatment but not as equal as the results from the control group. The experiment proved that treatment with PLGA-JFP can stabilize abnormal counts of erythrocytes, leukocytes, and leukocytes' differentiation and also be able to improve the spleen histology effectively in STZ-induced rats.

The result is supported by the experimental research done by Kim et al. [19] noted that PLGA-covered polyethyleneglycol (PEG) could enhance blood circulation. Other experiments showed that treatment with Umbelliferone β-D galactopyranoside (UFG) PLGA nanoparticles increased erythrocytes level in diethylnitrosamine-induced rats [20]. Leukocytes' count was also lowered in sheep erythrocytes antigens-induced mice when treated with curcumin-PLGA nanoparticles [21]. Treatment with PLGA-JFP did

not cause any allergy effect, and it can be seen in the ANOVA results of basophils and eosinophils counts that showed both parameters did not have any significant difference in each treatment (Table 2). This is only because basophils would only be reacted while antigens were invading the body [22]. Meanwhile, eosinophils have the function of maintaining allergen inflammation [23].

In type 2 diabetes mellitus patients, the lowered level of neutrophils and the increased level of lymphocytes showed that there is an implication of inflammation in the disease [24]. When treated with jengkol fruit peel, STZ-induced rats achieved a lowered level of lymphocytes and an increased level of neutrophils (Table 1) because of the flavonoids and tannins contained in jengkol fruit peel ethanol extract that can act as the anti-inflammatory [25].

The spleen organ, with its white pulp, has the function of antibody producers, and its red pulp is used to remodel the old or abnormal phagocytosed erythrocytes [7]. In this study, the spleen histology of diabetic rats is improved by PLGA-JFP treatment as observed from the narrowed size of the white pulp diameter and the red pulp area. This showed that the antioxidant and anti-inflammation properties of jengkol fruit peel extract could improve the white pulp diameter and the red pulp area. The antioxidant property of jengkol fruit peel ethanol extract is proven by the narrowed size of white pulp diameter and red pulp area as it suppresses the free radical number, antibody production, and erythrocytes remodeling activity in the red pulp of the spleen. The phytochemicals contained in PLGA nanoparticle-JFP, which include polyphenols, flavonoids, tannins, alkaloids, saponins, glycosides, quinones, and steroids [10], proved that it could be used as a diabetic treatment as it offered the ability to stabilize the hematological profile and improved the spleen histology of diabetic rats.

The mechanism of flavonoids in maintaining erythrocytes from oxidative stress is by inhibiting the lipid peroxidation caused by the production of H_2O_2 and preventing protein degradation [26]. Flavonoids account for improving diabetic complications caused by lipid abnormality and insulin resistance by modulating lipid metabolism, improving peripheral insulin resistance [27], improving glucose tolerance, maintaining the activity and expression of carbohydrate metabolism's enzyme, and affecting the insulin signaling pathway [28]. The antioxidant property of flavonoids showed by the ability to inhibit free radical interaction with cell membrane with electron transfer [29], stabilizing the free radicals by donor the flavonoids' highly reactive hydroxyl group [30], and scavenge the free radicals emanated from peroxide [26]. Therefore flavonoids can protect the lipoprotein and protein of the cell membranes [31]. The tannins contained in jengkol fruit peel ethanol extract are one of the antioxidants that account for improving the pathological condition of diabetes mellitus [32] as it possesses the ability to scavenge the free radical molecules and lowered oxidative stress activity in diabetic people, so it allows the ability to maintain blood glucose levels [33]. Hypoglycemia in diabetic people is also achieved by tannin with its ability to improve the glycogenesis process [34]. Another antioxidant contained in jengkol fruit peel is polyphenols which can function as hydrogen donors and muffle oxygen singlets [35].

Other than the phytochemicals contained in jengkol fruit peel, the PLGA-encapsulated nanoparticles affected the hematological profile and the spleen histology of the diabetic rats. Ethanol extract from the jengkol fruit peel was made in nanoparticles size as it helps to effectively enter through the blood capillary as the smallest diameter size of the blood capillary is 5-6 µm [36]. PLGA nanoparticle of ethanol extract helps maintain the integrity of the extract by minimizing the degradation possibility from digestive enzymes, and it helps to ease the absorption so then it enhances the efficacy and potentiates the therapy of diabetes mellitus [37, 38]. PLGA nanoparticle of ethanol extract enables the possibility to efficiently emanate the ethanol extract on the target organ. The efficiency of PLGA nanoparticles of ethanol extract as the diabetes mellitus treatment can be seen as it effectively stabilized the hematological profile and improved the spleen histology in diabetic rats. Nonetheless, the results given were not capable to reach the normal value.

CONCLUSION

Based on the results of the study, it can be concluded that the ethanolic extract of jengkol fruit peel (*Archidendron pauciflorum*) encapsulated in PLGA can improve the hematological profile and spleen histology of streptozotocin-induced diabetic female Wistar rats (*Rattus norvegicus*), indicated by the significant increase of erythrocytes and leukocytes counts, decrease in lymphocytes and neutrophils percentage, and smaller size of splenic white pulp diameter and red pulp area.

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