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Ethanol Extract of Pirdot (*Saurauia vulcani* Korth) as Immunostimulant in Rats (*Rattus norvegicus*)

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Abstract

BACKGROUND: Ethanolic extract of Pirdot leaves (*Saurauria vulcani* Korth) (EES) has bioactive compound to decrease glucose and increase insulin levels.

AIM: To know of effect of ethanolic extract of Pirdot leaves of immunoglobulin G and lysozyme activity in rats treated with *Sheep Red Blood Cell* (SRBC).

METHODS: Experimental design used in this research was Completely Randomized Design (CRD). There were 24 white rats to be classified into four groups: a control group with water (G_0), a group treated with 0.1 ml sheep red blood cell (SRBC) (G_1), a group treated with 500 mg/kg ethanol extract pirdot leaves (EES) (G_2), and a group treated with 500 mg/KgBW ethanol Extract pirdot leaves (EES) + 0.1 ml SRBC (G_3). Immunostimulant activity was evaluated by analyzingthe levels of immunoglobulin G with ELISA method, lysozyme with ABX Micros 60 and histophatology kidney with *Hematoxylin-Eosin stainning*.

RESULTS: The lysozyme activity in rats were treated with only SRBC () is higher and significantly different compared with a group of control (). Necrosis was not found in a group control () and rats treated EES 500 mg/kg ().There were no significant change in histophatology of kidney in rats treated EES 500 mg/kg. Disruption in bowman space with glomerulus was found in G_1 (SRBC). Antigen induced disruption in G_1 (SRBC) whereas giving extract ethanolic Pirdot leaves concurrently with antigen SRBC can protect kidney from disruption in G_3.

CONCLUSIONS: A significantly effect of immunoglobulin G, lysozyme activity and histophatology kidney in rats after given by ethanolic extract of pirdot leaves.

Introduction

Immunodrugs have been used to treat immune-related diseases but have serious side effects [1]. Modern medicine nowadays also uses active compounds isolated from plants that have a potential to treat diseases [2]. Most medicinal plants have been utilized effectively in elevating human immune system [3]. Phytochemicals, such as resveratrol, curcumin, genistein, quercetin, epigallocatechin-3-galate, camptothecin, and 3,3′-diindolymethane, are responsible for plants' immunomodulating properties and allow plants to play a main role in disease prevention [4].

The ethanol extract of Pirdot (*Saurauia vulcani* Korth) everolimus-eluting stents (EES) has bioactive compounds, including polyphenol, flavonoid, steroid, saponin, and tannin. The flavonoid compounds of Pirdot including isoflavone genistein, decrease glucose, reduce glucose tolerance, heal wounds, and increase insulin levels. Agents that induce the components of the immune system are called immunostimulants [5]. Pirdot leaves have bioactive compounds that tend to be an immunostimulant in increasing the defense of the body. EES enhances erythrocyte and lymphocyte values [6].

Immunostimulant activity was tested by evaluating the parameters of innate and adaptive immunities [7]. The ability of specific response in an antigen depends on the communication between innate and adaptive systems. The immunostimulant activity of the leaves of Buas can enhance IgG, IgM, and lysozyme [8]. Lysozyme activity is an important value of innate immune system because it has lytic activity against pathogen and activates complement system and phagocytes. Furthermore, immunoglobulins bind antigen in the recognition and effector phases of humoral immunity. Sheep red blood cell (SRBC) was used as antigen to trigger immunoglobulin production and stimulate specific antibodies. SRBC (0.1 *mL*) was administered to obtain high antigen levels and form detected antibodies [2], [9].

The histopathology of kidney is also a parameter that can indicate immunostimulant activity. The decreased immune response in uremia is caused by the reduced phagocyte function of polymorphonuclear neutrophils and monocytes or macrophages. These components are the main component of the immune system and are key targets for immunomodulatory drugs [10].

Therefore, the measurement of immunoglobulin level, lysozyme activity, and renal histopathology aimed to evaluate immunostimulant activity on this research.

Methods

Preparation plant material and extraction procedure

Fresh Pirdot leaves (S. vulcani Korth) were obtained from North Tapanuli (North Sumatera, Indonesia). The leaves were dried in an oven, blended, and soaked in 95% ethanol. The extracts were separated using a filter paper and were placed on water bath for 4 days. The ethanol extract [11], [12], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25] of Pirdot leaves was administered orally in white rats with a dose of 500 mg/kg/day for 30 days [6], [26].

Preparation of animals

Twenty-four white rats (*Rattus norvegicus*) aged 3 *month*s with an average weight of 150–200 *g* were obtained from the pharmacy of Institut Teknologi Bandung and acclimatized at 24 ± 27°C for a week in the pharmacy laboratory of Universitas Padjadjaran. Subsequently, the rats were treated for 30 *day*s and fed with feeds and water *ad libitum*. The treatment of rats received ethical approval from the Ethics Committee of Health Research of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan (Number: 497/KEPH- FMIPA/2017).

Preparation of SRBC

Sheep blood antigens were obtained from Lembang Laboratory of Veterinary Bandung, Indonesia. Sheep blood was placed on 3 *mL* vacuum tube and centrifuged at 3500 *rpm* for 5 *min*. Serum was transferred on 1.5 *mL* microtube and stored in a refrigerator at −4°C [27], [28].

Enzyme-linked immunosorbent assay

Blood samples obtained by decapitation on day 31 were collected in a tube, added with anticoagulant (EDTA), and analyzed using ABX Micros 60. Serum was separated and subjected to enzyme-linked immunosorbent assay to analyze IgG levels.

Lysozyme activity evaluation

Serum lysozyme activity was measured following the factory procedure (Sigma Cat Number L7651). Lysozyme activity was measured based on lysis suspension bacteria Micrococcus lysodeikticus [29]. Micrococcus lysodeikticus (Sigma) (0.15 mg/mL) was dissolved in 66 mM PBS (*pH* 6.2). Serum (50 µL) was added to 1 mL of bacterial suspension. The decrease in absorbance was recorded between 0.5 and 4.5 min for 4 min on a spectrophotometer with a wavelength of 450 nm. One unit of lysozyme activity is defined as the 0.001 L/min decrease in absorbance [2], [8].

Experimental design

The experimental design used in this research was completely randomized. The 24 white rats were classified into four groups: A control group treated with distilled water (G_0), a group treated with 0.1 mL of SRBC (G_1), a group treated with 500 mg/kg EES (G_2), and a group treated with 500 mg/kg EES, and 0.1 mL of SRBC (G_3). The data were analyzed by least significant difference test through SPSS version 22 with 95% accuracy. The animals were treated for 30 *day*s, and groups G_2 and G_3 were given SRBC on days 8 and 15 of the treatment [30], [31].

Histological analysis

A portion of the kidney tissue was placed in 10% formalin. The tissues were washed in running tap water, dehydrated in descending grades of isopropanol, cleared in xylene, and soaked in molten paraffin wax. The tissues were sliced into 10 µm thickness and stained with hematoxylin–eosin. Tissue slices were viewed under a light microscope for histopathology [32].

Results and Discussion

Measurement of IgG activity

Measuring IgG level is one of the parameters to evaluate the immunostimulatory activity of Pirdot leaves because IgG is the most abundant protein in human immunoglobulins and accounts for approximately 75% of the total immunoglobulin. The data are shown in Figure 1. The highest IgG level was found in G_3 (EES + SRBC), whereas IgG levels were lowest in G_1 (SRBC). G_2 (EES) had a higher IgG level than G_0 (control). IgG levels were elevated in the treatments with EES, such as in *group*s G_3 (EES + SRBC) and G_2 (EES).

Bioactive compounds, such as flavonoid, saponin, and tannin, found in Pirdot leaves stimulate the production of IgG. The ability of saponin can stimulate the cell-mediated immune system in enhancing antibody production [3], [33].

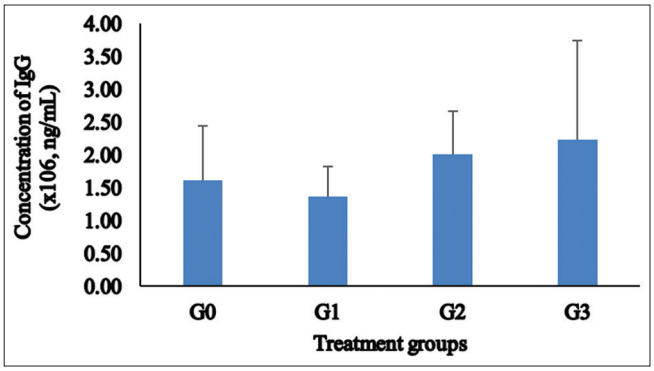

Figure 1: Measurement of IgG level (G_0 = control, G_1 = SRBC, G_2 = everolimus-eluting stents [EES], G_3 = EES + SRBC)

Effect of EES on lysozyme activities

Lysozymes are ubiquitous enzymes that have specific hydrolytic activity against foreign organisms. Lysozymes are the main factors of innate immunity due to their antibacterial, antiviral, antitumor, and immune modulatory activities [*3*], [33]. Lysozyme activity is one of the parameters that can measure immunostimulant activity. The previous studies have reported that the oral administration of 5–25 mg⁄kg nimbidin significantly inhibits the relocation of macrophage in rats in response to inflammation in the peritoneal cavity. Nimbidin attenuates the degranulation of neutrophils in releasing lysozyme [1]. *Oreochromis mossambicus* treated *Eclipta prostrata* has a significantly elevated non-specific immune response and lysozyme activity [33].

Lysozyme activities of the four groups of rats are shown in Table 1. The rats given with EES (500 mg⁄kg) had a significant elevated lysozyme activity. The rats treated with EES + SRBC had the highest lysozyme activity and followed by the rats treated with EES only. The lysozyme activity of rats treated with only SRBC was significantly higher than that of rats in the control group.

Table 1: Effect of the ethanol extract of Pirdot leaves on lysozyme activities

Treatment	µ/mg (mean±SD)
G_0 (control)	0.02±0.005
G_1 (SRBC)	0.04±0.015
G_2 (EES)	0.06±0.015
G_3 (EES+SRBC)	0.12±0.013

EES: Everolimus-eluting stents, SRBC: Sheep red blood cell.

Pirdot plant is a medicine plant used to treat some diseases. Pirdot leaves have bioactive compounds, such as flavonoid, saponin, tannin, and steroid. The flavonoid compound of Pirdot leaves includes isoflavone genistein. Pirdot has biologically active compounds that act as inflammation inhibitor and antibacterial agent [5], [6], [34]. A previous study has reported that Pirdot extract effectively accelerates wound healing. The flavonoid of Pirdot accelerates wound healing by rectifying the epithelialization process. Saponin stimulates lysozyme activity. Saponin isolated from Pirdot leaves also can stimulate the cell-mediated immune system to elevate antibody production and induce lysozyme activities [2], [8].

Effect of EES on renal histopathology

Most toxicant administration cause severe renal damage (apoptosis [35], [36], [37], [38] and necrosis [39]) by producing highly reactive free radicals [22], [40]. Plants have bioactive compounds, such as flavonoids, steroids, and alkaloids, which considerably affect nephroprotective and diuretic activities. For example, *Eurycoma longifolia* has antioxidant [29] and anti-inflammatory effects, in which the quassinoid of E. longifolia has anti-inflammatory activity that provides a protective effect against paracetamol-induced toxicity [15].

Kidney is most often targeted by the immune response against pathogens and autoimmune systems. Kidney failure can lead to the dysfunction of the intestinal barrier, systemic inflammation, and immunodeficiency, which is related to the morbidity and mortality of patients with kidney disease. Giving bangun-bangun 500 mg⁄kg body weight does not affect kidney weight, and renal function is within the normal range [2], [6].

The effect of EES on renal histopathology was observed. Figure 2 shows that necrosis was not detected in the group control and the rats treated with EES only. No significant change in renal histopathology was observed in the rats treated with 500 mg⁄kg EES alone.

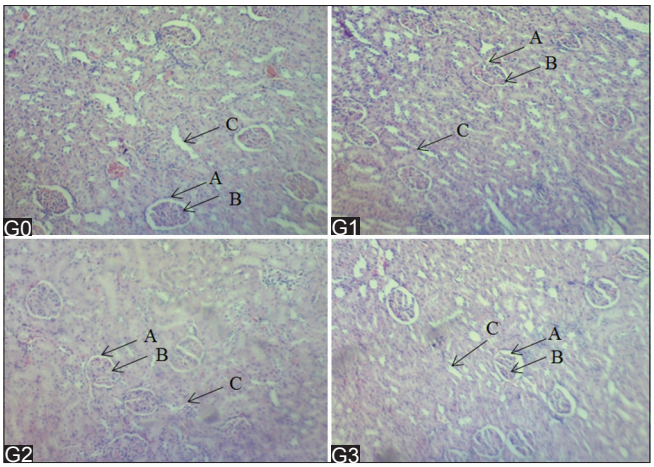

Figure 2: Renal histopathology in rats. G0: Control group, G1: Rats treated with SRBC, G2: Rats treated with everolimus-eluting stents (EES), G3: Rats treated with EES + SRBC; A: Bowman capsule, B: glomeruli; C: tubules

Giving antigen (SRBC) in G3 (EES + SRBC) and G1 (SRBC) induced changes in nephrons. Disruption in Bowman space with glomerulus was found in G1 (SRBC). Antigen induced a disruption in G1 (SRBC), whereas giving EES concurrently with antigen could protect kidney from disruption, as shown in G3 (EES + SRBC). This protection comes from the flavonoid content in the extract. Extracts containing flavonoids aid in kidney healing and protection [41], [42]. The flavonoid content is antioxidant and repairs renal glomerular structure damage thereby increasing glomerular filtration rate [42].

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