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Noni Simplistic Effect with Chicken Shank Gelatin Film on White Rat Spleen Exposed to Dexamethasone

(EFEK SIMPLISIA MENGKUDU DENGAN FILM GELATIN CEKER AYAM TERHADAP LIMPA TIKUS PUTIH YANG TERDEDAH DEKSAMETASON)

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ABSTRACT

Dexamethasone is a corticosteroid drug belong to glucocorticoid group. Dexamethasone is immunosuppressant and anti-inflammatory in various inflammatory conditions. Side effects of its use can cause cell apoptosis in various organs such as the spleen. The immunosuppressant effect of dexamethasone can reduce and inhibit peripheral lymphocytes and macrophages until the death of lymphoid cells in the white pulps of the spleen. The simultaneous effect of administering chicken shank gelatin and noni has the potential to improve the structure of the spleen. This study was aimed to prove and obtain effective and safe dose of chicken shank gelatin and noni on the spleens of rats exposed to dexamethasone. The research was carried out experimentally in the laboratory with a completely randomized design (CRD). A total of 25 heads male rats were grouped into five treatments and each treatment consist of five repetitions. There was a treatment group P1 as a negative control, P2 as a positive control (dexamethasone 5 mg/kg BW), P3-P5 (dexamethasone 5 mg/kg + gelatin 1.585 mg/kg + noni simplicia 50; 112; 250 mg/kg BW). The results of the study showed an increase in the area of white pulps and a decrease in the percentage of necrotic cells in the spleen, however, it did not increase the relative weight of the spleen and serum albumin levels (P>0.05). In conclusion, the effective and safe dose for the spleen organs of rats exposed to dexamethasone is 250 mg/kg BW.

Key words: dexamethasone; chicken shank gelatin; spleen; noni

ABSTRAK

Deksametason merupakan obat kortikosteroid dari golongan glukokortikoid. Deksametason bersifat immunosuppressant serta antiinflamasi pada berbagai kondisi radang. Efek samping penggunaannya dapat menyebabkan apoptosis sel pada organ-organ tubuh seperti limpa. Efek imunosupresan deksametason dapat menurunkan serta menghambat limfosit dan makrofag perifer hingga kematian sel limfoid pada pulpa putih limpa. Pengaruh simultan pemberian gelatin ceker ayam dan mengkudu berpotensi meningkatkan perbaikan struktur limpa. Penelitian ini bertujuan untuk membuktikan dan mendapatkan dosis efektif serta aman dari gelatin ceker ayam dan mengkudu terhadap limpa tikus yang terdedah deksametason. Penelitian dilakukan secara eksperimental di laboratorium dengan Rancangan Acak Lengkap (RAL). Sebanyak 25 ekor tikus jantan dikelompokkan kedalam lima perlakuan dan setiap perlakuan terdiri atas lima ulangan. Terdapat kelompok perlakuan P1 sebagai kontrol negatif, P2 sebagai kontrol positif (deksametason

5 mg/kg BB), P3-P5 (deksametason 5 mg/kg + gelatin 1.585 mg/kg + simplisia mengkudu 50; 112; 250 mg/kg BB). Hasil dari penelitian menunjukkan adanya peningkatan luas pulpa putih dan penurunan persentase sel nekrosis pada limpa, akan tetapi, belum meningkatkan bobot relatif limpa dan kadar serum albumin (P>0.05). Simpulan penelitian ini adalah, dosis efektif dan aman terhadap organ limpa tikus yang terdedah deksametason adalah 250 mg/kg BB.

Kata-kata kunci: deksametason; gelatin ceker ayam; limpa; mengkudu

INTRODUCTION

Immune disease is caused by a decrease in the structure and function of the body. The immune disease can be caused by several factors, one of which is the use of drugs containing corticosteroids (Lane, 2019). Dexamethasone is a corticosteroid drug that has side effects on other vital organs of the body like the spleen organ.

Gelatin is one of the biomaterials that can be used as an antiinflammation and immunostimulation. Kuntana *et al*. (2020) state that chicken shank gelatin has stimulated collagen synthesis so gelatin could be an environmental-friendly product to prevent immune diseases. Other herbal ingredients are needed to support the optimization of gelatin.

Noni is a plant that has a high antioxidant content. High antioxidant levels can restore the spleen organ from oxidative stress (Guo *et al*., 2020). One of the compounds contained in noni is flavonoids. Flavonoids can stimulate the proliferation of bone marrow mesenchymal stem cells (BMSC) and osteoblastogenesis in bone cells. This indicates that noni can be used as an herbal remedy for immune diseases like osteoporosis (Hussain *et al*., 2016).

The spleen is an organ that is sensitive to exposure to foreign substances. The structure and function of the spleen can be an indicator of the safety of providing gelatin films and noni simplicia. There is no further research regarding the safety of gelatin and noni on the spleen. The study was aimed to observe the effect of giving noni simplicia with gelatin film. The outcome will be observed in the spleen of white rats that exposure to dexamethasone.

RESEARCH METHODS

Methods

This research was conducted experimentally in the laboratory, using a completely randomized design (CRD) with five treatments and each treatment consist of five replications. The test animals used in the study were 25 male Wistar strain white rats (*Rattus norvegicus*) aged 2-3 months and weighing 180-220 g (coefficient of diversity ≤ 10%). A total of 25 male Wistar rats were grouped into five treatments and five repetitions in each group. Treatment of P1 as the negative control, P2 as the positive control (dexamethasone 5 mg/kg BW), P3- P5 (dexamethasone 5 mg/kg + gelatin 1.585mg/kg + noni simplicia 50; 112; 250 mg/kg BW). Injection of dexamethasone was given subcutaneously for seven days. Noni and gelatin were administered orally for 14 days. Spleen parameters included relative spleen weight, white pulp surface area, percentage of necrotic spleen cells, and serum albumin level.

Preparation of Noni Simplicia

Noni fruit is obtained from the yards of residents' houses around the Faculty of the Mathematic and Natural Science, Department of Biology, Umiversitas Padjadjaran, Jatinangor, Sumedan. First of all is washing the noni fruit so that the fruit becomes clean, then cutting the noni fruit crosswise and thinly is carried out. The noni fruit slices were then placed under the sun to dry for 72 hours. The dried noni fruit is then mashed using a blender to become powder. The noni simplicia was sieved using a 40 mesh to get a finer powder.

Preparation of Chicken Shank Gelatin Film

The chicken shank used in this study was obtained from the Resik Jatinangor Market. Chicken shanks are soaked in water at 50-60°C for 30 minutes. The chicken shank is then skinned to remove the bones. The chicken shank is cut into about 5-10 cm pieces, followed by the soaking process in the acid solution. Soaking each solution was repeated three times with a ratio composition of chicken claw skin (shank) and immersion solution of 1:5. The result was carried out alternately for 40 minutes, starting with immersion in 0.1% NaOH solution, sulfuric acid (H_2SO_4), and 0.4% citric acid ($C_6H_8O_7$). This washing process is carried

out after immersion in an acid solution. This washing process uses running water to restore the collagen pH to its original state. Afterward, the collagen enters the extraction process in a water bath at 65°C for seven hours.

The extracted gelatin liquid is placed in an aluminum container and dried in an oven at 60°C for eight hours. The result obtained is in the form of gelatin in the form of sheets. The gelatin sheets are then mashed to form gelatin powder. Gelatin powder 1,585 mg/kg and noni according to each research dose were weighed and then dissolved using distilled water and 2% food grade technical glycerol plasticizer. Gelatin films were stirred for 30 minutes until homogeneous (Muttaqien *et al*., 2013).

Data Analyze

Data were analyzed using the parametric statistical test Analysis of Variance at the 95% confidence level (α=0.05) and continued with Duncan's Multiple Range Test by using Statistical Package for Social Sciences (SPSS) 21.0 for Windows.

RESULTS AND DISCUSSION

Spleen Relative Weight

The results showed that the treatment of gelatin film and noni simplicia had no significant effect on increasing the relative weight of the spleen.

White Pulp Area

Treatment of noni and gelatin significantly increased the white pulp area of the spleen of rats. The results of this study are also in line with research reported by Al-Niaeem *et al.* (2015), they proposed spleen responds to gelatin by increasing the white pulp area in the spleen.

The white pulp is part of the spleen, which contains many lymphocyte cells. B and T lymphocytes are present in the spleen's white pulp. The white pulp on the histological picture can be onserved in the purple area. The Periarteriolar lymphoid sheath (PALS) area is visible outside the central artery in the P1 treatment group (negative control). There is also a marginal zone that delimits the white pulp and red pulp at the periphery of the white pulp of the spleen. This limit was oberved in the P1 group. In the P2 group (positive control), there was a decrease in the area of the white pulp. The boundary between the white and red pulp zones is unclear. The red pulp zone looks more extensive compared to the other groups. Spleen follicles were looked smaller than the other treatment groups. Treatment of gelatin films and noni simplicia significantly increased the white pulp area of the spleen in groups P4 and P5. The marginal zone was observed more clearly in the rat group after treatment. The highest increase in the white pulp area close to the negative control was in the P5 treatment group (gelatin film + noni 250 mg/kg). The white pulp zone looks broader and more precise.

Table 1. Relative weight of spleen organs in male white rats (*Rattus norvegicus*) after dexamethasone injection and combination treatment of noni and gelatin

Treatment	Spleen Relative Weight ($\bar{x}$ ± SD (%))
P1	0,39 ± 0,10
P2	0,32 ± 0,13
P3	0,37 ± 0,11
P4	0,41 ± 0,16
P5	0,52 ± 0,13

Note: Means in the same column with different superscripts differ insignificantly (P>0.05). P1=negative control with aqua dest + food grade glycerol 2%; P2=positive control with dexamethasone 5 mg/kg; P3-P5 = (dexamethasone 5 mg/kg + gelatin 1.585mg/kg + noni simplicia 50;112;250 mg/kg)

Table 2. White spleen pulp area of male white rat (*Rattus norvegicus*) after dexamethasone injection and combination treatment of noni and gelatin

Treatment	White pulp area ($\bar{x}$ ± SD (μm^2))
P1	123.543,33 ± 4.686,29^{c}
P2	101.458,40 ± 6.282,66^{a}
P3	104.080,71 ± 2.998,72^{a}
P4	110.114,81 ± 5.305,85^{b}
P5	121.904,67 ± 1.991,89^{c}

Note: Means in the same column with different superscripts differ significantly (P<0.05). P1=negative control with aqua dest + food grade glycerol 2%; P2=positive control with dexamethasone 5 mg/kg; P3-P5=(dexamethasone 5 mg/kg + gelatin 1.585mg/kg + noni simplicia 50;112;250 mg/kg)

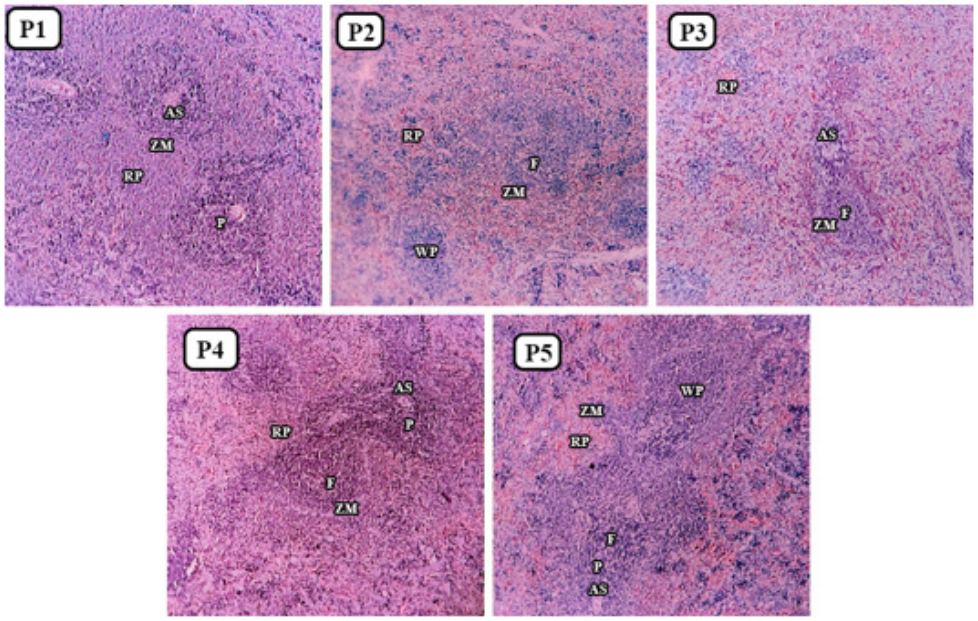

Figure 1. Histology of white rat spleen after treatment with dexamethasone injection and a combination treatment of noni and gelatin. (Hematoxylin-Eosin staining, 100 times magnification) (P1= negative control (aqua dest + glycerol); P2= positive control (dexamethasone + glycerol); P3= (dexamethasone + noni 50 mg/kg + gelatin); P4= (dexamethasone + noni 112 mg/kg+gelatin); P5= (dexamethasone+ noni 250 mg/kg + gelatin); AS = Central Artery; ZM= Marginal Zone; WP= White Pulp; RP= Red Pulp; P= PALS; F= Follicle)

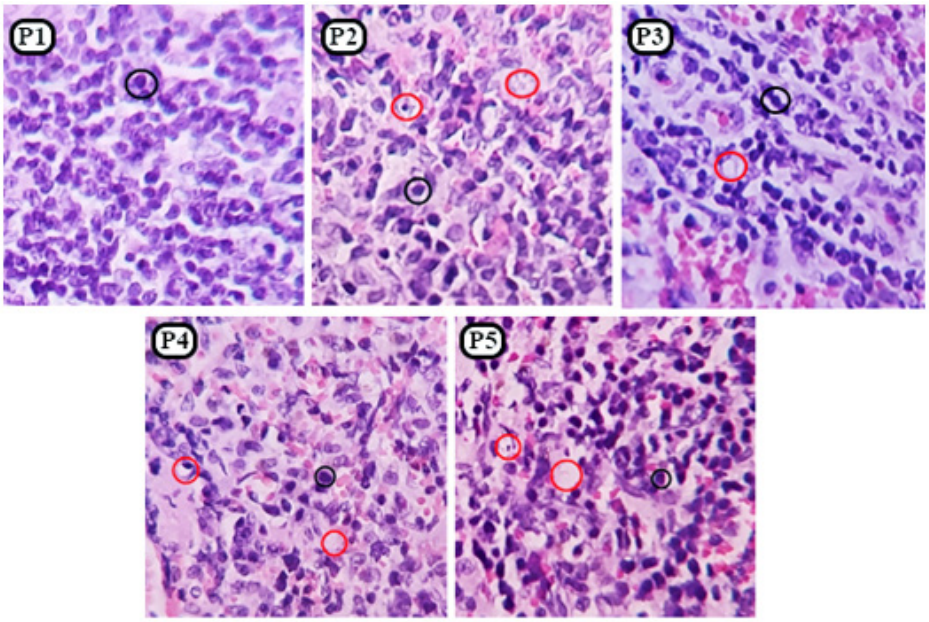

Figure 2. Histology of white rat spleen after treatment with dexamethasone Injection and a combination treatment of noni and gelatin. (Hematoxylin-Eosin staining, 400 tims magnification) (P1= negative control (aquadest + glycerol); P2= positive control (dexamethasone + glycerol); P3= (dexamethasone + noni 50 mg/kg + gelatin); P4= (dexamethasone + noni 112 mg/kg + gelatin); P5= (dexamethasone+ noni 250 mg/kg + gelatin); Dark circles = normal cells; Red circle = necrotic cells)

Table 3. Percentage of spleen cell necrosis in male white rats (*Rattus norvegicus*) after dexamethasone injection and combination treatment of noni and gelatin

Treatment	Percentage of Splenic Necrotic Cell ($\bar{x}$ ± SD (%))
P1	3,88 ± 1,01[a]
P2	21,72 ± 3,11[d]
P3	11,38 ± 0,82[c]
P4	9,32 ± 0.79[bc]
P5	8,72 ± 1,02[b]

Note: Means in the same column with different superscripts differ significantly (P<0.05). P1=negative control with aqua dest + food grade glycerol 2%; P2=positive control with dexamethasone 5 mg/kg; P3-P5=(dexamethasone 5 mg/kg + gelatin 1.585mg/kg + noni simplicia 50;112;250 mg/kg)

Table 3. Serum albumin levels of male white rats (*Rattus norvegicus*) after dexamethasone injection and combination treatment of noni and gelatin

Treatment	Serum Albumin Level ($\bar{x}$ ± SD (g/dL))
P1	4,00 ± 0,09
P2	3,66 ± 1,14
P3	4,09 ± 0,62
P4	3,51 ± 0,34
P5	3,65 ± 0,46

Note: Means in the same column with different superscripts differ significantly (P<0.05). P1=negative control with aqua dest + food grade glycerol 2%; P2=positive control with dexamethasone 5 mg/kg; P3-P5=(dexamethasone 5 mg/kg + gelatin 1.585mg/kg + noni simplicia 50;112;250 mg/kg)

Percentage of Necrotic Spleen Cell-

Treatment of gelatin film and noni simplicia significantly reduced the percentage of necrotic spleen cells of white rats injected with dexamethasone . The P1 treatment group (negative control) has the lowest percentage of necrotic spleen cells, which is indicated by the percentage of necrosis below 10%. The highest percentage of necrotic spleen cells were in the P2 treatment group (positive control). A low percentage of necrotic cells are characterized by an increased percentage of necrosis above 20%.

The stages of necrotic cells in the spleen histology were pyknosis, karyorrhexis and karyolysis. A shrunken, dark-colored nucleus characterizes pyknosis. Karyoriexis is characterized by the nucleus breaking into fragments. Karyolysis is characterized by nuclei that cannot be stained or lost.

Serum Albumin Level

The results showed that the treatment of gelatin films and noni simplicia had no significant effect on increasing serum albumin levels in the body. The results showed that gelatin film and noni simplicia in the three test groups P3 (50 mg/kg), P4 (112 mg/kg), and P5 (250 mg/kg) were at safe doses because the serum albumin levels of the three test groups are within normal limits.

Dexamethasone, as an immunosuppressant, can cause oxidative stress. Oxidative stress happens because immune cells in the body are damaged or do not reach the maturation stage. The incomplete maturation of splenic cells causes a severe reduction of the number of cells of the spleen. Dexamethasone inductions also reduce the area of the white pulp of the spleen. According to Rousdy and Wardoyo (2018), changes in the area of the white pulp can be indicated as changes in the number of lymphocyte cells in the spleen. If there is a change in the white pulp spleen's area, it could be stated that there is a change in the activity of the immune system that occurs in the body. Mitevska *et al*. (2015) stated that dexamethasone induction led to a decrease in lymphocyte proliferation in the white pulp area. The decrease in lymphocyte proliferation causes the area of the white pulp to become small.

The relative weight of the spleen is strongly influenced by lymphocyte activity. These cells include macrophages, granulocytes, T lymphocytes, and B lymphocytes.

Noni fruit can cause the proliferation of lymphocyte cells in the white pulp of the spleen. Flavonoid compounds contained in noni fruit can increase the production of IL-2 and the proliferation and differentiation of T and B cell lymphocytes in the spleen (Parlinaningrum *et al.,* 2014). Lymphocyte cell proliferation will affect CD4+ cells and activate Th1 cells so that immunity will increase.

Noni has active compounds in the form of flavonoids. Flavonoids can stabilize free radicals in the body. Free radical compounds

that bind to flavonoids can restore the body from oxidative stress. Noni, as an immunostimulatory, works to increase T cell proliferation ability. An increase in the number of T lymphocyte cells can trigger an increase in macrophage activation (Wahyuni *et al*., 2019), which can be observed from the white pulp area of rats after treatment with gelatin film and noni simplicia. This study's results align with Afiqoh *et al*. (2017), which stated that flavonoids could increase the area of the white pulp. Lymphocyte cells that experience proliferation cause the weight of the spleen to increase.

According to Kumar and Pandey (2013), flavonoids can combat Reactive oxygen species (ROS) or free radicals, which are abundant in the body. Flavonoids donate hydrogen atoms and electrons to free radicals through the configuration of the hydrophilic group of ring B. Unpaired free electrons can be bound by flavonoids and then become compounds that are stable and harmless to the body. The bond between ROS and flavonoids causes a decrease in free radical levels. A decrease in ROS levels can also cause a decrease in the percentage of cells that experience necrosis. This study's results align with Goein *et al*. (2020), who stated that noni fruit extract (*Morinda citrifolia*) had been proven to reduce the number of necrotic hepatocyte cells.

The increase in the white pulp area was due to the immunostimulant activity carried out by gelatin. Gelatin increases the non-specific immune response (B cells) and antibody production. The proliferation of B cells causes an increase in the area of the white pulp of the spleen. Increased B cell proliferation can also be an indicator of an increased immune system.

Gelatin has various amino acids contained in it. Higuera *et al*. (2016) stated that gelatin contains antioxidant compounds. The change from collagen to gelatin through the hydrolysis process can increase the antioxidants in gelatin. The triple helix structure of collagen opens, and collagen is split into peptide chains when heated. The antioxidant activity in gelatin can reduce levels of malondialdehyde (MDA). The MDA is one of the free radicals resulting from lipid peroxidation. Decreasing levels of free radicals in the body can restore the body's state from a state of oxidative stress. When the body returns from a state of oxidative stress, the percentage of spleen cell necrosis can also decrease.

According to Spinella *et al*. (2015), one of the functions of albumin is as an antioxidant and scavenger of free radicals in the blood. The spleen is one of the alternative organs in cell hematopoiesis. Albumin will bind with bilirubin in the spleen and then go to the liver for further synthesis. Normal albumin levels indicate that the physiology of the spleen in the process of hematopoiesis of red blood cells is still running normally.

CONCLUSION

Based on the results, it can be concluded that the combination of noni (*M. citrifolia*) simplicia with chicken shank gelatin can increase the area of the spleen white pulp and decrease the percentage of necrotic spleen cells in white rats. The effective dose of noni (*M. citrifolia*) simplicia with gelatin film shank of broiler chickens, which can improve the structure of the spleen organs of white rats, is 250 mg/kg. However, the treatment has not been able to increase the spleen relative weight and serum albumin levels in white rats.

SUGGESTION

A proximate test process is needed on gelatin and antioxidant analysis on noni to complete the contents of the two test materials. Research on other spleen functions, such as hemoglobin and bilirubin levels, is necessary.

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