Original Article

The effect of ethanol extract oak gall (Quercus infectoria G. olivier) on the cellular immune response of mice

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ABSTRACT

Oak gall (Quercus infectoria G. Olivier) has many benefits. It is used as natural astringen material for treating infections such as candidiasis and also as a burn remedy to help heal the skin and prevent infection. The purpose of this research was to determine the characteristic of simplicia, phytochemical screening and the ethanol extract effect of oak gall on cellular immune response of mice. Determination of simplicia characterization was carried out on dry powder oak gall simplicia. Phytochemical screening was performed to simplicia and extract. The extract was macerated with 80% ethanol then evaporated using rotary evaporator to get a thickened extract and dried using freeze dryer. Ethanol extract oak gall was tested for its cellular immune response in mice by counting the total number of white blood cells (leukocytes) and differential count of leukocytes. The results of characteristics' examination of dried powder simplicia oak gall showed a water content 7.97%, water soluble extract 56.46%, ethanol soluble extract 60.59%, total ash content 1.60% and acid soluble ash content 0%. Phytochemical screening result showed that ethanol extract oak gall positively contains alkaloids, glycosides, flavanoids and tannins. Leukocytes count decreased after cyclophosphamide induction and increased after administration of ethanol extract oak gall at dose of 50, 75 and 100 mg/kgBW. The differential leukocyte count obtained the neutrophil cells, lymphocytes and monocytes. Ethanol extract oak gall (quercus infectoria g. olivier) has an effect on the cellular immune response of mice and it showed a dose-dependent manner.

Keywords: Oak Gall, Leukocyte, Mice, Cyclophosphamide.

Introduction

Herbal medicine has been used for a long time in worldwide, according to World Health Organization (WHO), 65% population of developed countries and 80% population of developing countries use herbal medicine as alternative remedies. WHO support the campaign "back to nature", by giving recommendations for using herbal medicine, which include maintaining health and diseases' prevention, especially infectious, chronic degenerative diseases and cancer [1-6].

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Indonesia Health Profile 2010 showed that the prevalence and case number of infectious diseases in Indonesia are higher compared with ASEAN countries (Association of Southeast Asian Nations). The status of immunity becomes important in maintaining health, because one of the causes of infection is decreased immune system [2,7-10].

Immune system is the body's defense against infectious. Prevention of infectious diseases can be done by improving the individual's immune system. Immune system is affected by white blood cells (leukocytes). Leucocytes are one of the blood cells' component that play a role in the defense system of the body, which is partially formed in bone marrow and tissues lymph. There are five different types of leukocytes normally found in the blood that are neutrophils, eosinophils, basophils, monocytes and lymphocytes. These cells are formed and then transported to blood toward various body parts to use. Most of leukocytes are specifically transported to the infected and inflammation areas [3,11].

One of the medicinal plants is oak gall (Quercus infectoria G. Olivier) that has been used as natural remedies. Initial

research showed that oak gall was used for postpartum care. Arabian, Persian, Indian, Malaysian, and Chinese have traditionally used it to treat vaginal candidiasis and post-infection labor. Moreover, it can be used to treat various diseases, including the diseases related to the immune system [4,12,13].

The main component of oak gall is tannin (50-70%) especially the galicine acid that is formed of tannin acid, gallic acid (2-4%), ellagic acid, siringat acid, calcium oxalate, resin and starch [5].

Blood examination is a parameter that can be used to describe the condition of the body. Effectiveness of oak gall to the immune system can be observed through examination the blood including the total count leucocyte and differential cells [6]. Based on the above description, researchers are interested in determining the characteristic of simplicia, phytochemical screening, and effects ethanol extract oak gall on the cellular immune response.

Material and Methods

Plant Material

Oak gall sample was collected from a traditional medicine store Sambu Market at Medan, Northern Sumatra, Indonesia.

Extraction of oak gall

An amount of 1800 g dried material plant samples were crushed in a blender, then macerated in ethanol 80% for 5 days, thereafter countinued to remecerate for 2 days. The solvent was evaporated at low pressure with a temperature of not more than 40°C using a Rotary evaporator, then dried using freeze dryer [7].

Preparation of Simplicia Characterization

Simplicia characterization includes macroscopic examination, microscopic examination, determination of water content, determination of total ash content, determination of ash content not soluble in acid, determination of ethanol soluble extract and determination of water soluble extract [8].

Animals and blood sample

Animals used in this study were 25 male mice, weighing 20-30 g, which were divided into 5 groups, each group consisting of 5 mice. The blood sample was collected from tail vein.

Preparation suspension of ethanol extract of oak gall 0.5%

An amount of 125 mg ethanol extract of oak gall was crushed in mortar, then a 0.5% CMC suspension up to 25 ml was added.

Determination of leukocytes count and differential of leukocytes

25 male mice divided into 5 groups, each group consists of 5 mice. The group I was a normal control without treatment, groups II, III, IV, V were induced with 0.5% cyclophosphamide at a dose of 30 mg / kgBW by intra-peritoneal. After 30 hours of cyclophosphamide administration, groups III, IV, V were given ethanol extract oak gall at a dose of 50 mg / kg BW, 75 mg / kg BWand 100 mg / kg BW respectively, for 7 days. After that, the blood was collected from the mouse's tail and put in microtub to determine the total count of leukocytes and differential leukocytes.

Determination of leukocytes was performed by using Thoma's pipette. Blood sample given anticoagulants (EDTA) was drawn to the 0.5 mark with a pipette. Blood in pipette was immediately drawn to turk solution until reaching "11" mark while rotating the pipet to mix the specimen and diluent for 3-5 minutes, in order to obtain 1:20 dilution. The first 2-3 drops of blood were thrown away. Then the blood was dropped to counting chamber and was left for one minute to lysis the erythrocyte. The leukocytes were counted under 40 magnification microscope to 4 counting chamber. The number of leukocytes each millimeter cubed (mm3) is the number of cells multiplied by 50 [9].

Determination of differential leukocytes was done by blood smear method. One drop of blood was taken from each group and put in object glass. Using the corner of another object glass, the blood drop was spread. Blood smear preparations were fixed with methanol for 3-5 minutes, then allowed to dry. Blood smear is further stained with Giemsa solution for 15-20 minutes. After washing in tap water, the slides were air-dried and examined under 100 magnification.

Results and Disscusion

Phytochemical screening result of ethanol extract oak gall

Phytochemical screening result showed that ethanol extract oak gall positively contains Flavonoids, Alkaloids, Tanins and Glycosides.

Simplicia Characterization

Table 1: Determination of Simplicia Characterization of oak gall

Caracterization	% content
Water content	7.91
Total ash content	1.6
Ash content is not soluble in acid	0
Content of ethanol soluble extract	60.59
Content of water soluble extract	56.46

Immun Response Test Results

Total leukocytes count

Table 2: Leukocytes count

Experimental groups	leukocytes count (Cells/mm^3)					Total leukocytes count (Cells /mm^3)	Mean leukocytes count (Cells /mm^3)
	1	2	3	4	5		
Group I (normal Control)	11100	10700	9750	9500	9600	50650	10130
Group II (cyclophosphamide control)	4400	4200	3800	3800	2900	19100	3820
Group III (treatment group)	4650	4500	4500	4200	4000	21850	4370
Group IV (treatment group)	4800	4650	4500	4500	4200	22650	4530
Group V (treatment group)	5500	4800	4650	4650	4500	24100	4820

Leukocytes of the immune system are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system [10].

As Table 2 shows, group II-V have an amount of leukocyte cells lower than group I (normal group). Leukocyte has decreased by cyclophosphamide induced that work as immunosuppressant by killing activated lymphocyte cells and also depress bone marrow, which causes lymphopenia that decreases in number lymphocytes in the blood [11].

Group III-V that was induced by cyclophosphamide and given ethanol extract oak gall at doses of 50, 75 and 100 mg/kgBW respectively, have increased in number of leukocytes compared to group II that was given cyclophosphamide without any treatment. The phytochemical constituent of oak gall which has an immunostimulant effect is Gallic acid. Previous study revealed the immunostimulatory potential of gallic acid against two anticancer drugs, cyclophosphamide and cisplatin which induced immunosuppression in Swiss albino mice [12].

Gallic acid improved the antibody response effects, indicating that it may facilitate humoral immune response. The immunity involves interaction of B cells with the antigen and its subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effector of the humoral response by binding to antigen and neutralizing it or facilitating its elimination by cross-linking to form clusters that are more readily ingested by phagocytic cells [13]. The immunomodulatory activity ethanol extract of oak gall showed a dose-dependent manner.

Leukocytes differential count

Table 3: Percentage of neutrophil count

Experimental groups	Neutrophil Count (%)					Total Neutrophil Count (%)	Neutrophil Count (%)
	1	2	3	4	5		
Group I (normal Control)	55	64	60	65	65	309	61.8
Group II (cyclophosphamide control)	36	50	48	48	50	232	46.4
Group III (treatment group)	42	45	50	51	55	243	48.6
Group IV (treatment group)	47	50	55	59	65	276	55.2
Group V (treatment group)	55	59	65	65	70	314	62.8

Table 4: Percentage of lymphocyte count

Experimental groups	lymphocyte count (%)					Total lymphocyte count (%)	Mean lymphocyte count (%)
	1	2	3	4	5		
Group I (normal Control)	34	28	21	27	30	140	28
Group II (cyclophosphamide control)	9	17	8	11	13	58	11.6
Group III (treatment group)	42	42	42	40	41	207	41.4
Group IV (treatment group)	44	49	45	46	48	232	46.4
Group V (treatment group)	56	55	55	51	55	272	54.4

Table 5: Percentage of monocyte count

Experimental groups	Monocyte count (%)					Total Monocyte count (%)	Mean Monocyte count (%)
	1	2	3	4	5		
Group I (normal Control)	11	8	17	12	15	63	12.6
Group II (cyclophosphamide control)	6	6	10	7	11	40	8
Group III (treatment group)	8	7	11	10	12	48	9.6
Group IV (treatment group)	9	8	11	12	12	52	10.4
Group V (treatment group)	10	11	11	12	12	56	11.2

The leukocyte cell differential count only found neutrophil cells, lymphocytes and monocytes, whereas eosinophils and cells basophile were not found. Administration of ethanol extract of oak gall increaced the percentage of neutrophils, lymphocytes and monocytes. The neutrophil, lymphocyte and monocyte levels of the mice given 50 mg, 75 mg and 100 mg/kgBW garlic per day for seven days' duration were higher than the cyclophosphamide control animals.

Conclusion

Ethanol extract of oak gall at doses of 50, 75 and 100 mg /kgBW increased total leukocytes and differential count of mice which is induced by cyclophosphamide and it showed a dose-dependent manner.

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