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LYMPHOCYTE PROLIFERATION ON HYPERSENSITIVITY OF Balb/C MICE AFTER GIVEN ETHANOL EXTRACT TUBER OF *Dioscorea alata* L.

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ABSTRACT

Purple yam (*Dioscorea alata* L.) is a source nutritious tubers that has not been used optimally. Nutrients content in *Dioscorea* species are starch, essentials amino acid, minerals, polyphenol, glycoprotein, purin derivates such as allantoin, and steroid saponin which have biological activity such as immunomodulatory and antiallergic. The purpose of this research was to assess the absolute number of lymphocyte on hypersensitivity of mice after treated with ethanol extract of *D. alata*. Twenty one male Balb/C mice were used which were divided into seven groups: control (C), negative control (NC), treatments with ethanol extract of *D. alata* 0.17 g/kg, 2.01 g/kg, 10.04 g/kg (T I – T III), treatment with antihistamine drug 0,4 mg/mice/day (T IV) and treatment with diosgenin 200 mg/kg (T V). For 17 consecutive days the T I - T III groups were treated with ethanol extract of *D. alata* correspond to their doses, T IV group were treated with antihistamine drugs, and T V group were treated with diosgenin. On day 15, NC and T I – T V groups of mice were induced by ovalbumin 0,0483 mg/mice. Mice were sacrificed on day 18, and the lymphocyte was isolated from spleen, and the absolute number of lymphocyte was counted with Haemocytometer. Results showed that the absolute number of lymphocyte on mice hypersensitivity after treated with ethanol extract of *D. alata* L. were the lowest, while the highest absolute number of lymphocyte was found in the group treated with antihistamine drugs followed by the group treated with diosgenin on 200 mg/kg BW, negative control group and control group, respectively.

Keywords: Balb/C mice, *Dioscorea alata*, ovalbumin, hypersensitivity, lymphocyte

INTRODUCTION

Purple yam (*Dioscorea alata L.*) is a source of nutritious tubers that has not been used optimally. Nutrients content in *Dioscorea* species are starch, essentials amino Acid, mineral, polyphenol, mucilage (glycoprotein), purin derivates (such as allantoin) and steroid saponin (Wanasundera & Ravindran, 1994; Lape & Treche, 1994; Agbor-Egbe & Treche, 1995; Hikino *et al.*,1986, He *et al.*, 1994; Hu *et al.,* 1996; Hu *et al.*,1997; Yang *et al.*, 2003; Wang *et al.*, 2011; Yoon *et al.*, 2008). *D. alata* L. contains diosgenin (Cheng *et al.,* 2007), a main steroidal saponin steroid aglicon as intermediate steroidal in pharmaceutical manufacture. Steroidal saponin is the most important bioactive compound due to its biological functions such as anticarcinogenic, antithrombotic, antiviral, hemolitic, hypocholesterolemic, hypoglichemic, immunostimulatory, antitumorogenic, antimutagenic, immunomodulatory and antiinflammatory (He *et al.*, 1994; Hu *et al.*, 1996; Hu *et al.,* 1997; Yang *et al.*,2003). Results of previous studies showed that steroidal saponin has antiallergic activity (Zhang *et al.,* 2012). The purpose of the research was to assess the absolute number of lymphocyte on hypersensitivity of mice after treated with ethanol extract of *Dioscorea alata L.* tubers.

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MATERIALS AND METHODS

Experimental research on Balb/C mice with posttest only control group design. Independent variable is ethanol extract of *Dioscorea alata* tubers with different doses of 0.17 g/kg, 2.01 g/kg and 10.04 g/kg. Dependent variable is absolute number of lymphocyte.

Twenty one male Balb/C mice were divided into seven groups: control (C), negative control (NC), treatments with ethanol extract of *D. alata* L. tubers of 0.17 g/kg, 2.01 g/kg, 10.04 g/kg (T I – T III), treatment with antihistamine drug 0,4 mg/mice/day (T IV) and treatment with diosgenin 200 mg/kg (T V) (Huang *et al.,* 2010). For 17 consecutive days the T I-T III groups were treated with ethanol extract of *D. alata* correspond to with their doses, T IV group was treated with antihistamine drugs and T V group was treated with diosgenin. On day 15, the NC and T I – T V groups were induced with ovalbumin of 0,0483 mg/mice (Fischer *et al.,* 2005 modified by Diding *et al.*, 2008). Mice were sacrificed on day 18, the lymphocytes were isolated from spleen and the absolute number of lymphocytes from pellet suspension of lymphocyte was counted with haemocytometer 3 times on minor chamber. The formula for absolute number of lymphocytem is $N = A \times DF \times 10^4$sel/ml, with N= number of lymphocyte, A= number of a live/death cell mean/field of view, FP= Dilution Factor (10x).

RESULTS AND DISCUSSION

The results of measurements on absolute number of spleen lymphocytes of mice after treated with ethanol extract of *D. alata* tubers on various doses for 17 consecutive days and treatment model of allergic type hypersensitivity to ovalbumin on day 15 was shown in Table 1.

Results of this study indicated that the absolute number of lymphocytes of mice after treated with ethanol extract hypersensitivity of *D. alata* tuber was notably lower in the group with treatment dose of 10.04 g/kg bw, while the highest absolute number of lymphocytes was found in mice treated with antihistamines followed diosgenin 200 mg/kg bw, negative control,and the control groups, respectively.

On hypersensitivity allergic reactions, cells which are crucial in determining types of reactions are antigen presenting cells and mast cells, whereas lymphocytes play a role in the early phase and late phase allergic response (Abbas & Lichtman, 2011). However, results of this study indicated that in mice induced by allergic type of hypersensitivity to negative control group showed the absolute number of lymphocyte greater than the control group. Even in mice that induced allergies and were given with antihistamines, the absolute number of lymphocyte much larger, and so as to in mice induced by allergy and treated with diosgenin. The absolute number of lymphocytes was found in mice induced by allergic reaction and given the highest dose of ethanol extract 10.04 g/kb bw, followed by induced allergic mice given ethanol extract dose and 0.17 g / kb bw, and mice given the extract induced allergic and ethanol dose of 2.01 g/kb bw.

Diosgenin is the main active compound in *Dioscorea* species and are structurally similar to cholesterol (Basch *et al.,* 2003). Diosgenin levels vary among different species of *Dioscorea* (Datta *et al*., 1984; Huai *et al*., 1989). Diosgenin level varies on different species, growing techniques, harvesting, processing and storage conditions (Basch *et al*., 2003).

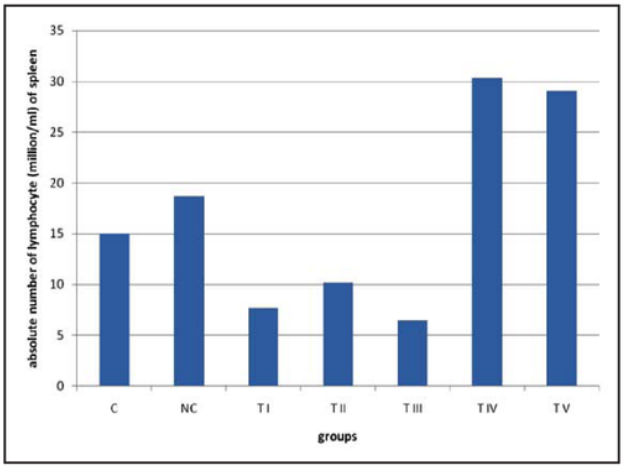

Figure 1. Absolute number of lymphocyte on spleen of hipersensitivity of Balb/C mice after treated with ethanol extract of *D. alata* L orally (EEDA) for 17 consecutive days.

Note: C: control group, NC: negative control group, T I: EEDA 0,17 g/kg; T II: EEDA 2,01 g/kg, T III: EEDA 10,04 g/kg, T IV: antihistamine drugs 0,4 mg/mice, T V: diosgenin 200 mg/kb.

Saponin as adjuvant has the unique ability to boost immunity (Iqbal *et al*., 2007). Saponin as adjuvant also has the ability to modulate cell-mediated immune system and increases the production of antibodies and has the advantage that it requires only a low dose of the adjuvant activity (Oda *et al*., 2000). Saponin adjuvant induces a strong effect on T cell-dependent antigen or antigens that are not dependent on T cells. Saponins also induce CD8 + cytotoxic lymphocyte responses are strong and provide a response to mucosal antigens (Kensil, 1996). Saponins not only have an effect on components of specific immunity stimulatory, but also have non-specific immune reactions, such as inflammation (de Oliveira *et al.,* 2001; Haridas *et al*., 2001) and lymphocyte proliferation (Delmas *et al*., 2000; Yui *et al.,* 2001).

Cellular immune response are activated to eliminate the allergen ovalbumin as allergic agents. Allergen provocation affects lymphocytes as cells that have the ability to regulate immune responses through modulation of T cells and local tissue inflammation. Exposure to antigen presenting cells (APC) by ovalbumin will initiate the activation of CD4 + cells are Th2 and IgE synthesis, better known as the stage of allergic sensitization. The exposure of the next allergen ovalbumin orally will lead to the recruitment and activation of inflammatory cells and release of mediators that will cause an allergic response to both fast and slow phase. In the early stages of an allergic response, a few minutes after contact with an allergen occurs accompanied by the release of mast cell degranulation of inflammatory mediators. These mediators include histamine, leukotriene and cytokines that will increase the permeability of blood vessels, smooth muscle contraction, and mucus production. Chemokines produced by mast cells and other cells will directly lead to the recruitment of inflammatory cells. These chemokines will contribute to the slow phase of the allergic response characterized by the number of cells CD4 + Th2 and eosinophil (Holdgate & Polosa, 2008).

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