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Immunological adjuvant activities of saponin extracts from the pods of *Acacia concinna*

Ratiya Kukhetpitakwong [a], Chariya Hahnvajanawong [b], Preecha Homchampa [c], Vichai Leelavatcharamas [d], Jarunee Satra [e], Watcharee Khunkitti [a,*]

[a] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[c] *Department of Clinical Immunology, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*
[d] *Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
[e] *Veterinary Biologics Assay Center, Bureau of Veterinary Biologics, Department of Livestock Development, Ministry of Agriculture and Cooperatives, Pakchong, Nakornratchasima 30130, Thailand*

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Abstract

Pods of *Acacia concinna* (Leguminosae) contain several saponins. In this study, four saponin fractions which were acetone fraction (AAC), aqueous fraction (WAC), hydromethanolic fraction (HAC) and methanolic fraction (MAC) were generated and their haemolytic activities and surface activities were determined in comparison with *quillaja* saponin (QS). There were no significant differences between the haemolytic activities of MAC and QS. However, the surface tensions of MAC was significantly lower than QS ($p<0.001$). Furthermore, the immunomodulatory effect and the adjuvant potential of MAC on the cellular and humoral immune response of BALB/c mice against ovalbumin were investigated. The splenocyte proliferations induced by MAC were significantly higher than QS at the concentrations of 200, 400, 800 and 1000 μg/ml ($p<0.05$). BALB/c mice were immunized subcutaneously either with OVA 20 μg alone or with OVA 20 μg combining with QS (10 μg) or MAC (10 and 40 μg). Ten days after the second immunization, concanavalin A (Con A)-, pokeweed mitogen (PWM)-, and OVA-stimulated splenocyte proliferation and OVA-specific antibodies in serum were measured. The results suggested that MAC (40 μg) could activate T and B cells. In addition, OVA-specific IgG, IgG1 IgG2a and IgG2b antibody levels in serum were significantly enhanced by MAC (40 μg) as compared with OVA control group ($p<0.001$). This finding suggested that MAC might be effect on Th1 and Th2 helper T cells. In conclusion, the results indicated that MAC at a dose of 40 μg could be used as vaccine adjuvant to increase immune responses.

Keywords: *Acacia concinna*; Saponins; Antibody; Immune responses; Splenocyte proliferation

1. Introduction

Saponins are the crude extracts from natural products that are a promising source of adjuvants. They are chemically a heterogeneous group of sterol glycosides and triterpene glycosides. Having amphiphilic structure consisting of a hydrophobic aglycone and hydrophilic sugar chains, they appear to adsorb at the various interfaces between solids, liquids and gases resulting in changes in the nature of interface which are termed surface active agent [1–3]. At the present, saponins

* Corresponding author. Tel.: +66 43 362092; fax: +66 43 362092.
E-mail address: watkhu@kku.ac.th (W. Khunkitti).

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extracted from the bark of the South American tree *Quillaja saponaria* Molina have been used as an adjuvant to increase the immune responses to a variety of antigens [4,5]. Recently, the immunomodulating activity of saponins from other sources has been investigated, such as seeds of *Chenopodium quinoa* (quinoa) [6], leaves of the plant *Hedera taurica* (Crimean ivy) [7], *Panax ginseng* [8], *Panax notoginseng* [9], and *Polygala senega* [10].

Acacia concinna (Leguminosae), a well-known medicinal plant of Southeast Asia, is commonly known as Sompoi in Thailand. The pod of this plant has been used as a skin tonic, shampoo, an expectorant, a purgative and an emetic [11]. The pod of *A. concinna* has been reported to contain several saponins including flavonoids and monoterpenoids. The methanolic extract from the pod of this plant was found to contain kinmoonosides A, B and C. An alkaline hydrolysis of the crude saponin fraction of *A. concinna* was found to contain four monoterpenoids and five prosapogenins while an acid hydrolysis of an alkaline hydrolysate was found to be an acacic lactone [12]. However, the immunomodulating and adjuvant activities of the methanolic extract from *A. concinna* pod grown in Thailand have not yet been reported.

In this study, the haemolytic activity of four *A. concinna* saponin fractions and the immunomodulating activity of the methanolic fraction (MAC) were carried out. The adjuvant activity of MAC on the cellular and humoral immune responses of BALB/c mice against ovalbumin was also investigated.

2. Materials and methods

2.1. Materials

Ovalbumin (OVA; grade V), 3,3′,5,5′-tetramethylbenzidine (TMB) liquid substrate system for enzyme-linked immunosorbent assay (ELISA), concanavalin A (Con A) from *Canavalia ensiformis*, lectin from *Phytolacca americana* (PWM) and saponins from quillaja bark (QS) as partial purified extract with 25% of sapogenin content (Sigma®) were purchased from Sigma Chemical Co., USA. Goat anti-mouse IgG, IgG1, IgG2a or IgG2b horseradish peroxidase (HRP)-conjugates were obtained from Southern Biotechnology Associates, Birmingham, USA. RPMI 1640 medium powder (Gibco®) was purchased from Invitrogen Corp., California, USA. Fetal Bovine Serum (FBS, Biowest®) was purchased from Biowest, Nuaill, France. All other chemicals were of grade AR.

2.1.1. Animals

BALB/c mice, 6–8 weeks old, were purchased from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The animals were housed under standard conditions at 25±2 °C and were provided with chow pellets and tap water ad libitum. The procedures applied to the mice were approved by the Animal Ethics Committee of Khon Kaen University, in accordance with the requirements of the Ethics of Animal Experimentation of the National Research Council of Thailand.

2.1.2. Plant material

Dried pods of *A. concinna* were purchased from Tai-Un-Jun store in Bangkok, Thailand. A herbarium sample of this plant was identified and kept by the authorities at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

2.2. Extraction of *A. concinna* pods

The dried pods of *A. concinna* (2 kg) were refluxed with MeOH at 60 °C. The methanolic extract (670 g) was suspended in water and then extracted with hexane and butanol saturated with water, respectively. The butanolic extract (~ 230 g) was chromatographed on a Diaion® HP20 column using water, 50% MeOH, MeOH, and acetone consecutively to yield crude saponin fractions labeled as WAC (65 g), HAC (22 g), MAC (71 g), and AAC (2 g), respectively. The methanolic extract of *A. concinna* yield was about 45% of the dried pods. The fractions named WAC, HAC, MAC, and AAC were 3.25%, 1.14%, 3.60%, and 0.13% of the dried pods, respectively.

2.3. *A. concinna* saponin characteristics

2.3.1. Haemolytic activity assay

The haemolytic activity of *A. concinna* fractions or QS was tested according to the method of Santos et al. [13]. New Zealand white rabbit blood collected in Alsevers (Hemostat, Dixon, CA) was obtained from National Laboratory Animal Center, Mahidol University, Nakorn Pathom, Thailand. The blood was free from specific pathogens, and was washed three times with sterile normal saline solution (NSS) by centrifugation at 1600 rpm for 5 min to collect red blood cells (RBC). The RBC suspension was prepared by diluting the pellet to 0.5% with NSS. The RBC suspension was mixed with an equal volume of *A. concinna* fractions (WAC, HAC, MAC, and AAC) or QS containing 2.5, 5, 10, 25, 50, 100, 250, 500 and 1000 μg/ml of saponin extract. The mixtures were incubated at 37 °C for 30 min and centrifuged at 10,800 rpm for 2 min. The free haemoglobin in the supernatants was measured spectrophotometrically at 412 nm. Each experiment included triplicates at each concentration. The 50% haemolysis dose (HD_{50}) was defined as the concentration of saponin extracts inducing 50% of the maximum haemolysis.

2.3.2. Determination of surface activity

Solutions of *A. concinna* fractions (WAC, HAC, MAC, and AAC) or QS at the concentration of 1000 μg/ml in deionized water were prepared. Surface tension of the solutions was