Myanmar Health Sciences Research Journal, Vol. 30, No. 2, 2018

SHORT REPORT

Establishment of In-house Production of Phytohaemagglutinin (PHA) Reagents for Detection of Chromosomal Disorders

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Key words: Phytohaemagglutinin, Karyotyping, Chromosome, Mitogenic activity

Phytohaemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolus vulgaris*) (ဖြဴထောကိုပဲ), contains potent cell agglutinating and mitogenic activities. It possesses the ability to stimulate lymphocytes to undergo mitosis.[1] Lectins are carbohydrate-binding glycoproteins that can react specifically with human blood cells, preferentially agglutinate malignant cells, and undergo mitogenic stimulation of lymphocytes.[2] Lectin extraction is usually achieved using different methods of diffusion in aqueous solution and ammonium sulfate precipitation.[3] PHA-induced blastogenic response is a potentially useful assay for the detection of immunocompromized persons especially in patients suffering from squamous cell carcinoma.[4] Lymphocytes, cultured with PHA, can be used for karyotype analysis. PHA is one of the main reagents for karyotyping analysis of chromosomal diseases. It has enhancing activity of cellular mitosis. This study assessed the quality of in-house PHA extract from red kidney beans for karyotyping to analyze chromosomal diseases.

After getting approval from the Ethics Review Committee of Department of Medical Research, this laboratory-based study was carried out from January to October, 2016. Red kidney beans were collected from the market and extracted in-house PHA reagent from this bean by homogenate method. Red kidney beans (20 g) were washed with sterile water and softened in 400 ml 0.15 M NaCl for 24 hours at 4˚C, then homogenized in a blender. The homogenate was diffused for another 24 hours at 4˚C, then filtered with Whatman paper 1. The filtrate was centrifuged at 9168×g for 30 minutes, and the supernatant was fractionally precipitated with ammonium sulfate at 40%, 50%, 60%, and 70% saturation, respectively. The four pellets were combined, dissolved in 1 ml of water, and dialyzed against distilled water at 4˚C.[3]

The protein concentration of the extracted PHA reagent from red kidney beans was 180 mg/ml by Bradford's method in this study. This mitotic activity was found in these reagents by using one in ten dilutions of protein concentration of extracted in-house reagents. The protein concentration of in-house PHA reagents was used in 18 mg/ml. The protein concentration of commercial PHA reagent was 10 mg/ml. In-house PHA reagents was stimulated the effect of mitotic activity that was same activity of commercial PHA reagents. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed for the standardization of in-house PHA extracted from red kidney beans by the method of Laemmli. This method used 15% separating and 5% stacking gel to confirm the effectiveness of the purification.[5] A single band of in-house PHA reagent

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appeared by SDS-PAGE in this study. Specific activity was expressed by hemagglutinating activity assay. Serial two-fold dilutions of the lectin solution in microtiter v-plates (25 µl) were mixed with 25 µl 2% chicken red blood cell suspension in saline (pH 7.2). Readings were recorded after 30 minutes at room temperature, when the blank had fully sedimented. The hemagglutination titer was defined as the reciprocal of the highest dilution exhibiting hemagglutination.[3]

Mitogenic activity of the PHA (extracted from *Phaseolus vulgaris*) was compared with commercial PHA (SIGMA) using karyotyping method. After obtaining informed consent from ten normal healthy persons, 2 ml of venous blood from antecubital vein were collected into sterile heparinized tubes to determine comparative effect of in-house and commercial reagents. Ten normal healthy volunteers were cultured with both the in-house PHA reagent and commercial reagent to determine human chromosomes. The commercial PHA reagent was compared with in-house PHA reagents in these subjects.

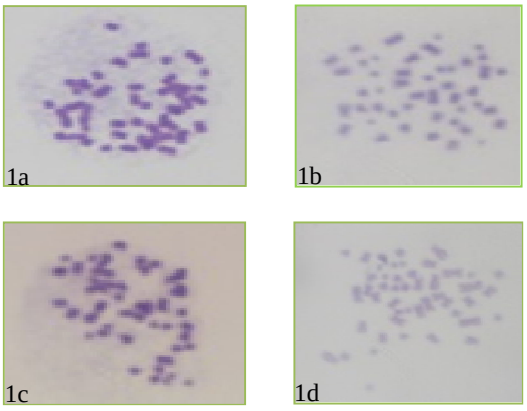

Fig. 1. Comparison between commercial PHA reagent (1a & 1b) and in-house PHA reagent (1c & 1d)

Mitogenic activity of the lectin was reasonably good compared with commercial PHA (extracted from *Phaseolus vulgaris*).[3] In this study, the number of human chromosomes was determined by using in-house PHA compared with commercial reagent (Fig. 1). This study found that the comparison between in-house PHA and commercial PHA reagent was good in mitogenic activity. The in-house PHA reagents have stimulation effect of individual cells (lymphocyte) for analysis of chromosomes (karyotyping) and it is useful for detection of autosomal defects in chromosomal disorders. PHA, a lectin extracted from red kidney bean, has been widely used as mitogen for chromosome study. In this study, preparation of indigenous in-house PHA reagent could be used for identification of chromosomal diseases. It is useful as commercial product, fresh, low cost and easily accessible. However, further investigation of in-house PHA reagent's properties should be performed.

ACKNOWLEDGEMENT

We would like to thank Dr. Kyaw Zin Thant, Director-General, Department of Medical Research for his permission and encouragement to conduct this study. We also express our thanks to Dr. Nay Win, Director of National Health Laboratory and all participants in this study.

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