ABSTRACT

Diphtheria is a disease caused by exotoxins from the bacterium *Corynebacterium diphtheriae* which is infected with a virus (*bacteriophage*). Until now, diphtheria still be a problem in developing countries. Antidiphtherial polyclonal antibodies are one of the therapeutic agents that can be injected into patients as passive immunizations. To produce antidiphtherial immunoglobulin, three stages are needed, first, *plasmapheresis* (plasma separation from whole blood), digestion with pepsin enzyme, and purification. One of the obstacles in the production of antidiphtheria immunoglobulin is that the *plasmapheresis* process still not optimal. The *plasmapheresis* method which is now widely used in industry is conventional *plasmapheresis* which uses gravity sedimentation techniques at room temperature. This method takes a long time, so there is a need for optimization of separation methods to increase the production and quality of immunoglobulin products. Another alternative method is using centrifugation. However, this centrifugation method has not yet been developed. Therefore, this study aims to develop alternative methods using centrifugation techniques in the production process of antidiphtheria immunoglobulin. Blood is drawn from horses that have been immunized with diphtheria toxoid, then separated by two methods, gravity sedimentation for 4 hours as a control, and the centrifugation method as a treatment group. For the control group, gravity sedimentation was carried out at room temperature and 4 ℃. For the treatment group, the speed of centrifugation used ranged from 500 to 4000 rcf (radians centrifugal force) with a centrifugation length of 5 and 10 minutes. Both the control and treatment groups tested several parameters including; hemolysis of red blood cells, total plasma protein levels before and after digestion of the pepsin enzyme, antibody titer values, and immunoglobulin F $(ab')_2$ fragments. Repetition is done twice for both groups. Calculation of protein levels is done by the BCA method (bicinchoninic acid). Antibody titer was determined by flocculation test, then Immunoglobulin F $(ab')_2$ fragments were analyzed by SDS-PAGE. The pepsin enzyme digestion was carried out at pH 3.2, with the concentration of the pepsin enzyme 0.6% (w/v), and the incubation temperature of 37 ℃. The results of this study indicate that, the viability of red blood cells (cells not hemolysis) for the control and treatment groups did not differ significantly, the average viability of red blood cells > 99%. Before the digestion process of the pepsin enzyme, the highest plasma protein level was found in centrifugation treatment with a speed of 500 rcf for 5 minutes (118.78 ± 13.87 mg/mL). The results of measurement of antibody titer values showed that the value of antibody titer in centrifugation treatments with speeds above 500 rcf was not significantly different from the 4 ℃ control (antibody titer 420 - 480 Lf/mL). Analysis of the ratio between antibody titers and total protein shows the highest value is found in the centrifugation treatment with a speed of 4000 rcf for 10 minutes, but the value is not significantly different from the 4 ℃ sedimentation. After the digestion process with the pepsin enzyme, total protein content and the results of immunoglobulin $F(ab')_2$ qualitative analysis showed no significant difference between sedimentation and control (centrifugation). From the results of this study it can be concluded that the centrifugation method with a speed range of 500 to 4000 rcf does not cause hemolysis of red blood cells. Before the digestion process with the pepsin enzyme, the speed and length of time of centrifugation had an effect on total plasma protein levels, antibody titer values, and the ratio between antibody titers and total protein. However, the effect of the centrifugation was not significantly different after the digestion process by the pepsin enzyme. Therefore it is necessary to conduct a quantitative study after the digestion process by the pepsin enzyme to determine the effect of centrifugation in the production process of antidiphtheria immunoglobulin.

Keywords: Diphtheria, plasma, centrifugation, *plasmapheresis*, antibodies, Immunoglobulin $F(ab')_2$