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Establishment of technique for isolation of phagocytes from peripheral blood of elephants (*Elephas maximus*)

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In elephants, both neutrophils (heterophils) and monocytes which are phagocytic white blood cells serve as the first line of defence. Measuring the phagocytic efficiency of the leukocytes could be an important parameter to evaluate the innate immunity in any animal. Such studies in elephants however, require laboratory techniques for isolating functionally active phagocytes from blood. If established, such laboratory test could detect possibly immune-suppressed elephants for treatment and special care. Eight venous blood samples (5 ml each) from 4 captive male elephants were used. After obtaining total blood counts and the differential counts, samples were centrifuged for 2000 *g* for 10 min. Plasma were separated, buffy coat and the 1st quarter of the red cell column was pipetted out, and was subjected to hypotonic lysis of red blood cells and isotonicity was restored with 2.7% phosphate buffered saline (PBS). After centrifugation, remaining cell pellet was washed 3 times with 0.8% PBS and subjected to Nigrosine (0.1%) dye exclusion test for viable counts. This method resulted in leucocyte isolations with negligible contamination of RBC with 99.3 ± 0.2 % viability. 39.8 ± 4.7% of the total leukocytes could be harvested by this method and out of that 65.5 ± 11.3% were phagocytes. Average 40% of heterophils and 55.5% monocytes showed positive phagocytosis when two of these isolates were incubated with opsonized bacteria (*E. coli* ATCC 25922). This procedure once improved, quantified and standardized, will provide a suitable technique to isolate phagocytes from elephant blood and a method to quantify their phagocytic ability.

Keywords: Asian elephant, leukocyte isolation, phagocytosis

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