

Supporting Information
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Immunosuppressive Phenolic Constituents from
***Hypericum montbretii* Spach.**

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Preparation of Opsonized Zymosan

The opsonization of zymosan particles was carried out as described earlier¹⁵ with some modifications. Briefly, zymosan (100 mg) was mixed in 5 mL phosphate buffer saline (PBS, pH 7.4), and 5 mL fresh pooled serum from healthy human volunteers. The mixture was incubated at 37 °C in shaking water bath for 30 minutes. Then centrifuged at 300 g for 20 minutes, and washed twice with PBS, and the pellet was finally re-suspended in 5 mL of PBS. The mixture was kept frozen at -20 °C till use, and was brought to room temperature immediately before the use.

Preparation of Luminol

The luminol (1.8 mg) was dissolved in 1 mL borate buffer and vortexed for 5-10 minutes. The solution was then further diluted up to 10 mL of HBSS⁺⁺ (with Ca and Mg) to give 180 µg luminol/ mL.

Isolation of Polymorphoneutrophils (PMNs)

The human whole blood was withdrawn from the antecubital veins of healthy volunteers into syringes containing 100 U/mL heparin, and placed in steril tubes. The heparinized blood was diluted with of HBSS⁻ (without Ca and Mg) (pH 7.4), and then mixed with one third of dextran (3 % in 0.9 % NaCl) solution for differential sedimentation, and removal of erythrocytes. After gentle mixing, it was kept at room temperature for 20 minutes undisturbed. The upper layer containing leucocytes was collected, and gently layered over equal volume of Lymphocytes Separation Medium (LSM), and then subjected to centrifugation at 400 g for 25 minutes at room temperature. After removal of the upper phase, neutrophils were collected and subjected to hypotonic lysis with sterile distilled water for one minute, and then washed twice with HBSS⁻. The purity of isolated PMNL was > 96 % (as determined by trypan blue dye exclusion by using light microscopy). Cells were re-suspended in Hank's balanced salt solution (HBSS⁺⁺) to give 1×10^6 .

Isolation of Murine Macrophages

Peritoneal macrophages were isolated by injecting fetal bovine serum into the peritoneal cavity of NMRI mice (20-30 g), as documented earlier¹⁶. Macrophages were collected after three days using RPMI-1640, 10% fetal bovine serum. The exudates was then subjected to centrifugation at 300 g at room temperature for 20 minutes, washed with serum RPMI 1640 medium, and macrophages were re-suspended in complete RPMI 1640 medium.