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Activity of Medicinal Herbs Extracts Against Bovine Respiratory Syncytial Virus Infection and Co-Infection with BRSV-*Pasteurella multocida* adherence on MDBK Cell

N. Lanniari[1*], B. P. Priosoeryanto[1], S. Setiyaningsih[1], M. Shofa[1], R. Wulansari[1], H. Pisestyani[1]

[1]Faculty of Veterinary Medicine, Bogor Agricultural University (IPB), Kampus IPB Dramaga 16680 Bogor, Indonesia

[*]Corresponding author email: lanni_nafrina@yahoo.com

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INTRODUCTION

Bovine respiratory disease complex (BRDC) in calves are responsible of major economic losses in both beef and dairy production (1,2). BRDC was caused by interaction of viral and or bacterial pathogens which are mutually in major cases, this combination includes bovine respiratory syncytial virus (BRSV), bovine herpes virus, bovine viral diarrhea virus, bovine parainfluenza-3 virus, *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma bovis*, and *Histophilus somni* (2). BRSV is a single negative stranded RNA virus belonging to the Paramyxoviridae family as a primary etiological agent of respiratory disease in calves of beef and dairy cattle (1,2,3). BRSV is capable of initial infection or facilitates secondary infection of the lower respiratory tract by bacteria (4,5). Recently, high density of the animals movement and extreme temperatures change causing stress and going to be respiratory problems. There is currently no specific treatment for BRDC and treatment is primarily of symptomatic. Specific treatment as an antivirals are urgently required. *Herbs* has known as a traditional medicinal plant used as an antiviral, anti-inflammatory and immune-stimulant (6). Given the traditional usage of herbs, this study sought to determine whether the principal bioactive ingredient of herbs possessed detectable anti-BRSV activities. However, information about antiviral treatment in Indonesia is limited. The aim of this study to determine bioactive ingredient of herbs have an anti-BRSV activities.

MATERIAL AND METHODS

Cell culture, virus and bacteria. MDBK cell preparation, BRSV and PM propagation were followed (7). Briefly, MDBK seeded at 1 × 10^5 cells/ml and cultured as a monolayer in DMEM, 10% FBS; 100 U/ml penicillin, 100 µg/ml streptomycin and 1 µg/ml amphotericin-B, maintained at 37°C/5% CO_2. BRSV RS-52 strain, propagated in HEp-2 cells and virus titter was checked in a plaque assay using MDBK cells. PM 2368 strain, capsular type B isolated, was grown at 37°C in Brucella broth. BRSV and PM were aliquoted, and kept at −80°C until use.

Herbs preparation. Herb A (*Andrographis paniculata*), herb B (*Phyllantus niruri*), herb C (*Curcuma aeruginosa*) and herb D (*Curcuma xanthorrhiza*) dissolved in vehicle 100% DMSO to a final stock concentration of 100 mM and stored at −80 °C. Compound was serially diluted to 1 and 10 µg/ml using DMEM 2% FBS. The final concentration of DMSO in media was less than 0.1%.

Herbs treatment in co-infection assay. MDBK cells were seeded in 12-well plate, >60% confluence, inoculated with BRSV (MOI = 1) and culture medium as a negative control. Inoculation time was 2 h and replaced with culture medium containing herbs and culture medium only as a control. After 72 h post infection (hpi), BRSV-infected and uninfected cells treated by herbs and culture medium were exposed with PM suspension for 2 h. Cells were dissociated by trypsinization and collected by centrifugation. Dissociated resulting bacteria plated on Brucella agar. Adherence of bacteria per cell was calculated from total counting of CFU with the total number of monolayer cell.

RESULT AND DISCUSSION

Morphological features of BRSV-infected and uninfected cells, BRSV-infected and treated with herb-A to herb-D were observed under microscope with 20× magnification. The normal morphology of MDBK maintained in DMEM medium is illustrated in Fig. 1. While BRSV-infected cell untreated with herbs shown the cytopathic effect (CPE) and cell detached. These condition was not occur in BRSV-infected cell treated with herb B. In contrast, herb A; herb C and herb D are not affected to BRSV-infected cell. Fig. 2 shows that treated 1 and 10 ug/ml of the herb B decreased significantly of PM adherence to the BRSV-infected depend on the untreated cells. This result suggested that herb B is inhibit the cell damage from BRSV infection and decrease the PM adherence to BRSV-infected cell. In another hand, BRSV infectivity will influences the PM adherence to the epithelial cell (7). Thus, promote an effective in herb B on pathways which would be antiviral

synergistically to kept the cell healthy from BRSV infection and through inhibition of adherence from PM.

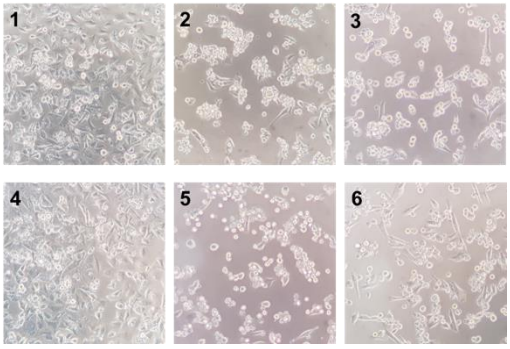

Fig. 1: Photograph BRSV-infected cell maintenance by herbs. 1) culture medium; 2) BRSV-infected cell; BRSV-infeced cell maintenance with herb A (3); herb B (4); herb C (5); and herb D (6).

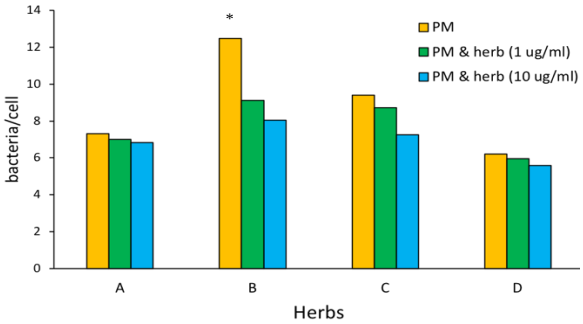

Fig. 2: Effect of herbs maintenance on BRSV-infected cell and treated with PM in co-infection assay.

CONCLUSION

In conclusion, we showed that maintenance by herb B effective to inhibit of cell damage from BRSV-infected MDBK cell and decreasing of PM attachment. Further studies are required to understand the action of herb B treating pathway *in-vitro* assay to control BDRC problem in Indonesia.

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REFERENCES

[1] Gershwin, L.J., Berghaus, L.J., Arnold, K., Anderson, M.L., Corbeil, L.B., 2005. Immune mechanisms of pathogenetic synergy in concurrent bovine pulmonary infection with Haemophilus somnus and bovine respiratory syncytial virus. Vet. Immunol. Immunopathol. 107,119–130. http://dx.doi.org/10.1016/j.vetimm.2005.04.004

[2] Beaudeau, F., Ohlson, a, Emanuelson, U., 2010. Associations between bovine coronavirus and bovine respiratory syncytial virus infections and animal performance in Swedish dairy herds. J. Dairy Sci. 93, 1523–1533. http://dx.doi.org/10.3168/jds.2009-2511.

[3] Meyer, G., Deplanche, M., Schelcher, F., 2008. Human and bovine respiratory syncytial virus vaccine research and development. Comp. Immunol. Microbiol. Infect. Dis. 31, 191–225. http://dx.doi.org/10.1016/j.cimid.2007.07.008.

[4] Larsen, L.E., Tegtmeier, C., Pedersen, E., 2001. Bovine respiratory syncytial virus (BRSV) pneumonia in beef calf herds despite vaccination. Acta Vet. Scand. 42, 113–121. http://dx.doi.org/10.1186/1751-0147-42-113.

[5] Tjønehøj, K., Uttenthal, Å., Viuff, B., Larsen, L.E., Røntved, C., Rønsholt, L., 2003. An experimental infection model for reproduction of calf pneumonia with bovine respiratory syncytial virus (BRSV) based on one combined exposure of calves. Res. Vet. Sci. http://dx.doi.org/10.1016/S0034-5288(02)00154-6.

[6] Chudapongse, N., Kamkhunthod, M., & Poompachee, K. (2010). Effects of Phyllanthus urinaria extract on HepG2 cell viability and oxidative phosphorylation by isolated rat liver mitochondria. Journal of Ethnopharmacology, 130(2),315–319. https://doi.org/10.1016/j.jep.2010.05.010

[7] Sudaryatma PE, Nakamura K, Mekata H, Sekiguchi S, Kubo M, Kobayashi I, Subangkit M, Goto Y, Okabayashi T, 2018. Bovine respiratory syncytial virus infection enhances Pasteurella multocida adherence on respiratory epithelial cells. Veterinary Microbiology 220, 33–38.