SHORT COMMUNICATION

EFFECT OF Cymbopogon nardus FRACTIONS IN COMBINATION WITH RIBAVIRIN ON MEASLES VIRUS

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Cymbopogon nardus (L.) Rendle is a local medicinal plant, traditionally used for post partum bath (Perry and Metzer, 1980). Scientific studies have proven C. nardus to possess several biological activities, such as antiviral (Nurul Aini et al., 2006), antibacterial (Hanina et al., 2002; Ahmad et al., 1993) and insect repellent (Jantan and Zaridah, 1998). Although measles is a vaccine-preventable disease (WHO, 2001), eradication efforts are constrained by inadequate vaccination campaigns in underdeveloped countries (Mulder et al., 2001) and problems in vaccination administration (Atkinson et al., 2007). The search of new antivirals is important as current drugs give adverse effects to patient such as toxicity (Grancher et al., 2004). Ribavirin has been proven to be effective against measles virus (Mims et al., 2004) but may cause teratogenicity (Hosoya et al., 2004) and anemia (Fernandez et al., 1986).

Synergistic effect from combination of antiviral drug with plant-based compound has been reported (Barquero et al., 1997). Combination therapy is a procedure in which two or more drugs at lower concentration are given concurrently with expectation of synergism to occur. Synergism is observed when the effect of combination treatment is up to threefold compared to the effect of single treatment (Ahmad, I.B. personal communication). In this study, standardized C. nardus fractions SWA13, SWA14, SWB6 and SWB9 were used individually and in combination with ribavirin against measles virus to observe synergism or antagonism. Two different protocols were adopted in this study to determine the prophylactic or therapeutic properties of the fractions.

Test fractions SWA13, SWA14, SWB6 and SWB9 were prepared as described in Nurul Aini et al. (2006). Cytotoxicity test was done on confluent Vero/SLAM cells (African green monkey, Cercopithecus aethiops, human receptor) according to Marini et al. (1998). The concentration of test fractions that kills 50% of the cell population or CC50 were obtained from the graph plotted using the optical density at readings from microplate reader (Labsystems Multiskan Multisoft, Finland) at A620.

Working solutions in the antiviral combination experiment were diluted to obtain concentrations of 0.5 CC50 and 0.1 CC50 for C. nardus fractions and ribavirin (RBV) at 0.01 CC50 and 0.005 CC50. Test materials involved four combinations of test fractions with RBV; 1. RBV (0.01 CC50): SW (0.1 CC50), 2. RBV (0.01 CC50): SW (0.5 CC50), 3. RBV (0.005 CC50): SW (0.1 CC50) and 4. RBV (0.005 CC50): SW (0.5 CC50). Test virus, measles Edmonston strain (Serum Institute of India Ltd) at 1000TCID50 was diluted into 10TCID50.

Two protocols were used in the experiment: Protocol I [(S+V)+A] involves inoculation of virus (V) to the cells (S), incubation for 20 minutes to allow virus adsorption and later treatment with test material (A). Protocol II [(S+A)+V], involved treatment of cells with test material first and subsequent virus inoculation. Experiment was done in quadruplicates. Cell viability was determined by optical density readings using microplate reader at A620. Data was analyzed using One-Way ANOVA and any significant data was analyzed with Tukey’s Test (p<0.05) to compare means between treatments, including mean of untreated inoculated cells treatment. Degree of effectiveness for combination treatment was determined by comparing cell
viable in combination treatment to viability in single treatment.

Cytotoxicity test showed CC50 values of 150 μg/mL for both SWA13 and SWA14, and 200 μg/mL for SWB6 and SWB9 that can be considered to be non-cytotoxic as the values are higher than 20 μg/mL (Gad 2000). There are four combinations which showed synergistic effect as shown in Table 1 with one combination of RBV with SWB6 using protocol I and three combinations of RBV with SWA13 and SWB9 were effective in Protocol II. The effectiveness of the fractions from *C. nardus* using Protocol II is suggestive of the potential to be used for prevention against viral infection as shown earlier in the study using single treatment against measles (Nurul Aini *et al.*, 2006).

However, it is difficult to conclude that the combination treatment is efficient as a preventive agent since there is also synergistic effect from combination in Protocol I. The efficacy of combination treatment rely heavily on the concentration of each antiviral used. The dose for each antiviral component needs to be optimized and standardized (Norris, 2005). Inappropriate combinations would result in nullifying the effects of components towards each other (Gilani and Atta, 2005). This would result in toxicity of the substances (Cowan, 1999) which will lead to cell death. An example of a poor combination was reported between combination therapy of St. John’s wort with indinavir (Tirona and Bailey, 2006). Constituents in herbs interact with nuclear receptors to enhance metabolizing enzyme and/or transporter activity leading to reduced drug concentrations. Therefore, proper concentration of each antiviral component in the fractions should be further evaluated for their activity, safety and their mechanism either singly or in combination at molecular level and in *in vivo* studies. As a conclusion, this preliminary study indicates the *in vitro* safety of all the test fractions. SWB6 fraction in combination with ribavirin has the potential in controlling measles virus infection. Fractions SWA13 and SWB9 in combination with ribavirin are suitable as preventive antiviral drug.

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**REFERENCES**


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