ANTHI-HERPESVIRUS ACTIVITY OF SOURSOP (Annona muricata) SEED EXTRACT: A STUDY USING MARèK’S DISEASE VIRUS IN EMBRYONATED CHICKEN EGG MODEL

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Introduction

Soursop (Annona muricata) is believed and widely used as herbal medicine to cure cancer (Mohanty et al. 2008). Herpesviridae belong to DNA virus group and often cause latent infection in human and animal. Marek’s disease virus (MDV) is a member of genus Alpha Herpesviruses of the Herpesviridae which can transform the infected cell and become a tumor/cancer cell (Chayavichitsilp 2009). It is therefore interesting to explore whether anti-cancer activity of the plant also prevents herpesviridae replication.

This research was conducted to study the ability of soursop seed extract to prevent herpesviridae infection using MDV infection in embryonated chicken eggs (ECE) model. In this research, two tests were conducted: toxicity test to measure the safe dose of soursop seed extract for ECE, and anti-virus test to study the antiviral effect of soursop seed extract against MDV.

Materials and Methods

Plant Materials
Soursop (Annona muricata) seed was collected from ripe fruits harvested in Sumedang, West Java, Indonesia. The plant species was confirmed by Herbarium Bogorensis

Seed Extraction
Soursop seed was sun dried for 3 days before grinded to become seed powder. Soursop seed extract was prepared using decoction method by adding 0.5, 1, or 1.5 g of seed into 100 ml of pre-heated distilled water (80°C) for 30 min. After that this suspension was filtrated using filter paper, and the aqueous extract was collected and stored in refrigerator until used in the subsequent biological assays.

Embryo Toxicity Test
Effect of the soursop extract on embryonated chicken eggs (ECE) as medium for Marek Disease Virus culture was tested by injecting soursop seed extract with various concentration (0.5%, 1.0%, 1.5%) and placebo (PBS+antibiotic) each ECE was injected by 0.1 ml of extract or placebo in chorioallantoic membrane. The ECEs were then incubated for 7 days before terminated for pathological examination.

Anti-MDV Activity Test
A total of 25 ECE divided randomly into 5 groups and given the following inoculation. Non-treated control MDV virus (positive control), Negative control (PBS+antibiotic), MD virus +1% soursop extract, and 1% soursop extract. The studied variables in anti-MDV test were embryo mortality rate, viral plaque formation in chorioallantois membrane, weight, and pathological lesion of the embryo.

Results and Discussion

Among the three concentrations, 1% soursop seed extract was the safest dose for the embryo with 0% mortality rate and no difference in embryonic condition compared to negative control ECE (Table 1). This concentration also exhibited the highest activities against the virus as evidenced by no embryo mortality and prevention of viral plaque formation in the chorioallantois membrane.

Table 1 Result of Embryo Toxicity Test

<table>
<thead>
<tr>
<th>Concentration of soursop seed extract (%)</th>
<th>No. of ECE</th>
<th>CET Mortality (%)</th>
<th>Embryo Weight (g)</th>
<th>Pathological Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5</td>
<td>0</td>
<td>11.23±1.363*</td>
<td>Normal</td>
</tr>
<tr>
<td>0.5%</td>
<td>5</td>
<td>80</td>
<td>11.69±1.759*</td>
<td>Haemorrhagic, ess feather</td>
</tr>
<tr>
<td>1.0%</td>
<td>5</td>
<td>90</td>
<td>11.07±1.367*</td>
<td>Normal</td>
</tr>
<tr>
<td>1.5%</td>
<td>5</td>
<td>100</td>
<td>7.13±0.688*</td>
<td>Haemorrhagic, No lesion</td>
</tr>
</tbody>
</table>

Figure 1 Comparison between three Anti-MDV Activity Test ECEs: (A) Virus inoculated ECE, (B) Virus + Soursop extract inoculated ECE, and (C) Control ECE. Notice the existence of viral plaque and hemorrhage (yellow arrow) in (B) which not exist in (A) and (C)

Table 2 Result of Anti-MDV Activity Test

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of ECE</th>
<th>Embryo Weight (g)</th>
<th>Liver Weight (g)</th>
<th>Spleen Weight (g)</th>
<th>Liver Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>15.30</td>
<td>0.313</td>
<td>0.105</td>
<td>Normal</td>
</tr>
<tr>
<td>Positive (Virus)</td>
<td>5</td>
<td>15.31</td>
<td>0.326</td>
<td>0.105</td>
<td>Normal</td>
</tr>
<tr>
<td>Negative (PBS)</td>
<td>5</td>
<td>15.68</td>
<td>0.298</td>
<td>0.101</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Weight of embryo in the ECEs given soursop seed extract were also significantly higher than ECE infected virus controls (P<0.05). The antiviral activity of soursop extract mainly contributed to glucosidase inhibiting imino sugars content such as caustegine 5%, deoxyxynojirimycin (DNS), 2R, 3R, 4R, 5R-2,5 dihydroyxymethyl-3,4, dihydroyxpyrrolidine (DMDP), and deoxyxymanojirimycin (DMJ). The inhibition of glucosidase will prevent the replication of the virus which resulted in the failure of infection (Mohanty et al. 2008).

Conclusion and Future Prospect

The soursop seed extract is proven to be able to prevent herpesviridae infection with MDV in ECE, as a model. Further research of this substance could open the possibility of finding a cure for herpesviridae infection in human.

This research could trigger further biomedical research in herpesvirus curing subject that eventually lead to the discovery of new herpesvirus curing substances from soursop extract as a new base development.

Selected References