Activity honey bee venom *Avis mellifera* to glucose transport membrane cell erythrocytes

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Abstract

Bee venom sting many biological activities and widely used as traditional medicine. The objective of this research is to determine the activity of bee venom on glucose transport and the integrity of erythrocyte cell membrane.

The result of this research showed than on 2, 4, 6, and 8 times of sting did not affect to the integrity of the membrane with hemoglobin as control (p<0.05).

Glucose transport analysis with glucose concentration of 5, 30, and 50 mM on 2, 4, 6, and 8 times of sting also did not show any inhibition and no significant differences statistically (p<0.05). However, the transport rate of glucose into erythrocyte cell for 15 seconds compared between without, 2 and 4 times of stings with glucose concentration of 5, 30, and 50 mM, it was discovered that there are significant differences between the treatment statistically (p<0.05).

Keywords: Honey bee venoms, glucose transport, erythrocyte cell membrane, hemoglobin

Introduction

The use of traditional medicine for the treatment of many deceases is associated to folk medicine from different parts of the word. Natural products from insect, fungi, bacteria and other organism, continue to be used in pharmaceutical preparation either as pure compounds or as extracts.  

Bee Venom therapy is becoming recognized and accepted for treating certain human ailments. Honey bee venom can be used in many different ways forms. The major constituent, of honey bee venoms, enzyme fosfolipase A-2(10%), hyaluronidase (2%), etc, protein complex, melitin (50%), peptide, histamine, amino acid, etc was determinates by Rothfeld, M.D., Glenn. 2002.  

Sting of insect of honey bee venom *Avis mellifera* to be used of traditional medicine combination and acupuncture for symptomatic therapy. A number of animals studies have shown honey bee venoms to be an effective immunomodulator (an agent that can effect the behavior of immune cells). While extensive human studies have not been done, several reports also suggest honey bee venoms has therapeutic effects on spinal arachnoiditis, and also for complication arachnoiditis – Tuberculoses Meningitis.  

Bee venom is a rich source of pharmaceutically active components. In twelve European countries, in the drug category, we can find twenty-four products containing bee venom. These products include creams, liniments, ointments, salves, injection forms for treating different human ailments. The are available by prescription or without a prescription in certain countries.

Blood is considered a connective tissue because it provides a transport system that connects different body tissues. Nutrients and chemical messengers are transported to all body tissues by blood, and wastes are transported from tissues to excretory organs through the blood. Blood is composed of red blood cells or erythrocyte, white blood cells, platelets, and plasma. Plasma is the extra cellular matrix with fiber that is observed only during blood clotting. Erythrocyte obtains their red color from hemoglobin. Hemoglobin contains a red pigment called heme that gives blood its red color. The plasma membrane (cell membrane) with its unique design is responsible for selectively allowing substances into and out of the cell either by active or passive transport processes. Active transport processes require ATP (stored energy) to move substances across the plasma membrane. Active transport is especially important for maximal recovery of sugars (glucose) from the intestine when the intestinal concentration of glucose is less than that in the blood.

The transport of glucose into cells is mediated by substrate specific transport proteins. The transport of blood glucose is generally by facilitated diffusion, as the intracellular concentration of glucose is typical less than 5 mmol/L (90mg/dl). In contrast, the transport of glucose from the intestine into blood involves both facilitated diffusion and active transport processes.

The objective of this research is to determine the activity of bee venoms on blood especially of glucose transport and the integrity of erythrocyte cell membrane.
Materials and Methods

Material

- Bee species (60 lebah) *Apis mellivera*
- Animals. Sting of honey bee venom *Apis mellivera* obtained from The Babusalam Ciburial Pesantren School, Bandung.
- Electric shock
- Honey bee venom (is colorless liquid).
- Cold Ethanol
- Becker glass
- Swirling flask
- Glass rod
- Whole blood from donator.
- NaCl 0.9 % : Weight 0.9 gram NaCl, add100 ml aquadest.
- Glucosa solution (5.30, 50)mM in phosphat buffer pH 7.4.
- Fiksasi cold solution: Add 2 mM HgCl2, 310 mM NaCl dan 1.25 mM KI keep in refrigerated (4°C).
- Centrifuges Janetzki T 100.
- Incubator Memert .
- pH-meter Corning Model 2051T.
- Stirrer “Super Mixer Leniel Scientific” and swirling flask for hemolytic the erythrocyte.
- “Waterbath Kottermann “, for incubation.
- Spektrofotometer Spektronik 21 digital Bausch & Lomb.

Preparation of Erythrocyte (Shahib M N., 1995)

1. Pipette 5 ml of whole blood with anticoagulant heparin (Centrifuge tube containing of anticoagulant).
2. Spin in clinical Centrifuges 2000 rpm along 15 minute
3. Carefully remove the clear serum with pipette (do not disturb the cloth/ erythrocyte).
4. Add 1:10 NaCl physiologist to the cloth again centrifuges 2000 rpm along 15 minutes (purification erythrocyte)
5. Purification of erythrocyte (4 x)

Erythrocyt dry determination

Add 0.1 ml erythrocyte to watch glass; keep in oven on temperature 120°C along 12 hours, and than weighing.

Experimental procedure for erythrocyte transport membrane cells

1. Set up the test tubes at room temperature as for standard essay conditions, with buffer phosphate pH 7.4
2. Add 1.0 ml erythrocyte to 10 ml glucose solution (100mM) in the tubes; incubate the tubes (37°C).
3. Pipette 1 ml along 1, 2.5, 5 and 10 minutes respectively add 10 ml cold solution in centrifuges tube.
4. Centrifuges 2000 rpm for 3 minutes respectively.
5. Carefully remove the clear solution.
6. The cloth is erythrocyte

Hemoglobin determination by Cyanmethemoglobin Method

In this determination, whole blood is diluted with potassium ferricyanide solution which oxidize hemoglobin to methemoglobin, which in turn is converted to cyanmethemoglobin. The intensity of the color is measured photometrically ($A_{540nm}$). The procedure is standardized with blood that has been analyzed for its iron content. The following values for the standard curve. Follow procedure shown in table above

Procedure for hemoglobin determination

<table>
<thead>
<tr>
<th>Additions (ml)</th>
<th>Tube Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>0.02</td>
</tr>
<tr>
<td>Water</td>
<td>0.02</td>
</tr>
<tr>
<td>K.ferricyanide</td>
<td>5.0</td>
</tr>
</tbody>
</table>
| Cover with parafilm; gently mix, incubate for 10 minute at room temperature, and read against the blank. Calculate the average for tubes 1 and 2 and report the data in gram percent.

Determination of glucose in erythrocyte before incubation

1. Add 0.1 ml of erythrocyte to 10 ml cold solution mix by swirling flask.
2. Centrifuges 2000 rpm along 3 minute
3. Carefully remove the clear solution
4. Hemolytic the erythrocyte; add 5 ml aquadest again mix by swirling flask “super mix”.
5. Allow in room temperature 20 minute.
7. Take absorbance reading at 546 nm.

Determination of glucose in erythrocyte after incubation

1. Add 5 ml aquadest to precipitate erytrocyt, mix by vortex “Super Mixer”.
2. Allow 20 minutes in room temperature.
3. Centrifuges at 2000 rpm and 15 minute.
4. Collect the Filtrate
5. Take absorbance reading at 546 nm.
6. Calculation concentration of glucosa

A sample/ A Standard x concentration standard x diluting factor x 1/ dry cell erythrocyte (gram).
Analysis data: by statistically distribusi t student.

Results and Discussion

Activity of the sting to integrity of hemoglobin was determinates\(^1\). The results of past research have shown that the venom sac of the honey bee has about 0.1mg, of dried weight content.

The result of this research showed than on 2, 4, 6, and 8 times of sting did not effect to integrity of the membrane with hemoglobin as control (p<0.05). The activity on 2, 4, 6, 8 times of stings of bee to integrity hemoglobin are expressed in Table 1 and Figure 1.

Hemoglobin is synthesized in the erythrocyte precursor cells-erythroblasts and reticulocytes- under a tight control dictated by concentration of heme, the synthesis of which involves the chelation of reduced ferrous iron (\(\text{Fe}^{2+}\)) by four nitrogen atoms in the center of a porphyrin rings. The main function of erythrocyte are transport of oxygen and the removal of carbon dioxide and hydrogen ion; as they lack cellular organelles, they are not capable of protein synthesis and repair. As a result, erythrocytes have a finite life span of 60-120 days before being trapped and broke down in the spleen. Hemoglobin, restricted to the erythrocytes, is responsible for the movement of \(\text{O}_2\) between the lungs and other tissues. This experiment presents that the sting did not effect.

Table 1 Activity of the Sting to Integrity of Hemoglobin

<table>
<thead>
<tr>
<th>Sting</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>0.497</td>
<td>0.486</td>
<td>0.444</td>
<td>0.455</td>
<td>0.434</td>
</tr>
</tbody>
</table>

Figure 1 Activity of the sting to integrity of hemoglobin.

The result of activity of the sting to velocity of glucose in membrane cell erythrocytes shown in Table 2 and Figure 2. A major role of membranes is to maintain the structural integrity and barrier function of cells and organelles. However membranes are not rigid or impermeable: they are fluid, and their components move around, are metabolized, and are subject to metabolic turn over. The turn over of membrane components is especially important for the cellular response to information from inside and outside the cell: recognized, transfer, amplification, and signal transduction processes occur in or on the membranes. Furthermore, both small and large molecules must pass through biomembranes. With few exceptions, these transport process are mediated by specific membrane proteins.

Activity of the sting to transport glucose from outside to inside membrane cells erythrocytes, shown in Table 3 and Figure 3.

Table 2 Activity of The Sting to Velocity of Glucose in Membrane Cell Erythrocytes

<table>
<thead>
<tr>
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<th>Sting 0</th>
<th>Sting 2</th>
<th>Sting 4</th>
</tr>
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<tr>
<td>5 mM</td>
<td>0.1823</td>
<td>0.074</td>
<td>0.14</td>
</tr>
<tr>
<td>30 mM</td>
<td>0.3954</td>
<td>0.216</td>
<td>0.215</td>
</tr>
<tr>
<td>50 mM</td>
<td>0.6361</td>
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Figure 2 Activity of the sting to velocity of glucose in membrane cell erythrocyte.

Table 3 Activity of the sting to transport glucose from outside to inside membrane cell erythrocytes

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Conclusions

1. The result of this research showed than on 2, 4, 6, and 8 times of sting did not affect to the integrity of the membrane with hemoglobin as control (p<0.05).
2. Glucose transport analysis with glucose concentration of 5, 30, and 50 mM on 2, 4, 6, and 8 times of sting also did not show any inhibition and no significant differences statistically (p<0.05).
3. The transport of glucose into erythrocyte cell for 15 second compared between without, 2 and 4 times of sting with glucose concentration of 5, 30 and 50 mM, it was discovered that there are significant differences between the treatment statistically (p<0.05).

References