Effects of Honeybee (Apis Mellifera L.) Venom Injection on the Growth Performance and Hematological Characteristics in Pigs

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Abstract

This study was conducted to evaluate effects of honeybee venom injection (VI) collected using bee venom collector compared to that of bee venom acupuncture (VA) on the body weight gain, growth rate and hematological characteristics of piglets. One hundred sixty two piglets from 15 sows were allocated in to three groups; honeybee venom subcutaneous injection groups (97 piglets from 9 sows), honeybee venom acupuncture-treated group (31 piglets from 3 sows), and non-treated control group (34 piglets from 3 sows). Honeybee venom treatment was administrated at 1, 3, 14, and 30 days after birth. Honeybee venom subcutaneous injection groups divided by a syringeful; group A (0.5, 1.0, 1.5, 2.0 \(\text{mg}\)), group B (1.0, 1.0, 1.0, 1.0 \(\text{mg}\)), and group C (1.0, 1.5, 2.0, 2.5 \(\text{mg}\)). During 60 days experiment, weight gain and survivability in VI and VA treatment of pigs were higher compared with control. Survival rate during the experiment period was 96.8\% in group C, 93.2 \% in VA and 86.7 \% in control. Weight gain and survivability were affected by VI and VA. WBC, RBC, lymphocytes, monocytes, serum total protein, and albumin concentration were not affected by VI and VA. Serum IgG concentration of VI and VA treatment s were greater than that of control. In conclusion,

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VI and VA were effective for improving growth performance and to increase the concentrations of blood IgG in growing pigs. No statistical differences were found for VI and VA. These results suggested that the treatment of honeybee venom injection collected using bee venom collector could be used effectively for the increase productivity.

Key words: Honeybee venom, Bee venom, Hematology collector, Pig, and Growth performance.


**Introduction**

Bee venom has long been used as one of folk remedies for the arthritis and gout \(^1, 2\). Bee venom known to be effective on the inflammatory diseases and pains is composed of complex mixture of various components. Of them, the peptides have anti-inflammatory\(^3, 4\), antibacterial\(^5\), and strong analgesic\(^6\) actions, and contribute to the enhancement of immune responses\(^7\). Melittin, a major component of the dried bee venom, stimulates the pituitary and adrenal glands to produce catecholamine and cortisone, and stabilize the cell membrane of the lysosome for the anti-inflammatory action\(^7)-9\). Like melittin, apamin is also known to stimulate the pituitary and adrenal glands to increase the cortisone for the anti-inflammatory action\(^3, 4\). Bee venom is an alternative approach to the arthritis drugs containing steroids and immunosuppressants, of which prolonged use causes serious side effects on the patients\(^10\).

To meet the demand on the safety issues, including dosage and residues, of drugs to cure the animal diseases, we urgently need to develop new drugs with high efficacy without problems on the continuity and resistance. As the well-being is currently emphasized, the customers require the supply of more hygiene and safe livestock products. In this regard, bee venom, a pure natural substance that has strong antibacterial and anti-inflammatory actions, and less risk of side effects and residues, is an ideal remedy for the domestic animal diseases. Recently, the bee venom therapy combined with acupuncture, which injects the natural bee venom to the meridian locus, has been used to cure the arthritis, the bacterial diarrhea, and agalactia in pigs\(^11)-13\).

However, the practical use of the bee venom acupuncture treatment using live honeybees is limited, because it is difficult to quantify the uniform amount of bee venom, together with an unskilled handling problem. Furthermore, the effect of the natural bee venom has not yet been systematically addressed. We used the electric shock method to isolate bee venom from the honeybees (Apis Mellifera L.) being raised in Korea, and analyzed the growth rate, hematological characteristics, and immune response to the isolated bee venom in pigs.

**Materials and Methods**

**Isolation of Bee Venom**

We used a bee venom collector (Cheongjin Tech, Korea) to isolate bee venom from the honeybees being raised in the institute, and used the Liquid Chromatography (AKTA explorer, Pharmacia, USA) to identify the isolated bee venom. 0.1 M ammonium formate (pH 4.5) was used as a developing solvent, together with Sephadex TM 200 column. We used melittin, apamin, and phospholipase A2 of the Sigma whole honeybee venom as the reference standards.

**Experimental Animal Feeding Management**

The pigs born from healthy 9 sows selected at random were included in the bee venom injection group. The pigs born from two groups of 3 sows were publicly invited and included respectively in the bee venom acupuncture group and the control group. For the experiment, all pigs were raised in Saewoori Farm in Icheon, Gyeonggi-Do (Table 1).

A litter of pigs were raised in the same pigsty freely providing feeds and water for each feeding stage. Among pigs publicly invited, 14 days old pigs were weaned, and 30 days old pigs were moved into the swinery.

Male pigs were castrated at 3 days of age. Based on the swine disease prevention program\(^14\), all pigs in both test group and control group had an injection containing iron at 3 and 10 days of age, and was vaccinated against microplasma at 1 week and 3 weeks of age, against hog cholera at 40 days.
of age, and against pleuropneumonia at 50 days of age.

The Bee Venom Injection and the Bee Venom Acupuncture Treatment

Both procedures were performed four times at birth, castration, weaning period, and after moving into the swinery. The dosage of bee venom for the injection was adjusted depending on the days of age, as shown in Table 1. Bee venom was injected under the skin of the anus, the mid part of tail, or pelvic arch. The acupuncture with two live honeybees above 15 days of age was applied to each meridian, including Hai-men and Bai-hui\(^{15}\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Sow (Pigs)</th>
<th>Dosage or acupoint of bee venom treatment (Days after birth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee venom injection (A)</td>
<td>3 (33)</td>
<td>0.5(^a)</td>
</tr>
<tr>
<td>(B)</td>
<td>3 (32)</td>
<td>1.0</td>
</tr>
<tr>
<td>(C)</td>
<td>3 (32)</td>
<td>1.0</td>
</tr>
<tr>
<td>Bee acupuncture</td>
<td>3 (31)</td>
<td>Hai-men, Du-kou, Jiao-chao</td>
</tr>
<tr>
<td>Control</td>
<td>3 (34)</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\): mg/head, \(^b\): No treatment

Table 1. Experimental design for growth performance according to different stage (per head)

Blood Analysis

We used K\(_3\)-EDTA Vacuum Tube (Vacutainer, BD, USA) to collect the blood from the jugular vein at 30 days after birth, and then used the Automatic Blood Analyzer (Hemat 8, SEAC, Italy) to analyze the blood cell. In addition, we used Serum Vacuum Tube (Vacutainer, BD, USA) to collect the blood, which was then centrifuged at 2,000xg for 30 minutes at 4\(^\circ\)C to isolate the serum. The isolated serum was referred to the Seoul Medical Science Institute for the biochemical analysis using the Automatic Chemistry Analyzer (ADVIA1650, Byer, USA; Cobas Inetra, Roche, Switzerland).

Statistical Analysis

For the statistical analysis of all data, we used Duncan’s t-tests of SAS (SAS enterprise guide 3.0) to perform the analysis of variance (ANOVA).

Results

Identification of Bee Venom

There was little difference in major components, including melittin, apamin and phospholipase A2, between bee venom isolated from the bee venom
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collector and the Sigma whole honeybee venom, as shown in Fig. 1.

Fig 1. Gel filtration of 100 mg freeze-dried whole honeybee venom on Sepadex TM200 10/300. Elution with 0.1 M ammonium formate buffer, pH 4.5. Determination of main components was compared with standard proteins by the optical density at 280 nm (A, Sigma whole honeybee venom; B, whole honeybee venom collected from bee venom collector; PA2, phospholipase A2; M, melittin; A, apamin).

**Body Weight**

We measured the body weight for each stage of growth to determine the effect of the acupuncture treatment using live honeybees, and the injection using bee venom isolated from the bee venom collector on the early growth in pigs (Fig. 2). The body weight was increased by 3% in the bee venom injection group, and the bee venom acupuncture treatment group, over the control group. However, there was no difference between the bee venom injection group, and the bee venom acupuncture treatment group. As the dosage of bee venom for the injection was increased depending on the days of age, it was evident that the higher days of age further increased the difference in body weight between the bee venom injection group and the control group.

![Body Weight Graph](image)

**Fig 2.** Changes of body weight in pigs of honeybee venom injection, acupuncture and control group.

**Survival Rate**

Table 2 shows the effects of bee venom injection and the bee venom acupuncture treatment on the survivability. The survival rate at 14 days of age during the weaning period was 96.8-100.0% in the bee venom injection group, and 96.8% in the bee venom acupuncture treatment group, more or less higher than 93.8% in the control group. The survival rate at 60 days of age until the completion of the swinery was 93.4-96.8% in the bee venom injection group. No pig died after 14 days, particularly, in the C group. The bee venom acupuncture treatment group showed 93.2% of survival rate, significantly higher than 86.7% in the control group. There was no significant difference between the bee venom injection group and the bee venom acupuncture treatment group \((p>0.05)\).
Table 2. Effects of honeybee venom injection and acupuncture treatment on survivability in piglets

<table>
<thead>
<tr>
<th>Groups</th>
<th>Survivability (%) after Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 14 days</td>
</tr>
<tr>
<td>Bee venom injection (A)</td>
<td>100.0</td>
</tr>
<tr>
<td>(B)</td>
<td>96.8</td>
</tr>
<tr>
<td>(C)</td>
<td>96.8</td>
</tr>
<tr>
<td>Bee venom acupuncture</td>
<td>100.0</td>
</tr>
<tr>
<td>Control</td>
<td>93.8</td>
</tr>
</tbody>
</table>

Changes in Hematological Characteristics

To address the changes in hematological characteristics, we collected the blood to analyze the blood cell and serum at 60 days of age after administering bee venom. Although there was numerical difference in the contents of WBC, RBC, lymphocytes and monocytes, there was no statistical difference among the bee venom injection group, the bee venom acupuncture treatment group, and the control group, as shown in Table 3. On the contrary, in the bee venom injection (C) group, the WBC, lymphocytes and monocytes were significantly higher than those in the bee venom acupuncture treatment group and the control group ($p>0.05$).

Table 3. Changes of hematological values in piglets treated with honeybee venom injection and acupuncture

<table>
<thead>
<tr>
<th>Item</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bee Venom Injection</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>WBC (k/$\mu$l)</td>
<td>15.6±2.2</td>
</tr>
<tr>
<td>RBC (M/$\mu$l)</td>
<td>6.2±0.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>56.7±3.8</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>5.2±0.1</td>
</tr>
</tbody>
</table>

Discussion

Various outcomes have been reported from the studies on bee venom that has long been used to cure the diseases in a body. The recent findings indicated that bee venom was composed of 23 components: 11 peptides, including melittin accounting for over 40% of the dried bee venom, 5 enzymes, 3 physiologically active amines, and 4 non-peptide component. Bee venom is known to have major physiological activations, including antibacterial, anti-inflammatory, and analgesic...
actions, the enhancement of immune response, and the protection from radiation\textsuperscript{16, 17}. Many studies are being carried out to use bee venom for the treatment of incurable diseases, including the arthritis and neuralgia. Some good results have already been published\textsuperscript{18, 19}.

Bee venom is known to stimulate the immune system, and fight against the disease in mammals. In other words, it stimulates the living system of an organism to increase the defensive strength\textsuperscript{20}. In Korea, the bee venom acupuncture treatment has recently been applied to the domestic animals for the rise in productivity, the improvement of feed efficiency, and the prevention and treatment of animal diseases\textsuperscript{21), 22). The bee venom acupuncture treatment that applies live honeybees to a certain meridian in pig at 1 day, 3 days (after castration), 14 days and 30 days of age improved the daily weight gain and the feed requirement by 14.1\% and 11.3\% respectively\textsuperscript{15). In addition, the survival rate was improved by 10.0\%, better than the improvement by probiotics or antibiotics. However, it is difficult to standardize and commercialize the bee venom acupuncture treatment, because it shall handle the live honeybees, and there is a lack of studies on the systematic procedures. In this study, we injected the dried bee venom collected from a bee venom collector into pigs to assess the effect of bee venom injection over that of the bee venom acupuncture treatment. Bee venom collected from a bee venom collector maintained a uniform composition. After diluting with saline, we administered the following dose of bee venom to three groups of pigs: 0.5 \text{mg} at birth (1 day of age), 1.0 \text{mg} at castration (3 days of age), 1.5 \text{mg} during the weaning period (14 days of age), and 2.0 \text{mg} at 30 days of age in group A; 1.0 \text{mg} at each stage in group B; 1.0 \text{mg} at birth, 1.5 \text{mg} at castration, 2.0 \text{mg} at 14 days of age, and 2.5 \text{mg} at 30 days of age in group C. Based on 0.3 \text{mg} of bee venom, which is usually secreted by one honeybee, the dosage was determined in accordance with the bee venom acupuncture treatment\textsuperscript{15), 23). Although the dosage of bee venom did not significantly contribute to the weight gain, the group C administering more amount of bee venom than any other group showed relatively higher weight gain. However, there was no significant difference between the bee venom injection group and the bee venom acupuncture treatment group. The weight gain was increased by 12.5-17.8\% in the groups treated with the bee venom injection and the bee venom acupuncture, over the control group. Like the weight gain, there was no significant difference in the survival rate between the bee venom injection and the bee venom acupuncture treatment, though it was also increased by 3.0-10.0\% in the treated groups, over the control group. However, 96.8\% of high survival rate was maintained at 60 days of age in the group C administering more amount of bee venom. This study indicated that the bee venom acupuncture treatment using the bee stings improved the weight gain and the survival rate in early productivity of pigs, as reported earlier, and suggested that the bee venom injection using bee venom isolated from a bee venom collector also remarkably improved the weight gain and the survival rate. It was recommended to increase the dosage of bee venom

### Table 4. Effect of honeybee venom injection and acupuncture on total protein, albumin and IgG in piglets

<table>
<thead>
<tr>
<th>Item</th>
<th>Bee Venom Injection</th>
<th>Bee Venom Acupuncture</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.94±0.30</td>
<td>6.06±0.30</td>
<td>6.06±0.32</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.90±0.10</td>
<td>4.10±0.34</td>
<td>4.30±0.40</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>315.20±41.30</td>
<td>318.40±36.80</td>
<td>320.00±60.20</td>
</tr>
</tbody>
</table>
based on the days of age in the bee venom injection. More dosage improved more weight gain and survivability. To address the changes in hematological characteristics, we analyzed the blood collected from the pigs with 60 days of age. The experiment found that there was no significant hematological difference in the WBC, RBC, lymphocytes and monocyte among the bee venom injection group, the bee venom acupuncture treatment group, and the control group. The group C showed more or less higher lymphocytes and monocytes. From the analysis of serum to address the change in the contents of the total protein, albumin and IgG, there was no significant difference in the total protein and albumin between the treated groups and the control group, though the value was generally higher in the treated groups. The IgG was high both in the bee venom injection group and the bee venom acupuncture group. Our results were inconsistent with the study results by Choi, et al.\(^24\) who reported that there was no hematological change in the blood collected in 3, 6 and 12 hours after administering bee venom. However, Cho, et al.\(^25\) presented the study results similar to ours by demonstrating that the concentration of IgG was increased in the bee venom acupuncture treatment group of the pigs with atrophic rhinitis (AR) over the control group. There were other studies suggesting that bee venom generally increased the contents of immune factors, including IgG, albumin and lymphocytes, in the blood, though it had less effect on organs, i.e., kidneys and liver, in pigs\(^24\),\(^26\). Accordingly, the study shall further be made to address the correlation between bee venom and immunity.

As a result, there was no difference in the improvement of body weight and survivability between the bee venom injection using the dried bee venom collected from a bee venom collector, and the bee venom acupuncture treatment applying the live honeybees to the meridians in pigs. The increased dosage of bee venom further improved the productivity in the bee venom injection. In addition, there was no difference in the hematological characteristics, and the immune responses in the blood between the bee venom injection and the bee venom acupuncture treatment. With the advantages that can maintain uniform components, adjust the dosage of bee venom, and easily being applied to the domestic animals, the bee venom injection is expected to greatly contribute to the production of safe livestock products, and the revenue growth of the pig farmers and beekeepers. We think that further study shall be made on the prevention and treatment of various animal diseases in the future.

**Conclusion**

The study compared the effect of the bee venom injection using the dried bee venom isolated from a bee venom collector to that of the bee venom acupuncture treatment using the live honeybees on the weight gain, survival rate and the hematological characteristics in pigs. We divided one hundred sixty two pigs from 15 sows into three groups: the bee venom injection group (97 pigs from 9 sows), the bee venom acupuncture treatment group (31 pigs from 3 sows), and non-treated control group (34 pigs from 3 sows). Bee venom was administrated at 1, 3, 14, and 30 days after birth. The bee venom injection group was divided into three groups based on the dosage of bee venom: A (0.5, 1.0, 1.5, 2.0 \(\text{mg}\)), group B (1.0, 1.0, 1.0, 1.0 \(\text{mg}\)), and group C (1.0, 1.5, 2.0, 2.5 \(\text{mg}\)). Bee venom was subcutaneously injected, and the bee stings were applied to the general meridian in pigs.

There was no difference in body weight between the bee venom injection group, and the bee venom acupuncture treatment group. Although the body weight was increased by 3% in both treated groups over the control group, there was no significant difference in body weight. There was no difference in survival rate between the bee venom injection group A & B and the bee venom acupuncture treatment group, but the survival rate was 96.8% at
60 days of age in the bee venom injection group C, higher than 93.2% in the control group. There was no significant hematological difference in the contents of the WBC, RBC, lymphocytes and monocyte among the bee venom injection group, the bee venom acupuncture treatment group, and the control group, though the value was higher in the group C. The IgG was high both in the bee venom injection group and the bee venom acupuncture group, but there was significant difference between the treated group and the control group.

In conclusion, the bee venom injection similarly or further improved the body weight and survival rate, and similarly changed the hematological characteristics in pigs, as compared to the bee venom acupuncture treatment. Therefore, the bee venom injection, which has the advantage of uniform components and easy use, is expected to greatly contribute to the improvement of pig production in the future.

References


