HEMATOLOGICAL ALTERATIONS IN ALBINO RATS AFTER ADMINISTRATION OF HONEYBEE VENOM

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Abstract: Bee venom is a complex mixture of proteins (enzymes and peptides) with unique pharmacological activities. The main enzymes in bee venom are hyaluronidase and phospholipase A2. Melittin in high concentrations also has caused hemolysis of red blood cells. This study was conducted to evaluate effects of honeybee venom on hematological characteristics of albino rats. Rats were divided in 3 groups viz., A - (Naive) group; B - mammalian saline treated group and group C - experimental Group treated with bee venom. The last group was divided in 3 subgroups (a - 200µg dose, b - 500µg dose and c - 600µg dose). Rats showed primary symptoms of allergy. Hemoglobin and RBC were depleted and WBC lymphocytes and monocytes were increases. In conclusion, these results suggested that the venom peptides altered blood compositions.

Key words: Honeybee venom, Hematology, Rat

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 [8,9]. Bee venom is an alternative approach to the arthritis drugs containing steroids and immunosuppressant. However, its prolonged use causes serious side effects to patients [10]. Further, bee has more potent venom during the summer [11,12]. Venom of Apis dorsata contains the enzyme, phospholipaseA-2. This enzyme hydrolyzes the phospholipids to free fatty acids and lyoplipids and thus initiates the biosynthesis of eicosanoids and platelet–activating factor, potent mediators of inflammation, allergy, apoptosis, and tumorigenesis.

Melittin is the another principal component of bee venom. It is 100 times more potent than hydrocortisone [13,14]. Melittin also stabilizes the lysosome cell membrane to protect against inflammation [7,9]. It is the major component of the dried bee venom, stimulates the pituitary and adrenal glands to produce catecholamine and cortisone. The objective of present study is to analyze the toxic effect of venom on rat blood composition.

MATERIALS AND METHODS

Collection of honeybee venom: Honeybees (Apis dorsata) are collected from beekeepers in a basket. Then each honeybee was stimulated to sting on a clean glass plate. With a sting a little drop of venom came out on the site of sting. The venom drops on the glass plate were allowed to dry. The air dried venom was collected in a clean vial and stored at low temperature (-40 °C). Extracted venom of Apis
*dorsata* was used for further experimentation.

**Experimental model:** The present study was carried out on Wistar rats *Rattus norvegicus* weighting 120 ± 5 gms. The rats were procured from National Institute of Nutrition (NIN), Hyderabad. The experimentation was conducted according to “INSA-Ethical Committee.” The animals were housed in polypropylene cages in the adequately ventilated room. The rats were fed standard feed and water *ad libitum* throughout the course of the study. After recording initial body weight, they are divided into three groups. As referred in literature, the LD$_{50}$ dose of honeybee venom for human beings is 3 mg/ kg body weight. Accordingly a sub lethal dose for albino rats weighing 120 gm was calculated and selected three sub lethal doses (200 µg, 500µg and 600µg) for the present study.

**Naive:** Rats not administered with either water or venom.

**Control:** Rats administered with 0.5 ml mammalian saline.

**Group a:** Rats administered with 200 µg/0.5 ml dose of bee venom.

**Group b:** Rats administered with 500 µg/0.5 ml dose of bee venom.

**Group c:** Rats administered with 600 µg/0.5 ml dose of bee venom.

Naïve and control male albino rats were kept at atmospheric conditions in animal cages. Experimental albino male rats were used for different doses. Blood sample were collected after 4-6 h. of venom administration.

**Collection of blood sample:** The venous blood was obtained from the orbital sinus (retro-orbital vein) of the control and experimental albino rats.

**Estimation of hemoglobin:** The blood was added to 0.1 N hydrochloric acid to convert hemoglobin to brown colored acid hematin. The resulting color after dilution is compared with standard brown glass reference block of a Sahli hemoglobinometer.

By using a pasture pipette 0.1 N hydrochloric acid was added in the tube up to the lowest mark (20%). Blood was drawn up to 20 µl mark in the Hb pipette. Adjust the blood Coolum carefully without bubbles. Then blood was transferred to the acid in the graduated tube, the pipette was rinsed well, mixed the reaction mixture and allowed the tube to stand for at least 10 minutes. The solution was diluted with distilled water by adding few drops at a time carefully and by mixing the reaction mixture, until the color matched with the glass plate in the comparator. The matching was done only against natural light. The level of the fluid was noted at its lower meniscus and the reading corresponding to this level on this level on the scale recorded in g/dL.

**Total erythrocyte count:** Blood was drawn up to 0.5 marks in RBC pipette. Carefully wiped the excess blood outside the pipette by using cotton. Diluting fluid was drawn up to 101 mark. The pipette was rotated rapidly by keeping it horizontal during mixing. After five minutes, by discarding few drops from the pipette and holding it slightly inclined small volume of the fluid was introduced under the cover slip which is placed on type counting chamber. Settle the cells for 2 to 3 minutes. The red blood cells in the four corners square and in the centre square were counted.

**Total leucocyte count:** Blood was drawn up to 0.5 marks in a WBC pipette. Wiped out excess blood outside the pipette by using cotton. Diluting fluid was drawn up to 11 mark. The contents were mixed carefully in the pipette and after five minutes by discarding few drops the counting chamber was filled and allowed the cells to settle for two to three minutes. Focused on one of the 16 square areas by turning objective to low power (10x). Cells were counted as per standard rules.

**Differential leucocytes counting:** A drop of blood was taken on one end of microscope slide and a thin film of blood was prepared with the help of another slide. A known quantity of Leishman’s stain was put on the blood film and let to stay for about 3 minutes. An equal volume of distilled water was added to stain and mixed with the help of pipette. The smear was left to stain for about 7-10 minutes until a greenish metallic scum seen on the surface of dilute stain. The stain was then drained off and the film was washed for about 10 seconds under running tap water. The film stained pink rose color. The different types of leucocytes present in the blood are counted and each type of cell was expressed as a percentage of the total number of cells counted (100). Absolute counts can be calculated by multiplying by the total leucocytes count.
RESULTS

Albino rats were intraperitonially administered with 200 µg, 500 µg, 600µg raw honeybee (*Apis dorsata*) venom. The control rats received an injection of 0.5 ml vehicle. Hemoglobin (gm %) was estimated in albino rats (control as well as experimental) and the results are given in table 1. In control rats the hemoglobin content in blood was 14.9 gm%. However, rats administered with 200µg/0.5ml dose of honeybee venom, exhibited moderate (p < 0.05) depletion in Hb content. In rats administered with 500 µg/0.5ml and 600 µg/0.5ml of bee venom 15.43 % and 29.53 % respectively depletion was recorded which is highly significant (p < 0.01).

Erythrocyte count was found to be decreased after envenomation at all doses selected (Table 1, Fig. 2). The decrease was highly significant (p<0.01) in animals treated with venom dose of 500 µg. Intraperitonial administration of 600 µg/0.5ml honeybee venom also resulted in decrease in erythrocyte count (42.82%).

Contrary to erythrocyte, honeybee venom elevated in total WBC count in the experimental rats (Table 1, Fig, 3). The rise in count was 8.92 %, 14.28% and 35.71% after a dose administration of 200 µg, 500 µg, and 600 µg honeybee venom respectively. The increase in WBC count after administration of 600 µg bee venom was highly significant (p < 0.01).

The results of differential leucocyte count in control as well as experimental rats are shown in table 1 and Figs. 4-8. Neutrophils are reported to be depleted in number (35.18%) after 600 µg dose of bee venom. However, lymphocyte and eosinophil count was elevated in all the experimental rats significantly (P<0.01). Lymphocyte number was increased by 51.42 % and eosinophil count was elevated by 33.33% after intraperitonial administration of 600 µg dose of honeybee venom.

DISCUSSION

In the present investigation significant decrease in RBC count is seen following toxic action in rat due to administration of 600 µg dose of honey bee venom. This might be because of the action of PLA-2 and melittin on red cell membrane provoking hemolysis.

Melittin is the main pain inducing compound, which functions by altering membrane integrity. PLA-2 works in concern with melittin; it has no catalytic effects by itself (15,16). These two components are responsible for red cell hemolysis. Once melittin has disrupted the cell membrane, PLA-2 cleaves bonds in the fatty acid portion of the bilipid membrane layer. Eventually PLA-2 reacts with the cell membrane phospholipids to produce lysophosphatides, which

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Control+ Vehicle (0.5 ml)</th>
<th>200 µg/0.5 ml</th>
<th>500 µg/0.5 ml</th>
<th>600µg/0.5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin gm%</td>
<td>14.9 ± 0.82</td>
<td>14.9 ± 0.76</td>
<td>13.00 ± 0.86* (12.75)</td>
<td>12.6 ± 0.92** (-15.43)</td>
<td>10.50 ± 0.58** (-29.53)</td>
</tr>
<tr>
<td>R.B.C. 10^6 cell/cu mm</td>
<td>4.25 ± 0.09</td>
<td>4.26 ± 0.08</td>
<td>4.04 ± 0.15 NS (-9.94)</td>
<td>3.47 ± 0.16** (-18.35)</td>
<td>2.43 ± 0.21** (-42.82)</td>
</tr>
<tr>
<td>W.B.C. 10^3 cell/cu mm</td>
<td>5.6 ± 0.50</td>
<td>5.6 ± 0.36</td>
<td>6.1 ± 0.55* (8.92)</td>
<td>6.4 ± 0.22 (14.28)</td>
<td>7.6 ± 0.36** (35.71)</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>54</td>
<td>51</td>
<td>41 (-24.07)</td>
<td>39 (-27.77)</td>
<td>35 (-35.18)</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>35</td>
<td>38</td>
<td>44 (25.71)</td>
<td>49 (40.00)</td>
<td>53 (51.42)</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>06</td>
<td>06</td>
<td>08 (33.33)</td>
<td>07 (16.66)</td>
<td>08 (33.33)</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>04</td>
<td>04</td>
<td>06 (50)</td>
<td>04 (00)</td>
<td>03 (-25)</td>
</tr>
<tr>
<td>Basophil %</td>
<td>01</td>
<td>01</td>
<td>01 (00)</td>
<td>01 (00)</td>
<td>01 (00)</td>
</tr>
</tbody>
</table>

Table 1: Alterations in Haematological parameters of male albino rats after intraperitoneal administration of honeybee venom. Values are ± SE of 6 replicate from each group.*p< 0.05, **p<0.01, NS – Not significant. Figures in parenthesis indicate percent change over control.
Fig. 1: Alteration in Hb content of male albino rats after administration of different doses of honeybee venom

Fig. 2: Alteration in RBC count of male albino rats after administration of different doses of honeybee venom

Fig. 3: Alteration in WBC count in male albino rats after administration of different doses of honeybee venom

Fig. 4: Alteration in Neutrophil % count in male albino rats after administration of different doses of honeybee venom

Fig. 5: Alteration in lymphocyte % count in male albino rats after administration of different doses of honeybee venom

Fig. 6: Alteration in Eosinophil% count in male Albino rats after administration of different doses of honeybee venom

Fig. 7: Alteration of Monocyte count of male albino rats after administration of different doses of honeybee venom

Fig. 8: Alteration in Basophil % count in male albino rats after administration of different doses of honeybee venom
cause even more disruption of the RBC membrane [17]. Ultimately, there is damage to the cell membrane enzyme system also. Melittin thus is known to work synergistically with PLA2 on many biological membranes, causing myonecrosis and lysis of erythrocytes [18,19].

Melittin mimics both the cytotoxic and mitogenic actions of TNF [20]. Many phosphatidylcholines and phospholipids are present in plasma of albino rat. PLA-2 from honeybee venom thus can act on phosphatidylcholines and phospholipids to liberate lysolecithin which can in turn act on erythrocyte and destroy them. It is the inherent property of albumin to bond with lysolecithin so that hemolytic action of lysolecithin is inhibited. But in the absence of albumin, lysolecithin becomes active and burst opens the red blood cells [21].

The hemolytic ingredients of honeybee venom have strong affinity for phosphatidyl-ethanolamine and phosphatidyl-ethanolamine and phosphatidyl serine present in the erythrocyte membranes [22]. Hence this might also have accelerated the lysis of erythrocyte resulting into depletion of total erythrocyte count in the envenomated albino rats. Thus, in the present investigation, decrease in erythrocyte counts observed due to the complex mechanism in which the membrane of erythrocyte rupture leading to leakage of hemoglobin and many ions including K⁺, Na⁺ etc. The liberated hemoglobin might have in turn metabolized by the proteolytic enzymes of the honeybee venom leading to its depletion. 6 hours after envenomation (600µg), total WBC count was increased significantly (p<0.01) with significant rise in eosinophils (33.33 %) and lymphocyte (51.42 %).

These results are also indicating an immunological response as in every immune response towards the entry of foreign material lymphocytes and eosinophils are proliferated. In the present investigation depleted neutrophils count has been observed which might have resulted due to immune mechanism when the excess of antibodies produced lysed the granulocyte in the presence of PLA-2 [23].

**CONCLUSION**

The blood was used to study the alterations in hematological profile of envenomated rats. 2 controls were kept, one normal rats without any administration i.e. naïve and other, rats administered with 0.5 ml mammalian saline (vehicle used for dilution of venom).

RBC count and hemoglobin gm % was depleted in all the envenomated rats; however this depletion was highly significant at a dose of 600 µg.

Total WBC count was significantly increased in all the envenomated rats. The DLC shows, significant increased of lymphocytes and Eosinophils and significant depletion in neutrophils count at 600 µg dose.

**REFERENCES**