EFFICIENCY ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF HONEY-BALM ON EXPERIMENTAL BURN WOUNDS

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Abstract

The antibacterial activity of honey-balm and silver sulphadiazine applied onto burn wounds in pigs of Biała zwisłoucha breed was compared. The efficiency assessment was based on the clinical and antimicrobial examination. It was demonstrated that honey-balm showed higher antimicrobial activity than did sulfadiazine.

Key words: swine, honey, sulfadiazine, antimicrobial activity, burn wounds.

Technological progress leads to the increase in the exposure of people to the formation of burns and, at the same time, to the intensification of research on new efficient methods for the treatment of these ailments.

Researches relating burns show that most frequent reason of death is infection. Wide burns are an infection gate for many microbes and arising necrosis of tissues is convenient environment for their multiplication. Number and types of microbes existing in after-burn wounds are significant for the running of biological processes of which final effect should be wound healing (4).

Nowadays, the treatment of burns relies, among other things, on surgical removal of necrotic tissues, supplementation of liquids and wound protection from infection with microbes (8, 9, 10, 13, 14, 19). Preventive local use of antibiotics does not always ensure the sterility of the wound. This is caused by the inactivation of some antibiotics or development of resistance of microbes (6, 7).

Such resistance makes possible secondary infection development or widening of already existing. Therefore, so important is the suitable selection of applied treatment. If efficient antimicrobial medications are not used, further development of microbes follows and deep penetration of them into the tissues takes place. If defensive mechanisms of the organism are weakened or inefficient and the number of bacteria in wound exceed 10⁷/g of tissue, the infection of not-burned tissues is going to develop (5).

Taking into consideration the above mentioned conditions, research on new efficient biogenic or biosynthetic therapeutic preparations appear to be intentional (15).

Among present conventional methods of treatment, more and more attention is brought to the pharmacologically active fractions received from honey bee products showing healing properties.

One of most precious newly discovered bee products are comb cappings. This is a natural product synthesized by bees from wax, propolis and honey. Basic component of cappings is honey. It is composed of over 300 components belonging to different groups of chemical compounds, determining its of antibacterial, detoxical, nutritious, preservative, anti-allergic, regenerative, cardioprotective and calming activity.

In view of the difficulty in the treatment of burn wounds, our goal in this study was to define the efficiency of antimicrobial activity of apipharma-therapeutic – honey-balm in relation to bacterial flora isolated from burn wounds in „Biała zwisłoucha” breed pigs.

Material and Methods

The antimicrobial activity of honey-balm, applied on burn wounds in 2 pigs, 30-40 kg of weight and 15-16 week-old was analysed. The model of the burn wound was prepared according to Hoekstra standard (2). The experiment was in agreement with the rules of Good Laboratory Practice and was accepted by the Regional Ethical Board for Experiments on Animals.

Each pig was wounded at 9 places on each side with hot (170⁰C) brass brick weighing 450 g and applied for 10 s. The surface of each burn wound was
5x3 cm and did not exceed 10% of the body surface. Before burning the pigs were put to general anesthesia with 1 mg/20 kg b.w. of atropine sulphate, 0.1 ml/kg b.w. of 2% xylazine (Sedazin) and 10 mg/kg b.w. of ketamine hydrochloride (Bioketan).

In the first swine, the burn wounds were treated once a day with the honey- balm. In the second one, the wounds were treated with silver sulfadiazine (SSD), applied also once a day.

For microbiological examination, the swabs from the burn wounds were taken on 1, 3, 5, 10 and 15 d of the experiment. The samples were analysed quantitatively and qualitatively by culture, microscopic examination and biochemical tests (API-bioMérieux), according to NCLS standards. In the same time microbiological control of skin and honey balm was performed.

Results

Clinical observations. Results of clinical observations, concerned on general condition of animals and healing wound process are presented in Table 1. In the clinical assessment of the healing process, the following features were taken into account: the appearance and size of wound area, appearance of surrounding skin and the process of granulation and formation of the crust.

For the first 24 h the analgesic medications have been administered to eliminate an eventual pain feeling in animals. On next days of the experiment the animals showed a normal reaction to the environment and revealed no symptoms of suffering due to after-burn wounds.

Bacteriological examinations. Microbiological examinations in all subgroups concerned 4 microorganisms: *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus* sp. and *Candida albicans*.

In the group I, in the first 24 h, *Staph. aureus*, *Ent. faecalis*, *Bacillus* sp. and *Candida albicans* were isolated. Their growth lasted for next days of the experiment until day 5. On the 10th d the growth of *Bacillus* sp. and *Candida albicans* was not observed. On day 15 only *Staph. aureus* was isolated.

<table>
<thead>
<tr>
<th>Day</th>
<th>Honey balm</th>
<th>1% Silver sulfadiazine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 3</td>
<td>Clinical symptoms were similar. Inflammation with large quantity of exudation and swelling of skin surrounding the place of scald was accompanied by necrotic changes. On the next days the appearance of wounds was different in all subgroups.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Areas of wounds were clear, crust was correctly formed, skin in area of burn showed no pathological changes.</td>
<td>Wounds were coated with not completely formed crust, skin around was cracked, reddened and swollen.</td>
</tr>
<tr>
<td>10</td>
<td>At wound edge (under the crust) pink scar was seen, what pointed at advanced epidermalization.</td>
<td>Wounds were dried and coated with crusts.</td>
</tr>
<tr>
<td>15</td>
<td>Areas of wounds were markedly reduced (up to 0.5x1.5 cm) and were coated with scar. The wounds were recognized as healed.</td>
<td>Crusts were still adhered to the surface of wounds. On edges they came off from wound surface. In these places the epithelium appeared with a red tint what indicated that the scar organisation was created. The area of wounds was not diminished.</td>
</tr>
<tr>
<td>20</td>
<td>Wounds were healed.</td>
<td>Crusts came off from the surface of wounds. Under the crusts there were reddened scars. Size of the wounds was not changed.</td>
</tr>
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</table>

In the group II in the first 24 h of the experiment the growth of *Staph. aureus* and *Ent. faecalis* was observed. These species were isolated also on days 3, 5 and 10 of the experiment. However, on the 15th d a lack of growth of above-mentioned organisms was observed with exception of *Staph. aureus*.

Simultaneously quantitative examinations of bacterial flora in the after-burn wounds were conducted. In the group I, the number of microbes existing on 1 cm² of the wound amounted to 7x10⁵ in the first 24 h and increased up to 1x10⁶ on the 3rd d. This number was stable till the 5th d of the experiment, however, on day 10 it decreased considerably to the value of 1x10⁵. This value was smaller than initial one, what showed that after the use of honey balm the number of microbes was smaller than in the first 24 h of the experiment. Next, on the 15th d the number of microbes considerably decreased and reached the value of 2x10⁴.
In the group II, in the first 24 h the number of microbes amounted to $2 \times 10^4$ and gradually increased during the following days to the value of $6 \times 10^5$ on day 5. In the 10th d the number of microbes began imperceptibly to decrease until day 15, when reached the value of $2 \times 10^5$. Results of microbiological examinations are shown in Fig. 1.

![Fig. 1. Total number of microorganisms isolated from burn wounds.](image)

Honey accelerated processes of granulation and epithelialization of tissues and diminished wound swelling.

We observed in our experiments that the wounds treated with honey-balm were healed after 20 d. At the same time in SSD treated wounds crusts came off from the surface of wounds and under the crusts there were reddened scars. On the 15th d the total number of isolated microorganisms was higher ($2 \times 10^5$) when SSD was applied as compared to wounds treated with honey-balm ($2 \times 10^4$).

Subrahmanyam showed, that in the patients treated with honey, 91% of wounds were redered sterile within 7 d. In the patients treated with silver sulfadiazine, 7% showed control of infection within 7 d (16, 17).

Pharmacological activity of honey-balm observed in the treatment of after-burn wounds points at its more profitable influence in comparison with effects obtained with the use of honey. It should be supposed that this is a result of composition and obtaining process of honey-balm. The balm contains comb cappings as active substance and honey supplemented with wax, propolis and secretion of bee’s saliva glands.

The described profitable activity of honey-balm and honey confirms their status of pharmacopeal resources, which in frames of adaptation of Polish Pharmacopea to European Norms will take a place in its contents. It should be supposed, that further researches on pharmacological activity of these resources will confirm their influence on cell proliferation and stimulation of angiogenesis.

Discussion

Therapeutic efficiency of apitherapeutics, which pharmacological activity results from physico-chemical properties of honey or propolis, have been confirmed in clinical research by Molan (11). Also Ndayisaba et al. (12) showed the efficiency of honey usage in the treatment of wounds of different aetiology.

Profitable activity of honey in the treatment of 50 infected surgical wounds (caesarian operation, hysterectomy) has been reported by Al-Wali (1). Results of these studies have pointed on quicker elimination of bacteria, reduction of hospitalization period, acceleration of healing process of wounds and decreasing thescar size after the use of the honey.

Willix et al. (18) showed, that growth of the most of bacterial strains was completely stopped by honey already in 11% concentration. They showed also a clinical efficiency of honey in the treatment of skin infections (18).

In 1998 Efem (3) tried to use honey in the treatment of wounds and ulcerations in 59 patients where conventional methods of therapy did not bring awaited effects. When honey was applied, wounds, formerly infected, became sterile after about a week.
References


