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Study on anti inflammatory effect of subcutaneous honey bee venom injection and dermal application of cream containing honey bee venom in adjuvant-induced arthritic rats

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ABSTRACT

Honey bee sting is used in some societies as a treatment of inflammation in joint diseases such as arthritis. To investigate the effect of honey bee venom in reducing inflammation, we selected 30 male Wister rats which were divided in to 6 groups. Except group 1, all remaining groups received 0.5 ml of complete Freund's adjuvant to induce arthritis. After 9 days, all animals that received adjuvant were suffering from acute inflammation in their joints especially in knee joint (tibia-tarsal region). Group 2 was not received any treatment. Group 3 was received only saline (0.05 ml) by subcutaneous injection at the site of inflammation. Group 4 received cream without any honey bee venom. Group 5 was received cream containing 200µg honey bee venom/gram of cream. Group 6 was received 0.05 ml solution containing freshly prepared 7 µg honey bee venom through subcutaneous injection at the site of inflammation. The parameters determined were, arthritis index score (redness, edema, stiffness in movement) and joint diameter, all the parameters were noted before and during experiment. Results obtained in this experiment shows that all animals that received complete freund's adjuvant, suffered from acute inflammation, redness and difficulty in movement. Treatment by honey bee venom at mentioned dose could not reduce either the inflammation or difficulty in movement. This study brings a great doubt for using honey bee venom as an anti inflammatory drug.

Keywords: Apitherapy, Bee venom**,** Rheumatoid arthritis, Inflammation

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology that is characterized by progressive joint destruction, deformity, disability and premature death in most patients (Emery & Symmons 1997). Medication therapy used in most cases utilizes various kinds of medicine such as NSAIDs, adrenal cortical hormone, anti-rheumatic drugs and immunosuppressive agents, which can cause severe adverse effects such as depression, peptic ulcers, enterohemorrage, liver malfunction and renal disorders (Dinant & Dijkmans 1999, Emery & Symmons 1997, Firestein 2005). Arthritis resembling human rheumatoid arthritis is produced in rats either by immunization with type 2 collagen

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or injection of complete freund's adjuvant. (John *et al* 1988) Adjuvant-induced arthritis (AA) in rats has been shown to be similar to rheumatoid arthritis (RA) in many respects, and it is widely used as a model for detection and evaluation of compounds with anti-inflammatory or anti-rheumatic activity (Nakajima *et al* 1991). Published evidence is in favor of the hypothesis that adjuvant arthritis is a delayed hypersensitivity response to some antigen derived from the mycobacterium. Rat adjuvant arthritis has been shown to be inhibited by many steroidal and non steroidal anti-inflammatory agents, and the inhibition correlates well with their clinical effects in man (Nakajima *et al* 1991). Several studies suggest that the effects of BV depend on the locations injected; acupuncture points exert much stronger effects than non-acupoints (Kwon *et al* 2001, Seo *et al* 2003, Kim *et al* 2003). The effects of bee venom might be intensified by acupuncture stimulations, which may help in reaching therapeutic goals. The number of controlled studies of BV in arthritis is quite small, and their quality is limited (Myeong *et al* 2008). The purpose of the present study was, therefore, to evaluate the data from experimental studies and to explore further ways to better comprehend the effectiveness of BV on arthritis.

MATERIALS AND METHODS

Bee Venom was extracted from honey bees using electric shock plate, lyophilized and preserved at -20 °C until use (Seo *et al* 2003). Size of the joint in knees was determined using collies in both the directions ventralposition at the start point of experiment and every alternate day once. To induce Rheumatoid arthritis, 30 Wister rats were selected; rats were divided in to 6 groups. Except group1 all the animals received 0.5 ml of complete frond's adjuvant by subcutaneous injection in tibia-tarsal joint region. Every 3 days size of joint was determined till day 9 (Kwon *et al* 2001, Marja-Leena *et al* 1982). To determine the anti-inflammatory effect of honey bee venom on day 9 the five remaining groups who developed rheumatoid arthritis were used for the honey bee venom treatment. Group 2 did not receive any treatment. Group 3 received only saline (0.05 ml) by subcutaneous injection at the site of inflammation.

Group 4 received cream without any honey bee venom. Group 5 received cream containing 200µg honey bee venom/gram of cream. Group 6 received 0.05 ml solution containing freshly prepared 7 µg honey bee venom through subcutaneous injection at the site of inflammation. The parameters determined were, arthritis index score (redness, edema, stiffness in movement) and joint diameter (Marja-Leena *et al* 1982). Statistical analysis done using student unpaired T test.

Table 1. Arthritis index score in accordance with signs observation in each rat.

Score	Signs
0	No sign
1	Redness without edema
2	Redness with mild edema
3	Redness with severe edema
4	Redness, severe edema &stiffness in movement

RESULTS

Signs and symptoms of adjuvant arthritis such as red ness and inflammation appeared within 3 days after the injection of complete frond's adjuvant. The average size of tibia-tarsal joint region in all the groups except group 1, before receiving complete frond's adjuvant was 0.6 ± 0.04 cm and within 9 days it increased significantly (P> 0.001) up to 1.23 cm. All the animals that received complete Freund's adjuvant developed AA except one rat that was removed from the group. Undevelopment of arthritis in one rat could be due to some defect in injection of adjuvant to the animal.

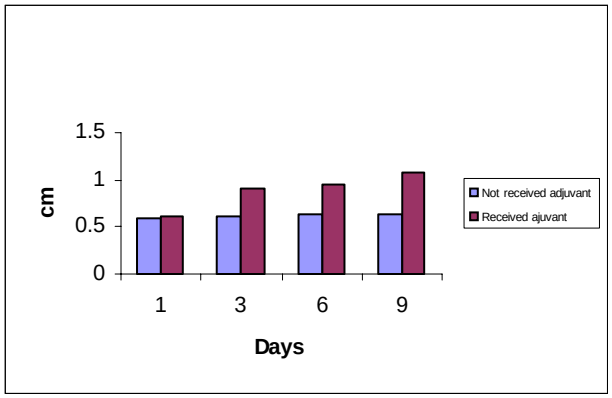

Figure 1. Adjuvant arthritis induction in rats using complete freund's adjuvant.

The size of tibia-tarsal joint region in group 2 which did not received any treatment after AA development on day 9 till day 21 did not change significantly. However a slight increase was observed which could be due to normal increase in the weight of animals during 21 days of experiment. The changes in size of tibia-tarsal joint region in group 3, 4, 5 and 6 animals which received saline, cream, cream containing venom and venom injection treatment after AA development was not significant.

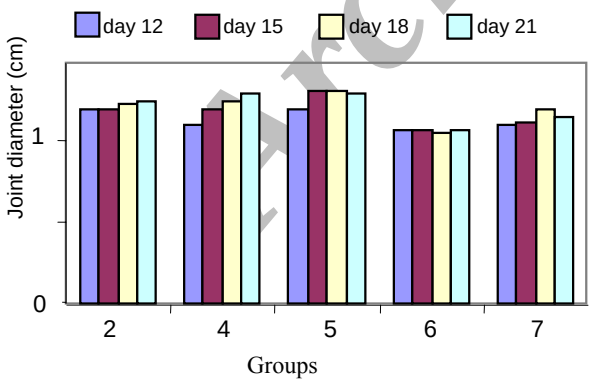

Figure 2. Changes in joint diameter in adjuvant induced arthritis after honey bee venom treatment

Arthritis index in all the animals showed no significant change after treatment with honey bee venom using either cream containing BV or by injection rout.

DISCUSSION

Adjuvant arthritis in experimental rats was induced by complete Frundś adjuvant. This is in accordance with previous research works (Marja-Leena *et al* 1982). Size of the tibia-tarsal joint region was increased significantly (2 folds) till the day 9. The treatment with bee venom started on day 9 by two ways cream application and subcutaneous injection of the solution containing honey bee venom (Kwon *et al* 2001). The inflammation increased till day 9 and it remained in almost stationary state during the experiment. We considered day 9 as the day for starting the bee venom treatment because the condition of arthritis was at its maximum and no significant change was observed after day 9. This experiment is in accordance with other research works (Marja-Leena *et al* 1982). However some research workers started the treatment on day first along with adjuvant (Kalpana *et al* 2007). It was because they wanted to see the preventing effect of drugs. Since we wanted to observe the anti-inflammatory effect of bee venom we started the bee venom treatment on day 9 which was the maximum inflammation.

One of the most important sign and symptoms that are considered as an indicator for AA is inflammation at the tibia-tarsal joint region. Therefore we used this indicator as a reference for the affectivity of our treatment. In this experiment unfortunately we could not analyze the pain. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. Chronic inflammation involves the release of number of mediators like cytokines (IL-IB and TNF), GM-CSF, interferon's and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can lead to severe disability

(Kwon,YB *et al* 2001, Kalpana & Patil 2007).The very expansion of the bee venom therapy of rheumatic diseases started only in the twenties of this century and soon the use of bee venom spread throughout Europe. Nevertheless, bee venom treatment had also its period of decline, both in Eastern Europe and the Western countries. Thus in the majority of classic handbooks on rheumatology many authors consider it a dubious method, admitting at most its histamine-like and counter-irritant effect (Myeong *et al* 2008).

Table 2. Changes in score of Arthritis Index (average sum of two knee joints) after honey bee venom treatmen.

Days Groups	Mean±SE day 12	Mean±SE day 15	Mean±SE day 18	Mean±SE day 21	P>0.05
2	5±0.71	5±0.62	5.1±0.68	5.2±0.63	N.S
3	5.3±0.38	5.5±0.39	5.6±0.40	5.6±0.42	N.S
4	5.2±0.62	5.3±0.60	5.3±0.35	5.2±0.65	N.S
5	5.2±0.64	5.2±0.55	5.1±0.32	5.2±0.59	N.S
6	5.2±0.51	5.3±0.49	5.5±0.32	5.4±0.35	N.S

The disrepute brought upon this method is due to the fact that whereas the pharmacological and chemical research of bee venom developed along strictly scientific and laboratory lines, the clinical research remained encumbered, on the one hand, by an almost mystical enthusiasm and, on the other hand, by a stubborn unwillingness of clinicians to believe blindly in the natural forces hidden in the bee-sting. The number of controlled studies of BV in arthritis is quite small, and their quality is limited. Results of this study shows no significant improvement in reducing the edema and RA index score in RA induced rats. Some of the research works indicate that the honey bee venom can be effective when it is used as pretreatment (Seo *et al* 2003). In our study we did not use the bee venom as pretreatment. Hence this can be a factor that no positive results obtained by us.

Several studies suggested that the effects of bee venom were intensified by acupuncture stimulations, which may help in reaching therapeutic goals (Kim *et al* 2003, Kwon *et al* 2001, Seo *et al* 2003). In our study we did not considered the acupoint for the injection of the bee venom. This may be another reason that no positive results obtained in this study. Some reports indicate that the positive results of apitherapy are to relief the pain (Seo, *et al* 2003). However we could not determine the grade of pain in rats, hence we are not able to draw a conclusion on this part of subject.

However one of the components in the bee venom is found to be phospholipase A2 activating protein (PLAP). Phospholipase A2-activating protein induces the synthesis of IL-1 and TNF in human monocytes. Phospholipase A2-activating protein can also be found in high concentrations in joint (synovial) fluid from patients with rheumatoid arthritis, and injection of PLAP into animal joints results in an inflammatory, rheumatoid-like lesion. Further, results of their study also demonstrated that PLAP may regulate cytokine synthesis and thus perpetuate an immune or inflammatory response (Bomalaski *et al* 1995). It means, injection of bee venom in an over dose or at wrong site (other than acupuncture specific site) not only dose not suppress the inflammation but even it may stimulate it. In conclusion based on this study, at least in animal model no positive results obtained for reducing the edema caused by RA after treatment with either using cream or injection of honey bee venom. This study also shows that even if there is any positive results using HBV as a treatment for RA there are many limitations for this way of treatment. May be rigorous trials with large sample size and adequate design are needed to drew a correct conclusion on the role of BV for these indications. In addition, studies on the optimal dosage and concentration of BV are recommended for future trials.

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