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Study on anti inflammatory effect of scorpion (*Mesobuthus eupeus*) venom in adjuvant-induced arthritis in rats

Ahmadi[1], M., Zare Mirakabadi[*2], A., Hashemlou[1], M., Hejazi[1], M.

1. Department of Chemistry, Razi Vaccine and Serum Research Institute, Kraj ,Iran
2. Department of venomous animals and anti venom production, Razi Vaccine and Serum research Institute, Karaj, Iran

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ABSTRACT

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints as well as other organs in the body. Adjuvant-induced arthritis models in inbred rats serve as relevant models for RA, having many clinical similarities to this disease. Using honey bee venom as a treatment for Rheumatoid arthritis is an ancient therapy in various parts of the world. However scorpion venom neurotoxins are responsible for toxicity and pharmacological effects**.** Twenty-five Wistar male rats weighing 110-130 g were divided in 5 groups and Arthritis was induced in them, using Freund's adjuvant, except in group 1. In group 2 after the induction of arthritis no treatment was given. Group 3 received Betamethasone as an anti-inflammatory medicine. Venom (5µg/rat) was used in group 4 as a treatment and Group 5 received crude venom (10µg/rat) as treatment, after R.A induction, all the animals received treatment near the site of tibio-tarsal joint subcutaneously. The clinical features of the adjuvant induced arthritis like difficulty in movement and edema in joint appeared 3 days after inoculation of adjuvant. The onset of inflammation was explosive occurring 13-15 days post inoculation with a peak onset at day15. After the treatment of rats, there was a significant reduction in score of arthritis index in all treated animals. The changes in size of tibio-tarsal joint region in groups 4 and 5 which received crude scorpion venom and group 3 with Betamethasone treatment after arthritis development decreased. At the end of experiment, blood collection for WBCs count was carried out. In 2 (untreated rats) and Betamethasone treated rats, there was a significant rise in WBCs count. However in venom treated rats the rise in WBCs was not significant as compared to group 1 rats. The present study demonstrated that the scorpion (*Mesobuthus eupeus)* venom could be effective as anti-arthritis agent in animal model of acute inflammation. More studies are needed to be carried out to find the mechanism of the venom and exact therapeutic doses of the venom for acting as anti-arthritis agent.

Keywords: *Mesobuthus eupeus*, Scorpion venom, anti-arthritic effect, anti-Inflammation, adjuvant-induced arthritis

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints as well as other organs in the body. Autoimmune diseases are illnesses that occur when the body tissues are mistakenly attacked by its own immune system. Patients with autoimmune diseases have antibodies in their blood that target their own body tissues, where they can be associated with inflammation (Wolfe 1996). Adjuvant-induced arthritis (AA) in rats has been shown to be similar to R.A in many respects, and it is widely used as a

[*]Author for correspondence. E-mail: A.zare@rvsri.ir

model for detection and evaluation of compounds with anti-inflammatory or anti-rheumatic activity (Nakajima *et al* 1991). Using honey bee venom as a treatment for Rheumatoid arthritis is an ancient therapy in various parts of the world. However scorpion venom neurotoxins are responsible for toxicity and pharmacological effects. They are active in sodium and potassium channels leading to an increase in the release of neurotransmitters, such as glutamate (Maeulo *et al* 2002). Recently the researchers used the venom's characteristics as an initial guide to develop a chemical with gentle, more therapeutic properties. From a study that started originally by looking at how scorpion venom inactivated cell functions, it is synthesized the chemical TRAM-34 and found it suppressed T-cell function without affecting other biochemical processes in the cell. This study brought a hope to find new ways to keep the immune system from attacking itself in certain diseases or from rejecting transplanted organs (Wulff 2002). Hence in this study we attempt to find out if there is anti inflammatory activity by the venom of scorpion *Mesobuthus eupeus* in the adjuvant-induced arthritis model in rats.

MATERIALS AND METHODS

Twenty-five wistar male rats weighing 110-130 g were divided in 5 groups and used in this study. Arthritis was induced in rats, except group 1, by subcutaneous injection of killed Mycobacterium tuberculosis suspended in mineral oil (Freund's complete adjuvant) 0.05 ml in tibio-tarsal joint region (Kongtawelert *et al* 1998). All the animals were given food and water and kept in an animal room at a constant temperature of 22 °C with a 12 h alternating light-dark cycle (Castro *et al* 1981). The animals were injected 0.05ml of the venom weekly as following: Group 1 did not receive Freund's adjuvant and considered as negative control group, therefore no treatment was given to this group. In group 2 AA was induced, but no treatment was given hence this group was considered as positive control group. Group 3 received Betamethasone (0.05 ml) as standard treatment after induction of AA. Venom (5µg/rat) was used in group 4 as a treatment after induction of AA. Group 5 received crude venom (10µg/rat) as treatment, after AA induction. All the animals were received treatment near the site of tibio-tarsal joint subcutaneously. Clinical observations such as weight, Arthritis Index, severity of swelling of ankle joints (tibio-tarsal) were assessed weekly after day 15 to day 35. The severity of swelling was determined by determination of diameter of ankle joints. At the end of experiment blood collection carried out and level of WBCs were counted. Statistical analysis was carried out using analysis of variance.

RESULTS

The clinical features of the adjuvant induced arthritis, like difficulty in movement and edema in joint, appeared 3 days after inoculation of Adjuvant. The onset of inflammation was explosive occurring 13-15 days post inoculation with a peak onset at day15 in all the groups except group 1.

Table 1. Arthritis Index score in accordance with signs observations 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 and stiffness in movement

The tibio-tarsal joint swelling could also be easily quantified by measuring the diameter of the ankle (table 2). As shown in table 2 the ankle diameter significantly increased indicating inflammation in all groups except group 1. The size of tibio-tarsal

joint region in group 2 (positive control), which did not receive any treatment after R.A. development on day 15 till day 35, although increased slightly but it was not significant.

Table 2: Effect of venom and corticosteroid drug on the diameter of tibio-tarsal of adjuvant induced arthritis rats

Days	Group 1 (cm)	Group 2 (cm)	Group 3 Betamethasone (cm)	Group 4 Venom 5µg/rat (cm)	Group 5 Venom 10µg/ml (cm)
Day 1	0.54	0.55	0.542	0.526	0.538
Day 15	0.56	1.3	1.2	1.3	1.5
Day 21	0.57	1.44	1.128	1.29	1.38
Day 28	0.59	1.61	1.07	1.18	1.1
Day 35	0.61	1.79	0.96	1.09	1
P Value	N.S.	N.S	P<0.01	P<0.05	P<0.05

Note: The comparison was between day 15 and day 35 in all groups

N.S.: The P value is not significant

The results in group 2 animals were compared between day 35 with day 15.

The changes in size of tibio-tarsal joint region in groups 4 and 5, which received crude scorpion venom, and group 3, with Betamethasone treatment after R.A. development decreased. Signs and symptoms of R.A. such as difficulty in movement and edema appeared within 3 days after the injection of complete Freund's adjuvant. The peak of edema, redness and stiffness in movement was on day 15. Score of arthritis showed in table 3.

Table3. Changes in score of Arthritis Index after treatment with venom and corticosteroid drug

Days	Group 2	Group 3	Group 4	Group 5
Day 15	5	4.8	4.8	4.6
Day 21	5.2	4	4.6	4.4
Day 28	5.4	3.4	4.6	4
Day 35	5.6	2.4	4.1	3.5
P Values	N.S.	P<0.005	P<0.05	P<0.005

N.S.: The p value is non significant

The score of arthritis when compared from day 15 to day 35, except in group 2 which did not change significantly there was a significant reduction in score of arthritis index in all the treated animals in groups 3,4and 5.

Table 4. Changes in WBC count in different groups on last test day (cells/mm)

Groups	Mean ± S.E.M.	P Value
1	7200 ± 74.5	No P value
2	16960 ± 1541.3	P<0.005
3	19280±1381.8	P<0.005
4	12340 ± 3320.9	N.S.
5	12100 ± 2212.2	N.S.

N.S.: Not significant

No P Value: all the groups were compared with Group 1 as control group. Hence no P value could be calculated for group 1 animals.

As shown in table 4 the total WBC count were remarkably increased in adjuvant-induced rats and Betamethasone group (groups 3, 4 & 5 respectively).

Table 5. Changes in body weight in adjuvant induced arthritis in rats

Groups	On induction day (gm)	On last day (gm)	Mean changes (gm ± S.E.M.)	P Value
1	152	198.4	46.4 ± 7.7	P<0.005
2	132.6	145	12.4 ± 4	N.S.
3	137.8	161.2	23.4 ± 7.2	P<0.05
4	140	165.2	25.2 ± 5.1	P<0.05
5	129	171.2	45.4 ± 6.3	P<0.005

Results were expressed as mean ± S.E.M. The significance of difference between means was determined by the students t-test and results were regarded as significant at P<0.05. Comparison was between day 1 and day 35.

WBC count was not increased significantly in groups 4 and 5 as compared to control group (group 1) on the last day of experiment. In group 1 there was a highly significant increase (p<0.005) in body weight during the

experiment. The significant increase ($P<0.05$) was also observed in all the treated animals. However in group 2 the increase in body weight was not significant (Table 5).

DISCUSSION

In this study we used complete Freund's adjuvant to induce arthritis, and induction of inflammation occurred on day 3 and reached to its peak on day 15. Adjuvant induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable to those seen in human rheumatoid arthritis (Shinde *et al* 1999). Rats with adjuvant-induced arthritis have been used for several years to evaluate anti-inflammatory activity of drugs (Gouret *et al* 1976, Newould 1963). Changes in body weight have been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs (Winder *et al* 1969). In group 1 rats (negative control group) the average increase in body weight was 30% during the experiment while in group 2 (positive control group) it was only 9.2%. The changes in body weight in group 5 animals was almost similar to negative control group. The loss of the body weight during arthritic condition was also supported by earlier observation on alterations in the metabolic activities of diseased rats (Walz *et al* 1971). Earlier findings suggest that absorption of ^{14}C-glucose and ^{14}C-leucine in rats intestine was reduced in the case of inflamed rats (Somasundaran *et al* 1983), but on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation (Somasundaran *et al* 1983). The increased body weight during treatment of crude scorpion venom may be due to the restoration of absorption capacity of intestine. Rise in corticosteroids which can cause the increase in the body weight, was observed in scorpion venom poisoning (Zare et *al* 1994). This may be one of the reasons for rising body weight in rats that received the venom as a treatment. In this study rise in WBC was observed in all the groups as compared to negative group However in group 4 and 5 which received venom as treatment the increase in WBC was comparatively less than **in** other groups. In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-IB inflammatory response IL-IB increase the production of both granulocyte and macrophages colony stimulating factor (Kalpana *et al* 2007). In the present study, the migration of leukocytes into the inflamed area is suppressed by crude scorpion venom as seen from the decrease in total WBC count. However Betamethasone therapy causes a transient increase in maternal leukocyte count. It accelerates WBC release from bone marrow and reduces clearance from the circulation (Vaisbuch *et al* 2002). Injection of the crude venom reduced the paw edema which was induced by complete Freund's adjuvant as measured by the ankle swelling on the injected site. Scorpion venom contains a vast number of biologically active substances such as peptide toxins with varied ion-channel specificities, enzymes, nucleotides, lipids, mucoproteins, biogenic amines, glycosaminoglycans and histamine (Zlotkin *et al* 1978). Whole scorpions, scorpion tails or their extracts have been found to be effective in treating some neural diseases such as apoplexy, epilepsy, facial paralysis, hemiplegia besides being used to soothe the nerves and relieve pains caused by meningitis, cerebral palsy and rheumatism (Liu *et al* 2003). From the findings of the test it may be inferred that the venom is effective in the delayed immunological response to the constituents of the mycobacterium tuberculosis. From a study that started originally by looking at how scorpion venom inactivated cell functions, Heike Wulff, synthesized the chemical TRAM-34 and found it suppressed T-cell function, without affecting other biochemical processes in the cell, "said Wulff". If TRAM-34

proves effective in humans, it may be an effective way to keep the immune system from attacking itself in certain diseases or from rejecting transplanted organs (Heike 2000, Joiner *et al* 1997, Grissmer *et al* 1993, Jensen et *al* 1999). The present study demonstrated that scorpion (*Mesobuthus eupeus)* venom could be effective as anti-inflammatory agent in animal model of acute inflammation. More studies are needed to be carried out to find the mechanism of the venom and exact therapeutic doses of the venom for acting as anti-inflammatory agent.

References

Castreo, C., Sutter P. DE., Gybels J., Vaheens J. Adjuvant-induced arthritis in rats: a possible animal model of chronic pain. *Pain* 1981, 10, 173-186.

Gouret, C., Mocquet G. Raynaud G. Use of Freund's adjuvant arthritis test in anti-inflammatory drug screening in the rat: value of animal selection and preparation at the breeding center. *Laboratory animal Science*., 1976, 26, 281-287.

Grissmer, S, Nguyen AN, Cahalan MD. Calcium-activated potassium channels in resting and activated human T lymphocytes. Expression levels, calcium dependence, ion selectivity, and pharmacology. *Journal of General. Physiology*., 1993, 102, 601-630.

Heike, W. Scorpion venom research may lead to new drugs that treat immune disorders, aid transplants. University of California at Irvine College of Medicine Research. *Proceeding of National. Academy of Science. USA*, 2000, 97, 8150-8151

Jensen, BS, Odumn N, Jorgensen NK, Christophersen P, and Olesen SP Inhibition of T cell proliferation by selective block of Ca (2+)-activated K (+) channels. *Proceeding of National. Academy of Science. USA* , 1999, 96, 10917-10921

Joiner WJ, Wang LY, Tang MD, Kaczmrek LK., hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proceeding of National. Academy of Science. USA*, 1997, 94, 11013-11018

Kalpana, S. Patil, Jayaprakash Suryavanshi, Phcog MAG. Effect of Celastrus paniculatus Willd. seed on adjuvant induced arthritis in Rats. *Pharmacognosy Magazine* , 2007, 3, 177-181.

Kongtawelert, P., Francis D.J., Brooks P.M., Ghosh P., Aplication of an enzyme-linked immunosorbent-inhibition assay to quantitate the release of KS peptides into fluids of the rat subcutaneous air-pouch model and the effects of chondroprotective drugs on the release process. *Rheumatology International*, 1998, 9, 77-83.

Liu, Y.F., Ma, R.L., Wang, S.L., Duan, Z.Y., Zhang, J.H., Wu, L.J. and Wu, C.F., Expression of an antitumor-analgesic peptide from the venom of Chinese scorpion Buthus martensi Karsch in Escherichia coli. *Protein Expression and Purification* 2003, 27(2), 253–258

Maeulo, V. M., Lebrun I.V., Dorce A C. Effect of scorpion *Tityus serrulatus* venom toxin TS-8F on rat learning and memory. *Journal of Venomous Animals and Toxins*, 2002, 8,74-87

Nakajima, H., Takamori H., Hiyama Y., Tsukada W. The effect of treatment with recombinant γ-interferon on adjuvant-induced arthritis in rats. *Agents and Actions*, 1991, 34, 1-2.

Newould, B.B., Chemoterapy of arthritis induced in rats by mycobacterial adjuvant. *British Journal of Pharmacology and Chemotherapy*, 1963, 21, 127-130.

Shinde U.A., Phadke A.S., Nair A.M., Mungantiwar A.A., Dikshit V.J., Saraf M.N., Studies on the anti-inflammatory and analgesic activity of Cedrus deodora (Roxb.) Loud. Wood oil. *Journal of Ethnoparmacology*, 1999, 65, 21-27.

Somasundaran, S., Sadique J., Subramonia A. Influence of extra-intestinal inflammation on the in vitro absorption of 14C-glucose and the effects of anti-inflammatory drugs in the jejunum of rats. *Clinical and Experimental Pharmacology and Physiology*. 1983, 10, 2, 147-152

Somasundaran, S., Sadique J., Subramoniam A. In vitro absorption of [14c] leucine duringinflammation and the effect of anti-inflammatory drugs in the jejunum of rats. *Biochemistry Medicine*., 1983, 29, 2, 259-264.

Vaisbuch E., Levy R., Hagay Z. The effect of betamethasone administration to pregnant women on maternal serum indicators of infection. Journal of Perinatal Medicine,*2002, 30, 287-291*

Walz, D.T., Dimartio M.J., Misher A. Adjuvant-induced arthritis in rats. II. Drug effects on physiologic, biochemical, and immunologic parameters. Journal of Pharmacology and. *Experimental Therapy*, 1971, 178, 1, 223-231

Winder, C.V., Lembke L.A., Stephens M.D. Comparative bioassay of drugs in adjuvant-induced arthritis in rats:

flufenamic acid, mefenamic acid and phenylbutazone. *Arthritis Rheumtology*, 1969, 12, 5, 472-482

Wolfe, F. The natural history of rheumatoid arthritis. *Journal of Rheumatology*., Supp. 1996, 44, 13-22.

Wulff, J. Design of a potent and selective inhibitor of the inter-mediate-conductance. . *Proceeding of National Academy of Science USA*, 2000, 97, 8151-8156

Zare, MA., Murthy KRK., Haghnazari L. Scorpion venom poisoning in experimental animals. *Archives of. Razi Institute*, 1994, 44, 67-72.

Zlotkin, E., Miranda, F. and Rochat, H.,. Chemistry and pharmacology of Buthinae scorpion venoms. In: Bettini, S., Editor, *Handbook of Experimental Physiology* vol. 48, 1978.