Effect of bee venom on rat glucocorticoid receptor beta: a therapeutically model of rheumatoid arthritis

A.T.Sh. Al-Hassnawi[1], R.K. Mahdi[1] and H.M. AL-Rubaei[2]

[1] Department of Biology, College of Science, [2]Department of Biology, College of Science for Women, University of Babylon, Babil, Iraq

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Abstract

This study aim to use bee venom as alternative medicine for treatment of rats induced with rheumatoid arthritis. Forty rats used for this purpose which divided into four groups, three groups induced with rheumatoid arthritis and one group considered as control group that subdivided into control negative and control positive (rheumatoid group). All the groups induced with rheumatoid arthritis injected with bee venom with different doses (high 40 µg/kg and low dose 10 µg/kg) and different times (after 5 days and after two weeks from CFA injection and along with CFA injection). Glucocorticoid receptor beta used as a biomarker which suggested function as negative regulator determine glucocorticoid sensitivity in target tissues and as an endogenous inhibitor for glucocorticoid action. The high and low dose showed significantly decrease in GCRβ as compared with control group and non-significant between rheumatoid and both along CFA and after 5 days of CFA injection. The pre-treatment high and low dose revealed significant decrease in GCRβ compared with Rheumatoid group and non- significant as compared with control group in low dose bee venom treatment. Also, depending on hand paw edema assessment, a weak evidence about anti-inflammatory effects of bee venom has shown. From our data we concluded that bee venom prevents GCRβ elevation especially in pre-treatment group this may result assess to anti-inflammatory effect but the safety of this toxin still needed for another study. Clinically no evidence about the treated effect of bee venom on rheumatoid arthritis in rat.

Keywords: Glucocorticoid receptor beta, Bee venom, Rheumatoid Arthritis
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تاثير سم النحل على المستقبل القشري نوع بيتا في الجرذان: نموذج علاجي لالتهاب المفاصل الرثوي

علاء طارق شاكر الحسناوي[١]، رشا كاظم مهدي[١] و هادي مزعل الربيعي[٢]

[١] قسم علوم الحياة، كلية العلوم، [٢] قسم علوم الحياة، كلية العلوم للبنات، جامعة بابل، بابل، العراق

الخلاصة

هدفت الدراسة الحالية الى استخدام سم النحل كأحد البدائل الدوائية لعلاج داء المفاصل الرثوي المستحث في ذكور الجرذان حيث استخدم لهذا الغرض اربعين جرذ مقسمة الى اربع مجاميع وهي ثلاث مجاميع تم استحثاث الروماتزم الرثوي فيها ومجموعة واحدة اعتبرت كمجموعة سيطرة تم حقن المجاميع المستحثة فيها المرض في سم النحل بجرع مختلفة (عالية ٤٠ مايكروغرام /كغم وواطئة ١٠ مايكروغرام /كغم) واوقات مختلفة (بعد استحثاث المرض بخمسة ايام و بعد اسبوعين من اسحثاث المرض ومع استحثاث المرض مباشرة) استخدم المستقبل للستيرويدات القشرية نوع بيتا كمؤشر حيوي والذي يعمل كمثبط لعمل وحساسية الاستيرويدات القشرية (ونعتقد ان هذه اول دراسة على حسب علمنا في استخدام هذا الموشر مع سم النحل). أظهرت كل من الجرعة العالية والمنخفضة انخفاضا كبيرا في GCRβ بالمقارنة مع مجموعة السيطرة وغير معنوية بين مجموعة الروماتويد الرثوي ومجموعة الجرذان المعالجة بالسم النحل مع وبعد خمسة ايام من الاستحثاث. وكشفت الجرعة العالية والمنخفضة قبل المعالجة انخفاضا كبيرا في المستقبل نوع بيتا GCRβ مقارنة مع مجموعة الروماتويد وغير معنوية بالمقارنة مع مجموعة السيطرة عند استخدام جرعة السم المنخفضة. أيضا. المعايير السريرية أظهرت أدلة ضعيفة حول الآثار المضادة للالتهابات كنتيجة لاستخدام سم النحل. من بياناتنا نستنتج أن سم النحل يمنع ارتفاع المستقبل نوع بيتا

GCRβ خاصة في مجموعة ما قبل المعالجة وهذا قد يؤدي إلى تقييم لتأثير مضاد للالتهابات. ولكن على الرغم من ذلك يبقى هناك حاجة ماسة لمعرفة سلامة هذا السم على وظائف الاعضاء. سريريا الشواهد ضعيفة على تأثير المعالجة لسم النحل على التهاب المفاصل الروماتويدي في الجرذان.

Introduction

For a long time, bee venom was used in eastern traditional medicine for the treatment of several inflammatory diseases such as rheumatoid arthritis (1).

Glucocorticoid receptors (GR) have a many physiological, immunological, cell fate determination, mitotic progression and chromosomal segregation (2). GR one of the receptors that belong to the nuclear receptor superfamily that surrounded, among others, peroxisome proliferators–activated receptor and estrogen receptor, all of glucocorticoids natural and synthetic bound to GR and caused extensive variation in conformation which resulted in nuclear translocation of transcription factors involving nuclear factor-kB, most of the resultant of glucocorticoids actions were intermediated by GR and concluded in a modification of the synthesis and expression of crucial mediators of innate and adaptive immunity (3).

There are two types of GR which are isoforms described as GC receptor alpha (GRα) and GR beta (GRβ) originating from the same gene by alternative splicing of the GR primary transcript (4).

The protein structure of them (amino acid sequencing) have the same up to 727 but deviate from this site in GRα and in addition 50 amino acids and in GRβ a supplementary different 15 amino acids (5).

Glucocorticoid receptor beta suggested that function as negative regulator determine the glucocorticoid sensitivity in the target tissues and as an endogenous inhibitor for glucocorticoid action because didn't bind glucocorticoids and didn't have the ability to modulate the expression of GC responsive genes via binding to specific glucocorticoid response elements (GREs) as in GRα (6).

As well-known bee venom is Apitoxin, which extract from by honey bees, *Apis melifera* (7). Bee venom is composed of several polypeptides such as melittin, apamin, and adolapin; enzymes such as phospholipase A2 (PLA2), hyaluronidase; mast cell degranulating peptides (MCD), and nonpeptide components (histamine and norepinephrine) (8).

Experimental Rheumatoid arthritis induction was used in this work as a model of autoimmune disease disorder distinguished by synovitis and chronic inflammation of the peripheral joints especially hands and feet (9).

Despite the widespread use bee venom as a folk medicine for many diseases, but still, pharmacological effect is controversial, also the pathway of bee venom work still not clear. The beneficial and harmful effects of bee venom already estimated depend on clinical signs and by using a different type of biomarkers (interleukins, cytokines, growth factor, hormone, tumor marker and so on). This paper amid to sought out for give one explanation of bee venom effect pathway, where we are using new bio-indicator (GRβ) to find out the effect of bee venom as alternative medicine in one hand, and in another hand to know the relationship between rheumatic disease and rat GRβ expression.

Material and methods

Animal

Forty adult male albino rats (*Rattus rattus*) with body weight ranging from 200-250± gm. and the age 8-12 weeks. These animal purchases from the animal house at the University of Babylon, college of science. The animals were maintained in a temperature and light cycle-controlled environment at temperature 25 ºC and humidity 50±. Food and water were available ad libitum. The animals randomly assigned and grouping into four groups (10 rats for each group) these were: control group, subdivided into control positive injected with CFA alone and the second considered as control negative or placebo injected with D.W. only. High dose (40 µg/kg) and Low dose (10 µg/kg), both of them injected with 0.1 ml of complete Freund's adjuvant (CFA) in right hind paw and subdivided into two groups the first one treated with bee venom i.p. for 4 weeks in the same time of CFA injected and the second one treated with bee venom after five days from CFA injection for 4 weeks. pre-treatment group which subdivided into high (40 µg/kg) and low (10 µg/kg) dose injected with bee venom for two weeks before induction of rheumatoid arthritis by CFA and after injection of CFA also continued treatment with bee venom for 4 weeks.

Anesthesia

The rats were anesthetized with ketamine (100 mg/Kg) intramuscular (I.M) (small mammal manual).

Arthritis induction

Subcutaneous single injection of Complete Freund's adjuvant (100µl) into the plantar surface right hind paw to induction rheumatoid arthritis (10). Complete Freund's adjuvant was obtained from Santa Gruz Biotechnology that each ml contained 1 mg of *Mycobacterium tuberculosis*; heat killed and dried, 0.85 ml paraffin oil and 0.15 ml mannidemonooleate.

Bee venom

Bee venom was purchases from (CN Lab Company, China) that contained 17% of melittin, where 100mg of BV was dissolved in 200 of sterile distilled water and kept in darkness at 4 ºC until used for required doses (40 µg/kg and 10 µg/kg) according to (11).

Right hind paw measurements

The measurement of the right hind paw was evaluated daily by using digital vernier caliper.

Blood collection

The samples were taken after 28 days for both high and low dose along with CFA and after 34 days in after five days from CFA injection (both high and low dose), control and rheumatoid groups and after 44 days in pre-treatment both high and low dose. The blood collected by heart acupuncture and then gel tubes were used for samples collection. Serum was separated by centrifugation for 5 minutes at 3000 rpm. The serum was stored at -20ºC in three replicate until dissolving for assay.

Glucocorticoid receptor beta (GCRβ) measurement

Quantitative Sandwich ELISA technique was used to measure Rat glucocorticoid receptor beta (GCRβ). The commercial ELISA kit purchased from Elabscience company (Rat GCRβ ELISA Kit Catalog No.E-EL-R0424). The lower limit of sensitivity of this assay for serum samples was (< 9.38 pg /ml).

Statistical Analysis

Completely randomized design, mean ±standard deviation and least significant differences (L.S.D) were used. All Statistical Analysis was performed using SAS System (2012) software. Values of $p < 0.05$ were considered statistically significant.

Results

Glucocorticoid receptor beta (GCRβ)

Experimental A: Effect of high dose bee venom (40µg/kg) on GCRβ in rheumatoid rats: The using high dose bee venom both along with CFA and after 5 days from CFA injection showed increasing in GCRβ significantly as compared with control group and non-significant in comparison with Rheumatoid group as showed in figure (1-A and B) at $p<0.05$.

Experimental B: Effect of low dose bee venom (10µg/kg) on GCRβ in rheumatoid rats: Our results of low dose bee venom (both along with CFA and after 5 days from CFA injection) revealed non-significant differences in GCRβ in comparison with Rheumatoid group and significant increase as compared with negative control as shown in figure (2-A and B) ($p<0.05$).

Experimental C: Effect of pre-treatment (high and low) dose bee venom (40 and 10µg/kg) on GCRβ in rheumatoid rats: The pre-treatment high and low dose bee venom revealed significant decreasing in GCRβ as compared with Rheumatoid group and non-significant as compared with negative control as shown in figure 3 (A and B) ($p<0.05$).

Right hind paw measurement

The measurement of right hind paw showed non-significant differences as showed in figure (4, 5, 6 both A and B).

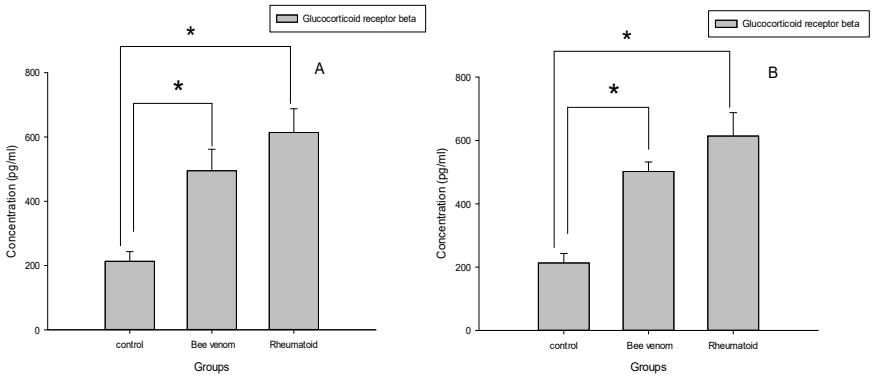

Figure 1: Effect of injected high dose bee venom (40 µg/kg) along with CFA injection (A) and after 5 days of CFA injection (B) on mean ± S.D of Rat Glucocorticoid beta receptors (pg/ml).

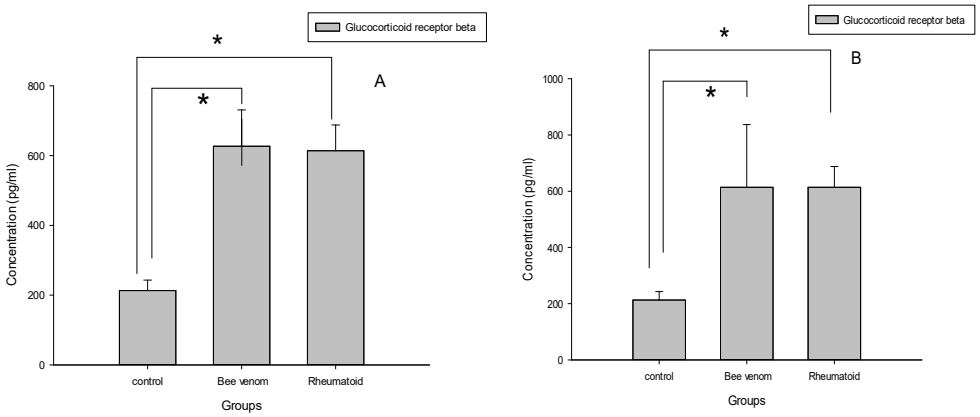

Figure 2: Effect of injected low dose bee venom (10 µg/kg) along with CFA injection (A) and after 5 days of CFA injection (B) on mean ± S.D of Rat Glucocorticoid beta receptors (pg/ml).

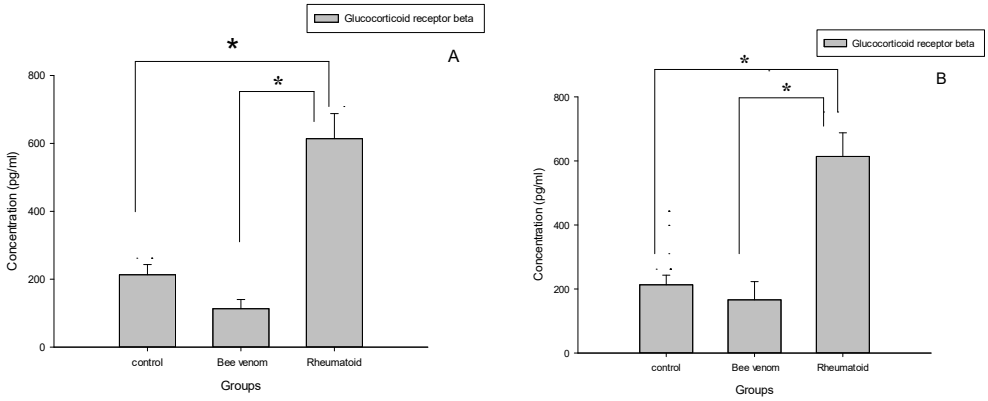

Figure 3: Effect of high (A) and low (B) dose bee venom injection (40 and 10 µg/kg) on mean ± S.D of Rat Glucocorticoid beta receptors (pg/ml) in pre-treatment group.

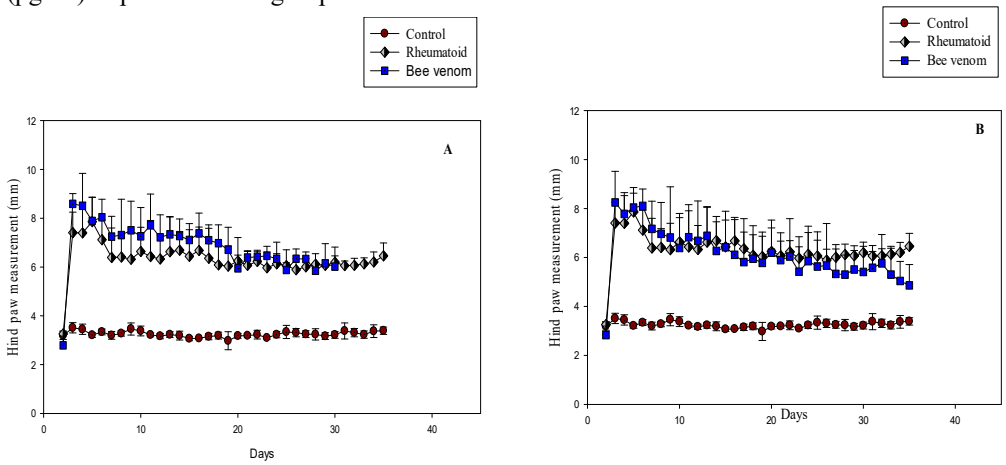

Figure 4: Effect of injected high dose bee venom (40 µg/kg) along with CFA injection (A) and after 5 days of CFA injection (B) on mean ± S.D of Right hind paw measurements (mm).

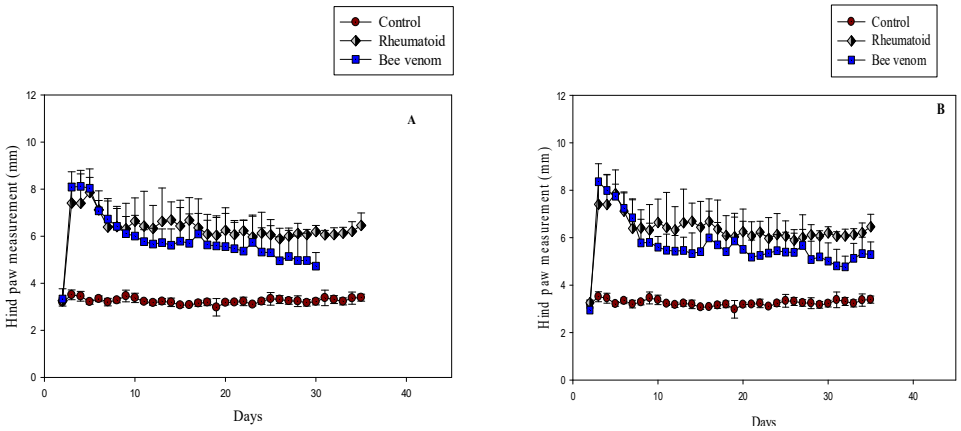

Figure 5: Effect of injected low dose bee venom (10 µg/kg) along with CFA injection (A) and after 5 days of CFA injection (B) on mean ± S.D of Right hind paw measurements (mm).

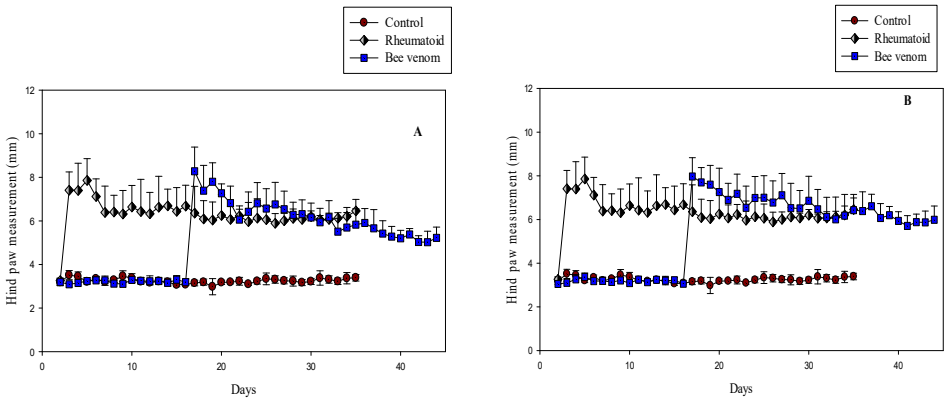

Figure 6: Effect of high (A) and low (B) dose bee venom injection (40 and 10 µg/kg) on mean ± S.D of Right hind paw measurements (mm) in pre-treatment group.

Discussion

For our knowledge this work the first one that showed the bee venom effectiveness on GCRβ. Here we are use a different dose of bee venom toxin and acute rheumatoid arthritis rat model. Based on several studies that showed BV exhibit as anti-arthritis activity. However, the pathway of this activity is still not clear (12). Many studies suggest that anti-arthritis effect of BV and melittin occur via targeting the inhibition of NF-kB, thereby the direct binding to the p50 subunit and inhibit its translocation to the nucleus and a consequent reduction the transcription of the inflammatory genes (13,14). The inhibits activity of NF-kB and MAPK (mitogen-activated protein kinase) by suppressing NO production and decreased the iNOS and COX-2 expression (15). Also, another study showed the synovial membrane of hind paw of rats with RA-induced by CFA treated with BV revealed significantly decreased in the expression of both NF-kB and TNF-α (16). The novel things here may bee venom decreased GCRβ and because it's anti-inflammatory activity may give us the new explanation of bee venom effectiveness pathway. The high and low dose of bee venom presented here don't show any effect on GCRβ whereas pre-treatment injection showed the sharp decrease on GCRβ. Dose and time are very important things for arthritis score treatment, for example, the activation of NF-kB in human cell line by pro-inflammatory cytokines such as TNF-α and IL-1β produce significantly increasing in GCRβ depending on dose and time (17). The main question here and according for our data why bee venom causes markedly reduced GCRβ in pre-treatment group than after induction of rheumatoid or along with CFA injection? The answer to this question really may lead to another question, whether BV is suitable

as a treatment for example to RA or not? Regardless of harmful use as medicine. The model animal, type of chronic or acute of RA induction and adjuvant model of arthritis also, the dose of RA material such as complete Freund's adjuvant (CFA), adjuvant arthritis (AA), collagen typeII-induced arthritis (CIA), and incomplete Freund's adjuvant(IFA) may collectively affected in therapeutic interventions and can be more easily accepted when taken this things into account (18). Moreover, the location of BV (low dose) administration, for example, the zusali acupoint showed greatest anti-arthritic effect than injection into anon-acupoint located in the back of the rodent model of RA (19). The anti-nociceptive and anti-inflammatory effects of the water-soluble fraction of bee venom (BVA), which particularly important to this effects includes several polypeptides (such as melittin, adolapin, and mast cell degranulating peptide) and enzymes (i.e. Phospholipase A2), showed significant reduction of inflammatory symptoms of RA includes the increasing levels of IL-6, paw edema, and radiological changes (20). The score of rheumatoid also affected by different kind of cytokine such as of TNF-α, IL-6, IL-1β, IL-8, monocyte chemoattractant protein -1 on different cell types and G-CSF, also the pathogenesis of RA is related with IL-17, which have a role in activation of the immune system by numerous activities of pro-inflammation cytokines (9). These cytokines really have kinetic expression according to days, a score of arthritis and type of animal model (21).

Also, anti-inflammatory cytokines (IL-10) increasing after bee venom treated were published (22). What we would say here, there are several pathways that may affect the response of animals to a particular type of drug or alternative drug (such as bee venom) one of this animal itself, cytokine (pro and anti-inflammatory) upregulation and so on.

This observation was also confirmed by our data (not published). Were the unexpected result of pro-inflammatory cytokines in positive and negative control. Our finding showed GCRβ may be used as a biomarker for drug assessment because of its importance as mention above. The complete Freund`s adjuvant injection into right hind paw stimulate paw edema initially in the ipsilateral limb and later in the contralateral limb (19). Swollen paw considered as an indicator to evaluate the anti-arthritic activity of different drugs. Paw edema is simple and quick procedure to assess the degree of inflammation and the therapeutic effect of treatments. Our results showed non-significant differences in paw edema after treatment with bee venom this may be not affected in suppression the pro-inflammatory cytokines which resulted from activation of macrophages that result in producing of different cytokines such as IL-1, IL-6 and interferon-ϒ and α (23).

Based on our results presented here we can be concluded that GCRβ suppression by using bee venom may be one of explanations or pathways effect of bee venom as the anti-inflammatory. Also, GCRβ suppression by bee venom can cause significant adverse effects leading to unsatisfactory therapeutically outcomes, especially, it has been reported that GCRβ suppression can cause cancer (24). Also, depending on hand paw edema assessment, a weak evidence about anti-inflammatory effects of bee venom has shown. Although bee venom prevents GCRβ elevation especially in pre-treatment group this may relate with the anti-inflammatory effect but the safety of this toxin is still needed for another study.

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