Research Article

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THERAPEUTIC ACTIVITY OF BEE-STINGS THERAPY IN RHEUMATOID ARTHRITIS CAUSES INFLAMMATION AND OXIDATIVE STRESS IN FEMALE PATIENTS

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Received on: 02/01/13 Revised on: 20/02/13 Accepted on: 10/03/13

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DOI: 10.7897/2277-4343.04303
Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT
Here the present study aimed to evaluate the therapeutic activity of bee venom acupuncture in rheumatoid arthritis (RA) which causes inflammation and oxidative stress in female patients. 75 female patients were divided into 5 groups as control, bee venom acupuncture, rheumatoid arthritis, treated rheumatoid arthritis and rheumatoid arthritis stung with bee venom groups. Serum rheumatoid factor, erythrocyte sedimentation rate, C-reactive protein, prostaglandins E2 and F2α, lipid peroxidation, nitric oxide, glutathione and total antioxidant capacity levels were determined in all groups. Rheumatoid arthritis in female patients was resulted in a significant elevation in serum rheumatoid factor, erythrocyte sedimentation rate, C-reactive protein, prostaglandins E2 and F2α, lipid peroxidation and nitric oxide levels ($p < 0.05$) compared to control group. In addition, rheumatoid arthritis caused a significant reduction in serum glutathione and total antioxidant capacity levels. On the other hand, bee venom stings alleviated rheumatoid arthritis inflammation and oxidative stress effects, where all investigated parameters were statistically significant compared to rheumatoid arthritis group. Moreover, bee venom therapy was more potent than the routine treatment of rheumatoid arthritis in patients treated group. Bee venom acupuncture in RA patient may have therapeutic, anti-inflammatory and antioxidant activities.
Keywords: Rheumatoid arthritis; Bee venom therapy; Inflammation; Oxidative stress.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune and progressive inflammatory disease that involves the joints and its progression leads to their destruction. The prevalence of RA is 0.5%–1.0% in the general population worldwide. Females are nearly three times more likely than males to develop the disease and can start at any age, although the mean age at the onset is 40 to 60 years. The precise cause of RA is unknown; like other autoimmune diseases it arises from a variable combination of genetic susceptibility, environmental factors and the inappropriate activation of the immune responses that eventually result in the clinical signs of arthritis.[1]

Rheumatoid arthritis is a systemic disease characterized by progressive, erosive, and chronic polyarthritis. Cellular proliferation of the synoviocytes and neo-angiogenesis leads to formation of pannus which destroys the articular cartilage and bone. Studies of Karatas et al.[2] and Kamanli et al.[3] provide evidences for the involvement of free radicals/ reactive oxygen species in the pathogenesis of RA. A study of Karatas et al.[2] indicated that increased oxidative stress and/or defective antioxidant status contribute to the pathology of RA. This study showed raised levels of malondialdehyde and low levels of endogenous antioxidants in patients of RA. Another study reported an impaired glutathione reductase activity in synovial fluid in RA patient.[4] In active RA and juvenile idiopathic arthritis, increased oxidative stress and decreased levels of antioxidants have been reported.[3]

Bee venom, as a therapeutic modality in use since at least the second century BC in Eastern Asia, has been extensively researched and practiced in Korea, focusing on clinical applications as a meridian therapy. Herbal acupuncture is a new method of acupuncture where a distilled herbal decoction is extracted, and purified to be administered on acupoints for stimulation. Bee venom acupuncture (BVA) is a kind of herbal acupuncture taking advantage of diluted bee venom instead of distilled herbal decoction.[5] The bee venom once extracted and processed is utilized on the relevant sites according to specific diseases or acupoints. BVA simultaneously exerts pharmacological actions from the bioactive compounds isolated from bee venom and mechanical actions from the acupuncture stimulation. BVA has been considered as a promising therapeutic method for various diseases, especially in Korean medicine. In complementary and alternative medicine (CAM), acupuncture is one of the most common therapies used to treat a number of human inflammatory diseases including RA and osteoarthritis.[6]

BVA is capable of producing anti-nociception and anti-inflammatory actions in several animal models.[5-6] Several studies suggested that the effects of bee venom were intensified by acupuncture stimulations, which may help in reaching therapeutic goals. The anti-nociceptive property of BVA may be explained by the process of counter irritation; that is, when noxious stimuli are applied to body regions, these stimuli increase the pain thresholds and reduce pain rating scores through the body. For centuries, pain has been relieved by counter-irritation methods such as moxibustion (a method of burning herbs to stimulate acupuncture points) on arthritic limbs.[5]

The purpose of the present study was, therefore, to evaluate the effectiveness of BVA therapy on arthritis female patients.

MATERIALS AND METHODS

Source of Data

The present study comprises of 85 normal and RA female patients from Cairo, Egypt clinically diagnosed of active rheumatoid arthritis and they aren't smokers. They do not have diabetes, hypertension, inactive rheumatoid arthritis and arthritis other than RA. The study was undertaken between September 2010 to August 2011. All the patients were in the age group of 40-60 years and were not on any nutritional supplements. Ten of the participants were dropped out at the end of the selection, as they did not like the idea of giving blood or suffer from bee stings and represented irritation.

The selected cases were divided then into five groups as following:

Group I: Served as control and haven't any systemic disease.

Group II: Served as bee venom stings group which stung with honey bee two times per week for 3 successive months.

Group III: Served as RA patients group which early diagnosed as RF +ve and they didn't receive treatment.

Group IV: Served as RA treated patients group which received a routine treatment of NSAID.

Group V: Served as RA bee venom sting patients group which stung with honey bee two times per week for 3 successive months and they didn't receive any other treatment.

Ethics approval

All participants provided written informed consent prior to study enrollment, and this research was reviewed and approved by the Research Ethics Committee of Ain Shams University, Cairo, Egypt "Approval Number: 148/04/01/2010".

Collection and Storage of Blood Sample

5 ml of blood was collected from the cases. Samples were also collected from the controls, under aseptic precautionary measures by using disposable syringe. All samples were placed immediately on ice. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min.

Measurement of autoantibodies

All samples were tested for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP2) autoantibody. RF (IU/ml) was measured by nephelometry using the Dade Behring system; anti-CCP2 (U/ml) was measured using anti-CCP2 ELISA assay (Axis-Shield Diagnostics, Ltd., Scotland, UK). A dichotomous cut-off for each RF assay was established according to 1987 American College of Rheumatology (ACR). RA criteria specifying a 'positive' RF if present in <5% of control subjects, by determining a <5% cut-off in 30 blood donor controls. Anti-CCP2 was considered positive if greater than the kit cut-off of 5 U/ml, which corresponded to a <2% cut-off in the same 30 blood donor controls.

Measurement of C-reactive protein

Serum samples from the first RF positive were tested for high sensitivity C-reactive protein (hsCRP, mg/l). hsCRP was tested by a nephelometric assay (BN II Nephelometer, Dade Behring, Deerfield, Illinois, USA).

Measurement of erythrocyte sedimentation rate

The erythrocyte sedimentation rate was measured over a period of 1 hour; normal values were considered to be, 10 mm in the first hour.

Oxidative stress markers

Lipid peroxidation (LPO) in serum of different groups were determined by reaction of thiobarbituric acid [7]. Similarly, those serums were used to determine nitrite/nitrate (nitric oxide; NO), glutathione (GSH) and total anti oxidant capacity (TAC).

Serum cytokines assessment

The same serum were used to determine prostaglandin E2 (PGE2), and prostaglandin F2α (PGF2α) using enzyme-linked immunosorbent assay "ELISA" kits obtained from Abcam Company, Cambridge UK.

Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as post hoc test according to the statistical program statistical package program (SPSS version 17.0).

RESULTS

Results indicated a significant ($P < 0.05$) increase in the RF, ESR, CRP and anti-CCP concentrations in serum of RA patients compared to the control group (Figure 1). While, the bee venom-sting therapy alleviated RA effects and all those markers were significantly reduced but still above the values of control group. Moreover, BV-sting therapy in RA patients was more potent than the routine treatment in group IV. Moreover, treatment with BV-sting alone did not cause significant effects on the markers of rheumatoid in normal patients.

DISCUSSION

In recent years, there are growing evidences of possible role of highly reactive products of oxygen and nitrogen termed as free radicals, in the pathogenesis of RA as well as other degenerative diseases.[8] These reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced endogenously during aerobic metabolism and at the site of chronic inflammation. ROS such as super oxide radical, hydroxyl radical and hypochlorous acid contribute significantly to tissue injury in RA. Several mechanisms are involved in the generation and action of ROS in the joint of RA patient, including increased pressure in synovium cavity, reduced capillary density, vascular changes and increased metabolic rate of synovial tissue. In addition activated leukocytes also produce ROS.[3]

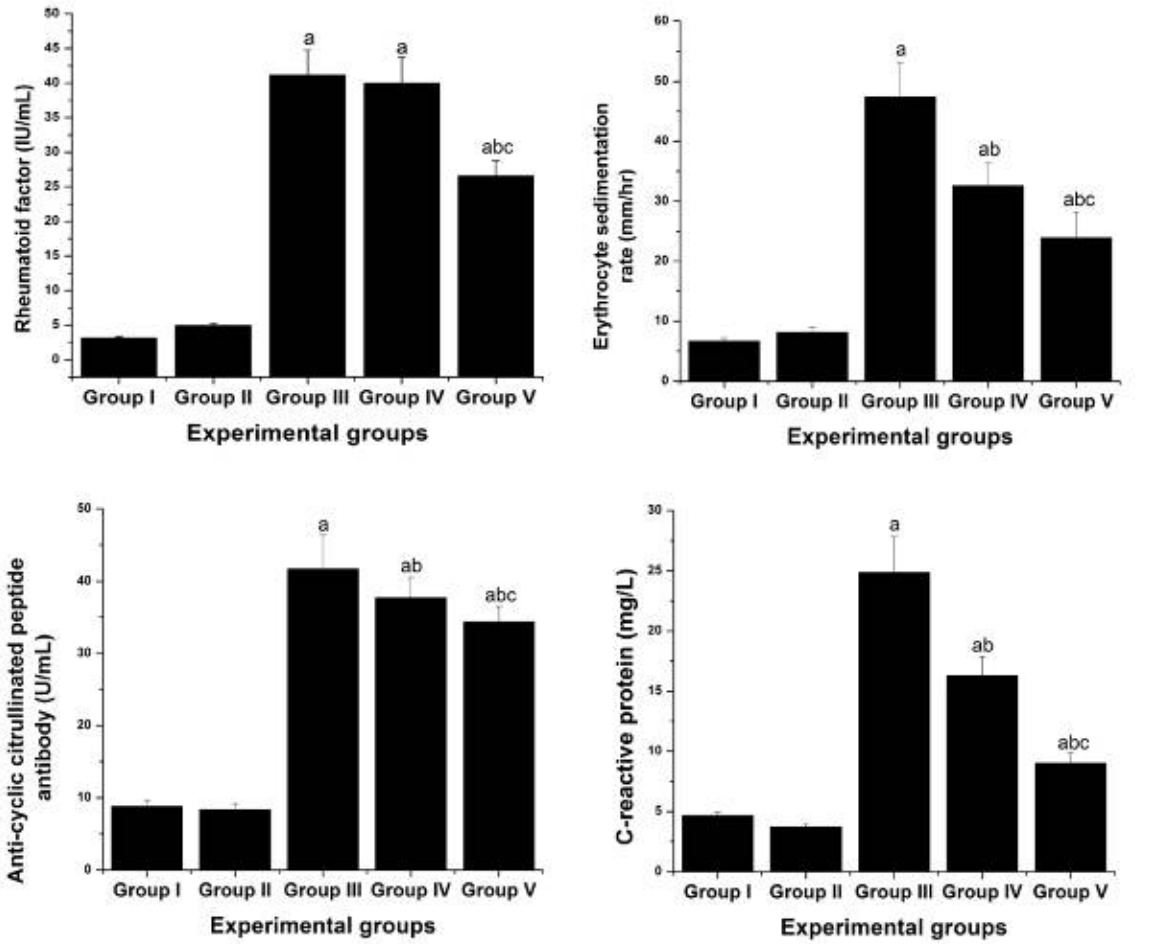

Figure 1: The levels of rheumatoid factor, erythrocyte sedimentation rate, anti-cyclic citrullinated peptide antibody and C-reactive protein in bee venom and/or rheumatoid arthritis patients. Values are means ± SEM (n=15). [a]$p<0.05$, significant change with respect to Group I; [b]$p<0.05$, significant change with respect to Group III; [c]$p<0.05$, significant change with respect to Group IV for Duncan's post hoc test. Group I: Control; Group II: Bee venom; Group III: RA; Group IV: RA treated and Group V: RA stung with bee venom

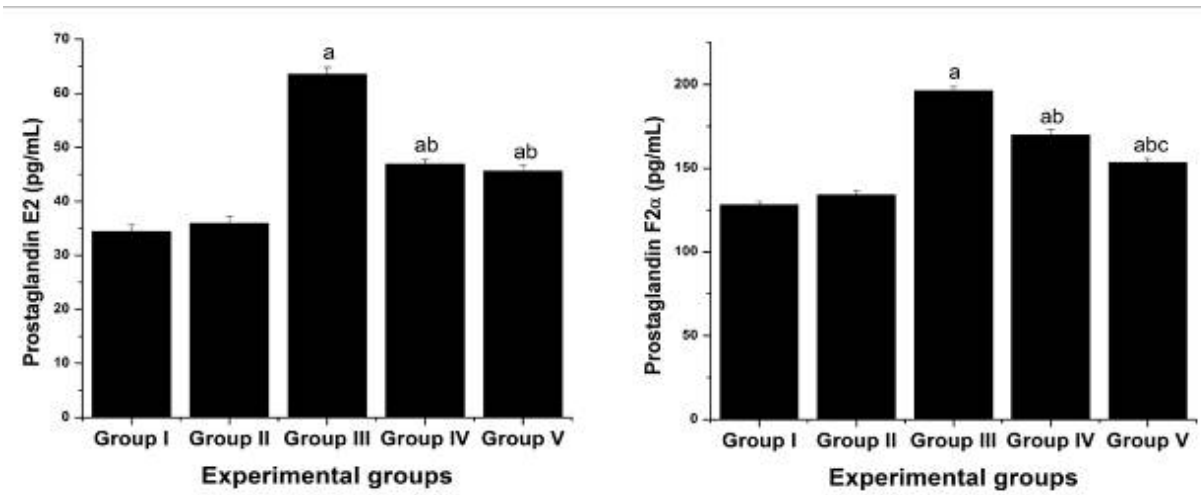

Figure 2: The levels prostaglandins E2 and F2α in bee venom and/or rheumatoid arthritis patients. Values are means ± SEM (n=15). [a]$p<0.05$, significant change with respect to Group I; [b]$p<0.05$, significant change with respect to Group III; [c]$p<0.05$, significant change with respect to Group IV for Duncan's post hoc test. Group I: Control; Group II: Bee venom; Group III: RA; Group IV: RA treated and Group V: RA stung with bee venom

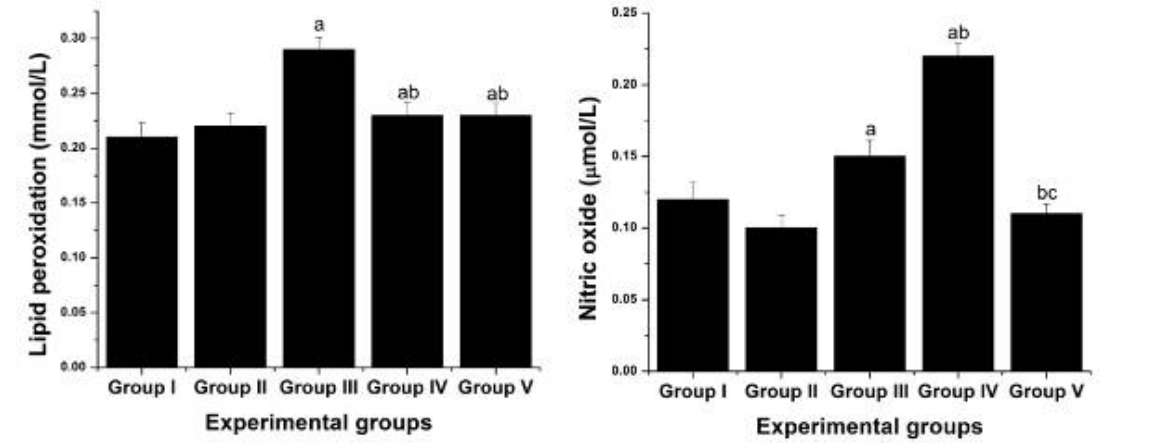

Figure 3: The levels of lipid peroxidation and nitric oxide in bee venom and/or rheumatoid arthritis patients. Values are means ± SEM (n=15). [a]*p*<0.05, significant change with respect to Group I; [b]*p*<0.05, significant change with respect to Group III; [c]*p*<0.05, significant change with respect to Group IV for Duncan's post hoc test. Group I: Control; Group II: Bee venom; Group III: RA; Group IV: RA treated and Group V: RA stung with bee venom

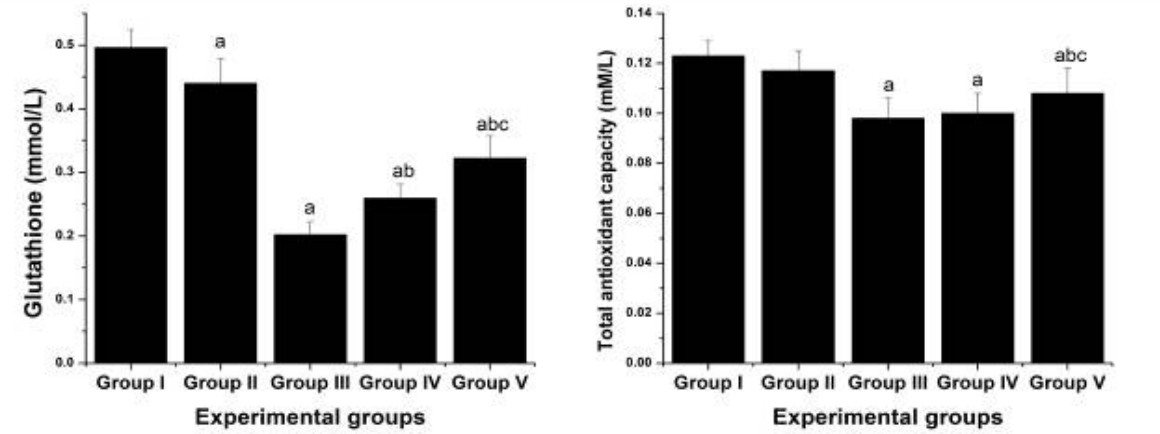

Figure 4: The levels of glutathione and total antioxidant capacity in bee venom and/or rheumatoid arthritis patients. Values are means ± SEM (n=15). [a]*p*<0.05, significant change with respect to Group I; [b]*p*<0.05, significant change with respect to Group III; [c]*p*<0.05, significant change with respect to Group IV for Duncan's post hoc test. Group I: Control; Group II: Bee venom; Group III: RA; Group IV: RA treated and Group V: RA stung with bee venom.

In the present study, mean level of LPO was increased significantly in RA cases compared to controls. Our findings are in accordance with the research of Shaabani et al.[9] and Vasanthi et al.[10]. Gambhir et al.[11] reported markedly increased concentrations of LPO in patients as compared to controls. Enhanced lipid peroxidation may occur as a result of imbalance between scavenging mechanisms and free radical generation process.

Data in Figure 2 showed significant ($P < 0.05$) increase in serum PGE2 and PGF2α in patients suffer from RA compared to the control patients. While, in the group of RA stung with BV, significant reductions in these tested cytokines were observed and BV-sting was alleviated the negative effects of RA in group V on these parameters. In addition, BV-sting was caused significant decline in PGF2α compared with Group IV.

Suffering from RA in female patients resulted in significant ($P < 0.05$) elevation in LPO and NO levels in the serum (Figure 3), this elevation in oxidative markers were associated with significant reduction in the glutathione and total antioxidant capacity contents compared to the control group (Figure 4). Stings with BV caused significant reductions in LPO and NO with significant elevation in GSH content when compared to the control patients group. However, BV alone had a negative effect on GSH content of the serum.

Lipid oxidation probably contributes to accelerated atherosclerosis in RA.[12] Persistent local and systemic elevation of inflammatory cytokines promotes lipolysis, and the systemic release of free fatty acids contributes to the dyslipidemia seen in RA. Oxidative stress arising from inflammatory reactions leads to the oxidation of local LDL.

Several studies in patients with RA have documented evidence for increased endogenous NO synthesis, suggesting that overproduction of NO may be important in the pathogenesis of RA. The inflamed joint in RA is the predominant source of NO.[13] Several investigators

found correlations between serum nitrite concentration and RA disease activity or radiological progression while others did not find such correlations.[14-15] Nitric oxide Synthase (NOS) polymorphism has been observed in RA. Inducible NOS is regulated at the transcriptional level, while endothelial NOS and neuronal NOS are regulated by intracellular Ca^{2+}. Several different cell types are capable of generating NO in the inflamed synovium, including osteoblasts, osteoclasts, macrophages, fibroblasts, neutrophils and endothelial cells.

The glutathione redox enzymatic cycle represents the most important intracellular defense against toxicity induced by oxygen free radicals. The cycle includes glutathione (GSH), and the enzymes glutathione peroxidase (GPx) and glutathione reductase (GRd). The GPx enzyme uses GSH as a substratum in reactions that catalyze reduction of H_2O_2, of fatty acids, and organic hydroperoxides into water and hydroxylated fatty acids.[16] During the reduction of peroxides, oxidized GSH is produced. The GRd enzyme reduces oxidized GSH, thus regenerating GSH. Under oxidative stress, there is an excess glutathione redox cycle, and thus an increase in the concentration of oxidized GSH. As a consequence, excess GSH is eliminated through the bile. The decrease in the levels of these non-enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in RA.[4]

Because of the limitations and risks of conventional therapy, people are exploring alternative measures to treat the disease. Commonly used alternative approaches include dietary modifications, nutritional supplements and botanicals. The response to these treatments varies from patient to patient. Alternative treatments have been used both as adjunct and an alternative to conventional therapy. Most of the treatments are relatively free of side effects.[6]

Several investigators have previously reported that BV is a useful therapeutic agent to manage arthritis-induced edema in experimental animal models. For example, the induction of arthritic inflammation by adjuvant injection into the base of the tail is successfully suppressed by bilateral intramuscular BV injection (0.5 mg/kg) into the hind limb.[6]

Individual components of BV have only been tested to verify the anti inflammatory effect of BV treatment on RA. BV consists of a variety of different peptides including melittin, apamin, adolapin and mast cell degranulating (MCD) peptide.[17] Although adolapin (20 mg/kg) and purified MCD peptide (1 mg/kg) have inflammatory activity,[17] these substances are present in very small quantities (1±2%) in whole BV. Saini et al.[18] reported that melittin is a major component of BV (50% of dry weight) and that it binds to secretory PLA2 and inhibits its enzymatic activity. Because PLA2 is a major inflammatory trigger (i.e. it causes arachidonic acid release) whose activity is enhanced in RA, it is possible that the formation of melittin-PLA2 complex by BV injection is able to suppress some of the symptoms associated with the development of arthritis. In the present study, we also observed that rheumatoid markers and inflammation were significantly suppressed by BV stings.

Varanda and Tavares,[19] reported on a great interest in the production of *Apis mellifera* venom in the USA. The venom would be used both for desensitization of hypersensitive individuals and for the treatment of RA. According to Hyre and Smith[20], the efficacy of bee venom for the treatment of RA has been extensively reported, although the precise mode of action is not yet known. Reports on the literature say that the venom induces production of corticosterone, release of histamine and exhibits anti-inflammatory activity. Billingham et al.[17] isolated the MCD peptide of BV which is believed to be responsible for the anti-inflammatory activity.

CONCLUSION

Our study demonstrated that BV has an anti-inflammatory activity by inhibiting NO and PG production. The antioxidant activity was associated with the anti-arthritis effect of BV, suggesting that the anti-arthritis effects of BV might be related to its anti-inflammatory and antioxidant effects. It thus seems that BV treatment may serve an effective therapy for RA.

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Cite this article as:
Abdel-Rahman M., Elebiary A.S., Hafez S.S., Mohammed H.E., Abdel Moneim A.E. Therapeutic activity of bee-stings therapy in rheumatoid arthritis causes inflammation and oxidative stress in female patients. Int. J. Res. Ayurveda Pharm. 2013;4(3):316-321

Source of support: Nil, Conflict of interest: None Declared