Chronic anti-inflammatory effect of bees' honey on Freund's Complete Adjuvant induced arthritis in rats

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Abstract

Bees' honey has been used to treat in many diseases in traditional medicine. The usage of bees' honey as a medicine is referred to in the most ancient Ayurveda medical records. Therefore, this study was design to evaluated the chronic anti-inflammatory effect of bees' honey using adjuvant-induced arthritis in experimental rat model.

Healthy male Wistar rats were randomly assigned into 4 groups (n=6 in each). Arthritis was induced by a single intra-dermal injection of Freund's Complete Adjuvant (FCA) containing *Mycobacterium butyricum* suspension into a foot pad of the left hind paw of all groups of Wistar rats except healthy control group (Group I). Group II - arthritic rats were received distilled water. Group III-arthritic animals treated with a standard drug Celecoxib (5mg/kg) and Group IV-arthritic animals were received BH (4ml/kg). Following induction of arthritis, daily oral treatment was started on day 14 and continued up to day 28. Body weight, ankle joint thickness and foot pad thickness were measured in all animals using dial calliper on Day 0 and on Day 3,7,10,14,17,21,24 and 28 after the injection of adjuvant. Full blood count was tested on day 28. Induction of arthritis significantly increased FPT, AJT and loss of BW. Treatment with Bees' honey and standard drug Celecoxib in the arthritic animals produced significant reductions respectively $p<0.05$, $p<0.001$ in FPT, AJT, WBC count, reducing erythema and oedema in the ankle joints and foot pad of the AIA rats and normalized BW. This study provided transitional medicinal evidence for BH used in the treatment of chronic inflammation in traditional medicine.

Keywords: Arthritis, Bees' honey, Chronic anti-inflammatory effect

Introduction

For centuries, Bees' honey (BH) has been used as effective and well-deserved reputation healing agent for various diseases in different traditional systems of medicine. Vitamins and trace elements contained in honey have a positive impact on all the vital processes of the human body. BH was prescribed by the physicians of many ancient races of people for a wide variety of ailments.

According to the Charaka Samhita, honey is of four types; *Makshika, Bhramara, Kshaudra* and *Paittaka. Makshika*, the best type of honey is produced by reddish variety of honey bee. This type of honey is the color of *Tila taila* (sesame oil). *Bhramara* honey is produced by the *Bhramara* type of bee. It is Guru (heavy) and is of white color. *Kshaudra* honey is produced by a small type of honey bee and is brown in color. *Paittaka* honey is produced by a large type of bee and is of the colour of ghee[1]. According to Susrutha Samhita, honey is of eight types[2].They are *Makshika* (Bees' honey), *Bhamara* (honey produced by Bumble) , *Argya* (honey produced by Wasp), *Pouthika* (honey produced by tiny insect call *Kannei*), *Ouddhalaka* (honey collected in anthill), *Kshaudra* (honey produced by species of tiny bees), *Dala* (honey collected in flower petals), *Chatra* (honey collected by a certain kind of bees whose hive like an umbrella).

Out of these eight honeys, the variety produced by honey bees is the most commonly referred type of honey. BH is recommended as an *Anupana* (vehicle) for in pediatrics age group in Ayurveda[3, 4]. According to the Ayurvedic authentic texts, BH was widely used in the treatment of ophthalmic disorders, jaundice, piles, tuberculosis, asthmatic conditions and, respiratory disorders. Old bees' honey helps to reduce over weight. Literature survey revealed that BH was most useful as an *Anupana* (vehicle*)* in Ayurvadic medicine against inflammation but no scientific evidence is available for its chronic anti-inflammatory potential.

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Thus, this present study was focused to evaluate the chronic anti-inflammatory effect of BH in experimental rat model.

MATERIALS AND METHODS

Bees' honey

Fresh BH was collected from Millaniya division in Kalutara and authentication was conducted with standard BH by chromatographically at the Department of *Dravyaguna Vignana*, Institute of Indigenous Medicine, University of Colombo.

Animals

Healthy, unused male Wistar rats (200-250g) were purchased from Medical Research Institute (MRI), Colombo, Sri Lanka. The animals were kept in plastic cages (two per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light "per day" relative humidity, 50–55%) at the animal house of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka with access to food and water ad *libitum*. A period of one week was given for acclimatization to animal house conditions prior to the commencement of the experiments.

Animals were euthanized under anesthesia following completion of the experiment with an overdose of aesthetic ether. All animals were humanely treated in accordance with WHO and Federation of European Laboratory Animal Science Associations (FELASA) guidelines for animal care.

All experiments in rats were carried out in accordance with the recommendation of the guidelines for care and use of laboratory animals and the project proposal was approved (No.591/11) by the Ethics Review Committee of the Faculty of Medical Sciences of the University of Sri Jayewardenepura, Sri Lanka.

Chronic anti-inflammatory effect

Chronic anti-inflammatory activity of BH was analyzed using adjuvant-induced arthritis rats model[5]**.** Arthritis was induced by a single intra-dermal injection of 0.1 ml of Freund's Complete Adjuvant (FCA) containing 0.05% w/v *Mycobacterium butyricum* suspension in sterile paraffin oil into a foot pad of the left hind paw of all rats with help of glass syringe and 26 G needles after the rats were subjected to light diethyl ether anesthesia[6]. The healthy control group was injected with a single dose of 0.1 ml of normal saline into a foot pad. The animals were randomly divided to four groups of 6 rats each (Table 1).

Drug administration

On the 14th day, each group was treated with medication by intragastric administration once daily for next 14 days[7]. All the rats were orally treated for 14 consecutive days from the day 14 to till 28th day.

Body weight examination

Body weight (BW) of the rats was measured on day 0, 4, 8, 12, 16 and 20, 24, and 28 after induced. The body weight gain of each animal was calculated using the following formula[8].

$$\text{Body weight gain} = \text{Body weight on day N} - \text{Body weight on day 0}$$

Measurement of ankle joint thickness (AJT) and foot pad thickness (FPT) of the rats

The hind paw ankle joint thickness (AJT) and Foot pad thickness (FPT) of all animals were estimated on day 0 (before injection of FCA emulsion) and on day 3, 7, 10, 14, 17, 21, 24 and 28 after the injection of adjuvant. Dial Caliper (Mitutoya, Japan) was used for measuring the AJT and FAT[9].

Percentage inhibition of change in ankle joint thickness and food pad thickness was calculated by using the following formulas;

$$\text{AJT (\%)} = \frac{(\text{Treated group}_{AJT} - \text{Healthy control}_{AJT})}{\text{Healthy control group}_{AJ}} \times 100$$

$$\text{FPT (\%)} = \frac{(\text{Treated group}_{FPT} - \text{Healthy control}_{FPT})}{\text{Healthy control group FPT}} \times 100$$

Sample collection

All animals were sacrificed on day 29th and blood was collected in EDTA-coated vials: for Full blood count (FBC) by automated haematology analyzer (Dirui BCC-3000 B, China).

Table 1: Treatment groups and the drugs the rats were given

	Group Name	Drug
Non arthritic rats	Healthy control	Distilled water (2.5 ml)
Adjuvant Induced Arthritis rats (AIA)	Arthritic control	Distilled water (2.5 ml)
	Standard	Celecoxib (5 mg / kg)
	BH	Bees' honey (4ml/kg)

Statistical analysis

The data were expressed as arithmetic mean ± SEM. Untreated arthritic rat group was compared with healthy control animals and the treated arthritic groups were compared with untreated arthritic animals. The significance level was determined using student's t-test and considered extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Results

Body weight of rats

Following FCA emulsion injection, there was no significant changes in the body weight as observed on Day 0 and 7 in arthritic rats. The body weight of negative control rats was significantly ($p<0.001$) compared with healthy control animals. But arthritic rats treated with standard drug Celecoxib and BH showed significant weight gain after day 14, as compared to negative control animals. After 14 days, weight gain of rats was gradually increased in treated groups (Figure 1).

Effect on ankle joint thickness (AJT)

At Day 0, no significant differences were found among the AJT of all studied groups. A significantly enhanced in AJT was found in the FCA injected group of animals on Day 3 (first swelling phase). Thereafter, swelling slowly subsided until seventh day and then began to increase again when disseminated arthritis appeared. After the initiation of drug administration on Day 14, significant reductions of AJT in AIA group of animals on Day 17, 21, 24 and 28 were observed as compared to the arthritic control rats. The test groups Bees' honey exhibited significant ($p < 0.05$) reductions of the AJT. Celecoxib showed extremely significant ($p < 0.001$) reductions of AJT on Day 28 as compared to the arthritic control rats. The rats treated with BH have also shown ($p<0.05$) significant reduction of AJT following the oral administration (Figure 2).

Effect of foot pad thickness

On Day 0 beginning of the experiment, no significant differences were found in the rat FPT among all the groups. A significant increase in FPT was observed for the adjuvant injected group on Day 3. Swelling and redness developed over a 24-h period in the hind paw injected with CFA and reached maximum intensity on day 3 (first swelling phase). Thereafter, swelling slowly subsided until the seventh day and then the FPT began to increase again when disseminated arthritis appeared (second swelling phase, which was greater than the first one and peaked on days 21–24). Foot pad thickness of AIA rats were observed on Day 7, 10, 14, 17, 21, 24 and 28 as compared to the healthy control rats and showed statistically reduction ($p < 0.001$). There were no significant differences ($p> 0.05$) between AIA rats on Day 4, 7 and Day 14.

After 14^{th} day, treatments were started with the standard drug celecoxib and BH. Significantly reduced of foot pad thicknesses were observed on Day 17, 21, 24 and 28. Extremely significant effects ($p < 0.001$) were observed in celecoxib group on Day 21, 24, and 28; whereas in significant differences of FPT were observed after 14^{th} day BH group as well. The reduction of foot pad thickness taken as a marker of disease recovery in AIA rats is shown in Figure 3.

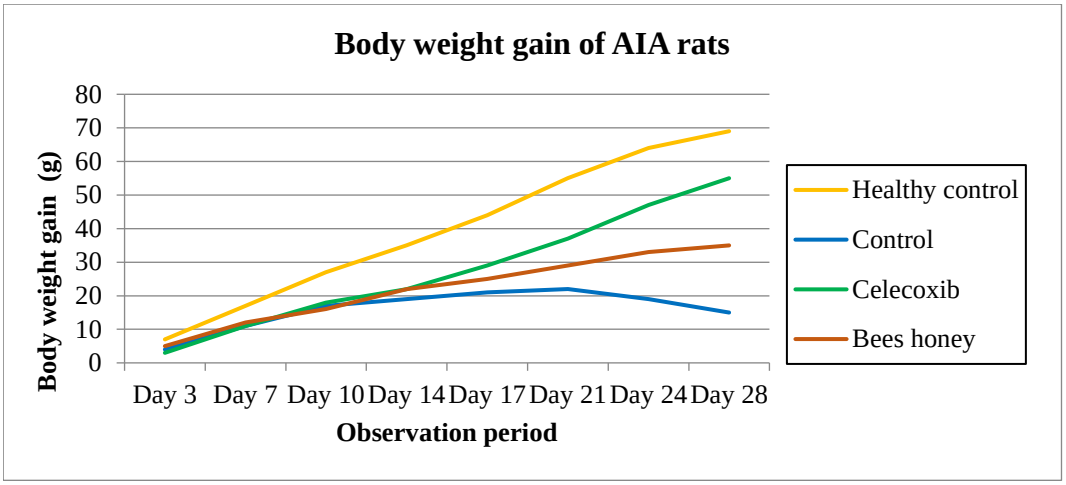

Figure 1: Effect of drugs on body weight gain of AIA.
The data are expressed as mean ± SEM (n=6 per group). $p<0.05$; and not significant ($p>0.05$)

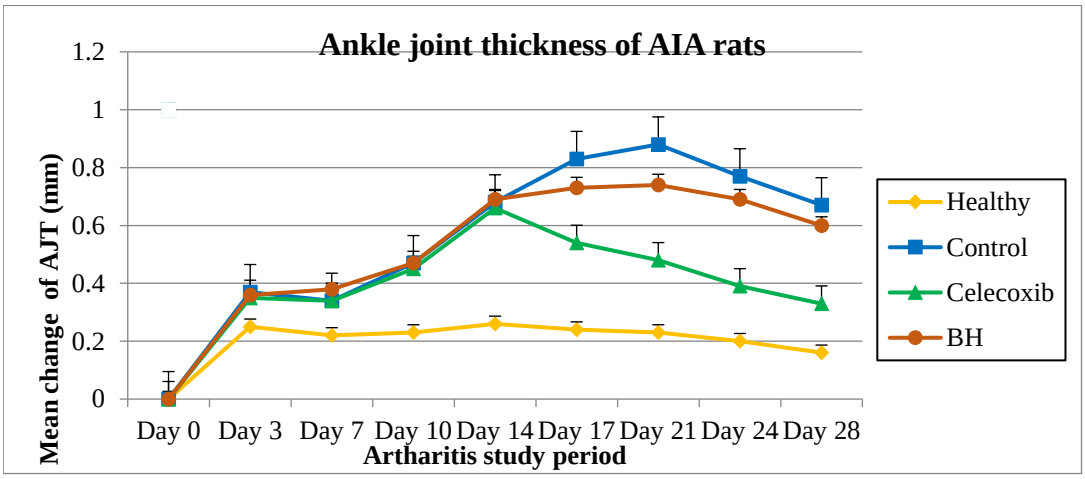

Figure 2: Effect of drugs on ankle joint thickness (AJT)

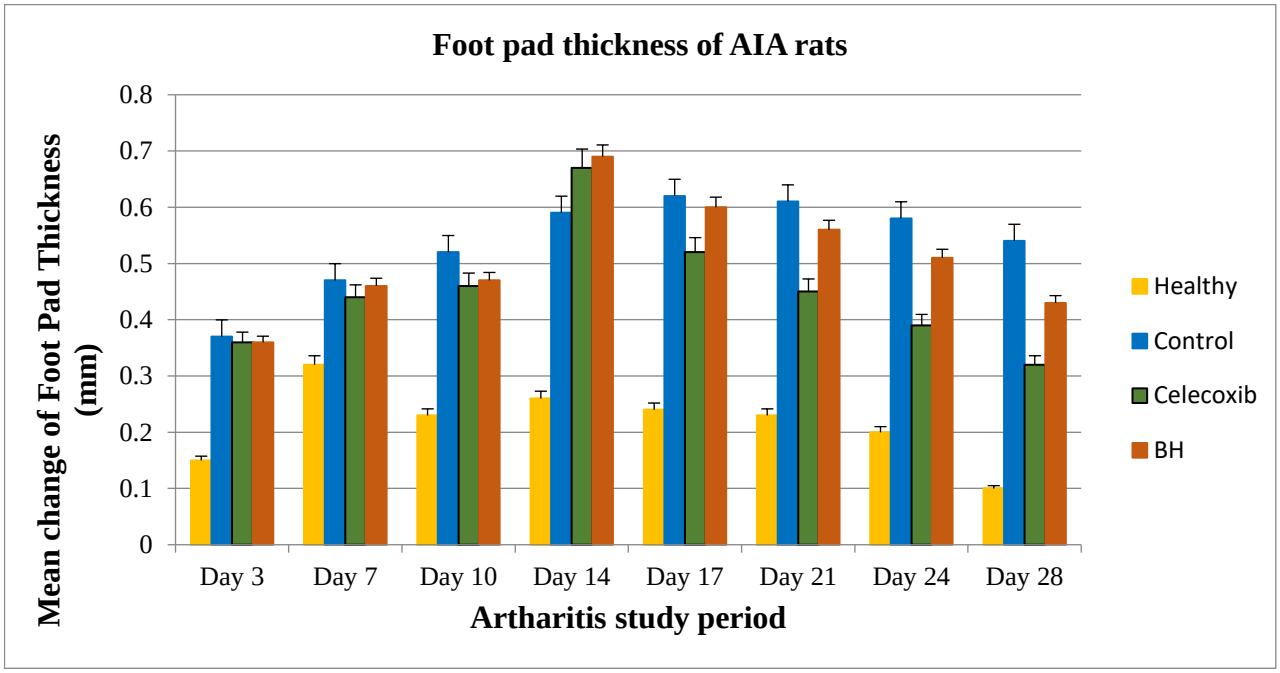

Figure 3: Effect of foot pad thickness in adjuvant-induced arthritis model

Table 2: Effect of drugs treatment on haematological indices in arthritic rats

Blood parameters	Groups			
	Healthy	Control	Celecoxib	BH
White blood cells (WBC) (109/L)	8.2 ± 0.2	18.2 ± 0.2	10.6 ± 0.3***	16.2±0.5*
Neutrophil (%)	31.9 ± 3.6	13.1± 1.4	22.3 ± 0.9***	16.1 ± 2.9
Lymphocytes (%)	55.8 ± 2.0	81.2 ± 1.3	71.1 ± 0.8***	75.4 ± 2.1*
Monocyte (%)	4.9 ± 1.7	5.8 ± 0.8	6,6 ± 0.6	8.4±0.9
Red blood cells (RBC), (109/L)	8.1 ± 0.5	6.3 ± 0.4	8.3 ± 0.5**	6.95 ± 0.3
Hemoglobin (Hb) (g/dl)	14.9 ± 0.3	13.3 ± 0.3	14.2 ± 0.3	13.5 ± 0.2
Packed cell volume (PCV) (%)	51.3 ± 2.3	31.7± 3.0	37.7 ± 1.8	33.7± 3.1
Platelets (109/L)	836.6 ± 3.5	832.6 ±9.2	866.6 ± 11.0*	852.6 ± 11.8

The data are expressed as mean ± SEM (n=6 per group). Symbols represent statistical significance: extremely significant (***) $p<0.001$; highly significant (**) $p<0.01$; significant (*) $p<0.05$; and not significant ($p>0.05$).

Effect of drugs on haematological indices of AIA rats

There was a significant ($p<0.01$) decrease observed in Heamoglobin (Hb) and Red blood cells (RBC) concentrations in AIA control group, when compared to the healthy control rats. Moreover, white blood Cells (WBC), neutrophils and lymphocytes also showed significant differences when compared to the healthy control group.

In the AIA rats treated with Celecoxib was significant ($p < 0.001$) reduction in the WBC and lymphocytes and significant ($p < 0.01$) increase in RBC (Table 2). In the group given Celecoxib, there was a significant ($p<0.001$) increase in PLT when compared with the AIA control group. Bees' honey had the capacity to decrease WBC and lymphocytes ($p <0.05$).

Discussion

Adjuvant-induced arthritis (AIA) in rats is a useful tool to study the pathophysiology of Rheumatoid Arthritis (RA), especially because the experimental model and the human disease share various signs and symptoms[10]. Therefore, in the present study an intra-dermal injection of FCA containing heat-killed cells of *Mycobacterium butyricum* was used to induce inflammation and arthritic lesions in the animals.

Anti-arthritic potency of drugs was determined by reversal of the altered arthritic parameters. The result of Figure 3 shows that the foot pad thickness (FPT) increased in adjuvant- challenged animals. Drug administration suppressed severity of clinical arthritis, as demonstrated by decreased FPT in rats.

Previous report showed that there was significant body weight loss, the day following injection of the adjuvant[11]. The result of the present study also indicates that there is close relationship between the extent of inflammation and loss of BW. The body weight of negative control rats was significantly decreased compared with healthy control rats. The data suggested that oral treatment of Celecoxib and BH recovered inflammatory body weight loss in arthritic animals. The body weight of the rats treated with BH significantly ($p<0.01$) increased compared with negative control animals after starting the oral treatment.

Similarly, body weight in arthritic animals was enhanced by Celecoxib as well as BH administration as shown in Figure 1. The observed changes in AJT of the experimental groups of animals are shown in Figure 2.

The percentage of AJT was significantly enhanced in the negative control group of arthritic animals as compared to healthy control rats. Recovery from increased AJT in adjuvant injected rats, BH treated animals showed better results in certain extent similar to that of standard drug Celecoxib.

Adjuvant-induced arthritis study was carried out to investigate the chronic anti-inflammatory activity of BH in rheumatoid arthritis rats. In this study, adjuvant injected paw was illustrated by a rapid onset of inflammation evident within 24 h of adjuvant injection and continued for 28 days.

Administration of FCA led to increase in the total blood leucocyte count suggesting the involvement of WBCs in response to antigen-mediated arthritic[12]. A significant increase in WBC count was observed in the AIA control rats when compared to the healthy control rats. However, there was a significant difference ($p<0.05$) of the WBC counts observed in the BH treated group when compared with the baseline values and this observation needs further investigation.

According to the findings of the present study Bees' honey has proven its potent chronic anti-inflammatory effect.

Conclusions

This study revealed that the promising chronic anti-inflammatory effect of BH in rats which was reported scientifically for the first time. As found in the present study, bees' honey exerts chronic anti-inflammatory effects that are closely similar to standard drug Celecoxib. This study provided transitional medicinal evidence for BH used in the treatment of chronic inflammation in traditional medicine.

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