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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF KUKKUTANDATVAC BHASMA IN ACUTE AND CHRONIC INFLAMMATION MODELS

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Abstract

Kukkutandatvakbhasma (Hen Egg shell bhasma) which is known as calcinated Hen egg shell is used in the treatment of leucorrhea in the form of bhasma since ancient times in Ayurveda system of medicines.It is known to have anti-inflammatory, anti-arthritic, rejuvenative for heart and aphrodisiac property.Hence, the objective of present study was to explore its anti-inflammatory activity of Kukkutandatvakbhasma through appropriate animal models.The Kukkutandatvakbhasma of both types of hen egg shell i.e. modern (KKBM-7)and gavran (KKBG-7) was prepared separately using *Putapaka* method in three steps – Shodhan (purification), Marana and Bhavana. Anti- inflammatory activity of KKBM-7 and KKBG-7 was evaluated using carrageenan inducedpaw edema and Adjuvant-induced chronic arthritis models.Treatment with KKBM-7 (300 and 500 mg/kg) and KKBG-7 (300 and 500 mg/kg) reducedcarrageenan induced rat paw edema volume. Treatment with KKBM-7 (300 and 500 mg/kg) and KKBG-7 (300 and 500 mg/kg) significantly reduced paw volume compared to control group from day 3 till day 21 in Adjuvant-induced chronic arthritismodel.thus, it was concluded that KKBM-7 and KKBG-7 have significant anti-inflammatory activity in acute as well as chronic model of inflammation.

Keywords: *Kukkutandatvakbhasma, Anti- inflammatory activity, Shodhan, Bhavana*

Introduction

Inflammation is a defense mechanism aimed to remove theinjurious stimuli and initiate the tissue healing process. However, prolonged inflammation can lead to numerousdiseases including rheumatoid arthritis (RA), psoriasis, and inflammatorybowel disease. RA, a chronic inflammatory diseasewhich is characterized by immune-mediated inflammatory sinusitisinvolving cartilage and bone destruction, joint malformation,functional impairment results into continued swelling around thejoint, pain, synovial hyperplasia, pannus formation, and morphologicalchanges.The only available medicine in modern practiceare cyclooxygenase (COX) inhibitors i.e. NSAIDs and opioids. Theuse of these classical medicine for long term treatment such as incase of RA, may produces severe adverse effects such as gastrointestinaldisturbances and renal damage. [1]

Nowadays, although the synthetic anti-inflammatory drugs are dominating the market, the element of toxicity from these drugs cannot be ruled out. Many drugs (both nonsteroidal anti inflamatory drugs (NSAIDs) and corticosteroids) have been developed but their safety profile studies have shown that none of them is clearly safe.[2]Due to adverse reactions of synthetic and chemical medicines i.e. causing gastrointestinal irritation and reappearance of symptoms after discontinuation, herbal medicines have made a comeback to improve our basic health needs.[3]Hence, the traditional medical practitioners and scientists are turning towards medicinal plants and traditional system of medicine-- Ayurveda to reduce the side effects and toxicity.

In current scenario, Nanomedicines are most popular therapeutic applications with more efficacy and lesser side effects.[4]Bhasma, the ancient concept of nanomedicine is used for treatment of various chronic ailments since 7th century BC.[5]

Kukkutandatvakbhasma (Hen Egg shell bhasma) which is known as calcinated Hen egg shell is used in the treatment of leucorrhea in the form of bhasma since ancient times in Ayurveda system of medicines.[6]It is prescribed by practitioners in sperm deficiency, amenorrhea, leucorrhoea and polyuria.[7] It is known to have anti-inflammatory, anti-arthritic, rejuvenative for heart and aphrodisiac property. Eggshell contains high percentage of calcium and very low phosphorous. Eggshell contain calcium carbonate (94%), calcium phosphate (1%), organic compounds (4%), and magnesium carbonate (1%).[8]Eggshell calcium is more effective in increasing bone mineral density.[9]

Literature survey revealed that Kukkutandatvakbhasmahas not been explored for its anti-inflammatory activity till date. Hence, the objective of present study was to explore its anti-inflammatory activity through appropriate animal models.

Methods

Two types of hen egg shell (modern and deshi) were selected and procured from local market in Pune.

Method of Preparation

The Kukkutandatvakbhasma of both types of hen egg shell (modern and gavran) was prepared separately using *Putapaka* method in three steps – Shodhan (purification), Marana and Bhavana.[6,7]

Shodhan (purification)

Egg shell was immersed in saindhavjala for 24 hrs to remove the foreign matter and contaminants. Saindhavjala was prepared by mixing 1 part of saindhav in 16 parts of water (1/4:1). In this jala, 4 parts of egg shell was added. For purification of 1 kg of egg shell, 250 gm of saindhav was added to 4 liters water. Shell was rubbed in the saindhavjala for removal of adhering dirt and then kept for 24 hrs. After 24 hrs.the shell was removed from the jala and exposed to bright sunlight for dryness. Later, the inner shell membrane was removed.

Marana

The purified egg shell was placed in between two sharav (earthen pots) which were sealed by multanimatti. A pit of one gaja (king's arm) length, breadth and height (2' X 2' X 2') was dug. Its inner surface was smoothened. 50 cow dung cakes were arranged in a pit and the sealed sharavs were placed over them. It was covered with the 25 cow dung cakes. The cow dung cakes were ignited and then the sharav was allowed to cool. It was then removed from the pit. The inner material was collected and it was labeled as Kukkutandatvakbhasma (KKBM and KKBG)

Bhavana

The collected KKBM and KKBG was macerated with korphad rasa. It was dried and kept in between two

sharavs and subjected to gajaputa. The obtained material was labeled as KKBM-1 and KKBG-1. It was taken out, mixed with korphad rasa, dried and placed in sharav& then subjected to gajaputa. Seven such putas were given which is labeled as KKBM-7 and KKBG-7.

Animals

Adult male Wistar albino rats weighing 150-200 g, were kept under standard conditions such as temperature at 24 ± 10°C, relative humidity at 45-55% and 12:12 h light:dark cycle. The animals were fed divided in the six groups of 6 animals each. Group I serve as a 'Negative control' which received only suspension of gum acacia (4%) in normal saline solution. Group II received 'Reference standard' Prednisolone (10mg/kg), Group III, IV, V and VI received KKBM-7 (300mg/kg), KKBM-7 (500mg/kg) and KKBG-7 (300mg/kg), KKBG-7 (500mg/kg).After 1 hr. Carrageenan (0.05mL of 1% w/v) in saline was injected subcutaneously into the left hind paw of each animal. The paw volume of each rat was measured before a carrageenan injection and then at hourly intervals up to 5 hours usingPlethysmometer.[10]

Adjuvant-induced chronic arthritis

Experimental arthritis was induced in rats according to the method propose by Newbould[78] with somemodifications. The right footpad of each rat was injected (s.c.) with 0.1mL of complete Freund's adjuvant (CFA) with Mycobacterium butyricum 1% suspension in mineral oil. Rats in the test groups were treated with KKBM-7 (300mg/kg), KKBM-7 (500mg/kg) and KKBG-7 (300mg/kg), KKBG-7 (500mg/kg) and then with the daily treatment until 21 days after the CFA challenge. The paw volume of each rat was measured on day 1,3,6,9,12,15,18 and 21 using plethysmometer.[10]

Results

In the present investigation, anti- inflammatory activity of KKBM-7 and KKBG-7 was evaluated using carrageenan inducedpaw edema and Adjuvant-induced chronic arthritis models. Carrageenan inducedpaw edema is an acute model of inflammationwhile Adjuvant-induced chronic arthritis is a chronic model of inflammation. Inflammation induced by carrageenan, originally described is acute, non immune, well-researched, and highly reproducible.[11,] [12]In carrageenan with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiments. All the experiments on animals were performed with the approval from IAEC.

Anti-inflammatory and antiarthritic activity

Carrageenan-induced rat paw edema.

It was performed according to the method of Winter et al. (1962). On the day of experiment, all the animals were

inducedpaw edema test, carrageenan induced significant increase in paw volume with increasing time intervals, with maximum paw volume at 3 hr interval. Treatment with KKBM-7 (300mg/kg), KKBM-7 (500mg/kg) and KKBG-7 (300mg/kg), KKBG-7 (500mg/kg) reduced paw edema volume significantly at 3 hr interval as shown in Table 1.Thus, it can be concluded that KKBM-7and KKBG-7 possess significant anti-inflammatory activity in acute model of inflammation.

Adjuvant-induced chronic arthritis

Adjuvant arthritis in rats exhibits many similarities to humanrheumatoid arthritis which is one of chronic inflammatory disease. An injection of complete Freund's adjuvant into the rat paw induces inflammationas primary lesion with a maximum after 3 to 5 days.Secondary lesions occur after a delay of approximately 11 to 12 days which are characterized by inflammationof non-injected sites (hindleg, forepaws, ears, noseand tail), a decrease of weight and immune responses.Perper et al (1971)experimented using Adjuvant-induced chronic arthritis model to differentiate between anti-inflammatory andimmunosuppressive activity.[13]It was observed that anti-inflammatory compounds do not inhibit secondarylesions, which are prevented or diminished by immunosuppressiveagents.[14]Injection of adjuvant (Mycobacterium butyricum suspended in mineral oil) into rats produces an immune reaction that characteristically involves inflammatory destruction of cartilage and bone of the distal joints with concomitant swelling of surrounding tissues. Adjuvant-induced arthritis in rats is commonly used to evaluate compounds that might be of potential use as drugs for treatment of rheumatoid arthritis and other chronic inflammatory conditions.[1]

In present study we used this model to investigate anti- inflammatory activity of KKBM-7, KKBM-7,KKBG-7 and KKBG-7 in chronic inflammation.In present study, an injection of CFA induced significant increase in paw volume in control group after 3 days. Treatment with KKBM-7 (300 and 500 mg/kg) and KKBG-7 (300 and 500 mg/kg) for 21 days significantly reduced paw volume compared to control group from day 3 till day 21. However, it was observed that reduction in paw volume with KKBM-7 (500 mg/kg) and KKBG-7 (500 mg/kg) was more significant KKBM-7 (300 mg/kg) and KKBG-7 (300 mg/kg). The results are shown in Table 2. Thus it can be concluded that KKBM-7 and KKBG-7 possess significant anti- inflammatory activity against chronic inflammation.

Conclusion

From the results of present study, it was concluded that KKBM-7 and KKBG-7 have significant anti-inflammatory activity in acute as well as chronic model of inflammation as demonstrated by significant reduction in paw volume in carrageenan inducedpaw edema test and adjuvant-induced chronic arthritis model respectively. Thus, Kukkutandatvakbhasma of modern and gavran hen egg shell found to be one of the alternative treatments for acute and chronic inflammatory diseases like rheumatoid arthritis.

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Table 1. Changes paw edema volume in Carrageenan-induced paw edema model in rats

Group (Dose in mg/ kg)	Change of paw edema volume (ml)				
	1h	2h	3h	4h	5h
Control	0.30±0.03	1.32 ± 0.12	1.84 ±0.20	1.82 ±0.11	1.78 ±0.15
Prednisolone (10mg/kg)	0.17 ± 0.04	0.49 ± 0.13*	0.44 ± 0.09*	0.57 ± 0.16*	0.58 ± 0.20*
KKBM-7 (300)	0.12± 0.03*	0.53 ± 0.14*	0.90 ± 0.20*	1.19 ± 0.15*	1.12± 0.08*
KKBM-7 (500)	0.09 ± 0.03*	0.66 ± 0.01*	0.89 ± 0.10*	1.15 ± 0.13*	1.10 ± 0.07*
KKBG-7 (300)	0.13 ± 0.06*	0.55 ± 0.14*	0.90 ± 0.21*	1.06 ± 0.01*	1.15 ± 0.09*
KKBG-7 (500)	0.11 ± 0.02*	0.77 ± 0.09*	0.88 ± 0.31*	1.04 ±0.14*	1.15 ± 0.20*

Values are mean ± SEM, n=6 in each group

*P<0.01 compared to control (ANOVA followed by Dunnett test)

Table 2. Changes paw edema volume in Adjuvant-induced chronic arthritis model in rats

Time (days)	Normal	Control	Indomethacin (1)	KKBM-7 (300)	KKBM-7 (500)	KKBG-7 (300)	KKBG-7 (500)
Edema volume of paw							
1	0.65 ±0.07	1.56 ±0.78	1.34 ±0.41	1.42 ±0.51	1.38 ±0.45	1.43 ±0.52	1.37 ±0.43
3	0.78 ± 0.13*	1.94 ±0.08	1.52 ±0.16*	1.60 ±0.06	1.53 ±0.05*	1.59 ±0.07	1.54 ±0.03*
6	0.94 ±0.09*	2.28 ±0.05	1.56 ±0.26*	1.77 ±0.06*	1.59 ±0.04*	1.77 ±0.03*	1.58 ±0.10*
9	1.03 ±0.10*	2.68 ±0.05	1.32 ±0.22*	1.82 ±0.06*	1.72 ±0.08*	1.80 ±0.05*	1.70 ±0.6*
12	1.08 ±0.16*	2.93 ±0.07	1.84 ±0.11*	1.99 ±0.13*	1.88 ±0.15*	1.97 ±0.11*	1.88 ±0.10*
15	1.09 ±0.20*	3.11 ±0.08	1.59 ±0.39	1.56 ±0.79	1.35 ±0.50	1.57 ±0.72	1.36 ±0.25
18	1.07 ±0.11*	3.28 ±0.12	1.46 ±0.19*	1.59 ±0.09*	1.33 ±0.07*	1.59 ±0.10*	1.32 ±0.02*
21	0.92 ±0.12*	3.53 ±0.16	1.20 ±0.17*	1.24 ±0.11*	1.12 ±0.10*	1.23 ±0.10*	1.15 ±0.09*

Values are mean ± SEM, n=6 in each group

*P<0.01 compared to control (ANOVA followed by Dunnett test)

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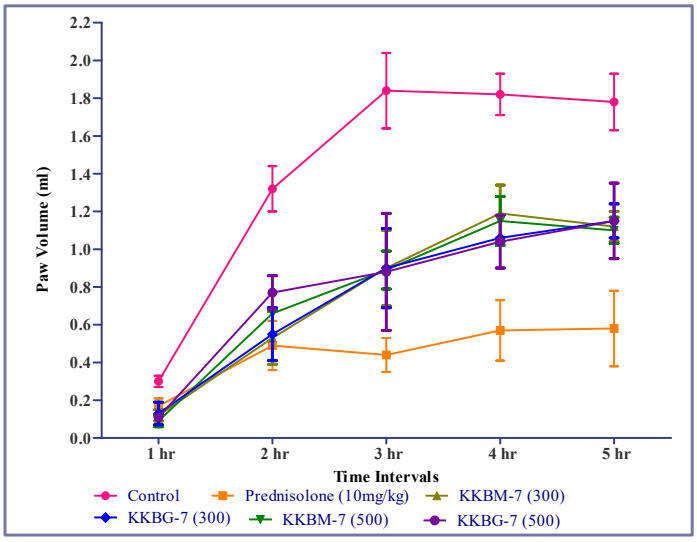
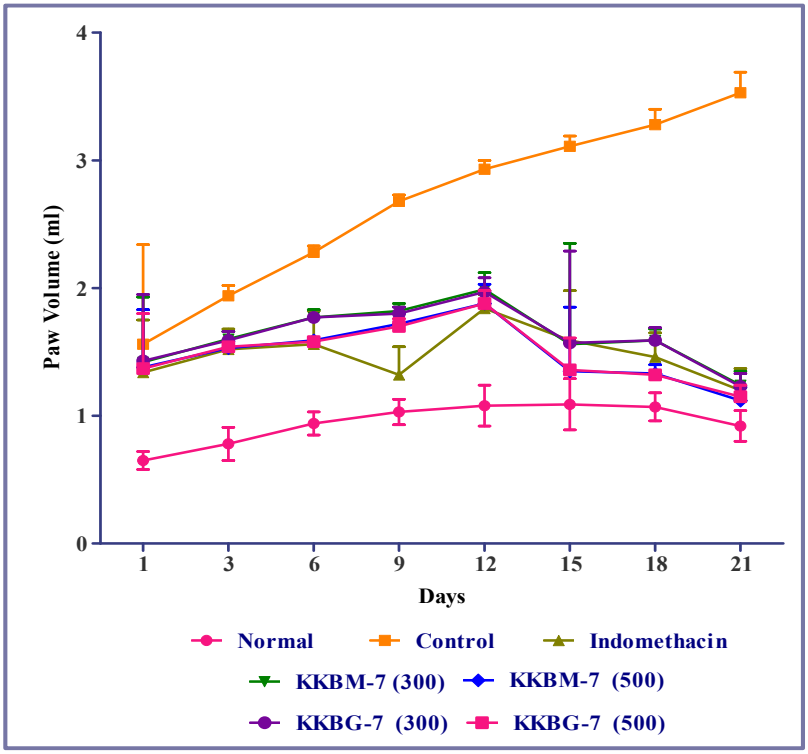
Figure 1. Changes paw edema volume in Carrageenan-induced paw edema model.



Values are mean ± SEM, n=6 in each group
*P<0.01 compared to control (ANOVA followed by Dunnett test)

Figure 2. Changes paw edema volume in Adjuvant-induced chronic arthritis model.



Values are mean ± SEM, n=6 in each group
*P<0.01 compared to control (ANOVA followed by Dunnett test)