



## Anti-inflammatory, Anti-arthritic and Analgesic Effect of the Herbal Extract Made from *Bacopa monnieri*s, *Cassia fistula* and *Phyllanthus polyphyllus*

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**Abstract** – Anti-inflammatory, anti-arthritic and analgesic activity of each herbal extract, which is extracted from *Bacopa monnieri*s, *Cassia fistula* and *Phyllanthus polyphyllus*, respectively. The treatment of herbal extract exhibited anti-inflammatory effect as a dose-dependent manner, from 1.25 mg/kg to 12.5 mg/kg, in acute inflammatory models (carrageen and egg-albumin induced rat hind paw edema). It also elicited significant anti-inflammatory activity in chronic inflammatory models (cotton pellet granuloma and Freund's adjuvant induced polyarthritis in rat). In cotton pellet granuloma test, the extract exhibited the inhibitory effect of 23 and 57% at the dose of 6.25 and 12.5 mg/kg, respectively. In Freund's adjuvant induced model, the treatment of the extract of 1.25, 6.25 and 12.5 mg/kg showed the inhibitory effect of 23, 56 and 66% at 8 days, respectively. In the acetic acid-induced model, the extract significantly reduced abdominal writhing in mice when compared to the control group, reducing the mean number of writhing from  $41 \pm 2$  in the control group to  $17 \pm 3$  and  $15 \pm 2$  at the dose of 6.25 and 12.5 mg/kg. From these experiments, the extract, which was extracted from the combination of *Bacopa monnieri*s, *Cassia fistula* and *Phyllanthus polyphyllus*, (w/w/w = 1/2/1) is surprisingly found a significant analgesic and anti-inflammatory activity.

**Keywords** – *Bacopa monnieri*s, *Cassia fistula*, *Phyllanthus polyphyllus*, Anti-inflammatory

### Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. Symptom are generally redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs (nonsteroidal anti-inflammatory drugs). Unfortunately, these drugs cause increased a lot of risk case in clinical. Lots of the research publications have recently strengthened its warning that NSAIDs cause an increased risk of heart attack and stroke, especially in higher doses. They have also been known to cause stomach bleeding. It is important that you are aware of potential side effects.<sup>1-5</sup>

To reduce these side effect, many researchers have been screened the lead compound from so many synthesis compounds made from new drug synthesis technological methods. Unfortunately, optimal compounds reduced those side effect were not led until now a day.

As the result of the inherent problems associated with

the current non-steroidal as well as steroidal anti-inflammatory agents, there is continuous search especially from natural sources for alternative agents.<sup>6</sup>

A large number of herbal extract as well as the products being employed in the treatment of inflammatory diseases. According to lots of publications, most of herbal plants have been reported on own their anti-inflammatory activity, including antioxidant and free radical scavenging effects.

Each herbal extracts of *Bacopa monnieri*s, *Cassia fistula* and *Phyllanthus polyphyllus* have been used in many disorders such as bronchoconstriction, rheumatism, asthma and septic shock in India and Srilanka.<sup>7-9</sup> Herein, with this background of information, the effect of the herbal plant has been evaluated on various animal models of inflammation such as carrageenan and egg-albumin induced rat hind paw edema, cotton pellet granuloma and Freund's adjuvant polyarthritis including the analgesic effects.

### Experimental

**Animal** – Albino mice of Swiss strain (weight 20 – 30 g)

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and Wister strain rats (weight 110 – 150 g) were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). The animal housing conditions maintained were 25 °C ± 1 °C, 65 ± 10% relative humidity and 12 h light and dark cycle under conventional conditions in accordance with the institute's animal care guidelines. Food (5L79, Orient-bio Co., Ltd., Korea) and water were available *ad libitum*. All experiments are designed with 5 heads per each experimental group. The experimental design and research plan along with animals handling and disposal procedure were reviewed with the animal ethics committee and approved by the committee (KJAEC-20160307).

**Chemicals and herbal plants** – Carrageenan, egg albumin and Freund's adjuvant were from Sigma Chemical (Louis, USA), and the dried herbal plants (*Bacopa monnieriis*, *Cassia fistula* and *Phyllanthus polyphyllus*) from China were purchased by Essell Bio (Seoul, Korea) and authenticated by Prof. Ki-Heung, Lee from Kong-Ju University. The voucher specimens were deposited in Kong-Ju University.

**Preparation of the extract** – Coarsely powdered, shade dried whole plants of *Bacopa monnieriis*, *Cassia fistula* and *Phyllanthus polyphyllus* were composed as a follow ratio; *Bacopa monnieriis*, *Cassia fistula* and *Phyllanthus polyphyllus* = 13.3 g/13.3 g/13.3 g, 20 g/10 g/10 g, 10 g/20 g/10 g, 10 g/10 g/20 g, and were extracted with absolute ethyl alcohol for 72 h in a stirred extractor. The dark colored extract filtered and the solvent recovered by distillation in vacuo. The residue so obtained was used for subsequent experiments. The w/w total yield in terms of the extract was 9 ± 1.2%. The dried extract was suspended in 5% Carboxyl methyl cellulose (CMC) for animal administration.

**Screening of lead extract** – The determination of the optimum ratio for the herbal extraction was carried out to screen various extracts by Egg-albumin induced model. Albumin edema was induced by injecting subcutaneously 0.1 ml of 2% (%) bovine albumin prepared in normal saline, into the planter region of the hind paw of rats.<sup>10</sup> The paw volume was measured at 0 min ~ 60 min after the injection of undiluted fresh egg white using a plethysmometer. Edema was expressed as the increase in paw volume (ml) after carrageenan injection relative to the pre-injection value for each animal. The samples were administered by oral route, at 1 h before carrageenan injection. Diclofenac sodium (12.5 mg/kg) was used orally as standard anti-inflammatory drug for positive control.

**Carrageenan and Egg-albumin induced model** – In this test, 0.1 ml (w/v) carrageenan 0.1% was injected subcutaneously into the right hind paw.<sup>11</sup> Paw volume

was measured with a plethysmometer (Ugo-Basile, Varese, Italy) immediately prior to the injection of carrageenan and thereafter at hourly intervals for 8 h. The experimental condition of Egg-albumin induced model is the same protocol above the contents of screening of lead extract.

**Cotton pellet granuloma** – The sterilized cotton pellets (50 ± 1 mg) were soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg), and then inserted one in each axilla. At the point of the end of experiment, the animals (Swiss albino rat) were anaesthetized on the tenth day the pellets together with granuloma were removed surgically and dried at 60 °C to constant weight. The increments in the dry weight taken as measure of granuloma formation.<sup>12</sup> The samples and Diclofenac sodium were administered in oral route for 10 days.

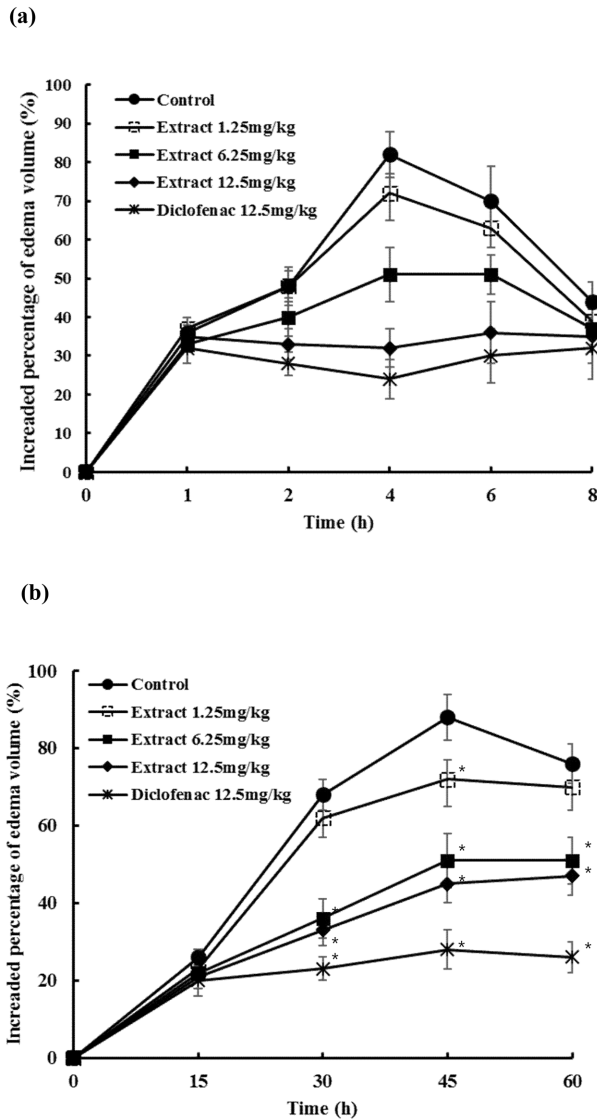
**Freund's adjuvant induced model** – Rats were injected subcutaneously 0.1 ml of complete Freund's adjuvant into the planter region of the left hind paw. The changes in the paw volume (left and right) were measured on various days up to the end of experiment following Freund's adjuvant injection. The samples and Diclofenac sodium were administered in oral route for 20 days.<sup>13</sup>

**Acetic acid induced model** – A group of mice were administered 0.1 ml/10 g of 0.3% (v/v) acetic acid by abdominal. The mice exhibiting the writhing episodes were selected for the experiment. The samples and Diclofenac sodium were administered in oral route 1 h prior the acetic acid injection. The number of writhing episodes were counted for 30 min following acetic acid administrations.<sup>14</sup>

**Statistical analysis** – Data were presented as means ± SE for the indicated number of independently performed experiments. Statistical significance ( $p < 0.05$ ) was assessed by one-way analysis of variance (ANOVA) coupled with Dunnett's *t*-tests.

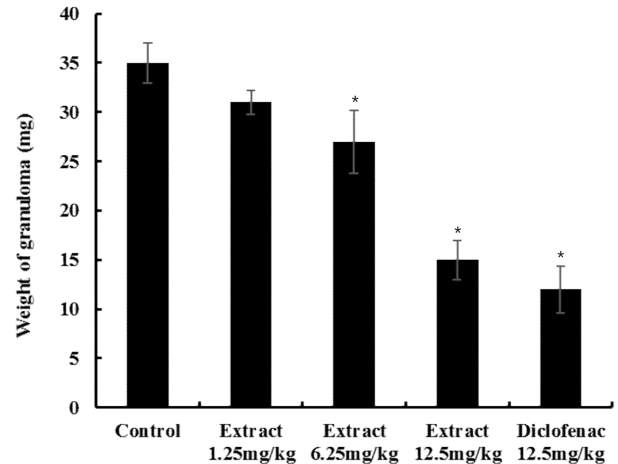
## Result and Discussion

In this study, the anti-inflammatory, anti-arthritis and analgesic effects of the herbal formulate that consists of three herbs, which were *Bacopa monnieriis*, *Cassia fistula* and *Phyllanthus polyphyllus*, were evaluated by various arthritis animal models. To lead the optimum ration of the combination of each material, the primary screen was conducted in Egg-albumin induced model against the extracts prepared by different weight ratios. As a result, the formulate that is extracted from the combination of 10 g/20 g/10 g exhibited optimum effect in screening model. Therefore, an optimum combination ratio was 1/2/



**Fig. 1.** Edema inhibitory effect of the herbal extract on carrageenan (a) and egg-albumin (b) induced rat paw edema. Values represent mean  $\pm$  SE (n = 6). \* $P \leq 0.05$  vs. control.

1 (w/w/w) (data not shown). Further testing is carried out with the extract made by this ratio. To determine the anti-inflammatory effects of the extract, which was extracted from the combination of *Bacopa monnieri*s, *Cassia fistula* and *Phyllanthus polyphyllus*, its effect of anti-inflammation was evaluated by the experimental animal model that was induced by various boosting chemicals. As shown Fig. 1a., after following carrageenan administration, the optimum inflammation response was observed at 4 h by 0.1 % carrageenan administration from 4 h to 6 h. Compared to PBS treatment group, increased edema volume was detected in the range from 70 to 82%, so we determined the activity of the extract at this time 4 and



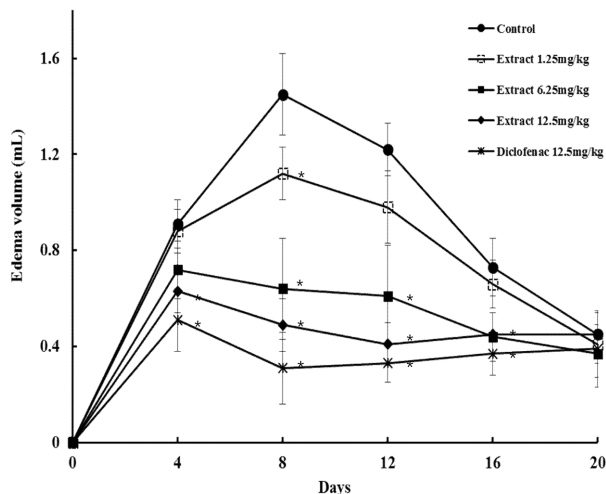
**Fig. 2.** Inhibitory effect of the herbal extract on cotton pellet-induced granuloma in albino Wistar rats. Weight of granuloma is difference of dry cotton pellet weight and initial cotton pellet weight. Values are expressed as mean  $\pm$  SE (n = 6). \* $P \leq 0.05$  vs. control.

6 h, which is reached at assessable edema volume. However, the extract (6.25 and 12.5 mg/kg) treated on animals showed a significant decrease in paw edema at time points of 4 and 6 h as compared to the control group. That is, Diclofenac (12.5 mg/kg) as positive drug also significantly suppressed carrageenan-induced paw edema at 2, 4 and 6 h.

As shown in the result of egg-albumin edema formation (Fig. 1.b.), the maximum inflammation response was observed at 45 min by 2% egg-albumin administration. At the 45 min, all treatment groups of the extracts and diclofenac was detected the significant inhibition of edema. The treatment of the extract on the concentration of 12.5 mg/kg showed the inhibitory effect of 49% at 45 min. However, diclofenac treatment of 12.5 mg/kg reduced edema of 68% at 45 min.

In cotton pellet granuloma (Fig. 2), the treatment of extract by oral inhibited significantly the weight of granuloma at the experimental doses of 6.25 and 12.5 mg/kg. The percentage of inhibition were 23 and 57% at the dose of 6.25 and 12.5 mg/kg, respectively. There is no significance between the treatment of the extract and diclofenac of 12.5 mg/kg, so the extract of 12.5 mg/kg has the similar activity with the same dose of diclofenac.

In complete Freund's adjuvant (CFA) induced model test (Fig. 3), joint swelling by CFA was exhibited on day 8 maximally, after which there was gradually decreased during the end of the experiment, in specific, joint swelling is decreased at about 50% in 16 days. At 8 and 12 days, inhibition of edema volume by treatment of the extracts was significantly showed at all doses compared to control

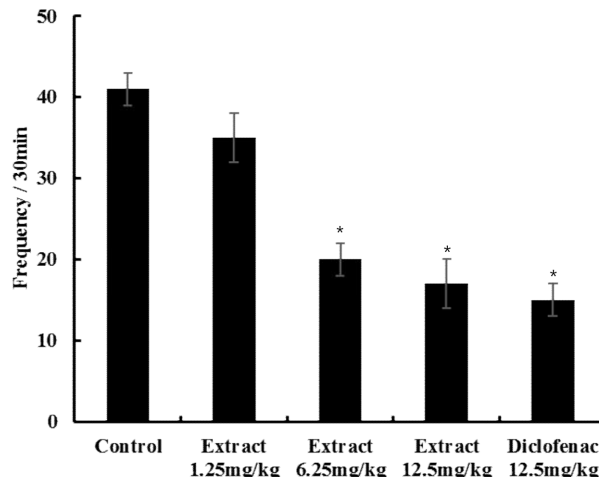


**Fig. 3.** Change of edema volume (ml) by the treatment of the herbal extract on Freund's adjuvant induced arthritis in rats. Values are expressed as mean  $\pm$  SE (n = 6). \*  $P \leq 0.05$  vs. control.

group. The treatment of the extract of 1.25, 6.25 and 12.5 mg/kg showed the inhibitory effect of 23, 56 and 66% at 8 days, respectively. However, although, diclofenac treatment of 12.5 mg/kg reduced edema of 79% at 8 days, it was not significant between 12.5 mg/kg of the extract and diclofenac. In 16 days, only 12.5 mg/kg of the extract and diclofenac was shown the significant inhibition of edema.

The effect of the extract on the acetic acid-induced abdominal constrictions in mice is presented in Fig. 4. The result shows that the extract (6.25 and 12.5 mg/kg) and diclofenac (12.5 mg/kg) significantly reduced abdominal writhing in mice when compared to the control group, reducing the mean number of writhing from  $41 \pm 2$  in the control group to  $17 \pm 3$  and  $15 \pm 2$  at the dose of 6.25 and 12.5 mg/kg. Also, all doses of the extracts caused a dose dependent increase in inhibition of abdominal writhing, increasing it from 0% in the control group to 15, 51 and 59% of the dose of 1.25, 6.25 and 12.5 mg/kg.

Diclofenac, used in our study as positive control, is a powerful anti-inflammatory substance of inhibiting cyclooxygenase and reducing the levels of prostaglandins A and E22.<sup>15</sup> This anti-inflammatory action was clearly observed in the evaluation of the analgesic effect of diclofenac and the herbal extract on carrageenan and egg-albumin induced animal model, cotton pellet granuloma, Freund's adjuvant induced animal models and acetic acid-induced animal model. From these our results that is obtained by *in vivo* test, this herbal extract is determined a potential anti-inflammatory effect as end-point. So, further study is necessary to find a clear mechanism of this herbal extract. However, this present experimental finding of



**Fig. 4.** Analgesic effects of the herbal extract by acetic acid writhing in mice. Values are expressed as mean  $\pm$  SE (n = 6). \*  $P \leq 0.05$  vs. control.

pharmacological parameters suggests that the herbal extract made from three herbal plants, *Bacopa monnieri*, *Cassia fistula* and *Phyllanthus polyphyllus*, is a promising anti-inflammatory effect in the treatment of inflammatory conditions. Hence, it is necessary to evaluate its anti-inflammatory activity in human clinical conditions.

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