ANTIPYRETIC EFFECT OF THE ETHANOLIC EXTRACT OF *FICUS RACEMOSA* ROOT IN RATS

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ABSTRACT: *Ficus racemosa* (Ma-Due-Au-Thum-Porn), an herbal plant, has long been used in many countries for the management of several diseases including diabetes, dysentery and pyresis. In this study, we investigated the antipyretic activity of the ethanolic extract of *Ficus racemosa* root (EFR) compared to that of acetylsalicylic acid (ASA) using lipopolysaccaride (LPS) and brewer's yeast-induced fever in rats. Pyresis was induced in animals with an intramuscular injection of LPS (50 µg/kg) 1 h after oral administration of 2% Tween 80, ASA 300 mg/kg or various doses of EFR (50-400 mg/kg). Rectal temperature was measured before the pretreatment and at 1 h intervals for 7 h after LPS injection. All doses of EFR significantly (p<0.001) reduced the increased rectal temperature produced by LPS and were found to be as potent as ASA. In the Brewer's yeast-induced pyrexia model, eighteen hours after subcutaneous injection of 20% brewer's yeast (10 ml/kg) rats were orally administered 2% Tween 80, ASA or various doses of EFR (50-400 mg/kg). Rectal temperature was measured each hour for 7 h after the extract administration. All doses of EFR significantly (p<0.05) reduced the pyrexia induced by yeast and EFR doses of 200 and 400 mg/kg were equally potent as ASA

Keywords: *Ficus racemosa*, lipopolysaccaride-induced fever, yeast-induced pyrexia, antipyretic effect

INTRODUCTION: *Ficus racemosa* L., belonging to the family Moraceae and known locally in Thailand as 'Ma-Due-Au-Thum-Porn', is grown in every regions of Thailand. Several parts of *F. racemosa* such as the leaf, stem bark, fruit and root have demonstrated numerous pharmacological effects. The root has been used for the treatments of dysentery and pyresis in Thailand, and for diabetes in other countries**.** *F. racemosa* root is one of the five herbal roots in the 'Ben-Cha-Lo-Ka-Wi-Chian' herbal formula, an antipyretic drug for both children and adults in Thailand. Ben-Cha-Lo-Ka-Wi-Chian powder (100, 200, and 400 mg/kg) has demonstrated antipyretic activity in a baker's yeast-induced fever model[1)]. Additionally, the single root powder of *F. racemosa*, orally administered at a dose of 40 mg/kg, also significantly decreased the rectal temperature in rats made pyretic[1)].

Although the antipyretic effect of the methanolic extract of *F. racemosa* bark was documented in the yeast-induced fever model[2)] and the root powder was capable of lowering rectal temperature in baker yeast-induced fever model in rats[1)], the root extract has not been tested in any animal models. Therefore, we were interested in investigating the antipyretic activity of the ethanolic extract of *F. racemosa* root in comparison with acetylsalicylic acid (ASA) utilizing lipo-polysaccaride (LPS)-induced fever and yeast-induced pyrexia models to provide evidence for a potential role of the ethanolic extract of *Ficus racemosa* root in the treatment of fever.

MATERIALS AND METHODS:

Plant material and preparation of plant extract

The *F. racemosa* root was collected during September, 2007 from Karnchanaburi, Thailand. The sample was identified as *F. racemosa* L. by one of the authors (N.R.). The Voucher specimen was deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Roots were washed, air-dried, and powderized. The dried-root powder was successively extracted with absolute ethanol by maceration at room temperature, and the filtrate was evaporated to dryness with an evaporator. The paste appeared dark-brown color (yield 4.27% w/w). The ethanolic extract of *Ficus*

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racemosa root (EFR) was kept in a tight container at 2-8°C until the time of treatment. A weighed amount of the EFR was suspended in 2% (w/v) aqueous Tween 80 solution and used for the study.

Animals

Male Wistar rats (140-180 g) obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornprathom, Thailand were used in the study. The animals were housed in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under standard conditions of temperature (25 ± 2ºC) and 12 h/12 h light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments and allowed food and water *ad libitum*. At the end of each experiment, the animals were sacrificed with carbon dioxide asphyxiation. Eight animals were used in each treatment group. The study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Drugs and chemicals

Acetylsalicylic acid (ASA; Sigma Chemical Co., USA) was suspended in 2% (w/v) aqueous Tween 80 solution. Lipopolysaccharide from *Escherichia coli* (LPS; Sigma Chemical Co., USA) was dissolved in sterile pyrogen-free normal saline at 500 µg/ml. Brewer's yeast (Sigma Chemical Co., USA) was suspended in normal saline.

Antipyretic activity test

Lipopolysaccharide-induced fever

The method of Santos and Rao[3] was modified and used for the assessment of the antipyretic activity of the EFR. Animals (N=56) were fasted overnight before the experiments, and on the day of testing, animals were kept singly in restrainers for 30 min to acclimate to their new environment. The animals were pretreated for 1 h with the EFR doses of 50, 100, 200 and 400 mg/kg, 2% Tween 80 solution or ASA (300 mg/kg), all administered orally before the administration of LPS. Fever was induced with 50 µg/kg of LPS injected intramuscularly into the right thigh of the rats. Rectal temperature was measured before the pretreatment of animals and at 1 h intervals for 7 h after the administration of the bacterial endotoxin with a lubricated digital thermometer (YSI Precision ™ 4000A) inserted 3-4 cm deep into the rectum of the rats. The rectal temperature of normal rats was also measured at 1 h intervals for 7 h. The control arm of the experiment involved animals treated with 2% Tween 80 solution plus LPS. All experiments were carried out between 08.00 h and 18.00 h in a quiet laboratory with an ambient temperature of 25 ± 2ºC.

Yeast-induced pyrexia

Fifty-six male Wistar rats (140-180 g) were randomly divided into 7 groups and fasted overnight before the experiment with free access to water. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. Fever was induced according to the method described by Smith and Hambourger[4]. The animals were trained to remain quiet in a broom-style restrainer for 30 min before treatment. A lubricated thermometer probe was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. Temperature was measured on digital thermometer (YSI Precision™ 4000A). After measuring the basal rectal temperature, animals were injected subcutaneously with 10 ml/kg of 20% w/v brewer's yeast in NSS in the dorsum of the rats. Rats were then returned to their housing cages.

Eighteen hours after brewer's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only rats that showed an increase in temperature of at least 1°C were used for this study. The EFR at the doses of 50, 100, 200 and 400 mg/kg were administered orally to four groups of animals. The control group received an equivalent volume of vehicle (2% Tween 80 solution) and the positive-control group received acetylsalicylic acid (ASA, 300 mg/kg) orally. Rectal temperature was measured at 1h intervals for 7 h after the extract/drug administration. The rectal temperature of normal rats (normothermic rats) was also measured at 1 h intervals for 7 h. The results are expressed as percentage of the pre-drug

temperature recorded for the same animals using the formula of Makonnan *et al.*[5].

$$\% \text{ Reduction} = \frac{\text{yeast-induced pyrexia} - \text{post treatment temperature}}{\text{yeast-induced pyrexia}} \times 100$$

Analysis of Data

The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test for multiple comparisons. Statistical significance was assessed as $p<0.05$.

RESULTS:

Lipopolysaccharide-induced fever

Lipopolysaccharide (LPS; 50 µg/kg) injected intramuscularly produced a time-dependent increase in rectal temperature in vehicle pretreated rats starting from 1 h and this effect was maintained for 7 h after LPS injection. The maximum increase in rectal temperature was reached at 4 h (1.84°C) giving the maximum mean rectal temperature of 38.44 ± 0.15°C after which there was a decrease (Figure 1). During the same period, the maximum mean rectal temperature of normothermic rats was 36.89 ± 0.03°C. Thus, LPS significantly ($p<0.001$) increased the rectal temperature.

ASA 300 mg/kg significantly ($p<0.001$) prevented the increase in rectal temperature produced by LPS over a period of 7 h with a maximum reduction at 4 h. The maximum mean rectal temperature in the presence of ASA was 36.81 ± 0.10°C. All doses of the ethanolic extract of *F. racemosa* root (EFR; 50, 100, 200 and 400 mg/kg) also significantly prevented the increase in rectal temperature produced by LPS ($p<0.001$) with a maximum reduction at 5, 3, 7 and 5 h, respectively. The antipyretic effect of EFR was noted as early as 1 h and the effect was maintained for the full 7 h after LPS injection. The maximum mean rectal temperature produced by LPS in the presence of 50, 100, 200 and 400 mg/kg of the EFR were 36.99 ± 0.16°C, 36.94 ± 0.14°C, 36.89 ± 0.12°C and 37.04 ± 0.18°C, respectively. All doses of the EFR used in this study were found to be as potent as ASA (Figure 1).

Yeast-induced pyrexia

Results of the antipyretic effect of the EFR are presented in Figure 2. Eighteen hours after subcutaneous injection of brewer's yeast, a significant increase in rectal temperature by 2.36 ± 0.14°C was observed in control animals ($p<0.001$) producing a mean rectal temperature of 38.92 ± 0.18°C. The mean rectal temperature of

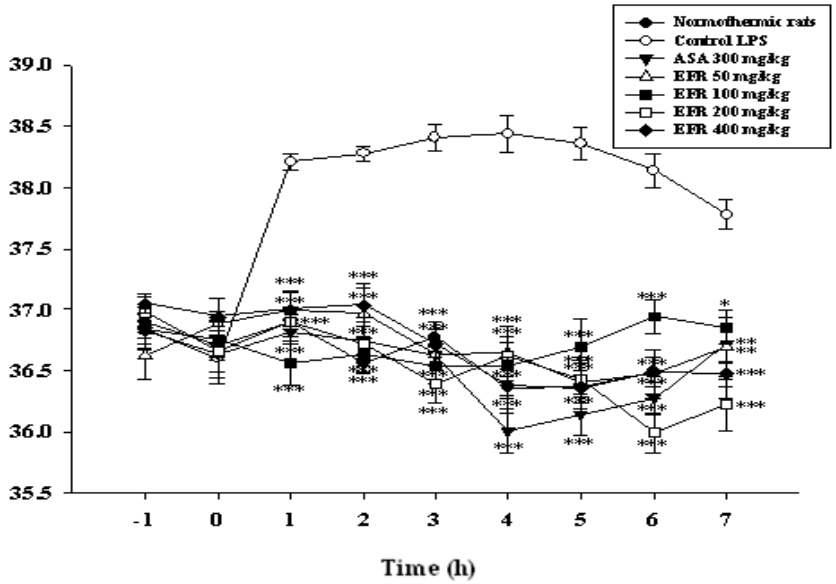


Figure 1 Lipopolysaccharide-induced fever. Changes in rectal temperature after oral administration of 2% Tween 80 (control LPS), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *F. racemosa* root (EFR; 50-400 mg/kg) to febrile rats. Fever was induced by intramuscular injection of lipopolysaccharide (LPS; 50 µg/ml) at 0 h. All drugs were administered 1 h prior to LPS, n = 8 for all groups. *$p<0.05$, **$p<0.01$, ***$p<0.001$ significantly different compared to Control LPS animals.

saline-injected normal rats was 36.59±0.15°C demonstrating that no febrile responses occurred. Treatment with ASA 300 mg/kg significantly (p<0.001) reduced fever induced by brewer's yeast at 1 h after oral administration by approximately 2.09°C, and decreased the rectal temperature of rats at all time tested. Rectal temperature of the ASA group was reduced to normal temperatures within 1 h after ASA administration.

Treatment with EFR at 50 mg/kg decreased rectal temperature significantly (p<0.05, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.01, respectively) at 1, 2, 3, 4, 5, 6 and 7 h after oral administration. Treatment with EFR 100 mg/kg significantly (p<0.01, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.01, respectively) decreased rectal temperature at 1, 2, 3, 4, 5, 6 and 7 h after administration. Similarly, treatment with EFR at the doses of 200 and 400 mg/kg significantly (p<0.001) decreased the rectal temperature at all time tested. The antipyretic effects of all doses of the EFR were noted as early as 1 h and the effect was maintained for the full 7 h after oral administration. Rectal temperature of EFR groups at the doses of 50, 100, 200 and 400 mg/kg was reduced to normal temperature at 6-7 h after oral administration (Figure 2).

The percent reduction in fever by ASA at 1, 2, 3, 4, 5, 6 and 7 h after oral treatment were 5.37%, 5.71%, 5.93%, 5.32%, 5.68%, 5.69% and 5.92%, respectively. Treatment with ASA demonstrated the maximum % reduction of fever at 3 h after oral administration. The maximum % reduction in fever after oral treatment with EFR at the doses of 50, 100, 200 and 400 mg/kg were 4.68%, 5.01%, 6.14% and 5.69%, respectively. The maximum antipyretic effects of EFR were observed at 6-7 h after oral administration. Treatment with EFR at the doses of 200 and 400 mg/kg was equally potent to ASA (Figure 3).

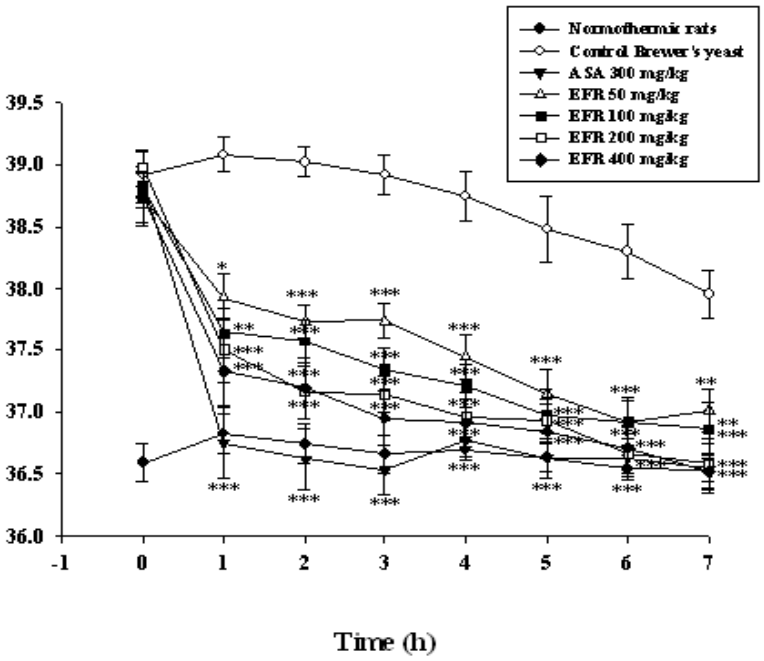


Figure 2 Brewer's yeast-induced pyrexia. Changes in rectal temperature after oral administration of 2% Tween 80 (Control Brewer's yeast), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *F. racemosa* root (EFR; 50-400 mg/kg) to rats with pyrexia. Pyrexia was induced by subcutaneous injection of 20% brewer's yeast (10 ml/kg) and 18 h later the rats were treated as above. All drugs were administered at 0 h, n = 8 for all groups. *p<0.05, **p<0.01, ***p<0.001 significantly different compared to control Brewer's yeast animals.

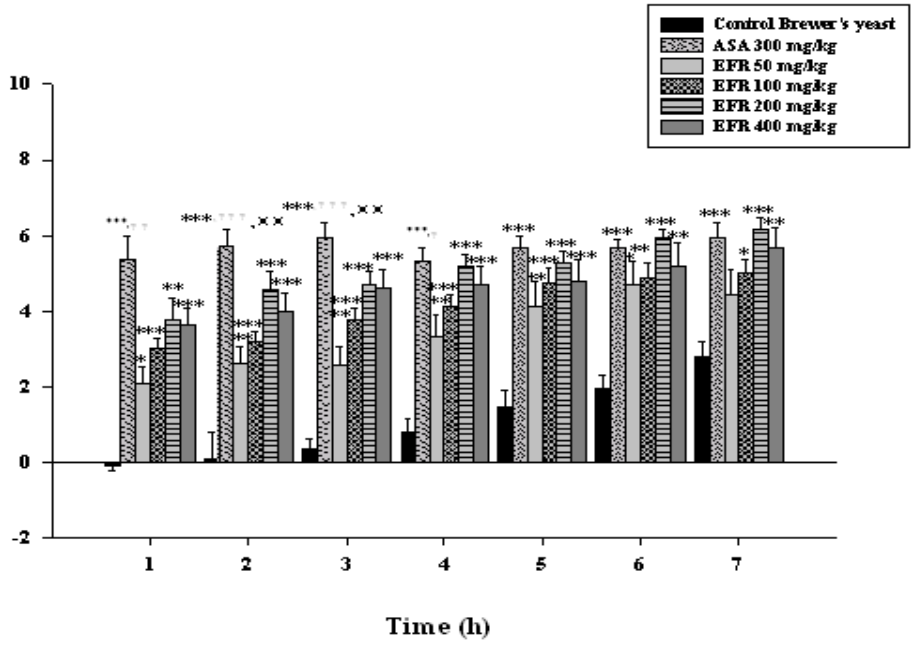


Figure 3. Percent reduction in fever. Percent reduction in fever after oral administration of 2% Tween 80 (Control Brewer's yeast), acetylsalicylic acid (ASA; 300 mg/kg) and various doses of the ethanolic extract of *F. racemosa* root (EFR; 50-400 mg/kg), n=8 for all groups. **p*<0.05, ***p*<0.01, ***p <0.001 significantly different compared to 2% Tween 80 (Control Brewer's yeast). ▲*p*<0.05, ▲▲*p*< 0.01, ▲▲▲*p*< 0.001 significantly different compared to EFR 50 mg/kg. ✖✖*p*< 0.01 significantly different compared to EFR 100 mg/kg.

DISCUSSION AND CONCLUSION:

Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins[6]. LPS has been shown to produce fever in laboratory animals such as guinea pigs and rabbits by stimulating the production of endogenous TNF-α[6, 7]. Prostaglandin synthesis can be activated by TNF-α or phospholipase A_2[8]. Brewer's yeast induces both TNF-α and prostaglandin synthesis[6].

For better characterization of the antipyretic activity of the EFR, two models of pyresis including LPS-induced and brewer's yeast-induced fever were employed in this study. Antipyretics such as ASA and other nonsteroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β, while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E_2 (PGE_2)[8].

In the LPS-induced fever model, orally administered ASA, the positive control, significantly attenuated fever in LPS treated rats at all time tested likely due to the inhibition of cyclooxygenase and therefore interference with the cascade of the synthesis of prostaglandin which induces fever (Figure 1). The oral administration was chosen in order to imitate the normal human consumption of 'Ben-Cha-Lo-Ka-Wi-Chian', an antipyretic herbal formula. All doses of the EFR showed antipyretic activity in LPS-induced fever model of rats at all times tested. Additional studies are needed to determine if the antipyretic effects of EFR are due to suppression of TNF-α and inhibition of TNF-α and prostaglandin synthesis. The antipyretic effect of all doses of EFR occurred shortly within 1 h after LPS injection and was sustained for up to 7 h, similar to that seen with ASA treatment (Figure 1). The efficacy of all doses of EFR used was comparable to that of ASA.

All doses of the EFR also significantly attenuated the fever induced by brewer's yeast.

Additional studies are needed to determine if the antipyretic mechanism of the EFR occurred through inhibition of both TNF-α and prostaglandin synthesis. The onset of action of all doses of the EFR started within 1 h after oral administration with duration of action of 7 h, similar to that for ASA (Figure 2). The high doses of EFR (200 and 400 mg/kg) had comparable efficacy to that of ASA. The efficacy of all doses of the EFR increased with time (Figure 3).

In this study brewer's yeast induced increases in rectal temperature in rats by approximately 2.24°C, while LPS increased the rectal temperature by approximately 1.84°C. Therefore, the lower doses of EFR (50 and 100 mg/kg) had less efficacy in reducing rectal temperature in the brewer's yeast-induced pyrexia model compared to the LPS-induced fever model. It can be concluded that the EFR at all doses tested demonstrated antipyretic effect. Additional studies are required to determine if their mechanism of action involves the inhibition of TNF-α and prostaglandin synthesis.

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