# Effect of *Thunbergia laurifolia* Lindl. Extract on Anti-Inflammatory, Analgesic and Antipyretic Activity

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**Background:** Thunbergia laurifolia Lindl. belongs to the family Acanthaceae commonly known as Rang jeud in Thailand. This plant is traditionally used in Thailand for centuries as an antidote for several poisons and drug overdose. **Objective:** This research aimed to study the anti-inflammatory, analgesic and antipyretic activities of T. laurifolia water extract by using animal models.

*Material and Method:* The anti-inflammatory study was conducted by 3 experiments in animal models namely ethyl phenylpropiolate (EPP)-induced ear edema, carrageenan or arachidonic acid-induced paw edema and cotton pellet-induced granuloma formation. The analgesic activity was studies using 2 methods of pain induction including acetic acid and heat induced pain. Finally, the antipyretic activity study was performed by yeast-induced hyperthermia.

**Results:** The results showed that the administration of T. laurifolia extract possessed acute anti-inflammatory effects in EPPinduced ear edema and carrageenan or arachidonic acid-induced paw edema. The extract showed the analgesic activity by reducing acetic acid-induced writhing response and heat-induced pain as well as showed antipyretic activity by decreasing body temperature of hyperthermic rats induced by brewer's yeast.

**Conclusion:** The study indicates that the T. laurifolia extract possesses the anti-inflammatory, analgesic and antipyretic activities in animals.

Keywords: Thunbergia laurifolia extract, Anti-inflammatory activity, Analgesic activity, Antipyretic activity

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*Thunbergia laurifolia* Lindl. belongs to the family Acanthaceae commonly known as Rang jeud in Thailand. The leaves, barks and roots are used as an antidote for insecticide, drug, arsenic, strychnine and alcohol, and for treating food poisoning and chemical toxic.

The pharmacological activity of *Thunbergia laurifoliac* Lindl. has been reported for several treatments. These are the decreases of blood glucose level in diabetic rats<sup>(1)</sup>, antimutagenic<sup>(2)</sup>, antimicrobial<sup>(3)</sup>,

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hepatoprotective<sup>(4)</sup>, antinociceptive and antiinflammatory<sup>(5)</sup>, antioxidant activities<sup>(6,7)</sup> and detoxifying<sup>(8)</sup>. The aim of this study was to evaluate the anti-inflammatory, analgesic and antipyretic activities of *T. laurifolia* leave extract in animal models.

## **Material and Method**

## Animals and ethical approval

Male ICR albino mice (30 to 40 g) and male Sprague Dawley (SD) rats (40 to 60 g, 100 to 120 g, and 200 to 220 g) were used in this study. The animals were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The animals were acclimatized in the house for 7 days before the start of experiments. The house was a temperature-controlled room  $(25\pm1^{\circ}C)$  and 12 h light-

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dark cycle. The animals were provided with standardized pelleted feed and water given ad libitum. The protocol of this study was approved by the Animal Ethics Committee of Faculty of Medicine, Thammasat University, PathumThani, Thailand (AE 012/2014).

## Plant materials and extracts

*Thunbergia laurifolia* dry leaves (50 kg) were boiled in water (500 L) for 2 h and filtered. The residue from the filtration was boiled (100°C) and filtered again with the same procedure. The collective filtrate was spray dried. The quality of dry extract was controlled by using parameters according to Thai herbal pharmacopoeia such as the physical appearance, and the percentage of loss on drying. The quantity of total phenolic compounds and flavonoids contents were assessed by using chromatographic fingerprints<sup>(9)</sup>.

# *Ethyl phenylpropiolate (EPP)-induced ear edema in* $rats^{(10)}$

Male SD rats (40 to 60 g) were divided into 3 groups of 3 animals each. The ear edema was induced by topical application of EPP (1 mg/20 mL/ear) to the inner and outer surface of both ears by using an automatic microliter pipet. *T. laurifolia* extract (0.5, 1, 2, 4 mg/ear), phenylbutazone (1 mg/ear), and vehicle (acetone) were applied in the same manner and with the equal volume of 20 mL just before EPP application. The edema thickness was measured by using the vernier caliper at 0, 15, 30, 60, and 120 min after EPP induction.

## Carrageenan-induced paw edema in rats<sup>(11)</sup>

Male SD rats (100 to 120 g) were divided into 5 groups (n = 6). *T. laurifolia* extract (300, 600, and 1,200 mg/kg), aspirin (300 mg/kg) and distilled water (5 mL/kg) were orally given 1 h prior to paw edema induction. Acute inflammation as paw edema was induced by intradermally injection of 0.05 ml of 1% carrageenan (in sterile normal saline solution, NSS) into the plantar surface of the right hind paw of an unanesthetized rat. The volume of paw was measured by means of volume displacement technique using the plethysmometer (model 7140, Ugo Basile, Italy) before the injection and at 1, 3 and 5 hours after carrageenan injection.

# Arachidonic acid (AA)-induced paw edema in rats $^{\left( 12\right) }$

Male SD rats (100 to 120 g) were divided into 3 groups (n = 6). *T. laurifolia* extract (1,200 mg/kg), phenidone (5 mg/kg) and distilled water (5 mL/kg) were

orally given 1 h prior to paw edema induction. The paw edema was induced by injecting 0.1 mL of 0.5% AA in 0.2 M carbonate buffer (pH 8.4) intradermally into the plantar surface of the right hind paw. The paw volume was determined by means of a volume displacement technique using the plethysmometer (model 7150, Ugo Basile, Italy) before and at 1 h after AA injection.

## Cotton pellet-induced granuloma formation<sup>(13)</sup>

Male SD rats (180 to 200 g) were divided into 4 groups of 6 animals each. Leaving a normal group, the other 3 were subject to cotton pellet-induced granuloma formation. T. laurifolia extract (1,200 mg/ kg), aspirin (300 mg/kg), prednisolone (5 mg/kg) and distilled water (5 mL/kg) as a control group, were orally given to each of the 3 groups 1 h prior to chronic inflammatory induction. The abdominal skin of rat was shaved and disinfected with 70% alcohol. Then, two sterile pellets  $(20\pm1 \text{ mg})$  were implanted subcutaneously, one on each side of an abdomen in all groups except in the normal group, under light ether anesthesia and sterile technique. The suture was made after that and the animal was allowed to recover. Each test substance was administered three times daily for 7 days. On the 8th day after cotton pellet implantation, all rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). Blood was collected by cardiac puncture technique for determination of the ALP and total protein. The implanted cotton pellets were removed and weighed for the wet weight, then dried at 60°C for 18 h and weighed for the dry weight. The granuloma weight, transudative weight, and percentage of granuloma inhibition were calculated. The chest was opened and the thymus was cut out and weighed immediately for the wet weight. Then it was dried at 60°C for 18 h and weighed again for its dry weight.

# Acetic acid-induced writhing test in mice<sup>(14,15)</sup>

Male ICR mice (30 to 40 g) were divided into 5 groups (n = 6). *T. laurifolia* extract (300, 600, 1,200 mg/kg), aspirin (300 mg/kg) and distilled water (5 mL/kg) were orally given 1 h prior to 0.75% acetic acid injection in a volume of 0.1 mL/10 g body weight into the peritoneal cavity. The animals were then kept in transparent cage individually for observation of the number of writhes (the response consisting of contraction of an abdominal wall, pelvic rotation followed by hind limb extension). The number of writhes was counted during continuous observation for 15 min beginning from 5 min after acetic acid injection.

## Tail-flick test in rats<sup>(16)</sup>

Male SD rats (180 to 200 g) were divided into 6 groups (n = 6). T. arjuna extract, aspirin and codeine were prepared by dissolving in distilled water. T. laurifolia extract (300, 600, 1,200 mg/kg body weight), aspirin (300 mg/kg body weight), codeine (120 mg/kg body weight) and distilled water (5 mL/kg body weight) were orally given 1 h prior to placing the tail of rat (3 cm from tip) on a flush mounted photo cell window of the tail-flick apparatus (model 7360, Ugo Basile, Italy) with infrared lamp (50 W bulb). When the rat felt pain and moved (flicked) its tail away from the heat, then the reaction time was presented on a digital display. The voltage was adjusted to give a normal reaction time of 2 to 4 sec. The cut-off time of 10 sec was a maximum time for the rat that did not move its tail away from the heat to avoid tissue damage. The reaction time was determined before and at 1, 2, and 3 h after each test substance administration.

## Yeast-induced hyperthermia<sup>(17)</sup>

Male SD rats (200 to 250 g) were divided into 5 groups (n = 6). The rectal temperature ( $^{\circ}$ C) was measured by using the twelve-channel electrical thermometer (LETICA, model TMP 812 RS, Panlab SL, Spain). The basal rectal temperature was recorded at 1 h after probe insertion. The hyperthermia was induced by subcutaneous injection of 25% yeast in sterile NSS in a volume of 1 mL/100 g body weight. At 18 h after yeast injection, the rectal temperature was measured again and those rats that showed rises in rectal temperature >1°C were used. T. arjuna extract and aspirin were prepared by dissolving in distilled water. T. laurifolia extract (300, 600, and 1,200 mg/kg body weight), aspirin (300 mg/kg body weight) and distilled water (5 mL/kg body weight) were or ally given and the rectal temperatures were then recorded at 30, 60, 90, 120, and 180 min.

## Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA), Dunnett test and Student's t-test. The p-values less than 0.05 were considered significant.

#### Results

The percentage yield of T. laurifolia extract was 29.20% weight of raw materials. The values of quality control and quantity of chemical compounds of T. laurifolia extract were remained within the normal ranges (Table 1).

In EPP-induced ear edema model, T. laurifolia extract at the doses of 0.5, 1, 2 and 4 mg/ear and

| Physical appearance  | The color of the powder is brown  |
|--|---|
|  |   |
| <ul> <li>% Loss on drying</li> <li>Solubility</li> <li>Total phenolic content (% g GAE<sup>1</sup>)</li> <li>Total flavonoid content (% g QAE<sup>2</sup>)</li> <li>% Caffeic acid (HPLC)</li> <li>% Cosmarinic acid (HPLC)</li> </ul> | <ul><li>5.98</li><li>Freely soluble in water slightly soluble in 95% ethanol</li><li>18.51</li><li>3.29</li><li>0.14</li><li>0.24</li></ul> |

<sup>1</sup>Gram gallic acid equivalent per 100 gram dried extract, <sup>2</sup>Gram quercetin equivalent per 100 gram dried extract

Table 1. Monograph of T. laurifolia extract

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phenylbutazone at the dose of 1 mg/ear showed significant inhibitory activity on the ear edema formation at all time-points (Table 2).

In carrageenan-induced paw edema model, aspirin (300 mg/kg) and *T. laurifolia* extract (600 and 1,200 mg/kg) could significantly inhibit paw edema at all time-points (Table 3).

In arachidonic acid–induced paw edema model, phenidone (5 mg/kg) and *T. laurifolia* extract (1,200 mg/kg) could significantly inhibit paw edema at assessment time (Table 4).

In cotton pellet-induced granuloma formation model, *T. laurifolia* extract (1,200 mg/kg) could not reduce transudative weight and granuloma formation (Table 5). The group treated with aspirin (300 mg/kg) and prednisolone (the steroidal anti-inflammatory drug) at the dose of 5 mg/kg showed a marked inhibition on both parameters (21.21% and 40.15% glanuloma inhibition, respectively). Moreover, both *T. laurifolia* extract and aspirin did not affect the body weight gain, thymus weight of animals and alkaline phosphatase activity. On the contrary, prednisolone significantly reduced those parameters (Table 6 and 7).

In the writhing response test, aspirin (300 mg/kg) and *T. laurifolia* extract (300, 600 and 1,200 mg/kg) possessed inhibitory effect on writhing response (Table 8).

In tail flick test, aspirin (300 mg/kg) and codeine (120 mg/kg) could significantly increase test reaction time at 1, 2 and 3 hour. *T. laurifolia* extract at the doses of 1,200 mg/kg could significantly increase test reaction time at 3 hour (Table 9).

In yeast-induced hyperthermia, aspirin (300 mg/kg) could significantly reduce hyperthermia at all time-points. *T. laurifolia* extract, at the dose of 300, 600 and 1,200 mg/kg, could significantly reduce hyperthermia at 120 min (Table 10).

#### Discussion

Anti-inflammatory activity was studied in

| Table 2. | Effect of 7 | T. laurifolia | extract on | EPP-induced | ear edema in rats |
|----------|-------------|---------------|------------|-------------|-------------------|
|----------|-------------|---------------|------------|-------------|-------------------|

|                             | Time                  | Time after topical application of EPP/ear edema (mm) |                       |                      |  |  |
|-----------------------------|-----------------------|--|-----------------------|----------------------|--|--|
|                             | 15 min                | 30 min   | 1 h                   | 2 h                  |  |  |
| Control (Acetone) 20 ml/ear | 128.33 <u>+</u> 16.62 | 170.00 <u>+</u> 16.93                                | 203.33 <u>+</u> 11.74 | 193.33 <u>+</u> 8.43 |  |  |
| Phenylbutazone 1 mg/ear     | 43.33+5.58*           | 76.67 <u>+</u> 2.11*                                 | 80.00 <u>+</u> 3.65*  | 76.67 <u>+</u> 2.11* |  |  |
| T. laurifolia 0.5 mg/ear    | 88.33 <u>+</u> 1.67*  | 106.67 <u>+</u> 8.43*                                | 113.33±6.15*          | 105.00±5.00*         |  |  |
| 1.0 mg/ear                  | 73.33 <u>+</u> 4.22*  | 90.00±3.65*  | 113.33±5.58*          | 105.00±5.00*         |  |  |
| 2.0 mg/ear                  | 66.67 <u>+</u> 7.60*  | 86.67 <u>+</u> 7.60*                                 | 98.33 <u>+</u> 3.07*  | 93.33 <u>+</u> 4.22* |  |  |
| 4.0 mg/ear                  | 58.33 <u>+</u> 4.01*  | 81.67 <u>+</u> 3.07*                                 | 95.00 <u>+</u> 5.63*  | 88.33 <u>+</u> 3.07* |  |  |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p < 0.05

| Table 3. | Effect of T. | <i>laurifolia</i> extract | on carrageenan-inc | luced | hind | paw ed | lemain rats |
|----------|--------------|---------------------------|--------------------|-------|------|--------|-------------|
|----------|--------------|---------------------------|--------------------|-------|------|--------|-------------|

|   |  | Edema volume (mL)   |  |
|---|--|---|--|
|   | 1 h  | 3 h   | 5 h  |
| Control (distilled water)<br>Aspirin 300 mg/kg<br>T. Jaurifolia | $\begin{array}{c} 0.35{\pm}0.03 \\ 0.14{\pm}0.01{*} \end{array}$                   | $\begin{array}{c} 0.78 \pm 0.04 \\ 0.23 \pm 0.02 * \end{array}$ | $\begin{array}{c} 0.76{\pm}0.05\\ 0.27{\pm}0.02{*} \end{array}$                      |
| 300 mg/kg<br>600 mg/kg<br>1,200 mg/kg                           | $\begin{array}{c} 0.30{\pm}0.06\\ 0.24{\pm}0.04{*}\\ 0.23{\pm}0.03{*} \end{array}$ | $0.61\pm0.04*$<br>$0.54\pm0.05*$<br>$0.48\pm0.04*$              | $\begin{array}{c} 0.45{\pm}0.04{*}\\ 0.44{\pm}0.04{*}\\ 0.43{\pm}0.04{*}\end{array}$ |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p < 0.05

| Group                     | Dose (mg/kg) | Edema volume (ml)    |
|---------------------------|--------------|----------------------|
| Control (distilled water) | -            | 27.17 <u>+</u> 2.44  |
| Phenidone                 | 5            | 13.22 <u>+</u> 1.66* |
| T. laurifolia extract     | 1,200        | 19.11 <u>+</u> 2.18* |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p<0.05

| Group  | Granuloma<br>wet weight<br>(mg)   | Granuloma<br>dry weight<br>(mg)                                  | Transudative<br>weight<br>(mg)                                       | Granuloma<br>weight<br>(mg/mg cotton)  | GI<br>(%)                |
|--|---|--|--|--|--------------------------|
| Control (distilled water)<br>Prednisolone 5 mg/kg<br>Aspirin 300 mg/kg<br><i>T. laurifolia</i> 1,200 mg/kg | $\begin{array}{c} 354.92{\pm}18.08\\ 233.88{\pm}11.76{*}\\ 275.08{\pm}8.55{*}\\ 362.38{\pm}17.63 \end{array}$ | $72.74\pm2.74 \\ 51.68\pm1.51* \\ 61.55\pm1.69* \\ 74.03\pm2.84$ | $282.18\pm16.04 \\182.19\pm10.60* \\213.53\pm7.49* \\288.36\pm14.90$ | $\begin{array}{c} 2.64{\pm}0.14\\ 1.58{\pm}0.08{*}\\ 2.08{\pm}0.08{*}\\ 2.70{\pm}0.14 \end{array}$ | -<br>40.15<br>21.21<br>0 |

Values are expressed as mean  $\pm$  SEM (n = 6). GI = Granuloma inhibition \* Statistically significant difference from the control group, *p*<0.05

| <b>Table 0.</b> Effect of <i>I. tuur fottu</i> extract offoory weight in cotton penet-induced granuformation in |
|---|
|---|

| Group                     | Body we              | Body weight (g)       |                      |
|---------------------------|----------------------|-----------------------|----------------------|
|                           | Initial              | Final                 | weight (mg/100g)     |
| Control (distilled water) | 180.00 <u>+</u> 0.00 | 201.67 <u>+</u> 1.67  | 26.37 <u>+</u> 1.56  |
| Prednisolone 5 mg/kg      | 185.00 <u>+</u> 2.24 | 190.00 <u>+</u> 2.58* | 10.55 <u>+</u> 0.47* |
| Aspirin 300 mg/kg         | 183.33 <u>+</u> 3.33 | 198.33 <u>+</u> 3.07  | 22.82 <u>+</u> 0.34  |
| T. laurifolia 1,200 mg/kg | 185.00 <u>+</u> 3.42 | 205.00 <u>+</u> 5.00  | 27.62 <u>+</u> 2.16  |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p < 0.05

various animal models including EPP-induced ear edema, carrageenan or arachidonic acid-induced paw edema, cotton pellet-induced granuloma formation.

The model for screening and evaluation the anti-inflammatory activity of the extract is EPP-induced ear edema<sup>(10)</sup>. The inflammatory mediators released in EPP-induced ear edema model are histamine, serotonin, bradykinin and prostaglandins (PGs). These mediators are capable to promote vasodilatation and increase vascular permeability as well as synergistically producing edema<sup>(10,18,19)</sup>. The studies showed that *T. laurifolia* extract exhibited inhibitory effect on ear edema formation. The result indicates that *T. laurifolia* 

extract possesses an anti-inflammatory activity in the acute phase of inflammation via inhibition of the release and/or formation of inflammatory mediators involved in edema formation.

Carrageenan-induced paw edema model is useful to detect active anti-inflammatory agents, especially COX inhibitors<sup>(11)</sup>. Several inflammatory mediators, such as histamine, serotonin, kinins, PGs, complement, and pro-inflammatory cytokines play a major role in paw edema caused by carrageenan<sup>(20,21)</sup>. The initial phase (0 to 2.5 h after carrageenan injection) is caused by the release of histamine, serotonin, and kinins, whereas the second phase (2.5 to 6 h) is

| Table 7. | Effect of T. laurifolia extract of | on alkaline phosphatase ac | tivity in cotton r | pellet-induced granuloma | a formation in rats |
|----------|------------------------------------|----------------------------|--------------------|--------------------------|---------------------|
|          | 2                                  | 1 1                        | 2 1                |                          |                     |

| Group                     | Alkaline phosphatase<br>(units/l) | Total protein<br>(g/dl) | Serum alkaline phosphatase<br>activity<br>(U of enz./mg of serum<br>protein x 10) |
|---------------------------|-----------------------------------|-------------------------|---|
| Normal                    | 95.00+7.24                        | 5.53+0.11               | 17.13+1.15  |
| Control                   | $112.50 \pm 7.21$                 | $5.45 \pm 0.08$         | $20.68 \pm 1.40^{a}$  |
| Prednisolone 5 mg/kg      | $106.83 \pm 5.68$                 | 6.33 <u>+</u> 0.06      | $16.86 \pm 0.86^{\text{b}}$   |
| Aspirin 300 mg/kg         | 141.67 <u>+</u> 4.56              | 5.12 <u>+</u> 0.06      | 27.70 <u>+</u> 0.89   |
| T. laurifolia 1,200 mg/kg | 126.67 <u>+</u> 9.67              | 5.52 <u>+</u> 0.16      | 22.87 <u>+</u> 1.34   |

Values are expressed as mean  $\pm$  SEM (n = 6)

 $^{\rm a}$  Statistically significant difference from the normal group,  $*p{<}0.05$ 

 $^{\rm b}$  Statistically significant difference from the control group, \*p<0.05

| T 11 0    | $\mathbf{T}^{\mathbf{C}\mathbf{C}}$ $(\mathbf{C}^{\mathbf{T}\mathbf{C}}1)$ $(\mathbf{C}^{\mathbf{T}\mathbf{C}}1)$ |                 |            | · 1 1      | ·             | •          | •     |
|-----------|---|-----------------|------------|------------|---------------|------------|-------|
| Table X.  | Effect of L lauritol  | a extract on ac | efic acid- | -induced v | writhing resi | oonse in   | mice. |
| I HOIC OF | Liter of 1. with you  | a charact on ac | otre aera  | maacca     | " maning resp | Joinse III | mee   |

| Group         | Dose (mg/kg) | Number of writhes | % inhibition |  |
|---------------|--------------|-------------------|--------------|--|
| Control       | _            | 21.83+1.08        | _            |  |
| Aspirin       | 300          | 4.83+0.60*        | 77.86        |  |
| T. laurifolia | 300          | 16.33+1.48*       | 25.19        |  |
| 0             | 600          | 12.33+2.19*       | 43.51        |  |
|               | 1,200        | $11.83 \pm 1.99*$ | 45.80        |  |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p < 0.05

| Table 9. | Effect of T. | laurifolia | extract on | tail flic | c test in rats |
|----------|--------------|------------|------------|-----------|----------------|
|----------|--------------|------------|------------|-----------|----------------|

| Group                 |                 | Reaction time (sec) |                    |                     |  |  |
|-----------------------|-----------------|---------------------|--------------------|---------------------|--|--|
|                       | Baseline        | 1 h                 | 2 h                | 3 h                 |  |  |
| Control               | 2.65+0.28       | 3.32+0.48           | 3.21+0.37          | 3.08+0.25           |  |  |
| Aspirin 300 mg/kg     | $2.73 \pm 0.24$ | $4.23\pm0.16*$      | $4.38\pm0.29*$     | $4.21 \pm 0.31^{*}$ |  |  |
| Codeine 120 mg/kg     | $2.85\pm0.21*$  | $6.61\pm0.37*$      | $6.09\pm0.30*$     | $7.04\pm0.42*$      |  |  |
| T. laurifolia extract | $2.78 \pm 0.26$ | $3.43\pm0.18$       | 3.61 <u>+</u> 3.43 | $0.20 \pm 0.23$     |  |  |
| 300 mg/kg             | $2.81 \pm 0.18$ | $3.45\pm0.11$       | $3.59\pm0.27$      | $3.04\pm0.17$       |  |  |
| 600 mg/kg             | $2.74 \pm 0.04$ | 4.08 <u>+</u> 0.25  | 3.99 <u>+</u> 0.33 | 4.29 <u>+</u> 0.19* |  |  |
| 1,200 mg/kg           | $2.65 \pm 0.28$ | 3.32 <u>+</u> 0.48  | 3.21 <u>+</u> 0.37 | 3.08 <u>+</u> 0.25  |  |  |

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p<0.05

correlated with prostaglandins (PGs), oxygen-derived free radicals, and the local neutrophil infiltration<sup>(22-25)</sup>. In this study, the *T. laurifolia* extract possessed antiinflammatory activity at both phases. Thus, *T. laurifolia* extract could act via the inhibition of inflammatory cytokines releasing and could possibly inhibiting PGs biosynthesis as well.

Arachidonic acid-induced paw edema model was used to evaluate the anti-inflammatory activity of the substance in acute inflammation via LOX-inhibitors<sup>(12)</sup>. The study showed that *T. laurifolia* extract could also inhibit paw edema formation in this

| Group                                 | 18 h after yeast                                   | Rectal temperature (°C)   |  |   |   |  |
|---------------------------------------|--|---|--|---|---|--|
|                                       | injection  | 30 min  | 60 min   | 90 min  | 120 min   |  |
| Control<br>Aspirin                    | 38.60 <u>+</u> 0.12                                | 38.62 <u>+</u> 0.15   | 38.48 <u>+</u> 0.17                                | 38.42 <u>+</u> 0.14   | 38.47 <u>+</u> 0.16   |  |
| 300 mg/kg<br><i>T. laurifolia</i>     | 38.75 <u>+</u> 0.20                                | 38.20 <u>+</u> 0.14*  | 37.90 <u>+</u> 0.15*                               | 37.52 <u>+</u> 0.10*  | 37.47 <u>+</u> 0.08*  |  |
| 300 mg/kg<br>600 mg/kg<br>1,200 mg/kg | $38.68\pm0.18$<br>$38.65\pm0.17$<br>$38.63\pm0.14$ | $\begin{array}{c} 38.50 \pm 0.21 \\ 38.48 \pm 0.18 \\ 38.50 \pm 0.21 \end{array}$ | $38.28\pm0.20$<br>$38.20\pm0.16$<br>$38.13\pm0.20$ | $\begin{array}{c} 38.10 \pm 0.19 \\ 38.07 \pm 0.13 \\ 37.98 \pm 0.16 \end{array}$ | $\begin{array}{c} 38.00 \pm 0.18 * \\ 38.02 \pm 0.14 * \\ 37.82 \pm 0.17 * \end{array}$ |  |

 Table 10.
 Effect of T. laurifolia extract on yeast-induced hyperthermia in rats

Values are expressed as mean  $\pm$  SEM (n = 6)

\* Statistically significant difference from the control group, p < 0.05

model. Thus, *T. laurifolia* extract may be exhibit antiinflammatory activity via LOX-inhibition.

Cotton pellet-induced granuloma formation was an in vivo test model for chronic inflammation. A subcutaneously implanted cotton pellet has been reported to involve in 3 phases of inflammatory responses, including transudative, exudative and proliferative phases. In these chronic inflammatory responses, there is the persistence of inflammatory cells leading to the release of pro-inflammatory mediators and oxygen-derived free radicals, as well as lysosomal enzymes such asalkaline phosphatase, which causes subsequent tissue injury. The study showed that aspirin as a slightly inhibited all observing signs of chronic inflammation where as steroidal anti-inflammation drugs showed a strong inhibition. The positive effect of the model in this study was similar to the other<sup>(13)</sup>. T. laurifolia extract did not produce any inhibitory effect on both body weight gain and thymus weight in the present study. Moreover, prednisolone could normalize serum alkaline phosphatase activity by stabilizing the lysosomal membrane, whereas T. laurifolia extract and aspirin did not affect this enzyme. The results indicate that T. laurifolia extract does not share steroidal-like activity.

The acetic acid-induced writhing test model was used for evaluation of both centrally and peripherally analgesic activities<sup>(26)</sup>. Since the acid induced the synthesis and release of pro-inflammatory mediators such as bradykinin, serotonin, histamine, PGs, and substance P, which irritated the pain nerve endings or nociceptors<sup>(14,15)</sup>. On the contrary, the tail-flick test model was used to evaluate on the centrally analgesic activity<sup>(16)</sup>. The results showed all three doses of *T. laurifolia* extract orally given to mice exhibited

the analgesic effecton the writhing test whereas the only dose, 600 mg/kg, was able to delay the tail flicking time when rats were orally given the extract 3 h after the initial dose. The results indicated that *T. laurifolia* extract affected both centrally and peripherally analgesic activities. There was also a clue of the prolonged effect on the later test which might emphasize the inhibition of a certain pro-inflammatory cytokine release in rats.

The yeast-induced hyperthermia model was used for the evaluation of antipyretic activity. High body temperature involves the stimulation of the release of local PGs which resets in the hypothalamic thermal set point<sup>(27)</sup>. In the present study, *T. laurifolia* extract (300, 600 and 1,200 mg/kg) showed antipyretic activity by decreasing body temperature of hyperthermic rats induced by brewer's yeast injection at 120 min assessment times. Thus, the antipyretic activity of *T. laurifolia* extract may be due to the inhibition of the synthesis and/or release of local PGE<sub>2</sub> into the hypothalamus.

## Conclusion

The anti-inflammatory effect of *T. laurifolia* extract was evidenced by the significant reduction of acute inflammatory reaction as proved by EPP-induced ear edema and carrageenan or arachidonic acid-induced rat hind paw edema. The results indicate that the anti-inflammatory effect of *T. laurifolia* extract probably mediates via inhibition of COX pathway and partly inhibition of LOX pathway. *T. laurifolia* extract did not reduce transudative and proliferative phases, body weight gain and thymus weight in cotton pellet-induced granuloma formation. Therefore, this finding suggests that anti-inflammatory activity of *T. laurifolia* extract

does not possess steroidal-like effect. The analgesic activity of *T. laurifolia* extract may be via both peripheral acting and partly centrally acting. Antipyretic study showed that *T. laurifolia* extract possessed significant reduction of rectal temperature probably due to its inhibition of PG synthesis/release in hypothalamus.

## What is already known in this topic?

*Thunbergia laurifolia* Lindl. (Rang jeud) leaves, barks and roots have been used to treat in several conditions. It has antidiabetic, antimutagenic, antimicrobia, hepatoprotective, antinociceptive, antioxidant activities and detoxifying.

### What this study adds?

Extract of *T. laurifolia* leaves shows antiinflammatory effect, analgesic effect and anti-pyretic effect. It significantly reduces acute inflammatory reaction as shown by reducing EPP-induced ear edema and carrageenan or arachidonic acid-induced rat hind paw edema. This anti-inflammatory effect probably mediates via inhibition of COX pathway and partly inhibition of LOX pathway. The analgesic activity extract may be via both peripheral acting and partly centrally acting. *T. laurifolia* extract can reduce temperature probably due to its inhibition of PG synthesis/release in hypothalamus.

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#### Potential conflicts of interest

None.

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ผลของสารสกัดรางจืดในการตา้นการอักเสบ ระงับปวดและลดไข้

อุรารัตน แน่นหนา, ณัฏฐกานติ์ จิรัณธนัฐ, กาญจนา ใจจ้อย, ปียนุช โรจน์สง่า, สีวบูรณ์ สิรีรัฐวงศ์

ภูมิหลัง: Thumbergia laurifolia Lindl. อยู่ในวงศ์ Acanthaceae ประเทศไทยเรียกว่า รางจืด ใช้เป็นยาต้านพิษของสารพิษและการใช้ยาเกินขนาด วัตถุประสงค์: เพื่อศึกษาฤทธิ์ต้านอักเสบ ระงับปวด และลดไข้ของสารสกัดน้ำรางจืดในสัตว์ทดลอง

วัสดุและวิธีการ: การศึกษาฤทธิ์ด้านการอักเสบใช้ 3 แบบจำลองการทดลองในสัตว์คือ การเหนี่ยวนำการบวม ของใบหูหนูด้วยสาร ethyl phenylpropiolate (EPP) การเหนี่ยวนำการบวมของอุ้งเท้าด้วยคาร์ราจิแนน หรือกรดอะราซิโดนิ และการฝังกอนสำลีเพื่อเหนี่ยวนำให้เกิด granulation การศึกษาฤทธิ์ระงับปวดใช้ 2 การทดลองในการเหนี่ยวนำให้เกิดการปวดคือการใช้การอะซิติกและความร้อน นอกจากกนี้ยังทำการศึกษา ฤทธิ์ลดไข้โดยการเหนี่ยวนำให้หนูเกิดไข้โดยใช้อีสต์

**ผลการศึกษา:** สารสกัดรางจืดมีฤทธิ์ด้านการอักเสบเฉียบพลันจากผลการทดลองในการเกิดการบวมของใบหูหนูโดยใช้สาร EPP การบวมของอุ้งเท้าหนู จากการเหนี่ยวนำด**้วคารร์ราจิแนนหรือกรดอะราซิโดนิก สารสกัดน้ำราง**จืดยังแสดงฤทธิ์ระงับปวดในการทดลองเหนี่ยวนำความปวดด้วยกรดอะซิติก และความร้อน นอกจากนี้สารสกัดน้ำรางจืดยังมีฤทธิ์ลดไขโดยสามารถลดอุณหภูมิร่างกายของหนูที่มีอุณหภูมิร่างกายสูงจากการเหนี่ยวนำด้วยยีสต์ สรุป: จากผลการวิจัยสามารถสรุปได้ว่า สารสกัดรางจืดมีฤทธิ์ด้านการอักเสบ ระงับปวดและลดไขในสัตว์