

Investigation of *In Vivo* Antipyretic Activity of *Thottea dependens* leaves

Ahmed QU, Radhiyah I, Siti Zaiton MS

Department of Pharmaceutical Chemistry, Kulliyah of Pharmacy, International Islamic University Malaysia (IIUM), 25200 Kuantan, Pahang DM, Malaysia

ABSTRACT

Introduction: Leaves of *Thottea dependens* have been used as folk medicine in Malaysia for the treatment of several conditions involving pain and inflammation with accompanying pyrexia. However, there is no scientific evidence for its effectiveness to treat fever. Hence, the purpose of this study was to evaluate the anti-pyretic activity of methanol (MeOH) and aqueous (Aq) extracts of *T. dependens* leaves in albino mice (ICR strain). **Methods:** Thirty-six albino mice of both sexes, randomized into six groups (n=6) were used. Group I served as the control and received only the vehicle, normal saline. Group II received paracetamol 150 mg/kg body weight (b.w.) orally, groups III and IV received 100 and 200 mg/kg b.w. of the MeOH extract while groups V and VI received 100 and 200 mg/kg b.w. of the Aq extract orally. Pyrexia was induced by a subcutaneous injection of 10 mL/kg b.w. of 15% (w/v) yeast suspended in 0.5% (w/v) methylcellulose solution. LD₅₀ of both extracts were determined according to the method of Organisation for Economic Co-operation and Development guidelines. Phytochemical screening was carried out qualitatively for the presence of different class of biologically active compounds. **Results:** LD₅₀ values for both extracts were found to be greater than 1000 mg/kg b.w. indicating the wide margin of safety of *T. dependens* leaves. The MeOH extract at 200 mg/kg b.w. showed statistically significant (P<0.01) dose dependent reduction of brewer's yeast induced pyrexia in albino mice. Flavonoids, saponins and phenolic compounds were found to be major biologically active phytoconstituents in MeOH extract. **Conclusion:** The study showed that *T. dependens* leaves possess significant anti-pyretic activity, thus justifying its ethnomedicinal use in Malaysia to treat fever.

KEYWORDS: *Thottea dependens*, Brewer's yeast induced pyrexia, albino mice

INTRODUCTION

Fever or pyrexia, is a common symptom of many illnesses and occurs in up to 75% of adults who are extremely sick as well as in children. It occurs in 19% to 30% paediatric emergency visits, and these are always associated with great panic among parents.¹ Non-steroidal anti-inflammatory drugs (NSAIDs) are currently the most popular antipyretics drugs which includes acetaminophen, aspirin and celecoxib. However, these drugs have been reported to manifest many harmful side effects. Gastric irritation, ulcers and bleeding of the stomach mucosa may occur following long term exposure to the NSAIDs.^{2,3} In addition, acetaminophen has been reported to show toxicity to the liver at a dose slightly above the therapeutic dose.⁴ Selective cyclooxygenase-2 (COX-2) inhibitors

namely celecoxib, valdecoxib and rofecoxib have been reported to display increased cardiovascular risk along with several other side effects including insomnia, abdominal pain, flatulence, headache, nausea and diarrhoea. Hence, valdecoxib and rofecoxib were removed from the market in 2004 and 2005, respectively, due to their excessively increased risk for strokes and heart attacks with long term use.⁵ In respect of the aforementioned side effects, research on the discovery of new antipyretic agents that are safe, more effective and affordable is highly encouraged.⁶ Medicinal plants found in our environment should be the best solution to tackle this problem with respect to discover new and safe antipyretic agents. Plant based medicines used by the population are generally considered as cheaper alternatives to conventional orthodox medicines.⁷ Herbal medicines are booming in the current era and are regarded as important to the public as well as to the scientific community.⁸

The World Health Organization (WHO) reported that 70% to 80% of the world population depend on mainly on herbal sources for their primary medicines and almost 80% of Asian populations use herbal therapies to maintain their health and fight against many disorders.⁹ According to the WHO, nearly 25% of current drugs prescribed in the United States of America have been derived from different plants. No less than 7,000 medicinal compounds in the modern

Corresponding author:

Qamar Uddin Ahmed, PhD;
Department of Pharmaceutical Chemistry,
Kulliyah of Pharmacy,
International Islamic University Malaysia (IIUM),
25200 Kuantan, Pahang DM, Malaysia;
Email: quahmed@iium.edu.my;
Tel: +609-5714932; Fax: +6-095706775

pharmacopoeia are based on natural products and among the 120 active compounds have currently been isolated from the higher plants and extensively used in modern medicine today, 80% display a affirmative correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.^{10,11} Plant derived medicines have many benefits, being relatively safer and affordable as well as their use for the treatment of various ailments.¹²

One such plant used traditionally in the treatment of conditions involving pyrexia is *Thottea dependens* (Planch.) Klotzsch (or locally known as Telinga Beruang). It is an erect shrub that belongs to the family known as Aristolochiaceae. It is up to 2 m tall and smooth. The leaves are elliptical or obovate in shape, with a size of 12-29 cm x 5-15 cm, acuminate apex, hairless above, hairy beneath and with 2 pair basal veins.

The bark is rubefacient in nature. *T. dependens* is used by Temuan Tribe in Malaysia to treat fever. The leaves are pounded for skin complaints and rubbed behind ear to treat fever.^{13,14} In this study, we set out to investigate the anti-pyretic activity of *T. dependens* with a view to establishing a basis for its use in conditions involving pyrexia of different causes. An extensive search of the literature revealed no scientific reports on the antipyretic activity of this plant. Hence, present investigation was an attempt to scientifically prove the traditional claim of the anti-pyretic effect of *T. dependens* leaves through estimation of its *in vivo* antipyretic effect of methanolic and aqueous extracts of the leaves using mice.

MATERIAL AND METHODS

Collection and identification of plant material

Fresh leaves (7 kg) of *T. dependens* were collected from Tasik Kenyir located in the state of Terengganu, Malaysia and authenticated by Dr. Norazian Hassan (Taxonomist), Kulliyah of Pharmacy, IIUM. The voucher specimen (NMPC-QRI414) has been deposited in the herbarium of KOP for future reference.

Preparation of methanolic (MeOH) and aqueous (Aq) extracts of *T. Dependens* leaves

The leaves (7 kg) were first inspected for deterioration and the presence of foreign materials and then dried in a PROTECH laboratory air dryer (FDD-720-Malaysia) at 40°C for 2 days and powdered through Fritsch Universal Cutting Mill-PULVERISSETTE 19-Germany to get 750 g dried powder. It was then stored in a desiccator at 2 °C until further use.¹⁵

For the preparation of methanolic extract (MeOH), the coarsely grounded dry powder [0.5 kg] was taken and subjected to maceration extraction process for 3 cycles (each 24 h soaking) of methanol extraction at room temperature followed by 4 cycles of heat under reflux (2 h each) over boiling water bath. The extract was filtered and concentrated through rotary evaporator (Buchi Rotary Evaporator, R-210, Switzerland) to yield 40 g (8%) MeOH extract upon freeze drying process.¹⁵

For the preparation of aqueous extract, 0.2 kg powdered (pulverized) material was soaked in 2L of sterile double-distilled water in a tightly closed round bottom flask at room temperature for a period of 24 h and filtered through Buchner funnel. The whole process was repeated three times and further heated at 95 °C on water bath for 2 h to ensure maximum yield of water soluble compounds. The extract was freeze-dried to give a final yield of 20 g Aq extract (10%).¹⁵

Drugs and reagents

Chemicals and reagents used included methanol, paracetamol syrup 125 mg/5 mL, Brewer's yeast, gum acacia and normal saline. All the reagents and chemicals used were of analytical grade and were directly purchased from Merck Sdn Bhd, Kuantan. Paracetamol syrup 125 mg/5 mL (GlaxoSmithKline) was obtained from Lim Pharmacy, Kuantan.

Phytochemical screening

Phytochemical screening for methanolic (MeOH) and aqueous (Aq) extracts of *T. dependens* leaves was carried out qualitatively for the presence of different class of biologically active compounds viz. tannins, steroids, terpenoids, flavonoids, phenolics, saponins, alkaloids and glycosides.¹⁵

Experimental animals

Healthy albino mice (Institute of Cancer Research (ICR) strain, USA) of either sex weighing 28-36 g were used in this study and obtained from the Sapphire Enterprise, Selangor, Darul Ehsan Malaysia. The animals were housed in disinfected metal cages padded with saw dust, placed on metal shelves in a fully ventilated room at controlled temperature (25 ± 2°C) and maintained in a 12 hour dark-light cycle. The mice were given free access to feed and clean tap water ad libitum. The drinkers containing clean tap water for the animals were washed and refilled daily. The use of experimental mice was permitted by the Institutional Animal Care & Use Committee (IACUC-IIUM), Kuantan. Ethics clearance for the *in vivo* experimental work was obtained from the IACUC-IIUM prior to experimental procedure.

Acute toxicity study of Aq and MeOH extracts of the leaves of *T. dependens*

A separate experiment was conducted to know whether any toxic effect could be produced by Aq and MeOH extracts of the leaves on normal healthy albino mice of either sex. The mice were allowed to acclimatize for a period of 7 days before the determination of LD₅₀ which was carried out according to the method of Organisation for Economic Co-operation and Development (OECD) guidelines.¹⁶ All mice starved for 12 h were randomly divided into two groups (n=10) and were fed orally with both Aq and MeOH extracts separately starting with the graded doses of 100, 500 and 1000 mg/kg body weight (b.w.), respectively. Animals were dosed individually and observed continuously for 24 h and their behavioural and neurological changes were vigilantly observed for any sign of acute toxicity by following up and down

method (OECD).¹⁶ All mice were allowed to pellet diet and tap water ad libitum, and the mortality caused by the extracts within this period of time was also observed. Any acute toxic symptoms were observed and recorded systematically in 1, 2, 4, 6 and 24 hours after extract administration. The number of animals that survived or died in each group within 24 hours was observed and recorded. The observation of acute toxic symptoms included but is not limited to skin changes, restlessness and aggressiveness, rubbing of mouth and nose on the surface of the cage, sensitivity to sound and pain, as well as respiratory movements and sedation. The acute toxic effect of the extract was assessed on the basis of mortality, which was expressed as LD₅₀. Finally the LD₅₀ value was determined as the arithmetical mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where if deaths happened).

Induction of pyrexia

The animals were allowed an acclimatization period of 7 days before the commencement of experiment for the evaluation of antipyretic activity to be carried out. Fever was induced by a subcutaneous injection of 10 ml/kg b.w. of 15% (w/v) yeast suspended in 0.5% (w/v) methylcellulose solution. Mice were then carefully put back to their housing cages and initial rectal temperatures of the experimental animals were recorded with the help of clinical thermometer by inserting it 1.5 cm deep into the rectum, and holding it in place for one minute. This was done in order to ensure that the mice were normothermic before administering the brewer's yeast. After 18 hours, the rectal temperature of the mice were determined via the same procedure and recorded. Animals that indicated a rise in body temperature of at least 0.6 °C were considered eligible for carrying out further experiments.¹⁷

Antipyretic activity

Thirty-six experimental animals eligible for the

experiment were randomly divided into 6 groups of 6 mice each (n=6). Group I received 5 ml/kg body weight of 2% (v/v) aqueous normal saline solution orally (vehicle control group), as negative control. Group II received standard drug viz. paracetamol 150 mg/kg body weight orally. Groups III and IV received MeOH extract and groups V and VI received Aq extract orally at 100 and 200 mg/kg body weight, respectively. The MeOH extract prepared in the form of emulsion (oil: water, 6:4) and Aq extract in double-distilled sterilized water, were administered to the experimental animals by gastric intubation using forced feeding needle. Temperature was noted down at 1 h intervals up to 22 h after giving yeast injection and the rectal temperatures of the mice were recorded at 0, 1, 2, 3 and 4 hours.¹⁷ The percent reduction in pyrexia was calculated by the following formula: Percent reduction = $\frac{B - Cn}{B - A} \times 100$; Where, B represents temperature after pyrexia induction; Cn temperature after 0, 1, 2, 3 and 4 h and A represents normal body temperature.¹⁷ Statistical analysis. The data values were shown as mean±standard error of mean (S.E.M) and analysed using one way ANOVA followed by Dunnett's test. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Dried leaves of *T. dependens* yielded 40 g (8%) and 20 g (10%) methanol (MeOH) and aqueous (Aq) extracts, respectively. Phytochemical screening revealed for the occurrence of saponins, flavonoids, tannins and phenolic compounds as biochemical constituents in appreciable amount in MeOH extract. However, Aq extract revealed for the presence of phenolics, saponins and glycosides only. Both extracts showed negative results for the presence of alkaloids as well as cardiac glycosides. The results are shown in Table I.

Table I. Preliminary phytochemical analysis of methanolic (MeOH) and aqueous (Aq) extracts of *Thottea dependens* leaves

Chemical analysis		MeOH extract of leaves	Aqueous extract of leaves
Phenolics	FeCl ₃ test	+++	+
	Lead acetate test	+++	+
Flavonoids	NaOH test	+++	-
	Shinoda test	+++	-
	H ₂ SO ₄ test	+++	-
Alkaloids	Dragendorff's test	-	-
	Wagner's test	-	-
	Mayer's test	-	-
Tannins	Ferric chloride test	++	-
	Gelatin test	+	-
Saponins	Foam test	+++	+
Steroids	Liebermann test	+	-
Terpenoids	Salkowski test	+	-
Glycosides	Borntrager's test	+	++
	Legal's Test	-	-

Note: +++ (good intensity); ++ (moderate intensity); + (weak intensity); - (absence)

Acute toxicity study of Aq and MeOH extracts of the leaves of *T. dependens*

Acute toxicity study clearly revealed the non-toxic and safe nature of both MeOH as well as Aq extracts of the leaves of *T. dependens*. Nomortality was observed in the extracts treated mice and behavior of the treated mice also appeared normal in most of the animals. Neither lethality nor any toxic reactions were found at any dose selected until the completion of the study. The LD₅₀ of the Aq and MeOH extracts as per OECD guidelines falls under class 4, as no signs of acute toxicity and mortality were observed up to 1000 mg/kg b.w. Hence, the dosages for both extract to determine antipyretic activity were fixed at 100 mg/kg b.w. and 200 mg/kg b.w., respectively.

Antipyretic activity

It was witnessed that the MeOH extract at 200 mg/kg b.w. instigated lowering in normal rectal body temperature up to 4 h following its administration.

This effect was highest at 4 h (Table II). It was found that the subcutaneous injection of yeast suspension distinctly elevated the rectal temperature of mice following 18 h of its administration. Standard drug paracetamol (150 mg/ kg b.w.) reduced the yeast-induced elevation of body temperature. However, at 100mg/kg b.w., MeOH extract had no effect in reducing the temperature. The same results were manifested by Aq extract at 100 and 200 mg/kg b.w., whereby no temperature subsiding was observed.

Among six groups, standard Paracetamol (150 mg/kg b.w.) and MeOH extract of 200 mg/kg b.w. showed temperature reduction by more than 20%. Based on the result and analysis, MeOH extract at 200 mg/kg b.w. significantly exhibited antipyretic activity but not at lower dose viz. 100 mg/kg b.w. However, Aq extract at both doses viz. 100 and 200 mg/kg b.w. did not show any antipyretic effect in mice.

Table II: Antipyretic effect of methanolic and aqueous extract of *Thottea dependens* leaves on mice induced hyperthermia.

Group	Treatment	Dose, mg/kg b.w.	Initial Rectal Temp. in °C before Yeast Injection	Rectal Temperature in °C after 18h of Yeast Injection				
				0hr	1hr	2hr	3hr	4hr
I	Normal Saline	5 mL/kg	37.75 ± 0.33	38.67 ± 0.38	38.83 ± 0.41	39.17 ± 0.33	38.90 ± 0.49	38.90 ± 0.4
II	Standard Paracetamol	150 mg/kg	37.18 ± 0.75	38.08 ± 0.32	37.7 ± 0.43*	37.48 ± 0.47*	37.58 ± 0.43*	37.8 ± 0.47*
III	MeOH extract	100 mg/kg	37.38 ± 0.23	38.57 ± 0.47	38.57 ± 0.56	38.63 ± 0.33	38.85 ± 0.57	38.83 ± 0.50
IV	MeOH extract	200 mg/kg	37.63 ± 0.30	38.58 ± 0.43	38.23 ± 0.37	38.10 ± 0.60	37.97 ± 0.52*	37.75 ± 0.66*
V	Aq extract	100 mg/kg	36.18 ± 1.12	38.33 ± 0.37	39.02 ± 0.44	38.85 ± 0.30	38.72 ± 0.40	38.83 ± 0.39
VI	Aq extract	200 mg/kg	36.8 ± 0.58	38.33 ± 0.19	38.65 ± 0.34	38.68 ± 0.48	38.78 ± 0.43	38.85 ± 0.52

Data are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks(*) indicated statistically significant values from control. *P<0.01.

DISCUSSION

Plants have been used for centuries for medicinal purposes by various cultures and ethnicities around the world and continue to be important curative agents for various disorders even with the revolution in antibiotics and other synthetic agents in modern scientific world. Different mechanisms of action of phytoconstituents from medicinal plants have been proposed. They may impede different metabolic processes or may modify gene expression and signal transduction pathways or

may obstruct microorganisms' growth.¹⁸ Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs owing to their unswerving effectiveness in the treatment of pain, fever, inflammation and rheumatic disorders.¹⁹ Since these drugs have toxic effect to the various organs of the body, a search for safe drugs especially safe herbal remedies with potent antipyretic activity has received great momentum recently.²⁰ Fever is a multifaceted

physiologic response elicited by aseptic stimuli or various infections. Rise in body temperature arises when the concentration of prostaglandin E2 (PGE2) upsurges inside the hypothalamic region of the brain. Such an elevation creates substantial alteration in the firing degree of neurons that regulate the thermoregulation process in the hypothalamus.²¹ It is now evident that most of the antipyretic drugs manifest their effect by impeding the enzymatic activity of cyclooxygenase and subsequently decrease the levels of PGE2 within the hypothalamic region of brain.²² A natural antipyretic agent with less or no toxicity is therefore, considered vital.²³ Since antipyretic activity is normally cited as a characteristic of drugs or agents, which possess an inhibitory property on prostaglandins biosynthesis, the yeast induced hyperpyrexia in rat model was employed to determine the antipyretic effect of MeOH and Aq extracts of the leaves of *T. dependens*. Yeast induced pyrexia is known as pathogenic fever which is elicited because of the formation of prostaglandins (PGE2) that regulate the thermoregulatory center at an elevated temperature.²⁴

T. dependens, which possess the biologically active phytoconstituents like flavonoids, steroids, saponins and terpenoids is an important medicinal plant indigenous to South Asian countries, whose medicinal properties are reported in traditional systems of medicine.^{13,14} The current study was carried out to evaluate and compare the *in vivo* antipyretic activity of the MeOH and Aq extracts of the leaves of *T. dependens* by yeast induced pyrexia method. The results showed that MeOH extract possesses a significant antipyretic effect in yeast induced elevation of body temperature in experimental mice. It was observed that the extract shows dose dependent antipyretic activity. At a dose of 200mg/kg b.w., it showed significant antipyretic activity. At this concentration, normalization of body temperature was maintained for sufficient period of time. Flavonoids are known to target prostaglandins which are involved in the pyrexia. These polyphenolics have been reported to elicit antipyretic action through inhibiting arachidonic acid peroxidation as well as subduing TNF- α , which result in reduction of prostaglandin levels thus helping to reduce the fever and pain.²⁵ Hence, the presence of greater amount of flavonoids in the methanolic extract of *T. dependens* leaves might be responsible for its antipyretic activity. The MeOH extract showed more pronounced effect in lowering the hyperthermia than the Aq extract, but found to have similar effect as the standard drug paracetamol at 3rd hour of administration. The extract is likely to reduce pyrexia by decreasing brain concentration of prostaglandin E2 especially in the hypothalamus owing to its effect on COX-3 or by augmentation of the production of the body's own antipyretic substances like arginine and vasopressin.²⁶ Antipyretics have been reported to subdue fever by retarding the activity of prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or subduing the escalation of interleukin-1 α production subsequent to interferon production.²⁷ This study also correlates with the study

that suggested about flavonoids and saponins to act synergistically to exert the antipyretic effect.²⁸ The results of present study indicate that the MeOH extract of *T. dependens* leaves possesses significant antipyretic activity compared to the activity of Aq extract on yeast induced hyperthermia in mice. This could be credited for the presence of flavonoids and saponins in leaves that might be responsible for the inhibition of prostaglandin synthesis. Moreover, there are several intermediaries accentuating the pathogenesis of fever. Reducing the activity of these intermediaries could further reduce fever significantly.

CONCLUSION

MeOH extract of *T. dependens* leaves at higher dose do exhibit antipyretic activity and provides evidences for the antipyretic activity of *T. dependens* which could partly contribute to its traditional medicinal use. However, further investigation is still required to isolate the active constituents responsible for the antipyretic activity as well as to elucidate the exact mechanism of action.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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