ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE WATER EXTRACT FROM *TERMINALIA CHEBULA* REZT.

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Abstract

Background: In ayurvedic and Thai traditional medicine, the fruit of *T. chebula* is useful in arthritic disorders, inflammation, tumor, pains, chronic and recurrent fever. The study investigated the analgesic and anti-inflammatory activities in animal models.
Materials and methods: The water extract of *T. chebula* fruit was prepared and pain induced in mice by 0.1% formalin, before testing for the analgesic activity of the extract. The anti-inflammatory study was conducted in rats using four experimental models; ethyl phenylpropiolate or arachidonic acid-induced ear edema, carrageenan-induced paw edema and cotton pellet-induced granuloma formation.
Results: The *T. chebula* extract decreased licking times in mice injected with 0.1% formalin in both the early and late phases. Moreover, the extract inhibited rat ear edema induced by ethyl phenylpropiolate as well as in carrageenan-induced paw edema. In contrast, the extract did not have any inhibitory effect on arachidonic acid-induced ear edema in rats. The *T. chebula* extract did not reduce granuloma weight, body weight gain and thymus dry weight in cotton pellet-induced granuloma formation.
Conclusion: These results likely suggest that *T. chebula* water extract possess both analgesic and anti-inflammatory activities. The main mechanisms of action of *T. chebula* water extract may be due to the inhibitory effect on the synthesis and/or release of pain or inflammatory mediators

Keywords: *Terminalia chebula* Retz., analgesic activity, anti-inflammatory activity

Introduction

Terminalia chebula Retz, family Combretaceae, is known as "Sa Maw Thai" in Thailand. *T. chebula* tree is medium-sized or large, attaining a height of up to 30 meter, with wide spreading branches and a broad roundish crown. The leaves are glabrous with a yellowish pubescence below. The flowers are monoecious, dull white to yellow. They are terminal spikes or short panicles and have a strong unpleasant odor. The fruits are usually smooth or frequently 5-ridged, ellipsoid to ovoid drupes, and yellow to orange brown in color, (Department of Medical Sciences, 2000) as shown in Figure 1.

Figure 1: *Terminalia chebula* Retz.

In ayurvedic and Thai traditional medicine, the fruit of *T. chebula* has been extensively used for astringent, carminative, expectorant, laxative, and tonic agents (Department of Medical Sciences, 2000). They are useful for treating arthritic disorders, inflammation, tumor, pains, chronic and recurrent fever. The water extract from dried fruits of *T. chebula* has several pharmacological activities in both *in vitro* and *in vivo* tests, such as antioxidant (Naik et al., 2004; Cheng et al., 2003; Lee et al., 2005, 2007), antibacterial (Malekzadeh et al., 2001; Bonjar 2004; Kim et al., 2006),

antifungal (Dutta et al., 1998), antiviral (Yukawa et al., 1996; el-Mekkawy et al., 1995), and antimutagenic activities (Naik et al., 2004). Moreover, the oral administration of the *T. chebula* water extract did not produce both acute and chronic toxicities in female and male rats (Panunto et al., 2011). However, analgesic and anti-inflammatory activities of this plant have not been experimentally reported. Therefore, the objective of this study was to evaluate the analgesic and anti-inflammatory activities of the water extract from dried fruit of *T. chebula* in animal models.

Materials and methods
Plant material and Preparation of plant extract

Ripe fruits of *T. chebula* were collected from the forest at a joint in Soengsang district, Nakorn Rajsrima province and Prakam district, Buriram province, Thailand. The plant was identified and a voucher specimen number (PBM 00485) was deposited at Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The method of preparation was described as follows: 68 kg of *T. chebula* fruits were boiled for 1 hr, filtered, and then repeated 3 times. The extract was spray dried to remove trace of water *T. chebula* water extract was stored at -20 °C after preparation.

Standardization of plant extract

The water extract of *T. chebula* was tested for quality control such as physical appearance, percentage of loss on dryness, total ash, acid insoluble ash, microbial test, aflatoxin test, heavy metal, and quantity of chemical compounds (percentages of tannins, total carbohydrate, uronic acid, and gallic acid), according to Thai Herbal Pharmacopoeia (Department of Medical Sciences, 2000). The extract was standardized by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Experimental animals

Male Sprague-Dawley rats, weighing 40-60, 100-120, and 200-250 g as well as male ICR mice weighing 30-40 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. They were housed under standard environmental conditions and temperature at 24±1 °C under a 12 hr dark-light cycle, and allowed free access to drinking water and standard pellet diet. Rats were deprived of food except water 16-18 hour prior the experiments. The Animal Ethics Committee of Faculty of Medicine, Thammasat University approved all experimental protocols (No. 0001/2006).

Formalin test (Hunskaar and Hole, 1987)

Mice weighing (40-60 g) were used in this test. Each group of mice was orally administered with *T. chebula* extract at the doses of 150, 300 and 600 mg/kg, and aspirin (reference drug, 300 mg/kg). An hour later, the early phase assessment was conducted by subcutaneously injecting 20 μl of 1% formalin in saline solution into the right dorsal hind paw of the mouse. Then, between 0 and 5 min after formalin injection, the time in seconds the mouse spent for intensive licking the right dorsal hind paw was recorded. In the late phase assessment, another set of mice was used. The formalin was injected after the test drug administration for 40 min and the licking time was determined between 20 and 30 min after the formalin injection.

Ethyl phenylpropiolate (EPP)-induced ear edema in rats (Brattsand et al., 1982)

Male rats of 40–60 g were divided into 4 groups (n=6). The EPP was dissolved in acetone. Ear edema was induced by topical application of EPP at the dose of 1 mg/20 μl/ear to the inner and outer surfaces of both ears using an automatic microliter pipette. *T. chebula* extract dissolved in a mixture of dimethylsulfoxide and acetone (1:1) was administered topically at the dose of 1, 2 and 4 mg/20 μl/ear before the EPP application. Phenulbutazone (reference drug) was administered topically at the dose of 1 mg/20 μl/ear. The thickness of each ear was measured using vernier calipers before and at 15, 30, 60 and 120 min after EPP induction.

Arachidonic acid (AA)-induced ear edema in rats (Young et al., 1984)

The procedure was similar to that previously described in EPP-induced ear edema model. *T. chebula* extract was administered topically at the dose of 1, 2 and 4 mg/20 μl/ear. The reference drugs, phenulbutazone and phenidone, were administered topically at the dose of 1 mg/20 μl/ear. AA was dissolved in acetone, and the ear edema was induced by topical application of AA at a dose of 2 mg/20 μl/ear. The thickness of each ear was measured at 60 min after AA induction.

Carrageenan-induced hind paw edema in rats (Winter et al., 1962)

Male rats weighing 100–120 g were divided into 5 groups of 6 rats. Paw edema was induced by an intradermal injection of carrageenan (1% in normal saline solution) into the plantar surface of the right hind paw of the rats at a volume of 0.05 ml. The edema volume was determined using a plethysmometer (model 7140, Ugo Basile, Italy) prior to and 1, 3 and 5 hr after carrageenan injection. *T. chebula* extract at the doses of 150, 300 and 600 mg/kg and aspirin (reference drug, 300 mg/kg) were given 1 hr prior to carrageenan injection.

Cotton pellet-induced granulation formation in rats (Swingle and Shideman, 1972)

Male rats weighing 200–250 g were divided into 4 groups of 6 rats. The *T. chebula* extract (600 mg/kg), aspirin (300 mg/kg) and prednisolone (5 mg/kg) were orally given 1 hr before subcutaneous implantation of the cotton pellet. Under ether anesthesia, sterile cotton pellets (20±1 mg) were implanted subcutaneously in both the axilla region of each rat through a single needle incision, one on each side. Test substances were given once daily for seven consecutive days. On the 8th day, the rats were sacrificed and the pellets covered with granulation tissue and thymus were dissected out, and weighed immediately for the wet weight. Cotton pellet and thymus were dried at 60 °C for 18 hr and their dry weight was determined. The change in body weight from the first and last day of experiment was also recorded. The increase in dry weight of the pellets was taken as the measure of granuloma formation.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical significance was determined by one-way analysis of variance (ANOVA) and Dunnett test P values less than 0.05 were considered significant.

Results
T. chebula water extract

The contents of quality inspection items such as total ash, acid-insoluble ash, loss on drying, tannins and gallic acid were analyzed in both raw material and the water extract of *T. chebula* fruit as shown in Table 1. The extraction yield as the percentage of the weight of the crude extract to the raw material was 9.62%. Chemical compounds screening found flavonoids, hydrolysable tannin, terpenes, and blue fluorescence compounds. Both raw material and the extract had no contamination of aflatoxin, pesticide, heavy metal and microbes.

Table 1: Quality control status of the raw material and the water extract of *T. chebula* fruit

	Raw material	Water extract
% Loss on drying	7.8548	2.9592
% Total ash	3.5615	3.9694
% Acid insoluble ash	0.4094	0.1552
% Tannins	24.67	60.00
% Gallic acid	0.67	4.37

Effect on formalin test in mice

T. chebula extract at the doses of 150, 300 and 600 mg/kg body weight significantly inhibited the licking response in the early phase and markedly in the late phases. As positive control, aspirin at a dose 300 mg/kg as well as morphine (10 mg/kg) could intensively inhibit the licking response in both phases. Although the effect of aspirin in the early phase was slight, the licking time was significantly reduced (Table 2).

Table 2: Analgesic activity of *T. chebula* extract on the formalin test in mice

Group	Dose (mg/kg)	Licking time (sec)	% inhibition
Early phase			
Control	-	93.7±2.9	-
Aspirin	300	79.0±5.5*	15
Morphine	10	0.0±0.0*	100
T. chebula extract	150	70.7±4.7*	24
	300	66.8±5.7*	29
	600	57.0±7.5*	39
Late phase			
Control	-	64.5±17.0	-
Aspirin	300	0.0±0.0*	100
Morphine	10	0.0±0.0*	100
T. chebula extract	150	35.5±15.2*	45
	300	22.2±8.0*	66
	600	12.0±9.2*	81

Data represent mean ± S.E.M. (n=6). *Significantly different from the control group, p<0.05

Effect on EPP or AA-induced ear edema in rats

T. chebula extract at the dose of 1, 2 and 4 mg/ear exerted an inhibitory effect on the ear edema formation induced by EPP (Table 3), but not on the AA-induced ear edema (Table 4).

Table 3: Effects of *T. chebula* extract on ethyl phenylpropiolate (EPP)-induced ear edema formation in rats

Group	Dose (mg/ear)	Time after topical application of EPP			
		15 min	30 min	60 min	120 min
Control	-	91.7 ± 4.8	153.3 ± 8.8	185.0 ± 13.3	128.3 ± 6.5
Phenylbutazone	1	33.3 ± 11.1*	46.7 ± 8.0*	68.3 ± 6.5*	55.0 ± 5.0*
T. chebula extract	1	61.7 ± 6.0*	101.7 ± 12.2*	115.0 ± 12.3*	81.7 ± 13.5*
	2	50.0 ± 4.5*	85.0 ± 12.3*	91.7 ± 9.4*	60.0 ± 8.2*
	4	43.3 ± 2.1*	63.3 ± 4.2*	81.7 ± 7.5*	50.0 ± 5.2*

Data represent mean ± S.E.M. (n=6). *Significantly different from the control group, *p*<0.05
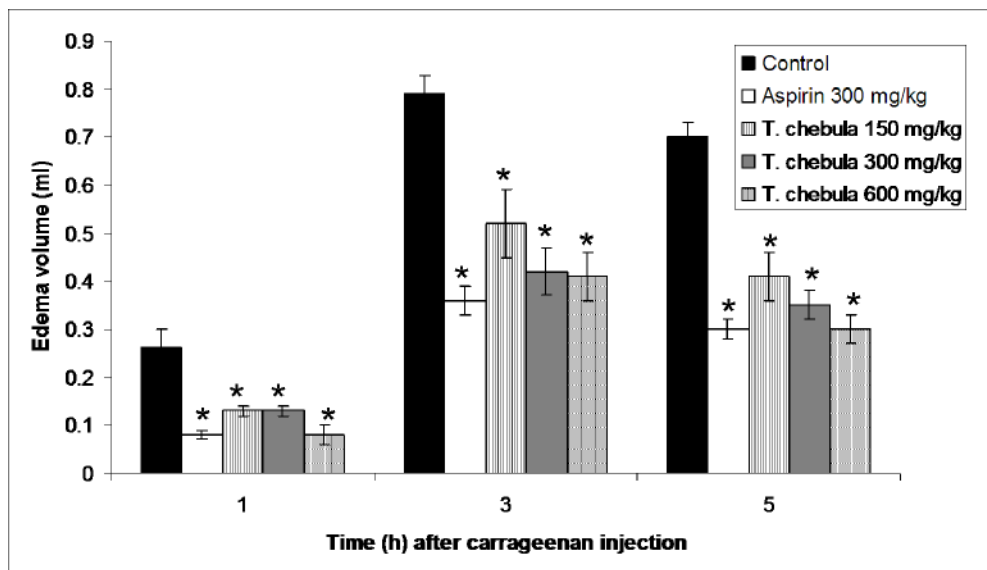
Table 4: Effects of *T. chebula* water extract on arachidonic acid (AA)-induced ear edema formation in rats

Group	Dose (mg/ear)	Edema thickness (μm)
Control	-	128.3 ± 17.0
Phenylbutazone	1	66.7 ± 15.8*
Phenidone	2	56.7 ± 9.2*
T. chebula extract	1	96.7 ± 18.9
	2	103.3 ± 10.5
	4	128.3 ± 14.2

Data represent mean ± S.E.M. (n=6). *Significantly different from the control group, $p<0.05$

Effect on carrageenan-induced paw edema in rats

As illustrated in Figure 1, *T. chebula* water extract at all doses of 150, 300 and 600 mg/kg reduced the paw edema after carrageenan injection for 1-5 hr. The reference drug, aspirin at the dose of 300 mg/kg, also showed a significant inhibitory effect of the paw edema.

Figure 2: Effects of *T. chebula* water extract on carrageenan-induced paw edema in rat. *Significantly different from the control group, $p<0.05$.

Effects on cotton pellet-induced granuloma formation in rats

T. chebula water extract at 600 mg/kg and aspirin (300 mg/kg), dosage did not reduce transudative weight, granuloma formation, body weight gain and thymus weight whereas the group treated daily with prednisolone (5 mg/kg) for 7 days elicited a marked inhibition of these parameters (Tables 6 and 7).

Table 6: Effect of *T. chebula* water extract on transudative weight and granuloma formation of cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
Control	-	466.1 ± 9.4	77.4 ± 2.8	388.9 ± 8.3	2.87 ± 0.1
Prednisolone	5	350.9 ± 7.1*	63.7 ± 2.2*	287.1 ± 5.7*	2.19 ± 0.1*
Aspirin	300	449.7 ± 12.2	75.9 ± 2.1	373.7 ± 11.0	2.80 ± 0.1
T. chebula extract	600	447.2 ± 17.4	74.3 ± 4.5	372.8 ± 14.4	2.72 ± 0.2

Data represent mean ± S.E.M. (n=6). *Significantly different from the control group, $p<0.05$

Table 7: Effect of *T. chebula* water extract on body weight and dry thymus weight of cotton pellet-induced granuloma formation in rats

Group	Dose (mg/kg)	Body weight (g)			Dry thymus weight (mg/100g)
		Initial	Final	Gain	
Control		240.8 ± 18.2	269.1 ± 15.7	28.3 ± 3.5	47.7 ± 4.5
Prednisolone	5	205.8 ± 17.3	221.6 ± 13.2*	15.8 ± 4.3*	35.1 ± 4.2*
Aspirin	300	234.1 ± 14.2	253.3 ± 14.9	19.1 ± 2.3	50.0 ± 5.9
T. chebula extract	600	202.5 ± 11.1	230.8 ± 12.0	28.3 ± 3.0	52.2 ± 3.1

Data represent mean ± S.E.M. (n=6). *Significantly different from the control group, $p < 0.05$

Discussion

In ayurvedic and Thai traditional medicine, the fruit of *T. chebula* has been reported for astringent, carminative, expectorant, laxative, and tonic effects. This plant has been long used for the treatment of arthritic disorders, inflammation, tumor, pains, chronic and recurrent fever. In our study, the analgesic and anti-inflammatory effects of the water extract from the fruit of *T. chebula* were demonstrated using several experimental models in rats and mice.

The formalin test is a reliable model of pain which involves two distinct phases. A neurogenic pain corresponds to the early phase, followed by an inflammatory pain that is conducted by the release of inflammatory mediators designated as late phase (Dubuisson and Dennis, 1977; Hunskaar et al., 1985; Hunskaar and Hole, 1987). In this study, *T. chebula* water extract exhibited the analgesic activity in both phases of the formalin test, but was more remarkable on the late phase. These results suggested that the principal mechanism of analgesic effects of *T. chebula* extract may be due to the inhibition of the synthesis and/or release of inflammatory pain mediators such as prostaglandins and other mediators at peripheral nociception sites. Moreover, the analgesic activity of *T. chebula* water extract may be partly active as well.

Inflammation occurs as a result of defensive response to eliminate the initial cause of cell injury as well as the necrotic cell and tissue resulting from the original assault. Inflammatory reaction produces the synthesis and release of the inflammatory mediators, such as histamine, bradykinin, PGs, IL-1 and TNF-α, with effects are related to pain and fever (Engblom et al., 2002; Ivanov and Romanovsky, 2004).

Rat ear edema induced by EPP or AA is generally a useful model for rapid *in vivo* screening of anti-inflammatory activity. EPP causes the release of many inflammatory mediators such as kinin, serotonin and PGs (Brattsand et al., 1982). AA-induced ear inflammation in mice has been reported to be sensitive in detecting the anti-inflammatory action of lipoxygenase inhibitors (Young et al., 1984; Carlson et al., 1985). In this study, *T. chebula* extract inhibited the ear edema induced by EPP, but not on the AA-induced ear edema. These results suggested that *T. chebula* possibly inhibits the inflammatory mediators of the acute phase of inflammation, but does not involve the lipoxygenase pathway.

Carrageenan-induced paw edema is a predictive test for orally active anti-inflammatory agents acting by the mediators of acute inflammation (Di Rosa et al., 1971; Mossa et al., 1995). This model involves the synthesis or release of mediators at the injured site including prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin (Asongalem et al., 2004). *T. chebula* water extract exhibited a significant inhibitory effect on the paw edema induced by carrageenan. This observation indicated that *T. chebula* extract can be effective to alleviate acute inflammatory disorders. The possible mechanism of action of *T. chebala* extract may result from the inhibition of prostaglandins synthesis and the other inflammatory mediators.

The inflammatory granuloma is a typical feature of established chronic inflammatory reaction (Spector, 1969). The subcutaneously implanted cotton pellet in rat is generally used to evaluate the interfering capacity of agents on the proliferative phases of inflammatory process. The response to a subcutaneously implanted cotton pellet has been reported to involve a host inflammatory response (Remes and Williams, 1992; Tang and Eaton, 1995; Hu et al., 2001), which can be divided into at least three phases, transudative, exudative and proliferative phases (Swingle and Shideman, 1972). Non-steroidal anti-inflammatory drugs, such as aspirin, elicit only a slight inhibition whereas steroidal anti-inflammatory drugs have a strong inhibition on both transudative and proliferative phase of inflammation (Swingle and Shideman, 1972). In this study, *T. chebula* extract did not reduce transudative weight, granuloma formation, body weight gain and thymus weight. The obtained results indicated that *T. chebula* water extract does not have the steroidal-like activity.

In conclusion, the water extract from the fruits of *T. chebula* significantly reduced both the licking time in formalin test and the formation of edema induced by ethyl phenylpropiolate and carrageenan. The *T. chebula* extract exhibited analgesic and anti-inflammatory activities. The main mechanisms of action of *T. chebula* extract may be due to the inhibitory effect on the synthesis and/or release of pain or inflammatory mediators and the analgesic activity may be partly centrally acting.

Acknowledgements

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