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Full Length Research Paper

Analgesic, antipyretic and anti-inflammatory effects of *Tacca chantrieri* Andre

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Tacca chantrieri **Andre is an indigenous perennial of the tropics which is used by local healers to relieve pains of the body and stomach, and as an antidote for food poisoning. The present study was undertaken to investigate the analgesic, antipyretic and anti-inflammatory activities of *T. chantrieri* as claimed in traditional medicine. The ethanolic extract of the plant's rhizome was prepared and tested in experimental animals. It was found that the extract significantly inhibited pain caused by acetic acid injection in the writhing response test in mice and the tail flick test in rats. This finding suggests that the extract exerts analgesic effect through both peripheral and central mechanisms. The analgesic effect was not antagonized by pretreatment with naloxone, an opiod antagonist and this signifies a mechanism other that of the opioid system was utilized. The extract also significantly decreased the yeast-induced hyperthermia in rats. Anti-inflammatory effect of *T. chantrieri* extract was demonstrated in ethylphenylpropiolate-induced ear edema and formalin tests in mice. These findings indicate that the ethanol extract of *T. chantrieri* possesses analgesic, antipyretic and anti-inflammatory effects, which is in accord with its use in traditional medicine.**

Key words: *Tacca chantrieri* Andre, analgesic, anti-pyretic, anti-inflammatory.

INTRODUCTION

Tacca chantrieri Andre (Taccaceae) is an indigenous perennial of the tropics. The plant can be ornamental due to its queer looking flower that is shaped like a flying bat. The decoction of *T. chantrieri* rhizomes, alone or in combination with other herbs, is used by local healers in South-east Asia to relieve pains of the body and stomach, and as an antidote for food poisoning. Phyto-chemical studies of the rhizomes of *T. chantrieri* have yielded a wide array of saponins and glycosides (Yokosuka et al., 2002; 2005), and some compounds (spirostanol saponins and diarylheptanoid glucosides) have shown cytotoxic properties.

However, there has been no pharmacological study to support the beneficial effect of *T. chantrieri*. As per the traditional use, we hypothesized that the plant possesses analgesic antipyretic and anti-inflammatory activities. Therefore, the aim of the current study was to investigate theses activities of the plant in experimental animals. Crude ethanolic extract of *T. chantrieri* (TCE) was prepared from the plant's rhizome. The TCE was tested for analgesic activity using writhing responses in mice and tail flick test in rats. Anti-inflammatory activity was evaluated by formalin test in and ethyl phenylpropiolate-induced ear edema in rats. Lastly, the TCE was tested for the antipyretic activity using yeast-induced hyperthermia in rats.

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MATERIALS AND METHODS

Plant material and extraction

The rhizomes of *T. chantrieri* were collected from Payao province in April 2009. The plant was authenticated by one of the authors (Rujjanawate) and the voucher specimen (no. 143) has been deposited at the school of Health Science, Mae Fah Luang University, Thailand. The air dried powdered rhizome was

macerated with 95% ethanol overnight and filtered. The filtrate was concentrated in vacuo at 55°C and lyophilized to obtain a dry ethanolic extract (15.5% yield) which from now on is referred as TCE. The TCE was subsequently reconstituted in water to required concentrations for the experiments.

Animals

Male Sprague-Dawley rats weighing 150 - 200 g (or stated otherwise) and Swiss albino mice weighing 25 to 30 g were purchased from the National Laboratory Animal Center, Salaya Mahidol University, Thailand. They were acclimatized for at least 7 days in an animal room where the temperature was maintained at 22 ± 3°C and there was a 12 h light-dark cycle. The food was supplied by Pokphan Animal Feed Co. Ltd. Bangkok. The bedding was autoclaved. The animals had free access to food and water. All animals received humane care in compliance with the ethics in the use of animals issued by the National Research Council of Thailand 1999.

Writhing responses in mice

This is a basic screening test for analgesic activity. Briefly, a typical writhing response in mice was produced by an intraperitoneal injection of 0.75% acetic acid aqueous solution at a dose of 0.1 ml/10 g body weight. The TCE was administered intraperitoneally at 30 min before the acetic acid injection (Nakamura et al., 1986). Indomethacin and morphine at a dose of 10 mg/kg were served as positive controls for locally and centrally acting, respectively. Five minutes later, the number of writhes was counted over a period of 15 min. In the other two groups, mice were pretreated with an opiod antagonist naloxone (5 mg/kg, i.p.), 15 min before administration of the TCE (500 mg/kg, i.p.) or morphine (10 mg/kg i.p.).

Tail flick test in rats

The response to thermal pain was evaluated according to the tail flick test described by D'Amour and Smith (1941). The rat was placed on the tail-flick unit (Ugo Basile), so that the tail occluded a slit over a photocell. Heat was applied by a 100-W lamp mounted in a reflector. The apparatus was arranged so that when the operator turned on the lamp a timer was activated. When the rat felt pain and flicked its tail, light fell on the photocell then the timer was automatically stopped. The light intensity was adjusted to give a normal reaction time of 2 to 4 s. A 10 s cut-off time was used in order to prevent tissue damage. Two control readings, taken 30 min apart, were averaged and constituted the control reaction time. The extract was administered (i.p.) immediately after this step, and 30 min later, the post-extract reaction time was measured. The analgesic response was calculated as a percentage of the maximum possible response time. Morphine at a dose of 10 mg/kg was served as a positive control.

Formalin test in rats

The formalin test comprised the early phase and the late phase assessment of the analgesic effect which were performed separately according to the method of Dubuisson and Dennis (1977). Rats were injected intraperitoneally with the TCE. Thirty minutes later, they were administered 50 µl of a 2.5% solution of formalin, subcutaneously under the plantar surface of the left hind paw. They were then placed in an observation chamber and monitored for 1 h. The number of flinches indicated the severity of pain. Analgesic effect was determined in two phases: early phase (first 5 min) and late phase (last 45 min) with a 10 min lag period in between phases. Acetyl salicylic acid (ASA) at a dose of 150 mg/kg was served as a positive control.

Ethyl phenylpropiolate-induced ear edema in rats

The method was modified from that of Brattsand et al. (1982). Male rats weighing 30 to 50 g were used. Ear edema was induced by topical application of ethyl phenylpropiolate (EPP) dissolved in acetone (50 mg/ml) in a volume of 10 µl to the inner and outer surfaces of both ears (20 µl/ear). The test sample was topically applied to the ear just before the irritant. The thickness of each ear was measured with a vernier caliper at 15, 30, 60 and 120 min after the edema induction. The edema thickness of the sample-tested group was compared to that of the vehicle group using phenylbutazone (PHBZ) at a dose of 1 mg/ear as a positive control.

Yeast-induced hyperthermia in rats

Rats were restrained individually in a plastic cage. A probe (model it-rr4) of an EXACON electronic thermometer (model MC8940, EXACON Scientific Instrument Aps, Denmark) was inserted to a depth of 2 cm into the rectum in order to record the initial rectum temperature. The animals were then fevered by injection of 20% suspension of Brewer's yeast in 0.9% NaCl at a dose of 10 ml/kg subcutaneously in the back below the neck. Eighteen hours later, the rectal temperatures were recorded again. Those animals with rectal temperatures lower than 38.0°C were excluded from the experiment. The TCE was administered intraperitoneally and the rectal temperature was then again recorded every 30 min for 2 h.

Statistical analysis

Data were subjected to statistical analysis using ANOVA and statistical comparison was done using Duncan Multiple Range Test. The value exceeding 95% confidence limits was considered to be significant.

RESULTS

Given intraperitoneally, TCE at doses of 250 and 500 mg/kg significantly inhibited acetic acid-induced writhing responses as shown in Table 1, which also shows the similar effect of reference compounds, indomethacin and morphine. Pretreatment of the animals with naloxone could significantly reverse the analgesic effect of morphine but not that of the TCE. Table 2 shows that TCE at a dose of 50 mg/kg could not significantly inhibit the pain response in the tail flick test while at higher doses (125, 250 and 500 mg/kg) the extract significantly prolonged the reaction time as did morphine, the positive control of this test.

In the formalin test, intraperitoneal administration of TCE at doses of 125, 250 and 500 mg/kg significantly lowered the number of flinches in both the early and late phases while TCE at a dose of 50 mg/kg could significantly lowered the number of flinches only in the late phase (Table 3). TCE showed significant inhibition in the EPP-induced rat ear edema model as shown in Table 4. The information obtained also indicated that TCE is

Table 1. Effect of *Tacca chantrieri* ethanolic extract (TCE) on acetic acid-induced writhing response in mice.

Treatment (i.p)	No. of writhes	Inhibition (%)
Morphine 10 mg/kg	0 ± 0*	
Naloxone 5 mg/kg + Morphine 10 mg/kg	± 1	

Data are expressed as mean ± S.E.M. (n = 8). *significant different from control ($p < 0.05$).

Table 2. Effect of *Tacca chantrieri* ethanolic extract (TCE) on radiant heat-induced tail flick in rats.

Treatment (i.p.)	Tc (sec)	Tr (sec)	Inhibition (%)
Control	2.5 ± 0.1	2.5 ± 0.1	0
Morphine 10 mg/kg	3.1 ± 0.2	9.5 ± 0.5	93
TCE 50 mg/kg	2.9 ± 0.2	3.8 ± 0.3	13
TCE 125 mg/kg	3.6 ± 0.2	6.1 ± 0.3*	39
TCE 250 mg/kg	3.2 ± 0.1	9.4 ± 0.3*	91
TCE 500 mg/kg	3.1 ± 0.2	9.4 ± 0.6*	91

Data are expressed as mean ± S.E.M. (n = 10). Tc = control reaction time; Tr = reaction time after injection of test drugs. * Significantly different from Tc ($p < 0.05$).

markedly more potent than phenylbutazone (PHBZ), the reference drug in this test. In the yeast-induced hyperthermia in rats, it was found that TCE at doses of 125, 250 and 500 mg/kg could produce significant anti-pyretic effect (Table 5).

DISCUSSION

The analgesic effect of the CAF was evaluated using the acetic acid-induced writhing, the tail flick and the formalin (early and late phases) tests. These procedures are used to detect central and peripheral analgesia and to distinguish analgesic from anti-inflammatory properties. The writhing test is used for screening of the analgesic activity regardless of the central or peripheral causes. In the tail flick test, which uses a thermal stimulus, an increase in reaction time is generally considered as an important parameter of central analgesic activity (Chang and Lewis, 1989). The formalin test (Hunskaar et al., 1985) is sensitive to NSAIDs and other mild analgesics. The test employs a chemical nociceptive stimulus that elicits a spontaneous response indicative of pain. The test has two different phases, possibly reflecting different types of pain (Dubuisson and Dennis, 1977; Hunskaar et al., 1985). The early phase may be due to direct effects on nociceptors and can be inhibited by centrally acting analgesics such as morphine. In contrast, the late phase may be due to an inflammatory response partly mediated by prostaglandins and can be inhibited by NSAIDs and steroids, as well as by the centrally acting drugs. As inflammation occurs at the site of formalin injection, it is possible to elucidate the role of inflammation on the responses in the two phases. It was previously shown that ASA and indomethacin are anti-nociceptive through partially different modes of action in this test (Hunskaar et al., 1986).

It was also shown that ASA does not have any delay of onset of its action in the early phase compared to morphine (Hunskaar and Hole, 1987). Both of these studies suggested that ASA has some effects which cannot be attributed to the inhibition of prostaglandin synthesis alone.

In this study, the analgesic effect of the extract was demonstrated in the writhing response in mice and tail flick tests in rats. The TCE at doses of 250 and 500 mg/kg was active in both the writhing response and the tail flick tests. This finding suggests that TCE exerts analgesic effect through both peripheral and central mechanisms. The finding that pretreatment of the mice with an opiod antagonist naloxone could not reverse the analgesic effect of the TCE in acetic acid-induced pain model indicates

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Table 3. Effect of *Tacca chantrieri* ethanolic extract (TCE) on the formalin test in rats.

Treatment (i.p.)	No. of flinches	
	Phase I	Phase II
Control	30 ± 4	213 ± 26
ASA 150 mg/kg	21 ± 3	102 ± 11*
TCE 50 mg/kg	23 ± 2	130 ± 6*
TCE 125 mg/kg	11 ± 2*	56 ± 13*
TCE 250 mg/kg	7 ± 3*	34 ± 15*
TCE 500 mg/kg	0 ± 0*	0 ± 0*

Data are expressed as mean ± S.E.M. (n = 8). * Significantly different from control ($p < 0.05$)

Table 4. Effect of topical application of *Tacca chantrieri* ethanolic extract (TCE) on EPP-induced ear oedema in rats.

Treatment	edema ear thickness (μm)				% inhibition			
	15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
Control	60 ± 5	162 ± 20	205 ± 15	228 ± 18				
PHBZ 1 mg/ear	12 ± 4*	105 ± 16*	130 ± 18*	142 ± 14*	80	35	37	38
TCE 0.5 mg/ear	21 ± 3*	98 ± 10*	120 ± 17*	134 ± 10*	65	40	41	41
TCE 1 mg/ear	7 ± 4*	45 ± 13*	102 ± 24*	98 ± 13*	88	72	50	57

Data are expressed as mean ± S.E.M. (n = 10). * Significantly different from control ($p < 0.05$); PHBZ = phenylbutazone.

Table 5. Effect of *Tacca chantrieri* ethanolic extract (TCE) on yeast-induced hyperthermia in rats.

Treatment (mg/kg i.p.)	Rectal temperature (˚C)					
	Initial value	18 h after yeast injection	Minutes after TCE injection			
			30	60	90	120
Control	36.9 ± 0.1	38.5 ± 0.1	38.5 ± 0.1	38.4 ± 0.1	38.4 ± 0.1	38.3 ± 0.1
TCE 125	37.1 ± 0.2	38.6 ± 0.2	37.2 ± 0.2*	36.9 ± 0.2*	36.9 ± 0.2*	37.0 ± 0.2*
TCE 250	37.0 ± 0.1	38.1 ± 0.3	37.4 ± 0.3*	36.9 ± 0.3*	36.4 ± 0.4*	36.2 ± 0.4*
TCE 500	36.7 ± 0.1	37.9 ± 0.1	36.7 ± 0.3*	35.7 ± 0.3*	34.7 ± 0.3*	34.1 ± 0.4*

Data are expressed as mean ± S.E.M. (n = 10). *Significantly different from control ($p < 0.05$).

formalin test in mice was conducted to distinguish analgesic from anti-inflammatory properties (Hunskaar et al., 1985). The finding that TCE as well as ASA exerted a marked analgesic activity in the late phase of the formalin test suggests an effect on acute inflammation. Results from the ear edema test using EPP as an inducer of inflammation confirm this suggestion.

Finally, the antipyretic effect of the extract was demonstrated in yeast-induced hyperthermia in rats. The production of prostaglandins appears to be a final common pathway responsible for fever production induced by several pyrogens (Milton, 1982). Therefore, it is reasonable to assume that the inhibition of prostaglandin biosynthesis may take part in the anti- inflammatory and antipyretic activities of the TCE. In conclusion, this study confirms the ethnomedical use of *T. chantrieri* as an analgesic, antipyretic and anti-inflammatory agent. It's worth noting that steroidal saponins have been isolated from the rhizomes of the plant (Yokosuka et al., 2002; 2005) but it is not clear at present whether these substances are responsible for the pharmacological properties of TCE.

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