Original Research

Preliminary evaluation of the analgesic and anti-inflammatory effects of *Tacca integrifolia* in rodents

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Summary. This is a preliminary investigation of the ethyl acetate extract of the leaf of *Tacca integrifolia* (TIE) for the analgesic activity using writhing response in mice, tail flick test in rats and for anti-inflammatory activity using ethyl phenyl propiolate (EPP)-induced ear edema, carrageenan- and arachidonic acid-induced hind paw edema, as well as cotton pellet-induced granuloma formation in rats. The results showed that TIE (200 mg/kg, PO) significantly inhibited pain caused by acetic acid injection (65.9%) but did not exhibit effect in tail flick test in rats. These findings suggest that analgesic mechanism of TIE may act via peripherally pathway. The study of anti-inflammatory effect showed that TIE significantly inhibited ear edema induced by EPP. TIE (200 mg/kg, PO) inhibited paw edema induced by carrageenan (55.5%) and arachidonic acid (48.6%) but had no effect on cotton-induced granuloma formation in rats. In conclusion, the ethyl acetate extract of leaf of *T. integrifolia* possessed anti-inflammatory activity in acute inflammation and analgesic activity.

Industrial relevant. Plants of the genus Tacca have been reported to possess many activities such as analgesic, anti-inflammatory and, antipyretic activities. Many species have been used to treat high blood pressure, burn, gastric ulcer, and hepatitis. The scientific studies supporting the traditional uses of *Tacca integrifolia* for some of the alleged activities are still lacking. The screening test for analgesic and anti-inflammatory effect of the ethyl acetate extract of the leaf of *Tacca integrifolia* provides scientific data to confirm the potentials of *T. integrifolia* as an analgesic and anti-inflammatory medicinal plant. In addition, the outcomes may be useful to develop a new analgesic and anti-inflammatory drug in the future.

Key words. *Tacca integrifolia*; Taccaceae; ethyl acetate extract; analgesic activity; anti-inflammatory activity

INTRODUCTION

Inflammation is an important body response to inducers including infection, physical injury and some diseases. Although such reaction has a critical impact in the regulation of cells, tissues and organs functions, in certain cases, prolonged, uncontrolled or exacerbated inflammatory reaction provokes the damages to the body (Rang et al., 2003). Although the effective anti-inflammatory drugs are available, prolonged use may produce undesired adverse effects. Therefore searching for new drugs with low side effect is still needed. Plants are well known as major sources of biological active compounds that can be developed into therapeutic agents for various indications (Fabricant et al., 2001).

Plants in Taccaceae family have long been used not only as food, but also in medicine role. Several species of plants in the Taccaceae family have been reported and used as traditional medicines for alleviation of pain, inflammation, fever, incised...
the ability of an agent to inhibit the proliferative component of the subchronic and chronic inflammatory process. The pellets were placed in the subplantar of the rats in the same manner as above. The paw volume was measured prior to and at 1 h after AA injection. The percent edema inhibition of each test compound was calculated.

Carrageenan (0.05 mL of 1% in NSS) was injected intradermally into the plantar of the right side of hind paw of an unanesthetized rat restrained in a plastic cage. Foot volume of animal was determined by means of a plethysmometer (model 7140, Ugo Basile, Italy). The paw volume was measured prior to and at 1, 3 and 5 h after carrageenan injection. The percent edema inhibition of each test compound was calculated.

Arachidonic acid (AA)-induced hind paw edema in rats (DiMartino et al., 1987). TIE (50, 100 and 200 mg/kg), diclofenac (3 mg/20 µL/ear), diclofenac (3 mg/20 µL/ear) and vehicle were applied in the same manner just before application of EPP. Before and at 15, 30, 60, and 120 min after edema induction, the thickness of each ear was measured with a digital vernier caliper. The edema thickness at time from baseline was calculated and the percent edema inhibition was calculated and compared with that of the control group.

Carrageenan-induced hind paw edema in rats (Chan et al.,1995). TIE (50, 100 and 200 mg/kg), diclofenac (10 mg/kg), and vehicle were orally given 1 h prior to carrageenan injection into 5 groups of 6 rats (100-120 g) each, respectively. Carrageenan (0.05 mL of 1% in NSS) was injected intradermally into the plantar of the right side of hind paw of an unanesthetized rat restrained in a plastic cage. Foot volume of animal was determined by means of a volume displacement technique using a plethysmometer (model 7140, Ugo Basile, Italy). The paw volume was measured prior to and at 1, 3 and 5 h after carrageenan injection. The percent edema inhibition of each test compound was calculated.

Arachidonic acid (AA)-induced hind paw edema in rats (DiMartino et al., 1987). TIE (50, 100 and 200 mg/kg), diclofenac (3 mg/20 µL/ear), diclofenac (3 mg/20 µL/ear) and vehicle were applied in the same manner just before application of EPP. Before and at 15, 30, 60, and 120 min after edema induction, the thickness of each ear was measured with a digital vernier caliper. The edema thickness at time from baseline was calculated and the percent edema inhibition was calculated and compared with that of the control group.

Cotton pellet-induced granuloma formation in rats (Ashok et al., 2010). This experiment was performed to investigate the ability of an agent to inhibit the proliferative component of the subchronic and chronic inflammatory process. The pellets of adsorbent cotton wool (20 ± 1 mg) were sterilized in a hot air oven (model 600, Memmert, Germany) at 120 °C for 2 h. Two pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anesthesia and sterile technique. TIE (200 mg/kg), diclofenac (5 mg/kg), prednisolone (5 mg/kg), and vehicle were orally administered into 4 groups of 6 rats (180-200 g) each, respectively in once daily throughout the experimental period of 7 days. On the eighth day after cotton pellets implantation, rats were anaesthetized and the blood was collected for determination of the amount of alkaline phosphatase (ALP) and total protein. Thereafter, the rats were sacrificed and the implanted pellets were dissected.
out and carefully removed from the surrounding tissues and weighed immediately for the wet weight. Moreover, the thymus was also dissected out. Both the cotton pellets and the thymus were dried at 60 °C for 18 h and their dry weights were determined. The transudative weight, granuloma weight and the percent granuloma inhibition of the test substance and the reference were calculated.

Statistical analysis. The results from the experiment were expressed as mean ± standard error of the mean (S.E.M.). Statistical comparisons between groups were analyzed by using one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. P values less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Writhing response in mice. The analgesic activity of TIE was evaluated using both chemical (acetate acid-induced writhing response) and thermal (tail-flick test) methods. The acetate acid-induced writhing response in mice is commonly tested for detection of peripheral and central analgesic acting of drugs. The acetate acid injection induces tissue damage and causes the release of several endogenous substances such as bradykinin (BKs), prostaglandins (PGs), and serotonin (5-HT) and contributes to the process of inflammation and increased sensitivity of nociceptors. These endogenous substances sensitize peripheral nerve terminal (peripheral sensitization), leading to phenotypic alteration of the sensory neurons and increased excitability of the spinal cord dorsal horn neurons (central sensitization) (Fields, 1987; Raj, 1996). In addition, peripheral sensitization also plays an important role in the development and maintenance of central sensitization (Boyce-Rustay et al., 2010). In analgesic evaluation using the acetate acid-induced writhing response model, the number of writhing responses of groups received TIE (50, 100, and 200 mg/kg) and diclofenac (10 mg/kg) was significantly decreased when compared with that of the control group (Table 1). The pain-relieving action of diclofenac is cyclooxygenase (COX) enzymes inhibition which diminishes production of PGs; the PGs heighten the sensitivity of primary afferent nerve fibers leading to pain (Okuse K., 2007). The results show that pre-treatment with various doses of TIE significantly inhibited the writhing response caused by acetate acid injection in dose-dependent manner. The obtained data imply the analgesic activity of TIE.

Table 1 Effect of Tacca integrifolia extract (TIE) on acetate acid-induced writhing response in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>nWR</th>
<th>%WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Tween80</td>
<td>-</td>
<td>21.5 ± 0.8</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>7.0 ± 0.8**</td>
<td>67</td>
</tr>
<tr>
<td>TIE</td>
<td>50</td>
<td>12.8 ± 1.1**</td>
<td>40</td>
</tr>
<tr>
<td>TIE</td>
<td>100</td>
<td>10.7 ± 1.3**</td>
<td>50</td>
</tr>
<tr>
<td>TIE</td>
<td>200</td>
<td>7.3 ± 1.2**</td>
<td>66</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n = 6). ** P < 0.001 compared to control group (5% Tween80).

%WI = (nWR of control group - nWR of test group)/nWR of control group x 100

Tail-flick test in rats. The tail-flick test in rats is a measure of acute cutaneous thermal pain and is generally considered to be a measure of nociceptive threshold (Kohn, 1997). It is well known that pain produced in the tail-flick test is sensitive and inhibited by centrally acting analgesic drugs such as morphine and codeine. In the tail-flick test, the reaction time of rats received TIE or diclofenac was not significantly different from that of the control group (Table 2). On the other hand, codeine showed a marked inhibitory effect on the tail-flick response in rats. The results obtained from acetate acid-induced writhing response and tail-flick test therefore suggest that the analgesic effect of TIE may involve the peripherally pain pathway rather than the centrally pain pathway similar to that of diclofenac.

Table 2 Effect of Tacca integrifolia extract (TIE) on heat-induced tail flick in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Tb</th>
<th>Tr</th>
<th>%MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Tween80</td>
<td>-</td>
<td>2.69 ± 0.27</td>
<td>3.22 ± 0.17</td>
<td>7</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>2.96 ± 0.26</td>
<td>3.48 ± 0.26</td>
<td>7</td>
</tr>
<tr>
<td>Codeine</td>
<td>200</td>
<td>2.58 ± 0.08</td>
<td>5.55 ± 0.28**</td>
<td>40</td>
</tr>
<tr>
<td>TIE</td>
<td>50</td>
<td>3.03 ± 0.25</td>
<td>3.51 ± 0.29</td>
<td>7</td>
</tr>
<tr>
<td>TIE</td>
<td>100</td>
<td>3.00 ± 0.21</td>
<td>3.55 ± 0.20</td>
<td>8</td>
</tr>
<tr>
<td>TIE</td>
<td>200</td>
<td>3.07 ± 0.19</td>
<td>3.58 ± 0.17</td>
<td>7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n = 6). ** P < 0.001 compared to control group (5% Tween80).

TP is baseline reaction time. Tr is reaction time after administration of test drugs. %MR is percent maximum possible response. %MR = [(Tr - Tb)/(10 - Tb)] x 100, 10 = cut-off time of 10 sec.

EPP-induced ear edema in rats. EPP-induced ear edema is a useful model for screening and investigating the anti-inflammatory activity of test substances on acute phase of inflammation. This model is a rapid and simple test, requires small amount of substances and provides well-reproducible results (Winyard, 2003). EPP application causes the release of various inflammatory mediators including histamine, 5-HT and PGs and thereby induces the vasodilation, increases vascular permeability and produces edema (Carlson et al., 1985). Moreover, the application of EPP has been reported to cause epidermal hyperplasia and inflammation (Cameron et al., 1991). For anti-inflammatory screening test using the EPP-induced ear edema model, it was found that TIE and diclofenac at a dose of 3 mg/ear inhibited ear edema at all of the assessment time (Table 3). Therefore, TIE may possess the anti-inflammatory effect.
Carrageenan-induced hind paw edema in rats. The inflammation induced by carrageenan injection into the rat paw is an acute, non-immune, well-researched and highly reproducible method (Winter et al., 1962; Winyard and Willoughby, 2003). Carrageenan-induced hind paw edema in rats is a useful model in evaluating the contribution of mediators involved in vascular changes associated with acute inflammation. This inflammatory model is commonly used for determining the anti-inflammatory activity of test compound of which its mechanism involves COX inhibition (Guay et al., 2004). Oral administration of TIE at doses of 100, 200 mg/kg and diclofenac at a dose of 10 mg/kg significantly inhibited carrageenan-induced paw edema at all of the recorded time. TIE at a dose of 50 mg/kg significantly inhibited paw edema formation at the 1st and the 3rd h but had no effect at 5th h after carrageenan injection (Table 4).

Edema induced by carrageenan consists of three phases. The first phase, during the first 1.5 h, is mediated by histamine and 5-HT; the second phase (1.5-2.5 h) is mediated by BKs; and the third phase is attributed to local production of PGs from 2.5 to 6 h after carrageenan injection (Di Rosa et al., 1971). The results show that TIE and diclofenac exhibited edema inhibition at all assessment times. Moreover, the percent inhibition of TIE on the edema formation was gradually increased as the dose increased. It is suggested that TIE exhibits anti-inflammatory effect and may involve the mediators which are associated in inflammation and pain.

Table 4 Effect of Tacca integrifolia extract (TIE) on carrageenan-induced hind paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Edema volume (mL)</th>
<th>% Edema inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Tween80</td>
<td>-</td>
<td>0.26 ± 0.03</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.14 ± 0.02*</td>
<td>0.27 ± 0.04**</td>
</tr>
<tr>
<td>TIE</td>
<td>50</td>
<td>0.16 ± 0.03*</td>
<td>0.33 ± 0.03*</td>
</tr>
<tr>
<td>TIE</td>
<td>100</td>
<td>0.14 ± 0.02*</td>
<td>0.32 ± 0.02*</td>
</tr>
<tr>
<td>TIE</td>
<td>200</td>
<td>0.12 ± 0.01**</td>
<td>0.30 ± 0.04*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n = 6). * P < 0.05, ** P < 0.001 compared to control group (5% Tween80).

% Edema inhibition = [(ET of control group - ET of test group)/ET of control group] x 100

Arachidonic acid (AA)-induced hind paw edema in rats. AA-induced paw edema in rat is a potentially useful model for detecting anti-inflammatory of lipoxygenase (LOX) inhibitors with a mechanism of action different from COX inhibitors. In the AA-induced paw edema, TIE at a dose of 50 mg/kg and diclofenac at a dose of 10 mg/kg could not inhibit paw edema, however, TIE at doses of 100, 200 mg/kg and prednisolone at a dose of 5 mg/kg significantly inhibited paw edema (Table 5).

Edema produced by AA is extremely sensitive to inhibition by corticosteroids (e.g., prednisolone, dexamethasone), dual inhibitors of AA metabolism (e.g., phenidone), and LOX inhibitors (e.g., zileuton) but is insensitive to COX inhibitors (DiMartino et al., 1987). In this study, oral administration of prednisolone, and TIE significantly inhibited AA-induced edema. Moreover, the percent inhibition of TIE at all doses on the edema formation was gradually increased in dependent manner. Taken together with the results from carrageenan-induced paw edema, it indicates that anti-inflammatory effect of TIE may be related to dual inhibition of AA metabolism.

Table 5 Effect of Tacca integrifolia extract (TIE) on arachidonic acid (AA)-induced hind paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Edema volume (mL) at 1 h</th>
<th>% Edema inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% Tween80</td>
<td>-</td>
<td>0.169 ± 0.02</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.137 ± 0.01</td>
<td>19</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>5</td>
<td>0.085 ± 0.02*</td>
<td>50</td>
</tr>
<tr>
<td>TIE</td>
<td>50</td>
<td>0.122 ± 0.02</td>
<td>28</td>
</tr>
<tr>
<td>TIE</td>
<td>100</td>
<td>0.097 ± 0.01*</td>
<td>43</td>
</tr>
<tr>
<td>TIE</td>
<td>200</td>
<td>0.087 ± 0.02*</td>
<td>49</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. (n = 6). * P < 0.05 compared to control group (5% Tween80).

Cotton pellet-induced granuloma formation in rats. The cotton pellet-induced granuloma formation in rats is a typical model which has been widely employed to access the transudative, exudative and proliferative components of subacute inflammation (Ashok et al., 2010). The inhibitory effect of test drug is evaluated for its ability to reduce the deposition of granulation tissue around implanted cotton pellets. In this model, the high dose of TIE that possessed the anti-inflammatory effect in previous model was used to evaluate the anti-inflammatory effect on chronic inflammation. The results show that TIE at a dose of 200 mg/kg could not reduce transudative weight and granuloma weight in cotton pellet-induced granuloma formation in rats when compared to those of the control group which received 5% Tween80 (Table 6). Both diclofenac and prednisolone reduced transudative weight and granuloma formation. The dry thymus weight of TIE- and diclofenac-treated groups did not change when compared to that of the control group. On the other hand, prednisolone significantly reduced dry thymus weight.

The serum ALP activity in the control group (33.27 ± 3.50 unit x 10^-3/mg) was significantly increased when
The results obtained from inflammatory models suggest that TIE had no effect on ALP activity and inhibition of the migration of the inflammatory cells into the inflammatory sites (Salmon and Higgs, 1987). The mechanism of analgesic effect may be due to the inhibition of peripherally mediated nociception and the mechanism of anti-inflammatory effect may be via the inhibition of COX and LOX pathways. Moreover its effect does not share the steroidal-like effect.

The response to subcutaneously implanted cotton pellet in rats has been divided into transudative, exudative and proliferative phases, respectively. The transudative phase is defined as the increase in the wet weight of the granuloma whereas the proliferative phase is defined as the increase of dry weight of the granuloma. The thymus weight of rodents. In the present study, TIE and diclofenac did not influence the thymus weight of the rats whereas prednisolone showed a marked reduction of the thymus weight. Therefore, the anti-inflammatory activity of TIE which found in acute inflammation did not share the steroidal-like effect.

The migration of leukocytes to the injury site occurs during chronic inflammation. Leukocytes accumulation leads to the release of lysosomal enzymes and oxygen radicals at inflammatory site (Salmon and Higgs, 1987). In cotton pellet-induced granuloma formation, the activity of lysosomal enzyme such as ALP in serum, is markedly elevated on the 7th day after implantation (Nishikaze O, 1980) and can be normalized by NSAIDs and steroids through the stabilization of lysosomal membrane and inhibition of the migration of the inflammatory cells into the inflammatory sites (Salmon and Higgs, 1987). The results obtained from inflammatory models suggest that TIE had no effect on ALP activity.

CONCLUSIONS

The ethyl acetate extract of T. integrifolia leaves possesses analgesic and anti-inflammatory effect on acute inflammation. The mechanism of analgesic effect may be due to the inhibition of peripherally mediated nociception and the mechanism of anti-inflammatory effect may be via the inhibition of COX and LOX pathways. Moreover its effect does not share steroidal-like action.

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REFERENCES


