

Analgesic and Anti-Inflammatory Activities of Methanol Extract from *Desmodium triflorum* DC in Mice

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Abstract: In this study, we evaluated the analgesic effect of methanol extract from *Desmodium triflorum* DC (MDT) by using animal models of acetic acid-induced writhing response and formalin test. The anti-inflammatory effect of MDT was investigated by λ -carrageenan-induced paw edema in mice. In order to study the anti-inflammatory mechanism of MDT, we detected the activities of glutathione peroxidase (GPx) and glutathione reductase (GRd) in the liver, the levels of interleukin-1 β (IL-1 β), tumor necrosis factor (TNF- α), malondialdehyde (MDA) and nitric oxide (NO) in the edema paw tissue. In the analgesic test, MDT (0.5 and 1.0 g/kg) decreased the acetic acid-induced writhing response and the licking time on the late phase in the formalin test. In the anti-inflammatory test, MDT (0.5 and 1.0 g/kg) decreased the paw edema at the 3rd, 4th, 5th and 6th hour after λ -carrageenan administration. On the other hand, MDT increased the activities of SOD and GRd in liver tissues and decreased the MDA level in the edema paw at the 3rd hour after λ -carrageenan-induced inflammation. MDT also affected the levels of interleukin-1 β , tumor necrosis factor- α , NO and MDA which were induced by λ -carrageenan. The results suggested that MDT possessed analgesic and anti-inflammatory effects. The anti-inflammatory mechanism of MDT might be related to the decreases in the level of MDA in the edema paw via increasing the activities of SOD and GRd in the liver, and the NO level via regulating the IL-1 β production and the level of TNF- α in the inflamed tissues.

Keywords: *Desmodium triflorum*; Anti-Inflammation; Analgesia; Malondialdehyde; Nitric Oxide; Tumor Necrosis Factor- α ; Vitexin.

Introduction

Desmodium triflorum (abbrev. DF) DC (Fabaceae), a medicinal plant in Taiwan, is a popular medicine for relieving dysmenorrhea, muscle spasms, cough and pain. In India, the leaves are used as a remedy for diarrhea, dysentery, and convulsions; the roots are used for coughs, asthma, and applied to wounds and abscesses (Ghosal, 1971).

Synonymous Latin binomials for this plant are *Hedysarum triflorum*. In Taiwan, this herb, commonly known as San-Dam-Jin-Cao, is called “wing of fly” among Taiwanese by the morphology of the leaves (Huang and Sankarasubramanian, 1998). Ursolic acid, vitexin, genistin (Chio and Huang, 1995), fucosterol, 2-O- β -xylosylvitexin (Sreenivasan *et al.*, 1984) and a rare diholosylflavone, 2''-O-glucosylvitexin (Adinarayana and Syamasundar, 1982) had been isolated from DF. The alkaloids of DF include hypaphorine, N, N-dimethyltryptophan, betaine, choline, β -phenethylamine (a minor constituent), N, and N-dimethyltryptamine oxide (Ghosal *et al.*, 1971). DF possesses antioxidative activity (Mao *et al.*, 2007). Different extracts of DF exhibit analgesic and anti-inflammatory activities (Kawshik *et al.*, 2005). However, the exact mechanisms of DF have never been studied.

The previous reports show that λ -carrageenan-induced inflammatory effect is associated with free radicals. Free radicals, prostaglandin and NO will be released when administrating with λ -carrageenan for 1–6 hours (Dudhgaonkar *et al.*, 2006). The edema effect maximized at the 3rd hour (Kirkova *et al.*, 1992). Malondialdehyde (MDA) production was due to the free radical attacked plasma membrane (Janero, 1990). Thus, inflammatory effect would result in the accumulation of MDA (Lu *et al.*, 2007). Therefore, the aims of this study were to investigate the effect of MDT on the pain induced by acetic acid and formalin, and on the inflammation induced by λ -carrageenan in mice. In order to evaluate the mechanisms of analgesic and anti-inflammatory effects of DF, we also analyzed the levels of interleukin-1 β (IL1 β), tumor necrosis factor- α (TNF- α), MDA and NO in the edema paw tissues and the activities of SOD, GPx and GRd in the liver at the third hour after λ -carrageenan injection.

Materials and Methods

Plant Material and Crude Extract Preparation

DF was collected from the garden and grassland in Taichung, Taiwan as described by Flora of Taiwan. The plants were identified by professor Chung-Chuan Chen, Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan. A plant specimen has been deposited in the Institute. After cutting into small pieces, the materials were dried and crushed into a coarse powder. The coarse powder of DF (1,800 g) was extracted with methanol 3 times. The methanol extract was evaporated under reduced pressure using a rotavapor to yield 253 g (14.0% yield r) of crude methanol extract (MDT). The extract was stored under light protection before the experiment.

Chemicals

The following chemicals and reagents, λ -carrageenan, indomethacin, Griess reagent, etc., were purchased from Sigma-Aldrich Chemical Co.. Formalin was purchased from Nihon Shiyaku Industry Ltd. The SOD, GPx, GRd and MDA activity assay kits were purchased from Randox Laboratory Ltd. The NO assay kit was purchased from Cayman Chemicals Co. Chemicals and enzyme immunometric assay kits for mouse IL-1 β and TNF- α were obtained from Assay Designs, Inc. All of the other reagents used were analytical grade.

Phytochemical Analysis of MDT by HPLC

The HPLC fingerprint profile was established for the standard (Vitexin) and MDT. HPLC analysis was performed on a Waters HPLC 2695 separation module. Chromatographic separation was carried out on LiChroCART RP-18 endcapped column (250 \times 4.6 mm, i.d., 5 μ m pore size) with a injection of 10 μ l using an elution of 0.1% formic acid: acetonitrile (81:19) solvent at a flow rate of 0.8 ml/min. Peaks were detected at 260 nm with 2996 PDA detector.

Experimental Animals

Male ICR mice (20–25 g) were purchased from the BioLasco Charles River Technology, Taipei, Taiwan. The mice were kept in the animal center of China Medical University at 22 \pm 1°C, relative humidity 55 \pm 5%, and light and dark cycles of 12-hour (08:00 to 20:00) for 1 week before the experiment. Animals were provided with rodent diet and clean water *ad libitum*. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved under the code 96-195-S by the Committee on Animal Research, China Medical University. All tests were conducted under the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

Acetic Acid-Induced Writhing Response

The writhing test in mice was carried out by the method of Koster *et al.* (1959). The writhes were induced by intraperitoneal injection of 1.0% acetic acid (v/v, 0.1 ml/10 g body weight). Two different doses (0.5 and 1.0 g/kg) of MDT were administered orally to each group of mice, 60 min before chemical stimulus. Indomethacin, as the positive control, was administered 30 min prior to the acetic acid injection. The placebo groups were administered orally with 0.1 ml/10 g BW saline. The number of muscular contractions was counted for 10 min after acetic acid injection. That number represented the total numbers of writhes for 10 min and was expressed as writhing numbers.

Formalin Test

The method used was similar to that described previously (Shibata *et al.*, 1989). Twenty microliters of 5% formalin was injected subcutaneously into the right hind paw of the mice.

The time (in sec) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (early phase) and 20–30 min after formalin injection (late phase). MDT (0.1, 0.5 and 1.0 g/kg, p.o.) was administered 60 min before formalin injection. Indomethacin (10 mg/kg, i.p.) was administered 30 min before formalin injection. The control group received the same volume of saline by oral administration.

λ -Carrageenan-Induced Mice Paw Edema

The anti-inflammatory activity of MDT was determined by the λ -carrageenan-induced edema test in the hind paws of mice. Male ICR mice (10 per group), were fasted for 24 hours before the experiment with free access to water. Fifty microliter of a 1% λ -carrageenan suspension in saline was injected into the plantar side of right hind paws of the mice (Winter *et al.*, 1962). Paw volume was measured at 1-, 2-, 3-, 4-, 5- and 6-hours after the administration of the λ -carrageenan by using a plethysmometer. The degree of swelling was evaluated by the delta volume ($a-b$), where a and b were the volume of the right hind paw after and before the λ -carrageenan treatment, respectively. Indomethacin, as the positive control compound (Mascolo *et al.*, 1989), was administered at 90 min after λ -carrageenan injection. MDT was administered at 120 min after λ -carrageenan injection. In the secondary experiment, the whole right hind paw tissues and liver tissues were taken at the 3rd hour. The right hind paw tissue was rinsed in ice-cold normal saline, and immediately placed in its four volumes of cold normal saline and homogenized at 4°C. Then the homogenate was centrifuged at 12,000 \times g for 5 min. The supernatant was stored at -20°C for interleukin-1 β , tumor necrosis factor- α , NO and MDA assays. The whole liver tissue was rinsed in ice-cold normal saline, and immediately placed in an equal volume of cold normal saline and homogenized at 4°C. Then the homogenate was centrifuged at 12,000 rpm for 5 min. The supernatant was stored at -20°C for the antioxidant enzymes (SOD, GPx, and GRd) activity assays.

NO Assay

NO was measured according to the method of Moshage *et al.* (1995). For nitrite determination, NO_3^- was converted into nitrite after enzymatic conversion by nitrate reductase; NO_2^- was measured by using the Griess reaction (Green *et al.*, 1982). Values obtained by this procedure represented the sum of nitrite and nitrate.

MDA Assay

MDA was evaluated by the thiobarbituric acid reacting substance (TRARS) method (Tatum *et al.*, 1990). In brief, MDA reacted with thiobarbituric acid at a high temperature and formed a red-complex TBARS. The absorbance of TBARS was determined at 532 nm. Protein was measured by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Measurements of Antioxidant Enzymes Activity

The following anti-oxidative enzymes were analyzed to detect the antioxidant activities of MDT by the methods given below. Superoxide dismutase (SOD) enzyme activity was determined according to the method of Misra and Fridovich (1972) at room temperature. One hundred microliters of tissue extract was added to 880 μl (0.05 M, pH 10.2, 0.1 mM EDTA) carbonate buffer. Twenty microliters of 30 mM epinephrine (in 0.05% acetic acid) were added to the mixture at 480 nm for 4 min on a Hitachi U 2000 Spectrophotometer. The enzyme activity was expressed as the amount of enzyme that inhibited the oxidation of epinephrine by 50% which was equal to 1 unit. Glutathione peroxidase (GPx) enzyme activity was determined according to the method of Flohe and Gunzler (1984) at 37°C. A reaction mixture was composed of 500 μl phosphate buffer, 100 μl 0.01 M GSH (reduced form), 100 μl 1.5 mM NADPH and 100 μl GRd (0.24 units). One hundred microliters of the tissue extract were added to the reaction mixture and incubated at 37^{circ}C for 10 min. Then 50 μl of 12 mM *t*-butyl hydroperoxide were added to 450 μl tissue reaction mixture and were measured at 340 nm for 180 s. The molar extinction coefficient of 6.22×10^{-3} was used to determine the enzyme activity. One unit of the activity was equal to the mM of NADPH oxidized/min per mg protein. GRd enzyme activity was determined following the method of Carlberg and Mannervik (1985) at 37°C. Fifty microliters of NADPH (2 mM) in 10 mM Tris buffer (pH 7.0) were added in cuvette containing 50 μl of GSSG (20 mM) in phosphate buffer. One hundred microliters of tissue extract were added to the NADPH-GSSG buffered solution and were measured at 340 nm for 3 min. The molar extinction coefficient of 6.22×10^{-3} was used to determine GRd enzyme activity. One unit of the activity was equal to the mM of NADPH oxidized/min per mg protein.

Measurements of IL-1 β and TNF- α

IL-1 β and TNF- α were measured by enzyme-linked immunosorbent assays. Assays were carried out in accordance with manufacturer's instructions (Ataoglu *et al.*, 2002). The amount of IL-1 β and TNF- α were determined by reference to standard curves (0–1000 pg/ml) constructed in each assay. The concentration of IL-1 β and TNF- α in each sample were expressed as picogram per milligram protein (pg/mg) for cytokine concentration.

Statistical Analysis

Data were represented as the mean \pm SEM. Data were analyzed by oneway ANOVA followed by Scheffe's multiple range test. The criterion for statistical significance was $p < 0.05$.

Results*Phytochemical Analysis of MDT*

The vitexin was used as a maker component for the standardization of flavonoids ingredients of MDT by using HPLC. The retention time of vitexin was found at 11.19 min in the MDT. The HPLC fingerprint of MDT is show in Fig. 1.

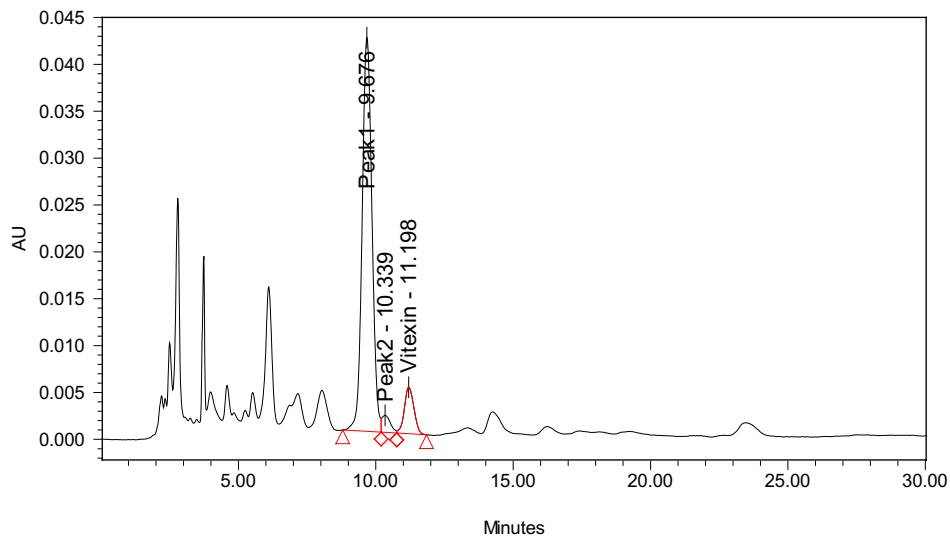


Figure 1. HPLC fingerprint profile of MDT.

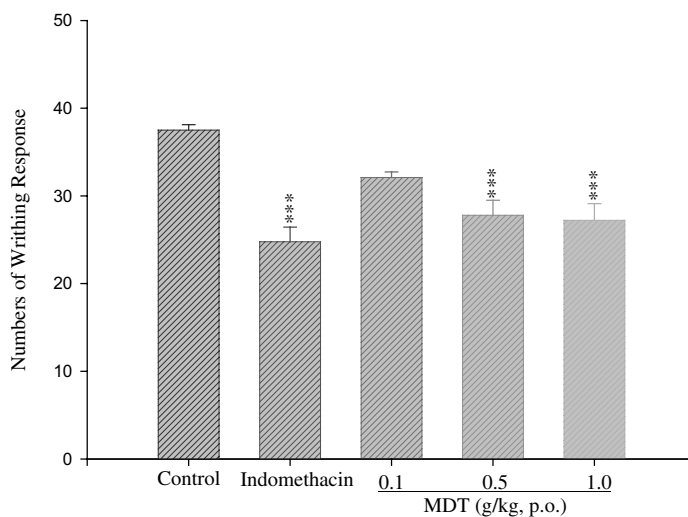


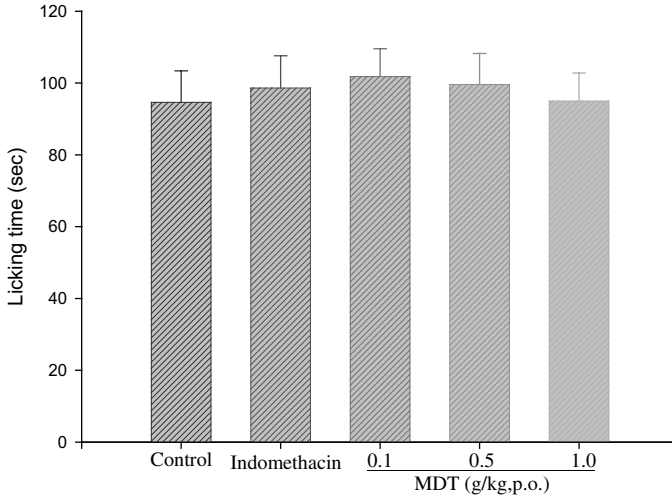
Figure 2. Analgesic effects of the methanol extract of the root of MDT and indomethacin on acetic acid-induced writhing in mice. Each value was represented as mean \pm SEM. *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

Effect of MDT on Acetic Acid Induced Writting Response

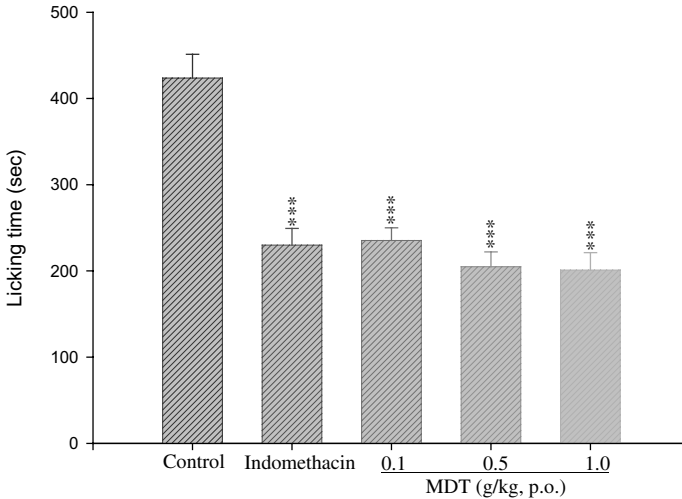
The results of acetic acid-induced writhing responses in mice which indicate the analgesic activity of the methanol extracts of MDT were presented in Fig. 2. It was found that the extract and indomethacin at the assayed doses caused a significant ($p < 0.001$) inhibition on the writhing responses induced by acetic acid when compared to the control.

Formalin Test

MDT demonstrated a dose-dependent relationship in late phase of the formalin induced pain. In the early phase, there were no significant inhibition at the doses of 0.1, 0.5, 1.0 g/kg MDT compared to the control group (Fig. 3A). In the late phase, the doses of 0.1, 0.5 and 1.0 g/kg significantly reduced the nociception similar to indomethacin (10 mg/kg) (Fig. 3B).



(A)



(B)

Figure 3. Effects of the methanol extract of MDT and indomethacin on the (A) early phase and (B) late phase of formalin test in mice. Each value was represented as mean \pm SEM. *** $p < 0.001$ when compared to the control group (one-way ANOVA followed by Scheffe's multiple range test).

Effects of MDT on λ -Carrageenan-Induced Mice Paw Edema

λ -Carrageenan-induced paw edema was significantly reduced in a dose-dependent manner by the treatment of MDT at the 3rd, 4th, 5th, and 6th hour after λ -carrageenan treatment. A significant anti-edema effect of MDT was observed at the 3rd, 4th, 5th, and 6th hour after λ -carrageenan injection ($p < 0.01$). At the third hour, the effect of anti-inflammatory of MDT reached its maximum after λ -carrageenan injection ($p < 0.01$). A significant anti-inflammatory effect of MDT at the dose of 0.5, 1.0 g/kg was similar to indomethacin (Fig. 4).

Effects of MDT on NO Level

In our assay data, the NO level in the edema paw induced by λ -carrageenan was largely increased. There is a significant effect in the NO level when treating with MDT (0.1, 0.5 and 1.0 g/kg). The treatment with 10 mg/kg indomethacin significantly decreased the NO level (Fig. 5).

Effects of MDT on MDA Level

The MDA level in the edema paw induced by λ -carrageenan increased significantly. However, the MDA level was largely decreased when treated with 0.1, 0.5 and 1.0 g/kg MDT as well as 10 mg/kg indomethacin (Fig. 6).

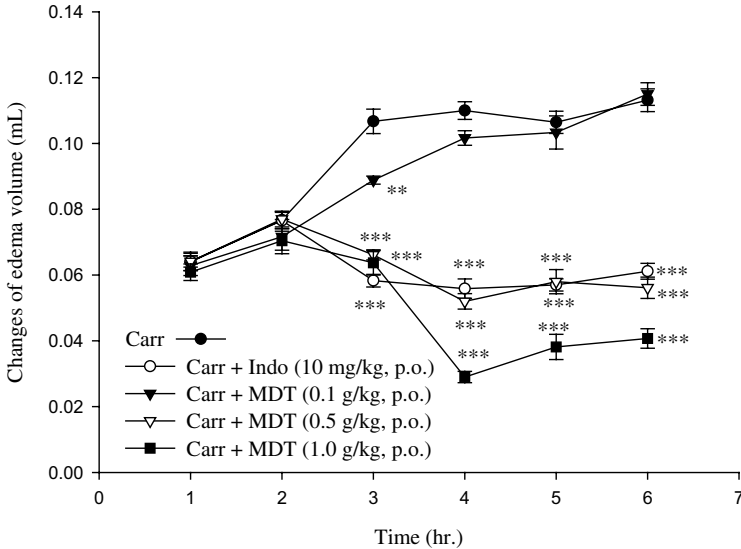
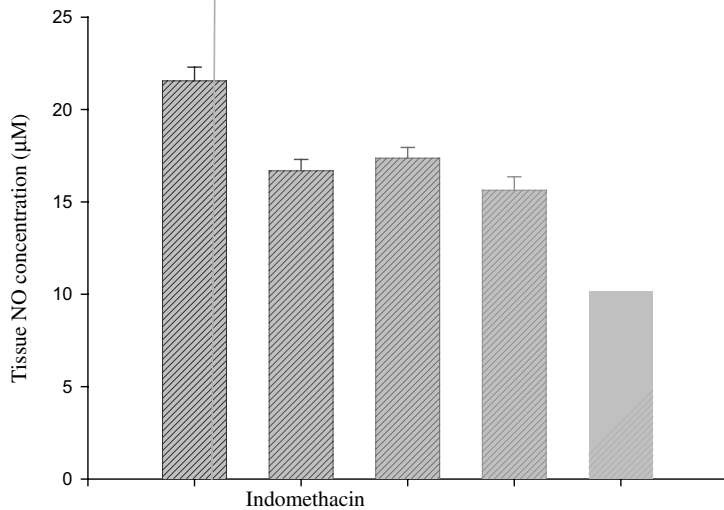


Figure 4. Effects of the methanol extract of MDT and indomethacin on hind paw edema induced by λ -carrageenan in mice. Each value was represented as mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$ when compared to the λ -carrageenan (Carr) group (one-way ANOVA followed by Scheffe's multiple range test).



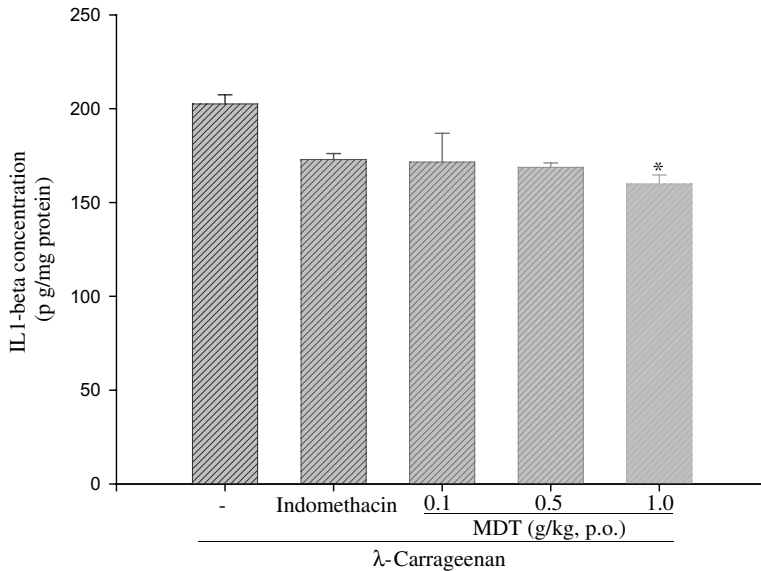


Figure 7. Effects of the methanol extract of MDT and indomethacin on the tissue IL-1 β concentration of paw in mice. Each value was represented as mean \pm SEM. * $p < 0.05$, when compared to the λ -carrageenan group (one-way ANOVA followed by Scheffe's multiple range test).

Effects of MDT on IL-1 β Level

In assay data, the IL-1 β level in the edema paw induced by λ -carrageenan increased significantly. There is a significant effect of MDT (0.5 and 1.0 g/kg) on the IL-1 β level (Fig. 7).

Effects of MDT on TNF- α Level

In assay data, the TNF- α level in the edema paw induced by λ -carrageenan increased significantly. There is a significant effect of MDT (0.1, 0.5 and 1.0 g/kg) on the TNF- α level (Fig. 8).

Effects of MDT on the Activities of Antioxidant Enzymes

Liver tissues, collected at the 3rd hour following the intra paw injection of λ -carrageenan, were also analyzed for the biochemical parameters, such as SOD, GPx and GRd activities (Table 1). SOD activity in liver tissue was decreased significantly by λ -carrageenan administration. SOD activity was increased significantly after treated with 0.5, 1.0 g/kg MDT and 10 mg/kg indomethacin ($p < 0.001$). λ -Carrageenan administration markedly decreased GPx and GRd activities in the liver. GRd activities of the liver were increased significantly by MDT (0.1, 0.5, 1.0 g/kg), as well as indomethacin (10 mg/kg).

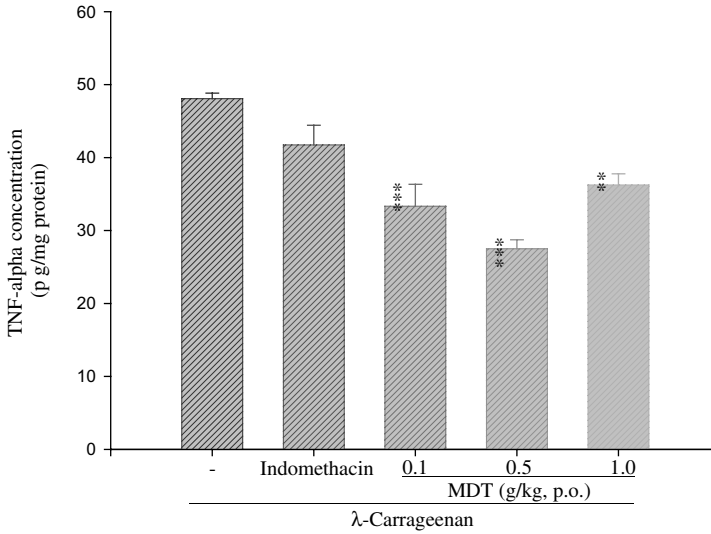


Figure 8. Effects of the methanol extract of MDT and indomethacin on the tissue TNF- α concentration of paw in mice. Each value was represented as mean \pm SEM. ** $p < 0.01$, *** $p < 0.001$ when compared to the λ -carrageenan group (one-way ANOVA followed by Scheffe's multiple range test).

Table 1. Effects of the Methanol Extract of *D. triflorum* (MDT) and Indomethacin (Indo) on SOD, GPx, and GRd Activities in the Livers of Mice

Groups	SOD (U/mg Protein)	GPx (U/mg Protein)	GRd (U/mg Protein)
Carr	26.99 \pm 2.52	1.03 \pm 0.04	0.067 \pm 0.007
Carr + Indo	41.41 \pm 2.33***	1.17 \pm 0.05	0.089 \pm 0.009*
Carr + MDT 0.1	29.87 \pm 1.51	1.09 \pm 0.10	0.118 \pm 0.025***
Carr + MDT 0.5	33.55 \pm 1.35***	1.18 \pm 0.04*	0.133 \pm 0.01***
Carr + MDT 1.0	41.47 \pm 2.14***	1.13 \pm 0.07	0.091 \pm 0.005**

Discussion

The acetic acid-induced abdominal writhing is a sensitive method to evaluate peripherally acting analgesics. As shown in Figs. 2 and 3B, it is suggested that MDT possessed analgesic effects in both models of acetic acid-induced writhing and formalin tests. In acetic acid-induced abdominal writhing, the visceral pain model, released arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis played a role in the nociceptive mechanism (Franzotti *et al.*, 2000). This model of response is thought to be mediated by peritoneal mast cells (Ronaldo *et al.*, 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathway. Results of the present studies show that MDT produced significant analgesic effect which may be due to the inhibition of the synthesis of the arachidonic acid metabolite.

Recent studies have shown that the early phase of formalin induced pain reflects the direct effect of formalin on nociceptors, whereas the late phase reflects that inflammatory

pain appeared to be attributable to prostaglandin synthesis (Shibata *et al.*, 1989; Tjølsen *et al.*, 1992). Our result showed that the MDT exerted significant inhibitory effect on nociceptive response of the late phase of the inflammatory pain model in the formalin test. The formalin test may be a more useful model of clinical pain in which the late phase was dependent on peripheral inflammation and changes in central processing (Tjølsen *et al.*, 1992). The histamine, serotonin, prostaglandins, nitric oxide and bradykinin are involved in the late phase of the formalin test (Tjølsen *et al.*, 1992). There is strong evidence that peripheral inflammatory procedure is involved in the late phase. The inhibitory effect of MDT on nociceptive response in the late phases of formalin test suggested that the anti-nociceptive effect of MDT could be due to its peripheral action.

λ -Carrageenan-induced paw edema as an *in vivo* model of inflammation is the most frequently used method to evaluate the anti-edematous effect of natural products. In different animal models of acute inflammation, λ -carrageenan-induced inflammation has been applied to study the free radical generation in liver tissues after inflammatory states (Lu *et al.*, 2007). In our study, MDT and indomethacin showed anti-inflammatory effects in λ -carrageenan-induced mice paw edema. A previous study indicated that the 3rd hour of the edema induced by λ -carrageenan, in which the edema effect reached its maximum (Kirkova *et al.*, 1992), is characterized by the presence of prostaglandins and other compounds of slow reaction (Spector and Willoughb, 1963). Ueno *et al.* (2000) found that the injection of λ -carrageenan into the rat paw induced the liberation of bradykinin, and then further induced the biosynthesis of prostaglandin and other autacoids. However, in the λ -carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism (Nantel *et al.*, 1999).

Experimental results suggested that the mechanism of action of MDT may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of indomethacin in the inhibition of the inflammatory process induced by λ -carrageenan (Di *et al.*, 1971). Furthermore, the classification of anti-nociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system (Planas *et al.*, 2000).

In the studies of mechanism on the inflammation, L-arginine-NO pathway has been proposed to have an important role in the λ -carrageenan-induced inflammatory response (Salvemini *et al.*, 1996). Our present results also confirmed that λ -carrageenan-induced paw edema model resulted in NO production. The expression of the inducible isoform of NO synthase has been proposed as an important mediator of inflammation (Cuzzocrea *et al.*, 1997). In this study, the level of NO was decreased significantly by treatment with 0.1, 0.5 and 1.0 g/kg MDT (Fig. 4). We suggested the anti-inflammatory mechanism of MDT may be through the L-arginine-NO pathway because MDT did significantly inhibit the NO production.

Current studies indicated that inflammatory effect induced by λ -carrageenan is associated with free radicals. The λ -carrageenan-induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as superoxide, hydroxyl radicals and hydrogen peroxide, as well as due to the release of other

neutrophil-derived mediators (Dawson *et al.*, 1991). Free radical, prostaglandin and NO will be released when administrating with λ -carrageenan for 1–6 hours (Dudhgaonkar *et al.*, 2006).

Janero (1990) indicated that MDA production is due to free radical attack in the plasma membrane. Therefore, inflammatory effect would result in the accumulation of MDA. Glutathione is known as an oxyradical scavenger. Enhancing the level of glutathione then reduces the MDA production. Cuzzocrea suggested that endogenous glutathione plays an important role against carrageenan-induced local inflammation (Cuzzocrea *et al.*, 1999). In this study, there was a significant increase in SOD and GRd activities with MDT treatment. Furthermore, there was a significant decrease in MDA level with MDT treatment. Therefore, we assumed that the suppression of MDA production was probably due to the increases in SOD and GRd activities.

Phytochemical investigations showed that the leaves of DF have a total alkaloid content of 0.01–0.15%, and the roots have a total alkaloid content of 0.01–0.018% (Ghosal *et al.*, 1971). Flavonoids are very important components in curing the diseases because of their potent antioxidant medical properties. Flavonoids are reported to be good antioxidants and anti-inflammatory agents. The above investigations showed that the DF contained alkaloids and flavonoids. In recent studies, it was reported that alkaloids and flavonoids possess analgesic and anti-inflammatory effects (Govindarajan *et al.*, 2007).

Phytochemical analysis also shows the presence of about 0.34% vitexin in the MDT. Many studies indicated that vitexin exhibited various biological and pharmacological activities, such as hypotensive, anti-inflammatory, anti-spasmodic (Prabhakar *et al.*, 1981), antimicrobial, antioxidant and radioprotective effects (Fu *et al.*, 2007). Vitexin has potent anti-inflammatory effects compared to its anti-histaminic, anti-bradykinin and anti-serotonin properties (Prabhakar *et al.*, 1981). Therefore, vitexin may be an active constitute of anti-inflammation in MDT.

Deraedt *et al.*, (1980) described an increase in the levels of prostaglandins PGE2 and PGF2 α during the first 30 min after acetic acid injection in writhing test. The intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Duarte *et al.*, 1988). It appears that the carrageenan-induced edema is related to the production of histamine, leukotrienes, platelet-activating factors, and possibly cyclooxygenase products. Thus, the result obtained for the writhing test using acetic acid is similar to those obtained for the edematogenic test using carrageenan, since MDT was effective in inhibiting the writhing response in mice. On the other hand, MDT decreased the production of inflammatory cytokines, IL-1 β and TNF- α . Therefore, we suggested that the anti-inflammatory mechanism of MDT may be related to the decreases in the production of IL-1 β and TNF- α .

In conclusion, MDT possessed analgesic and anti-inflammatory effects. The anti-inflammatory mechanisms of MDT might be related to the decreases in the levels of MDA and NO in the edema paw via increasing the activities of SOD, GPx and GR in the liver and decreasing the levels of IL-1 β and TNF- α in the serum of mice. MDT may be used as a pharmacological agent for preventing and treating diseases in which free radical formation is a pathogenic factor.

Acknowledgments

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