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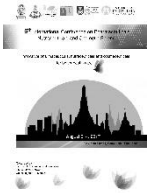

Antioxidative and Anti-inflammatory Activity Effects of *Datura metel* Leave Extract

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Introduction

Datura metal L. (Solanaceae family) is an annual herb that grows in the wild and the warmer regions of the world. Recently, *D. metal* is being widely cultivated worldwide for its chemical, pharmacological, and ornamental properties. Its leaves have been used as anesthetics in surgery, a fumigant in bronchial ashma, and anti-contractive agents in the stomach ulcers.[1] In Thailand, *D. metal* was called Lum-Pong and have been used in ethnomedicinal practices for treatment of hemorrhoids.[2] The withanolide-type steroids have been reported as the major chemical constituents of *D. metal*. Besides, several megastigmane sesquiterpenes and amide alkaloids from *D. metal* were also reported.[3] In this study, we have focused on the effect of this plant on antioxidation and hemorrhoids-related inflammatory factors inhibition. Antioxidants are found in many natural plant and various natural phytochemicals are extremely influential agents in health promotion and disease prevention. They catch oxidant effectively or terminate the free radical chain-reaction and reduce the oxidative stresses from external and intrinsic resources in human physical condition.[4] The exposure to oxidative stress induces inflammation of healthy organs and tissues.[5] Hemorrhoids, one of the most common anorectal inflammation around the world, are the vascular cushions within the anal canal usually found in three main location: left lateral, right anterior and right posterior portions.[6] Several factors are involved in causing hemorrhoids including irregular bowel function (constipation, diarrhea), exercise, gravity, low fiber diet, pregnancy, obesity, high abdominal pressure, prolonged sitting, genetic factors, and aging.[7] Symptoms of hemorrhoids include bleeding, mucosal or fecal soiling, itching and, occasionally, pain, which, if left untreated, continue to cause physical and social problems to patients.[8] The inflamed hemorrhoid can leak mucus that can cause inflammation of the skin surrounding the anus causing burning, bleeding and itching. Most importantly, these symptoms might be the sign of colon cancer. During inflammatory process, the inflammatory biomarkers are highly produced such as reactive oxygen species (ROS), reactive nitrogen species (RNS), tumornecrosis factor-α (TNF-α), interleukin (IL)-1, IL-6, cyclooxygenase (COX)-2. COX-2 is an inducible isoform of prostaglandin synthase found in activated phagocytes that are responsible for inflammation. In addition to TNF-α, IL-1 and IL-6 also play roles as endogenous pyrogens by stimulating the release of prostaglandins catalyzed from COX.[9] In the case of inflammation in the response to pathogen invasion, there are several free radical generations for pathogen elimination, the RNS such as nitricoxide (NO) is produced from inducible nitric oxide synthase (iNOS), and ROS such as superoxide ($O2^-$) is generated.[9] Multiple topical and oral preparations are available for management of hemorrhoids as medical treatment including steroids, antiseptics, analgesics, vasoconstrictor and diltiazem. Due to several limited modern pharmacological therapeutic options available for treatment, the herbal medicines remain the choice of therapy. Flavonoid components have also been used in treatment of this disease.[10]

In Thai traditional medicine, *D. metel* have been used for treatment of hemorrhoids. They were used as single herbal drug. However, their anti-inflammatory mechanism on expression have not been established. Therefore, in the present work, semi-quantitative RT-PCR technique was used to determine anti-inflammation via the expression of IL-6, iNOS and TNF- α genes in murine RAW264.7 macrophages. Moreover, antioxidant

activities and total phenolic content of this plant were evaluated *in vitro* using DPPH and Folin-Ciocalteau method, respectively.

Methods

Preparation of plant extracts

The mature leaves of *Datura metal* were collected from Sam-Rong District, Ubon Ratchathani Province, Thailand. The plant was identified and authenticated by Somkao, P (taxonomist, lecturer on Thai pharmacy from Faculty of Thai Traditional and Alternative Medicine). The samples were dried, pulverized and macerated in 50% ethanol for 5 days, then filtered through thin cloth and centrifuged at 3000 g, 25°C for 10 min using laboratory centrifuge (Kubota, Japan). The clear supernatant was concentrated using a rotary evaporator (ETERA, Japan) at 45-50°C, then freeze-dried (Christ, Germany). The ethanolic extract of *D. metal* was obtained and called DME.

Determination of total phenolic content and DPPH radical scavenging activity

The total phenolics content was determined by the Folin-Ciocalteu method.[11] The sample solution was mixed with 0.25 mL of the 1N Folin-Ciocalteau reagent and 1.25 mL of 20% sodium carbonate. After mixing and standing for 40 minutes at the room temperature, the absorbance was measured at 725 nm. The total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g dry extract.

The radical scavenging activity was determined by DPPH method.[12] Briefly, the reaction was performed in 96 well-plates and the extract was dissolved in 50% ethanol to various concentrations and then the extract solutions were mixed with 20 µl of DPPH solution (1 mM in methanol). The mixture was incubated at room temperature in the dark for 30 min. The mixture absorbance was measured at 515 nm using a UV spectrophotometer (Shimadzu, Japan). The DPPH solution alone in methanol was used as a control. All the tests were performed in triplicate. Ascorbic acid (Vit.C) and Tocopherol (Vit.E), two positive controls for the DPPH method, were used as a standard antioxidant.

Cell culture and Cytotoxicity test

Macrophage RAW 264.7 cells were treated with various concentrations of extract and then incubated at 37ºC in the humidified atmosphere with 5% CO_2 for 24h. Cell viability was analyzed by using MTT assay[13] and the absorbance measured at 570 nm. The results were calculated for % inhibition and expressed as 50% inhibitory concentration (IC_{50}).

Determination of gene expression

The cells were overnight cultured in 12-well plate and treated with various concentrations of extract and positive control. After incubation at 37ºC in the humidified atmosphere with 5% CO_2 for 22 hr, the LPS was added then further incubated for 2 hr. Total RNA was extracted from the treated cells by using a GE Healthcare extraction kit. The first-strand cDNA was synthesized from total RNA (40ng) with Omniscript reverse transcriptase kit. The primers were used for amplifying the respective fragments. Polymerase chain reaction (PCR) was performed by incubation of each cDNA sample with the primers, Taq polymerase, and deoxynucleotide mix. Amplification was completed for 30 cycles and the conditions for PCR amplification followed previous reports.[9] The PCR products were then analyzed on 1.5% agarose gel, visualized by NovelJoice staining and RT-PCR product densities measured by Gel Documentation and System Analysis machine. The inflammatory-related gene expressions were calculated for the relative mRNA expression level compared with β-actin.

Results

Phenolic content and antioxidative activity of DME

The DME with 15.50% yield exhibited moderate antioxidant activity (IC_{50} of DPPH at 98.59± 3.04 µg/mL) and phenolic contents as shown in Table 1.

Table 1 Phenolic content and antioxidant activity of DME

Test sample	Yield (%)	Total phenolic content* GAE (µg/mg extract)	DPPH IC_{50}* (µg/mL)
DME	15.50	153.89 ± 0.37	98.59±3.04
Ascorbic acid	-	-	3.21±0.10
Tocopherol	-	-	6.99±0.12

* Values are expressed as mean±SD (n=3)

Toxicity effect of DME on RAW 264.7 cells

The effect of DME on the viability of RAW264.7 cells was determined using MTT assay. The cells were treated for 24 hr with various concentrations of DME at 0-300 µg/mL. Figure 1 showed toxic effects on RAW264.7 cells as IC_{50} value (128.67±10.20 µg/mL). Based on these results, we evaluated the effect of DME on anti-inflammation at doses lower than its IC_{50} value.

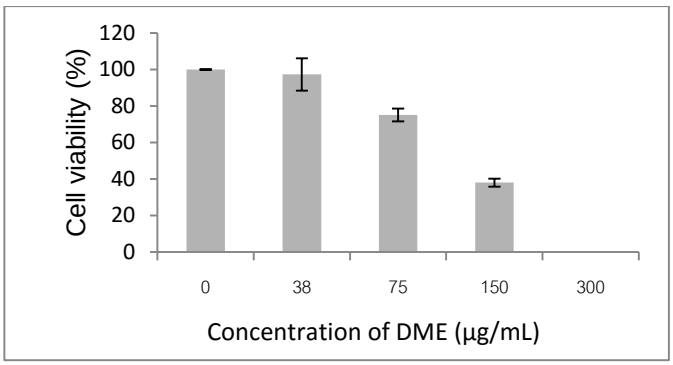

Figure 1: Effect of DME on RAW264.7 cells viability. Each value is a mean±SD compared to control from three individual experiments.

Effect of DME on the pro-inflammatory gene expression

The expression of the pro-inflammatory gene were not changed when treatment with DME alone but were up-regulated after treatment with LPS compared with control. The gene expression of IL-6 was significantly suppressed by DME in a higher level than that of indomethacin on IL-6 gene expression (Figure 2A). Similarly, DME also exhibited significant suppressive effect on the expression of iNOS (Figure 2B) and TNF-α (Figure 2C).

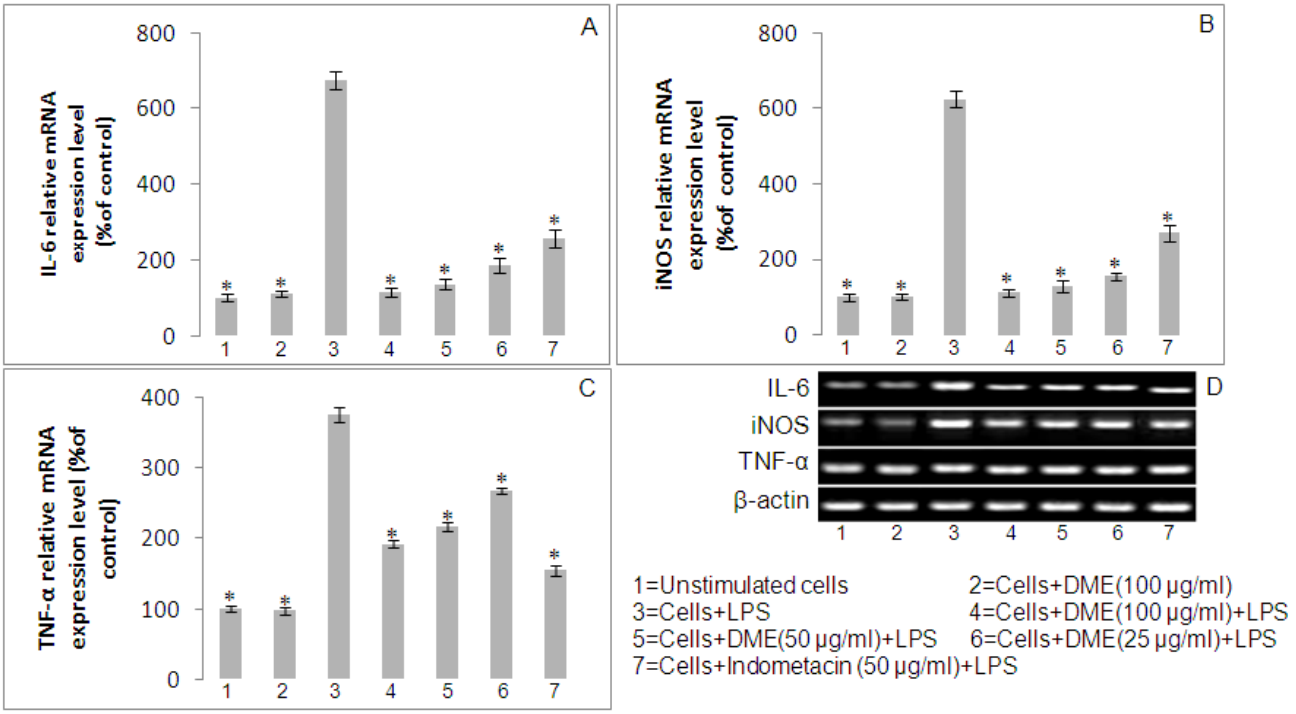

Figure 2: Inhibitory effect of DME on mRNA expression of pro-inflammatory mediators, relative mRNA expression of IL-6 (A), iNOS (B), TNF-α (C) compared with β-actin mRNA expression and the amplified bands of IL-6, iNOS, TNF-α (D). * Significant difference from LPS treatment alone ($p<0.05$)

Discussion

As shown in Table 1, DME exhibited moderate phenolic contents and antioxidant activity. Phenolic is strongly associated with antioxidant activity and antioxidant is related to anti-inflammation.[14] The exposure to oxidative stress induces inflammation of healthy organs and tissues. Therefore, in this research we have evaluated *in vitro* antioxidant activity using DPPH and anti-inflammatory activity in macrophages RAW 264.7 cells. Macrophages are the first line of host defense against inflammation and play an important role in adaptive immune response.[15] Macrophages exposed to stimulating agents such as bacterial lipopolysaccharides (LPS) release several inflammatory cytokines and other substances including interleukin-1 (IL-1), IL-6, IL-8 and tumor necrosis factor-α (TNF-α).[16] Our finding found that DME also exhibited anti-inflammation. *In vitro* RT-PCR revealed the significant dose-dependent inhibitory effects of DME on several pro-inflammatory genes expressions including IL-6, iNOS and TNF-α in LPS-induced RAW264.7 murine macrophage cells (Figure 2). The results were consistent with the hypothesis because *D. metal* was selected for this study due to its ethnopharmacological effects and chemical constituents. Its chemical compounds, withanolide-type steroids, have shown several remarkable pharmacological effects, including inhibition of Nitric Oxide production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Besides, several megastigmane sesquiterpenes and amide alkaloids which related to anti-inflammation were also reported from *D. metal*.[1] Moreover, Nine new withanolides from *D. metel* exhibited inhibition of nitrite production and antioxidant.[17] However, *D. metal* showed high toxicity on RAW264.7 murine macrophage cells (IC_{50} value was 128.67±10.20 µg/mL). Therefore, the dose uses of this plant must be concerned.

Conclusion

In conclusion, the present study demonstrated moderate phenolic content and antioxidant activity of ethanolic extract of *D. metal* leave extract. In addition, the results revealed the potential effects of the *D. metal* leaves extract on anti-inflammation activities. Our finding is the first molecular evidence to explain the anti-inflammatory effect of these plants which could at least be a part support the effectiveness of traditional utilization of these plants on hemorrhoids treatment.

Acknowledgements

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References

1. Mai NT, Cuc NT, Anh HLT, Nhiem NX, Tai BH, Yen PH, Quang TH, Minh CV, Kim KW, Kim YC, Oh H, Kiem PV. Two new guainane sesquiterpenes from *Datura metal* L. with anti-inflammatory activity. Phytochemistry Letters. 2017;19:231-6.
2. Sang-arun C. 2014. Thai Pharmacy: General Traditional medical texts, pharmaceutical fields, Division of health professionals. E-book.
3. Kuang HX, Yang BY, Xia YG, Wang QH. Two new withanolide lactones from flos daturae. Molecules. 2011;16: 5833–9.
4. Li WJ, Lin YC, Wu PF, Wen ZH, Liu PL, Chen CY, Wang HM. Biofunctional constituents from *Liriodendron tulipifera* with antioxidants and anti-melanogenic properties. International Journal of Molecular Sciences. 2013;14:1698-1712.
5. Lee CC, Chen YT, Chiu CC, Liao WT, Liu YC, David Wang HM. *Polygonum cuspidatum* extracts as bioactive antioxidation, anti-tyrosinase, immune stimulation and anticancer agents. Journal of Bioscience and Bioengineering. 2015;119(4):464-9.
6. Dey YN, Wanjari MM, Kumar D, Lomash V, Jadhav AD. Curative effect of *Amorphophallus paeoniifolius* tuber on experimental hemorrhoids in rats. Journal of Ethnopharmacology. 2016;192:183-191.
7. Mosavat SH, Ghahramani L, Sobhani Z, Haghighi ER, Chaijan MR, Heydari M. The effect of leek (*Allium iranicum* (Wendelbo)) leaves extract cream on hemorrhoid patients: A double blind randomized controlled clinical trial. European Journal of Integrative Medicine. 2015;7:669-73.
8. Gupta PJ, Kalaskar S. Radiowave ablation and mucopexy for prolapsing hemorrhoids-A pilot study. International Journal of Surgery. 2009;7:223-7.
9. Fangkrathok N, Junlatat J, Sripanidkulchai B. *In vivo* and *in vitro* anti-inflammatory activity of *Lentinus polychrous* extract. Journal of Ethnopharmacology. 2013;147:631-7.
10. Alonso-Coello P, Zhou Q, Martinez-Zapata MJ, Mills E, Heels-Ansdell D, Jahanson JF, Guyatt G. Metha-analysis of flavonoids for the treatment of haemorrhoids. British Journal of Surgery. 2006;93:909-920.
11. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin cioalteau reagent. Methods in Enzymology. 1999;299:152-178.
12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992;40:945-8.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assay. Journal of Immunological Methods. 1983;65:55-63.
14. Eom HJ, Kang HR, Kim HK, Jung EB, Park HB, Kang KS, Kim KH 2016. Bioactivity-guided isolation of antioxidant triterpenoids from *Betula phatyphylla* var. *japonica* bark. Bioorganic Chemistry. 2016;66:97-101.
15. Verstovsek S, Maccubbin D, Mihich E. Tumoricidal activation of murine resident peritoneal macrophage by interleukin-2 and tumor necrosis factor. Cancer Research. 1992;52:3880-5.
16. Keller R, Keist R, Frei K. Lymphokines and bacteria that induce tumoricidal activity, trigger a different secretory response in macrophages. European Journal of Immunology. 1990;20:690-8.
17. Yang BY, Guo R, Li T, Wu JJ, Zhang J, Liu Y, Wang QH, Kuang HX. New anti-inflammatory withanolides from the leaves of *Datura metal* L. Steroids. 2014;87:26-34.