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# Anti-rheumatoidal effects of *Uncaria Tomentosa* and *Maytenus* by a prolonged application

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## SUMMARY

*Uncaria Tomentosa* and *Maytenus* are known to have anti-inflammatory and anti-rheumatoidal effects after either a single application or application over a short-term period. We applied these natural products to Wister rats every day for two weeks and investigated the effects of this long-term application on inflammation. This was done by measuring footpad edema, which was induced by a locally injected carrageenan. There was a dramatic reduction in edema in both *U. Tomentosa*- and *Maytenus*-treated rats; furthermore, the reduction lasted as long as three days for rats treated with *U. Tomentosa*. When the Balb/C mice underwent similar treatment for one month, the level of IgM in the blood of *U. Tomentosa*-treated mice decreased while the level of IgG in *Maytenus*-treated mice increased. This suggests that the long lasting effects of *U. Tomentosa* may be related to a low level of IgM and the subclass switch from IgM to IgG. Since the anti-inflammatory effects of *U. Tomentosa* lasts for three days, it may prove useful in treating rheumatoid arthritis when applied for an extended period of time, especially since this product is known to have minimal side effects.

**Key words:** *Uncaria Tomentosa; Maytenus;* Footpad edema; IgM; IgG

## INTRODUCTION

Chronic rheumatoid arthritis (RA) is an inflammatory disease that affects the entire body, which is characterized by multi-inflammation of a joint. It is an autoimmune disease that leads to the deformation and destruction of joints. Major symptoms include complication-arthritis, such as neovascularization, lymphocyte infiltration of CD+ T-cells, abnormal proliferation of synovial cells, and bone destruction. Although numerous attempts to research this disease have been made, the cause of RA is still unknown. Currently, RA is treated with anti-rheumatoid drugs, non-steroid anti-inflammatory agents, adrenal cortex steroids, or immune modulators; however, the success of these drugs is somewhat tempered by their unavoidable side effects. Thus, the search for an effective anti-rheumatoid drug with minimal side effects continues.

We have chosen two natural products, *Uncaria Tomentosa* and *Maytenus*, which are known to have anti-inflammatory effects. The chemical ingredients of *U. Tomentosa* include the following: 6 alkaroids

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(Isopteropodine, Pteropodine, Isomitraphylline, Mitraphylline, Isorhynchophylline, Rhynchophylline); kinovic acid (an accelerating agent of anti-inflammation); and polyphenolic compounds (anti-ulcer agents and/or antioxidants) such as phenol glucoside, 2-hydroxybenzoic acid, folphenidin. The anti-inflammatory effects of these ingradients have been documented in literature. Yepez *et al.* reported that beta-sitosterol acid (one of the ingredients present in *Uncaria Guianensis*) and plant steroids (antioxidants) have anti-inflammation effects. They also found that quinovic acid glycosides (a newly found plant chemical from the bark and root of *Uncaria Guianensis*) are the ingredients that possess anti-inflammatory properties (Yepez *et al.*, 1991). Aquino *et al.* confirmed that the above described active ingredients, which are also found in *Uncaria Tomentosa*, have 46 - 69% anti-inflammatory functions, regardless of the conditions inside or outside of the body (Aquino *et al.*, 1988, 1990, 1991; Senatore *et al.*, 1989; Yasukawa *et al.*, 1989; Yepez *et al.*, 1991; Recio *et al.*, 1995). Dicarlo *et al.* observed an enhanced immunity in *Maytenus*-treated mice, an immunity that was associated with a dramatic increase of phagocytosis (Dicarlo *et al.*, 1964). Gonzalez *et al.* also observed the anti-inflammatory effects of triterpene and other anti-oxidants extracted from the bark of *Maytenus*, and they concluded that the ingredients of *Maytenus* clearly contribute to anti-inflammation, radiation protection and anti-cancer functions (Gonzalez *et al.*, 1982). It is important to note that the aforementioned anti-inflammatory effects were observed after either a single or short-term application of the products or ingredients.

In the present study, we applied either *Uncaria Tomentosa* or *Maytenus* to laboratory animals every day for 2 - 4 weeks and investigated the effects of this long-term application on footpad inflammation. We observed strong anti-inflammatory effects from these two products. We also observed that the effects last for 3 days in the case of *U. Tomentosa*. Therefore, we believe that *U. Tomentosa* might prove useful in treating inflammatory diseases like rheumatoid arthritis, especially since the side effects of drugs currently being used to treat such diseases are unacceptable.

## MATERIALS AND METHODS

### Footpad edema test

Wister male rats (4-week-old) were fed lab chews and tap water and kept in an environment maintained at 22 ± 3°C with 60 - 70% humidity. All rats were housed for a week prior to experiments. Each group consisted of 6 rats that were daily injected with either *U. Tomentosa* or *Maytenus* (500 ml/kg) for 14 days. These injections were forced into the stomach through the use of a catheter. The 6 control rats were also injected with distilled water. Following the 14 days of injection, 1.5% carrageenan (Wako, 039-09691) solution dissolved in PBS was subcutaneously injected into the footpad of the left hind leg (0.1 ml each). The subsequent swelling was measured with edema measuring equipment (Ugo Basile BM Co., Ltd.). Foot volume (ml) and edema ratio (%) were determined every hour for up to 4 h, and then every 12 h for up to 72 h following the carrageenan injection. The edema ratio (Dropsy ratio) was calculated from the following formula:

$$\text{Edema ratio (\%)} = ((Vt - Vo)/Vo) \times 100$$

where Vt is the foot volume (ml) at a given time after carrageenan injection and Vo is the foot volume (ml) immediately after carrageenan injection.

### Blood immunoglobulin determination

Balb/C mice (5-week-old) were fed lab chews and tap water and kept in an environment maintained at 22 ± 3°C with 60 - 70% humidity. All mice were housed for a week prior to experiments. Each group consisted of 6 mice that had either *U. Tomentosa* or *Maytenus* (500 ml/kg) injected daily into the stomach (via catheter, like the rats) for a

month. Mouse blood samples were then collected from the edge of an eye using a Haematokrit-Kappillaren capillary (75 mm/75 μl). Using a Kubota centrifuge, the serum was separated from the blood by spinning at 1,000 rpm for 10 min. Then the contents of IgE, IgG, and IgM in the serum were determined.

**Total IgE measurement**

The IgE-Mouse-Assay kit (RPN2704) from the Biotrak company was used. A diluted standard solution (100 μl) was placed in each well of a microtiter plate. Then, a sample solution (100 μl) was added. The plate was incubated with a cover plate for 30 min at room temperature (23°C) and washed 3 times using a wash buffer. Then, a TMB substrate solution (100 μl) was added and incubated for 15 min at room temperature for a color development. A stop solution (100 μl) was added, and the intensity of a color development was measured at 450 nm with a microplate reader (TOYOSODA Co., Ltd. Microplate-MPRA4).

**Total IgG and IgM measurements**

Mouse IgG ELISA quantification kit (E90-131), Mouse IgM ELISA quantification kit (E90-101), and ELISA starter accessory package (E101) from BETHYL Laboratories, Inc. company were used. A 96-well plate was pre-conditioned with a coating solution (100 μl) for 60 min at room temperature and washed 2 times, and then conditioned again with a postcoat solution (200 μl) for 30 min and washed 2 times. A diluted standard solution (100 μl) was placed in each well and then a sample solution (100 μl) was added. This multiwell plate was incubated for 60 min at room temperature with a cover plate and washed 4 times using a wash buffer. Then, a diluted goat anti-mouse IgG(IgM)-Fc-HRP solution (100 μl) was added, incubated for 60 min at room temperature, and washed 4 times. For a color development, a TMB substrate solution (100 μl) was added and incubated for 15 min at room temperature. A stop solution (100 μl) was added and the intensity of color development was measured at 450 nm with a microplate reader (TOYOSODA Co., Ltd. MPRA4).

## RESULTS

**Footpad edema test**

Following the injection of an inflammatory agent (carrageenan), footpad edema of the control rats increased for the first 12 h and then subsided thereafter (Table 1). The variation of edema measurement was smaller than 5%. For the rats treated with either *U. Tomentosa* or *Maytenus*, the

**Table 1.** Footpad Volume (ml)

| Time after carrageenan injection (hr) | Control | *U. Tomentosa* | *Maytenus* |
|---|---|---|---|
| 1 | 6.47 ± 0.15 | 6.52 ± 0.14 | 6.03 ± 0.12* |
| 2 | 6.47 ± 0.08 | 6.57 ± 0.23 | 6.17 ± 0.19 |
| 3 | 7.01 ± 0.11 | 6.92 ± 0.26 | 6.85 ± 0.34 |
| 4 | 7.33 ± 0.10 | 7.18 ± 0.30 | 7.15 ± 0.45 |
| 12 | 8.86 ± 0.18 | 8.28 ± 0.23* | 8.28 ± 0.24* |
| 24 | 8.23 ± 0.21 | 7.32 ± 0.30* | 7.31 ± 0.20** |
| 36 | 7.80 ± 0.29 | 6.73 ± 0.31* | 7.30 ± 0.18 |
| 48 | 7.61 ± 0.24 | 6.82 ± 0.36 | 7.49 ± 0.31 |
| 60 | 7.57 ± 0.16 | 6.52 ± 0.25** | 7.40 ± 0.28 |
| 72 | 7.54 ± 0.25 | 6.40 ± 0.28** | 7.48 ± 0.25 |

Each group is 6 rats. Data represent means ± SE (ml), and were analyzed by Student's t-test. Significant differences were detected between the control group and the two administration groups on 12 and 24 h (**$P < 0.01$, *$P < 0.05$).

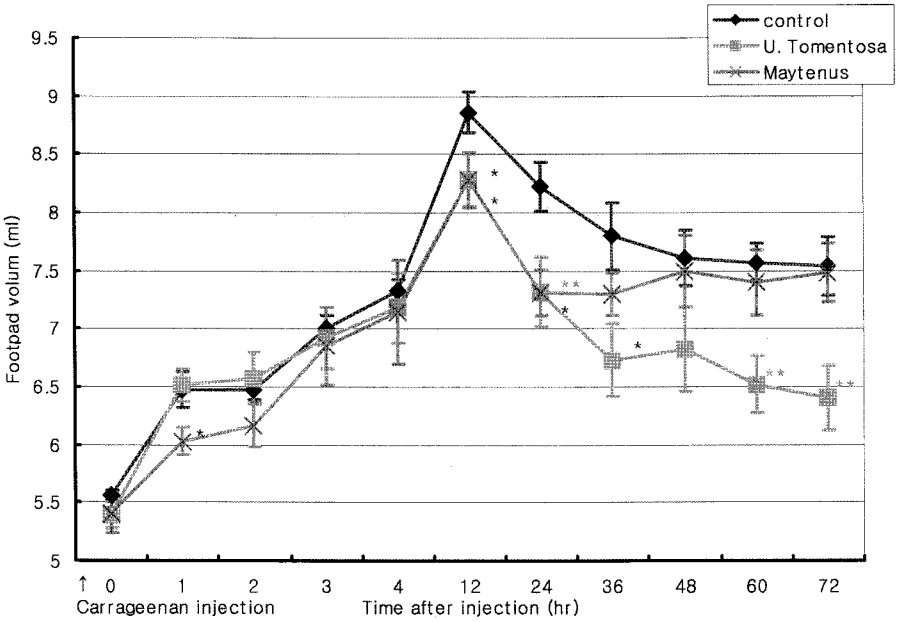


**Fig. 1.** This is the image of rat Footpad Volume (ml). Significant differences were detected between the control group and the two administration groups on 12 and 24 h (**$P$ < 0.01, *$P$ < 0.05).

initial increase in edema was very similar to the control group, but the edema 12 h after carrageenan injection was clearly smaller than the control group ($P$ < 0.05). This reduced state of edema was well maintained for another 60 h in the case of *U. Tomentosa*-treated rats, but only lasted for 12 h in the case of *Maytenus*-treated rats. As shown in Fig. 1, the Dropsy (edema) ratio for *U. Tomentosa*-treated rats not only significantly reduced ($P$ < 0.01) but also lasted for almost 3 days ($P$ < 0.05). The ratio for *Maytenus*-treated rats significantly reduced ($P$ < 0.05) as well, but lasted less than a day. Thus, we confirmed the anti-inflammatory effects of these two natural products when applied for a prolonged period of time. In addition, we observed a long lasting effect (nearly 3 days) in the case of *U. Tomentosa*.

**Blood immunoglobulin measurements**

For the study of immunoglobulin, the use of mice was inevitable because of available test kits in the market. When total blood IgE contents were determined, there was no difference among 3 different test. However, IgM contents significantly increased for *U. Tomentosa*-treated mice ($P$ < 0.05) while IgG contents did not. Interestingly, the situation is completely reversed for *Maytenus*-treated mice, where IgG contents significantly increased ($P$ < 0.01) while IgM contents did not groups (Table 2).

**Table 2.** Effect of *Uncaria Tomentosa* and *Maytenus* on total IgG and IgM in mouse serum

| Groups | Total IgG (ng/ml) Mean±SE | IgM (ng/ml) Mean ± SEM |
|---|---|---|
| Control | 368 ± 22.6 | 732 ± 36.8 |
| Uncaria Tomentosa | 387 ± 14.9 | 784 ± 40.5* |
| Maytenus | 412 ± 28.6* | 721 ± 47.3 |

*Significant difference ($P$ < 0.05) between control group and *Uncaria Tomentosa* and *Maytenus* group by Duntt's test.

## DISCUSSION

By using two natural products (*Uncaria Tomentosa* and *Maytenus*) for an extended period of time (2 weeks), we observed dramatic anti-inflammatory effects in the footpad of Wister rats (Table 1). We also observed that these effects last a long time (almost 3 days) in the case of *Uncaria Tomentosa* (Fig. 1). This observation is quite encouraging, and points towards a promising alternative for the treatment of inflammatory-associated diseases, like rheumatoid arthritis (RA). Use of *Uncaria Tomentosa* over a prolonged period of time may prove to be an effective alternative treatment for RA, with minimal side effects, which will be a better alternative to the drugs currently being used as treatment, whose side effects outweigh the intended anti-inflammatory effects.

Chronic rheumatoid arthritis (RA) is characterized by multi-inflammation of a joint. It is an autoimmune disease that leads to the deformation and destruction of joints. Common observations for RA are neovascularization, lymphocyte infiltration of CD4+ T-cells, abnormal proliferation of synovial cells, and bone destruction (Mosmann *et al.*, 1986). As reported in literature, various cytokines play complicated roles at the lesion of RA. For instance, inflammatory Th1-type cytokines (TNF-alpha, IL-1, IL-6, IL-12) and anti-inflammatory Th2-type cytokines (IL-4, IL-13) are often observed together with chemokines (Feldmann *et al.*, 1996a, b). T-helper (Th) cells produce IL-2 and IFN-gamma, and consist of Th1-type cells for cellular immunity and Th2-type cells for humeral immunity and IgE-mediated allergy response while producing IL-4 and IL-5 (Lanzavecchia, 1985; Feldmann *et al.*, 1996b). These Th1/Th2-type cytokines are often unbalanced in many immune diseases. For example, chronic autoimmune diseases such as type 1 diabetes and Hashimoto thyroiditis are associated with Th1-type cytokines. Total body autoimmune diseases such as Erymatodes are associated with Th2-type cytokines (Miossec and van den Berg, 1997). Among other cytokine cascade intermediates, TNF-alpha, IL-1, IL-6, and GM-CSF are closely associated with the development of RA. For example, collagen-induced arthritis (CIA) is enhanced by injecting these cytokines, but then inhibited by neutralizing antibodies. Furthermore, a TNF-alpha transgenic mouse (Keffer *et al.*, 1991) and an IL-1 humoral antagonist deficient mouse with excess function of IL-1 can develop arthritis similar to RA and became an animal model for new arthritis. These incidents indirectly support the possibility that excess function of cytokines is involved in the progression of arthritis. The fact that IL-4 and IL-10 are injected to treat CIA by inhibiting Th1-type cellular function, and that injections of anti-IL-10 and anti-TGF-beta antibodies can further deteriorate arthritis, may implicate a natural defensive function of IL-10 and TGF-beta that are internally produced (Miossec and van den Berg, 1997). In the present study, we observed altered contents of total blood IgG and IgM (Table 2). This difference could be related to the duration of anti-inflammatory effects (Fig. 1). Therefore, further studies exploring the relationship between various cytokines and the anti-inflammatory effects of *U. Tomentosa* and *Maytenus* may be warranted.

The production of IgG and IgM is associated with B cells in the blood. Once infected, a primary defense is placed by basophil and macrophage, and exposed B cells present the antigen to Th2 cells involving the class II MHC (Lanzavecchia, 1985). These antigen-associated Th2 cells can release IL-4 and IL-13, and the released IL-4 can enhance differentiation and proliferation of Th0 cells. At the same time, B cells further differentiate and proliferate, while Plasmacyte produce an antigen-specific IgG and promote opsonization of the IgG. As a result, neutrophil-detecting ability sharply increases, thereby producing pus from dead neutrophils and tissues that are under attack. In regards to the subclass switch from IgM to IgG, Kopf *et al.* emphasized that IL-4 plays an important

role in the switch, based on the fact that the contents of IgG1 and IgE are very low in the IL-4 knockout mice (Kopf *et al.*, 1993). Further, IFN-gamma induces the production of an isotype IgG2a (Stevens *et al.*, 1988) that inhibits the production of IgG3, IgG1, IgG2b and IgE. On the contrary, IL-4 induces IgG1 and IgE (Vitetta *et al.*, 1985; Coffman and Carty, 1986), which inhibit the production of IgG3, IgG2a, and IgG2b (Noma *et al.*, 1986). These observations may be summarized as Th2 cells are activated to promote allergy and Th1 cells to suppress allergy, since Th1 cells produce IgG2a upon the activation of IFN-alpha and Th2 cells produce IgE upon the activation of IL-4. In the present study, we observed a significant decrease in IgM for *U. Tomentosa*-treated mice while a significant increase in IgG for *Maytenus*-treated mice following one month long applications (Table 1). If the subclass switch from IgM to IgG is functional in the mice, it is highly probable that the long lasting anti-inflammatory effects observed for *U. Tomentosa*-treated mice are related to the subclass switch, in which Th2 cells might be activated for the promotion of allergy suppressing cytokines. It is an open question what cytokines are produced and how these cytokines are inter-related to result in a long lasting suppression of inflammation.

In addition, as a result of virulence test of *U. Tomentosa* and *Maytenus*, the toxicity was not recognized (Yamashita and Gu, 2005).

## REFERENCES


Aquino R, De Simone F, Pizza C. (1988) New Quinovic Acid Glycosides from Uncaria Tomentosa. *J. Nat. Prod.* **51**, 257-261.

Aquino R, De Simone F, Vincieri FF, Pizza C, Gacs-Baitz E. (1990) New polyhydroxylated triterpenes from Uncaria tomentosa. *J. Nat. Prod.* **53**, 559-564.

Aquino R, De Feo V, De Simone F, Pizza C, Cirino G. (1991) Plant metabolites. New compounds and anti-inflammatory activity of Uncaria tomentosa. *J. Nat. Prod.* **54**, 453-159.

Coffman RL, Carty J. (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. *J. Immunol.* **136**, 949-954.

Dicarlo FJ, Haynes LJ, Sliver NJ, Phillips GE. (1964) Reticulo endothelial system stimulants of botanical orgin. *J. Reticuloendothel. Soc.* **64**, 224-232.

Feldmann M, Brennan FM, Maini RN. (1996) Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* **14**, 397-440.

Feldmann M, Brennan FM, Maini RN. (1996) Rheumatoid arthritis. *Cell* **85**, 307-310.

Gonzalez JG, delle Monache G, delle Monache F, Marini-Bettolo GB. (1982) Chuchuhuasha - a drug used in folk medicine in the Amazonian and Andean areas. A chemical study of Maytenus laevis. *J. Ethnopharmacol.* **5**, 73-77.

Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. (1991) Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* **10**, 4025-4031.

Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. (1993) Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* **362**, 245-248.

Lanzavecchia A. (1985) Antigen-specific interaction between T and B cells. *Nature* **314**, 537-539.

Miossec P, van den Berg W. (1997) Th1/Th2 cytokine balance in arthritis. *Arthritis Rheum.* **40**, 2105-2015.

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348-2357.

Noma Y, Sideras P, Naito T, Bergstedt-Lindquist S, Azuma C, Severinson E, Tanabe T, Kinashi T, Matsuda F, Yaoita Y, *et al.* (1986) Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. *Nature* **319**, 640-646.

Recio MC, Giner RM, Manez S, Rios JL. (1995) Structural requirements for the anti-inflammatory activity of natural triterpenoids. *Planta Med.* **61**, 182-185.

Senatore A, Cataldo A, Iaccarino FP, Elberti MG. (1989) Phytochemical and biological study of

Uncaria tomentosa. *Boll Soc. Ital. Biol. Sper.* **65**, 517-520.

Stevens TL, Bossie A, Sanders VM, Fernandez-Botran R, Coffman RL, Mosmann TR, Vitetta ES. (1988) Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* **334**, 255-258.

Vitetta ES, Ohara J, Myers CD, Layton JE, Krammer PH, Paul WE. (1985) Serological, biochemical, and functional identity of B cell-stimulatory factor 1 and B cell differentiation factor for IgG1. *J. Exp. Med.* **162**, 1726-1731.

Yamashita T, Gu YH. (2005) Anti-rheumatic, Anti-inflammatory and Analgetic Effects of *Cat's-claw, Iporuru, Chuchuhuasi, and Devil's-claw* Combination. *Med. Bio.* **149**, 204-210.

Yasukawa K, Takido M, Takeuchi M, Nakagawa S. (1989) Effect of chemical constituents from plants on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice. *Chem. Pharm. Bull.* **37**, 1071-1073.

Yepez AM, de Ugaz OL, Alvarez CM, De Feo V, Aquino R, De Simone F, Pizza C. (1991) Quinovic acid glycosides from Uncaria guianensis. *Phytochemistry* **30**, 1635-1637.