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Analgesic and Antiinflammatory Activity of *Morinda citrifolia* L. (Noni) Fruit

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M. citrifolia **is a tropical plant with a long tradition of medicinal use in Polynesia and tropical parts of eastern Asia and Australia. One of its favorite uses is the treatment of painful inflammatory conditions, such as arthritis. The analgesic activity of Noni fruit puree on mice was investigated using the hot plate test. A 10% solution of freeze concentrated Noni fruit puree in the drinking water of mice reduced the pain sensitivity comparably to the central analgesic drug tramadol. This effect was only partly reversed by the application of the morphine antagonist naloxone. An alcohol extract of noni fruit puree also caused an inhibition of MMP-9 release from human monocytes after stimulation with LPS. This effect was comparable to hydrocortisone (10^{-5} M). The findings suggest that preparations of noni fruits are effective in decreasing pain and joint destruction caused by arthritis. Copyright © 2009 John Wiley & Sons, Ltd.**

Keywords: Noni; *Morinda citrifolia*; analgesic; antiinflammatory; metalloproteinase; MMP-9.

INTRODUCTION

Morinda citrifolia L. is a plant growing in almost all tropical areas of the world. The robust plant grows as a shrub or tree depending on conditions of soil and climate. On Polynesian islands and Hawaii, noni plants grow wild as well as in plantations, on beach areas and inland up to a height of 600 m. Flowers and fruits at all phases of ripeness can be observed on the plant all year. Noni plants proliferate very rapidly and bear fruits already in the first year after seeding.

Noni plants are very important in Polynesian folk medicine. Fruits and leaves have been used for more than 2000 years for the treatment of arthritis, to heal wounds, to stop or reduce all kinds of pain as well as to cure many diseases. This tradition is still alive (Dixon *et al.*, 1999; McClatchey, 2002; Pande *et al.*, 2005). During the past 10 years, juices prepared from ripe noni fruits became famous as a wellness drink worldwide. In 2003 noni fruit juice was approved as a novel food by the European Commission (Commission, 2003). No adverse effects of noni juice have been observed in extensive toxicological studies including tissue culture work, animal experiments and a human clinical trial (West *et al.*, 2006; Westendorf *et al.*, 2007).

The ancient Polynesians used noni fruits and fruit juice for the treatment of wounds and arthritis (Li *et al.*, 2003). This tradition is still vivid in the South Pacific area. A recent epidemiological analysis of the beneficial effects of noni reported by inhabitants of the Fiji islands revealed that reductions of pain from arthritis and other reasons are among the most frequent benefits (Pande *et al.*, 2005).

After the introduction of noni juice as a wellness drink in industrial countries after 1996, numerous reports on the health benefits of noni juice became available on the internet, most of which are private testimonials. A follow up study with about 2000 consumers of noni juice in Europe is currently being analysed by our group. Beside improvements of energy and overall well being, beneficial effects on arthritic conditions are the most prominent (unpublished results).

The aim of the present investigation was to confirm the analgesic effect of noni fruit juice in a standardized animal model and to study possible biochemical mechanisms responsible for the antiinflammatory activity.

MATERIALS AND METHODS

Plant materials. The ripe fruits of *M. citrifolia* were harvested from different islands in French Polynesia. The fruits were pureed while the seeds and the skin were removed. The fruit puree was either completely freeze dried or freeze concentrated by removing almost 90% of the water, leaving a dark viscous liquid. Alcohol extracts of the freeze dried noni fruit puree were prepared by shaking 1 g solid puree with 50 mL of ethanol in a rotating incubator at 37 °C for 16 h. Insoluble material (660 mg) was removed by filtration through a paper filter and the solvent was removed by evaporation. The residue (340 mg) was dissolved in 1 mL of DMSO. The Noni freeze concentrate was diluted 1:10 with water and supplied to the mice as drinking water for the hot plate assay.

Chemicals. Ethanol abs. and DMSO (ultra pure) were purchased from Merck, Darmstadt, Germany. All other chemicals were purchased from Sigma-Aldrich, Taufkirchen, Germany, if not stated otherwise.

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Animals. Male NMRI mice were purchased from Charles River, Sulzfeld, Germany. The animals were housed in groups of ten in macrolon cages at 22 °C and a light/dark cycle of 12 h with unlimited access to food and water.

Isolation and culture of human monocytes. Monocytes were isolated from pooled human blood cell suspensions from different donors (Bayerisches Rotes Kreuz, Germany) by a double density gradient centrifugation method using Ficoll- and following 46%-Percoll-gradients (Biochrom, Berlin, Germany). Subsequently, yielded cells were seeded in 24-well plates (BD Labware NJ, USA) at a density of 1×10^6 cells/mL. For attachment the cells were cultured overnight in modified McCoy's 5a medium supplemented with 15% FCS, 1% penicillin/streptomycin, 1% non-essential amino acids and 1 mM L-glutamine (Biochrom, Berlin, Germany) in a 6% CO_2 humidified atmosphere at 37 °C (Hera cell incubator, Kendro, Hanau, Germany). Assays were performed on day 1 after seeding in a final volume of 1 mL/well.

Elucidation of analgesic activity in mice. The hot plate test, first reported by Woolfe and MacDonald (Woolfe and MacDonald, 1944) was used to test the antinociceptive activity of noni fruit. The test was performed with an apparatus, Model 7280, of Ugo Basile Inc., Milan, Italy. The performance of this animal test was approved by the Veterinary Department of the State of Hamburg, Germany. Mice were set on a metal plate heated to 56 °C. This temperature, although uncomfortable, does not cause any tissue damage. As soon as the animals were signaling a pain reaction by licking the paws or jumping, they were removed from the plate. The time between the first contact with the plate and the reaction was registered and stored in a computer. Six groups of ten mice each were used for the first experiment. Group 1, serving as a negative control received 1 mL of 0.9% NaCl-solution (saline) per 100 g BW by s.c. injection, 1 h before the test. Group 2, the positive control, received a s.c. injection of 30 mg/kg of the central analgesic tramadol in 1 mL of saline per 100 g BW. Group 3 received 1 mg/kg of naloxone by s.c. injection, 1 h before the test. Group 4 received 10% noni fruit puree concentrate dissolved in their drinking water for the last 4 days before the experiment. One hour before the test they received a s.c. injection of saline according to the negative control. Groups 5 and 6 were treated like groups 2 and 4, but they additionally received naloxone according to group 3. The treatment of the negative control and the noni group with saline, 1 h before the experiment, was done in order to prevent a different bias between these groups and groups 2, 3, 5 and 6, resulting from stress.

In a second experiment metamizol (200 mg/kg) was given to the mice by s.c. injection as a positive control. All the other treatments were identical to the first experiment.

Inhibition of MMP-9 release from human monocytes. Inhibition of LPS-induced MMP-9 release from monocytes was analysed as described previously (Grimm *et al.*, 2006). Briefly, fresh medium containing different dilutions of Noni-extract dissolved in DMSO (concentration of vehicle in final assay volume ≤1%) was added to each well. After 1 h, the cells were treated with 10 ng/mL LPS (lipopolysaccharides (rough strains) from Salmonella Minnesota Re 595, Sigma-Aldrich) and kept incubated at 37 °C for 24 h. Control experiments for each Noni-extract dilution were treated with vehicle, i.e. DMSO. Supernatants were harvested, diluted 1:25 and assayed for total MMP-9 protein concentration using a sandwich-ELISA method (Quantikine™ assay, R&D Systems, Minneapolis, USA) according to the manufacturer's protocol.

Data analysis. Statistical analysis of data received from the MMP-9 inhibition was performed using the software GraphPad PRISM® (Graph-Pad software, Inc., San Diego, CA, USA). Calculation of the statistical significances (p value) for the hot plate test was done by using Student's t-test with Bonferroni correction. A value of $p < 0.01$ indicated a significant difference of the reaction time compared with the control.

RESULTS

Antinociceptive activity of noni fruit puree

The results from the hot plate test are shown in Tables 1 and 2. Application of the central analgesic drug tramadol (30 mg/kg) significantly increased the reaction time (i.e. decreased the thermal sensitivity) compared with the

Table 1. Antinociceptive activity of Tramadol and Noni puree concentrate in the hot plate test with NMRI mice (10 mice per group)

Treatment	Reaction time (s)	p
Control	6.9 ± 1.11	
Tramadol (30 mg/kg)	8.9 ± 0.9	0.00068
Noni puree concentrate (10%)	9.2 ± 1.45	0.001
Naloxone (1 mg/kg)	4.7 ± 0.8	0.00022
Tramadol (30 mg/kg)	5.0 ± 1.4	0.003
Naloxone (1 mg/kg), Noni puree concentrate (10%) Naloxone (1 mg/kg)	5.8 ± 1.0	0.05

Statistical comparisons between each treatment and associated control were carried out by Student's t-test followed by a Bonferroni correction. Values of $p < 0.01$ were considered as significant.

Table 2. Antinociceptive activity of metamizol and Noni puree concentrate in the hot plate test with NMRI mice (10 mice per group)

Treatment	Reaction time (s)	p
Control	7.17 ± 0.98	
Metamizol (200 mg/kg)	9.63 ± 0.88	0.000013
Noni puree concentrate (10%)	9.12 ± 1.04	0.0004
Naloxone (1 mg/kg)	5.46 ± 1.27	0.0034
Metamizol (200 mg/kg)	7.71 ± 0.49	0.136
Naloxone (1 mg/kg), Noni puree concentrate (10%) Naloxone (1 mg/kg)	7.42 ± 0.97	0.573

Statistical comparisons between each treatment and associated control were carried out by Student's t-test followed by a Bonferroni correction. Values of $p < 0.01$ were considered as significant.

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control from 6.9 to 8.9 s ($p = 0.00068$). The application of 10% of the noni fruit puree concentrate in the drinking water of the mice for a period of 4 days prior to the experiment resulted in a reaction time of 9.2 s ($p = 0.001$). The morphine antagonist naloxone increased the sensitivity of the mice by reduction of the reaction time to 4.7 s ($p = 0.00022$). Naloxone also completely reversed the effect of tramadol to 5.0 s ($p = 0.003$). It also reduced the effect of noni fruit puree to a reaction time of 5.8 s. This reduction was less pronounced compared with tramadol, however, the difference between both effects was not statistically significant.

The experiment was repeated with the peripheral analgesic metamizol as a positive control to elucidate whether the reduction of the antinociceptive activity by naloxone was specific for central analgesics. As shown in Table 2, the results were similar to the first experiment. Metamizol increased the reaction time from 7.2 s to 9.6 s ($p = 0.000013$). In contrast to tramadol, this effect was only partly reversed by naloxone and remained above the control level. The effect of noni fruit puree was again well pronounced and only slightly lower than metamizol. Combination of the noni fruit concentrate and naloxone did reduce the reaction time of the mice comparably to the combination of metamizol and naloxone.

Inhibition of MMP-9 secretion from human monocytes

The LPS-induced secretion of matrix metalloproteinase 9 (MMP-9) was significantly inhibited compared with the untreated controls (Fig. 1). Noni-extract dilutions of 1:100 and 1:200 revealed a comparable inhibition of MMP-9 secretion down to 56.31 ± 1.03% and 50.75 ± 4.66%, respectively. As expected, the 1:500 dilution of the noni extract displayed a weaker reduction of MMP-9 secretion from LPS-stimulated monocytes (70.38 ± 6.69). As a positive control the effect of 10^{-5} M hydrocortisone was analysed. This active treatment reduced the LPS-induced MMP-9 secretion down to 65.35 ± 2.02%. Thus, the effect of noni extract (dilution 1:500) was only slightly lower compared with the antiinflammatory actions of hydrocortisone. The dilutions of 1:100 and 1:200 were even slightly more effective on inhibition of MMP-9 release than hydrocortisone.

DISCUSSION

Painful chronic inflammatory processes affecting the joints are very frequent in our society. It was reported that they affect about 46 million citizens of the USA, which is over 20% of the population (Akihisa *et al.*, 2007). Acute phases of osteoarthritis are characterized by swollen and heated joints often accompanied by heavy pain. Beside the inflammation, a progressive destruction of the cartilage takes place in arthritic joints. This process is triggered by the formation of tissue degrading metalloproteinases (MMP), which are secreted by immune-functional cells during the inflammation process (Nagase and Kashiwagi, 2003). Osteoarthritis is mostly treated with non steroidal antiinflammatory drugs (NSAID). These compounds inhibit the formation of prostaglandins from their precursor arachidonic acid by the enzymes cyclooxygenase I and II, which trigger the inflammation process (Vane and Botting, 1998). Although quite effective, these drugs have often severe side effects on the stomach, liver, kidneys and other organs (Pirmohamed *et al.*, 2004; Schlondorff, 1993). The destructions caused by the MMPs are not reversed by the drugs used for the treatment of arthritis and responsible for the progression of the disease, characterized by a permanent loss of the functionality of the joints.

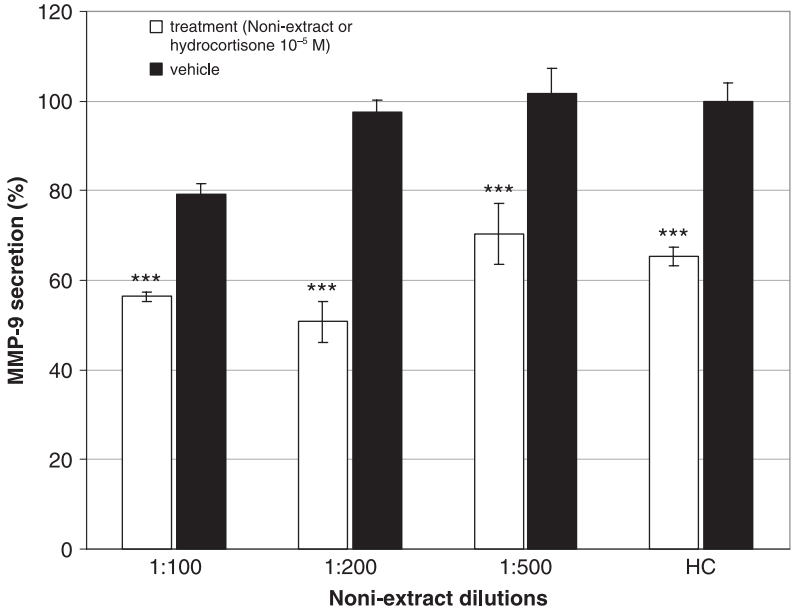

Figure 1. Inhibition of total MMP-9 release from freshly isolated human monocytes. Cells were pretreated for 1 h with Noni extract diluted in medium or hydrocortisone 10^{-5} M (HC; positive control) or vehicle. Then the cells were stimulated with 10 ng/mL LPS for 24 h and the MMP-9 concentration was measured in the supernatants. Black columns represent % of vehicle controls in reference to untreated control, white treatment columns are in % of associated vehicle controls and all values as mean ± deviation ($n = 3$). Statistical comparisons between each treatment and associated control were carried out by ANOVA followed by a Bonferroni multiple comparison test, respectively. Values of $p < 0.05$ were considered as significant (*** $p < 0.001$).

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Possible alternatives to the use of synthetic antiinflammatory drugs with a great spectrum of adverse effects may come from plant derived medicine. Several medicinal plants have demonstrated their effectiveness in reducing symptoms of arthritis. Among these are devil's claw (*Harpagophytum procumbens*) (Brien *et al.*, 2006), cat's claw (*Uncaria tomentosa*) (Hardin, 2007) and ginger (*Zingiber officinale*) (Grzanna *et al.*, 2005).

The use of the fruits of the noni plant (*Morinda citrifolia*) for the treatment of inflammatory diseases such as arthritis was common in ancient South Pacific populations (Dixon *et al.*, 1999; McClatchey, 2002). An epidemiological investigation which was recently performed on Fijian islands showed that this tradition is still alive (Pande *et al.*, 2005). Recent investigations have demonstrated that the inhibition of cyclooxygenases (COX-1 and COX-2) and lipoxygenases (LOX-5) could be responsible for this activity (Palu *et al.*, 2004; Su *et al.*, 2001).

Another possible pathway for the reduction of arthritis symptoms is the inhibition of the cartilage destruction by metalloproteinases, which are excreted by immune competent cells, such as monocytes (Nagase and Kashiwagi, 2003). Articular cartilage covers the ends of the bones in the joints and guarantees a smooth movement. Its degradation results in direct bone to bone contact causing a progressive destruction of the joint architecture accompanied by inflammation and pain. Patients with osteoarthritis reveal significantly higher MMP-9 levels in serum compared with healthy control subjects and even 50-fold higher concentrations of MMP-9 in the synovial fluid (Hulejova *et al.*, 2007). It has been shown that plant extracts of devils claw inhibit the expression and excretion of metalloproteinases in human monocytes and thus inhibit the cartilage destruction during arthritis (Brien *et al.*, 2006). The present investigations demonstrate that the release of MMP-9 from human monocytes after an inflammatory stimulus is also inhibited by an alcohol extract of noni fruits. The inhibition was very pronounced and was observed at dilutions of 1:500 of an alcohol solution prepared by extraction of 1 g dry noni fruit matter with 1 mL of ethanol. The effect is comparable to that of the endogenous antiinflammatory compound hydrocortisone (cortisol). Other antiinflammatory effects of noni fruit extracts may be due to a dual inhibition of cyclooxygenases and lipoxygenases (Palu *et al.*, 2004). It has been hypothesized that such a dual inhibition of enzymes of the arachidonic acid pathway is more effective than inhibitors of cyclooxygenases only (Leval *et al.*, 2002). A dual inhibition of arachidonic acid metabolism together with an inhibition of the excretion of metalloproteinases could be a very potent mechanism of antiinflammatory effects. The lack of typical side effects of NSAIDs after use of noni juice may be due to the less pronounced inhibition of functional prostaglandins by this combinatory effect.

Noni juice is also known as a 'pain killer' in ethnic folk medicine (Dixon *et al.*, 1999; McClatchey, 2002). Experimental evidence for an antinociceptive activity of noni fruit extracts does come from animal experiments (McKoy *et al.*, 2002; Punjanon and Nandhasri, 2005). In the present investigation mice were treated with noni fruit juice in the drinking water for a period of 4 days prior to a hot plate test. A highly significant reduction of the thermal sensitivity was observed, which was comparable to that of the central analgesic tramadol and the peripheral analgesic metamizol. Simultaneous treatment of the animals with noni juice and the endorphin receptor antagonist naloxone only resulted in a partial reduction of the antinociceptive activity of noni fruit puree, whereas the effect of tramadol was totally abolished. Other authors reported only a partial reduction of the analgesic effect by tramadol (Bamigbade and Langford, 1998).

In a second experiment with metamizol as a positive control noni juice showed almost the same activity as metamizol. The addition of naloxone was equally effective in the inhibition of the antinociceptive effect of metamizol and noni fruit puree. This reduction can be explained by the inhibition of the endogenous antinociceptive system by naloxone as demonstrated by the reduction of the reaction time of the control mice in the hot plate test. The mechanism of the analgesic activity of metamizol is not fully understood. It seems that peripheral and central analgesic mechanisms are involved (Mazario and Herrero, 1999). Moreover, the activation of the endogenous antinociceptive system has been demonstrated after injection of metamizol into the periaqueductal gray matter in rats (Vazquez *et al.*, 2005). This effect was inhibited by application of naloxone. Whether the analgesic action of metamizol and noni fruit juice is mediated by similar mechanisms remains unknown. The lack of side effects caused by the peripheral inhibition of prostaglandins in both metamizol and noni fruit juice suggests similar mechanisms of action. Further experiments are warranted to answer these questions.

In conclusion, the present investigations support the hypothesis of beneficial effects of noni fruit juice on painful inflammatory diseases, such as arthritis, suggested by its use in ethnic folk medicine and recent epidemiological observations. Clinical trials are now warranted to further confirm this hypothesis.

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