Why you and your family should use Divine Noni?

Reason 7

Pulmonary anti-inflammatory and spasmolytic effect of noni juice on lung cells improve the function of lung cells and respiration condition in microbial infected persons

Scientific Reference:

Pulmonary anti-inflammatory effects and spasmolytic properties of Costa Rican noni juice (Morinda citrifolia L.)

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ABSTRACT

Ethnopharmacological relevance: Morinda citrifolia L. (Noni) is a medicinal plant used in Polynesia for many properties such as anti-inflammatory, anti-diabetic and antineoplastic effects. Recent studies showed that noni juice have anti-oxidant and acute anti-inflammatory activities likely due to poly-phenols, iridoids and vitamin C content. The present study was undertaken to evaluate chronic anti-inflammatory and spasmolytic effects of noni juice.

Materials and methods:

Therefore, we evaluated the effect of oral or intraperitoneal administrations of noni juice in vivo on the lung inflammation in ovalbumin (OVA) sensitized Brown Norway rat (with prednisolone 10 mg/kg intraperitoneously as reference compound) and the ex vivo effect of noni juice on BaCl\textsubscript{2} (calcium signal) or methacholine (cholinergic signal) induced spasms in jejunum segments.

Results: We found that noni juice (intraperitoneously 2.17 mL/kg and orally 4.55
mL/kg) reduced the inflammation in OVA-sensitized Brown Norway rat with regard to the decreased number of in-flammatory cells in lung (macrophages minus 20–26%, lymphocytes minus 58–34%, eosinophils minus 53–30%, neutrophils minus 70–28% respectively). Noni juice demonstrated a dose-dependent NO scavenging effect up to 8.1 nmol of nitrates for 50 mL of noni juice. In addition noni juice inhibited (up to 90%) calcium and cholinergic induced spasms on the jejunum segments model with a rightward shift of the concentration response curve.

Conclusion: We describe for the first time that noni juice demonstrate (1) a chronic anti-inflammation activity on sensitized lungs along with (2) a spasmolytic effect integrating a calcium channel blocker activity component.

1. Introduction

Morinda citrifolia L. (Rubiaceae) is a small tropical tree that grows widely in Polynesia. Commonly called noni, the fruits have been used as folk medicine in Polynesia, Australia, China and Ha-waii, for 2000 years for its medicinal properties (Krauss, 1993; Earle, 2001; Wang et al., 2002). In traditional Pharmacopeia (Chang and But, 1987; Barr et al., 1988; Tu et al., 1992), therapeutic claims include: anti-diabetic, anti-hypertensive, anti-inflammation (Wang et al., 2002), anti-asthma (Whistler, 1992), analgesic, stimulation of the immune system and anti-cancer (Earle, 2001; Wang et al., 2002; Chan-Blanco et al., 2006). Currently, the market of noni juice is continuously increasing and noni fruits have been reported to exhibit antioxidant, anticancer activities and anti-inflammation activities (Dussossoy et al., 2011; Hirazumi and Furusawa, 1999; McKoy et al., 2002). In addition, recently a case report of the effect of Noni juice on Crohn’s disease, a chronic inflammatory disorder was published (West, 2013). Furthermore Noni was demonstrated to be analgesic in a mouse model of colic pain suggesting central effect. This model involving intestinal spasms, peripheral analgesic effects through spasmolytic activity may contribute to pain relieve (Wang et al., 2002).

The aim of the present study was to evaluate the anti-inflammation activities of Noni juice in a chronic inflammatory model and its spasmolytic activities. Various animal model of inflammatory bowel diseases have been developed but most of them are characterized by high levels of pain for the animals (Mizoguchi, 2012). Therefore, for ethical reasons, we chose a much less drastic model of chronic inflammation, the ovalbumin sensitized Brown Norway rat.

In our previous study published in Journal of Ethnopharmacology (Dussossoy et al., 2011), we characterized finely Noni juice. The phytochemical analysis of noni juice
identified 12 compounds and 9 were quantified. We performed these analyses using HPLC-DAD-MS for analysis and quantification of Noni juice compounds. We established the presence of phenolic compounds: coumarins (scopoletin, esculetin), flavonoids (rutin, quercetin, quercetin de-rivative, isoquercitrin and kaempferol rutinoside), phenolic acid (vanillic acid), vanillin and iridoids (asperulosidic acid and dea-cetylasperulosidic acid). Deacetylasperulosidic acid and asperulosidic acid were the major compounds in noni juice, with 159.1 and 71.6 mg loganic acid equivalent/100 g of fresh weight, respectively. The major polyphenolic compound was rutin, with 4.63 mg/100 g of fresh weight, followed by scopoletin with 1.33 mg/100 g of fresh weight. Our results matches and complete previous reports (Potterat et al., 2007; Potterat and Hamburger, 2007). Theses molecules were shown to bear anti-oxidative, anti-inflammatory, anti-cancer and spasmodic activities. Indeed, Knekt et al. (Knekt et al., 2002) reported that the incidence of asthma was inversely correlated to the intake of quercetin, nar-ingenin and hesperetin. Some studies showed an inhibition of lung inflammatory and/or bronchial hyperresponsiveness by quercetin (Jung et al., 2007; Moon et al., 2008; Park et al., 2009; Rogerio et al., 2007), isoquercitrine (Rogerio et al., 2007), rutin (Jung et al., 2007) and a kaemferol glycoside (Medeiros et al., 2009) on ovalbumin (OVA)-sensitized animal models. The anti-asthmatic activ-ity of a coumarin (umbelliferone) and an iridoids (verproside) were proved on ova-sensitized mice models by Vasconcelos et al. (2009) and Oh et al. (2006) respectively.

Flavonoids are known to have a relaxation activity on intestinal smooth muscle (Hammad and Abdalla, 1997) but also on aortic (Herrera et al., 1996; Chan et al., 2000), tracheal (Leal et al., 2003) and uterine smooth muscles (Revuelta et al., 2000). Similarly, scopoletin presents an aortic smooth muscle relaxant activity by inhibition of intracellular calcium mobilization from the noradrenaline-sensitive storage (Oliveira et al., 2001).

Regarding the presence of such biological components in the Costa Rican noni juice, the aim of this study was to evaluate, for the first time, antiasthmatic and spasmodic properties of noni juice.

2. Materials and methods

2.1. Plant material

Noni fruits used in this study were collected from an experimental plantation established by EARTH University in the humid tropical region of Limón (Costa Rica) on February, March 2010. A voucher specimen is deposited at EARTH University of Costa Rica and at the “Droguier” in the Faculty of Pharmaceutical Sciences of Montpellier (France) (Dussossoy et al., 2011).

2.2. Chemical
All solvents were HPLC grade, purchased from Carlo Erba (Val de Reuil, France). Folin–Ciocalteu reagent was purchased from Carlo Erba (Val de Reuil, France). Ascorbic acid, quercetin, vanillin, vanillic acid, scopoletin, Tris (2-carboxyethyl) phosphine hydro-chloride (TCEP) and metaphosphoric acid (MPA), fluorescein, 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxilic acid (Trolox), 2,2-diphenyl-1-picyrylhydrazyl (DPPH), were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Rutin, kaempferol and loganic acid were purchased from Extrasynthese (Genay, France). 2′-2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, USA). RPMI 1640 with glutaMAX®, foetal bovine serum, streptomycin and penicillin were purchased from Gibco, Invitrogen (Cergy Pontoise, France). Recombinant murine IFN-γ was purchased from Genzyme (Tebu Bio, Le Perray en Yvelines, France). Ovalbumin grade V, methacholine, aluminium hydroxide, fluorescein, sodium nitroprusside, atropine were purchased from sigma aldrich (Saint Quentin Fallavier, France). NaCl, KCl, MgCl$_2$·7H$_2$O, CaCl$_2$·2H$_2$O, NaHCO$_3$; Glucose, BaCl$_2$, KH$_2$PO$_4$, Na$_2$HPO$_4$, CaCl$_2$, Hemacolor® was purchased from VWR international (Strasbourg, France).

2.3. Sample preparation

The fresh fruits were washed and disinfected using chloride solution (100 ppm). Puree of noni juice was produced as described by Brat et al. (2012) mimicking traditional preparation. It was then submitted to an enzymatic treatment with 150 mL/L of Klerzyme®-DSM during 150 min at 35 °C to reduce the viscosity and the suspended solids content of the juice. Puree of noni was then pressed with a press cloth with an hydraulic rack at 25 T. A tangential microfiltration was performed according to Vaillant et al. (1999). The pilot-scale microfiltration unit used featured a tubular ceramic membrane (Membralox® 1P19–40, Pall Exekia, Bazet, France) with the following attributes: 19 channels with an internal diameter (d¼1/4 _ 10$^{-3}$ m), length (L¼1.02 m), average pore diameter (θ¼0.2 mm), and a total effective filtration area (A¼0.22 m$^2$). The process conditions were a flow velocity of 5 m/s, temperature of 35 °C and applied a transmembrane pressure of 150 kPa. Tangential microfiltration represents an alternative to high temperature treatment. Microfiltration allows the production of a microbiologically stabilized clarified juice (Revuelta et al., 2000). Noni juice was stored at _20 °C until used.

Microfiltered noni juice was successively partitioned with hexane, ethyl acetate and n-butanol. 50 mL of noni juice was firstly delipidated with 3 _ 50 mL of hexane and then, successively ex-tracted with 3 _ 50 mL of ethyl acetate (ethyl acetate extract, EAE) and 3 _ 50 mL of butanol (butanol extract, BE). Extracts were fi-nally evaporated to dryness in a rotavapor and redissolved in methanol–chloroform (1:1, v/v).
2.4. Physico-chemical analysis

As previously described, (Dussossoy et al., 2011). Noni juice dry weight (DW) was determined by gravimetric after a 3 h period at 50 °C in a drying chamber followed by a 12 h period at 60 °C in a steam room under vacuum. Total soluble solids were measured in the juice with an Atago refractometer (Japan) at 20 °C. The results were reported as Brix degree. The pH was measured with a Schott pH-meter. Titratable acidity was determined by titration with 0.1 N NaOH to pH 8.2. The results were expressed as citric acid equivalent per 100 mg fresh weight (FW).

Sugar analysis was performed on a DX-600 ion chromatograph (Dionex, France) equipped with a GP50 pump and an ED50 electrochemical detector. The separation was performed using a 4 mm _ 250 mm CarboPac MA1 analytical column (Dionex, France). The solvents were water for solvent A and NaOH 0.8 M for solvent B. Sugars were analyzed using the following gradient: 100% B for 10 min, from 100% to 75% B in 10 min, from 75% to 100% B in 10 min and 100% B for 10 min at the flow rate of 0.4 mL/min. The injection volume was 10 μL. Identification was achieved via comparison with standards: sucrose, fructose, and glucose. The analysis was performed in triplicate.

2.5. Total polyphenol content

Noni juice total polyphenol content was determined by the Folin–Ciocalteu method optimized by George et al. (2005). The results were expressed as mg of gallic acid equivalent per 100 g FW. The analysis was performed in triplicate (Dussossoy et al., 2011).

2.6. Ascorbic acid and dehydroascorbic acid content

Ascorbic acid (AA) was measured by the HPLC method. Noni juice was diluted to 1/5 in a solution of 4% metaphosphoric acid to stabilize the ascorbic acid. A calibration curve was established with ascorbic acid in a dose range of 10–200 mg/L diluted in 4% metaphosphoric acid. The samples were then filtered through a 0.45 mm filter (Millipore). The HPLC analysis was carried out on a Dionex liquid chromatograph equipped with model P680 pumps, an ASI 100 autosampler and a UVD 340U diode array detector coupled to a HP ChemStation (Dionex, France). The separation was performed at 30 °C using a 250 mm _ 4.6 mm, 5 mm particle size, endcapped reversed-phase Lichrospher ODS-2 (Interchim, Mon-tluc-on, France) in isocratic mode with 0.01% sulphuric acid so-lution (pH 2.6) as mobile phase. The flow rate was 0.8 mL/min. The injection volume was 20 μL and detection was carried out at 245 nm.

The dehydroascorbic acid (DHAA) was quantified according to Wechtersbach and
Cigic (2007) with some modifications. After reduction of DHAA to AA by the Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) solution at 10 mM in 4% metaphosphoric acid (MPA), the AA was quantified. The DHAA content was calculated by the difference between the peak area of AA with and without reduction. The analysis was performed in triplicate (Dussossoy et al., 2011).

2.7. HPLC-DAD-MSn analysis of noni juice compounds

Samples were filtered through a 0.45 mm filter (Millipore). The HPLC analysis was carried out on a Waters 2690 HPLC system equipped with a Waters 996 DAD (Waters Corp., Milford, MA) and Empower Software (Waters). The separation was performed at 30 °C using a 250 mm _ 4.6 mm i.d., 5 mm, endcapped reversed phase Lichrospher ODS-2 (Interchim, Montlucon, France). The solvents were water/acetonitrile/formic acid (99.195:0.8:0.005, v:v:v) for solvent A and 100% acetonitrile for solvent B. Phenolic compounds and standards were analyzed using the following gradient: 5% B for 5 min, from 5% to 35% B in 40 min, from 35% to 100% B in 3 min and 100% B for 10 min at a flow rate of 0.7 mL/ min. The injection volume was 10 mL, and detection was carried out between 200 and 600 nm. After passing through the flow cell of the diode array detector, the column eluate was split and 0.25 mL/min was directed to an LCQ ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA). Experiments were performed in both negative and positive ion modes. The scan range was 100–2000 and the scan rate 1 scan/ s. The desolvation temperatures were 250 and 300 °C in positive and negative ion mode, respectively. High spray voltage was set at 5000 V. Nitrogen was used as the dry gas at a flow rate of 75 mL/ min. MS2 and MS3 were carried out using helium as the target gas, and the collision energy was set at 25–35% and 50%, respectively. Identifications were made based on the ion molecular mass, MSn and UV–visible spectra (Dussossoy et al., 2011).

2.8. HPLC-DAD quantification of noni juice compounds

Phenolic compounds and iridoids were quantified in EAE and BE extracts. Samples and standards (rutin, scopoletin, kaempferol, quercetin, isoquercitrin, vanillin, vanillic acid, loganic acid) were filtered through a 0.45 mm filter (Millipore). The HPLC analysis was carried out on a Dionex liquid chromatograph equipped with model P680 pumps, an ASI 100 autosampler and a UVD 340U diode array detector coupled to a HP ChemStation (Dionex, France), with the same column as described above. The separation was performed at 30 °C. The solvents were water/acetonitrile/ formic acid (99.195:0.8:0.005, v:v:v) for solvent A, and acetonitrile 100% for solvent B. Phenolic compounds and standards were analyzed using the following gradient: 5% B for 7 min, from 5% to 35% B in 38 min, from 35% to 100% B in 3 min and 100% B for 2 min at a flow rate of 1 mL/min. The injection volume was 20 mL and detection was carried out at 240, 280 and 330 nm. In order to quantify the amount of each
compound in both extracts, calibration curves were prepared with the standards: rutin, scopoletin, quercetin, vanillin, vanillic acid, kaempferol, isoquercitrin and loganic acid dissolved in methanol–chloroform (1:1, v/v). Conjugated forms of quercetin and kaempferol glycosides were quantified as their corresponding aglycones. All calibration curves showed good linearity in the studied concentration range (Dussossoy et al., 2011).

2.9. Induction of asthma in Brown Norway rats and drug administration

Ten-week-old Brown Norway rats were purchased from Jan-vier® (France) and maintained under constant conditions (temperature: 22 ± 1 °C, 12 h light/12 h dark cycle).

Rats were randomly divided in five groups of eight animals and airway inflammation was induced by ovalbumin in three groups. The sensitization of Brown Norway rats was performed as described by Roumestan et al. (2007). Briefly, each rat was sensitized to ovalbumin as follows: ovalbumin (1 mg/mL) was emulsified with aluminium hydroxide (100 mg/mL) in saline prior to intraperitoneal (IP) injection of 1 mL per rat at day 1, 2, 3 and 16. From day 22 to 29, three groups of asthma-induced rats were treated with oral administration of noni juice (4.55 mL/kg) or with IP injection of noni juice (2.27 mL/kg) or prednisolone (10 mg/kg intraperitoneally), 15 min prior nebulisation with a 1% (w/v) ovalbumin solution during 20 min. Untreated rats were nebulised with a 1% (w/v) ovalbumin. Unsensitized controls received IP injections of aluminium hydroxide alone and were then nebulised with saline from day 22 to 29.

2.10. Collection of bronchoalveolar lavage (BAL) fluid

On day 30, rats were anaesthetized with pentobarbital and exsanguinated by catheterization of the abdominal aorta to avoid contamination of BAL fluid with red cells. BAL fluid was collected by lavaging the lung via the trachea with 3 times of 4 mL of phosphate buffered saline. The total number of cells in BAL fluid was immediately determined by counting on Malassez chamber. The different cell types were distinguished and counted after cytocentrifugation, fixation and staining with hemacolor®.

2.11. Measurement of antioxidative status of BAL

Antioxidative status of BAL was carrying out by ORAC (Oxygen Radical Absorbance Capacity) method. ORAC assays were performed as described by Huang et al. (2002). We used a microplate spectrofluorometer TECAN Infinite 200 (TECAN Austria GmbH) in 96-well polypropylene plates. Briefly, the excitation and emission wavelengths were 48579 nm and 520 720 nm respectively. Solutions were all prepared with 75 mM Phosphate buffer (pH 7.4). Each well was filled with 160 μL of a 78.75 nM fluorescein solution and 20 mL of buffer or ¼ dilute BAL. The plate was
incubated at 37 °C during 15 min before 20 mL of a 178 mM AAPH solution were added. After the AAPH addition, the fluorescence decay was measured every minute during 60 min. The ORAC values were expressed as the net area under the fluorescein decay curve (net AUC). The area under the curve (AUC) and the net AUC were calculated as follow:

\[
AUC = 0.5 + f1/f0 + f2/f0 + f3/f0 + \ldots + f59/f0 + 0.5 (f60/f0)
\]

where \(f0\) is the initial fluorescence reading at 0 min and \(fi\) the fluorescence reading at time \(i\). net AUC = AUC sample – AUC blank

2.12. Nitric oxide (NO) scavenging effect

The scavenging effects of noni juice (dilute ½ to 1/640) on NO were measured according to the reported method (Meng et al., 2008). Sodium nitroprusside (SNP) (2.5 mM) was incubated alone or in combination with different dilutions of noni juice. SNP is an inorganic complex where NO is found as NO\(^+\) and light irradiation is necessary for the release of NO. Therefore, an incubation of 60 min under a daylight lamp at room temperature was carried. Then nitrite levels were determined by Griess reaction.

2.13. Antispasmodic model: animals, prelevement and mounting

The effect of noni juice on the smooth muscles of rat jejunum was studied. Male Wistar rat, weighting 300–350 g were purchased from Janvier and maintained under constant conditions (temperature: 2271 °C, 12 h light/12 h dark cycle). Rats were sacrificed by a blow to the head. The jejunum were immediately removed and cleaned of connective tissue. Segments (about 10 mm) of smooth muscle of jejunum were cut and suspended in organ baths containing Tyrode's buffer (NaCl: 137 mmol/l, KCl: 2.7 mmol/l, MgCl\(_2\) \(_7\)H\(_2\)O: 1 mmol/l, CaCl\(_2\) \(_2\)H\(_2\)O: 1,8 mmol/l, NaHCO\(_3\): 11.9 mmol/l et Glucose: 5.5 mmol/l) at 32 °C and aerated continuously with a mixture of 95% \(O_2\), 5% \(CO_2\). A resting tension of 1 g was applied and responses of segments were recorded by an isometric transducer (Emka\(^a\)) connected to a biometric amplifier and analyzed by informatics system (Mac Lab V3.6).

A 40 min equilibration period during which Tyrode's solution was changed every 10 min was done.

2.14. Spasmolytic activity

After obtaining control responses of the rat jejunum (Acetylcholin 10\(^{-5}\) M and BaCl\(_2\) 4 \(_{10}^{\text{-4}}\) M) and reequilibration of jejunum, a BaCl\(_2\) (0.4 mM) or methacholin (3 mM) contraction was done. A steady state of contraction was reached approximately in 5 min. At this time, different doses of noni juice (30 mL, 60 mL or 90 mL of noni juice/mL of buffer) were added. Inhibitory percentage of contraction was calculated as follows:
Inhibitory (%) = \left( \frac{\text{Tension (g) after adding Noni Juice } - \text{Tension (g) before adding Noni Juice}}{\text{Tension (g) before adding noni juice}} \right) \times 100.

2.15. Anticholinergic effect

After a 40 min equilibration period, segments were exposed to methacholine added in a cumulative manner (10^{-8}–3 \times 10^{-4} M) to obtain a concentration-response curve (CRC). After another 30 min equilibration period in tyrode buffer, the effect of a ten minutes pretreatment of noni juice (30 mL/mL, 60 mL/mL or 90 mL/mL) or muscarinic antagonist (atropin: 1 nM, 10 nM or 100 nM) was ex-amined on a second methacholine CRC. CRC of methacholine, in presence or absence of noni or atropin, were compared.

2.16. Calcium blocker effect

After a 40 min equilibration period, strip were placed in free calcium tyrode's solution (NaCl: 91 mmol/L, KCl: 50 mmol/L, MgCl_2 \cdot 7H_2O: 1 mmol/L, NaHCO_3: 11.9 mmol/L et Glucose: 5.5 mmol/l) containing EDTA (0.1 mM) during 30 min (change of free calcium tyrode's solution containing EDTA every 10 min) then in free calcium tyrode's solution during 30 min (change of free calcium tyrode's solution every 10 min). Then, strips was exposed to CaCl_2 added in a cumulative manner (10^{-5}–3 \times 10^{-2} M) for ob-tain a CRC. After another equilibration period in free calcium tyr-ode's solution containing EDTA and in free calcium tyrode's solu-tion alone, the effect of a ten minutes pretreatment of noni Juice (30 mL/mL, 60 mL/mL or 90 mL/mL) or calcium channels blocker (Nifedipin: 10 nM, 100 nM, 1000 nM) was examined on a second calcium CRC. CRC of CaCl_2, in presence or absence of noni or ni-fedipin, was compared.

2.17. Statistical analysis

Values were given as means±standard error of the mean (sem). Statistical analysis was performed using an unpaired Student's t-test. Differences were considered significant when p<0.05.

2.18. Animal care and use

These experiments were carried out in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US Na-tional Institutes of Health. Our laboratory practice and protocols were approved on February 8th, 2012, by the legal institution "Comité d'Ethique pour l'Expérimentation Animale Languedoc-Roussillon" with the bioethical approval code CEEA-LR-13015 for the University of Montpellier.

3. Results

3.1. Qualitative and quantitative physico-chemical analysis
Physico-chemical characteristics of noni juice are presented in Tables 1–3 as previously described (Dussossoy et al., 2011).

The major dry weight (DW) components (7.4% of the juice) were sugars, with glucose and fructose at 2.07 and 2.44 g/100 g FW, respectively (Table 1).

HPLC-DAD-MSn was used for analyzing the minor components of noni juice. UV-visible characteristics and LC–MS data are given in Table 2. Protocatechuic acid, vanillin, vanillic acid, quercetin, kaempferol, rutin, scopoletin and isoquercitrin were formally identified by co-injection of the corresponding standard compounds. UV-visible characteristics, LC–MS and the MSn fragmentations of the predominant positive and negative ions were used to confirm these identifications and to identify some aglycone derivatives. Identification of the individual peaks was performed according to data presented in Table 2.

The MS spectrum of peak 23 in EAE extract showed an [M–H]– at m/z 301 with MS2 data at m/z 179 and m/z 151. These data are characteristics of quercetin (Mullen et al., 2003). The identification was confirmed by co-injection with authentic standard. UV spectra of peak 16 in EAE extract and peaks 12 and 13 in BE extracts are similar to those of quercetin glycosides. Peak 16 (EAE) and peak 13 (BE) showed an [M–H]– at m/z 609 with MS2 data at m/z 301 and MS3 data at m/z 179 and m/z 151. The MSn fragmentation pattern matched a quercetin derivative. MS2 showed a loss of 308 mass units from the parent compound that might represent the loss of a hexose and a deoxyhexose, probably rutinose. The identification as rutin was confirmed by co-injection with the authentic standard. Peak 12 in BE extract showed [M–H]– at m/z 741 with MS2 data at m/z 609 and MS3 data at m/z 301. The loss of 132 mass units could represent a pentose, and the loss of 308 mass units a hexose and a deoxyhexose. The attachment position of the sugars could not be determined. In the same way, peak 18 in EAE extract (m/z 463) showed a fragmentation pattern matching a quercetin derivative. MS2 showed a loss of 162 mass units from the parent compound, potentially represented by a hexose. Peak 18 (EAE) was identified as isoquercitrin by coinjection with the authentic standard. UV spectra of peak 14 in BE extract was similar to those of kaempferol glycosides. Peak 14 showed an [M–H]– at m/z 593 with MS2 data at m/z 285, the MS2 fragmentation matching a kaempferol rutinoside. Indeed, MS2 showed a loss 308 of mass units from the parent compound that could represent the loss of an [hexose+rhamnose] group. Even if the strong hypsochromic shift at 345 nm betrays substitution at position 4 or 3, the attachment position of sugars could not be unambiguously determined.

Table 1: Physicochemical characteristics of noni juice.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dry weight&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
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<td>7.37  7  0.06</td>
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Soluble solids\(^b\) 5.8 7 0.0
pH 3.4 7 0.1
Titrable acidity\(^c\) 1.76 7 0.01
Glucose\(^d\) 2.07 7 0.01
Fructose\(^d\) 2.44 7 0.02
Total polyphenol\(^e\) 47.6 7 2.0
Total vitamin C\(^f\) 97.1 7 2.3
Ascorbic acid\(^f\) 71.1 7 1.4
Dehydroascorbic acid\(^f\) 26.0 7 0.8

Values are means±sem of three analyses.
\(a\) %/fresh weight (fw).
\(b\) Brix degree.
\(c\) g of citric acid/100 g fw.
\(d\) g/100 g fw.
\(e\) mg of equivalent gallic acid/100 g fw.
\(f\) mg/100 g fw.

Table 2: Identification of compounds in ethyl acetate (A) and butanol extract (B).

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rt (min)</th>
<th>UV data (nm)</th>
<th>MS</th>
<th>MS(^2)/MS(^3)</th>
<th>Tentative identification</th>
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<tr>
<td>A</td>
<td></td>
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<tr>
<td>1</td>
<td>4.3</td>
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<td>2</td>
<td>5.4</td>
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<td>6</td>
<td>16.4</td>
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<td>7</td>
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<td>(sh), 345</td>
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B

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<td>8</td>
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<td>431 ( _)</td>
<td>269, 251</td>
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Table 3: Quantification a (mg/100 g FW) of compounds in noni juice.

<table>
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<th>Compounds</th>
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<tr>
<td>Rutin</td>
<td>4.6370.04 a</td>
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<td>Quercetin</td>
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<tr>
<td>Quercetin derivative</td>
<td>0.4670.02 a</td>
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<td>Kaempferol derivative</td>
<td>tr b</td>
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<tr>
<td>Scopoletin</td>
<td>1.32 70.02 a</td>
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<td>Esculetin</td>
<td>0.2070.01 a</td>
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<tr>
<td>Vanillin</td>
<td>0.3570.01 a</td>
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<tr>
<td>Vanillic acid</td>
<td>0.2670.00 a</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>tr b</td>
</tr>
<tr>
<td>Isoquercitrin</td>
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<td>Asperulosidic acid</td>
<td>71.674.1 a</td>
</tr>
<tr>
<td>Desacetylasperulosidic acid</td>
<td>159.178.1 a</td>
</tr>
</tbody>
</table>

a Values are means ± SEM of three independent determinations.

b tr: traces (≤0.1 mg/100 g FW).

UV spectra of peaks 13 and 17 in EAE extract were characteristic of a coumarin derivative. Peak 13 showed a [M-H]⁻ at m/z 177 and peak 17 at m/z 191. Identification as esculetin and sco-poletin was established by co-injection with the...
authentic standards. Identification of peaks 5, 9 and 12 in EAE extract was confirmed by co-injection with authentic standards as being protocatechuic acid, vanillin and vanillic acid, respectively. Previous reports from the literature described the presence in noni of rutin (Akihisa et al., 2007), scopoletin (Potterat et al., 2007), quercetin and vanillin (Deng et al., 2007).

UV spectra of peak 7 in EAE extract and peaks 2 and 8 in BE extract are similar to iridoids UV spectra (~240 nm) (Chen et al., 2007). Peaks 7 in EAE extract and 8 in BE extract were similar and showed an [M-H] at m/z 431 with MS2 data at m/z 269 and m/z 251. This loss of 162 mass units represented the loss of glucose [M-Glc] and the loss of 180 mass units represented [M-Glc_H2O]. Those weight losses are found in iridoids compounds (Es-Safi et al., 2007). This data showed that peaks 7 (EAE) and 8 (BE) could be asperulosidic acid. Peak 2 in BE extract showed an [M-H] at m/z 389 with MS2 data at m/z 227 and m/z 209. Again, this loss of 162 mass units represented the loss of glucose [M-Glc] and the loss of 180 mass units represented [M-Glc_H2O]. This data showed that peak 2 could be deacetylasperulosidic acid. Those iridoids are commonly reported in noni juice (Akihisa et al., 2007; Potterat et al., 2007).

Twelve compounds were identified and 9 were quantified. We established the presence of phenolic compounds: coumarins (scopoletin, esculetin), flavonoids (rutin, quercetin, quercetin de-rivative, isoquercitrin and kaempferol rutinoside), phenolic acid (vanillic acid), vanillin and iridoids (asperulosidic acid and dea-cetylasperulosidic acid). Deacetylasperulosidic acid and asperulosidic acid were the major compounds in noni juice, with 159.1 and 71.6 mg loganic acid equivalent/100 g FW, respectively. The major polyphenolic compound was rutin, with 4.63 mg/100 g FW, followed by scopoletin with 1.33 mg/100 g FW. The quantification of phenolic compounds and iridoids is presented in Table 3.

3.2. Anti-inflammatory effect in sensitized rats

The chronic anti-inflammatory effect of noni juice was investigated in an animal model of allergic asthma. Brown norway rats were sensitized to ovalbumine (OVA) to trigger airway inflammation. Bronchoalveolar lavages of sensitized rats contained an increased number of total inflammatory cells, which was par-tially inhibited by the noni juice. The average total number of cells in the BAL of unsensitized rats was 0.27 millions of cells per millilitres. The BAL of OVA-sensitized rats contains 1.37 millions of cells per millilitres. As previously described, prednisolone in-traperitoneal treatment normalized the total number of cells in BAL (data not shown) (Roumestan et al., 2007). Noni juice treat-ment significantly decreased the number of cells in BAL at 0.88 million per millilitres for IP administration and 1.07 millions per milliliter for oral administration. Almost all cells present in BAL of non-sensitized rat were alveolar macrophages (485%). In the OVA-sensitized rats, BAL contained an increase of total
in-flammatotory cells which was decreased by noni juice treatment (oral or intraperitoneal). Analysis of leukocytes sub-population revealed that OVA sensitized rats' BAL contained an increase of eosinophils (24% of cells present in BAL were eosinophils) indicating that the OVA-sensitization induced an allergic airway eosinophilia and an increase of lymphocytes (21% of the cells present in BAL were lymphocytes) indicating an inflammation. The treatment by noni juice administered by IP or oral route decreases the number of macrophages (by 26% and 20% respectively) and have a more pronounced effect on inflammatory cells: eosinophils (by 53% and 30% respectively), lymphocytes (by 58% and 34% respectively) and neutrophils (by 28% and 70% respectively). (Fig. 1).

3.3. Antioxidant status of BAL in sensitized rats

ORAC assay in BAL showed an increase of antioxidative status in OVA-sensitized animals. The intraperitoneal administrations of noni juice lead to a decrease of the antioxidant status of the BAL which is not the case for the oral administrations (Fig. 1).

3.3.1. NO scavenging effect of noni juice

A solution of 2.5 mM of SNP (Sodium Nitroprusside) in PBS was incubated at room temperature for 60 min under a daylight lamp that generated nitrates (stable end-product of NO) which were significantly decreased by the presence of noni juice. This scavenging effect on NO° is dose-dependent: 0.25 mmol of SNP produce 9 nmol of nitrates. 50 mL of noni juice can scavenged 8.1 nmol of nitrates. (Fig. 1).

3.4. Effect of noni juice on BaCl₂ and methacholine induced jejunum contraction

BaCl₂ and methacholine induced a sustained contraction on jejunum segments. Relaxation of BaCl₂ and metacholine-induced contraction is dose-dependently induced by noni juice reaching a maximum of almost 90%. The BaCl₂ induced contraction is more inhibited than the methacholine induced contraction for the dose of 30 and 60 mL/mL (Fig. 2).

3.5. Effect of noni juice and atropin on a concentration response curve of methacholine

Metacholine induced a concentration-dependent contraction of the jejunum. The muscarinic receptor antagonist atropin at different doses caused a rightward shift of the methacholine CRCs without any impairment of the maximal response to methacholine (Fig. 3).

In the same conditions, noni juice (60 and 90 mL/mL) induced a rightward shift of the methacholine CRCs but with a marked decrease of the maximal response to methacholine (Fig. 3).

3.6. Effect of noni juice and nifedipin on a concentration response curve of calcium
Calcium chloride \((10^{-5}–3 \times 10^{-2} \text{ M})\) induced a concentration-dependent contraction of the jejunum. Nifedipin \((0.01–1 \text{ mM})\) induced a marked rightward shift of the calcium chloride-induced response with a dramatic impairment of the maximal response to CaCl\(_2\) (Fig. 4).

To a lesser extent, noni juice \((60–90 \text{ mL/mL})\) induced a non-parallel and rightward shift in the calcium chloride-induced response associated with a decrease of the maximal response to CaCl\(_2\) (Fig. 4).

4. Discussion

Physicochemical properties of noni juice are in accordance with previous reports (Akihisa et al., 2007; Deng et al., 2007; Dussossoy et al., 2011; Es-Safi et al., 2007; Mullen et al., 2003; Potterat et al., 2007) with expected qualitative and quantitative characteristics.

We have previously reported an anti-inflammatory activity of noni juice in acute inflammation with the inhibition of carrageenan-induced rat paw oedema. Inhibition of prostaglandin and NO\(^\circ\) production was identified as being part of the corresponding mechanism of anti-inflammatory effect (Dussossoy et al., 2011). On another hand, active molecules have been identified which could have played a significant role, such as polyphenols including ses-pletin, rutin, quercetin, as well as iridoïds with asperulosidic acid and desacétylasperulosidic acid, and finally vitamin C (Dussossoy et al., 2011; Potterat and Hamburger, 2007). Among the numerous biological properties attributed to the noni, spasmolytic properties have been claimed.

In fact, few studies and conflicting studies were performed. Mokkhasmit et al. (1971) reported an in vitro histaminergic and smooth muscle-stimulant activities of a noni fruit ethanolic extract in guinea pig ileum. But for Dixon et al. (1999), this action as other noni's properties reported during the 1970 s have not been corroborated by recent studies using more rigorous laboratory protocols. Cox et al. (1989) reported that the fresh fruit, at a concentration of 2 g/mL was inactive on guinea pig ileum vs electrical stimulation.
In this study, we studied anti-inflammatory effect of noni juice in chronic asthmatic inflammation in addition to its spasmolytic activity.

Asthma is a chronic inflammatory disease characterized by airway inflammation, remodeling, bronchial hyperresponsiveness, variable air flow obstruction and mucus hypersecretion. During the asthmatic inflammation, there is a migration of inflammatory cells into lung tissue, including eosinophils and lymphocytes. This migration plays an important role in the process of pathogenesis of asthma. In particular, eosinophils are effector cells in allergic diseases by releasing cytotoxic granule
proteins. The worldwide prevalence of allergic disease such as asthma has been increased for the last two decades. Interactions between environmental and genetic factors are involved in allergic disease. Recently, it has been suggested that the dietary change can increase the risk of asthma (Devereux and Seaton, 2005). Indeed, the consumption of fruits and vegetables can adduce, to the organism, vitamin C and other antioxidant compounds such as polyphenols that can oppose to the oxidative stress present in asthma. Costa Rican noni juice can adduce these antioxidants which have still prove to have anti-inflammatory effect.

In ovalbumin sensitized rats, the total number of cells, the number of eosinophils, neutrophils, lymphocytes and macro-phages in the BAL fluid were increased. The administration of noni juice at the oral dose of 4.55 mL/kg significantly decreased the total number of cells as well as the number of neutrophils and eosinophils. Macrophages and lymphocytes strived for decreasing. The intraperitoneal administration at the dose of 2.27 mL/kg significantly decreased the total number of cells, the number of eosinophils and lymphocytes. Macrophages and neutrophils strived for decreasing. These results strongly suggest that noni juice have an anti-inflammatory activity in the lungs of sensitized rats. The doses used (4.55 mL/kg oral route and 2.27 mL/kg IP route) are in the low range of usual juice intake reported in the literature (0.4–20 mL/kg) (West et al., 2009a, 2009b). If noni juice does not inhibit the whole inflammatory process, it is significantly part of its control. The intraperitoneal administration gave better results than oral administration. The
bioavailability of some molecules such as phenolic compounds might explain this difference.

In the physiopathology of asthma, NO plays an important role. NO, involved in the inflammatory process, is found in the exhaled air of asthmatics \( \text{Kharitonov et al., 1994; Nelson et al., 1997}. \) In this study, we found that noni juice was able to scavenge NO in vitro, which can probably explain a part of the antiasthmatic activity of noni juice in vivo.

ORAC assay in BAL showed an increase of antioxidative status in sensitized animals. This can be explained by the need of animals to protect themselves against oxidative stress present in asthma. By the intraperitonally administrations of noni juice, we observed a decrease of the antioxidant status of the BAL which was not the case when noni juice was orally administrated. The antioxidative status of BAL of animals treated by intraperitoneal administrations of noni juice strived for becoming as normal animals. We can suppose that the effective noni juice treatment could correct a part of the oxidative stress and could diminish the oxidative status of the BAL because it's not necessary to oppose to oxidative stress.

This anti-inflammatory activity of noni juice could be supported by the presence of flavonoids such as quercetin, rutin, isoquercitrin and kaempferol glycoside which have antiasthmatic properties \( \text{Jung et al., 2007; Moon et al., 2008; Park et al., 2009; Rogerio et al., 2007} \).
Intestinal spasms are due to a high smooth muscle contractions and cause important pains. We therefore evaluated the anti-spasmodic effect of noni juice using jejunum fragments of rats. We studied the effects of noni juice on BaCl$_2$ or methacholine contrac-tons. BaCl$_2$ caused a hyperpolarization of the smooth muscle cell membranes and induced the entry of calcium by voltage dependent calcium channel that causes the contraction of cells. Methacholine is a synthetic agonist of muscarinic receptor that causes contraction of cells. Noni juice had a marked spasmolytic effect: both types of contractions being inhibited by noni juice in a dose dependent manner. The effect of noni juice on BaCl$_2$ contraction was sig-nificantly more important than that on methacholine contraction, which could be due to a calcium channel blocker activity.

To establish an ex vivo bioassay for muscarinic receptors and calcium channel located on jejunum muscle, we first studied the effect of methacholine administered in a cumulative manner in presence or absence of the muscarinic receptor antagonist: atropin and then, the effect of calcium (in calcium free Tyrode's solution), administered in a cumulative manner, in presence or absence of a calcium channel blocker: nifedipin. Atropin antagonised contrac-tions produced by methacholine and caused a rightward shift of the CRC with no impairment of the maximal response to the methacholine. Nifedipin antagonised calcium induced contraction with a diminution of the maximal response of calcium.

As opposed to muscarinic antagonist, noni juice administered at different doses decreased the maximal methacholine response. Therefore, the spasmolytic activity of noni juice was not due to a competitive antagonism of muscarinic receptors.

As the calcium channel blocker, noni juice administered at different doses decreased the maximal calcium contraction sug-gest-ing a calcium channel blocker activity of noni juice. However, this effect was less important than observed with nifedipin and does not seem to be significant enough to explained the whole spasmolytic effect of noni juice, others mechanisms being prob-ably involved. The calcium channel blocker activity should be confirmed by additional experiments involving electro-physiological studies. It must be noted that recently, Gilani et al. (2010) showed that a noni root extract presented a spasmolytic activity in rabbit jejunum and a vasodilator activity in rabbit aorta, the vasodilator activity being mediated through blockade of vol-tage dependent calcium channels. But the chemical composition of the noni fruit juice and root extract may be profoundly different.

The spasmolytic activity of noni juice could be supported by the presence of flavonoids and coumarins which have smooth muscle relaxation activity (Hammad and Abdalla, 1997; Herrera et al., 1996; Leal et al., 2003; Revuelta et al., 2000; Oliveira et al., 2001).
5. Conclusion

Noni juice from Costa Rica demonstrated, for the first time, an anti-inflammatory effect on bronchial inflammation and a spasmo-lytic activity probably due to a calcium channel blocker activity. The presence of phenolic compounds such as flavonoids and coumarins, vitamin C and iridoids can partially explain these activities.

Consumption of noni juice can therefore reduce the severity of pathologies associating spasms and inflammation such as asthma and intestinal diseases by reduction of chronic inflammation and smooth muscles hyperactivity. The doses used in this study are in the range of effective (1–10 mL/kg) (Pandy et al., 2012; West, 2013) and safe (90 mL/kg) (West et al., 2009a, 2009b) oral route intake in humans.

Our results confirm the ethno-pharmaco-therapeutic uses described in the various traditional pharmacopoeia. However, it is now necessary to identify the molecule or the group of molecules that confers these activities to noni juice.

Acknowledgement

We would like to express our thanks to the animal care facility technicians for their help in this study.

References


phytochemistry, pharmacology, safety. Planta Med. 73, 191–199.


