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Synthesis and characterization of norbelladine, a precursor of Amaryllidaceae alkaloid, as an anti-inflammatory/anti-COX compound

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ABSTRACT

Rising ROS and systemic inflammation is often a serious concern in many disease conditions including obesity. Therefore, compounds with both anti-oxidant and anti-inflammatory activities are considered beneficial in preventing/treating several human chronic diseases. Norbelladine is an amine compound, a precursor for Amaryllidaceae alkaloids (e.g., belladine, crinamine, lycorine, and galanthamine) found in plants traditionally used for treating a variety of human diseases. However, little information is available about its potential health effects. Therefore, the amine was first synthesized, and its anti-oxidant and anti-inflammatory activities were investigated in this study. Also, the potential effects of the amine on NF-κB activation were investigated due to the critical involvement of ROS in the activation. Norbelladine was synthesized with more than 60% yield, analyzed by a HPLC method, and verified using NMR spectroscopic method. Then, its radical scavenging activity was investigated using DPPH- and superoxide radical assays. At the concentration of 10 μM, norbelladine was a compound able to quench DPPH-radical by 31% ($P < 0.05$) and reduce superoxide radicals from xanthine oxidase by 33% ($P < 0.05$). At the concentration of 0.25 μM, the amine also inhibited both COX-1 and COX-2 enzymes by 51% and 25% ($P < 0.05$), respectively. Furthermore, at the concentration of 10 μM, norbelladine inhibited NF-κB activation by 23% ($P < 0.05$). In summary, the data suggests that norbelladine may be a compound to quench radicals, inhibit COX enzymes as well as suppress NF-κB activation at relatively lower concentrations.

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Obesity is a serious health condition often associated with several chronic diseases such as diabetes, cardiovascular disease, hypertension and chronic kidney diseases.[1,2] Particularly, visceral obesity is strongly associated with increased reactive oxygen species (ROS) and subclinical systemic inflammation, which are considered as major causes for developing several obesity-induced chronic diseases.[3,4] In fact, ROS are normal by-products of mitochondrial respiratory chain activity.[5,6] However, in association with inadequate antioxidant capacity and some disease conditions, the elevated levels of ROS can cause significant consequence to cellular signals and components such as lipids, proteins, and DNA, thereby shifting cell homeostasis.[7,8] For instance, ROS activate nuclear factor kB (NF-kB) via IkB kinase (IKK) through phosphoinositide 3-kinase (PI3K) and other processes.[9,10] NF-kB with other proteins eventually leads to activating several transcription-associated factors such as AP-1 and CREB-binding protein, presumably promoting the expression of multiple inflammation-related proteins including cyclooxygenase-2 (COX-2). Especially related to COX-2 expression, several data even suggest that the ROS-modified molecules may act as endogenous inducers of COX-2 responsible for increasing prostaglandin production during inflammatory and immune responses.[9–12] Cyclooxygenases (prostaglandin H synthase or PGHS) are enzymes with both cyclooxygenase and peroxidase activities,[13–15] and there are two major forms of cyclooxygenases (COX-1 and COX-2). COX-1 is constitutively expressed in numerous cell types, meanwhile the expression of COX-2 is transiently induced by a variety of stimuli such as phorbol esters, lipopolysaccharides, and cytokines.[16,17] The COX enzymes catalyze the conversion of arachidonic acid to prostaglandin H2, the precursor of important biological prostanoid mediators such as prostaglandins, prostacyclin and thromboxane. By producing a variety of prostaglandins, the enzymes are deeply involved in pathophysiological processes such as inflammation and pain.[17] Since ROS and COX have been considered to play significant roles in the initiation and/or progression of several chronic human diseases, there has been considerable research effort in order to find effective compounds with both anti-ROS and anti-COX activities.

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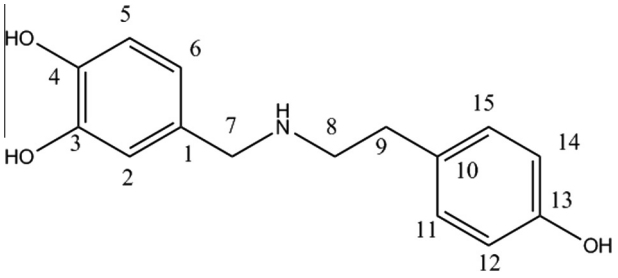

Figure 1. Chemical structures of norbelladine.

Norbelladine is a precursor for Amaryllidaceae alkaloids (e.g., belladine, crinamine, lycorine, and galanthamine) found in several plants such as *Galanthus nivalis*, *Leucojum aestivum*, *Narcissus tazetta*, *Nerine bowdenii*, and *Pancratium maritimum*.[18,19] Although the potential effects of the Amaryllidaceae alkaloids have been studied in a great number,[20,21] the potential health effects of norbelladine has not been investigated in detail. Therefore, in this study, norbelladine (Fig. 1) was prepared via the chemical synthesis method described in Supporting information section. The synthesis was relatively simple, and the yield was greater than 60%. The synthesized product was purified by HPLC, and analyzed using NMR spectroscopic methods (NMR data were provided in Supporting information section). A HPLC method was developed using the purified norbelladine as a standard. The amine was determined using the high-performance liquid chromatography (HPLC) equipped with Nova-Pak C_{18} column and an electrochemical detector. Since an electrochemical detector is used as a principal detector in this HPLC method, the electro-activities of the compound were measured using the detector. Several different buffer conditions were first tested to improve the resolution of the amine, and a gradient condition was selected for optimizing separation, detection and preparation of samples as described in Supporting information section. The chromatograms of the amine standard (100 pmol) are shown with two precursors (tyramine and protocatechuic aldehyde) in Figure 2.

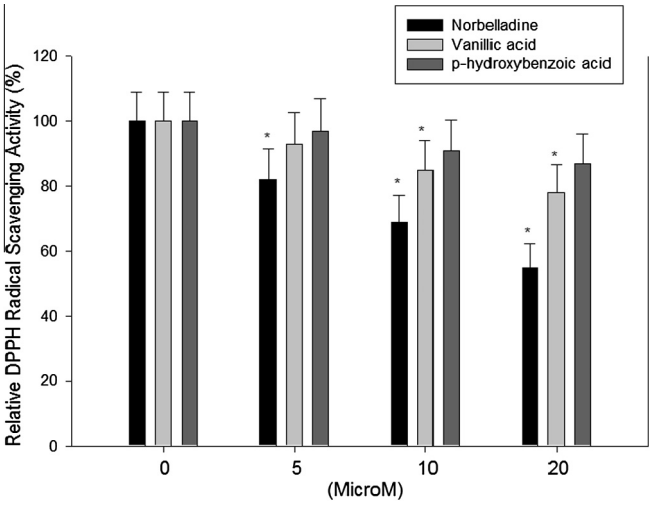

Figure 3. DPPH-radical scavenging activity of norbelladine. DPPH assay was performed at the concentrations of norbelladine, vanillic acid and *p*-hydroxybenzoic acid (0, 5, 10, 20 μM). Data are presented as mean ± SD ($n = 8$). *P* value was calculated using SigmaPlot 11.0 (Holm-Sidak method; ($P < 0.05$)). The mark (*) indicates a significant change compared to control samples.

The radical scavenging activity of norbelladine was determined using a stable radical DPPH assay. The DPPH radical is reduced to a colorless product as norbelladine scavenges the radical, and the reduction can be measured by a UV–visible plate reader. As shown in Figure 3, norbelladine showed relatively strong DHHP-radical scavenging activity. Interestingly, benzoic moiety with hydroxyl groups at the 3- and 4-positions showed better DHHP-radical scavenging activity than phenolics with one or modified hydroxyl group such as *p*-hydroxybenzoic acid and vanillic acid (Fig. 3). This suggests that intact hydroxyl groups at the 3- and 4-positions of benzoic moiety in norbelladine may be critical in scavenging DPPH-radical.

Since norbelladine was able to scavenge DHHP-radicals, its ability to quench superoxide radical was further investigated using

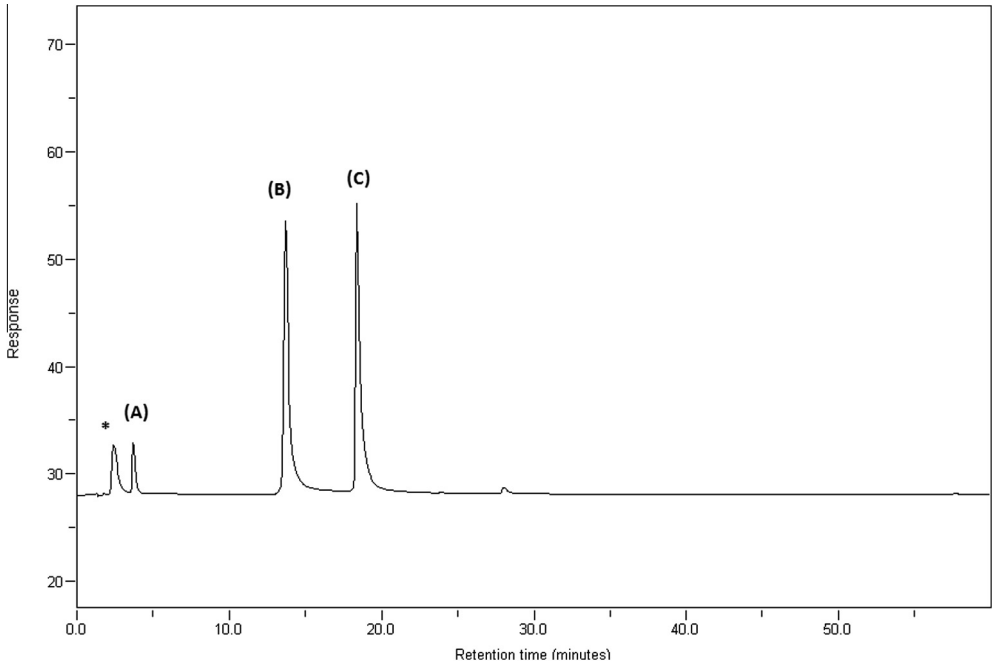

Figure 2. HPLC chromatograms of tyramine (A), protocatechuic aldehyde (B) and norbelladine (C). All three chemicals (100 ρmol) was injected into a high-performance liquid chromatography (HPLC), and detected by an electrochemical detector. The mark (*) indicates an injection peak.

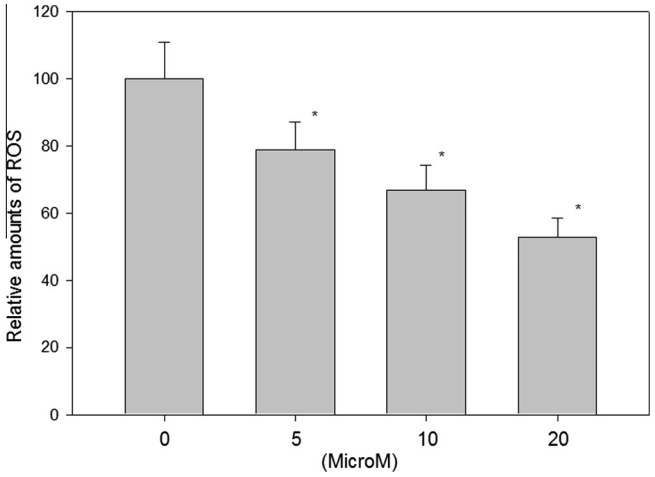

Figure 4. Effects of norbelladine on ROS from xanthine/xanthine-oxidase. ROS production was inhibited in a cell-free xanthine/xanthine-oxidase system using different concentrations of norbelladine (0, 5, 10, 20 μM). Data are presented as mean ± SD ($n = 8$). P value was calculated using SigmaPlot 11.0 (Holm-Sidak method; ($P < 0.05$)). The mark (*) indicates a significant change compared to control samples.

xanthine/xanthine oxidase system. Xanthine oxidase (XO) catalyzes hypoxanthine to xanthine and further catalyzes xanthine to uric acid.[22] During the catalysis, XO generates superoxide, a powerful reactive oxygen species, leading to cell degeneration and death. XO is found predominantly in the liver and intestine, but levels of circulating XO can increase dramatically with some disease conditions including diabetes and hypertension.[22] As shown in Figure 4, norbelladine was able to scavenge superoxide radical from xanthine/xanthine oxidase system by 33% ($P < 0.05$) at a concentration of 10 μM comparable to the control, confirming that the amine compound may be a good superoxide-radical scavenger. These data clearly indicated that norbelladine is able to scavenge superoxide-radicals generated from XO.

Potential effect of norbelladine on COX-1 was investigated as an inhibitory mechanism of inflammation, because COX-1 inhibitors

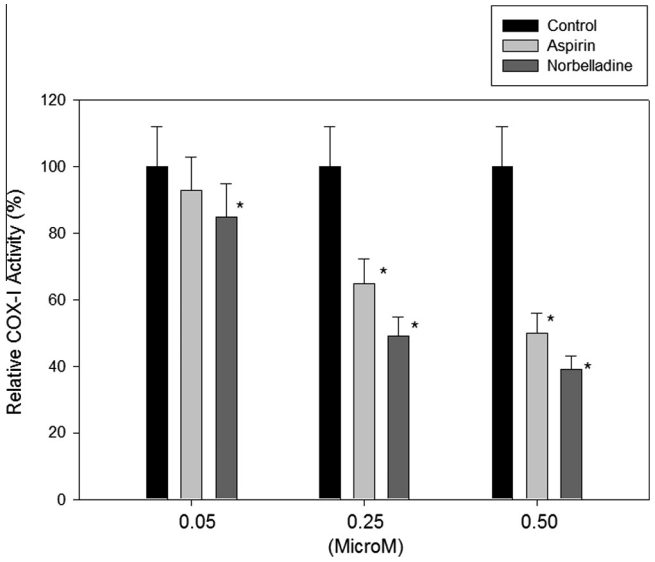

Figure 5. Effects of norbelladine on COX-1 enzyme. Control (1), Aspirin (2), and norbelladine (3). The amides or aspirin were added to the samples, and the reaction mixtures were incubated at room temperature (in the dark) for 10 minutes. Following the incubation, COX-1 activity was measured according to the kit's protocol using a luminometer. The mark (*) indicates a significant reduction compared to control samples.

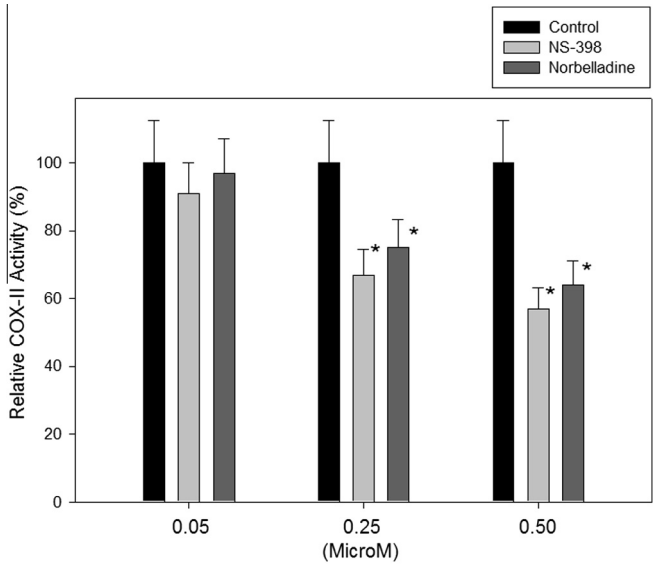

Figure 6. Effects of norbelladine on COX-2 enzyme. Control (1), NS-398 (2), and norbelladine (3). The amides or NS-398 were added to the samples, and the reaction mixtures were incubated at room temperature (in the dark) for 10 min. Following the incubation, COX-2 activity was measured according to the kit's protocol using a luminometer. The mark (*) indicates a significant reduction compared to control samples.

(e.g., aspirin) are known to inhibit inflammation and platelet activation via preventing the conversion of arachidonic acid (AA) to thromboxane A2 (TXA2) and others.[13–15] As shown in Figure 5, norbelladine was a compound able to inhibit COX-1 enzyme by 51% ($P < 0.05$) at the concentration of 0.25 μM. On comparison with a well-known COX-1 inhibitor (aspirin), norbelladine was able to inhibit COX-1 to a greater extent than the inhibitor. Although COX-1 enzyme is primarily involved in platelets, the effect of norbelladine on COX-2 was also investigated, because COX-2 is likely to be involved in many important physiological processes related to inflammation.[14,15] Like the COX-1, norbelladine was also a compound to inhibit COX-2 enzyme by 25% ($P < 0.05$) at the concentration of 0.25 μM. Compared to NS-398 (a COX-2 specific inhibitor), norbelladine was able to inhibit COX-2 as much as NS-398 (Fig. 6).

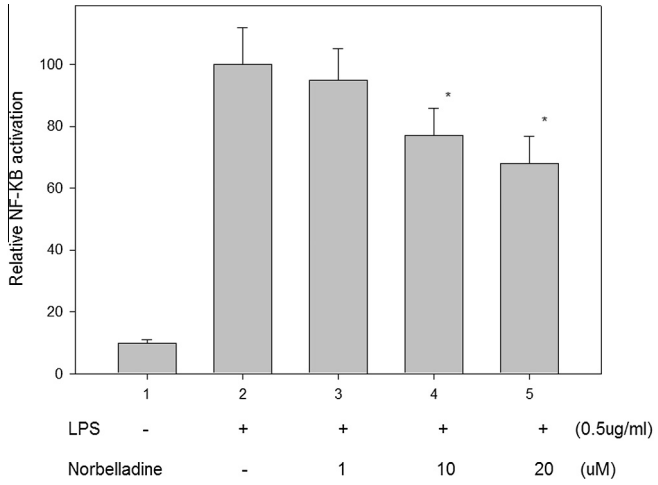

Figure 7. Effects of norbelladine on NF-kB activation. THP-1 cells (2×10^9) were incubated for 15 min with several concentrations of norbelladine (0, 1, 10, 20 μM), then treated with LPS (0.5 μg/ml) for 16 h. The cells were harvested and washed with PBS twice. The NF-κB assay was performed using Cayman's NF-κB (p65) transcription factor assay kit (Ann Arbor, Michigan). The mark (*) indicates a significant reduction compared to control samples.

These data suggest that norbelladine is a compound able to inhibit both COX-1 and 2 enzymes.

As mentioned previously, ROS is deeply involved in activating NF-κB and the activation is closely associated with expressing numerous inflammation-related proteins including cyclooxygenase-2 (COX-2).[9,10] Therefore, the potential effects of the amine on NF-κB activation were investigated. For the experiment, THP-1 cells (2×10^9) were treated with several concentrations of norbelladine (0, 1, 10, 20 μM), then treated with LPS as described in Supporting information section. As shown in Figure 7, norbelladine inhibited the NF-κB activation induced by LPS by 23% ($P < 0.05$) at the concentration of 10 μM. The data suggest that the amine may inhibit COX I and II enzymes as well as suppress COX II expression via inhibiting NF-κB activation. This study suggests that norbelladine may be a compound able to provide significant antioxidant and anti-inflammatory effects via scavenging radicals and inhibiting COX enzymes, furthermore inhibiting NF-κB activation at relatively lower concentrations.

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Disclosures: There is nothing to disclose about financial, consulting, and personal matters related to this Letter.

Supplementary data

Supplementary data (experimental details and compound characterization for norbelladine) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.10.051.

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