Characterization of Inhibitors of Postprandial Hyperglycemia from the Leaves of Nerium indicum

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Summary Nerium indicum is an India-Pakistan-originated shrub belonging to the oleander family. The ingestion of leaves of N. indicum before a meal is known to effect the lowering of postprandial blood glucose levels in Type II diabetic patients and this plant is now used as a folk remedy for Type II diabetes in some regions of Pakistan. In the present study, the hot-water extract of N. indicum leaves was found to reduce the postprandial rise in the blood glucose when maltose or sucrose was loaded in rats. It was also found that the extract strongly inhibited α-glucosidase, suggesting that the suppression of the postprandial rise in the blood glucose is due to the occurrence of some inhibitors of α-glucosidase in the leaves. We, therefore, tried to isolate the active principles from the leaf extract, using α-glucosidase-inhibitory activity as the index. Employing Sephadex G-15, silica gel and reversed-phase HPLC, we isolated two active compounds. The UV, mass and NMR spectrometric analyses established that the chemical structures of these compounds are 3-O-cafeoylquinic acid (chlorogenic acid) and its structural isomer, 5-O-cafeoylquinic acid. Both compounds were shown to inhibit α-glucosidases in a non-competitive manner. The authentic chlorogenic acid was found to suppress the postprandial rise in the blood glucose in rats and also inhibited the absorption of the glucose moiety from maltose and glucose in the everted gut sac system prepared from rat intestine. These results demonstrate that chlorogenic acid is one of the major anti-hyperglycemic principles present in the leaves of N. indicum. Furthermore, among polyphenol compounds tested, quercetin and catechins were shown to have strong inhibitory activity against α-glucosidase.

Key Words Nerium indicum, hyperglycemia, α-glucosidase inhibitor, chlorogenic acid, polyphenols

Nerium indicum (local name; Gul-e-Zangi) is an India-Pakistan-originated shrub belonging to the oleander family. One of the present authors (A.M.) observed that the ingestion of leaves of N. indicum effected the lowering of postprandial blood glucose levels in Type II diabetic patients and this plant is now used as a folk remedy for Type II diabetes in some regions of Pakistan.

Postprandial hyperglycemia plays an important role in the etiology of diabetes-related complications and outcomes. α-Glucosidase inhibitors such as acarbose, miglitol and voglibose are now widely used as means to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate-digesting enzymes and delaying glucose absorption (1, 2).

The purpose of this communication is to see if we can reproduce the postprandial hyperglycemia-suppressive effect of N. indicum leaves in rats and to characterize the inhibitory principles in the leaves.

MATERIALS AND METHODS

Materials. The materials in the present study were obtained from the sources indicated in the parentheses. Sephadex G-15 (Pharmacia, Sweden), AtlantisTM dC18 (5 μm, 100 Å) column (Waters Co., USA), ferulic acid (LKT Laboratories Inc., USA), chlorogenic acid (Tokyo Kasei Co., Japan), catechol, quercetin dihydrate, and acetonitrile for HPLC (Sigma Aldrich Co., USA), pyrogallol, catechin, epicatechin, catechin gallate, epigallocatechin, caffeic acid, trans-cinnamic acid, p-hydroxycinnamic acid, m-hydroxycinnamic acid, o-hydroxycinnamic acid, gallic acid hydrate, apigenin, Wako gel LP-60, and Glucose II-Test Wako (Wako Pure Chemical Ltd., Japan), and F-kit Glucose (Roche Diagnostic GmbH, Mannheim, Germany). Male Sprague-Dawley (SD) rats weighing about 250 g were purchased from Charles River Laboratories Inc., Japan. The leaves of N. indicum, grown in Sindh State of Pakistan, were col-

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lected and dried naturally under the sun. The dry leaves were pulverized with a coffee mill and stored at 5°C until use.

**Oral disaccharide tolerance test in rats.** Rats were fed on the laboratory diet and water ad libitum until use. After overnight (16 h) fasting, the rats were given aqueous solutions of the test compounds orally. After 10 min, a disaccharide (maltose or sucrose, 2 g/kg body weight) solution was administered orally. Blood samples were collected from tail veins before the administration and at intervals of 30 min up to 120 min, and immediately subjected to blood glucose analysis with the use of Glucose C II-Test Wako.

**Extraction of the active principles from the N. indicum leaves.** The pulverized N. indicum leaves were suspended in hot water at 80°C and allowed to stand for 1 h with stirring. After the hot-water extraction, the suspension was centrifuged at 10,000×g for 30 min. The pellet was mixed with hot water at 80°C for 1 h with stirring. After the hot-water extraction, the concentrate was subjected to reduced pressure at 50°C with an evaporator. 30 min, centrifuged and the extraction was repeated once more. The combined extracts were concentrated under reduced pressure at 50°C with an evaporator. The concentrate was used in the following experiments.

**Purification of the active principles from the N. indicum extract.** An aliquot of the extract, prepared as above, was applied to a Sephadex G-15 column (4.5×150 cm) equilibrated with water. The active fractions, possessing α-glucosidase inhibitory activity, were collected and concentrated under reduced pressure. The concentrate was then applied to a silica gel column (3.0×27 cm). The active components on the column were separated successively with the following systems: 1. chloroform: methanol: water (6 : 4 : 1. by vol.); 2. chloroform: methanol: water (5 : 5 : 1. by vol.); 3. chloroform: methanol: water (3 : 7 : 1. by vol.); 4. methanol; 5. methanol: water (1 : 1. v/v); 6. water; 7. acetic acid: water (1 : 19, v/v).

The active fractions were collected and concentrated under reduced pressure, and then separated by reverse-phase high-performance liquid chromatography (HPLC) on an Atlantis® dC18 column (3.9×150 mm) equilibrated with 0.05% trifluoroacetic acid. The HPLC was performed by elution with a linear gradient of acetonitrile (0–80%) in 0.05% trifluoroacetic acid for 80 min at a flow rate of 0.5 mL/min. The compounds eluted from the column were followed by measuring the absorbance at 325 nm, and the active fractions were subjected to structural elucidation with UV, NMR, and mass spectrometries.

**Assay of inhibitory activities for α-glucosidase.** The acetone powder, prepared from rat intestine, was suspended in 100 mM sodium phosphate buffer (pH 7.0) and centrifuged at 10,000×g for 15 min. The resultant supernatant was used as the source of α-glucosidase. The specific activities of maltase and sucrase in the supernatant were 0.8 and 0.12 U/mg, respectively. For the assay of inhibitory activities, the reaction mixture contained, in the final concentrations, 20 mM maltose or 200 mM sucrose, 100 mM sodium phosphate buffer (pH 7.0) and a given amount of various inhibitors in a total volume of 50 μL. The reaction was carried out at 37°C. After a 15-min incubation, the reaction was stopped by heating the mixture at 100°C for 5 min. The amount of glucose, produced by the action of α-glucosidase, was determined with the use of F-kit Glucose according to the manufacturer’s instruction. One unit is defined as the amount of enzyme that releases 1 μmol of glucose/min. Specific activity is expressed as units/mg of protein. In the experiments with water-insoluble inhibitors, such as quercetin and apigenin, the inhibitors were dissolved in ethanol, diluted 10-fold with 0.5% deoxycholic acid and used in the above assay. IC50 values were obtained by calculation of the concentrations of the active components at which they exhibit 50% inhibition of the α-glucosidase activity in comparison with that in the absence of the inhibitor.

**Spectrometries.** For measurement of UV spectra, the samples were dissolved in distilled water. UV spectra were taken with a Beckman Coulter DUK® 800 spectrophotometer. For NMR analyses, the samples were dissolved in methanol-d4 and their spectra were measured on a Bruker DX-300 NMR spectrometer (Bruker Elektronik GmbH, Germany). Mass analyses were carried out using a Finnigan TSQ-700 quadrupole mass spectrometer with an Ultrix-32 operation system (ion spray voltage, 5.5 V; ion multivoltage, 1.0 kV; flow rate of analyte solution, 5.0 μL/min; concentration of analyte solution, 1.0 μg/μL).

**Absorption of glucose by everted gut sac.** Rats were sacrificed under ether anesthesia. The intestine from the ileocecal junction to about 20 cm was excised and rinsed with the buffer solution. The composition of the buffer solution was (in mM) NaCl (119), NaHCO3 (21), KH2PO4 (0.6), K2HPO4 (2.4), CuCl2 (1.2), MgCl2 (1.2) and mannose (8.5), and was bubbled with 95% O2/5% CO2 (pH 7.4). The intestines were divided into segments about 6 cm long. The segments were everted, ligated at one end, filled with 0.2 mL of the buffer solution on the serosal side, and ligated at the other end to make gut sacs. The sacs were placed in the buffer solution containing 5.0 mM maltose or glucose with or without 1.0 mM chlorogenic acid. The incubation was carried out at 37°C for 1 h. The incubation, the concentration of glucose in the serosal side was measured with a Wako Glucose C II-Test Kit.

**Data analyses.** Each result is expressed as the mean±SE. Statistical evaluations were performed by Student’s t-test.

**RESULTS**

**Effect of N. indicum leaves on disaccharide tolerance test in rats**

We first examined if we could observe the postprandial hyperglycemia-suppressive effect of N. indicum leaves in rats in a similar manner as observed in human diabetic patients. The hot-water extract of N. indicum was prepared as described in Materials and Methods and the extract was stomach-fed before the oral administration of maltose or sucrose. As shown in Fig. 1, the
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extract markedly suppressed the rise in blood glucose levels after the loading of maltose or sucrose. When glucose was administered in place of maltose or sucrose, the extract exerted no effect on the tolerance curve (data not shown), indicating that the effect of the extract is specific to disaccharide loading.

This result suggests that N. indicum extract works by inhibiting α-glucosidases such as maltase and sucrase. Indeed, the extract had a strong inhibitory effect on the activities of maltase and sucrase in vitro (data not shown).

Isolation and characterization of α-glucosidase inhibitors from N. indicum leaves

We then proceeded to isolate and characterize the active principles from N. indicum leaves, taking advantage of the α-glucosidase inhibitory activity as the indicator and employing various chromatographic systems. The hot-water extract of N. indicum leaves was applied to a Sephadex G-15 column and eluted with water. A typical elution pattern is shown in Fig. 2. Three main active peaks (peaks A, B, and C) were observed. In the present study, we focused our effort to further purify peak C. The fractions corresponding to peak C were collected, and concentrated. The concentrate was applied to a silica gel column equilibrated with solvent system 1, described under Materials and Methods. The active components adsorbed on the column were eluted successively with the solvent systems described under Materials and Methods (Fig. 3). Fraction II obtained with solvent system 2 was found to inhibit α-glucosidases more strongly than fraction I obtained with solvent system 1. Therefore, fraction II was subjected to further purification.

Fraction II was concentrated and applied to an Atlantis™ dC18 column which separated the active components into peaks 1 and 2 with a linear gradient of acetonitrile (0–80%) in 0.05% trifluoroacetic acid. Peak 2 was eluted at the same position as the authentic chlorogenic acid (Fig. 4). Both components were found to inhibit maltase and sucrase, and both components were shown to be chromatographically pure by HPLC with various solvent systems.

The UV spectra of peaks 1 and 2 are shown in Fig. 5. The spectra of both peaks were found to be similar, having the maximal absorption wavelength (Amax) of 325 nm, but were not the same under close inspection. Of the two spectra, the spectrum of the peak 2 component exactly matched that of the authentic chlorogenic acid. Since peak 2 was found to be eluted at the same position as the authentic chlorogenic acid on the Atlan-
Inhibitors of Postprandial Hyperglycemia from Nerium indicum

It is very likely that peak 2 represents chlorogenic acid.

To substantiate this possibility, components 1 and 2 were hydrolyzed in the presence of 2-mercaptoethanol in 0.5 M HCl at 100°C for 4 h. When the hydrolysates were subjected to Atlantis™ dC18 column chromatography under the same conditions, a peak corresponding to the authentic caffeic acid was observed (Fig. 6). This result suggests that the two components contain a caffeic acid moiety. Mass analyses showed that the mass spectra of the two components are identical with that of the authentic chlorogenic acid and that the [M+H]+ of the components is m/z 355, which corresponds to that of chlorogenic acid. Furthermore, in high resolution mass analyses, the exact [M+H]+ masses of the components 1 and 2 were found to be 355.1032 and 355.1017, respectively. These molecular weights of the components were identical with that of authentic chlorogenic acid. These observations, taken together, indicate that component 2 is chlorogenic acid (3-O-caffeoylquinic acid) and component 1 may be a structural isomer of chlorogenic acid.

In order to elucidate the exact structures of the active components, components 1 and 2, together with the authentic chlorogenic acid, were analysed by NMR spectrometry (Fig. 7). The NMR spectrum of component 2 was identical with that of the authentic chlorogenic acid, but the spectrum of component 1 was slightly different. The two protons bound to the double bond in the caffeic acid moiety of the two components showed doublet signals at around 6.2 and 7.5 ppm (J=16 Hz), which means that caffeic acid has not cis-structure, but trans-structure. The NMR spectrum of the authentic chlorogenic acid showed the complex signals near 5.33, 3.72 and 4.16 ppm for the protons at C-3, C-4 and C-5, respectively, of its quinic acid moiety, and the spectrum of component 1 showed similar signals to those of the authentic chlorogenic acid. Comparison of these chemical shifts with those reported by Morishita et al. (3) supports the notion that the signals near 5.33, 4.16 and 3.64 ppm from component 1 may correspond to the protons at C-5, C-4 and C-3 of the quinic acid moiety of 5-O-caffeoylquinic acid. We thus conclude that...
component 1 is 5-O-caffeoylquinic acid, a structural isomer of 3-O-caffeoylquinic acid (chlorogenic acid).

We also examined the contents of components 1 and 2 in N. indicum leaves. The hot-water extract of the powdered leaves was analyzed by reverse-phase HPLC and the amounts of components 1 and 2 in the dried leaves were estimated to be 0.7, and 5.5 mg/g dry weight, respectively.

**Mode of inhibition of α-glucosidase by chlorogenic acid and related compounds**

We initially observed that the hot-water extract of N. indicum leaves strongly inhibited the activity of α-glucosidase from rat intestine when tested with maltose or sucrose as a substrate (data not shown). The mode of inhibition of α-glucosidase by the purified inhibitors, chlorogenic acid and related compounds, using maltose as the substrate, was investigated in detail. As seen in Fig. 8, Lineweaver-Burk plots of α-glucosidase activity in the presence of 5-O-caffeoylquinic acid or 3-O-caffeoylquinic acid (chlorogenic acid) show that both compounds inhibit the enzyme in a non-competitive manner. The IC50 values of chlorogenic acid and its isomer were found to be 2.99 and 3.12 mM, respectively. A number of polyphenolic compounds were examined with regard to inhibition of α-glucosidase activity with maltase and sucrase (Table 1). Caffeic acid, the aromatic moiety of chlorogenic acid, inhibited the α-glucosidase activity. Quinic acid, the sugar moiety of chlorogenic acid, did not show any inhibitory activity. Furthermore, pyrocatechol was found to be a strong inhibitor of α-glucosidase. Catechin derivatives also strongly inhibited α-glucosidase. In particular, catechin gallate with the IC50 value of 0.15 mM was found to be the most potent inhibitor of the polyphenolic compounds tested. Compounds like o-, m-, and p-coumaric acids and trans-cinnamic acid, which have no phenolic hydroxyl group at the benzene ring, did not inhibit α-glucosidase.

### Table 1. Inhibition of maltase and sucrase by polyphenolic compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 value (mM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Maltase</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>2.99 ± 0.38</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>8.28 ± 1.20</td>
</tr>
<tr>
<td>o-Hydroxycinnamic acid</td>
<td>No</td>
</tr>
<tr>
<td>m-Hydroxycinnamic acid</td>
<td>No</td>
</tr>
<tr>
<td>p-Hydroxycinnamic acid</td>
<td>No</td>
</tr>
<tr>
<td>trans-Cinnamic acid</td>
<td>No</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>No</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>No</td>
</tr>
<tr>
<td>Pyrocatechol</td>
<td>17.40 ± 2.70</td>
</tr>
<tr>
<td>Pyrogallocate</td>
<td>No</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.71 ± 0.08</td>
</tr>
<tr>
<td>Apigenin</td>
<td>No</td>
</tr>
<tr>
<td>Catechin</td>
<td>1.35 ± 0.34</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>1.75 ± 0.21</td>
</tr>
<tr>
<td>Catechingallate</td>
<td>0.15 ± 0.00</td>
</tr>
</tbody>
</table>

1 Values are mean ± SE (n = 3). No, not inhibited.
leaves, exerting an inhibitory effect on one of the major compounds, present in rats

Effect of chlorogenic acid on disaccharide tolerance test in rats

We have so far established that chlorogenic acid is one of the major compounds, present in N. indicum leaves, exerting an inhibitory effect on α-glucosidase. The inhibition of α-glucosidase would interfere with the digestion of dietary carbohydrates and thus delay the glucose absorption from the intestine.

We next tested if we can observe the postprandial hyperglycemia-suppressive effect of chlorogenic acid in a similar manner as observed with the hot-water extract of N. indicum leaves (Fig. 1). Twenty five mg/kg body weight of chlorogenic acid in aqueous solution was administered orally to rats 10 min before the oral loading of maltose or sucrose. As shown in Fig. 9, chlorogenic acid significantly suppressed the rise in blood glucose levels after loading of maltose or sucrose. On the other hand, chlorogenic acid showed no effect on the tolerance curve when glucose was orally loaded instead of maltose or sucrose (data not shown).

Effect of chlorogenic acid on the absorption of glucose by everted gut sac from rats

It is generally known that monosaccharides such as glucose and fructose can be transported across the intestinal mucosa whereas they are impermeable to disaccharides, and thus delay the glucose absorption from the intestine.

In order to verify that chlorogenic acid, by its inhibitory action against intestinal α-glucosidase, inhibits digestion and absorption of disaccharides, we constructed an everted gut sac from rats. The everted sac, prepared as described under Materials and Methods, was bathed in the buffer solution containing maltose, and the transported glucose concentration in the serosal side was measured. As shown in Fig. 10A, the addition of chlorogenic acid, at the concentration of 1.0 mM in the mucosal side, reduced the concentration of the transported glucose moiety from maltose to 46% of the control value. Chlorogenic acid also reduced the transport of glucose itself to 48% of the control value (Fig. 10B). Chlorogenic acid was thus found to inhibit the uptake of not only maltose but also glucose from the intestine, consistent with the antihyperglycemic effect of chlorogenic acid in vivo.

DISCUSSION

As described in the introduction, the ingestion of the leaves of N. indicum, grown in Pakistan, is effective in the lowering of postprandial blood glucose levels in Type II diabetic patients and this plant is now used as a folk remedy for Type II diabetes in some regions of Pakistan. The present study was undertaken to elucidate the mechanism of its action.

The hot-water extract of the leaves was found to reduce the postprandial rise in the blood glucose when maltose or sucrose was loaded in rats (Fig. 1), but the extract showed no effect when glucose was loaded in place of disaccharides (data not shown). It was also found that the extract strongly inhibited α-glucosidase activity, using maltose or sucrose as a substrate (data not shown). These observations suggest that the suppression of postprandial rise in the blood glucose is due to the presence of α-glucosidase inhibitor(s) in the leaves of N. indicum.

The chromatographic separation of the leaf extract on a Sephadex G-15 column, using α-glucosidase-inhibitory activity as the index, yielded 3 peaks of active compounds (Fig. 2). Further separation of one of the peaks (Fraction C) on a silica gel column yielded 2 active peaks (Fig. 3), and the subsequent chromatography of the more active peak on a HPLC column resolved 2 components (Fig. 4). The ensuing analyses of the chromatographically resolved components by UV, mass and NMR spectroscopies established that the chemical structures of the purified active components are 3-O-cafeoylquinic acid (chlorogenic acid) and its structural isomer, 5-O-cafeoylquinic acid. Both compounds inhibited α-glucosidase activities in a non-competitive manner, and the authentic chlorogenic acid suppressed the postprandial rise in the blood glucose in rats and also inhibi-
ited the absorption of the glucose moiety from maltose in the everted gut sac system prepared from rat intestine.

These results demonstrate that chlorogenic acid is one of the major anti-hyperglycemic principles present in the leaves of *N. indicum*. However, the leaves appear to contain active principles other than chlorogenic acid as evidenced by the presence of a number of active peaks in the chromatographic systems presently employed. It is possible that various active principles act synergistically against postprandial hyperglycemia.

Inasmuch as chlorogenic acid suppresses postprandial hyperglycemia by inhibiting α-glucosidase, its action resembles that of currently available α-glucosidase inhibitors such as acarbose, miglitol and voglibose (1, 2). Many other natural or synthetic α-glucosidase inhibitors of various chemical structures have been described (4–15). Matsui et al. (8) previously reported that chlorogenic acid inhibited maltase and sucrase with the IC\textsubscript{50} value of 18.1 and 3.1 mM, respectively. The difference between the IC\textsubscript{50} values obtained by the present study and by Matsui et al. (8) may be due to the difference in the assay methods employed. We assayed the α-glucosidase by the free enzyme system while Matsui et al. (8) used the immobilized enzyme system developed by Öki et al. (14). The significance of these two assay methods was discussed in a review article by Matsui et al. (15).

Chlorogenic acid is a polyphenolic compound and inhibits rat intestinal α-glucosidase in a non-competitive manner. A similar result was also described by Matsui et al. (15). The structure-inhibitory activity against α-glucosidases are presented in Table 1. Quinic acid, the sugar moiety of chlorogenic acid, showed no inhibitory activity, but esterification of caffeic acid with quinic acid to form chlorogenic acid resulted in an higher inhibitory activity, but esterification of caffeic acid with quinic acid to form chlorogenic acid resulted in an higher inhibitory activity than did the unesterified caffeic acid. Of phenolic compounds tested, the polyphenolic compounds with two hydroxyl groups at C-1 and C-2 of the benzene rings, such as pyrocatechol, catechin gallate and caffeic acid, showed strong inhibitory effects on α-glucosidase. However, hydroxycinnamic acids, cinnamic acid and apigenin, which have no or only one hydroxyl group on the benzene ring, exerted no inhibitory effect on the enzyme. These results clearly indicate that the catechol-type structure is essential for the manifestation of inhibitory activity against α-glucosidase.

Chlorogenic acid has been reported to occur widely in plants; coffee beans and blueberry leaves are particularly rich sources of chlorogenic acid (16). In particular, chlorogenic acid, present in coffee, has been implicated to be responsible for anti-hyperglycemic effects in humans (16). The leaves of *N. indicum* were found to contain a fair amount (5.5 mg/g dry weight) of chlorogenic acid, one of the factors responsible for the anti-hyperglycemic effect. However, as discussed above, other active principles in the leaves may also contribute to the efficacy of the leaves on the hyperglycemia of Type II diabetic patients. Characterization of other active principles, present in *N. indicum* leaves, is in progress.

Johnston et al. (17) recently reported that the consumption of coffee by humans reduced the rise of plasma glucose concentrations in a tolerance test and suggested that chlorogenic acid might have an antagonistic effect on glucose transport. Our experiments with everted gut sac showed that chlorogenic acid inhibited the uptake of glucose from the rat intestine (Fig. 10). This observation may suggest the possibility that chlorogenic acid inhibits the intestinal absorption of sugars both by the inhibition of α-glucosidase and by the attenuation of glucose transport in a synergistic manner.

*N. indicum*, belonging to the oleander family, is generally considered to be highly toxic due to the presence of toxic cardiac glycosides in the plant (18). However, the leaves of *N. indicum*, used in the present study, showed no toxicity toward rats. As described in the experiment on disaccharide tolerance test (Fig. 1), oral administration of 16 g dry leaves/kg body weight did not appreciably alter the behavior of rats during the 120-min test period. The long-term feeding of rats for more than 1 mo with the diet, containing 1% (w/w) powdered dry leaves, exerted no adverse effects on rats. Food intakes and weight gains were the same as for the control rats, fed the diet without the leaves, and no differences were observed in the blood chemistry (serum protein profiles and aminotransferase activities) or in the morphology of liver and small intestine after the feeding period (Inagaki E, Tsuji H, Natori Y, unpublished results). Further, the safe ingestion of the leaves by Type II diabetic patients in Pakistan was described in the Introduction. These observations suggest that the non-toxic *N. indicum*, grown in the State of Sindh, Pakistan, may belong to a different variety of the oleander family than that grown in other parts of the world. This requires further botanical studies.

There is considerable need for safe agents that can reduce risk for diabetes in at-risk subjects. Natural agents which slow carbohydrate absorption such as chlorogenic acid could be marketed as aids to good glucose tolerance and insulin sensitivity (19).

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