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Antiplatelet Activity of Coriander and Curry Leaf Spices

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Abstract

Thrombosis, an important event in cardiovascular diseases, can be fatal if platelet aggregation takes place in the narrowed lumen of arteries, causing an impairment of blood flow to the heart. Attempts have been made to study the antiplatelet activity of leaf spice extracts, as these are rich sources of natural antioxidants. Aqueous extracts of coriander leaf and curry leaf were tested on human platelets over a wide range of concentrations with agonists like adenosine diphosphate (ADP) (61 μM), epinephrine (76 μM), and collagen (0.005% in 0.1 N acetic acid). Both these leaf spice extracts inhibited human platelet aggregation. The IC_{50} values were 0.94, 0.65, and 0.58 mg for curry leaf and 0.55, 0.66, and 0.57 mg for coriander leaf with ADP, epinephrine, and collagen, respectively. A decrease in the malondialdehyde formed was used as a mechanism for accessing the function of the cyclooxygenase pathway in arachidonic acid metabolism at IC_{50} values of these extracts.

Keywords: Coriander, curry, platelets, thrombosis.

Introduction

Platelets are tiny cell fragments that measure about 2–4 μm in diameter and have a lifespan of 7–10 days. They traverse passively with other blood cells along the intact monolayer of endothelial lining of blood vessels (Marieb, 1998). In response to a vascular injury, a localized hemostatic plug is formed to stop blood loss (Hawiger, 1989) because platelets rapidly undergo the processes of adhesion, shape change, secretion, and aggregation through a series of exquisitely coordinated responses (Kroll & Schafer, 1989). When these responses take place in the blood vessels, it can result in pathological conditions associated with coronary or cerebrovascular problems (Jain & Apitz-Castro, 1994).

Spices, adjuncts in cuisine, have contributed to tang and flavor of foods, making them more palatable (Pruthi, 1976). Certain spices are also known to possess antidiabetic, anti-inflammatory, hypolipidemic, and antilithogenic effects as per the Ayurveda, the indigenous system of Indian medicine (Nadkarni, 1978). Spices are known to contribute to total dietary nutrients between 1.5% to 14.5% depending on their intake in the diet (Thimmayamma et al., 1983). Two of the most generously used spices are the curry leaf *Murraya koenigii* (Linn.) Spreng. (Rutaceae) and the coriander leaf *Coriandrum sativum* Linn. (Umbelliferae) for their distinct taste and enhanced acceptability of foods (Parrotta, 2001). Srivastava (1984), in a study with aqueous extract of the spices like onion, ginger and garlic, has shown the presence of component(s) that inhibit platelet aggregation with a variety of agonists.

The current study was carried out because there are no reports of the effect of aqueous extracts of leaf spices on human platelet aggregation.

Materials and Methods

Adenosine diphosphate (ADP), epinephrine, and collagen were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of pure analytical grade purchased locally.

Isolation of platelets

Venous blood was collected in 3.8% tri-sodium citrate (9:1 at least v/v) from healthy human volunteers who had not taken any medication for the past 10 days. This citrated blood was centrifuged at 1100 rpm for 20 min to obtain platelet rich plasma (PRP), which was used within 3 h after collection. The residual blood was again centrifuged at 2500 rpm for 20 min to obtain the homologous platelet

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poor plasma (PPP). Platelet count was adjusted to 1.6×10^7 platelets per μl of PRP (Gerrad, 1982).

Preparation of leaf spice extracts

Fresh coriander leaf and curry leaf were purchased from a local vegetable market, cleaned, and ground (40 g each) into a fine paste. To each, 5 ml of water was added, and the slurries were left overnight at 4°C. The contents were centrifuged at 10,000 rpm for 15 min to obtain clear extracts of each of these leaf spices, which were used for inhibition studies of platelet aggregation. Volumes of extracts used were between 10 to 50 μl, which contained 0.22 and 0.24 mg dried material per milliliter of coriander and curry leaf extracts.

Platelet aggregation

The aggregation experiments were carried out turbidimetrically in a Dual Path Aggro-meter (Chronolog Corporation, Havertown, PA, USA). PRP of 0.45 ml was kept stirred at 1200 rpm in a glass cuvette at 37°C as aggregation was induced by agonists like ADP (61 μM), epinephrine (76 μM), and collagen (0.005% in 0.1 N acetic acid).

Similarly, PRP was preincubated with the extracts for the indicated time period followed by induction of aggregation by agonists like ADP, epinephrine, and collagen. The change in turbidity was recorded at least for 4 min in each case with reference to PPP using an omniscribe recorder in the presence and absence of extracts, and the slopes were calculated.

Estimation of malondialdehyde (MDA) in agonist challenged PRP

Platelets, after aggregation (0.45 ml), were taken, and 0.02 ml of 1% BHT in ethanol and 0.1 ml of 100% TCA in 3 N HCl were added. The pellet was centrifuged at 10,000 rpm for 10 min to obtain 0.45 ml of supernatant to which 0.1 ml of TBA reagent (0.12 M TBA in 0.26 M Tris-HCl) was added. The chromophore generated was measured at 532 nm and MDA (malondialdehyde) was calculated by using the following equation:

$$\text{Amount of MDA formed} = (\text{Absorbance}/156) \times (\text{Total volume}/\text{mg of protein}/\text{ml})$$

MDA was expressed in nmol MDA formed mg protein^{-1} h^{-1} (Maguire & Csona-Khalifah, 1987).

Protein concentration was determined in agonist-challenged platelets by modified Folin-phenol method as reported by Hartree (1972). Bovine serum albumin was used as the standard.

Results and Discussion

Aqueous extracts of these leaf spices inhibited platelet aggregation with the agonists ADP, epinephrine, and collagen at different concentrations and 1-min incubation (Figs. 1 and 2). The IC_{50} values (Table 1) show that the coriander leaf extract exhibited better inhibition with ADP as agonist, whereas with epinephrine and collagen the IC_{50} values were similar for both the extracts. Reports indicate that activation of platelets with agonists brings changes in the cytoskeletal structure, resulting in the loss of the normal diskoidal shape, and formation of pseudopodal projections can be observed (Castagna et al., 1982; Mukherjee et al., 1990). ADP induces platelet aggregation by inhibiting the increasing levels of intraplatelet cAMP and adenylate cyclase stimulation (Hawiger et al., 1980). On the other hand, collagen-induced platelet aggregation is associated with a burst in oxidant hydrogen peroxide, which contributes to

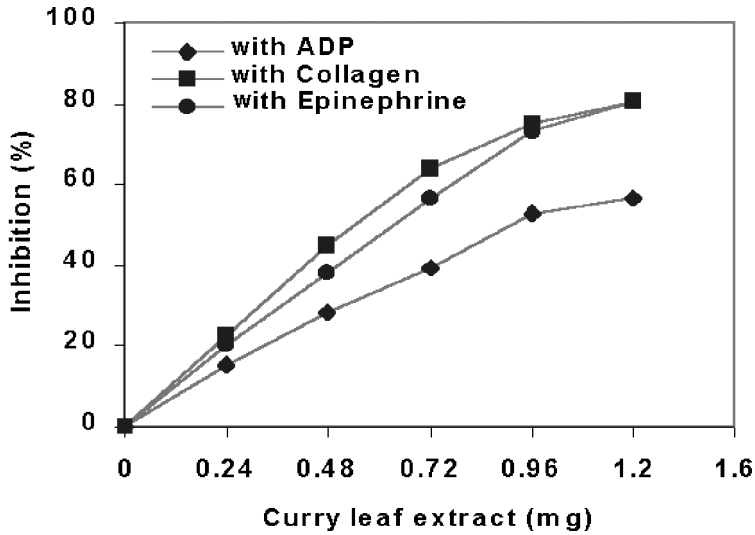

Figure 1. Effect of curry leaf extract on human platelets.

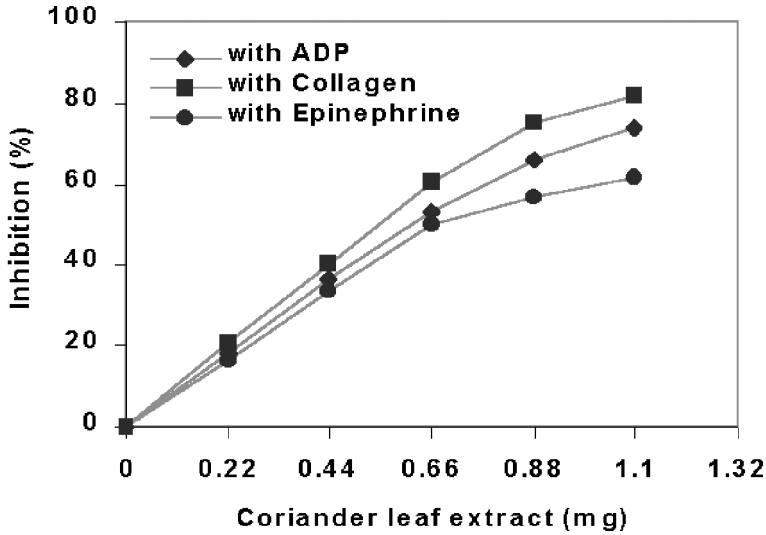

Figure 2. Effect of coriander leaf extract on human platelets.

platelet activation (Pignatelli et al., 1998) by stimulating the arachidonic acid metabolism and contributing to the platelet production of thromboxane A_2 (Pignatelli et al., 1999). For epinephrine-induced aggregation, there is a distinct receptor, which has been classified as a α_2-adrenergic receptor site (Figures et al., 1986).

Calcium ions are known to play an important role in platelet aggregation. A rise in the cytosolic calcium levels accompanies the activation of platelets through the stimulation of enzymes, which are not fully functional at low levels of calcium present as in resting platelets (Heemskerk & Sage, 1994). Drugs that are calcium antagonists usually do not bind to specific sites but may cause the thickening of platelet membranes due to the insertion of these drugs into platelet membrane bilayers, thus affecting the calcium mobilization (Blache et al., 1987). Hence, it may be suggested that the inhibition of human platelet aggregation may be due to the interaction of component(s) of these leaf spice extracts with the membrane bilayers, causing the thickening of membranes and thus affecting calcium mobilization. This may, in turn, effectively block the pathways of platelet aggregation due to the lack of stimulation of enzymes required for platelet aggregation. It may also be likely that they prevent ADP from inhibiting the increase in intraplatelet c-AMP levels and adenylate cyclase stimulation as well as possibly inhibit collagen-induced aggregation by blocking the release of hydrogen peroxide, which will otherwise stimulate arachidonic acid metabolism.

The IC_{50} values were used to determine the effect of incubation time on platelet aggregation. It was observed that as the duration of incubation was increased from 1 min to 2, 4, and 8 min, there was a significant increase in the inhibition by these leaf extracts with a maximum inhibition of up to 78.6% (Table 2). The increase in percentage inhibition indicates that a better uptake of these spice extract component(s) into the platelets was seen with longer interaction. Also, there may be a better incorporation of the leaf spice extract component(s) into the platelet membrane bilayers.

A decrease in the MDA formed was observed with these leaf spice extracts (Table 3). The MDA formed decreased up to 40% with curry leaf extract, whereas it was up to 45% with coriander leaf extract at IC_{50} values. This indicates that the lipid peroxides, which increase the

Table 1. Inhibition of platelet aggregation.

Extract	ADP	Epinephrine	Collagen
Curry leaf	0.94 ± 0.049	0.65 ± 0.042	0.58 ± 0.035
Coriander leaf	0.55 ± 0.045	0.66 ± 0.033	0.57 ± 0.031

IC_{50} values are mean $\pm$ SD of triplicates; Values are expressed in mg/ml. Time of incubation: 1 min.

Table 2. Effect of incubation time at IC_{50} values on inhibition of platelet aggregation.

	Inhibition (%)			
Agonist	1 min	2 min	4 min	8 min
Curry leaf extract				
+ ADP	49.3 ± 3.2	60.0 ± 3.9	68.4 ± 2.1	72.6 ± 1.8
+ Epinephrine	48.8 ± 4.2	58.7 ± 4.8	69.6 ± 4.6	78.3 ± 4.5
+ Collagen	50.6 ± 4.6	60.2 ± 2.8	69.5 ± 3.8	74.7 ± 3.0
Coriander leaf extract				
+ ADP	49.4 ± 1.6	62.2 ± 2.4	70.4 ± 2.9	78.6 ± 2.0
+ Epinephrine	48.3 ± 1.9	60.0 ± 2.6	66.7 ± 2.3	71.2 ± 3.1
+ Collagen	49.8 ± 3.2	57.8 ± 3.0	65.3 ± 2.8	73.9 ± 2.7

Values are mean $\pm$ SD of triplicates.

Table 3. Effect of leaf spice extracts on malondialdehyde formed in agonist-challenged platelets at IC_{50}.

Extract	ADP	Epinephrine	Collagen
Without extract	250.6 ± 22.5	138.9 ± 11.7	288.4 ± 20.2
Curry leaf	154.2 ± 16.4 (38.8%)	96.0 ± 7.4 (30.9%)	176.3 ± 17.8 (38.5%)
Coriander leaf	141.8 ± 18.6 (43.4%)	87.9 ± 8.1 (36.7%)	159.4 ± 18.9 (44.7%)

Values are mean ± SD of triplicates; Values expressed in nmol MDA formed mg protein^{-1} hr; Values in parenthesis are % inhibition.

platelet sensitivity to agonists thus causing coronary heart diseases (Neiva et al., 1999), were being effectively suppressed by the leaf spice extracts. It may also be likely that the cyclooxygenase pathway of arachidonic acid metabolism, where MDA is one of the end products (Rattan, 1988) being affected, thus causing a decrease in platelet aggregation.

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