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Research Article

Anti-Inflammatory Effect of *Syzygium cumini* on Chemotaxis of Human Neutrophils

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ABSTRACT

Various parts of the plant *Syzygium cumini (*synonyms: *Eugenia jambolana, Syzygium jambolana, Eugenia cumini, Szygium jambos*: commonly known as jamun in Hindi, black plum or Indian blackberry in English) have been used for treatment for various ailments and is notably known for its antidiabetic effect. Phytochemicals from this plant have been reported to have antidiabetic, antioxidant, antihyperlipidemic, antiulcer, antiallergic, antiinflammatory, antiarthritic and antibacterial properties as well as hepatoprotective and radioprotective activities. In the current study, aqueous seed extract of this plant was assessed for antiinflammatory properties using neutrophil chemotaxis as a model system. The seed extract significantly inhibited neutrophil chemotaxis towards a bacteria-derived chemoattractant (f-met-leu-phe). This result indicates that the seed extract has potential to elicit antiinflammatory effects.

Keywords: *Syzygium cumini*, neutrophil, phytochemical, chemotaxis, inflammation, f-met-leu-phe

INTRODUCTION

Syzygium cumini (syn. *Eugenia jambolana*) is known to have various pharmacological effects, including antidiabetic, antioxidant, antihyperlipidemic, antiulcer, antiallergic, antiinflammatory, antiarthritic, antibacterial, hepatoprotective and radioprotective activities[1,2]. According to the Ayurveda system of medicine, the seed of the plant possesses several medicinal properties[3]. Notably, seed powder of *S cumini* is known for its favorable effect on glucose control in non-insulin-dependent diabetic patients as well as in animal models of diabetes[4,5,6,7]. Both ethanolic and aqueous extracts of the seed powder have been shown to elicit effects on various enzymes involved in carbohydrate metabolism[8,9]. Active inflammation occurs in peptic ulcers and leads to disruption of mucosal integrity. Ethanolic extracts of *S. cumini* seeds protected rats against gastric ulcers induced by aspirin and the extract dosage was significantly below toxicity levels[10]. Flavonoids present in the seed extract are responsible for prevention of inflammation associated with ulcers[10].

Directed cell movement, also known as chemotaxis, is an integral component of the immune response. Chemotaxis of immune cells is regularly assessed when their mobilization and deployment to sites of inflammation are activated during early steps in the immune response[11]. Polymorphonuclear neutrophils have been well studied for chemotactic behavior relevant to their important role in inflammation and innate immunity[12]. Neutrophil chemotaxis is induced by chemoattractants such as chemokines (interleukin-8: IL-8), bacteria-derived agents (f-met-leu-phe: fMLP) and vasculature-derived components (complement proteins such as C5a)[11,13]. Reports[2,10] have indicated that seed extracts of *S.cumini* have antiinflammatory properties. For this reason, the focus of the current study was to assess the effect of *S. cumini* seed extract on neutrophil chemotaxis. Specifically, a fluorescent-based method[14] was used to determine effect of *S. cumini* seed extract on fMLP-induced neutrophil chemotaxis. In this assay, rapid and accurate evaluation of eukaryotic cell movement through a filter towards chemoattractant (fMLP) in the lower chamber was assessed. Briefly, *S. cumini* seed extract was incubated with neutrophils which were then added to the upper well of a chemotaxis chamber after fMLP had been placed in the lower wells (wells were separated by a nitrocellulose filter of defined pore size). After a designated incubation period, fluid was removed from the upper chamber, plate and filter assembly centrifuged to pellet chemotaxed neutrophils, filter discarded, fluorophore label (calcein-AM) added to the lower chamber and incubated for one hour to fluorophore-label neutrophils that had traversed the filter and entered the lower well. Fluorescence intensity of migrated cells was determined. Stated simply, *S. cumini* extract pre-treated neutrophils were assessed for movement through the filter and into the lower chamber and fluorescence intensity was the read-out for cell movement into the lower chamber.

Plant-derived compounds with anti-inflammatory potential have been reported and their actions in several pathophysiological conditions and disease models have been reported[15,16]. This report states results of screening the aqueous seed extract (ASE) of *Syzygium cumini* for antiinflammatory properties, specifically focusing on

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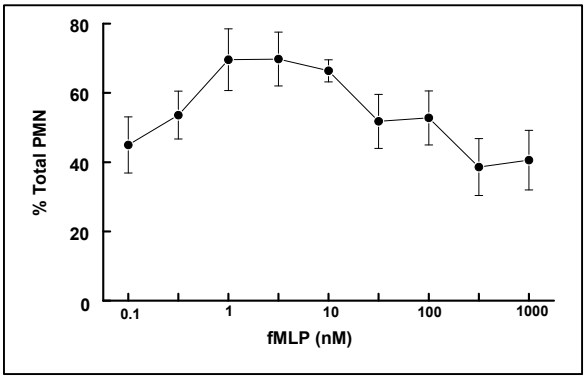

Figure 1: Concentration response curve of fMLP-induced chemotaxis of human neutrophils. n=5.

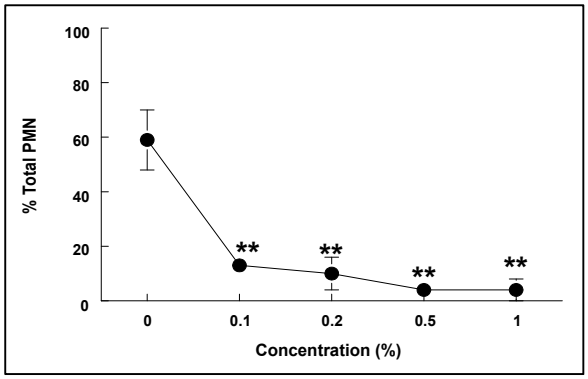

Figure 2: Effect of *S.cumini* seed extract on fMLP (0.5 nM)-induced chemotaxis of human neutrophils. n=3. ** = $p < 0.01$

inhibition of fMLP-induced neutrophil chemotaxis. Of importance, significant inhibition of chemotaxis was observed at very low doses of *S cumini* seed extract.

MATERIALS AND METHODS

Preparation of Seed Extract: *Syzygium cumini* seed powder was purchased from a commercial source (basic ayurveda jamun powder). Seed powder (10 gm) was added to distilled water (100 ml) and stirred overnight (50°C). Filtrate was centrifuged and supernatant was filtered and vacuum dried. The dried material was suspended in chemotaxis buffer (formulation below in Neutrophil Chemotaxis section) to a concentration of 10% stock solution. Effect of seed extract (0, 0.1%, 0.2%, 0.5%, 1%) on neutrophil chemotaxis was assessed.

Isolation of Human Neutrophils: Heparinized venous blood was obtained from normal healthy human donors after receiving informed consent as per an approved Institutional Review Board protocol. Neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque density gradient centrifugation[17]. Contaminating erythrocytes were removed by hypotonic lysis. Cell purity was determined by differential counts of Wright-Giemsa-stained cytospin preparations and neutrophil viability was determined by trypan blue exclusion.

Neutrophil Chemotaxis: Appropriate concentration of bacteria-derived chemotactic agent fMLP was determined empirically by performing concentration response curves of fMLP (Sigma-Aldrich, St. Louis, MO). Effects of *S. cumini* on neutrophils were determined by pretreatment (37°C, 30 min) with seed extract prepared in chemotaxis buffer (RPMI 1640 containing 1% heat-inactivated fetal calf serum). Buffer or chemoattractant was added to lower chambers and neutrophils (150,000/well) were added to upper chambers of 96-well microchemotaxis chambers (Neuroprobe, Cabin John, MD) with filters of 5 µm pore size (Neuroprobe) between the upper and lower chambers. After incubation (1 h, 37°C), fluid was removed from the upper chamber, plate and filter assembly were centrifuged to pellet chemotaxed PMN, filter was discarded, calcein-AM (EMD Millipore) was added to the lower chamber and incubated (1 h, 37°C) to fluorophore-label the neutrophils that traversed the filter and entered the lower well. Fluorescence intensity of migrated cells was then determined. Data were reported as percent of total neutrophil fluorescence as determined by the following calculation. Note that polymorphonuclear neutrophils were abbreviated to PMN in the calculation.

Percent total PMN = 100 x (mean total fluorescence of total PMN – mean total fluorescence of vehicle / mean total fluorescence of total PMN).

Statistical Analysis: All data were reported as the mean ± SEM. Comparisons of sample means were analyzed using repeated measures ANOVA followed by Dunnett multiple comparisons. Differences with $p < 0.05$ were considered significant.

RESULTS

fMLP Concentration Response Curve: Figure 1 shows results of the fMLP concentration response curve for the chemotaxis assay. Peak neutrophil movement was at 1 to 5 nM fMLP. Suboptimal concentrations were between 0.5 and 1 nM fMLP. Declining neutrophil chemotaxis was evident at 50-1,000 nM fMLP.

Inhibition of Neutrophil Chemotaxis by *S.cumini* Seed Extract: The suboptimal concentration of 0.5 nM fMLP was used for assessment of effects of phytochemical-pretreatment on neutrophils. Seed extract concentrations of *S.cumini* (0, 0.1%, 0.2%, 0.5%, 1%) were assessed to determine their effects on neutrophil chemotaxis. As shown in Figure 2, seed extract significantly inhibited fMLP-induced neutrophil chemotaxis at all concentrations tested with statistically significant inhibition at each point when compared to vehicle control (chemotaxis buffer). Trypan blue viability assays were performed in parallel with chemotaxis assays and the highest concentration tested (1% seed extract) showed no effect on cell viability. Additionally, neutrophil purity after isolation was assessed by Wright-Giemsa staining of cytospin prepared neutrophils and determined to be 98%.

DISCUSSION

A chemotaxis assay was used to demonstrate effect of *S.cumini* seed extract on inflammation. It was identified that *S. cumini* seed extract elicited an anti-inflammatory effect, specifically, it inhibited movement of human neutrophils towards a chemoattractant of bacterial origin (fMLP). Neutrophils are actively recruited by directional

movement (chemotaxis) towards chemoattractants, such as chemokines secreted by immune cells such as interleukin (IL)-8, complement break down products such as C5a or bacteria-derived agents such as f-met-leu-phe (fMLP). The concentration response curve of fMLP showed suboptimal (0.5 and 1 nM fMLP), peak (5 nM fMLP) and declining (50-1,000 nM fMLP) results of neutrophil chemotaxis. Use of suboptimal concentrations allowed for interpretation of augmentation or inhibition outcomes. Declining chemotaxis at higher fMLP concentrations was not used due to the desensitization of neutrophil surface receptors to fMLP at those concentrations.

In vitro assessment of inhibition of neutrophil chemotaxis is used as a physiologic indicator of anti-inflammatory potential[18]. Several studies have suggested that neutrophil movement and cancer cell movement share common mechanisms; such as, signal transduction, movement as a receptor-mediated event, and induction by chemokine chemoattractants[19,20]. Therefore, it is interesting to postulate that compound(s) that inhibit chemotactic movement of inflammatory cells towards chemoattractants may also inhibit metastatic cancer cell movement. Effects of S. cumini seed extract on glucose control of diabetes[21, 22], as an antiinflammatory agent (present study indicating its effect on neutrophil movement), and for postulated effects on cancer metastasis informs the scientific community that S. cumini seed extract is a very important compound and deserves further study to elucidate its effects. Future studies will focus on definition of mechanisms involved in *S. cumini* inhibition of chemotaxis and identification of the seed component responsible for this anti-inflammatory effect.

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