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Original Research

Date syrup–derived polyphenols attenuate angiogenic responses and exhibits anti-inflammatory activity mediated by vascular endothelial growth factor and cyclooxygenase-2 expression in endothelial cells

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ABSTRACT

Bioactive components such as polyphenols, present in many plants, are purported to have anti-inflammatory and antiangiogenic properties. Date syrup, produced from date fruit of the date palm tree, has traditionally been used to treat a wide range of diseases with etiologies involving angiogenesis and inflammation. It was hypothesized that polyphenols in date syrup reduce angiogenic responses such as cell migration, tube formation, and matrix metalloproteinase activity in an inflammatory model by exhibiting anti-inflammatory activity mediated by vascular endothelial growth factor (VEGF) and the prostaglandin enzyme cyclooxygenase-2 (COX-2) in endothelial cells. Date syrup polyphenols at 60 and 600μg/mL reduced inflammation and suppressed several stages of angiogenesis, including endothelial cell migration, invasion, matrix metalloproteinase activity, and tube formation, without evidence of cytotoxicity. VEGF and COX-2 expression induced by tumor necrosis factor–alpha at both gene expression and protein level was significantly reduced by date syrup polyphenols in comparison to untreated cells. In conclusion, polyphenols in date syrup attenuated angiogenic responses and exhibited anti-inflammatory activity mediated by VEGF and COX-2 expression in endothelial cells.

Abbreviations: ANOVA, analysis of variance; BME, basement membrane extract; COX-2, cyclooxygenase-2; DMEM, Dulbecco modified Eagle medium; ELISA, enzyme-linked immunosorbant assay; ESI-MS, electrospray ionization mass spectrometry; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GUSB, beta-glucuronidase; HECV, human vascular endothelial cell; HPLC, high-performance liquid chromatography; IL, interleukin; LC, liquid chromatography; MMP, matrix metalloproteinase; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PPDS, extracted date syrup polyphenol; TNF-α, tumor necrosis factor–alpha; TIMP, tissue inhibitor of metalloproteinase; VEGF, vascular endothelial growth factor.

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1. Introduction

Angiogenesis encompasses the formation of new blood vessels from preexisting vasculature and is a tightly regulated and coordinated process involving both pro- and antiangiogenic factors [1]. The development of new capillaries from preexisting microvessels is a critical event in wound repair and tissue regeneration [2] and is a time-controlled physiological process [3]. When the homeostatic balance between stimulation and inhibition is shifted, excessive angiogenesis ensues, resulting in inflammatory-associated angiogenesis. Inflammatory-associated angiogenesis is commonly associated with oxidative stress [4]. Inflammation is a paramount process in defense against pathogenic invasion, and it can induce adverse effects on tissue over time [5].

Key regulators involved in the angiogenic process include gelatinase matrix metalloproteinases (MMPs), which degrade the extracellular matrix of endothelial cells. MMP-2 and MMP-9 have been associated with inflammation and are key factors involved in inflammatory associated angiogenesis [6]. Another important physiological and pathological mediator is the cyclooxygenase (COX) enzyme COX-2. The COX-2 enzyme catalyzes prostanoid synthesis and is involved in the arachidonic acid pathway associated with prostaglandin E2 production [7]. COX-2 up regulates vascular endothelial growth factor (VEGF), another important factor promoting vascular development and therefore promoting inflammatory-associated angiogenesis [8].

Natural compounds have traditionally been used to prevent and treat various illnesses worldwide. As a result, there has been a growing interest in assessing the role of plant- and food-based bioactive compounds such as polyphenols with reported antioxidant [9,10], anti-inflammatory [11], and antimicrobial [12] activity. Antiangiogenic properties in endothelial cells have been described for several polyphenol compounds including quercetin [13], epigallocatechin gallate [14], curcumin [15], and resveratrol [16].

Date fruits, and date fruit products including date syrup from different cultivars, have traditionally been used as alternative medicine in the treatment of a range of ailments including stomach and intestinal disorders, fever, edema, and bronchitis and in wound repair [17]. Several bioactive compounds such as polyphenols have been identified within date syrup, which suggest a possible rationale for date syrup's perceived traditional medicinal application. Date syrup has been found to have a high content of polyphenol compounds such as flavonoids, tannins, carotenoids, and anthocyanins [18]. Given the increasing number of literature focusing on the role of bioactive compounds such as polyphenols as antiangiogenic and anti-inflammatory agents, the action of polyphenols in date syrup, in relation to endothelial cells' angiogenic and inflammatory responses, has not been investigated.

Hence, it was hypothesized that polyphenols derived from date syrup reduce angiogenic responses in an inflammatory model of endothelial cells and that this reduction is mediated by reduced expression of VEGF and COX-2.

To address this hypothesis, the objective of this study was to determine the effect of pretreating of date syrup polyphenols (60 and 600 μg/mL) on angiogenic responses associated with tube formation, cell migration, cell invasion, and MMP activity in human endothelial cells. Furthermore, the proinflammatory cytokine levels secreted by endothelial cells stimulated with tumor necrosis factor–alpha (TNF-α) and pretreated with date syrup polyphenols were determined. In addition, the study further determined whether changes in angiogenic and inflammatory responses in endothelial cells treated with date syrup polyphenols reduced the expressions of COX-2 and VEGF.

2. Methods and materials

2.1. Chemicals and reagents

XAD-2 resin, Folin-Ciocalteau reagent, and all polyphenol standards including gallic acid were obtained from Sigma (Sigma Aldrich, Dorset, United Kingdom). High-performance liquid chromatography (HPLC)–grade methanol and formic acid were obtained from Fisher Scientific (UK). TRIzol was obtained from Life Technologies.

2.2. Extraction and chemical analysis of date syrup polyphenol

Date syrup was produced from the date fruit cultivar Khadrawi, belonging to the family Arecaceae, genus *Phoenix*, and species *dactylifera*, during the wet seasons of 2012-2013. The date syrup was raw and unprocessed; it was stored at 4°C on receipt. Date syrup phenolic fraction was extracted according to the method described by Yao et al [19]. Unprocessed date syrup (50 g) was mixed with 250 mL of acidified water (pH 2) for 24 hours at room temperature; the mixture was filtered through cotton wool to remove undissolved solid particles. XAD-2 resin (Supelco) (approximately 47 g) was initially conditioned in 2 mol/L HCl for 1 hour and further conditioned by soaking in 1:1 methanol and water for preswelling overnight. The slurry was packed into a glass column (MBL), and the solution was removed to give an approximate bed volume of 1 × 50 cm^3 and rinsed with 1 L of deionized water. The date syrup solution was passed slowly at 1 mL/min through the packed resin column, followed by 250 mL of acidified water (pH 2) and deionized water (300 mL) (ELGA LabWater). Polyphenol fractions were finally eluted with 300 mL pure methanol. A 50-mL collected methanol extract was concentrated to dryness under vacuum at 40°C. The extract (extracted date syrup polyphenol [PPDS]) was stored at –80°C, subjected to chemical analysis, and dissolved accordingly for cell culture treatment in cell medium. The quantification of total phenolic content of date syrup was determined by the Folin-Ciocalteu colorimetric assay based on the procedure previously identified by Al-Farsi and colleagues [20]. Gallic acid was used as a spectrophotometric standard (0-100 mg/mL), and results were expressed and means ± SD mg of gallic acid equivalents per 100 g of date syrup. Measurements were taken in triplicate.

Extracted date syrup polyphenols were analyzed using HPLC based on the method by Weston et al [21]. Chromatographic analysis was carried out with an Agilent 1200 LC (Agilent, Berkshire, UK). Data were processed with Agilent ChemStation software. An Advanced Chromotography Technologies Ltd C18-300 column (250x7.75mm) was used to separate phenolic