

# The Dark Chocolate against Angiogenesis?

## Abstract

Aimed to study the connection of dark chocolate and different phases of angiogenesis resulting in cancer pathogenesis. Dark chocolate is derived from cocoa (cacao), may have speckled polyphenol and flavonoids contents retains different levels of antioxidant abilities. The existence of methylxanthines, peptides and polyphenols like flavonoids may synergistically augment or diminish antioxidant properties of dark chocolate (cocoa). The Dark chocolate has certain capacity to hamper tumors development and fighting against angiogenesis.

**Keywords:** Dark chocolate; Angiogenesis; Antioxidant

## Review Article

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**Sami Ullah Khan<sup>1\*</sup>, Abdul Qayyum<sup>2</sup>,  
Salahudin<sup>3</sup>, Izharullah<sup>4</sup>, Mahmood  
AkhtarKayani<sup>5</sup> and Faraz Arshad Malik<sup>5</sup>**

<sup>1</sup>Department of Biosciences, COMSATS Institute of Information Technology, Pakistan

<sup>2</sup>Department of Languages, Government Degree College, Pakistan

<sup>3</sup>Health Services Academy, Pakistan

<sup>4</sup>Department of Pharmacy, Malakand University, Pakistan

<sup>5</sup>Department of Biosciences, Comsats University of Information Technology, Pakistan

**\*Corresponding author:** Sami Ullah Khan, Department of Biosciences, COMSATS Institute of Information Technology, Islamabad, Pakistan, Tel: +79872167595; Email: skmwt2003@gmail.com

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## Introduction

Timeline of cocoa arose in 2,000 B.C, the date recognized by historians to hoariest swallowing cups and plates that has endlessly revealed in Latin America, Ulúa valley (Honduras), where cocoa has a vital role. Cacao is plagiaristic from Olmec and consequent Mayan languages kakaw, whereas chocolate-associated tenure cacahuatl is Nahuatl, Aztec language, resulting from Olmec-Mayan etymology [1]. An elusive tree, cacao is merely grown in rainy forests in tropics, ordinarily on large farmsteads, where it essentially sheltered from passionate sunlight and wind. Different parts of cacao tree have been consumed; explicitly cocoa beans primed as chocolate, cocoa butter, cocoa bark, cocoa pulp, cocoa flower and cocoa leaf. In 1505, Spanish fetched Cocoa to Europe. In 1653, in Europe cocoa used in terms of medicine comparatively than that of pleasant foodstuff. Practice of chocolate was renowned as an inspiring vigorous utility of spleen and other gastrointestinal functions. Revisions upon wellbeing profits of the cocoa food stuffs have been steered over past span with a topmost emphasis on deteriorating diseases. These doles could be in result of their momentous amounts of catechin and epicatechin (flavonoid monomers) which have copious favorable biological actions in anticipation of angiogenesis and cancer [2]. Most of revisions have scrutinized the charities of flavonoids in cocoa as well as cocoa foodstuffs towards health doles, but it should be renowned that cocoa and its foodstuffs are also ironic in methylxanthines (theophylline, caffeine, and Theo bromine) [3]. Revisions have confirmed which methylxanthines can retain equally positive and negative vigor effects (Table 1) [4].

**Table 1:** Components of dark chocolate [4].

Class	Sub Class	Polyphenol
<b>Anthoxanthins</b>	Flavonols	Myricetin, Fisetin, Quercetin, Kaempferol, Isorhamnetin
<b>Flavanols</b>	(Flavan-3-ols)	(+)-Catechin, (-)-Epicatechin, (-)-Epicatechin 3-gallate, Morin, (-)-Epigallocatechin, (-)-Epigallocatechin-3-gallate, (+)-Gallocatechin, Procyanidins, Prodelphinidins
<b>Tannins</b>		Catechin polymers, Epicatechin polymers, Ellagitannins, Proanthocyanidins, Casuarictin, Sanguin H6, Tannic acids

## Polyphenols

Dark chocolate prepared from cocoa that is opulent in polyphenols. Polyphenols are strong inhibitors of COX-2 [5]. COX-2 is known as an enhancer of carcinogenesis in various organs. Hereafter, assays of COX-2 expression may be used to monitor process of carcinogenesis, and suppression of COX-2 expression has become an important target for treatment and prevention of various types of cancers [6-8]. In 1909, vanDorsen and Ultée identified core polyphenol present in cocoa known as "Kakaool" [9]. Uphold studies specified that this was a catechin, which was inaccurately entitled as l-acacatechin found in cutch fabricating acacias [10]. In 1940, it believed that "Kakaool" possibly symbolized l-epicatechin also seen in Acacia catechu [11]. Cocoa and cocoa bean consists of four different brands of catechins of that (-)-epicatechin (92%) [12], un-fermented cocoa bean consists tannin and catechin [10]. Alongside these composites, cocoa has also leucoanthocyanins, which found as glycosides. Correspondingly, cocoa bean also consists of two cyanidin glycosides as well as at least 3-leucocyanidins (procyanidin) composites. Cocoa bean has also epicatechindimeric and leucocyanidin [12]. The foremost constituents of cocoa extracts are (-)-epicatechin, catechin, anthocyanins, leucocyanidins, chlorogenic acid, and p-coumarylquinic acid. Flavonols are the chief composites in cocoa and cocoa powder. Epicatechin is chief component in all chocolates, (in ration 1:0.1), related to catechin [13]. The antioxidant activities of polyphenols are due to their chemical structures [14]. Dark Chocolates has large amounts of polyphenol ingredients, like; Quercetin (including its glucoside), (-)-epicatechin (EC), (+)-catechin, deoxyclovamide, clovamide, trans-resveratrol its glucoside and procyanidin [14,15].

## Methylxanthines

Methylxanthines present in cocoa includes; caffeine, theophylline, and theobromine [3]. In dark chocolates,

methylxanthines are liable for cravings of chocolate [16]. Theobromine, the foremost methylxanthine found in cocoa, which is 4%, and caffeine is up to 0.2% [17]. The percentage of theobromine is higher than that of theophylline in cocoa beans [17]. Major Methylxanthine in cocoa and cocoa foodstuffs is caffeine [3]. The studies specified that bioactivity as well as importance of cocoa and cocoa foodstuffs were due to polyphenols [13]. It has been identified that theobromine has therapeutic results on cancer and angiogenesis, like; theobromine potentially obstruct angiogenesis prompted by ovarian cancerous cells through mechanism to reticence the production of VEGF (vascular endothelial growth factor) [18].

### Peptides

Cocoa contains large number of proteins. Peptides in cocoa are mainly accountable for flavor [19,20]. It consists of four different kinds of proteins, which are, albumins, prolamin, globulins, and glutelin. Among them albumin is in foremost protein ratio [21]. Protein Albumin is 52% whereas globulin is about 43% of protein present in cocoa bean [22].

### Angiogenesis

The process of developing new blood vessels resulting from pre-surviving blood vessels called as angiogenesis [23]. It is vigorous and common process in wound healing, development and growth. It is important for tumor conversion from resting condition into malignant, resulting in usage of angiogenesis inhibitors [24].

### Types

#### Sprouting angiogenesis

This type of angiogenesis carried out in different phases that are; biological signaling phase in which angiogenic factors motivate receptors located on surface of endothelial cells in blood vessels that are pre-existing, these motivated endothelial cells instigate to proclamate enzymes known proteases which worsen cellar membrane that allowing endothelial cells and seepage them from paternal vessel walls. This mechanism results in proliferation of endothelial cells into adjacent matrix to form sprouts, after which endothelial cells proliferate into adjacent matrix to form solid sprouts linking nearby vessels [25,26].

#### Intussusceptions angiogenesis

Intussusceptions angiogenesis is also called splitting angiogenesis. During this type of angiogenesis, capillary wall spreads into lumen results in splitting of single vessel into two. Its mechanism takes place in four different phases. In first phase of Intussuscepted angiogenesis, two conflicting capillary walls institute a region of communication followed by second phase in which the endothelial cell confluences are rationalized that allow vessel bilayer to Puncture to permit growth factor and thus cells are breach in lumen. In third phase, there is formation of core between two newly developed vessels at region of connection occupied by pericytes and myofibroblasts. Cells initiate to lay collagen givers within core to afford extra cellular for progression of vessel lumen [26].

## Mechanism of Angiogenesis

### Normal angiogenesis

In process of vasculogenesis, there is proliferation of angioblasts that merge in embryonic grid of vessels called as capillary plexus (primary). Endothelial cell matrix functions as a gibbet for angiogenesis generated by vasculogenesis [27]. There is development and splitting of new vessels resulting from pre-existing vessels in angiogenesis once capillary plexus (primary) formed. In embryo where is the most of normal angiogenesis happens and inaugurates primary vascular pyramid and tolerable vasculature to support growth and development of organs [28]. Angiogenesis process in adults carried out through ovarian cycle as well as in physiological repair mechanisms like; healing of wound [29]. There is minute endothelial cells revenue that happens in adult vasculature [30]. There are many sundry processes in micro vessels that results in fruition and renovation of newly bent micro vessels [29], pericytes should be detached from forking vessel to produce newly blood sprouts. Proteases like matrix metalloproteinases sullied as well as refashioned extracellular matrix and endothelial cell cellar membrane, thus new matrix produced via stromal cells [31]. This whole process results the flow of blood in newly synthesized vessels.

### Factors regulating normal angiogenesis [32]

The factors that regulate normal angiogenesis are consists of three main groups including soluble factors, membrane -bound proteins and biomechanical forces. There mechanisms are shown in Table 2a & 2b below.

### Tumor-persuaded angiogenesis

Tumors are inhabitants of host-imitative cells, which have vanished ability to legalize growth resulting in aberrant proliferate. Yet numerous landscapes differentiate them from non-renovated foils, many features of tumor cells that are analogous to normal ones [32]. One foremost resemblance is obligation for a sufficient hoard of oxygen as well as nutrients and an operative means to eradicate trashes in mandate for metabolic procedures to befall and being to be conserved. Propinquity to vascular stream fulfills these needs for mammalian cells. Ordinary cells and tissues trust on functional vasculogenesis and angiogenesis to deliver them with vasculature, which fulfills the metabolic needs [32].

### Tumor Vasculature

Tumors can begin their personal blood supply by numerous revenues. In tumor-persuaded angiogenesis, a tumor may provoke development of blood vessels resulting from pre-surviving capillaries. Furth more, tumors cells are capable to develop around a surviving vessel and later, at least primarily, do not want to persuade angiogenesis for passable vascularization [33]. In addition, (CEPs), angioblast-identical cells that result from bone marrow tissue but conveyed to be exist in adult circulation, have freshly been recommended to donate to a tumor-imitated blood vessels [34]. Among them numerous dearth functional pericytes [35], which are distended and elaborated, as well as they are remarkably permanent due to existence of fenestrae and trans-cellular slums and dearth of a thorough basement membrane [36]. Additionally, walls of tumor may be composed up of endothelial

cells as well as tumor cells mutually [37]. These structural aberrations in tumor’s vessels reproduce pathological flora of their generation, however their aptitude to upkeep cell growth

may also inspires use of physiological appliances of angiogenesis, which tumors requisition for their propagation [32] (Table 3).

**Table 2a:** Factors regulating normal angiogenesis.

Factor	Mechanism
Soluble Factors	
VEGF	Impedes endothelial cell apoptosis, Upturns endothelial cell permeability, Enhances endothelial cell migration, Motivates <i>in vivo</i> angiogenesis, Upturns endothelial cell permeability, Motivates endothelial cell proliferation, Motivates endothelial cell uPA/PAI-1 production
Angiopoietin-1 (Ang1)	Motivates <i>in vitro</i> endothelial cell sprout formation, Upturns girth and stability of endothelium
Angiopoietin-2 (Ang2)	Destabilizes endothelium Antagonizes Ang1 signaling
aFGF,bFGF	Motivates endothelial cell proliferation, Boosts endothelial cell migration, Motivates endothelial cell PA/collagenase production, Motivates endothelial cell tube formation, Motivates <i>in vivo</i> angiogenesis
PDGF	Motivates DNA synthesis in endothelial cells, Motivates endothelial cells to form chords <i>in vitro</i> , Motivates proliferation of smooth muscle cells and pericytes, Prompts vWF, VEGF, and VEGF receptor-2 expression in cardiac endothelial cells, Upturns capillary wall stability
TGF-β	Supports anchorage-independent growth of fibroblasts, Inhibits proliferation and migration of endothelial cells, Motivates/inhibits formation of endothelial cell tubes <i>in vitro</i> , Produces net anti proteolytic activity via modulation of uPA/PAI-1 expression levels, Inhibits production of other proteases/stimulates production of protease inhibitors, Motivates VSMA production by pericytes, Chemotactic for monocytes and fibroblasts, Motivates <i>in vivo</i> angiogenesis in presence of inflammatory response, Upturns vessel wall stability
TNF-α	Motivates angiogenesis <i>in vivo</i> , Motivates formation of endothelial cell tubes <i>in vitro</i> , Inhibits endothelial cell proliferation
EGF,TGF- α	Motivate endothelial cell proliferation, Motivate angiogenesis <i>in vivo</i>
G-CSF,GM-CSF	Motivate endothelial cell proliferation and migration
Angiogenin	Motivates angiogenesis <i>in vivo</i> , Supports endothelial cell binding and spreading
Angiotropin	Motivates random capillary endothelial cell migration, Motivate endothelial cell tube formation, Motivates <i>in vivo</i> angiogenesis
Tissue Factor	Contributes to development of yolk sac vasculature1
Factor V	Contributes to development of yolk sac vasculature
Prostaglandin	Motivates <i>in vivo</i> angiogenesis
Nicotinamide	Motivates <i>in vivo</i> angiogenesis
Monobutyryn	Motivates <i>in vivo</i> angiogenesis, Motivates endothelial cell migration <i>in vitro</i>

**Table 2b:** Factors regulating normal angiogenesis.

Membrane-Bound Proteins	
avb3 Integrin	Highly expressed on, activated endothelial cells, Mediates endothelial cell attachment, spreading, and migration Present on angiogenic capillary sprouts, Required for bFGF-stimulated angiogenesis <i>in vivo</i> , Localizes MMP-2 to capillary sprouts Suppresses endothelial cell apoptosis
avb5 Integrin	Essential for VEGF-stimulated angiogenesis <i>in vivo</i>
a5b1 Integrin	Essential for non-VEGF growth factor-stimulated, angiogenesis <i>in vivo</i>
VE Cadherin	May mediate permeability of endothelium, Required for <i>in vivo</i> angiogenesis, Prevents endothelial cell apoptosis
Eph 4B/Ephrin-B2	At the venous/arterial interfaces of developing embryo, Required for angiogenesis of head and yolk sac and for myocardial trabeculation
Ephrin A1	Essential for <i>in vivo</i> angiogenesis induced by TNF-a, Chemotactic for endothelial cells <i>in vitro</i>
Eph-2A	Essential for endothelial cell tube formation <i>in vitro</i>
Biomechanical Forces	
Blood Flow/Shear Stress	Increases endothelial stress fiber formation

**Table 3:** Factors regulate tumor angiogenesis [32].

Factor	Mechanism
Soluble Factors	
VEGF	Secreted by different tumor cells <i>in vitro</i> , Extremely unregulated in most human cancer, Expression compares with intra tumoral micro vessel density and poor prognosis in cancer patients, Retention decreases tumor vessel density and tumor growth 1
FGF	Inhibition suppresses generation of tumor vessels ( <i>invitro</i> and <i>in vivo</i> ) and tumor growth <i>in vivo</i> , Important for maintenance, vs. induction, of tumor angiogenesis, Synergizes with VEGF to promote angiogenesis <i>in vitro</i> and <i>in vivo</i> , Induces VEGF expression in tumor cells and VEGF receptor expression in endothelial cells
Heparanase	Stimulates invasion and vascular sprouting of endothelial cells, They Releases bFGF from extracellular matrix mRNA. The protein contents are augmented in the metastatic tumor cell lines and human tumors against normal tissues, Over expression renders no metastatic cell lines metastatic <i>in vivo</i> and increases tumor neovascularization
Angiopietin-2	Induced in endothelial cells of pre-existing vessels co-opted by a tumor, leading to vessel regression, Induced in endothelial cells of newly-formed vessels of tumor, leading to vessel plasticity and VEGF-mediated growth
IL-8	Mitogenic and chemotactic for Human Umbilical Vascular Endothelial Cells <i>in vitro</i> , Stimulates angiogenesis <i>in vivo</i> mRNA is upregulated in neoplastic tissues vs. normal ones <i>in vivo</i> ; expression correlates with extent of neovascularization, Over expression increases invasiveness, tumorigenicity, neovascularization, and metastatic potential of tumor cells Mediates stimulation of MMP-2 gene transcription
MMP-2	Directly modulates melanoma cell adhesion and spreading on extracellular matrix, Mediates tumor growth and neovascularization in CAM

## Working Mechanism of Chocolate Components

### Antioxidant effects

Reactive oxygen species (ROS) are potential carcinogens that facilitate mutagenesis, tumor promotion and progression [38]. Food derived products exist universally and are expected to be safe, they are highly interesting for development as chemo preventive agents to treat reactive oxygen free radicals that results in cancer pathogenesis [39,40]. Natural compounds has the ability to induce cytotoxicity thereby protects against cancer and many researcher's developing chemotherapeutic by based on its ability to induce apoptosis [41-44]. Well-known character of polyphenols in cocoa is their ability to show there action as antioxidants. Polyphenols (flavones and catechins) are powerful flavonoids for defensive the body alongside reactive oxygen radicals. Cells and tissues of body constantly endangered by the injury affected by free radicals as well as reactive oxygen radicals that are bent in usual oxygen metabolism or are persuaded by exogenous injury [45]. Sequences and mechanisms of trials thru which free radicals restrict with cellular roles are not entirely known but most significant trial appears to be lipid peroxidation resulting in damage of cellular membrane. This cellular damage causes a shift in the net charge of cell that changes the osmotic pressure, resulting in swelling and ultimately cell death. Free radicals can entice numerous inflammatory mediators, subsidizing to common inflammatory retort and tissue injury [32].

Living bodies have established numerous effective procedures order to prevent there selves from free oxygen radicals [46]. Antioxidant-defensive mechanisms of body comprise certain enzymes like; catalase, glutathione peroxidase, and superoxide dismutase, but have no enzymatic foils like; ascorbic acid, tocopherol, and glutathione. Neovascularization that includes angiogenesis is mandatory for advancement of metastasis [32].

### Anti-Angiogenesis Effects

Secretion of neovascularization/angiogenesis mediators via mast cells and motivation of mast cell migration by tumor-plagiaristic peptides specify that mast cells can be tangle in metastasis migration of tumor [47-49]. Persuasive retention of the mast cell stimulation and propagation by numerous flavonoids can also donate antitumor effects [49]. The mast cells discharge TNF that persuades molecule expression of endothelial adhesion [49,50]. There is reduction in stages of plasminogen activators, bFGF induction and their functional inhibitors [51]. bFGF motivates manufacturing of urokinase-type, plasminogen activator and PAI-1 in vascular-endothelial cells. Plasminogen synthesized plasmin that showing stepwise proteolytic deprivation of matrix protein. This is the key phase in mechanism of neovascularization.

### Conclusion

Components of dark chocolate analytically impulse numerous cellular, immunological and a series of biological measures related with angiogenesis, cancer development and growth, like; the cell proliferation, cell differentiation, apoptosis, and neovascularization. The studies *in vitro* have recognized a connotation between flavonoid-persuaded impulsion of MMP and protein kinase actions with tumor cell invasion, apoptosis and cellular proliferation conduct. Certain dark chocolate components, such as flavonoids pageant antitumor activity and reduce angiogenesis *in vivo*.

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