Review

A REVIEW ON ANGIOGENESISAND THEIR CONSEQUENCE ONVITIS VINIFERA (GRAPES)

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ABSTRACT

The current article deals with the review of angiogenesis, angiogenesis model especially as angiogenesis assay, anti-angiogenic activity of plants phytochemicals, drugs and food components with respect to anti-angiogenic properties, quantitative analysis, imaging of angiogenesis, botanical description of *Vitisvinfera*, medicinal importance,correlation between angiogenesis and *Vitisvinfera*. It briefly summerize the effect of Vitisvinfera on angiogenesis

KEYWORDS

ANGIOGENESIS, VITISVINFERA, ANGIOGENESIS ASSAY, ANTI-ANGIOGENIC ACTIVITY OF PLANTS PHYTOCHEMICALS

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1. INTRODUCTION

VITIS VINIFERA

The scientific name of grapes is Vitisvinifera. It is the oldest cultivated plant belonging to the family Vitaceae. Plant is a woody vine which requires solid support to grow. Fruit is a berry which grows in the form of clusters [1] . Color of fruit may be black, blue, red, purple, green or yellow. Main composition of grapes is sugar, dietary fibers, potassium, iron and polyphenolic compounds [2].

1.1. BOTANY:

Leaves of Vitisvinifera are alternate broad and 5-20cm long. Fruit is like a berry shape 6mm in diameter and up to 3 cm long. Plant usually exists in humid environment. Famously there are two species one is known as wild species (Vitissilvestris) and other one is known as domesticated or cultivated species (Vitisvinifera). Vitisvinifera have hermaphrodite flowers but wild species have dioecious (separate male & female flowers) flowers. Majority of the world wine is produced from Vitisvinifera[3].

Visible parts of grape vine above the ground are trunk, cordon, stem, leaves, flowers and fruit. Plant grows in the form of canopy to capture the sunlight completely for photosynthesis. Vine is the whole (trunk) of plant consists of stem, leaves and cordon. Height of canopy may be up to 30 feet. Cordon is infect the arms or branches of grapevine which extend from main trunk. Cordon is the part from where additional arms and leaves emerge. Leaves are the most visible structure of the vine. They are most important for physiologically for process of photosynthesis to create carbohydrates required for grapes formation [4].

Grapevine sap was used by European healers to cure skin and eye diseases. Leaves were used traditionally to stop bleeding, pain and hemorrhoids pain. Sore throat was treated by unripe grapes. Tuberculosis, constipation and thirst were treated by raisins. And ripe grapes were used for treatment of cholera, smallpox, skin infection, cancer, liver and kidney diseases. Ripened grapes contain many phytochemicals [5].

1.2. PHYTOPHARMACOLOGY:

The phytochemicals of grapes are polyphenols (Resveratrol) and anthocyaninse.g, catechins. Resveratrol and anthocyanins have inhibitory activity against heart diseases, degenerative nerve diseases, cancers, Alzheimer's disease and viral infections. As well as resveratrol have shown pronounced antiangiogenic activity in many in vivo models like mouse cornea, chorioallantoic membrane of chick and mesentery of rodents [6].

2. WHAT IS ANGIOGENESIS?

Blood vessels network proliferation is called Angiogenesis that penetrates the cancerous tissues, supply oxygen and nutrients along with removing wastes. Angiogenesis is a part and parcel of normal physiologic process as well as involved in tumor vascularization. It can be checked with equal comfort both in-vitro as well as in-vivo. Finding of suitable Angiogenesis assessing method was real difficulty for Researchers. Reliable, technically and easily quantifiable and clearly executable assay proves to be the best choice. Principle in vitro assays comprising those of study of endothelial cells differentiation, their proliferation and migration, and in vivo studies including, implantation of subcutaneous sponge, study of chamber of cornea, Zebra fish, Assay of CAM and tumor Angiogenesis were studied by them [7]

2.1. PROTAMINESULPHATE AND HEPARIN-CORTISONE ACETATE :

Here the Antiangiogenic agent's inhibitory effect on neovascularization & growth of CAM, and the possibility of development of new angiogenesis inhibiting assay were studied. The Anti-angiogenic activities of Protamine sulphate and combinations of heparin-cortisone acetate were well known, which impaired the growth of chorioallantois at dose which does not harm growth of embryos. The Agents inhibitory effect is presumed to be due to specific inhibition of endothelial cells growth forming chorioallantoic blood vessels based on the following results: There was found significant correlations between the CAM vessels length measured by an automatic image analyzer and the chorioallantois estimated volumes (correlation coefficient r = 0.94) and the (3H)-thymidine incorporation was inhibited by these agents into cultured endothelial cells at the dose having no effects on chick embryonic cells. MitomycinC, 5-fluorouracil, and paraformaldehyde the inhibitors of DNA synthesis suppressed both the CAM and embryo growth and resulted in early embryonic death, Nonspecific impairment of DNA synthesis by these agents might be the cause. It is indicated that present CAM assay could screen anti-angiogenic activity of these agents [8]

2.2. ABNORMAL ANGIOGENESIS:

Abnormal angiogenesis occurs due to Diabetes. A severe form of diabetic retinopathy occurs due to increased angiogenesis, myocardial ischemia decreases angiogenesis in patients of diabetes. Hyperglycemia's effect on formation of blood vessels in the CAM model was directly evaluated, which is used as a classical of active formation of new vessels. Diabetes was introduced in chick embryos of 7th day by injection of glucose in vitreous chamber. Either water or mannitol (osmotic control) was administered in control

embryos. Angiogenesis in this model got decreased by induction of diabetes from the day 5th. Hyperglycemia didn't alter the pattern of main vascular growth factors gene manifestation, assessed by semi quantitative polymerase chain reaction. The apoptosis of pericytes and endothelial cells get increased was observed 2 days after inducing hyperglycemia by electron microscopy. By Incorporation of bromo-deoxyuridine decreased endothelial cell proliferation in the meantime was assessed. Without altering the expression level of vascular growth factors, by inducing apoptosis and decreasing endothelial cells proliferation Hyperglycemia impairs the process of Angiogenesis

3. ANGIOGENESIS MODELS

3.1. SPONGE/ MATRIGEL ANGIOGENESIS ASSAY.

It is one of the famous in vivo assays, and has become the method of choice for studying in vivo angiogenesis in many studies. In this assay inducer of angiogenesis FGF or tumor cells are introduced in liquid Matrigel and injected subcutaneously. After its solidification Matrigel plug is penetrated by host cells and new blood vessels are formed. Angiogenesis assessment is done either by measuring hemoglobin or by scoring specific area for vascular density. Matrigel plug assay is most suitable because it allow the clear visualization of angiogenesis, histological analysis and photographic documentation and image analysis. It also illustrates the response to angiogenesis inducing factors e.g. FGF and VEGF along with anti-angiogenic agents like endostatin [9] (

3.2. MOUSE RETINA AN ANGIOGENESIS MODEL

Use of mouse retina as angiogenesis model has many advantage over other models. For example the developing vasculature of mouse retina was easily assessable for imaging and intervention without any difficulty. Usually developing mouse retina was used to assess the effect of VEGF expression on the process of angiogenesis. And the response of endothelial cells was studied to VEGF gradients. And mouse retina model of angiogenesis has advantage of giving ease for detection of abnormalities in normal angiogenic process. As organized vasculature development is required for other developmental processes along with development of retina. So observations made in mouse retina development also applies to other organs [10]

3.3 RAT MESENTERY ANGIOGENESIS ASSAY

Adult rat mesentery is the most suitable model for angiogenesis, because the phenomenon of angiogenesis involved is this model is sprouting angiogenesis, a main mechanism angiogenesis in human normal and tumor tissues. The angiogenesis is induced in mesenteric tissue by intraperitoneal injection of pro-angiogenic factor can be modified or modulated by treatment with s.c, i.v or oral administration of modifying agents. Advantages of using this system is that tissue is vascularized two dimensionally and network is microscopically assessed, and trauma induced angiogenesis ensures high sensitivity [11].

3.4. CAM AS ANGIOGENESIS ASSAY

An extra embryonic membrane known as CAM was used to study the development of new vessel as well as the inhibition of that process due to different factors in vivo. The extent of angiogenesis and anti-angiogenesis may be evaluated by Quantitative or semiquantitative methods. Factors inducing angiogenic and anti-angiogenic effects could be identified by using CAM system along with investigation of Angiogenesis in association with normal inflammatory & tumor tissues. Other good option of in vivo system was cornea of rabbit, but CAM was easy in handling and expense was low. Moreover, use of CAM has very few limitations [12]In the development of CAM, sprouting &intussusceptivemicrovascular growth (IMG) Contributions were identified. CAMs capillary bed was analyzed by light microscopy quantitatively for its growth and morphology. CAMs microvasculature perfusion by particles of colloidal gold, make capillaries uniquely different from the surrounding unlabeled region. Counting and measurement of the intercapillary tissue profiles become identifiable. Three developmental phases of CAM angiogenesis was shown by morphometric analysis. Sprouting is the main mechanism of growth of blood vessels in initial 5 to 7 days. From 8th to day 12 known as intermediary phase, IMG is prevailing, and in last stage ranging from 13 and 14, only expansion and a small increase in complexity occurs in CAM structure, these are important findings regarding use of the CAM as a medium to test factors of angiogenesis. Infect, care is required regarding misinterpretation of physiological age-related variations of the CAM vascular structure than particular responses to the tested compounds [13]

Different animal model systems used to explore angiogenesis mechanisms were studied by them, for the analysis of the angiogenic prospective of purified elements and complete cells, chick embryo models proves to be useful tools. The CAM is a specified tissue of the chick embryo with great supply of blood vessels, used to study the pro-angiogenic activities or anti-angiogenic activities of material under consideration [14].

An extra embryonic membrane CAM of chick embryo is a best biological model to study the process of angiogenesis. Present information about the architecture of CAM's capillary network is summarized by this review, debated the dispute about the presence of a single blood arc or network of capillary underlying the epithelium of chorion, presented a new classic of growth of vessels of CAM, also the use and boundaries of the CAM as a model for angiogenesis study were discussed [15].

A model CAM was actually made for the study of tumor angiogenic activity. Out of all in vivo assays the only one that could be conducted at any test site easily. The test substance effect on formation of vessels was examined on 10th day embryos by exposing to substance and patterns of formation of vessel of CAM were observed at day 12 or 13 [16].(Chick CAM is used as model for angiogenesis study. Process of synthesis of novel blood vessels from the previous ones is known as Angiogenesis. This process is controlled by initiation of intracellular mechanisms of endothelial cells. Angiogenesis blocking agents infect interfere with these processes. This model is used to study both topical and intravascular administration of study agents. This model is presented to evaluate pro as well as anti-angiogenic compounds [17].(

The chick CAM was used as best available example for study of tumor angiogenesis. Angiogenesis is the process of new blood vessels synthesis from existing ones, angiogenesis is essential for growth, progression and metastasis of tumor. Pre-clinical screening assays are required to develop such agents which can target tumor vasculature. The CAM assay has been extensively used as a substrate to study angiogenic process, side by side to anti-angiogenic process. Cigarette smoking is one of the known progressive toxicants in terms of all side effects. Prenatal and postnatal growth is adversely effected during pregnancy by maternal tobacco smoking, developmental and behavioral flaws risk get increase in early and late age. In an in vivo chicken embryo assay the impacts of different nicotine preparations and solution of smoke on movements of embryo were recorded during neonatal development by web camera. Nicotine proved to be hyperactive at low doses and hypoactive at higher doses accordingly; by applying 10µg nicotine and different smoke solution preparations caused a significant decreased movement. Embryonic movement decrease was observed dose-dependently, which remained persistent till the end. It is concluded in the end that embryonic movements were affect by nicotine [55].

4. FUNCTIONS OF CAM

Gases and nutrients get exchanged until hatching is mediated by CAM that is an extra embryonic membrane over chick embryo. Its dense capillary network is commonly used to study the process of new blood vessels formation under the control of normal tissues, compared to the cells of bioptic tumor, in vivo. This assay had been published in over 550 publications, during last 8 years. The angiogenic reply of CAM to numerous carcinoma cells and their replies to angiogenesis inhibitor molecules and FGF role in CAM vessels synthesis in detail are studied by her[18].

5. ANTIANGIOGENIC ACTIVITY OF PLANTS PHYTOCHEMICALS

Edible berries have Anti-angiogenic property. The prime area in investigation of tumor biology is the use of Anti-angiogenic techniques for prevention and treatment of cancer. In tumor vascularization main role is played by vascular endothelial growth factor. The vasculature of skin remains normally inactive. During inflammatory dermal diseases like psoriasis and skin cancer skin contains the potential of angiogenesis initiation. Berries effect on expression VEGF by human keratinocytes is identified by them. Extracts of six berries e.g. Bilberry, wild blueberry, cranberry, raspberry seed, elderberry, and strawberry and proanthocyanidins of grapes seed extract (GSPE) were analyzed. A multi-channel HPLC-coulArray approach was used to study uptaking of extracts by HaCaT (human keratinocytes). ORAC was used to study the antioxidant potential of extracts. Comparable ORAC values were observed by samples of berry seeds. The antioxidant capacity of other samples used to be significantly greater than these samples. The highest ORAC values are possessed by Wild bilberry and blueberry. The mechanism of action of each berry is the significant inhibition of hydrogen peroxide and expression of VEGF. This effect was commonly shared by pure flavonoids and not observed in case of other antioxidant such as α-tocopherol or GSPE. Edible berries cells showed the impairment of angiogenesis [19].

Delphinidine showed the cyclin-dependent inhibitory pathway in case of angiogenesis inhibition. Epidemiologic studies have shown that diets composed of vegetables and fruits have a positive protective effect against carcinomas and diseases of heart. Exact mechanism for these positive properties is not well known but it is evidenced that foods polyphenolic compounds seem to be having beneficial effects. Delphinidine potentially inhibits the migration and proliferation of HUVE cells. Delphinidine induced inhibition of proliferation related to the arrest of cell cycle in Go and G1 phase is demonstrated by flow cytometric analysis. The VEGF-induced decrease kinase inhibitor expression and the VEGF-induced increase of cyclin D1 and cyclin A is reversed by Delphinidine shown by western blot analysis, The G1-to-S transition is achieved by both inhibited functions of VEGF. Furthermore,

neovascularization is inhibited by Delphinidine in the model of CAM. So for the development of an anti-angiogenic therapy delphinidin appear to be encouraging as it is used to study angiogenesis in vivo as well as in vitro

In hemangioma edible berries showed the anti-angiogenic properties which were studied by them. For study of in vivo angiogenesis hemangioma proves to be an influential model. Angiogenesis is facilitated by monocyte chemotactic protein 1 & it is also responsible for macrophages recruitment to the sites of infection. The expression of VEGF factor and in vitro angiogenesis get suppressed by the use of edible berries extract. Wild blueberry and the powdered berries specifically suppress the expression of cell of endothelioma as compared with other berry extracts is observed by scientists. Therefore, to study their effect wild blueberry and berry powder were selected for experimental in vivo model of angiogenesis. Transcription of MCP-1 and inducible nuclear factor kappa B is clearly inhibited by berry powder shown in reporter's study. Previously treated cells of endothelioma with berry powders indicated fewer tendencies for hemangioma formation. Comparison of mice that got treatment to controls show marked decrease in infiltration of macrophages in hemangioma. The first in vivo evidence of edible berries anti-angiogenic property was provided by this experiment [20]

Pomegranate has the potential of inhibiting angiogenesis both in vitro model as well as in vivo model. Oxidation and synthesis of prostaglandin is retarded by pomegranate seed oil and polyphenols of fermented juice, pomegranate also prevents the propagation & invasion of carcinomas of breast, and promotes apoptosis of breast cancer cell. There are several ways to study the anti-angiogenic potential of these materials. Possible effects are checked on the regulation of angiogenesis by them thru measurement of VEGF, interlukin-4 and factor of migration inhibition. Pomegranate fractions shows significant anti-angiogenic potential by strongly down regulating VEGF in normal breast epithelial cells and estrogen sensitive breast cancer cells, and migration inhibitory factor is up regulated in estrogen resistant breast cancer cells. An ex-vivo model of pomegranate has shown an antiproliferative effect on angiogenesis observed in HUVEC, and fibroblasts of amniotic fluid. Finally the formation of new blood vessels was particularly decreased in CAM angiogenesis model. At the end antagonistic potential of pomegranate on Angiogenesis was studied by employing different models [58]

After studies the anthocyanin rich edible berries proved to be having anticarcinogenic, antioxidant and antiangiogenic potential. Berries rich in anthocyanins are having great anticarcinogenic potential. Anthocyanins are responsible for pigmentations of fruits, they also have antioxidative potential. Anthocyanins are responsible for protection of human genome DNA integrity. These compounds reduce age related oxidative stress and improve the functions of brain. This study was conducted on extracts of six berries to identify their antioxidation potential, potential for cytotoxicity and potential against angiogenesis. After undo efforts a synergistic combination of extracts of berries was prepared known as "optiberry". It has great capacity of scavenging oxygen radical. It proves to be highly effective more carcinogenic, highly antioxidant and less cytotoxic and highly antiangiogenic. These findings lead to optiberry extracts use in tumor cell biology studies. It is observed that optiberry extracts showed the capacity to decrease expression VEGF. As angiogenesis is regulated mainly by VEGF so optiberry proves to decrease angiogenic process. Pretreated endothelioma cells with optiberry extracts shows less development of hemangioma. These studies prove antiangiogenic, anti-carcinogenic, antioxidative properties of optiberry extracts [59].

Sphenocentrumjollyanum Pierre was proved to have anti-angiogenic and antioxidant effects. The oxidation inhibiting and angiogenesis inhibiting potential in methanolic extracts of Sphenocentrumjollyanum organs were assessed using DPPH and CAM assays respectively.in an anti-angiogenesis study it was proved that stem bark is the most active organ. After Further fractionation of the stem bark it is observed that the chloroform fractions of stem bark have the highest potential for anti-angiogenic effects at dose of 250 and 36.2 µg/pellet. Effects got increase by increasing dose. Results showed that further research should be conducted to identify active principles and to confirm the angiogenesis and oxidation inhibiting potential of plant [21].

The angiogenic and antiangiogenic activity of Chinese medicinal herbal extracts was studied. For angiogenic in vitro activity the aqueous extract of 24 Chinese herbal plants was taken which were used in treatment of ischemic heart disease. And another 24 plants were used to check their antiangiogenic potential in vitro by using their aqueous extracts traditionally for treatment of tumor and inflammatory process in Chinese clinics. To examine their effects on angiogenesis chicken CAM model is used and for cell proliferation studies cells of bovine aortic endothelium were used. Many herbal aqueous extracts were applied and few out of them particularly Dalbergiaodorifera showed the strong potential for angiogenesis on CAM model and endothelial cells. And some other e.g. Catharanthusroseus presented

the strong anti-angiogenic potential at the same dose [22].

Nasunin is an anthocyanin compound having antioxidant and antiangiogenicactivities found in bark of eggplant. Anthocyanin with antioxidant properties derived from bark of eggplant is known as nasunin, and it has presented an antiangiogenic potential as well. At higher 10 micro molar dose of it inhibits the outgrowth of microvessel in an in vitro model of angiogenesis. Nasunin's effect on different ex vivo models of angiogenesis was studied e.g. HUVECs. The suppression of HUVEC proliferation by Nasunin is directly related to dose (50-200µM). Nasunin can be used to prevent angiogenesis-related diseases, as it is proved by its antioxidant and antiangiogenicpotential[23].

. Even after promising clinical findings and major improvements, more effective and safer techniques are required. Identification and use of plants derived compounds is successfully practiced with some angiogenesis modulating plant components in ancient Chinese medicine (TCM) from long in past. In resemblance to European therapeutic practice, TCM imply mixture of extracts derived from plants, known as Fu fang, to boost effectiveness and lessen side effects. To potentiate further the efficacy of these botanical origined medicines in angiogenesis more evidenced based investigation on these compounds is required [24].

Edible berry juice suppresses the cancer cells propagation and suppresses the TNF-induced activation of NFkB. Several phytochemicals like phenolic acids, proanthocyanidins, anthocyanins and other flavonoids are present in berries. Edible berries have variety of potential chemopreventive activities. A variety of small berries cultivated or collected in the province of Quebec & Canada different varieties of berries were cultivated and assessed to have chemopreventive activity. Antiproliferative, antioxidant and anti-inflammatory activities are present in different berry fruits most commonly studied one are Strawberry, raspberry, black currant etc. They also induce apoptosis and arrest of cell cycle. Few kinds of berries e.g. raspberry, black currant, white currant juice strongly inhibits the growth of carcinomas of stomach, prostate, intestine and breast, but strawberry, high-bush blueberry, serviceberry, red currant, don't or slightly inhibits the growth of cancer cells. There is no correlation found between antioxidant and antiproliferative properties of berries. Berry juice never inhibit the cancer cells proliferation by apoptosis, but cause the blockage of cell-cycle, by down-regulating the cyclin D1 and D3 expression.13 berries were tested, 6 out of these considerably blocked the expression of activated cyclooxygenase and nuclear transcription factor activation. These outcomes indicate that juices of different berries have potentially different chemoprotective activities so variety of berries should be included in the diet to prevent the development of tumor [25]. The detected anthocyanin pigments were of 25 different kinds, many of them contain aglyconepelargonidin (Pg); few were also containing derivatives of cyanidin. The usual substituting sugars were Glucose and Rutinose, along with other identified sugars like arabinose and rhamnose; aliphatic acids get acetylated with few anthocyanins. The anthocyanin-derived pigments of anthocyanin origin named 5-carboxypyranopelargonidin-3-glucoside were detected along with few condensed pigments like flavanol and anthocyanins. The anthocyanin concentrations were found to be variable among same variety, reflecting a strong effect of the maturity level, environmental influences [26].

. It is also described that in the process of carcinogenesis the utilization of plant-based foods is related to cause the decrease in risk of carcinomas of epithelium. Vitamins, minerals and numerous micronutrients are the cytoprotectants present in fruits and vegetables. It is evidenced that cancer risk is decreased uniquely by the consumption of berries. Reason behind is that polyphenolic compounds like flavonoids and anthocyanins are present in berries which are believed to reduce the transfer of malignancies. Modulation of DNA damage biomarkers and malignant transformation indicators occurs by the consumption of phytochemicals present in extracts of berries both in vitro and in vivo. Anthocyanin components are potent anticarcinogens as identified by cell culture & animal models, and they appear to protect stability of genome. Anticarcinogenic mechanisms of berries include suppression of carcinogen activation and enhanced killing, decrease in binding of carcinogen to DNA, suppression of oxidative damage to DNA, alteration in signaling of cell and blockage of transformation, invasion and metastasis of malignant cells [27].

Phenethylisothiocyanate has inhibitory activity on angiogenesis both in animal and tissues models. The past data of lab findings mainly shows that components of edible vegetables recognized as Phenethylisothiocyanate has significant protective action against chemically induced cancer, and it can also inhibits the cancer cells growth by causing apoptosis (cell cycle arrest). This study have described that PEITC inhibits the Angiogenesis both in vivo and in vitro at nontoxic and therapeutic doses. The treatment with PEITC produced a decrease in activity of HUVEC depending directly on concentration and time. HUVEC also inhibited significantly the capillary like tube structure formation and invasion. The PEITC causes blockage of angiogenesis that was

related to the inhibition of VEGF release. The treatment with PEITC decreased the migration of carcinoma of human prostate, which was related to inhibition of VEGF expression, and release of colony stimulating factors. The treatment with PEITC blocked the in vitro angiogenesis studied in chicken egg chorioallantoic membrane assay. In conclusion, these studies suggest that angiogenesis inhibition by PEITC may be employed in cancer prevention [28]. The prophylactic effects are exhibited by juices of different plants on inflammation induced by lipopolysaccharides. The anti-inflammatory activities of berry, bitter melon, mulberry and loquat were hypothesized by them were due to cultures of murine peritoneal macrophages stimulated by lipopolysaccharide. Administration of juices concurrently with stimulated LPS was done to understand the mechanism. For experiment three models were prepared named as model A, B and C. in case of (model A) juices of vegetables and fruits were administered 24 h before stimulation of LPS. In case of (B model) administration of juice was 24 h after the treatment of LPS. And in (model C) both LPS and juices were co-administered with macrophages. Secretions of anti-inflammatory cytokines, tumor necrosis factor and pro-inflammatory cytokines induced by LPS were studied. The outcome indicated that bitter melon, strawberry, mulberry and loquat administration promoted anti-inflammatory cytokines production and they specifically ($p<0.05$) decreased the levels of proinflammatory cytokines [29].

The angiogenesis inhibiting activities of chalcones and flavonoids along with their mechanisms are discussed in this study. This study shows the antiangiogenic effect of flavonoids and chalcones. Generation of novel blood vessels from older ones is called angiogenesis a very significant process in normal development. Abnormal, uncontrolled Angiogenesis is involved in many diseases like asthma, diabetes, inflammatory disorders, cirrhosis, obesity, AIDS and bacterial infection and metastasis. That is also involved in proliferation, invasion and metastasis of tumor, because exchange of waste and supply of nutrients to tumor is done by Angiogenesis. So control of angiogenesis is of major therapeutic importance. Numerous plant based compounds are studied for their antiangiogenic properties. Many compounds importantly polyphenols found in fruits and vegetables have antiangiogenic properties and metastasis blocking properties. Polyphenols most importantly chalcones and flavonoids regulates the VEGF expression, metalloproteinase and EGFR inhibits the pathway of signaling, thereby inhibiting angiogenesis strongly [30].

Synthesis of novel blood vessels from pre-existing ones is known as angiogenesis. It's a necessary process in much pathology. Bilberries have conventionally been used in some countries for its antioxidant and cardioprotective potential. But present study is designed to evaluate the bilberry effect on CAM angiogenesis in vivo model. Results show that it inhibits angiogenesis process in concentration-dependent manner. And bilberries anti-angiogenic may be used in treating Angiogenesis-dependent human diseases [31].

It was known that Angiogenesis is reduced by bilberry extracts in both tissue & animal models. So the extract of Vacciniummyrtillus (VME) were tested to study their effects on angiogenesis. The matrix metalloproteinase GM6001 (0.1-100 μM) and VME (0.3-30μg/ml) inhibited both tube formation and migration of human umbilical vein endothelial cells (HUVECs) induced by vascular endothelial growth factor-A (VEGF-A) in a concentration dependent manner. In addition, phosphorylation induced by VEGF-A was inhibited by VME, but not that of phospholipase C γ (PLC γ). In an in vivo experiment VME was administered in vitreous chamber of mice for inhibition of oxygen induced retinopathies and it inhibited the formation of new blood vessels. Thus VME proved to be effective for inhibition of angiogenic process both in animal and tissue models [32].

Various plant derived compounds were supposed to have effect on Angiogenesis. They effect the formation of blood vessels. It was supposed that edible fruit plants extracts grown in Spain and Italy have an angiogenic effects. To prove that hypothesis Extracts were administered to cultured micro vascular human endothelial cells (HMEC-1), keratinocytes of human and human vascular endothelial cells; 96 extracts were analyzed an inhibitory effect was observed by half of them on HMEC-1 proliferation. In addition the HUVEC differentiation also experiences inhibitory effect. All the extracts showed inhibitory activity. This study indicated that local food plants extracts have potential anti-antigenic activity, and can be proved as excellent Antiangiogenic agent [33]

After 3 days administration of Indomethacin (18mg/kg, p.o) to mice, severe ulceration of stomach was observed with a great damage up to 3.46, reduction in size was observed by a 3-day treatment with the methanol extract of Myristicamalabarica (40mg/kg, p.o) to 0.95 and omeprazole (3 mg/kg, p.o) to 0.82, respectively. The healing action of test samples was observed to be due to their modulating action on PG synthesis and new vessel formation. Ulcer formation was found to be linked with VEGF, VWF and PGE (2) suppression, and an increase of endostatin and EGF levels. All the parameters responsible for its

healing activity got reversed after treatment with plant extracts. Omeprazole However couldn't affect these factors despite of providing similar healing [34].

6. DRUGS AND FOOD COMPONENTS ANTIANGIOGENIC PROPERTIES

CAM proves to be a valuable model for angiogenesis evaluation. Five days after fertilization, examination of Cytocalasin D and Suramin's mechanism of vascular development is performed with a purpose to characterize the CAM vessels formation, during this interval. Between days 5 and 6 a 20-folds increase in size of CAM occurs, mesodermal blood vessels migrate toward the ectoderm and new vessels from near the ectoderm. Density of mesodermal cells decreases and CAM become thinner between days 5 and 6. Different concentrations of both drugs were administered to CAMs of day 5 and on day 6 their effects were evaluated in order to determine mechanism of action of Antiangiogenic drugs. Formation of capillary plexus was inhibited by both drugs by inhibition of genesis of vessels and migration of mesodermal vessels toward ectoderm, altering the pattern of major blood vessels branching and decreasing area of major vessels. Compartmentalization of the plexus was also blocked by use of cytochalasin. Both drugs have suppressing effect at parallel doses. To observe the effect of inhibitor compounds on angiogenesis, day 5 to 6 CAM provides a useful assay [35].

Excessive Angiogenesis can be targeted with foods and neutraceuticals. Aiming at the process of angiogenesis is based on preventing and treating degenerative diseases. Because studies have proved that angiogenesis is a hallmark and essential factor for initiation and propagation of neoplastic & non-neoplastic diseases like all cancers, diabetes, inflammation, arthritis, macular degeneration and psoriasis. Angiogenesis is a complicated process involving all the complex enzyme system along with positive and negative regulators. Angiogenesis in healthy individuals remain under control. Targeting the process of new blood vessels formation (Angiogenesis) with safe antiangiogenic substances shows effect against novel blood vessels formation while sparing the older one. The rational of investigating functional food as antiangiogenic compound is due to their safety, effectiveness and reversible inhibition of angiogenesis and their prolonged use throughout life without toxicity. New techniques are based on treating angiogenesis with different neutraceuticals [36].

It is discovered that a dietary derived flavonoid Quercetin have an antiangiogenic potential. It is also discovered that dietary derived flavonoid Quercetin inhibits the tumor growth both in vivo and in vitro by inhibiting tyrosine kinase. It inhibits the very important events of angiogenic process such as propagation, immigration and duct formation of microvasculature of endothelial cells of human which is further proved by its effect on human umbilical vein endothelial cells. It is revealed that Quercetin has more inhibitory effect on endothelial cells proliferation than tumor cells proliferation. When Quercetin is used in chicken CAM assay it proved to be antiangiogenic in vivo due to its inhibitory effect on activity and expression of matrix metalloproteinase-2 which controls the proliferation, migration and tube formation, assayed by polymerase chain reaction technique. These results prove that Quercetin has anti-angiogenic property which is due to the influence on matrix metalloproteinase-2 [37].

7. ANTICANCER ACTIVITY OF PLANT DERIVED ANTHOCYANINS

The most common fruit & vegetable derived polyphenolic constituent is called Anthocyanins. Anthocyanins absorbs sun light at 500nm wave length and gives different colors to fruits, vegetables and cause sprouting of foliage in autumn season. They have studied that American residents consume 200mg of anthocyanin daily which is 9 folds of all other flavonoids. Anthocyanins are also tested both on cell culture and in animal models of tumor to describe their anticarcinogenic activity and molecular mechanism of action. They also suggested the reason for absence of correlation between anthocyanin efficacy in cell culture models and in human models. In future the studies will be aimed at improving anthocyanin absorption for their ultimate use in chemoprevention of human cancers [38]

. The berries anticancer activity is due to the abundance of bioactive phytochemicals found in these colorful fruits, including phenols like (flavonoids, proanthocyaninsetc). The present study has shown the anticancer activities of berries are due to their ability to repair damage done by the oxidative stress mechanism and inflammation. In addition, phytochemical substances present in fruit have the ability to control carcinogen particularly about their purified extracts, juices and freeze dried powder. This study also discusses the Potential mechanisms of anticancer action and availability of berry bioactive, and recommendations for future berry research [39].

The tumors in esophagus of rats are prevented by raspberry anthocyanins. The diet origined black raspberries (BRB) found to suppress the development of tumors in the esophagus of rat by inhibiting the expression of carcinogen derived protein. The present study was conducted to observe whether BRB anthocyanins have

chemopreventive activity in living organism. The experiment was designed to study activity of anthocyanins of BRB against cancerous tissues by using rats fed upon different diets containing either 5% whole BRB powder, or an anthocyanin-rich fraction, after two weeks of food supply Animals were killed at 30th week and esophageal tumors were examined. The anthocyanin treatments have shown equal activity in reducing tumor synthesis in the esophagus, indicating that the anthocyanins in BRB have chemoprotective potential. The process of angiogenesis, tumor proliferation and inflammation were inhibited by anthocyanin rich group of drug [40].

Anthocyanin is a plant pigment found in berry fruits has presented potential to postponed development of cancer in rat's models especially carcinomas of colorectal region. Extensive mechanistic studies have been done on the glycoside Anthocyanins and aglyconsAnthocyanidins mainly delphinidin and cyanidin. In tissue models, both anthocyanin and anthocyanidins have shown same potential as anti-neoplastic agents. Their mechanism was cell proliferation inhibitions and apoptosis induction of cell, also inhibition of transcription of oncogenic factors activity and tyrosine kinase. There are four isomers of Anthocyanins and anthocyanidins, their interconversion rely on access to light, temperature and ph. As compared to anthocyanin (glycosides), anthocyanidins are more decomposable chemically and survival is of few minutes only in vivo. The pharmaceutical issues like solubility parameter are very important determinants of potential development of chemoprotective drugs, which requires adequate attention. Studies have proved that Anthocyanins have higher chemopreventive activity in experimental groups and are more stable as compared to anthocyanidins. Hence anthocyanins seem to be the much better choice for development of drug [41].

. The ability of two anthocyanin rich mixtures was investigated for inhibition of the receptor tyrosine kinases (RTKs) EGRF, VEGFR-2, VEGFR-3, ErbB3 and ErbB2. Recombinant kinase activity was reduced by use of both mixtures at concentrations less than 12.9 micro grams per ml, with superior inhibition of EGFR & VEGFR-2 at less than 3.4 microgram per ml. Autophosphorylation of these RTKs in human vulva cancer was suppressed by both of mixtures, along with inhibition of VEGFR-3 as well as ErbB3. It is indicated by results that RKTs can be inhibited by anthocyanin-rich mixtures. The inhibitory activity is in order of greatest in vascular endothelial growth factors and less in endothelial cell proliferation against the tested RKTs. Considering those RKTs can have the important role in carcinogenesis [42].

Cancer patients must be treated by emphasizing on multiple physiological and biochemical pathways that control the growth of tumor and reduces toxicity of normal tissue. For cancer spread an angiogenesis is an important mechanism. Anti-tumor activity is shown by many plant extracts. Highly anti-angiogenic products are focused in this article. Molecular pathways are targeted by natural health products. The traditionally used anti-angiogenic and anticancer herbs through multiple interdependent processes include the curcuma longa, magnolia officinale, artemisiaannua and Resveratrol of grapes juice. Along with their multiple effects, these agents can be used for cancer therapy in synergistic combination with chemotherapy and radiation [43].

Wide range of fruits and vegetables are containing a reasonable quantity of natural agent Gallic acid. Studies have shown that it has an anticancer potential. In this study androgen -independent DU145 prostate cancer cells and androgen dependent-22Rv 1 human prostate cancer cells are used to evaluate the anticancer potential of Gallic acid in hairless mice. The apoptosis and viability of cells was determined. DU145 and 22Rv 1 xenografts were implemented to check the anticancer activity of Gallic acid in vivo. Both DU145 and 22Rv 1 cells viability was decreased largely by induction of apoptosis by use of Gallic acid in a dose dependent manner. The DU145 and 22Rv 1 prostate cancer cells xenografts growth was reduced by feeding Gallic acid in tumor studies of hairless mice. Both DU145 and 22Rv 1 models upon histochemical examination of tumors of hairless mice presented the inhibited proliferation, apoptosis induction and reduced microvascular density in tumor Xenografts from feeding of Gallic acid. In conclusion the anti-prostate cancer efficacy of Gallic acid is proved by this study. It also emphasizes on further evaluation of its anticarcinogenic potential [44].

It is proved from several epidemiological studies that fruits and vegetables consuming populations experience a decrease in incidence of cancer. The research efforts were supported by such encouraging observations from all over the world to focus on identification and characterization, of various phytochemicals to develop effective techniques for controlling variety of human carcinomas. Grapes products were one of dietary products that having known chemopreventive potential against cancer along with improving overall health. Studies from various sources have proven that grape products can be used as effective anti-cancer agents having beneficial effects in general population.

Bioactive phytochemicals of strawberry juice are responsible for its anticancer activity. The berry bioactive substances have the ability to counteract,

minimize and heal the damage occurs due to oxidative stress and inflammation, indicates their anticancer effects. Additionally regulation of carcinogen and xenobiotic metabolizing enzymes, inflammatory cytokines, growth factors and sub cellular signaling pathways of cancer cell proliferation and tumor angiogenesis are done by berry bioactives. SPIP (scanning probe image processor) was used for quantification of results to explore the role of angiogenesis. A marked anti-angiogenic effect was observed at 0.5% concentration of strawberry juice. The decrease in roughness and abbot curve was observed along with decrease in primary, secondary and tertiary diameter of blood vessels. In-vitro results showed that strawberry juice may be used as an anti-angiogenic agent [45].

8. MEDICINAL USES OF *VITIS VINIFERA* (RESVERATROL)

Red wine found to contain a well-known and safe plant derived compound resveratrol which proved to be anti-inflammatory and immune regulatory compound, and offers novel therapeutic utility in immune related diseases [46].

Epidemiological studies have shown that cardiovascular disease incidence decreases by use of wine, grapes, and other polyphenolic foods. Experiments indicated polyphenols of grapes origin could decrease cardiovascular diseases particularly fatty hardening could be reduced by grapes polyphenols by a many of mechanisms, e.g. suppression of LDL oxidation and other favorable effects on endothelial function enhancement, controlling hypertension, blockage of platelet aggregation, controlling inflammation etc. To confirm these effects more clinical studies are required. The available data, however, the grapes beneficial effect in decreasing cardiovascular diseases is strongly supported by data and helps to recommend that a diet of plant origin including grapes could reduce the risk for cardiovascular complexities [47].

The phytochemicals of plants origin like Resveratrol and Curcumin are known well for antioxidation, anti-inflammation, anti-microbial and anti-carcinogenic potentials. However anti-tumor activity is still not identified. This assay was conducted to evaluate the angiogenesis modulating activities of resveratrol and curcumin either separately or collectively with Carboplatin in ascites carcinoma of mice. By intradermal injection Tumors were induced in animal bodies. Microvascular density of such benign tumor was evaluated along with its intra-tumoral receptor type-2 (Flk-1) and VEGF. All values were calculated after 7, 14 and 21 days after inoculation. The microvascular density got decreased significantly after treatment with curcumin and resveratrol either alone or collectively with carboplatin. The plasma VEGF level also decreased greatly 7 days after treatment with them. And resveratrol and curcumin decreased fraction of Flk-1-rich tumor to the level of 42.9% and 28.6% respectively. Their collective administration with carboplatin further reduced the fraction of tumor to 28.6% and 14.3%. Studies showed strong collaboration of VEGF with microvascular density. The results demonstrated the antiangiogenic effects of resveratrol and curcumin as shown by decrease in microvascular density. Both have antiangiogenic effect by inhibiting VEGF and type 2 receptor. Outcomes have demonstrated the role of these phytochemicals in correlation with cancer treatment [48].

8.1. ANTIOXIDATION POTENTIAL

Grapes seed extract is known for its antioxidation potential which

Reduces hydroxyl free radicals, Breaks Lipid peroxidation chains. Delay initiation of peroxidation. Chelate free iron that is able to facilitate peroxidation. And reduce free radical generation by inhibiting xanthine oxidase.[49] (Murray et al. 1995) :

8.2. ANTICANCER POTENTIAL

Various researches have shown that grape extract has anticancer activity against various kind of cancers e.g. breast, lungs and gastric cancer. This property is due to polyphenolic compounds which have antitumor and chemopreventive potential. Aqueous and methanolic extracts of Vitisvinifera containing polyphenols is used in two in vitro assays 1) topoisomerase 1 relaxation assay and 2) Mitomycin C- induced DNA strand breakage. Polyphenol rich fractions of extract are inhibitors of topoisomerase making it anticancer. As well as grapes extract inhibits Mitomycin C-induced DNA strand breakage and making it chemopreventive, but polyphenols cause Mitomycin C-induced breakage, making it cytotoxic and anticancer [50]

8.3. CARDIOPROTECTIVE POTENTIAL

It is evidenced that grapes have cardioprotective potential after red wine consumption in moderate quantity. In this study they investigated the antihypertensive and vasodilator activity of alcohol free hydroalcoholic grapes skin extract obtained from skin of Vitisvinifera grapes in experimental rodent hypertension model. 55.5mg of grape seed extract have vasodilator effect on vasculature of rat mesentery and antioxidant effect was studied on lipid peroxidation of hepatic microsoma. Its oral administration reduced systolic and diastolic blood pressure. Bolus injection of grapes skin extract in norepinephrine treated isolate mesenteric vascular

blood cause endothelium dependent vasodilation. In conclusion antihypertensive effect of grapes skin extract was due to its vasodilator and antioxidant action[51].

9. ANTIANGIOGENIC ACTIVITY OF *Vitis vinifera* (RESVERATROL)

Endothelial cells growth is inhibited by Resveratrol a plant originedpolyphenolic compound also found in Grapes and other products of grapes origin act as an oral angiogenesis inhibitor. Resveratrol showed the Antiangiogenic property in many in vivo models like mouse cornea, chorioallantoic membrane of chick, curing of wound and model of tumor. Resveratrol dose dependently inhibited capillary endothelial growth. Resveratrol inhibits the chemotaxis as well as capillary endothelial cell growth which is mediated by FGF-2 and VEGF receptor. 100 µgm of resveratrol was able to induce a zone of vascular inhibition in the growing CAMs, and inhibition was observed in proportion to dose. 5.7µgm/ml concentration of Resveratrol was administered, could inhibit the growth of T241 fibrosarcoma in mice. Angiogenesis also takes place in healing of wound as it is essential for growth of tumor. At same dose administration of resveratrol as used against tumor would definitely delay healing of wound in mice which is estimated by sizes of wounds and percentage of healed animal. Resveratrol is proved as a novel angiogenesis inhibitor by these findings. Orally administered Resveratrol is proved as Angiogenesis inhibitors for the first time by their work. Angiogenesis-dependent diseases are prevented by the use of Resveratrol. However, the thing to be emphasized is that resveratrol's antiangiogenicpotential could do harm in situations of healing of wound [6]

The investigative studies have shown that progression of tumor and cardiovascular diseases are decreased by the consumption of red wine and green tea. Increase in blood supply causes the enlargement of developing tumor as well as enhanced atherosclerotic lesion, because nutrients and gaseous exchange to neighboring is done by new blood vessels development. So Antiangiogenic effects of green tea polyphenols (GTPs) and red wine polyphenolic compounds (RWPCs) are proved by various studies. The proposed mechanism of antiangiogenic effects of RWPCs and GTPs is thought to be inhibition of endothelial cells and vascular smooth muscle cells proliferation and migration, as well as VEGF and metalloproteinase-2 expression is suppressed. For in vivo studies CAM assay is employed for local application of RWPCs and GTPs. this also has described the antiangiogenic effects of both compounds. In conclusion the present study has indicated that GTPs and RWTCs have beneficial effects against heart diseases and cancer diseases [52].

. The infarct volume got specifically decreased with different resveratrol doses in both sexes (5 mg/kg for males and 1 mg/kg for females) applied 3 h after heart stroke. It also proved to be effective to decrease infarct volumes even after 6 h of administration. Expression of IL-1β and TNF-α and IL-1β, and production of ROS get suppressed in the ischemic cortex by use of resveratrol. The suppression of inflammation founds to be associated with Resveratrol partly with its protective effects on brain, and resveratrol can be proved as good drug for acute stroke of ischemia [53].

In this study rats were used. Rats were given the injection of normal saline to one group and resveratrol (10 mg/kg) to other group in case of acetic acid ulcer induction. The rats were killed 5 or 10 days post ulceration. Macroscopically ulcers wounds were evident in saline treated group of rats, while macroscopic scores of ulcer were low by treatment with resveratrol. Similarly RVT-administered groups were having lower ulcer score microscopically too. Both pre- and post-treatments of RVT increased again the depleted glutathione level in ulcer groups. Results demonstrate that resveratrol demonstrated both therapeutic and protective effects on gastric ulcer by inhibiting the activation of pro-inflammatory cytokines, neutrophils accumulation [54].

10. QUANTITATIVE ANALYSIS AND IMAGING OF ANGIOGENESIS

The clinical diagnostic imaging Technology's rapid development, related to medical angiogenesis research, introduces a major progress in healthcare. To study the physiological synthesis of blood vessels and recognized angiogenic and antiangiogenic compounds, the CAM assay was commonly used. In spite of the advancement, it was generally recognized that a major problem to be documented was the deficiency of appropriate quantifiable bioassay for angiogenesis. This problem is currently solved by novel image probing method, which showed great potential of measuring accurately even blood vessels of very small size along with detailed quantification of blood vessels and surface characteristics in a 3D manner in association with angiogenesis evaluation. The quantification in angiogenesis research can be done effectively by this technique [56].

Angiogenesis and tumor vessel architecture's Quantitative analysis was done by means of digital image analysis assisted by computer. Tumor microvascular bulk was affected by toxin of VEGF studied by them. Tumor growth depends particularly on angiogenesis. As a result

strategies for disruption of this method are seriously examined. Many angiogenic techniques are applied straight for comparison of angiogenic inhibitors efficacy. Still novel vascular (Angiogenesis) development's objective assessment has been tough to attain. The aim of the study was the quantification of the microvessel density in an unbiased manner achieved through the newly developed computer assisted image analysis. Athymic mice were used to grow human tumors, after a one week treatment tumor biopsies were taken. Sections of the frozen tumor were prepared and marked with antibodies like anti-CD-31 and images of vessels were taken with a special microscope. Vessel density was evaluated by an automated binarization and skeletonization protocol; morphological differences are further investigated from images. Total number of blood vessels, their length and branching points are important parameters of angiogenesis their estimation should be done by computrized process. The detection of differences in the angiogenic response within groups of control tumors and those treated with VEGF-toxin conjugate was done on the basis of these findings. More importantly computer-generated measurements appear to be similar to physical microvessel sums and particularly less variation were observed. A quick, reproducible, and alternate technique for the numerical calculation of tumor angiogenesis and vessel structure is the computer-assisted image analysis, proved by their result [57].

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