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REVIEW ARTICLE

Role of Naringin and Naringenin in Various Diseased Conditions - A Review

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ABSTRACT

In Citrus species, flavonones and glycosides occur in large amounts, plays a major role in maintaining various pathological conditions. Naringein and hesperidin, are the main constituents of the citrus fruits. Naringin gets converted into naringenin which is an aglycone part by the intestinal microorganism. They found to possess metal chelating effect, antioxidant, anti-microbial, anti-viral, anti-allergic, anti-estrogenic, antidiabetic, adipolytic activity, ischemic heart disease anti-inflammatory, anti-obesity, Hypoxia, hepatoprotective activity and anti-cancer activity. Because of all these pharmacological action, both Naringin and naringenin are gaining an importance to be used as a food supplement.

KEYWORDS

Citrus fruits, Naringin, Naringenin, Hesperidin, Metal chelation, Adipolysis

INTRODUCTION

India has an ancient heritage of traditional medicine .Indian traditional medicine is based on various systems including Ayurveda, Siddha and Unani. Evaluation of these drugs is mostly based on phytochemicals, pharmacological and allied approaches including various instrumental techniques like chromatography, microscopy and others. These traditional systems of Indian medicine have their uniqueness with no doubt but there is a common thread running through these systems in their fundamental principles and practices. With emerging interest in the world to adopt and study the traditional medicine and exploit their potentials based on different health care system, the evaluation of rich heritage of traditional medicine is essential.

The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, medicinal plants play a major role and constitute the back bone of the traditional medicine. Indian Materia Medica includes about 2000 drugs of natural origin. Almost all of which are derived from different traditional system and folklore practices. Out of these drugs derived from traditional system, 400 are of mineral and animal origin while the rest are of plant origin.

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Prospects of Herbal Research [1]

There is a worldwide “green” revolution, which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin. Digoxin and digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important example. Plants have also yielded molecules,

which are extremely valuable tools in the characterization of enzymes and the classification of receptors systems where phytostigmine, morphine, muscarine, atropine, nicotine and tubocurarine are important examples.

Therefore laboratories around the world are engaged in the screening of a plant for such study is traditional healer's claims for its therapeutics usefulness. It is such worth reflecting on the cultural environment in which traditional healers use plant remedies, as well as the methods of plant use, in order to strengthen the research design.

Need for the Usage of Plants as Medicine

The complete cure for cancer remains elusive despite the advancement of medical research and technology. Radical surgery, chemotherapy and radiotherapy which can bring about undesirable physical and psychological distress to the patients are the current cancer therapies. It is necessary for the continual global efforts in search for novel anticancer compounds that possess high therapeutic efficacy and fewer side effects compared to the existing anticancer drugs in the market. The mutagenic effect of plant has not yet been evaluated for its safety for consumption to date[2]. In developing countries medicinal use of herbs and spices has been increased gradually, which are generally considered to be safe and proved to be effective against various human ailments. High content of flavones, sulfur containing compounds and polyphenol derivatives has been reported to exhibit antioxidative and free radical scavenging abilities[3]. Many alkaloids had demonstrated for outstanding pharmacological potentials which exhibit antimicrobial, antiplasmodial and antitumor activities. Despites many pharmacological activities and potential benefits to human health, several flavonoids are described as mutagens[4].

Chemoprevention Property of Medicinal Plants

The scientific community has made an immense progress in acquiring the knowledge needed to prove cancer is curable, from the last three decades. To identify potentials cause of cancer, particularly environmental factors, diet and also to provide insight regarding their mechanism of action, pioneering research helped to greater extent. The accumulation of multiple sequential mutations and alternations in invasive neoplasm results in carcinogenesis. To either arrest or reverse cancer development by interfering with one or more steps in the process of carcinogenesis, promising inhibitors of cancer were identified and systematically evaluated for their potentials as chemo preventive agents[5]. To show the immense potential of medicinal plants used in traditional systems, focus on plant research has increased all over the world and a large body of evidences has been collected. Using the modern scientific approaches, various medicinal plants have been identified and studied, which revealed the potential medicinal plants in the area of pharmacology. Prevention of cancer, using specific agents to suppress or reverse the carcinogenic process is termed as chemoprevention. Many plant extracts had demonstrated potent cancer chemo preventive property and most of them are known to exert their effects by antioxidant mechanism, either quenching reactive oxygen species (ROS), or by inhibiting lipid peroxidation or by stimulating cellular antioxidant defense[6,7].

In pharmacological and nutritional effects, flavonones glycosides occur in large amounts in Citrus species plays a major role. In Japanese and Chinese Pharmacopeia. Formulations of some citrus species are used as crude drugs. As an appropriate medicinal use a number of citrus species have been recorded in the Chinese Pharmacopoeia the main constituents of the albedo of *Citrus grandis* osbeck is Naringin and minor amount of neo hesperidin. Aglycone part of Naringin is naringenin which is formed in the intestinal epithelia by enzymatic hydrolysis which inhibits cytochrome P450 mediated oxidation of some drugs[8.] In grape fruit and orange juice, Naringin and hesperidin are the main citrus flavonoid with physiological properties [9].

Isolation of Naringin from the *Citrus Grandis*

Fruits of *Citrus grandis* at various stages of maturation were obtained from the same tree during the entire season and stored for 2 or 3 days before proceeding with the analysis of the albedo. Albedo (40 g fresh weight) was cut into small pieces and homogenized for 5 minutes in a warming blender in 200 ml of 50 % methanol in water and 10 g celite was added during the homogenization. The homogenate was filtered immediately under vacuum through Buchner funnel lined with Whatman No.91 filter paper. The use of celite was crucial to avoid the formation of cloudy methanol and water solution. When working with albedo from mature fruit. A 10 ml aliquot of the solution was then added to 40 ml of 96 % ethanol and dried over the excess anhydrous sodium sulfate (three times) and then filtered through a non- sterile 0.2 µm PTFE [8].

Isolation of Naringin from Grape Fruit

2mm thick fresh grape fruit peel were finely chopped and then accurately weighed 1g. The weighed sample was extracted with 10 ml 99.7 % ethanol for 2 hours in an ultrasonic bath. The extract was then filtered through a filter paper. The extraction procedures was repeated three times. After filtered through 0.22 µm syringe cellulose acetate filter, the 80 µl of sample was diluted with 60 mmol l^{-1}, borate buffer to 1 ml. then it can be directly injected electro kinetically for analysis[10]. In the isolation and quantification of flavonoids several chromatographic techniques including HPLC have been used[11].

Pharmacological Actions of Naringin and Naringenin[12]

In most countries grape fruit is a part of their diet and it is usually consumed as juice. Sour flavor of the fruit is due to the presence of a chemical compound known as naringin which is a flavonones, rapidly gets transformed into naringenin by the action of enzymes such as α-rhamnosidase and β-glucosidase. As dietary antoxidant, naringin exhibit various pharmacological and therapeutic properties like antimicrobial, antimutagenic, anticancer, anti-inflammatory, cholesterol lowering, free radical scavenging and antioxidant effect.

Role of Naringenin in Ischemic Heart Disease

According to world health organization, ischemic heart disease (IHD) is the leading cause of morbidity and mortality in the western world and it will be the major cause of death in the world by 2020. Due to atherosclerotic disease of coronary arteries, an imbalance between myocardial oxygen supply and demand occurs which in turn results in myocardial hypoxia and accumulation of waste metabolites. Cell death occurs due to generation of oxygen derived free radicals by the ischemic tissues which in turn leads to chain reactions.

Polyphenolic compounds such as flavonoids which are widely distributed in food of plant origin such as vegetables, fruits, tea and wine have diverse in chemical structure and characteristic. In elderly men, regular ingestion of flavonoid containing food may protect against death from coronary artery disease, owing to their non- additive and nontoxic nature at traditional medicines, phytopharmaceuticals are gaining importance in allopathic medicine. For counteracting some problems and bolstering the body's defense against free radicals and cardiovascular disease, novel antioxidants might be effective.

Cardio vascular system is susceptible to many chronic diseases such as hypertension and myocardial infarction which is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Due to the generation of toxic reactive species such as superoxide radicals, myocardial cells gets damage. A common mechanism of molecular and cellular damage in cardio vascular disease is directly and indirectly related to oxidative stress. When compared to vitamin C and E, flavonoids have stronger antioxidant capacity. Orally administered Naringin gets hydrolyzed to a major metabolite naringenin by the intestinal microflora[13].

Free Radical

An important phenomenon in the cell metabolism is free radical production which is an enzyme catalyzed as well as electron transfer process. Lipid peroxidation in biological membrane occurs due to reaction of ROS with proteins, lipids and nucleic acid which in turn inhibit transcription and repair of DNA through the enzymatic process such as pump activity which ultimately results in cell death [13].

In Antidiabetic

The most frequent pathological features of diabetic complications appearing in around 50% diabetics are diabetic retinopathy, neuropathy, and nephropathy ad cardiomyopathy. Due to an array of factors including elevator hexosamine shunt, aldose reductase activation decrease in the nerve inositol content, an impaired neurotrophic support, activation of protein kinase C, impaired insulin peptide action, formation of advanced glycation end products (AEG) which modulate various intertwining biochemical pathways to orchestrate antioxidative glycosylation and polyol pathway leading to structure and functional aberration of peripheral neurons, spinal glial cells and nerve fibers, diabetic neuropathy is precipitated. In clinical trials treatment regimen with an antioxidant, anti-depressant, poly phenols, selective serotonin reuptake inhibitors, anti-arrhythmias, opioids and anticonvulsants for diabetic neuropathy has met with limited success. For the treatment of diabetic neuropathy, the chief lines of therapy are alpha-lipoic acid, acetyl – carnitine, benotiamine and methylcobalamine etc. In amelioration of various diseases, isolated bioactive moieties from the class of flavonoids are being recognized as promising free radical scavengers playing pivotal role. To scavenge free radicals easily hydroxyl groups should be attached to the aromatic ring structure of flavonoids which enable them to undergo a redox reaction. Wide spectrum of activities including anticancer anti-inflammatory and cardio protective activity was been evaluated for Naringin[14].

Periodontitis, a chronic inflammatory disease, which is characterized by the breakdown of periodontal ligament and resorption of alveolar bone affects a significant percentage of the human population. Conventional mechanical debridement followed by exogenous regenerative procedure such as guided tissue regeneration bone replacement grafts and growth factors and tissue engineering technologies are the methods that are currently used to reconstruct lost periodontal structures. To cure periodontitis or achieve predictable and optimal periodontal tissue regeneration, dentist have to access an increasing number of biomaterials and techniques for use in periodontal regenerative therapy but there is no ideal therapeutic approach is still available. Osteoclast and osteoblast are the two major responsible cell type which is present in alveolar bone which is a complex tissue continuously undergoing process of bone remodeling. Bone breakdown and overrides bone building, when resorption and formation of bone are not coordinated. Epidemiological and animal studies proved naringin a polymethoxylated flavonoid to have a possible protective effect against cardio vascular diseases and some type of cancer. It also found to inhibit osteoporosis which is induced by retinoic acid, in osteoblast cell line. Naringin could also induce the expression of estrogenic marker[15].

Metal Chelating Effect

In human and animals nickel salts are considered to be an occupational hazard and were reported to produce undesirable effects and as carcinogenicity. By affecting the T-cell system and suppress the activity of natural killer cells in rats and mice, nickel breakdown the immunity. Liver, kidney, brain, lung, testis are the major target organs of nickel toxicity. Depletion of glutathione and bonding to sulfydryl groups of proteins is the primary route for nickel toxicity[16].

Human body cannot metabolize nickel (Ni) which is a heavy metal with a density of atleast five times that of water, nickel salts are considered as an industrial health hazard.

Occupation, environmental exposure such as mining, extraction, refining, electroplating and food processing are the various occupations in which humans are exposed to nickel. In the toxio-kinetics of nickel, kidney plays a principle role because it serves as a major organ of nickel toxicity some of the nephrotoxic effects of nickel compounds are toxic nephropathy with proteinuria, amino aciduria and reduced renal clearance via Haber Weiss and Fenton reactions occurs due to accumulation of nickel which in turn accumulates iron which in turn generates ROS. In tissue increased level of ROS may cause damage to genetic materials.

Pharmacological and therapeutic properties o Naringin includes antioxidant, metal chelation, free radical scavenging and cholesterol lowering effect, inhibition of breast cancer cell proliferation and delay of mammary tumorigenesis. By up regulating the gene expression of superoxide dismutase, catalase and glutathione peroxidase, Naringin has been demonstrated to play an important role as an antioxidant [17].

Pathogenesis in Septic Shock

For several decades, the treatment for purpose of rescue from septic shock has been the subject of basic and clinical research. For pathogenesis in septic shock, endotoxin which is polysaccharides consisting of outer membrane of gram negative bacteria plays a critical role. Via endogenous mediators formed on interactions of lipopolysacchairde with cellular targets especially with macrophages, the pathogenic activities of lipopolysaccharides are induced. Tumor necrosis factor (TNF-α) produced by lipopolysaccharides elicited macrophage is the main pathogenic mediators in lethal shock. Reduction of TNF-α levels leads towards protection and rescue from lipopolysaccharides lethality. Number of lipids a derivatives have been synthesized to clarify the structure activity relationship and to discover antagonist because the active centre of lipopolysaccharides is the lipid a moiety. To suppress TNF- α release and lethal shock an inhibitory substance from cinnamon bark could bind directly to the lipid a moiety. Naringin, a flavonoid from the citrus fruit is able to inhibit the lipopolysaccharides induced TNF-α release and to block the lethal activity in D-galactosamine sensitized mice [18].

Antioxidant Activity

By donating or accepting electrons in various electron transfer reactions, iron is an indispensible trace element of cells which participates in various cellular process. A vital constituent o numerous enzyme including iron, sulphur and haem proteins of the respiratory chain, ribonucleotide reductase which catalyses the rate limiting step in DNA synthesis is iron, which has unique ability to alter its oxidation and redox status in response to ligandings. Due to generations of reactive oxygen species (ROS) during redox cycling this unique property renders iron potentially toxic in biological systems.

In the ground state, the triplet dioxygen can't directly react with biomolecules but the presence of iron or other transition metals can relieve the spin restriction of oxygen and enhance the oxidation rate of biomolecules dramatically. During normal conditions, the cell maintain the concentrations of free iron to a required minimum to avoid oxidative stress. In some circumstances the iron balance may be distributed either locally or systematically resulting in its participation in Fenton's chemistry and subsequently generating ROS. In several human disease, including anemias, heart failure, liver cirrhosis, fibrosis, gallbladder disorders, diabetes, arthritis, depressions, impotence, infertility and cancer, iron induced oxidative stress has been implicated.

In a variety of disease including cancer, atherosclerosis, acute hypertension, inflammatory disease, transplantation injury and aging, free radicals have been implicated. In eukaryotic cells, small amount of potentially toxic reactive oxygen species may be generated by normal oxidase action and also during the course of electron transport in mitochondria of microsome. During electron transport to molecular oxygen as well as various

hydroxylation reactions, the toxic reduction products of oxygen such as superoxide anion ($O2^{\cdot-}$) and hydrogen peroxide which are highly reactive and cause irreversible damage to various biomolecules in the presence of excess of iron are formed. Hydrogen radical an exceedingly strong and in discrimmates oxidant is formed through iron mediated reduction of H_2O_2 by superoxide anion.

Depending upon the target molecule hydrogen radical can abstract allylic hydrogen and hydroxylase or accept electrons. Lipid peroxidation is caused by hydrogen radical generated by Fenton's chemistry and lipid peroxides many undergo iron- mediated one electron reduction and oxygenation producing epoxyallylic peroxyl radicals which trigger lipid peroxidation, DNA damage and protein oxidation.

Against lipid peroxidation and other deleterious effect, eukaryotic cells are equipped with a repertoire of primary and secondary defenses. In excision/ repair of any lesions, primary defenses are mainly preventive whereas secondary defenses have "Back up" protective role. Before the initiation of lipid peroxidation, cyto protection relies on the scavenging/inactivation of reactive oxygen species or redox metal ions, superoxide dismutase (SOD) and glutathione peroxidase are the cytoprotective enzymes scavenge H_2O_2 efficiently at relatively lower concentrations whereas catalase can able to scavenge H_2O_2 more efficiently at relatively higher concentrations.

A common component that is present in the human diet which is obtained from the plant kingdom is flavonoids, which have been shown to have structurally dependent highly specific effect on a variety of enzyme and also able to interfere with numerous cellular process including growth and differentiation.

Naringin a glycoside, predominant flavonones found in the grape fruit, has metal chelation, antioxidant free radical scavenging properties. It is also reported that Naringin offers protection against mutagenesis and lipid peroxidation and also inhibit radiations induced depletion in GSH, GSHPX, SOD and Catalase [19].

Cholesterol Lowering Effect

In most hypercholesterolemia animals, probucol (4, 4(Isopropylidenedithio) bis [2, 6, tert- butyl phenol) which is a lipid soluble antioxidant shown to inhibit atherogenesis by limiting the oxidative modification of LDL and exerting other antioxidant properties. A large group of polyphenolic antioxidant that are present in a variety of food from vegetable source such as onions, apples, citrus, grapes, tea and wine are flavonoids capable of reducing the incidence of coronary heart disease. Naringenin aglycone of Naringin is reported as an antioxidant, antimicrobial, anti- cancer and cholesterol lowering agent [20].

Hypoxia

Hypoxia- ischemic brain injury, caroid artery pathologies, asphyxiation and shock etc are frequent incidence of cerebral ischemia that are seen in age- related disorders that are associated with great morbidity. During the cardiac or thoracic surgery, in certain clinical situations such as transient global cerebral ischemia anticipates or even induced latrogenically. To study the cerebral ischemia permanent ligation of bilateral common carotid arteries is a well-known model used. In diverse area of the brain, bilateral common carotid artery occlusion causes moderate and most likely permanent reduction of cortical and cerebral blood flow. In the pathogenesis of acute ischemia stroke, recent studies highlighted a pivotal role oxidative stress and mitochondrial dysfunction. In numerous neurodegenerative conditions including stroke, oxidative stress and associated mitochondrial dysfunction, excitotoxicity or neuroinflammation are implicated. Cerebral hypo perfusion induces the oxidative stress and during ischemic reperfusion insults reactive oxygen species predispose in the brain. Reactive oxygen species are produced after reperfusion. In normal physiological conditions, a homeostatic imbalance exists between the formation of oxygen free radicals and their removal by endogenous scavengers. To cause

neurotoxic effect and initiate a free radical mediated chain reaction causing additional damage to diverse areas in the brain. Reactive oxygen species namely superoxide, hydroxyl free radicals together with hydrogen peroxide have been proposed. During ischemia, oxidative reperfusion injury could be one of the possible cellular cascade affecting all organs and tissues. In experimental models of cerebral ischemia, antioxidants have been reported to be neuroprotective. As a dietary supplement, Naringin had recently received considerable attention [21].

Anti-inflammatory Conditions

In rat tracheal cultured cells, Naringin not only dose dependently decreased lipopolysaccharides- induced mucin secretion but also blockade activity of nuclear factor kappa B (NF-kB) p 65 by inhibiting phosphorylation of inhibitors KB-x which then significantly decreased lipopolysaccharides-induced tumor necrosis factor- α (TNF-x) secretion. By inhibiting the activity of NF-kB via EGFR-P13K-AKt /ERK MAP Kinase signaling pathway. Naringenin, aglycon of Naringin attenuates human neutrophil elastage (HNE) induced MUC 5 AC secretion. An activator protein-1 (AP-1) binding site and a NF-kB binding sites are the two different regions in which MUC5AC promoter was located.

In the onset of inflammation and tumor progression, a transcription factor which plays a central role in NF-kB which is kept as an inactive form in the cytoplasm. Through interactions with IKB proteins it causes NF-kB to localize in the cytoplasm and prevents its association with DNA mainly through the phosphorylation of IKB by inhibitors. By inhibiting kB Kinases and degradation by proteasome, activation of NF-kB occurs which in turn leads to NF-k/b p 65 heterodimer translocation to the nucleus and bind DNA.

AP-1 is another transcriptional activator of MUC 5 AC which is composed to homo and hetero dimmers of C- Jun and C- FOS proteins. AP-1 was activated by principle MAPK family members such as extra cellular signal regulated kinase (ERK). P38 mitogen activated proteins kinase (JNK) expression of C-Fos and C- Jun which were the components of the AP-1 complex is regulated via enhancing the downstream transcription factors including ELK-1, C- Jun ATF2 and C- Jun.

C- Terminal tail that contain specific tyrosine containing sequences is present in epidermal growth factors receptor, which has phosphorylated by tyrosine kinase after ligand such as EGF binding to EGP receptor dimerization. Through cooperatively between N-kB and Ap-1, three MAPK such as ERK-/2 P 38 MAPK and JNK as well as P13 K- AK-1 which are the downstream signaling cascades of EGF receptor activates the expression of MUC 5AC [22].

Apoptosis

In various models oxidative stress causes apoptosis which is attributed not only to disease but also the pharmacological actions of several drugs. Anticancer drugs cause oxidative stress induced apoptosis. Naringenin has been reported to exhibit antioxidant, inhibit lipid peroxidation in biological membrane, antiviral and antiallergic reduce the level of cytochrome P450±A2 protein, suppressed the cytotoxicity and apoptosis in mouse leukemia.P388 cells exposed to typical pro-oxidant, H_2O_2. It has extensive pharmacological activity and may affect the actions of anti-cancer drugs causing oxidative stress [23].

In the pathogenesis of disease including cancer apoptosis is an active form of cell suicide controlled by a network of genes which is an essential process as well as key role. By inducing apoptosis, numerous reports in the past decade have proven that many cancer chemotherapeutic agents kills the cancer cells. To approach in cancer therapies, clarification of the induction mechanism of cell apoptosis is useful [24].

Against Radiations

Since the dawn of time, humans have been exposed to natural background radiations.

During medical diagnostic procedures, space or air travel, cosmic radiations and use of certain electronic gadgets, humans are exposed to low level of radiations frequently. Moreover scientific and technological advancements have further increased radiations burden in humans. Radon in houses, contamination from weapon testing sites, nuclear accidents, radio therapy of cancer are the other sources of radiations exposure ,which produces cancer, death and loss of neural functions in humans and animals and induce killing, mutation and chromosomal aberrations in cells.

Energetic particles and electromagnetic radiations that are present in ionizing radiations can penetrate living tissues or cells and transfer the radiations to the biological materials which in turn break chemical bonds and ionizes different atoms and molecules including water and biologically important macro molecules such as nucleic acids, membrane lipids and proteins and leads to DNA and membrane damage via producing toxic free radicals. In aqueous media that is prevailing in the living system, most of the radiations induced damage to biomolecules is caused by formation of free radicals resulting from the radiolysis of water. In the pathogenesis of a number of disease including cancer, diabetes mellitus, rheumatoid arthritis, infectious disease, atherosclerosis and aging reactive oxygen species and lipid peroxide have been implicated with the repertoire of antioxidant enzymes and molecules. Cells are equipped to defend themselves against ROS.

To treat various disorders in man, the herbs have been commonly used. For their sustenance humans are dependent on herbs not only for the decimal use but also consume herbs and fruits. To reduce the deleterious effect of radiations, use of products from natural source could be a better choice. A predominant flavonones that is present in *Citrus paradise*, *Citrus sinensis*, *Citrus nushiu*, *Citrus mobilis*, *Citrus tachibana*, *Citrus junos*, *Artemisia selengensis* is naringin which has diverse and various pharmacological actions such as metal chelating, antioxidant and free radical scavenging properties, protection against mutagenesis and lipid peroxidation effect, increases the survival time of irradiated mice, reduce the radiations-induced micronuclei formation and chromosomal aberration in mice[25].

Application of High Pressure for the Bioconversion of Naringinase

When working on complex molecules, advantages based on chemi- region and stereo selectivity of enzymes and conditions, biocatalysts offers a number of key advantages over chemical synthesis. It is interesting to use to high pressure for the enzymatic synthesis of pharmacological molecules namely flavonoids which involves in modulation, uv protection, host defense in plants, health promoting effect in humans (against tumors), prevention of cardio vascular disease, inhibitor of enzymatic lipid peroxidation, anti-inflammatory and antithrombotic effect. Due to the activity as inhibitors of enzymes involved in the biotransformation of procarcinogens, flavonoids exhibit potential chemo preventive effects. naringenin which has important effects such as antioxidant, anti-ulcer, antimutagenic, anti-inflammatory, anti-thrombotic, vasodilator , anti-cancer effect, inhibition the proliferation of breast cancer and delaying mammary tumorigenesis is obtained by hydrolyzing Naringin by naringinase and α-rhamnopyranosidase.

To modulate both the stability and activity of several enzymes high pressure can be used. by changing the rate limiting step of modulating the selectivity of enzymes, catalytic behavior of enzymes can also be modified. Depending upon proteins involved and conditions, the pressure could either activate or inhibit enzymatic activities. An efficient statistical techniques for the modeling and optimization of multiple variables in order to predict the best performance conditions with minimum number of experiment is response surface methodology which is none conventional approach that has been successfully used for the optimization of enzymatic reactions, conditions and food preservation parameters. To compare the conjugative effect of temperature and pressure

on Naringin bioconversion by naringinase, central composite rotatable design (CCRD) and RSM were used [26].

Naringenin is a metabolite of Naringin, when administered orally shown to have good permeability across the blood brain barrier. *In vitro* and *in vivo* COX and NOS gene expression. In Naringin inhibits lipopolysaccharides (IP3) induced pro inflammatory cytokine response and COX and NOS gene expression. In the pathogenesis of neuro generative disorders, inflammatory response and their mediators plays a key role. In Kainic acid induced epileptic's models and 3-nitro prop ionic acid induced Huntington models, Naringin has reported to attenuate behavioral alternations and cognitive impairment. In colchicines and D- galactose induced learning and memory impairment models, Naringin administration improved cognitive deficits [27].

In Cancer

In worldwide cancer is the most distressing and life threatening disease that enforces several death. In advanced stages of cancer, current treatment such as surgery, radiotherapy and chemotherapy are mostly ineffective which are also often associated with severe side effects. There is an urgent need to develop therapeutic modalities with no or minimize side effects to normal organs, to avoid these side effects in cancer therapy. By protective DNA damage, inhibiting tumor development (Promotion), inhibiting tumor invasion (proliferation) flavonoids are potentially involved in the reduction of carcinogenesis. Through selective cytotoxicity, anti-proliferative action and by inducing apoptosis, anti-cancer effect of flavonoids has been established in eukaryotic cell models. Naringin is a flavonones present in citrus fruits and grape fruits possess three hydroxyl groups in its aromatic rings which are responsible for its potent antioxidant and wide spread pharmacological property such as anti-inflammatory, antimutagenic antiatherogenic, hepatoprotective and anticancer with low or intrinsic toxicity. In various human cancer cell lines naringenin has been reported to induce cytotoxicity and apoptosis. Due to low aqueous solubility which results in poor availability, poor penetrability, instability and extensive first pass metabolism before reaching systemic circulations and instability in physiological medium, despite great therapeutic potential of naringenin in a variety of experimental models, its clinical development is hindered.

By a process called "Enhanced permeation retention EPR mechanism, nano particles can escape from the vascular through leaky endothelial tissue that surrounds the tumor and accumulates in solid tumors. Due to small size, cellular and tissue targeting, improvement of oral bioavailability, sustaining the effect in the target tissues, solubilisation for intra vascular delivery and protection from enzymatic degradation especially in the stomach nano particles offers numerous advantages[28].

In cancer patients, metastasis is the main cause of death worldwide. Breast cancer is the second leading cause of cancer death among women. Depending upon the characteristic of tumor and the conditions of the patience, for most patients, the best treatment is surgery, in which the main problem is tumor metastasis local relapse. In most of the patients the metastases are not clinically detectable. To reduce the risk of metastasis and relapse chemotherapy might help but it has severe side effects on normal cells. To severe side effects of normal cells. To inhibit the outgrowth of metastasis, there is an urgent need to develop new adjuvant therapies that have low toxicity and high efficiency.

In struggling against cancers, the immune system has been shown to be extremely important. In immunogenic tumor models, immune cells can kill tumor cells and thus inhibit tumor can kill tumor cells and thus inhibit tumor growth and metastasis or even cause a primary tumor rejection where in patients and poorly immunogenic tumor models, most tumors are not rejected. Lack of effective recognition of tumors by T- cells are due to immunosuppressant induced by tumor derived molecules. In suppressing antitumor immune

response regulatory T- cells plays a critical role, there by inhibit T- cell activation and proliferation by producing immunosuppressive cytokines transforming growth factors- β (TGF-β) and IL-10. Due to tissue damage, anesthetic and analgesic drugs, hypothermia, blood loss, transfusion, pain, preoperative distress, surgery itself causes a generalized state of immune suppression. To improve the outcome of patients undergoing tumor surgery, they should be made relieved from immune suppression and their systemic antitumor immunity should be restored. Naringenin found to inhibit the production of regulatory T cells in vitro in 4T1 cell line [29].

DNA is primary intra cellular target of anticancer drugs due to interactions between small molecules and DNA which causes damage in cancer cells there by block the division of cancer cells which in turn result in cell death. It is necessary to understand the different binding modes, in order to develop a new anti-tumor drugs which specifically target DNA. Either a non-covalent or a covalent way metal complexes interacts with the double helix DNA. By intercalation, groove binding and external static electronic effect are the three binding modes by which metal complex interact non-covalently. The most important binding mode is intercalation which invariably leads to cellular degradation.

In vascular plants, flavonoids are broadly distributed class of naturally occurring pigments which are responsible for most of the colors in nature. By either stimulating or inhibiting a wide variety of enzymes, flavonoids could act as an antibacterial, anti-cancer, antioxidant anti-inflammatory and anti-allergic agents [30].

Worldwide lung cancer represents the leading cause of cancer and cancer related mortality. At the time of diagnosis only 25 % of patients are candidates for surgical treatment and in 75 % of patients, chemotherapy is the corner stone of treatment of small cell lung cancer. Treatment of lung cancer remains largely unsatisfactory with very low 5 years survival rates.

DNA damage and subsequent cell death is the target for the most of the anti-tumor agents. For the detection of carcinogens and for the evaluation of chemotherapy, DNA instability and deficiency in DNA repair mechanism are considered as a sensitive cytogenetic index. For the assessment of the cytostatic action of various therapeutic agents, delay in cell division which is assessed by the proliferation rate index (PRI) which is a valuable indicator of cytotoxicity has been used. Given the disappointing results and the toxicity of currently used antitumor agents, several other drugs have been investigated regarding their possible contribution in lung cancer treatment[31].

New approaches as treatment modalities for lung cancer have been tried *in vitro* and *in vivo*, in spite of recent progress in cancer chemotherapy. Treatment with monoclonal antibodies which recognizes an antigen which specifically expressed on the surface of lung cancer cells is one of the new approaches. In a clinical study, human Anti GM2 mab (IgM type) was used in melanoma patients which reacts with various GM2 positive human cancer cells including lung cancer through the anti-body dependent cell mediated cytotoxicity (ADCC) reaction in the presence of human mononuclear cells. ADCC of effectors cells must be examined in cancer patients for effective application of mab for the treatment of lung cancer [32].

Because of its early development of regional and distant metastases, small cell lung cancer is one of the most aggressive type of cancer. Various combinations of etoposide, cisplatinum, cyclophosphamide, doxorubicin and vincristin are the currently recommended and widely used regimens for patients with extensive small cell lung cancer [33]. Carcinoma that arises from the epithelium lining glands, ducts and surfaces of organs are the majority of human malignancies. Over the lost two decades the pathogenesis of cancer development and progression has advanced tremendously. Cancer is a disease due to genetic alterations and this fact has accepted through the process of identification and characterization of oncogens and tumor

suppressor genes. Carcinogenesis is influenced and controlled by cellular interactions derived from a complex relationship between stromal, epithelial and extra cellular matrix components. From the stroma of the corresponding normal tissue, the stromal micro environments of many human tumors are fundamentally different. Reactive stroma are characterized by modified extra cellular matrix composition.

Glial tumors/gliomass are nothing by neoplasm of neuroglian cells which arises from estrocytes or oligodendroglial cells or ependymal cells of the brain. In various human cancer cell lines such as breast cancer cell line MCF-7, stomach (KS70111 and MKN-&) and liver (HEP-3B and HUH 7) cancer cell lines Naringenin induced cytotoxicity. In human pancreatic cancer (PK-1), HL-60 (Promyelocytic leukemia) and colon cancer (Caco-2) naringenin showed inhibitory effect on cell growth. Against non- malignant vero cells, naringenin was found to non- toxic. Naringenin has to capacity to arrest cell cycle, inhibition of COX-2 activity because it plays an important role in regulating or promoting cell proliferation in some normal and neoplastically transformed cells. It also increases gap junction intercellular communication (GJIC) because cell to cell communication via gap junction plays an important role in the maintenance of normal cell growth. To identify anti-tumor- promoting chemo preventive drugs and anti-carcinogenic treatment, hypothesis for a rational approach is to use the prevention of the down regulation of GJIC by the tumor promoters and restoration of GJIC in neoplastic cells. In both 20 and 30 µg doses, naringenin supplemented C6 glioma cells has shown an increased level of dye coupling between the cells highlights the loss o tumerogenic potential of C6 cells[34]. For evaluating the proliferation of several tumors including glioma, PCNA functions as a co-factor of DNA polymerase, naringenin decreased the expression of PCNA which confirmed the effect of naringenin effect on replication. During the development and in various pathological process including brain tumors in rats. VEGE was originally identified as a highly potent endothelial cell mitogen that is closely correlated to vascularizeion and angiogenesis. Naringenin target AGNORs, a set of nuclear proteins that are necessary for ribosomal biogenesis which is a market of cell proliferation [35].

In worldwide, the second most common cancer in females is cervical carcinoma which is common among the women aged between 15 and 44 years. For the majority of the patients with early-stage cervical cancer, radical surgery or radiotherapy can be curative. For those patients with advanced cervical cancer where the prognosis remains very poor, chemotherapy or neo adjuvant chemotherapy is always the first choice.

Cancer incidence could be reduced by increasing the intake of fruits and vegetables because phytochemicals within them have been proposed as responsible for their protective effect. In the past few decades chemoprevention through dietary phytochemicals has become increasingly active area of research. In numerous epidemiological studies flavonoids have demonstrated to possess anticancer and chemo preventive property. Flavonoids have a broad range of effects and interactions at the cellular and molecular levels that contribute to their chemo preventive and anticancerogenic activity via modulating the cellular process such as cell cycle, apoptosis and anti-proliferation effect. In the molecular level flavonoid interact with receptors, enzymes and kinase.

Naringin was shown to kill human cervical cancer cells. Through both death receptor and mitochondrial pathway, Naringin apart from inhibiting cell proliferation, was also found to induce apoptosis in human cancer cells[24].

In Obesity

Excess of inflammatory adipokine including tumor necrosis factor (TNF-α) which are involved in the development of insulin resistance and type 2 diabetes are produced by obese adipose tissue. In obesity related insulin resistance TNF-α plays a role and expression is increased in the obese adipose tissue of both

humans and rodents. Through its direct ability to attenuate insulin receptor signaling, TNF-α and FFA are positively correlated. By impairing peripheral glucose utilization, chronically elevated plasma levels of FFa promote insulin resistance. In the improvement of insulin resistance, the reduction of excess of FFa level is important.

Through adipocytes lipolysis, TNF-α promotes FFA secretion, by down regulating the expression of antilipolytic genes such as perilipin and phosphodiesterase- 3 B (PDE313) on the surface of intra cellular triglycerides lipid droplets perilipin is located which in turn regulates the access of hormone- sensitive lipase which hydrolysis tri glycerides to FFA and glycerol. By insulin signaling the major hydrolytic enzyme of CAMP, PDE 3 phosphodiesterase is activated. Reduced activity or protein kinase a (pkA) occurs due to decreased intra cellular levels of C AMP, which phosphorylated and activates HSL. For TNF-α induced lipolysis, nuclear factor (kB(NF-kB and extra cellular signal- regulated kinase (ERK) are important because they regulate the expression of lipolysis- related genes including perilipin and phosphodiesterase. The mechanism by which TNF-α induces lipolysis is by regulating the antilipolytic gene expression through the activation of intra cellular signaling pathway.

In citrus fruit flavonoids such as hesperidins and naringenin are abundant and they exert antioxidant, anti-inflammatory and anti-proliferative effect. Intake of hesperidins and naringenin reduces the risk of chronic disease such as cerbro vascular disease and asthma. In mouse adipocytes, by inhibiting the NF-kB ad ERk pathways, hesperidins and naringenin block TNF-α from down regulating the transcription of two antilipolytic gene perilipin and phosphodiesterase 3 B ccurs during the inhibition of ERK pathway. FFA secretion is induced during the inhibition of NF-kB pathway which suppress the transcription of IL-6. Hesperidins and naringenin directly inhibits TNF-α stimulated FFA secretion and this finding may be useful for developing treatment to FFA- induced insulin resistance.

Hesperidins and naringenin inhibit both NF-kB and ERK pathways which in turn suppress TNF-α stimulated FFA secretion. For the recovery of perilipin and phosphodiesterase 313 m RNA, the inhibition of ERK pathway is required. Adipocytes function was regulated by Hesperian and naringenin. Adipocytes secrete bioactive factors involved in inflammation and metabolic disorders. Through the regulation of ERK-dependent antilipolytic gene expression and NF-kB dependent IL-6 synthesis, herperetin and naringenin inhibits TNF-α induced adipocytes lipolysis. Hesperidins and naringenin modulates the activity of a number of protein kinase that regulates various intra cellular signaling cascades like phosphoinotide 3 kinase (P13K), AKt/Protein kinase (PKC) and mitogen activated protein kinase (MAPK). Via binding of the flavonoid to the ATP binding sites on enzyme modulatory action is mediated.

In adipocytes hesperidins and naringenin regulates IL-6 synthesis. In obesity expression of IL-6 and TNF-α is elevated. An important strategy for the improvement of insulin resistance is the control of IL-6 expression because IL-6 the control of IL-6 expression because IL-6 impair insulin signaling and promotes insulin resistance. Hesperidins and naringenin inhibits TNF-α induced IL-6 synthesis and they found to exert inhibitory effect on IL-6 mediated metabolic disorders. Hesperidins and naringenin may be used for ameliorating insulin resistance because they inhibit TNF-α induced adipocytes lipolysis [36].

Antiestrogenic Activity

Among vegetarians there is a decreased risk for different types of cancer and this observation has spurred research on various structural classes of phytochemicals that may contribute anticarcinogenic effect. Major constituents of plant and vegetables are bioflavonoid, which elicits responses that may provide some protective effect. Consumption of bioflavonoid is high in Japan and China because of that there is a decreased incidence of breast cancer in women. Both in vitro and in vivo model bioflavonoid inhibit mammary cancer cell or

tumor growth. Different structural classes of bioflavonoid binds to the ER and induce diverse spectrum of estrogen- induced responses. Flavones apigenin, diidzen, kaempferol, quercetin are the bioflavonoid induce proliferation of estrogen- responsive MCF-7 human breast cancer cells and induce estrogen-inducible genes. In post-menopausal women, the dietary flavonoids have the potential to contribute to the growth of estrogen-dependent tumors whereas 17-β estradiol is limiting. Most bioflavonoid are weak ER agonist and sub-effective dose of these phytoestrogens may exhibit partial antiestrogenic activity. In mature 21- day-old female rats, bioflavonoid quercetin and luteolin inhibit E2- induced proliferation of MCF-7 human breast cancer cells and uterine wet weight. In rat uterus and in MCF-7 cells in culture, naringenin inhibit several estrogen induced responses. *In vitro* system naringenin is a weak ER agonist. At 15, 20, 30 and 40 mg/ rate doses, naringenin alone do not change rat uterine wet weight significantly. Along with E2 naringenin at 20, 30 and 40 mg/rat significantly inhibited E2- induced uterine wet weight[37].

CONCLUSION

Due to their capacity to scavenge free radicals flavonoids which are the major dietary groups of plant, polyphenol possess anti-cancer activity and they are also acting as anti-oxidants. Application of antioxidants usually results in the reduction of tumor cell proliferations because tumor cells display high levels of intra cellular oxidative stress than that of normal cells. By decreasing the LPO, naringenin could modify the cellular redox state and increase the activities of enzymatic and levels of non-enzymatic antioxidants which might be responsible for the reduction of in the expression of PKC and also NF-kB Naringin and naringenin has an important pharmacological activities such as antibacterial, anti-inflammatory, anti-estrogenic, adipolysis activity, antidiabetic , anti- Alzheimer's disease, apoptosis, antiviral, anti-allergenic, anti-obesity and anti-cancer activity against various cancers such as lung cancer, breast cancer, cervical cancer. Because of these above said pharmacological activities of Naringin and naringenin, Naringin had recently received considerable attention as a dietary supplement.

REFERENCES

1. Mukherjee Pulok K, "Quality control of herbal drugs", Business Horizons, New Delhi, India, 2002, 46-65.
2. Hui Meng Er, En- Hsiang Cheng, Ammu Kutti Radhakrishnan, "Anti-proliferative and mutagenic activities of aqueous and methanol extract of leaves from Pereskia bleo (Kunth) DC (Cactaceae)", Journal of Ethno Pharmacology, 2007, 113, 448-456.
3. Wongmekiat O, Leelarugrayub N, Thamprasert K, "Beneficial effect of shallot (Allium ascalonicum L) extract on cyclosporine nephrotoxicity in rats", Food and Chemical Toxicology, 2008, 46, 1844-1850.
4. Santos FV, Colus IMS, Silva MA, Vilegas W, Varanda. EA, "Assessment of DNA damage by extracts and fractions of Strychnos pseudoquina, A Brazilian medicinal plant with anti-ulcerogenic activity", Food and Chemical Toxicology, 2006, 44, 1585-1589.
5. Naghma Khan, Sarwat Sultana, "Chemo modulatory effect of Ficus recemosa extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats", Life Sciences, 2005, 7, 1194-1210.
6. Hikmet Keles A. Faith Fidan I. Hakki Cigerci, Ismail Kucukkurt, Erkan Karadas, Yilmaz Dundas, "Increased DNA damage and oxidative stress in chicken with natural Marek"s disease", Veterinary Immunology and Immuno Pathophysiology, 2010, 133, 51-58.
7. Rosa RM, Dinara Moura J, Ines SM, Rafael M, Marc SS, Richterm F, Bastos E, Pegas CJA, Heuriques, Ligia A, Ramos LDP, Saffi J, "Protective effects of Hibiscus tiliacens L methanolic extract to V79 cells against cytotoxicity and genotoxicity induced by

Hydrogen peroxide and tert- butyl hydro peroxide", Toxicology *in vitro*, 2007, 21, 1442-1452.

8. Caccamese S, Chillemi R, "Racemization at C-Z of naringin in Pummela (citrus grandis) with increasing maturity determined by chiral high performance liquid chromatography", Journal of Chromatography, 2010, 1217, 1089-1093.
9. Gorustein S, Huang D, Leoutowicz H, Lecutowicz M, Yamamoto K, Soliva Fortuny R, Bellose OM, Ayala ALM and Trakhtenberg S, "Determination of Naringin and hesperidin in citrus fruit by high performance liquid chromatography, the antioxidant potential of citrus fruit", Acta Chromatographica, 2006, 17, 108-124.
10. Ting WU, Oman Y, Jiannong YE, "Determination of flavonoids and ascorbic acid in grape fruit peel and juice by capillary electrophoresis with electro chemical detection", Food Chemistry, 2007, 100, 1573-1579.
11. Arnao MB, Casas JL, Del Rio JA, Costa MA, Garcia- Canovas F, "An enzymatic colorimetric method for measuring Naringin using 2,2' Azino- bix (3- ethyl benzthiazoline- 6 Sulphonic acid (ABTS) in the presence of peroxidase" Analytical Biochemistry, 1990, 185, 336-338.
12. Rajadurai M, Stanely P, Prince M, "Preventive effect of naringin on lipid peroxide and antioxidant in isoproterenol-induced cardio-toxicity in Wistar rats", Biochemical and histopathological evidences- Toxicology, 2006, 228, 259-268.
13. Rajadurai M, Stanely P, Prince M, "Preventive effect of naringin on lipid peroxide and antioxidant in isoproterenol-induced cardio-toxicity in Wistar rats: an in vivo and in vitro study", Toxicology, 2007, 232, 216-225.
14. Kandhare AD, Raygude KS, Ghosh P, Kumar EA, Subhash LG, Bodhanker, "Neuroprotective effect of naringin by modulation of endogenous biomarkers in Streptozotocin induced painful diabetic neuropathy", Fitoterapia, 2012, 83, 650-659.
15. Li-Li Chen, Li-Hong Lei, Pei- hui ding, Qi Tang, Yan-min Wu," Osteogenic effect of Drynariae rhizome extract and naringin on MG3T3- E1 cells and an induced rat alveolar bane resorption model", Archieves of Oral Biology, 2011, 56, 1655-1662.
16. Pari L, Amudha K, "Hepatoprotective role of Naringin on nickel induced toxicity in male Wistar rats", European Journal of Pharmacology, 2011, 650, 364-370.
17. Amudha K, Pari L, "Beneficial role off Naringin, a flavonoid in nickel induced nephrotoxicity in rats", Chemico- Biological Interactions, 2011, 193, 57-64.
18. Kawaguchi K, Kikuchi S, Hasegawa H, Maruyama H, Morita H, Kumazawa Y, "Suppression of lipoploysaccharide- induced tumor necrosis actor- release and liver injury in mice by Naringin", European Journal of Pharmacology, 1999, 368, 245-250.
19. Jagetia GC, Reddy TK, "Alleviation of Iron induced oxidative stress by the grape fruit flavonones naringin in vitro", Chemico-Biological Interactions, 2011, 190, 121-128.
20. Seon-Min jeon, Song- Hae Bok, Moon-Kyoo Jang, Yean-Hee Kin, Kyung- Tak Nam, Tae-Sook Jeong, Yong Bok Park, Myung-Sook Choi, "Comparison of antioxidant effects of Naringin and probucol in cholesterol–fed rabbits", Clinica Chimica Acta., 2002, 317, 181-190.
21. Gaur V, Aggarwal A, Kumar A, "Protective effect of Naringin against ischemic reperfusion cerebral injury; possible neuro behavioral, biochemical and cellular alterations in rat brain" European Journal of Pharmacology, 2009, 616, 147-154.
22. Yi-Chu Nie, Hao Wu, Pei-Bo-Li,Li-ming Xie, Yu-Long Lao, Jian-gang Shen, Wei-Wei Su, "Naringin attenuates EGF- induced MUC 5 AC secretion in A549 cells by suppressing the cooperation activities of MAPKS-AP-1 and IKKS-1 KB-NF-kB

signaling pathways" European Journal of Pharmacology, 2012, 690, 207-213.

23. Kammo SI, Hirata SRA, Ason K, Ishikawa M," Effects of Naringin on cytosine arabinoside (Ara-c) - induced cytotoxicity and apoptosis in p388 cells", Life Sciences, 2004, 75, 353-365.
24. Ramesh E, Alshatwi AA, "Naringin induces death receptor and mitochondria- m human cervical cancer cells", Food and Chemical Toxicology, 2013, 51, 97-105.
25. Jagetia GC, Reddy TK, "Modulation of radiations- induced alternation in the antioxidant status of mice by Naringin", Life Sciences, 2005, 77, 780-794.
26. Marques J, Vila-Real HJ, Alfaia AJ, Riberiro H, "Modeling of high pressure-temperature effect on Naringin hydrolysis based on response surface methodology", Food Chemistry, 2007, 105, 504-510.
27. Wang D, Gao K, Li X, Shen X, Chunuei MXZ, Qin C, Zhang L, "Long term Naringin Coonsumptioon reverse in glucose inake defect and improves cognitive deficits in a mouse model of AlZheimer's disease", Pharmacology, Biochemistry and Behavior, 2012, 102, 13-20.
28. Kumar NK, Sulfikkarali N, Prasad NR, Karthikeyan S, "Enhanced anti-cancer activities of naringenin- loaded nano particles in human cervical (HeLa) cancer cells", Biomedicine and Preventive Nutrition, 2011, 1 , 223-231.
29. Lei Qin, Lingtai Jin, Linlin Lu, Xiaoyan Lu, Chunling Zhang, Fayun Zhang, Wei Liang, "Naringenin reduces lung metastasis in a breast cancer resection model", Protein cell, 2011, 2 (6), 507-516.
30. Wang BD, Yang ZY, Wang Q, Cai TK, and Crewson P, "Synthesis, characterization, cytotoxic activities and DNA- binding properties of the La (III) complex with naringenin Schieff- base", Bioorganic and Medicinal Chemistry, 2006, 14, 1880-1888.
31. Mylonaki E, Manika K, Zarogoulidis P, DomVri K, Voutsas V, Zarogoulidis K, Mourelatos D, "In vivo synergistic cytogenetic effects of aminophylline on lymphocytes culture from patients with lung cancer undergoing chemotherapy", Mutation Research, 2012, 740, 1-5.
32. Parajuli P, Yanagawa H, Hanibuchi M, Takenchi E, Miki T, Sone SYS, "Humanized anti-ganglioside GM2 antibody is effective to induce antibody- dependent cell mediated cytotoxicity in mononuclear cells from lung cancer patients", Cancer Letters, 2001, 165,179-184
33. Matsumoto S, Kimura S, Segewa H, Kuroda J, Yuasa T, Sato K, Nogawa M, Tanaka F, Mackawa T, Wada H, "Efficacy of the third generation bisphonate zoledronic acid alone and combined with anti-cancer cell lines", Lung Cancer, 2005, 47, 31-39.
34. Sabarinathan D, Vanisree AJ, "Naringenin, a flavones alters the tumerogenic features of C6 glioma cells", Biomedicine and Preventive Nutrition, 2011, 1, 19-24.
35. Sabarinathan D, Mahanlakshmi P, Vanisree AJ, "Naringenin, a flavonones inhbits the proliferation of cerebrally implanted C6 glioma cells in rats", Chemio-biological Interactions, 2011, 189, 26-36.
36. Yoshida H, Takamura N, Shuto T, Ogata K, Tojunaga J, Kawai K, Kai H, " The citrus flavonoid hesperetin and naringenin block the lipolysis actions of TNF-α in mouse adipocytes" Biochemcial and Biophysical Research Communications, 2010, 394, 728-732.
37. Fruh M, Zacharewski T, Connor K, Howell J, Chen C, Sage S, "Naringenin, a weakly estrogenic bioflavonoid that exhibit antiestrogenic activity" Biochemical Pharmacology, 1995, 50(9), 1485-1493.

