Review Article **CODEN: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com

https://doi.org/10.36673/IJRPNS.2021.v10.i05.A37

AN OVERVIEW OF THE PROPERTIES OF THEAFLAVIN FLAVONOID

Rishika Singh[1], G. Lakshmi Devi*[1], Nandigama Prathyusha[1], J. Akhila[1], J. V. S. Sharma[1]

[1*]Department of Pharmaceutics, Joginpally B.R Pharmacy College, Yenkapally, Moinabad, Rangareddy, Hyderabad-75, Telangana, India.

ABSTRACT

Theaflavin is a flavonoid of low toxicity and multiple beneficial bioactivities. Published reviews all focused on the findings using eukaryotic cells, animal models, or epidemiological studies covering the pharmacokinetics, cancer chemoprevention, and drug interactions of theaflavin; however, no review is available on the various cancer effects of theaflavin. Theaflavins (TFs) are the dimers of a couple of epimerized catechins, which are specially formed during black tea fermentation. This review summarized various propertis of the theaflavin and its applications. The activity of TFs on anti-oxidation, anti-mutagenicity, hypolipidemic, anti-inflammatory, anti-cancer, anti-viral effect as well as the epidemiological cure were sorted. Role of theaflavin in skin cancer is highlighted in this review.

KEYWORDS

Catechins, Eukaryotic cells, Skin cancer and Theaflavins.

Author for Correspondence:

Lakshmi Devi G,

Department of Pharmaceutics,

Joginpally B.R. Pharmacy College,

Yenkapally, Moinabad, Rangareddy,

Hyderabad, Telangana- 5000075, India.

Email: lakshmi13bph@gmail.com

INTRODUCTION

Flavonoids are a type of natural product that is extensively found in fruits, vegetables, and some beverages. They are a type of plant secondary metabolite with a polyphenolic structure. They have a variety of beneficial biochemical and antioxidant properties that have been linked to cancer, Alzheimer's disease (AD), atherosclerosis, and other diseases[1]. Flavonoids are an essential component in a number of nutraceutical, pharmacological, medical, and cosmetic uses because they are linked to a wide range of health-promoting properties. This is due to their ability to control important cellular enzyme functions as well as their antioxidative,

anti-inflammatory, anti-mutagenic, and anti-carcinogenic capabilities. They're also known to block a number of enzymes, including xanthine oxidase (XO), cyclooxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase[2].

Flavonoid chemicals are plant extracts that can be found in many areas of the plant. Vegetables employ flavonoids to help them thrive and fight plaque. They are low-molecular-weight phenolic chemicals found throughout the plant kingdom. They are one of the most distinguishing types of chemicals found in higher plants. In most angiosperm families, several flavonoids are easily recognised as floral pigmentsThey can be found in all parts of plants, not just flowers. Dietary flavonoids are flavonoids that are plentiful in plant-based meals and beverages such fruits, vegetables, tea, chocolate, and wine. Chalcones, flavones, flavonols, and isoflavones are just a few of the subclasses of flavonoids. The key sources for these subgroups are different. Flavonols and flavones can be found in abundance in onions and tea, for example.

Plants, animals, and microorganisms all use flavonoids for a variety of biological purposes. Flavonoids are responsible for the colour and perfume of flowers, as well as the attraction of pollinators and, as a result, fruit dispersion to aid in seed and spore germination, as well as the growth and development of seedlings in plants[3]. Flavonoids work as UV filters, signal molecules, allopathic substances, phytoalexins, detoxifying agents, and antimicrobial defensive compounds, and protect plants from many biotic and abiotic stresses

Flavonoids have been linked to improved human and animal health, and their use in disease therapy and chemoprevention is currently being investigated. Approximately 6000 flavonoids contribute to the vibrant colours seen in fruits, herbs, vegetables, and medicinal plants.

Plant flavonoids and isoflavonoids were studied in depth by Dixon and Pasinetti[4], who discussed their implications in agriculture and human neuroscience. Kumar and Pandey discussed flavonoids' preventive effects against human diseases as well as their functions in plants.

This review summarized an overview of theaflavin. The activity of TFs on anti-oxidation, anti-mutagenicity, hypolipidemic, anti-inflammatory, anti-cancer, anti-viral effect as well as the epidemiological cure were described in this review.

CLASSIFICATION

The carbon of the C ring on which the B ring is bonded, as well as the degree of unsaturation and oxidation of the C ring, can be used to split flavonoids into subgroups. Isoflavones are flavonoids with the B ring connected at position 3 of the C ring. Neoflavonoids are those in which the B ring is joined in position 4; those in which the B ring is linked in position 2 can be further split into numerous subgroups based on the structural properties of the C ring. Flavones, flavonols, flavanones, flavanonols[5], flavanols or catechins, anthocyanins, and chalcones are the different subclasses. Figure No.1 depicts the classification of flavonoids.

Anthocyanins

Plants, flowers, and fruits contain pigments called anthocyanins, which give them their colour. The most prevalent anthocyanins investigated include cyanidin, delphinidin, malvidin, pelargonidin, and peonidin (Figure No.1). Cranberries, black currants, red grapes, merlot grapes, raspberries, strawberries, blueberries, bilberries, and blackberries are examples of fruits where they can be found in the outer cell layers. These compounds' stability, combined with their health benefits, allow them to be used in a range of applications in the food sector[6]. The anthocyanin's colour is affected by pH, as well as methylation or acylation of the hydroxyl groups on the A and B rings.

Chalones

Chalcones are a kind of flavone. The lack of 'ring C' of the basic flavonoid skeleton structure depicted in Figure No.1 distinguishes them. As a result, they are also known as open-chain flavonoids. Phloridzin, arbutin, phloretin, and chalconaringenin are examples of chalcones. Tomatoes, pears,

strawberries, bearberries, and some wheat products contain substantial levels of chalcones. Because of their multiple nutritional and biological benefits, chalcones and their derivatives have attracted a lot of attention. Table No.1 lists the food sources for all dietary flavonoids mentioned in this study, as well as bioactivity and research trends[7].

Flavonols

Flavonoids with a ketone group are referred to as flavonols. Proanthocyanins require them as building blocks. Flavonols can be found in large quantities in a wide range of fruits and vegetables. Kaempferol, quercetin, myricetin, and fisetin are the most widely investigated flavonols (Figure No.2). Flavonols can be found in a variety of foods, including onions, kale, lettuce, tomatoes, apples, grapes, and berries. Tea and red wine are additional good sources of flavonols, in addition to fruits and vegetables. Flavonol consumption has been linked to a number of health advantages, including antioxidant activity and a lower risk of cardiovascular disease.

Flavones

Flavones are a subclass of flavonoids that are important. As glucosides, flavones can be found in abundance in leaves, flowers, and fruits. Flavones can be found in a variety of foods, including celery, parsley, red peppers, chamomile, mint, and ginkgo biloba. This subclass of flavonoids includes luteolin, apigenin, and tangeritin (Figure No.2). The polymethoxylated flavones tageretin, nobiletin, and sinensetin are abundant in the peels of citrus fruits. They have a double bond between positions 2 and 3 on the C ring, as well as a ketone in position 4.

Flavanones

Another major class of compounds found in citrus fruits including oranges, lemons, and grapes are flavanones. This category of flavonoids includes hesperitin, naringenin, and eriodictyol (Figure No.2). Because of their free radical-scavenging properties, flavonones have been linked to a number of health advantages[8]. Citrus juice and peel contain these chemicals, which give them a bitter taste. Citrus flavonoids have pharmacological actions that include antioxidant, anti-inflammatory, blood lipid-lowering, and cholesterol-lowering properties.

Isoflavonols

Isoflavonoids are a subclass of flavonoids that is huge and distinct. Isoflavonoids are mostly found in soyabeans and other leguminous plants, and they have a limited distribution in the plant kingdom. Microbes have been found to contain certain isoflavonoids as well. During plant-microbe interactions, they are also discovered to serve as crucial precursors for the synthesis of phytoalexins. Isoflavonoids have a lot of potential in terms of disease prevention. Because of their oestrogenic action in certain animal models, isoflavones such as genistein and daidzein are widely classified as phyto-oestrogens.

Neoflavonoids

Neoflavonoids are a class of polyphenolic compounds. While flavonoids have a 2-phenylchromen-4-one backbone, neoflavonoids have a 4-phenylchromen backbone with no hydroxyl group substitution at position 2. The first neoflavone isolated from natural sources in 1951 was calophyllolide from *Calophylluminophyllum* seeds. It is also found in the bark and timber of the Sri Lankan endemic plant *Mesuathwaitesii*[9].

Theaflavin

Theaflavins are a type of polyphenol pigments generated during the fermentation of black tea. They are made up mostly of four components. After being identified from black tea in 1957, this group of polyphenol pigments has been added to a variety of novel compounds and researched extensively in terms of property[10], chemical structure, purification and assay methods, formation mechanism, and synthesis methods. Its pharmacological activity, which includes qualities as an antioxidant, anti-pathogenic agent, and cancer suppressor, has received a lot of attention in recent years.

In clinical trials, theaflavins have also been used to prevent coronary heart disease and treat diabetes. Theaflavins will be a very important type of natural medicinal material with enormous potential in the future, as the method of modelling the fermentation of black tea is constructed to produce theaflavins in

bulk. This report examined the current state of theaflavin research in China and internationally[11].

This review focuses on the anticancer effects of theaflavin as well as numerous applications of theaflavin.

Formation of theaflavins

A benzotropolone skeleton is formed by co-oxidation of appropriate pairs of catechins, one with a vic-trihydroxy moiety and the other with an ortho-dihydroxy structure, according to Sang *et al*. The intermediate proepitheaflagallin was isolated. Demonstrated that theaflavin is made from a bicyclo [3.2.1] octane intermediate. The underlying production process of theaflavin related to enzymatic acceleration, as well as physical-chemical transformation, was proposed based on this pioneering research, taking both endogenous and exogenous factors into account. Two catechin molecules were oxidised to quinone in equal amounts with the help of enzymes[12]. The dimerization of the quinones resulted in the formation of diphenolquinone. The polymerization of quinones, on the other hand, results in the creation of thearubigins and theabrowin. Proepitheaflagallin, a bicycle [3.2.1] octane-type intermediate, was produced sequentially. It's worth noting that proepitheaflagallin was an interacting quinone that might be reduced to bisflavanol. The motif of catechins have been linked to a drop in total TFs and an increase in TB levels[13]. A decrease in both types of catechins was followed by an increase in the concentration of theaflavins. At higher temperatures, the catechin levels dropped significantly faster. As a result, the fermentation period to attain maximal TF content was reduced.

The family of TFs had at least 25 members and was formed by the enzymatic oxidation of two catechin molecules. TF, TF1, TF2, and TF3 were the most common.

The production of these four dominant TFs was depicted graphically. The dimerization of EC, EGC, ECG, and EGCG as precursors resulted in the formation of TFs.

PREPARATION OF THEAFLAVINS

It is estimated that TFs make up between 2-6% of the dry weight of solids in brewed black tea. Biosynthesis would be a cost-effective way to obtain large amounts of TFs. Polyphenol oxidase (PPO) and peroxidase (POD) are well-known enzymes involved in pigment formation during the production of black tea. The biosynthesis of theaflavins was realised using model oxidation systems based on PPO and POD. Five critical variables, tea polyphenol concentrations[14], pH, aeration volumes, reaction time, and ratio of immobilised enzyme to substrate, were optimised via response surface methodology at five levels using an immobilised polyphenol oxidase system, and the highest theaflavin concentration obtained was 0.766mg/ml. TFs were bioprocessed on an immobilised tea PPO system in a cost-effective way with a conversion efficiency of 85 percent, a 14-fold increase over the maximum possible in typical black teas. Biosynthesis would be a low-cost method of obtaining vast quantities of TFs. Polyphenol oxidase (PPO) and peroxidase (POD) are two well-known enzymes involved in the pigment generation process in black tea production. The biosynthesis of theaflavins was achieved using PPO and POD-based model oxidation systems[15]. Using an immobilised polyphenol oxidase system, five critical variables, tea polyphenol concentrations, pH, aeration volumes, reaction time, and ratio of immobilised enzyme to substrate, were optimised at five levels using response surface methodology, with the highest theaflavin concentration obtained being 0.766mg/ml. TFs were cost-effectively bioprocessed using an immobilised tea PPO system with an 85 percent conversion efficiency, a 14-fold increase over the maximum feasible in conventional black teas.

Endogenous tea enzymes, PPO and POD, can be used in heterogeneous catalysis systems to successfully imitate the molecular changes that occur during tea manufacture Sang et al. reported the synthesis of 18 theaflavin derivatives using the horseradish POD/H2O2 system. Tea leaves were fermented using pectinase enzymes isolated from

Aspergillus spp., A. indicus, A. jluvus, and A. niveus. Crude enzymes, which included cellulase, hemicellulase (xylanase), proteinase, pectinase, and other enzymes, were found to be more effective than purified enzymes.

When it came to getting TFs, Collier *et al*. Were trailblazers. To remove the caffeine, tea samples were soaked in hot water, and the leach liquid was combined with methanol and extracted with chloroform. The solvent was then removed under vacuum, and the residue was extracted numerous times with ethyl acetate. Crude TFs might be obtained as an orange-yellow solid once the solvent was removed[16].

Previous research on TFs relied on mixtures due to their low abundance and difficult purifying technique. Chemical synthesis, on the other hand, could allow for the creation of vast amounts of pure chemicals for biological testing. Furthermore, bio-activity was discriminated between isomers of TFs and comparable branches bearing the basic motif of the benzotropolone skeleton. To determine the therapeutic efficacy of the monomers in the theaflavin family, extensive research was required. It is concluded that GTC in green tea is more stable than TF in black tea in terms of thermal and pH-dependent stability. The stronger the stability, the lower the pH of the sodium phosphate buffer. TF was considerably more unstable than GTC when incubated in the same pH buffer. TF3 and TF2 were shown to have slower rates of destruction than the other two TF derivatives[17]. Simultaneous detection of catechins and theaflavin was performed using reversed-phase high-performance liquid chromatography and capillary electrophoresis. Separation of TFs could be accomplished using high-speed counter current chromatography with gradient elution in conjunction with preparative HPLC. The phenolic chemicals in green tea, oolong tea, black tea, and coffee have also been studied using cyclic voltammetry.

CHEMICAL STRUCTURE AND SYNTHESIS OF THEAFLAVINS

Theaflavins are made up of four primary compounds: theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B), and theaflavin-3, 3'-digallate (TF2C) (TF3). TF1, TF2 (includes TF2A and TF2B), and TF3 have the chemical formulae C29H24O12, C39H28O16, and C43H32O20, respectively. Clark *et al*. (1998) modelled the chemical structures and conformations of TF1, TF2A, TF2B, and TF3. Some analogues have been discovered as the study of theaflavins progresses. Epicatechingallate (EC) was oxidised chemically using potassium ferricyanide, resulting in the formation of a new type of theaflavins known as theaflavate A. (Xiaochun *et al*, 1997). Black tea also contains theaflavate B, isotheaflavin-3'-o-gallate, and neotheaflavin-3'-o-gallate, all of which include a benzotropolone moiety (John *et al*, 1998). There are currently 12 different types of theaflavins. Theaflavins have the same hydroxy-substituted benzotropolone ring, which is a typical structure of theaflavins, despite their complex structure[18].

Catechin condensation between di- and tri-hydroxylated B rings of catechins can be used to make theaflavins. The oxidation of the B ring of catechins to quinines is followed by a Michael addition of the gallo catechinquinone to the catechinquinone, followed by a carbonyl addition across the ring and subsequent decarboxylation in the condensation step. In general, there are three ways to obtain theaflavins[19]: Theaflavins could be produced from black tea, as was done in the past. Because the content of theaflavins in black tea is so low, the process is usually quite expensive and not suitable for commercial application. Fermentation at 23°C for 80 minutes was the optimal condition for increasing the content of theaflavins in black tea. In the synthesis of theaflavins, a model of black tea fermentation in vitro was used, with chemical oxidation primarily using potassium ferricyanide (K3Fe(CN)6) and sodium bicarbonate (NaHCO3) as chemical catalyzers and enzymatic oxidation primarily using polyphenol oxidase (PPO) as a biocatalyzer (Xiaochun *et al*, 1997)[20]. Catechins

were oxidised by PPO in a model fermentation system under oxygen blowout, thanks to advances in fermentation engineering and enzyme engineering (Alastair and Derek, 1983). The yield of theaflavins can be increased even further by controlling the influence factor. Theaflavins are now synthesised using the third approach.

BIO-ACTIVITIES OF THEAFLAVINS

TFs were the first of the compounds isolated from tea to be proven effective in the treatment of hyperlipidemia and cardiovascular disease (CVD). *In vitro* and *in vivo* tests revealed that TFs can efficiently limit inter- and intra-cellular expressions of gene proteins, inhibit cell proliferation, and induce death. TFs demonstrated outstanding efficacy in areas such as antimicrobial, hypolipidemic[21], anti-inflammatory, anti-mutagenicity, and anti-cancer. Furthermore, epidemiological research revealed that TFs could be used as a medication.

RADICAL-SCAVENGING ACTIVITY

The conversion of catechins to TFs during the fermentation of black tea did not affect their free radical-scavenging activity significantly. Furthermore, both in the aqueous and lipophilic phases, TFs demonstrated the ability to scavenge free radicals. TFs were more effective than EGCG in preventing HPF-1 cells from H2O2-mediated damage, which was externalised as the ability to scavenge hydroxyl radicals. TF3 > TF2 = TF1 > EGCG > TF was the order of 2, 2-diphenyl-1-picrylhydrazyl scavenging ability[22]. TF also protected against t-BHP-induced oxidative stress, as shown by a decrease in protein carbonyl (PCO) and sulfhydryl group (-SH) content 1][23].

Arent and colleagues conducted a randomised, double-blind, crossover study to investigate the influence of TFs on human volunteers' responses to acute anaerobic interval training. Consumption of TF-enriched black tea extract increased recovery and reduced oxidative stress, as well as delayed onset muscular soreness responses to acute anaerobic periods, according to the findings.

HYPOLIPIDEMIC ACTIVITY

TFs were found to be attributable to inhibitory activities against lipase and amylase. TF suppressed the expressions of lipopolysaccharide-induced intercellular adhesion molecule and vascular cell adhesion molecule in intestinal epithelial cells by blocking NF-B and JNK activation

TFs, on the other hand, reduced the production of fatty acid synthase, a critical enzyme in lipogenesis, by down regulating the epidermal growth factor and receptor/PI3K/Akt/Sp-1 signal transduction pathway. Both of these findings confirmed the hypolipidemic effects of TFs[24].

TFs inhibited hepatic lipid accumulation by increasing AMP-activated protein kinase, which reduced lipid accumulation, suppressed fatty acid synthesis, and promoted fatty acid oxidation in the prevention of obesity. Oral administration of TFs resulted in increased energy expenditure and metabolic gene expression.

All of these findings point to TFs' potential use in functional meals and nutraceuticals for obesity management. Luo and colleagues found that theaflavin therapy reduced liver steatosis, oxidative stress, inflammation, and hepatocyte death. In fatty livers, TFs also demonstrated a protective effect against ischemia-reperfusion (1/R) injury[25].

ANTI-INFLAMMATORY ACTIVITY

The cytokine IL-6 can cause substantial tissue damage and apoptosis when it is expressed. TF compounds had anti-inflammatory properties by lowering the expression level of the cytokine IL-6 during viral infection TF2 reduced the 12-O-tetradecanoylphorbol-13-acetate-induced COX-2 gene expression, as well as TNF-, inducible nitric oxide synthase (iNOS), ICAM-1, and nuclear factor B (NF-B), according to Gosslau and coworkers[26]. TF3 enhanced the prevention of colonic inflammation by a similar way

Meanwhile, TFs protected neurons from cerebral I/R injury in ischemic brain by restricting leukocyte infiltration and ICAM-1 expression, as well as regulating upregulation of inflammatory-related

pro-oxidative enzymes (iNOS and COX-2) by lowering STAT-1 phosphorylation.

TFs reduced airway mucus hypersecretion by inhibiting the activation of the epidermal growth factor receptor and decreasing the level of mucin 5AC, which could be useful in the treatment of chronic airway inflammation Hosokawa and colleagues[27] studied the effects of TF3 on CXC chemokine ligand 10 production from human gingival fibroblasts, and found that TF3 prevented OSM-mediated CXCL10 synthesis in a dose-dependent manner.

Therapeutic reduction of neuro inflammatory in Parkinson's disease (PD) by TFs, according to Anandhan *et al*, could be a valuable therapeutic technique for the treatment of progressive neurodegenerative illness in the future. By the way, the capacity of TFs to promote the release of antimicrobial peptides called hBDs by oral epithelial cells showed that they could help prevent periodontal disease.

ANTI- CANCER ACTIVITY

When it came to tumour cells, TFs, particularly TF3, showed inhibitory effects on extracellular signal transmission and cell growth. TFs induced apoptosis in cancer cells such as mammary epithelial carcinoma cells and leukaemia cells by causing cell shrinkage, membrane blebbing, and mitochondrial clustering.

As previously reported, inhibiting NF-B via the p53-ROS interaction conferred anti-migratory effects on cancer cells[28]. Except for o-epicatechin, all tea polyphenols inhibited cell proliferation and activator protein activity significantly.

In A431 cells and mouse NIH3T3 fibroblast cells, TF3 may also suppress the phosphorylation of extracellular signal-regulated kinase, such as the epidermal growth factor and PDGF receptors. In the case of LNCap, TF3 inhibited it by inhibiting androgen receptor expression and lowering androgen-induced prostate-specific antigen production and fatty acid synthase protein levels. In other words, TF3 was identified as a possible prostate cancer chemoprevention drug.

QuicklyTF2 caused the up-regulation of p53 and BAX among a group of pro-apoptotic genes, implying that mitochondria are the primary target[29].

Based on the literature ascorbic acid increased apoptosis in human lung adenocarcinoma SPC- A-1 cells and esophageal cancer Eca-109 cells triggered by EGCG and TF3 via mitogen-activated protein kinases pathways. TFs indicated cytotoxicity of cells from the human oral cavity as a result of H2O2 production, which could be reduced with the addition of Co2+ or catalase. Fortunately, both malignant carcinoma cells and immortalised cells were more sensitive than normal cells

By the way, salivary esterases-mediated hydrolysis of theaflavin gallates suggested that theaflavins could be used to prevent oral cancer and dental caries. The suppression of A431 and A375 cell proliferation produced by TFs was found without harming normal human epidermal keratinocyte cells. And the chemical mechanism predicted that TFs would stop the cell cycle by translocating Bax and induce apoptosis through the mitochondrial death cascade.

ANTI-VIRAL EFFECT

TF3 was found to be an efficient inhibitor of 3CLpro in epidemiological investigations linked to severe acute respiratory syndrome (SARS)[30]. TF derivatives were found to have a direct effect on viral particle infectivity when tested with a neuraminidase activity assay, a hem agglutination inhibition assay, a real-time quantitative PCR assay for gene expression of HA, and a cytopathic effect reduction assay, which was consistent with the inhibitory effects against the influenza virus. TFs have also shown promise in the prevention of HIV sexual transmission, inhibiting infection by preventing it at the entry point.

Vaginal gel formulations based on theaflavin deriva-tives have been tested as a microbicide to prevent HIV sexual transmission. TF2 and TF3 contributed to the prevention of oxazolone-induced type IV allergy in male ICR mice via percutaneous and oral routes by inhibiting cytokine fluctuations and maintaining antioxidant status.

OTHER POTENTIAL ACTIVITIES

In the prevention of CVD, Lorenz *et al*. [701] found that TFs mostly compensated for the loss of catechins in black tea, resulting in stimulation of NO generation and vasodilation that were comparable to green tea. TF3 suppressed theormation and differentiation of osteoclasts by inhibition of Matrix metalloproteinases MMPs more efficiently than EGCG in suppressing actinring formation. The findings suggested that TF3 could be useful agents or lead compounds for treating bone resorption disorders. At the same time, TFs were found to protect against dimethylnitrosamine-induced liver fibrosis[23]. Park *et al* and coworkers revealed that TFs could shut the TJ pathway in Caco-2 cells, improving the intestinal barrier. As a result, TF consumption is predicted to be therapeutic for bowel disorders in the future. TFs could be created as natural AhR antagonists, according to study by Fukuda *et al*.

The majority of people now days are affected by cancer. Many anticancer synthetic medications have been produced, but they may have harmful and unwanted side effects on patients. Natural components such as flavonoids were employed to avoid harmful effects. The anticancer characteristics of the theaflavin are highlighted in this review.

WHAT IS CANCER?

Cancer is caused by uncontrolled cell proliferation, which results in the formation of a tumour. This occurs when cells are altered in such a way that the signals that control normal cell behaviour are disrupted, allowing for increased cell division. Other signalling pathways altered in cancer cells are crucial in homeostasis maintenance, such as metabolism, differentiation, survival, and angiogenesis[31]. Mutation of proto-oncogenes or tumor-suppressor genes causes dysregulated cell signalling and tumour development in cancer. An oncogene is created when a proto-oncogene becomes mutated. In general, a point mutation, localised reduplication, or chromosomal translocations leading to a gain-of-function mutation impact the conversion of a proto-oncogene to an oncogene. Point mutations can result in the production of proteins that aren't the same as normal proteins. With localised reduplication or chromosomal translocation, the protein product is usually overabundant, resulting in enhanced protein expression. Because these mutations are dominant, only one allele needs to be mutated to cause cancer.

THEAFLAVINS AS A POTENTIAL ANTI-CANCER AGENT

Many well-known chemotherapeutics come from natural sources. Taxanes like paclitaxel and docetaxel, which are used to treat a variety of malignancies, were originally isolated from the bark of the Pacific (Taxusbrevifolia) and European (Taxusbaccata) yew trees, respectively. The search for natural therapeutic solutions for human ailments, including cancer, has increased in recent years due to a focus on healthier food and living behaviours. Nutraceuticals are a type of supplement that combines nutrition and medications. Nutraceuticals, in general, are foods or dietary molecules that aid in the maintenance of normal physiological processes[32]. Many natural and dietary items have been proven to have anti-cancer properties, and nutraceuticals have the potential to lower cancer cell growth, inhibit cell proliferation, and induce cancer cell death.

Black tea has long been utilised as a nutraceutical since its components have been proven *in vitro* and *in vivo* studies to offer a number of health advantages, including anti-cancer actions. The presence of phenolic compounds, such as catechins and theaflavins, which account for the highest percentage of physiologically active substances in black tea extracts, is largely responsible for these benefits. Theaflavins are a category of polyphenolic chemicals found in black tea that make up 2–6% of the dry weight[33]. There are four primary varieties of theaflavin found in black tea: theaflavin (TF1), theaflavin-3-gallate (TF2a), theaflavin-3′-gallate (TF2b), and theaflavin-3, 3′-digallate (TF3). They are dimericcatechins generated by enzyme-catalyzed oxidation and polymerization of tea catechins (flavanols). A benzotropolone core

generated from the dimerization of a catechin and a gallo-catechin distinguishes the theaflavins structurally[34].

ANTI-CANCER PROPERTIES OF THEAFLAVINS

Theaflavins are effective against various types of cancers like breast cacer, prostate cancer, lungs cancer, leukemia. Ovarian cancer, cervical cancer, skin cancer, colon cancer and liver cancer.

In this article we manly focused on theaflavins skin cancer properties.

Treatment of A431 epidermoid carcinoma cells with 1-50 M TF1, TF2a, TF2b, or TF3 for 30 minutes each resulted in dose-dependent suppression of growth two days later. With an IC50 of 18 M, TF3 inhibited cell growth the most of the four theaflavins. Pre-treatment with TF3 followed by EGF inhibited EGF-induced EGFR activation in a dose-dependent manner, with 5 M inhibiting receptor kinase activity by 75% and 10 M completely inhibiting EGFR activation. TF3 pre-treatment and co-treatment for 30 minutes both reduced EGF binding to its receptor[35], as shown by a [125I] EGF binding assay. These findings imply that TF3 may contribute to black tea's anti-proliferative impact via lowering EGFR kinase activity.

Internalization of EGFR in EGFR-over expressing cells was determined using confocal microscopy after treatment of human A431 epidermoid carcinoma cells with 20 M TF3 for 1 hour. The TF3 therapy drastically lowered cell surface expression of EGFR to less than 50% of control in an EGF-binding assay. Western blotting of whole cell lysates revealed a time-dependent decrease in total EGFR levels, as well as a time-dependent increase in ubiquitinated EGFR levels, showing that TF3 is causing EGFR to be targeted for degradation. The TF3-induced EGFR down regulation was reversed in the presence of a proteasome inhibitor (MG132).

In a dose-dependent manner, treatment of A431 and A375 human skin cancer cells with theaflavins isolated from black tea resulted in decreased viability, enhanced apoptotic processes, and DNA fragmentation (Table No.7). A375 cells were found to be more numerous at the G0/G1 stage and less in the S stage, indicating cell cycle arrest. Under the same conditions, however, theaflavins failed to trigger apoptosis in normal human epidermal NHEK cells. Theaflavins boosted Bax translocation to mitochondria, decreased mitochondrial membrane potential, and increased cytochrome C accumulation in the cytoplasm in A375 cells. H2O2 levels were also elevated, which was accompanied by greater activation of caspase-3 and caspase-9, as well as higher levels of cleaved PARP[36].

In B16 murine melanoma cell lines, TF3, but not TF1, TF2a, or TF2b, inhibited melanogenesis (Table No.1). There were no differences in cell viability between the treated and untreated groups. Melanocyte-stimulating hormone (MSH)-induced tyrosinase, an enzyme implicated in melanin production, had its mRNA and protein expression inhibited by TF3.

Reduced cell survival, increased phosphorylated JNK and p38 MAPK, increased caspase-3 activity, and higher phosphorylation of MKK3, MKK6, MKK4, and ASK1 were all seen in A375 cells treated with theaflavins isolated from black tea (Table No.1). The effects of theaflavins on cell death, p-JNK, p-p38, MAPK, p-ASK1, and caspase-3 activity were all reversed when theaflavins were treated with NAC, a powerful antioxidant. Theaflavin-induced overexpression of p38 and JNK by ROS could be mediated by ASK-1, an upstream regulator of p38 and JNK. Table contains the details about the effect of theaflavin on skin cancer.

APPLICATION OF THEAFLAVINS

PREVENTION OF CORONARY HEART DISEASE AND ATHEROSCLEROSIS

Theaflavins have a wide range of medical applications due to their pharmacological action. Lipid peroxidation is linked to an increased risk of atherosclerosis and heart disease, as well as speeding up the ageing process (Prosenjit and Sukta, 2003).

Theaflavins are widely used to prevent coronary heart disease and atherosclerosis by regulating

blood fat levels[37], preventing lipid oxidation, clearing out oxygen free radicals, promoting fibre dissolution, restricting cell hyperplasia in the smooth muscle of the human aorta, and inhibiting the formation of lipid cake.

ANTIHYPERGLYCAEMIC ACTIVITY

Theaflavins are used to treat hyperglycemia in Japan, according to Mitsui Norin Co Ltd. (1993). Many studies afterwards attempted to discover the mechanism of theaflavins' antihyperglycemic action. There is evidence that theaflavins may protect -lymphocytes from the toxicity of streptozotocin (STZ) in mice with diabetes caused by STZ (Gomes et al., 1995)[38].

Furthermore, in an epididymal fat cell experiment (Anderson and Polansky, 2002), theaflavins could boost insulin activity *in vitro*. Although the mechanism of theaflavins' antihyperglycemic effect is unknown, theaflavins' antihyperglycemic efficacy is undeniable.

CANCER INHIBITION

Weisburge *et al*. (2002) recently discovered that theaflavins can prevent several cancers caused by lifestyle factors including[35] smoking and tobacco use. Theaflavins may also help to prevent cancers of the breast, colon, prostate, and pancreatic[39].

OTHER APPLICATION

Theaflavins can protect teeth by inhibiting the enzyme glucosyltransferase (GTF).

When TF2 was applied to the filter tip of a cigarette[40], Weisburge *et al*. (2002) found that theaflavins reduced the harm caused by smoking to the body. Hyperglycemia, atherosclerosis, heart disease, malignancies, and ageing are just some of the ailments that theaflavins can help prevent in our daily lives. As a result, theaflavins will become a new medical substance in the future. Various applications of theaflavin depicted in Figure No.2.

Table No.1: Effect of theaflavin on skin cancer

S.No	Cell Line/Animal Model	Treatment	Effects	Reference
1	A431	TF3 0.1–50 µM 10–30 min	↓Growth ↓EGFR kinase activity ↓EGF Binding	35
2	A431	TF3 20 µM 2 min–1 h	↑EGFR internalization ↓EGFR expression ↑EGFR ubiquitination ↓EGF-induced EGFR activation	36
3	A431 A375	0–100µg/mL Theaflavins (black tea extract) 0–48 h	↓Cell viability ↑Apoptosis ↑DNA fragmentation ↑Cell cycle arrest at the G0/G1 phase ↑Bax translocation to mitochondria ↓Mitochondrial membrane potential ↑Cytoplasmic cytochrome C ↑H_2O_2 ↑Cleaved caspase-3 ↑Cleaved caspase-9 ↑Cleaved PARP	37
4	Mouse B16 melanoma 4A5 cells	0–20µM TF1, TF2a, TF2b, TF3 0–72 h	↓Melanogenesis ↓Tyrosinase mRNA and protein	38
5	A375	0–100µg/mL Theaflavins (black tea extract) 0–48 h↓Cell viability	↓Cell viability ↑p-JNK ↑p-p38 MAPK↑Caspase 3 activity ↑p-MKK3 ↑p-MKK4 ↑p-MKK6 ↑p- ASK1 ↑ROS	39

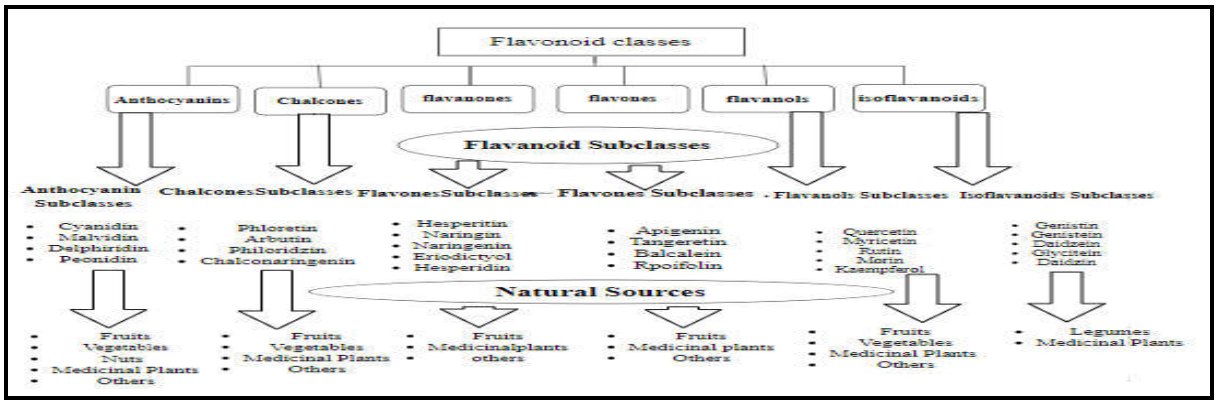

Figure No.1: Depicts the classification of flavonoids

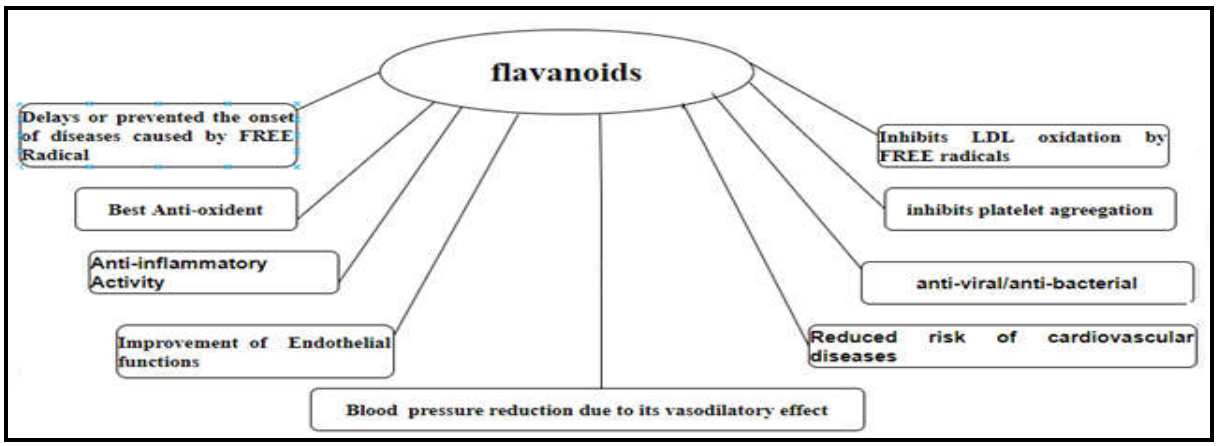

Figure No.2: Various applications of theaflavin

CONCLUSION

Flavonoids are a type of natural product that is extensively found in fruits, vegetables, and some beverages. They are a type of plant secondary metabolite with a poly phenolic structure. They have a variety of beneficial biochemical and antioxidant properties that have been linked to cancer, Alzheimer's disease (AD), atherosclerosis, and other diseases. Flavonoids are an essential component in a number of nutraceutical, pharmacological, medical, and cosmetic uses because they are linked to a wide range of health-promoting properties. Theaflavins are a type of polyphenol pigments generated during the fermentation of black tea. They are made up mostly of four components. After being identified from black tea in 1957, this group of polyphenol pigments has been added to a variety of novel compounds and researched extensively in terms of property. Theaflavins are effective against various types of cancers like breast cacer, prostate cancer, lungs cancer, leukemia. Ovarian cancer, cervical cancer, skin cancer, colon cancer and liver cancer.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmaceutics, Joginpally B.R Pharmacy College, Yenkapally, Moinabad, Rangareddy, Hyderabad-75, Telangana, India for providing necessary facilities to carry out this review work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Burak M, Imen Y. Flavonoids and their antioxidant properties, *Turkiye Klin Tip Bil Derg,* 19, 1999, 296-304.
2. Ovando C, Hernandez D, Hernandez E, *et al*. Chemical studies of anthocyanins: A review, *Food Chem,* 113(4), 2009, 859-871.
3. Lee Y, Yuk D, Lee J, *et al*. Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of β-amyloid generation and memory deficiency, *Br Res,* 1250, 2009, 164-174.
4. Metodiewa D, Kochman A, Karolczak S. Evidence for antiradical and antioxidant properties of four biologically active *N, N*, diethylaminoethyl ethers of flavanone oximes: A comparison with natural polyphenolic flavonoid (rutin) action, *Bio Mol Biol Int,* 41(5), 1997, 1067-1075.
5. Hayashi T, Sawa K, Kawasaki M, *et al*. Inhibition of cow's milk xanthine oxidase by flavanoids, *J Nat Prod,* 51(2), 1988, 345-348.
6. Walker E, Pacold M, Perisic O. Structural determinations of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine, *Mol Cell,* 6(4), 2000, 909-919.

7. Havsteen B. The biochemistry and medical significance of the flavanoids, *Pharmacol Ther,* 96(2-3), 2002, 67-202.
8. Dewick P M. Theshikimate pathway: Aromatic amino acids and phenylpropanoids In *Medicinal Natural Products: A Biosynthetic Approach*, *[Dewick PM, Editor], Chichester: John Wiley,* 2nd Edition, 2001, 137-186.
9. Griesbach R. Biochemistry and genetics of flower color, *Plant Breed Rev,* 25, 2005, 89-114.
10. Takahashi A, Ohnishi T. The significance of the study about the biological effects of solar ultraviolet radiation using the exposed facility on the international space station, *Biol Sci Space,* 18(4), 2004, 255-260.
11. Dixon R, Pasinetti G. Flavonoids and isoflavonoids: From plant biology to agriculture and neuroscience, *Plant Physiol,* 154(2), 2010, 453-457.
12. Kumar S, Pandey A K. Chemistry and biological activities of flavonoids: An overview, *Scientific World Journal,* 2013, Article ID: 162750, 2013, 16.
13. Giusti M, Wrolstad R. Acylatedanthocyanins from edible sources and their applications in food systems, *Biochem Eng J,* 14(3), 2003, 217-225.
14. Iwashina T. Flavonoid properties of five families newly incorporated into the order Caryophyllales (Review), *Bull Natl Mus Nat Sci,* 39(1), 2013, 25-51.
15. Hertog M G, Hollman P C, Van De P B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices, *J Agric Food Chem,* 41(8), 1993, 1242-1246.
16. Nishimura S, Taki M, Takaishi S, *et al*. Structures of 4-aryl-coumarin (neoflavone) dimers isolated from *Pistaciachinensis* BUNGE and their estrogen-like activity, *Chem Pharm Bull (Tokyo),* 48(4), 2000, 505-508.
17. Manach C, Scalbert A, Morand C, *et al*. Polyphenols: Food sources and bioavailability, *Am J Clin Nutr,* 79(5), 2004, 727-747.
18. Matthies A, Clavel T, Gutschow M, *et al*. Conversion of daidzein and genistein by an anaerobic bacterium newly isolated from the mouse intestine, *Appl Envrion Microbiol,* 74(15), 2008, 4847-4852.
19. Aoki T, Akashi T, Ayabe S. Flavonoids of leguminous plants: Structure, biological activity, and biosynthesis, *J Plant Res,* 113(4), 2000, 475-488.
20. Dixon R, Ferreira D. Molecules of interest: Genistein, *Phytochemistry,* 60(3), 2002, 205-211.
21. Linuma M, Tanaka T, Hamada K, *et al*. Revised structure of neoflavone in *Coutareahexandra*, *Phytochemistry,* 26(11) , 1987, 3096-3097.
22. Nishimura S, Taki M, Takaishi S, *et al*. Structures of 4-aryl-coumarin (neoflavone) dimers isolated from *Pistaciachinensis* BUNGE and their estrogen-like activity, *Chem Pharm Bull (Tokyo),* 48(4), 2000, 505-508.
23. Fatima M, Rizvi S I. Anti oxidative effect of black tea theaflavin on erythrocytes subjected to oxidative stress, *Natl Acad Sci Lett,* 38(1), 2015, 25-28.
24. Holt R R, Schramm D D, Keen C L. Chocolate consumption and platelet function, *JAMA,* 287(17), 2002, 2212-2213.
25. Stangl V, Dreger H, Stangl K, *et al*. Molecular targets of tea polyphenols in the cardiovascular system, *Cardiovasc Res,* 73(2), 2007, 348-358.
26. Duffy S J, Keaney J F, Holbrook M, *et al*. Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease, *Circulation,* 104(2), 2001, 151-156.
27. Friedman M. Overview of antibacterial, antitoxin, antiviral and antifungal activities of

tea flavonoids and teas, *Mol Nutr Food Res,* 51(1), 2007, 116-134.
28. Kuroda Y, Harar Y. Antimutagenic and anticarcinogenic activity of tea polyphenols, *Mutat Res,* 436(1), 1999, 69-97.
29. Yang Z, Tu Y, Xia H, et al. Suppression of free-radicals and protection against H_2O_2-induced oxidative damage in HPF-1 cell by oxidized phenolic compounds present in black tea, *Food Chem,* 105(4), 2007, 1349-1356.
30. Yang Z, Jie G, Dong F, *et al*. Radical-scavenging abilities and antioxidant properties of theaflavins and their gallate esters in H_2O_2-mediated oxidative damage system in the HPF-1 cells, *Toxicol In Vitro,* 22(5), 2008, 1250-1256.
31. Arent S M, Senso M, Golem D L, *et al*. The effects of theaflavin-enriched black tea extract on muscle soreness, oxidative stress, inflammation and endocrine responses to acute anaerobic interval training: A randomized, double-blind, crossover study, *J Int Soc Sport Nutr,* 7(1), 2010, 11.
32. Halder B, Pramanick S, Mukhopadhyay S, *et al*. Anticlastogenic effects of black tea polyphenols theaflavins and thearubigins in human lymphocytes *in vitro*, *Toxicol In Vitro,* 20(5), 2006, 608-613.
33. Manna S, Mukherjee S, Roy A, *et al*. Tea polyphenols can restrict benzo[*a*]pyrene-induced lung carcinogenesis by altered expression of *p53*-associated genes and *H-ras, c-myc* and *cyclin D1*, *J Nutr Biochem,* 20(5), 2009, 337-349.
34. Halder B, Pramanick S, Mukhopadhyay S, *et al*. Inhibition of benzo[*a*]pyrene induced mutagenicity and genotoxicity by black tea polyphenols theaflavins and thearubigins in multiple test systems, *Food ChemToxicol,* 43(4), 2005, 591-597.
35. Wang W, Sun Y, Liu J, *et al*. Protective effect of theaflavins on cadmium-induced testicular toxicity in male rats, *Food Chem Toxicol,* 50(9), 2012, 3243-3250.
36. Kusano R, Andou H, Fujieda M, *et al*. Polymer-like polyphenols of black tea and their lipase and amylase inhibitory activities, *Chem Pharm Bull,* 56(3), 2008, 266-272.
37. Song Y A, Park Y L, Yoon S H, *et al*. Black tea polyphenol theaflavin suppresses LPS-induced ICAM-1 and VCAM-1 expression via blockage of NF-κB and JNK activation in intestinal epithelial cells, *Inflamm Res,* 60(5), 2011, 493-500.
38. Yeh C W, Chen W J, Chiang C T, *et al*. Suppression of fatty acid synthase in MCF-7 breast cancer cells by tea and tea polyphenols: A possible mechanism for their hypolipidemic effects, *Pharmacogenom J,* 3(5), 2003, 267-276.
39. Lin C L, Huang H C, Lin J K. Theaflavins attenuate hepatic lipid accumulation through activating AMPK in human HepG2 cells, *J Lipid Res,* 48(11), 2007, 2334-2343.
40. Kudo N, Arai Y, Suhara Y, *et al*. A single oral administration of theaflavins increases energy expenditure and the expression of metabolic genes, *PLoS ONE,* 10(9), 2015, e0137809.

Please cite this article in press as: Lakshmi Devi G *et al*. An overview of the properties of theaflavin flavonoid, *International Journal of Research in Pharmaceutical and Nano Sciences,* 10(5), 2021, 341-354.