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Review Article

Anti-Carcinogenic Possessions of Citrus Peel Extracts and Flavonoids: A Review

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Abstract

Researchers and scientists synthesized numerous drugs and medications to treat cancer. These synthesized drugs related with many side reactions could deteriorate the quality of patient's life. Recently, various functional foods containing effective molecular components against cancer has gained much popularity as no side effects are associated with them and these are natural based product could be easily consumed and can left a long lasting health impression on the life of patients. Citrus peel enriched with flavonoids, polymethoxylated flavones and numerous bioactive components demonstrated anti-carcinogenic, anti-tumour, anti-proliferative and anti-inflammatory activities on skin cancer, gastric cancer, prostate cancer, oseophageal tumorigenesis, colon carcinogenesis, colorectal carcinoma cell, human squamous cell carcinoma and against human cancer cell lines. A novel functional natural product named Gold Lotion (GL) was formulated from citrus peel extract to inhibit the inflammation-associated tumorigenesis. Gold Lotion (GL) demonstrated a clear and promising strategy by *in vivo* analysis and result showed beneficial aspects to treat prostate cancer, skin cancer and colon cancer in mice. Six different orange peel extracts (OPEs) were prepared containing bioactive potential of polymethoxyflavones (PMFs) and hydroxylated PMFs (OH-PMFs). *In vitro* study of citrus flavonoids effects on the growth of a human squamous cell carcinoma cell line (HTB43) demonstrated the health benefit and anti-cancer effects by examination of four plant flavonoids (taxifolin, nobiletin, quercetin and tangeretin). Moreover, quercetin chalcone (QC) and pH-modified citrus pectin (MCP) are the modified substances of quercetin (a flavonoid) and citrus pectin (a polysaccharide found in the cell wall of plants). Earlier studies indicated that many antitumor properties due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumor cells; gene expression modification, anti-angiogenesis activity and apoptosis induction are exhibited by quercetin. Prostate cancer and melanoma metastases are also inhibited by modified citrus pectin (MCP).

Keywords: Citrus Peel; Polymethoxylated Flavonoids; OPEs; GL; Anti-Cancer

Introduction

Cancer is major fatal disease associated with many side effects, usually treated with synthesized drugs and medications. These side effects could be low blood counts (anemia, leucopenia, thrombocytopenia), hair loss, peripheral neuropathy (numbness and tingling of hands and feet), arthralgias and myalgias (pain in the joints and muscles), nausea and vomiting, diarrhea, mouth sores, hypersensitivity reaction (fever, shortness of breath) and blood clot [1].

Suzawa., *et al*. (2014) reported that Citrus peel enriched with polymethoxylated flavones, flavonoids and bioactive substances associated to treat malignant tumours and neoplasms. Dietary bioactive components are extracted from citrus peel as well as formulated the products to improve the quality of cancer patient's life and to inhibit the inflammation associated tumorigenesis [2].

Flavonoids

Flavonoids are the polyphenolic molecules containing 15 carbon atoms and appear as two benzene rings that are linked together by a three-carbon chain. They encourage several beneficial health effects. They are regarded as anti-oxidant, anti-viral, anti-carcinogenic, anti-tumour, anti-inflammatory and anti-proliferative [3].

Bioactive citrus peel flavonoids

Citrus peel contained flavonoids and polymethoxylated flavones are considered to be biologically active compounds against carcinogenesis and tumorigenesis. The major citrus peel flavonoids preventing cancer are nobiletin, quercetin, tangeretin, diosmin, hesperidin, apigenin, luteolin and morin as well as many bioactive substances are studied in this review [4].

Flavonoids nobiletin

Murakami., *et al*. (2000) consumption of citrus fruits has been proved to be a major cancer preventive measure. Nobiletin is extracted from citrus peel act as a functionally novel and chemopreventive substance in inflammation correlated tumorigenesis. The processes of epithelial carcinogenesis are directly related with the generation of nitric oxide (NO). NO production inhibitors in *Citrus unshiu* have been searched through various methods. An activity-guiding separation is the technique which was utilized to trace the vigorous substance. Furthermore, the combination of lipopolysaccharide and IFN-gamma with genetic makeup (RAW 264.7 cells) is used to induce the NO and superoxide O_2 generation in mouse macrophage, as well as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in differentiated human promyelocyte HL-60 is also involved. The production of NO and O_2 is intimately coupled with epithelial carcinogenesis. Western blotting is a technique through which expression of NO synthase and cycloxygenase 2 were detected. The in-vivo practices and methods are done to evaluate the antitumor and anti-inflammatory promoting activities by applying TPA to ICR mouth skin with several expressions of disease measurements. Nobiletin, a polymethoxyflavonoid inhibited the NO and O_2 generation and also suppressed the two stages of skin inflammation which is induced by applying double TPA [5].

Yoshimizu., et al. (2004) reported that citrus variety *depressa* hayata was utilized to extract nobiletin which is the citrus flavonoid and demonstrated the effects to avoid and prevent cancer and tumorigenesis Experiments revealed its effect on gastric cell lines TMK-1, MKN-45, MKN-74 and KATO-III. The action of nobiletin on these cells through various ways was analyzed by 3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, the TdT-mediated dUTP biotin nick-end labeling (TUNEL) method and cell-cycle analysis. The nobiletin acted by direct cytotoxicity, modulation of cell cycle and induction of apoptosis. Nobiletin efficacy by the combined treatment with unadventurous anticancer drug, CDDP was also analyzed as well as examined. To show the synergistic effect compared to the control, CDDP administration was made 24 h prior to demonstrate its treatment with nobiletin. The bioactive compound nobiletin showed a promising strategy for further investigation and through study nobiletin demonstrated the treatment of gastric cancer [6].

Kohno., *et al*. (2001) reported that polymethoxyflavonoid nobiletin was directly fed to male F344 rats, separated from citrus *unshiu* to demonstrate the modifying effects on the growth of azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF). Monoclonal antibody MIB-5 was utilized to observe the effects of nobiletin on cell proliferation activity of ACF. Rats were provided with the subcutaneous injections of AOM (15 mg/kg body weight) once a week for 3 weeks to encourage ACF. They also consumed the experimental diet starting ne week before the first dosing of AOM containing 0.01% or 0.05% nobiletin for 5 week. AOM introduction developed 139 ± 35 ACF/rat at the end of the study (week 5). Dietary management of nobiletin caused significant reduction in the frequency of ACF: 70 ± 15 (50% reduction, p ,0.001) at a dose of 0.01% and 63 ± 10 (55% reduction, p,0.001) at a dose of 0.05%. Nobiletin dietary administration significantly lowered MIB-5-index in ACF, decreased prostaglandin E2 content in the colonic mucosa, developed chemopreventive ability by the reduction of cell proliferating activity of ACF development [7].

Lin., *et al*. (2003) reported that in the mouse macrophage J774A.1 cell line and synovial human fibroblasts, nobiletin ability studied to develop cyclooxygenases (COXs), prostaglandin (PG) E_2, and proinflammatory cytokines. In human synovial cells in a dose-dependent manner (<6μM), interleukin (IL)-1-induced development of PGE_2 was suppressed by Nobiletin. Furthermore, COX-2, the IL-1-induced gene expression and development of proMMP-1/ procollagenase-1 and proMMP-3/ prostromelysin-1 in human synovial fibroblasts was downregulated by nobiletin and also interfered with the lipopolysaccharide-induced development of PGE2 and the gene expression of proinflammatory cytokines comprising IL-α, IL-1β, TNF-α and IL-6 in mouse J774A.1 macrophages. The development of the endogenous MMP inhibitor and the upregulation of TIMP-1 encouragement is a novel action of nobiletin [8].

Flavonoids diosmin and hesperidin

Tanaka., *et al*. (1997) reported that the dietary feeding of two flavonoids, diosmin and hesperidin initiated with azoxymethane

(AOM) to inhibit colon carcinogenesis utilized in combination during the initiation phases to investigate and analysis of cure effects experimented in male F344 rates. The diets of rats comprised of feeding at different concentration, containing diosmin (1000 ppm), hesperidin (1000 ppm) or diosmin (900 ppm) and hesperidin (100 ppm) for 5 weeks during initiation treatment or 28 weeks for post-initiation treatment. AOM was weekly injected at the dose of 15mg/kg of body wt which induces colon neoplasms. The study showed that the group of rats which were fed with AOM alone ($P<0.001$) have the large effect of multiplicity of neoplasms adenoma and adenocarcinoma) in the large intestine than those group of rats which were treated with the combination of diosmin and hesperidin. The consumption of diosmin and hesperidin used in combination showed the inhibition of development of colonic neoplasm as well as prevention of development of aberrant crypt foci. The 5´-bromodeoxy-uridine-labelling index and argyrophilic nuclear organizer region's number in crypt cells, colonic mucosal ornithine decarboxylase activity, and polyamine levels in the blood could also be reduced by dietary intake of diosmin and hesperidin and act as chemopreventive and antiproliferative agents to inhibit colon carcinogenesis. The result demonstrated no pathological alternations in rats treated with diosmin and hesperidin supplementation, individually or in combination as well as played role in reduction the expression of cell proliferation biomarkers (BrdU-labelling index and AgNORs number) of non-lesional oesophageal epithelium ($P<0.05$) and lower the blood polyamine attentiveness, also effective in suppression of oesophageal cancer development and inhibited the suppression of increased cell proliferation caused by MNAN in the oesophageal mucosa [9].

Dietary administration of diosmin and hesperidin

Flavonoids	Doses fed in F344 rats		Duration
	Initiation treatment	Post-initiation treatment	
Diosmin	1000 ppm	1000 ppm	5 weeks
Hesperidin	900 ppm	100 ppm	28 weeks
	Doses fed in male wistar rats		
	Initiation treatment	Post-initiation treatment	
Diosmin	1000 ppm	900 ppm	13 weeks
Hesperidin	1000 ppm	100 ppm	7 weeks

Table a

Flavonoids tangeretin

Hirano., *et al*. reported that human tumour cells are treated with various anticancer agents in order to encourage apoptosis. Normal tissue origin cells comprised of myelocytes and immunocytes are fundamentally cytotoxic by the induction of anticancer agents. There was observed no cytotoxicity against human peripheral blood mononuclear cells (PBMCs) by the treatment with tangeretin (5,6,7,8,4´-pentamethoxyflavone) which induces apoptosis in human promyelocytic leukaemia HL-60 cells. Tangeretin IC_{50} value range between 0.062 and 0.173 µM was used to suppress the growth of HL-60 cells *in vitro* evaluated by (^3H) thymidine incorporation or tetrazolium crystal formation.>2.7µM tangeretin was used to demonstrate the apoptosis of HL-60 cells *in vitro* evaluated by cell morphology and DNA fragmentation. The treatment of HL-60 cells with tangeretin contain low DNA content utilized to demonstrate the apoptotic cells by cytometric analysis and observed a reduction in G1 cells and a affiliated boost of S and/or G2/M cells. After the 24 hours of application with tangeretin, apoptosis was observed and DNA fragmentation was evaluated the tangeretin effect Ca^{2+}- dependent endonuclease activity was inhibited by the occurrence of Zn^{2+} attenuated by growth inhibition. Cycloheximide drastically caused reduction in the tangeretin effect on HL-60 cell growth. Dye exclusion test demonstrated that at higher concentration (27 µM) no cytotoxicity was observed by the application of tangeretin against HL-60 cells or mitogen-activated PBMCs. Furthermore, the flavonoid was ineffectual on growth of human T-lymphocytic leukaemia MOLT-4 cells or on blastogenesis of PBMCs. The result evident that tangeretin decreases the growth of HL-60 cells *in vitro*, moderately through apoptosis induction, devoid of severe side-effects on immune cells [10].

Pan., *et al*. 2002 reported that citrus peel is rich in tangeretin which is 5,6,7,8,4-pentamethoxyflavone that indicated through DNA flow cytometric analysis, cell cycle progression was blocked with tangeretin. Tangeretin demonstrated this effect in colorectal carcinoma COLO 205 cells at G_1 phase. The development of G_1 arrest and decrease in the degree of phosphorylation of Rb was experienced over a 24h exposure to tangeretin. It is showed that there is a reduction in the protein expression of cyclins A, D1 and E slightly under the same conditions. Immunocomplex kinase experiments showed that tangeretin prevented the cyclin-dependent kinase 2 (Cdk2) and 4 (Cdk4) in the cell-free system in a dose dependent

manner. It is occurred in the loss of both Cdk2 and 4 kinase activities gradually when cells were exposed to tangeretin (50μM) over 48h. the content of the Cdk inhibitor p21 protein was increased by tangeretin related with the p53 levels elevation. Moreover, within 18h, there is a increase in the level of Cdk inhibitor p27 protein by tangeretin. It is suggested through results that the growth-inhibitory effects through modulation of the activities of several key G1 regulatory proteins, such as Cdk2 and Cdk4 were exerted through tangeretin and it also mediates the increase of Cdk inhibitors p21 and p27 [11].

Gold lotion

Pan., *et al*. (2012) reported that a novel functional natural product named Gold Lotion (GL) was formulated from citrus peel extract to inhibit the inflammation-associated tumorigenesis. It is reported by experiment and analysis, Gold Lotion has inhibitory effects on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in mouse skin. The idea is obtained from TPA-induced skin inflammation model, in mouse skin the transcriptional activation iNOS and its mRNA and protein has been efficiently inhibited by the topical application of Gold lotion. It is found that, by decreasing inflammatory gene parameters the GL effectively prevented TPA-induced inflammation in mouse skin. Moreover, GL significantly repressed 7,12-dimethylbenez[a]anthracene (DMBA)/TPA-induced skin tumor development and prevented tumor occurrence, tumor influence and tumor diversity of papillomas at 20 weeks. These *in vivo* data have discovered that GL is an effectual anti-tumor agent that assists by down-regulating the protein levels of COX-2, ornithine decarboxylase (ODC), and vascular endothelial growth factor (VEGF) in mouse skin. Peels of *Citrus* genus comprised of polymethoxyflavonoids (PMFs), predominantly they are found in the peels of sweet oranges (*Citrus sinensis*) and mandarin (*Citrus reticulate*). Gold lotion (GL) is formed from the peels of 6 citrus fruits named as *navel oranges, citrus hassaku, citruslimon, citrus natsudaidai, citrus miyauchi* and *satsuma* accessible in Japan. GL was mainly developed as a cosmetic product to defend skin from UV irradiation. Patrons later reported subjective indication that depend on the topical application of GL to avoid melanoma, GL contained anti-cancer property and oral intake for prostate, lung and liver cancers. The efficacy of GL has the inhibitory possessions on TPA-induced iNOS, COX-2, and ODC expression in mouse skin; evaluated the anti-inflammatory activity of GL in mouse skin following TPA application was investigated and the inhibitory effect of GL on mouse skin tumorigenesis using a two-stage skin carcinogenesis model by the assessment of tumor occurrence, diversity and volume has been explored [12].

Suzawa., *et al*. (2014) reported that humans are facing two major lethal diseases, cancer is one of them. Researchers and scientists synthesized numerous drugs and medications but they are also related with many side reactions. These synthesized drugs can deteriorate the quality of patient's life. Lately, various functional foods containing effective molecular components against cancer has gained much popularity as no side effects are associated with them and these are natural based product could be easily consumed and can left a long lasting health impression on the life of patients. Citrus peels are very effective associated with numerous polymethoxyflavones and flavonoids to treat cancer. It has been reported that citrus peel extract contain health benefit and regarded to be as beneficial as pharmacological products. The overall results of these studies have proved to be effective by experiments and analysis and Gold Lotion (GL) was prepared. Various varieties of citrus peels were experimented and after the analysis of results, a effective health beneficial product Gold Lotion associated with the extracts of polymethoxyflavonoids was formulated. Gold Lotion (GL) demonstrated a clear and promising strategy by *in vivo* analysis and result showed beneficial aspects to treat prostate cancer, skin cancer and colon cancer in mice. The prevention and treatment of cancer by gold lotion has proved to be a access clinical trials and also demonstrated opportunities for the various researches in near future [13].

Effects of GL administration on the body weight and organ weight in a PC-3 xenograft model

Lai., *et al*. (2013) reported that prostate cancer is the most prevailing lethal disease among humans could be inhibited by the varieties of citrus peel extracts and flavonoids. Azoxymethane (AOM) induced colon tumorigenesis could be prevented by polymethoxyflavonoids obtained from citrus peel found in formulated product Gold lotion (GL). Human prostate xenograft tumor mouse model was utilized to predict the efficacy of Gold lotion (GL). GL caused the reduction in both weight (57-100% reduction) and volume (78-94% reduction) in tumour by both intraperitoneal injection and oral examination. Moreover, GL demonstrated down regulation of the protein levels of inflammatory enzymes (inducible nitric oxide

synthase, iNOS and cyclooxygenase-2, COX-2), metastasis (matrix metallopeptidase-2, MMP-2 and MMP-9), angiogenesis (vascular endothelial growth factor, VEGF), and proliferative molecules, as well as by the induction of apoptosis in prostate tumors [14].

Treatment	Group	No. of mice	Body weight (g)	Liver (mg)	Spleen (mg)	Lung (mg)
i.p.	control	8	16.17 ± 4.90	0.91 ± 0.35	0.07 ± 0.04	0.09 ± 0.01
	GL 1 mg kg^{-1}	6	16.36 ± 1.77	0.83 ± 0.07	0.05 ± 0.01	0.13 ± 0.06
	GL 2 mg kg^{-1}	6	23.18 ± 0.83	1.49 ± 0.07	0.07 ± 0.04	0.12 ± 0.02
Oral	control	8	20.41 ± 2.11	1.15 ± 0.12	0.08 ± 0.02	0.11 ± 0.02
	GL 2 mg kg^{-1}	6	21.22 ± 0.53	1.06 ± 0.09	0.09 ± 0.02	0.13 ± 0.08
	GL 4 mg kg^{-1}	6	21.34 ± 0.90	1.33 ± 0.21	0.08 ± 0.03	0.13 ± 0.07

Table b

Orange peel extracts

Gosslau., *et al*. (2014) reported that six different orange peel extracts (OPEs) were prepared containing bioactive potential of polymethoxyflavones (PMFs) and hydroxylated PMFs (OH-PMFs). The characterization and quantification of these peel extracts were done with the help of high performance liquid chromatography (HPLC). Utilizing a human cell-based TPA-induced monocyte-macrophage differentiation model which employs U-937 cells and inflammatory surrogate gene leads to the analysis of effects on inflammation by nutrigenomics. Dose response and kinetics analysis of OPEs with dissimilar chemical profiles exposed less cytotoxic effects of PMFs as compared to OH-PMFs as established by the MTT-method. It is worth mentioning here, a evaluation of two PMF members such as 3,5,6,7,3_,4_-hexamethoxyflavone (HexaMF) and 3,5,6,7,8,3_,4_-heptamethoxyflavone (HeptaMF) lasted less cytotoxic effects of HeptaMF as compared to HexaMF. A particular OPE enriched with HeptaMF, PMFs and OH-PMFs at low concentrations (10 _g/mL)appreciably down-regulated the appearance of a group of genes implicated in inflammatory rejoinder, together with *COX-2*, *TNF-α, ICAM-1, NFκB, IL-1β, IL-6*, and *IL-8* with an provocative catalog of −0.55.The strapping anti-inflammatory effects were then validated in a mouse carrageenan-induced paw edema model. Oral ingestion of OPE reduced paw edema drastically in a dose-dependent approach. A quantity of 250 mg/kg gavean has anti-inflammatory effect as compared to ibuprofen. A preface clinical study revealed that OPE was well tolerated showing no unsympathetic adverse reactions. Fortification of phyto extracts such as OPEs with definite polymethoxyflavones as anti-inflammatory bioactives is a gifted stratagem to find unsurprisingly derivative extracts that are effectual in opposition to ailments coupled with inflammation [15].

Lai., et al. (2011) reported that the molecular mechanism and chemopreventive effects of dietary administration of 0.01 and 0.05% hydroxylated PMFs in AOM-induced colonic tumorigenesis were investigated by institute of cancer research (ICR) in male mice at the age of 6 week twice weekly dose of 5mg/kg for 2 week. At 6 and 20 week, colonic tissues were collected from mouse. Dose-dependent dietary administration of hydroxylated PMFs feeding caused reduction in the number of aberrant crypt foci and tumours in colonic tissues, reduced the levels of inducible nitric oxide synthase, cyclooxygenase, cyclin D1 and vascular endothelial growth factor through interfering with Wnt/b-catenin and epidermal growth factor receptor/Ras/mitogen activated protein kinase signaling pathways as well as the activation of transcription factors NF-kB and STAT3 in colonic tissue by *in vivo* analysis [16].

Composition and contents of PMF and hydroxylated PMF in orange peel extract

OH-PMFs and PMFs	Concentration (mg/g OH-PMFs)
5-Hydroxy-3,6,7,8,3′,4′hexamethoxyflavone	254.78 ± 4.95
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone	396.42 ± 4.62
5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone	7.08 ± 0.11
5-hydroxy-6,7,8,4′-tetramethoxyflavone	74.96 ± 0.16
5-hydroxy-6,7,4′-trimethoxyflavone	115.52 ± 2.35
5-hydroxy-6,7,3′,4′-tetramethoxyflavone	44.5 ± 0.08
Nobiletin	10.41 ± 0.14
Heptamethoxyflavone	55.04 ± 0.46
Tangeretin	20.34 ± 0.62

Table c

Effects of dietary hydroxylated PMFs on AOM-induced ACF formation in ICR mice

Group	No. of mice	Body weight (g)	No.ACF/colon ACF	Large ACF	Incidence of ACF formation
AOM	15	37.9 ± 2.3	47 ± 3[b)]	23 ± 3[b)]	15/15 (100%)
AOM + 0.01% hydroxylated PMFs	15	38.4 ± 2.4	29 ± 2[b)]	13 ± 4[b)]	15/15 (100%)
AOM + 0.05% hydroxylated PMFs	15	38.5 ± 2.9	29 ± 6[b)]	10 ± 2[b)]	15/15 (100%)

Table d

a) All mice of each group were killed by decapitation at the end of wk 6. The colons were removed and fixed in 10% buffered
formalin. ACF in formalin-fixed colons were identified as crypts with increased methylene blue staining and expanded pericryptal spaces (n=15). The average number of ACF and large ACF (≥ 6 component crypts/focus) were expressed as mean ± SE *per* colon.

b) p< 0.01, compared with AOM-treated alone.

Effects of dietary hydroxylated PMFs on the AOM-induced colonic tumor in male ICR mice

Group	No. of mice	Body weight (g)	Liver (mg)	Spleen (mg)	Tumor multiplicity	Tumor incidence (%)
AOM	15	46.6 ± 3.2	2.5 ± 0.4	0.22 ± 0.04	7.8 ± 1.3[b)]	15/15 (100%)
AOM + 0.01% hydroxylated PMFs	15	40.6 ± 3.2	2.1 ± 0.4	0.23 ± 0.01	4.0 ± 0.8[c)]	15/15 (100%)
AOM + 0.05% hydroxylated PMFs	15	42.4 ± 4.2	2.2 ± 0.3	0.23 ± 0.02	1.5 ± 0.6[b)]	15/15 (100%)

Table e

a) All mice of each group were killed by decapitation at the end of week 20. Colon tissues were analyzed by H&E stain for microadneoma. The average numbers of tumor were expressed as mean ± SE per colon.

b) P <0.01, compared with AOM-treated alone.

c) P<0.01, compared with AOM-treated alone.

The data and analysis result demonstrated that dietary administration of hydroxylated PMFs treatment inhibited ACF formation, inhibited AOM induced iNOS and *COX-2* expression through down-regulating NF-kB and STAT3 signaling, prevent the AOM-upregulated EGFR and Ras signaling, inhibited the AOM induce colonic tumor formation.

Flavonoids quercetin

Hayashi., *et al*. (2000) reported that quercetin chalcone (QC) and pH-modified citrus pectin (MCP) are the modified substances of quercetin (a flavonoid) and citrus pectin (a polysaccharide found in the cell wall of plants). Earlier studies indicated that many antitumor properties due to immune stimulation, free radical scavenging, alteration of the mitotic cycle in tumor cells; gene expression modification, anti-angiogenesis activity and apoptosis induction are exhibited by quercetin. Prostate cancer and melanoma metastases has inhibited by MCP. The result examined the effects of MCP and QC on the size and weight of colon-25 tumors implanted in balb-c mice. Fifty mice were orally administered either 1 mL distilled water (controls), low-dose QC (0.8 mg/mL), high-dose QC (1.6 mg/mL), low-dose MCP (0.8 mg/mL) or high-dose MCP (1.6 mg/mL) on a daily basis, beginning the first day of tumor palpation (usually eight days post-implantation). A significant reduction in tumor size was observed at day 20 in all groups compared to controls. The groups given low-dose QC and MCP had a 29-percent

(NS) and 38-percent ($p<0.02$) decrease in size, respectively. The high-dose groups had an even more impressive reduction in size; 65 percent in the QC group and 70 percent in the mice given MCP (both $p<0.001$). MCP demonstrated the reduction in the growth of solid primary tumors, and QC has antitumor activity [17].

Relationship of citrus peel flavonoids to human cancers

Manthey., *et al*. (2000) reported that numerous types of phenols which are mainly comprised of hydroxycinnamates, flavonoid glycosides, and polymethoxylated flavones are found in rich quantity in citrus fruits as well as peel. Proliferation of a number of cancer cell lines is prevented by polymethoxylated flavones which are devoid of glycosidic linkages. Strong antiproliferative action was exhibited by synthetic methoxylated flavones which are proved to act similar activities and action as naturally found compounds. Experiments showed in various cases 10 micrometer occurred above IC50 values. It is demonstrated by various experiments, analysis and results that these polymethoxylated flavones, including hydroxylated flavones and flavanone exhibited antiproliferated as well as anticancer agents in humans [18].

Compound	µm	Lung	Colon	Breast ER-	Breast ER+	Prostate	Melanoma
(1) 3,5,6,7,8,3′,4′-heptamethoxyflavone	IC_{50}	4.6	2.1	0.9	0.2	1.8	9.9
	IC_{90}	14.5	8.8	2.8	2.3	7.4	16.4
(2) tangeretin	IC_{50}	3.2	1.6	1.3	0.34	0.54	0.27
	IC_{90}	6.7	4.0	3.5	2.7	2.7	3.8
(3) nobiletin	IC_{50}	3.5	4.7	1.2	2.9	1.0	0.50
	IC_{90}	10.1	13.9	3.7	6.2	2.2	2.0
(4) sinensetin	IC_{50}	13.7	9.5	3.9	5.5	16.5	10.8
	IC_{90}	23.6	13.9	7.4	9.7	22.5	17.9
(5) tetra-*O*-methylscutellarein	IC_{50}	21.5	6.3	0.80	0.53	3.9	2.9
	IC_{90}	39.4	13.7	2.9	2.4	8.1	7.6
(6) 5-desmethylnobiletin	IC_{50}	38.8	8.5	0.77	0.23	2.8	3.6
	IC_{90}	143	171	2.6	0.77	162	92
(7) tetra-O-methylisoscutellarein	IC_{50}	18.1	6.6	ND[a]	ND	2.6	11.3
	IC_{90}	25.8	19.7	ND	ND	11.5	19.7
(8) 5-desmethylsinensetin	IC_{50}	0.11	5.0	0.06	0.03	2.2	1.1
	IC_{90}	1.7	9.5	1.7	1.1	7.8	4.7
(9) quercetin 3,5,7,3′,4′- pentamethyl ether	IC_{50}	2.2	33	19	18	6.0	12.1
	IC_{90}	5.5	110	59	57	74	45
(10) quercetin 3,7,3′,4′- tetramethyl ether	IC_{50}	6.9	0.84	26	14	16	8.1
	IC_{90}	21	47	54	41	56	42
(11) limocitrin 3,5,7,4′-tetramethyl ether	IC_{50}	17	45	2.0	0.50	14	6.2
	IC_{90}	47	107	7.2	4.0	97	42
(12) quercetin 5,7,3′,4′-tetramethyl ether (S)*b*	IC_{50}	3.1	15.6	27	14.2	5.6	3.9
	IC_{90}	5.9	28	70	64	21	5.6
(13) limocitrin 3,7,4′-trimethyl ether (S)	IC_{50}	6.4	54	8.0	1.3	11	5.4
	IC_{90}	44	115	21	8.2	69	41
(14) quercetin 5,7,3′,4′-tetramethyl ether-3-acetate (S)	IC_{50}	21	14	8.5	3.0	20	14
	IC_{90}	27	22	20	15	27	21
(15) limocitrin 3,7,4′-trimethyl ether-5-acetate (S)	IC_{50}	2.3	18	2.1	0.11	2.6	5.3
	IC_{90}	17	69	8.0	1.6	23	20
(16) quercetin 3,7,3′,4′-tetramethyl ether-5-acetate (S)	IC_{50}	15	8.8	ND	ND	7.0	3.0
	IC_{90}	18	45	ND	ND	50	6.3
(17) 5,8-dihydroxy-3,7,3′,4′-tetramethoxyflavone (S)	IC_{50}	18	21	ND	ND	41	14
	IC_{90}	59	85	ND	ND	152	48

Table 1: Antiproliferative Activities (IC_{50} and IC_{90}) of Naturally Occurring and Synthetic Analogues of Polymethoxylated Flavones in Citrus against Six Human Cancer Cell Line.

Compound	µm	lung	colon	prostate	melanoma
(1) 3,5,6,7,8,3¢,4¢-heptamethoxyflavone	LC_{50}	48	41	126	34
	LC_{90}	>200	124	>200	142
(9) quercetin 3,5,7,3¢,4¢-pentamethyl ether	LC_{50}	79	47	110	47
	LC_{90}	>200	165	>200	191
(10) quercetin 3,7,3¢,4¢- tetramethyl ether	LC_{50}	47	41	>153	56
	LC_{90}	>200	>200	>200	>200
(11) limocitrin 3,5,7,4¢-tetramethyl ether	LC_{50}	50	47	111	47
	LC_{90}	>200	133	>200	190
(12) quercetin 5,7,3¢,4¢-tetramethyl ether	LC_{50}	6.4	7.8	117	6.9
	LC_{90}	44	35	>200	106
(13) limocitrin 3,7,4¢-trimethyl ether	LC_{50}	85	39	>100	39
	LC_{90}	>200	97	180	>200
(15) limocitrin 3,7,4¢-trimethyl ether-5-acetate	LC_{50}	24	22	104	23
	LC_{90}	171	72	>200	123
(16) quercetin 3,7,3¢,4¢-tetramethyl ether-5-acetate	LC_{50}	18	13	112	2.5
	LC_{90}	>200	87	>200	170
(17) 5,8-dihydroxy 3,7,3¢,4¢-tetramethoxyflavone	LC_{50}	>200	48	>200	>200
	LC_{90}	>200	189	>200	>200

Table 2: Toxicity ($LC_{50,90}$) of Polymethoxylated Flavones toward Six Human Cancer Cell Lines.

Compound	µm	lung	colon	Breast ER+	Prostate	melanoma
(18) rhamnetin	IC_{50}	38	76	85	22	25
	IC_{90}	174	>200	>200	158	>200
19) kaempferol	IC_{50}	115	42	>200	95	>200
	IC_{90}	>200	>200	>200	>200	>200
(20) chrysoeriol	IC_{50}	17	20	7	30	23
	IC_{90}	36	46	20	69	59
(21) apigenin	IC_{50}	41	29	22	37	41
	IC_{90}	100	85	85	93	125
(22) luteolin	IC_{50}	3.1	10.5	21	32	32
	IC_{90}	12	28	46	70	77
(23) quercetin	IC_{50}	59	40	73	86	>200
	IC_{90}	158	115	168	181	>200

Table 3: Antiproliferative Activities (IC_{50} and IC_{90}) of Hydroxylated Flavone Aglycons against Five Human Cancer Cell Lines.

compound	µm	lung	colon	Breast ER+	prostate	melanoma
(24) eriodictyol	IC_{50}	87	62	56	42	>200
	IC_{90}	>200	180	156	180	>200
(25) hesperetin	IC_{50}	181	149	181	181	>200
	IC_{90}	>200	>200	>200	>200	>200
(26) naringenin	IC_{50}	102	154	84	150	77
	IC_{90}	>200	180	>200	>200	158

Table 4: Antiproliferative Activities ($IC_{50,90}$) of Naturally Occurring Hydroxylated Flavanone Aglycons against Five Human Cancer Cell Lines.

Compound	lung	colon	Breast ER+	prostate	melanoma
(27) diosmin	>200	>200	>200	>200	>200
(28) naringin	>200	>200	>200	>200	>200
(29) isovitexin	>200	>200	17	>200	>200
(30) neohesperidin	>200	>200	>200	>200	>200
(31) prunin	>200	>200	>200	>200	>200
(32) quercetrin	>200	>200	>200	>200	>200
(33) isosakuranetin rutinoside	>200	>200	>200	>200	>200
(34) rutin	>200	>200	>200	>200	>200
(35) hesperetin trisaccharide	>200	>200	>200	>200	>200
(36) narirutin 4¢-glucoside	>200	>200	>200	>200	>200
(37) hesperetin 7-glucoside	42	>200	46	86	61
(38) hesperidin	106	77	51	101	>200
(39) neoeriocitrin	70	>200	>200	>200	>200
(40) rhoifolin	>200	>200	>200	>200	>200
(41) neodiosmin	>200	>200	>200	>200	>200
(42) margaretin	>200	>200	>200	>200	>200

Table 5: Antiproliferative Activities (IC_{50}) of Flavanone and Flavone Glycosides against Five Human Cancer Cell Lines.

Citrus peel flavonoids against human squamous cell carcinoma

Kandaswami., *et al*. (1991) reported that in vitro study of citrus flavonoids effects on the growth of a human squamous cell carcinoma cell line (HTB43) demonstrated the health benefit and anti-cancer effects by examination of four plant flavonoids (taxifolin, nobiletin, quercetin and tangeretin). For 3-7 days, cell cultures were treated with each flavonoid (2-8 µg/ml). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide could be utilized for cellular growth to determine the viability of cell by counting cells obtained from a colorimetric assay. The polymethoxyflavonoids quercetin and taxifolin demonstrated no significant prevention at any concentration tested in experiments while analysis showed that other flavonoids such as nobiletin and tangeretin remarkably inhibited cell growth on days 5 and 7 at all concentrations. On day 3, the cell growth prevention ranged from 70-72% at µg/ml. while on day 5, the inhibition observed was 61-88% at 2-4 µg/ml [19].

Flavonoids apigenin and quercetin

Caltagirone., *et al*. (2000) reported that numerous biological roles have been played by flavonoids containing chemoprevention and tumor growth reduction. *In Vivo* study was conducted in order to investigate and examine the effects of numerous polyphenols through comparison between their classes, on the metastatic potential and growth of B16-B16 melanoma cells. Quercetin, apigenin, (–) epigallocathechin-3-gallate (EGCG), resveratrol, and the anti-estrogen tamoxifen was compared to administrate intraperitoneal by the introduction of B16-B16 cells into syngeneic rats to inhibit the tumor growth without toxicity in a dose-dependent manner. The result reveals the descending order of potency was EGCG > apigenin = quercetin = tamoxifen > resveratrol > control. Moreover, a significantly potential anti-toxic dose of cisplatin was demonstrated to study inhibitory effect by polyphenols. The introduction of quercetin, apigenin, and tamoxifen (but not EGCG or resveratrol) potentiated resulted in the reduction of a number of B16-BL6 colonies in the lungs as well as decrease the invasion of B16-BL6 cells *in vitro*, dose-dependent delay of lung colonization. Quercetin and apigenin showed more affectivity as compared to tamoxifen. Furthermore, quercetin and apigenin decrease melanoma growth and invasive and metastatic potential and act as vital tool in the treatment of metastatic melanoma [20].

Flavonoid morin

Tanaka., *et al*. (1999) reported that flavonoid morin was dietary fed in male F344 rats to treat azoxymethane (AOM)-initiated colorectal carcinogenesis to determine the modifying effect during the initiation and post-initiation stages. Colorectal neoplasms were developed in a group of 55 animals by the injections of 15 mg/kg body wt AOM for 3 weeks. The dietary exposure of 500 p.p.m morin for 5 weeks during the initiation feeding and for 28 weeks during the post initiation feeding was carried. The result showed a less reduction of 43 and 29% in adenocarcinoma in the large intestine of rats fed with AOM combined with diet as compared to the rats provided with AOM alone (75%). Flavonoid morin caused reduction in the polyamine levels in colorectal mucosa and blood, prohibit the proliferative cell nuclear antigen-positive index in aberrant crypt foci, increased the enzymes glutathione S-transferase and quinone reductase activities in large bowel [21].

Future scope

This review summarizes the results of currently available data regarding *in vivo* and *in vitro* analysis of anti-cancer, anti-inflammatory and anti-proliferative activities of Citrus peel have multiple active molecular components including flavonoids, Provide opportunities for human clinical trials to deal with preventive and therapeutic effects. Formulation of numerous new products could be proved as effective and beneficial to deal with the fatal disease like cancer.

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