REVIEW

Molecular Mechanisms of Casticin Action: an Update on its Antitumor Functions

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Abstract

Casticin (3', 5-dihydroxy-3, 4', 6, 7-tetramethoxyflavone) is an active compound isolated from roots, stems, leaves, fruits and seeds of a variety of plants. It is well known for its pharmacological properties and has been utilized as an anti-hyperprolactinemia, anti-tumor, anti-inflammatory, neuroprotetective, analgesic and immunomodulatory agent. Recently, the anticancer activity of casticin has been extensively investigated. The resulkts showed that it exerts protective potential by targeting apoptosis, considered important for cancer therapies. In this article, our aim was to review the pharmacological and therapeutic applications of casticin with specific emphasis on its anticancer functions and related molecular mechanisms. Chemotherapeutic effects are dependent on multiple molecular pathways, which may provide a new perspective of casticin as a candidate anti-neoplastic drug. This review suggests that additional studies and preclinical trials are required to determine specific intracellular sites of action and derivative targets in order to fully understand the mechanisms of its antitumor activity and validate this compound as a medicinal agent for the prevention and treatment of various cancers.

Keywords: Casticin - flavonoid - natural compounds - cancer therapy - apoptosis

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Introduction

All over the 'realm of History', human beings relied on natural products as a primary source of medicine to cure many diseases and plants are vital source of novel natural medicines (Mukherjee et al., 2010). Natural medicines have been proven to be a central source of narrative agents with a pharmaceutical potential (Ji et al., 2009). Herbal medicine has held and still holds an important position in primary health care in China and western countries as a fertile source of novel lead molecules and constituted a pharmaceutical potential as part of modern drug discovery (Christen and Cuendet, 2012). One potential source of novel anticancer agents is natural plant products (Cragg and Newman, 2005). Flavonoids constitute a large family of the phytochemicals, including flavanols, flavones, flavonols, flavanones, anthocyanidins, proanthocyanidins and isoflavones (Leibowitz and Yu, 2010). The major sources of flavonoids are fruit and vegetables. This class of phytochemicals possesses various biological functions such as anti-cancer, anti-proliferative, antioxidant, pro-apoptotic, anti-inflammatory, and neuroprotective activities (You et al., 1998; Manosroi et al., 2005; Jiang et al., 2013; Zhu et al., 2013; Tan et al., 2014).

Casticin (3',5-dihydroxy-3,4',6,7-tetramethoxyflavone) is one of the bioactive flavonoids obtained from polyphenol plants, which are composed of a wide variety of molecules that are classified into several categories, according to their chemical type, such as phenolic acids, flavonoids, stilbenes, and lignans (Siasos et al., 2013). Casticin is a main active compound in roots, aerial parts, seed, wood, stems, leaves and fruits of variety of plants (Figure 1 and Table 1), has been reported to be responsible for a wide spectrum of biological and pharmacological activities including immunomodulatory (Mesaik et al., 2009; Ling et al., 2012), anti-hyperprolactinemia (Hu et al., 2007b; Ye et al., 2010), anti-tumor (Haidara et al., 2006; Shen et al., 2009; Ling et al., 2012; Zeng et al., 2012), neuroprotetective (Ling et al., 2012), anti-inflammatory (Lin et al., 2007b; Choudhary et al., 2009; Velpandian et al., 2013) and analgesic activities (Lee et al., 2012).

In addition, recent studies also reported that casticin can enhance efficiency in combination with chemotherapeutics drugs (Xia et al., 2013). This review article is an attempt to cover recent information available on the development of biological and pharmacological potential of casticin in the scientific literature compiled from databases such as PubMed, SpringerLink, ScienceDirect, Oncology and

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MEDLINE, further to provide comprehensive evidence insight into its natural sources, anticancer properties and mechanisms of action of this drug, which may provide a new perspective of casticin as a anti-neoplastic drug candidate for future cancer therapeutics.

Natural sources of casticin

Accumulated data indicate that casticin (Figure 1) has been isolated from many plants species, using ultra-high performance liquid chromatography diode array detector (UHPLC-DAD) in an ODS column under a mixed solvent system of acetonitrile and water, and microemulsion electrokinetic chromatography (MEEKC), structure was elucidated on the basis of NMR analysis and the compound was dissolved in dimethylsulfoxide (DMSO) to demonstrate the activities of casticin on various model system of different human diseases (Haidara et al., 2006; Hogner et al., 2013).

Further casticin, being a flavonoid natural compound is located in fruits, vegetables, nuts, seeds, herbs, spices, stems, and flowers (Jiang and Morgan, 2004; Miyahisa et al., 2006). It has also shown a variety of pharmacological properties of therapeutic interest such as anti-inflammatory and anticancer activities (Manthey et al., 2001; Touil et al., 2009). The summary of plants containing casticin, parts used, and biological/pharmacological activities, is shown in Table 1.

As shown in Figure 1, accumulated data indicate that casticin was isolated from many plant species such as, namely *Vitex agnus castus* (Choudhary et al., 2009; Mesaik et al., 2009; Webster et al., 2011; Righeschi et al., 2012; Hogner et al., 2013), *Daphne genkwa* (Xie et al., 2011), *Achillea millefolium* (Haidara et al., 2006; Csupor-Loffler et al., 2009), *Ficus microcarpa* (Wang et al., 2010), *Vitex rotundifolia* (Ono et al., 2002; Hu et al., 2007b; Shen et al., 2009; Ye et al., 2010; Koh et al., 2011), Fructus viticis (Hu et al., 2007b; Guan et al., 2010; Chen et al., 2011b; Yang et al., 2011; Zeng et al., 2012; Zhou et al., 2013a), *Vitex negundo* (Diaz et al., 2003; Kunwar et al., 2010; Velpandian et al., 2013), *Crataegus pinnatifida* (Kao et al., 2005), *Pavetta crassipes* (Mali and Dhake, 2011), *Nelsonia canescens*, *Butea frondosa Koen* , *Dalbergia odorifera* (Mali and Dhake, 2011), *Bryonia laciniosa* (Aggarwal et al., 2011), *Citrus unshu* (Mali and Dhake, 2011; Nagoor et al., 2011), *Centipeda minima*

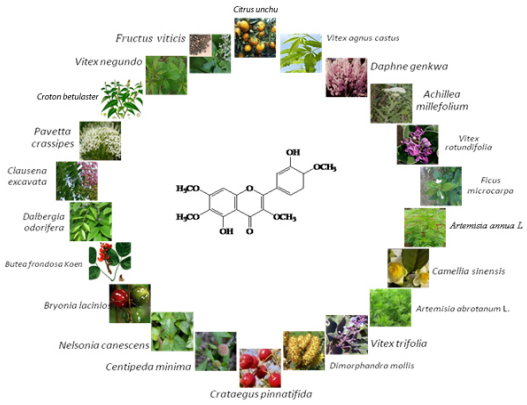

Figure 1. Chemical Structure and Natural Sources of Casticin

(Mali and Dhake, 2011), *Clausena excavate* (Manosroi et al., 2005), *Croton betulaster* (de Sampaio e Spohr et al., 2010; Freitas et al., 2011), *Dimorphandra mollis* (Freitas et al., 2011), *Artemisia abrotanum* L. (Hernandez et al., 1999), *Artemisia annua* L (Han et al., 2007), *Camellia sinensis* (Kunwar et al., 2010), and *Vitex trifolia* (Remberg et al., 2004; Ling et al., 2012).

Biological activity of casticin

Biological activity is the ethno-pharmacological approach's leading thread, its evaluation is necessary to validate traditional use of casticin. Based on the evidences related to casticin *in vitro* and *in vivo* activities have been made to investigate the biological properties ascribed to casticin. In the momentum it was held that, casticin has sound medicinal importance. Studies on casticin showed significant suppressive effect on the chemotaxic action at higher concentrations on fMLP (10^{-8}M) stimulated neutrophils. It also showed a potent suppressive effect on PHA stimulated T-cell (PMBC) (Mesaik et al., 2009). It inhibited eosinophil migration and activity of chemokines and adhesion of molecules involved in the inflammatory process of asthma by suppressing the NF-ϰB pathway (Koh et al., 2011). Casticin's biological effects have been reported in wide spectrum of indications (Table 1), including inflammation (Lin et al., 2007b; Koh et al., 2011; Lee et al., 2012), asthma (Koh et al., 2011), tumor (Ono et al., 2002; Hu et al., 2007b; Shen et al., 2009; Ye et al., 2010; Koh et al., 2011), pre-menstrual syndrome (Hu et al., 2007b; Webster et al., 2011), immunomodulation (You et al., 1998), headache (Choudhary et al., 2009; Mesaik et al., 2009; Webster et al., 2011; Righeschi et al., 2012; Hogner et al., 2013), rheumatoid arthritis (You et al., 1998), conjunctivitis (Remberg et al., 2004), trachoma, gonorrhoea, and toothache (Diaz et al., 2003; Kunwar et al., 2010; Velpandian et al., 2013).

On the basis of previous *in vitro*, *in vivo* and epidemiological studies, it has demonstrated that casticin have a great anticancer potential by targeting various signaling pathways related to the initiation, progression and metastasis of cancer. It appears that casticin hold great promise for cancer chemoprevention and treatment through anti-proliferation, blockage of the cell cycle, induction of apoptosis, inhibition of angiogenesis and elimination of drug resistance. This review summarizes the emerging data concerning bioactive compound with multidirectional mechanisms of action including caspase-mediated pathway and regulation of apoptosis-related proteins.

Targeting apoptosis pathways in cancer with casticin

Apoptosis is defined as an extremely synchronized mode of cell death. It is characterized by distinct morphological features, including chromatin condensation and nuclear fragmentation (Hengartner, 2000; Elmore, 2007a). The importance of signaling has been recognized in cell regulation during normalcy and disease (Hanahan and Weinberg, 2000; Evan and Vousden, 2001). Chemopreventive agents are apoptotic and induce death

Table 1. Plants Containing Casticin with their Biological Functions

Name of plant					
Botanical name	Common name	Chinese name	Part used/extract	Disease/function	References
Vitex agnus castus	Chaste Tree (Vitex)	--	Fruit/whole plant	Pre-menstrual syndrome, inflammation, headache, anxiety, immunomodulation,	(Choudhary et al., 2009; Mesaik et al., 2009; Webster et al., 2011; Righeschi et al., 2012; Hogner et al., 2013)
Daphne genkwa	--	--	Aqueous	Edema, asthma, anticancer	(Xie et al., 2011)
Achillea millefolium	yarrow	--	Aerial part, Whole plant	hardness of the uterus, anti-tumor	(Haidara et al., 2006; Csupor-Loffler et al., 2009)
Ficus microcarpa	Chinese Banyan	--	Aerial/roots/leaves	Chronic bronchitis, enteritis	(Wang et al., 2010)
Vitex rotundifolia	Beach vitex	Dan ye manjing	Fruit/Aqueous	Inflammation, asthma, antitumor	(Ono et al., 2002; Hu et al., 2007a; Hu et al., 2007b; Shen et al., 2009; Guan et al., 2010; Ye et al., 2010; Chen et al., 2011a; Koh et al., 2011; Zeng et al., 2012; Zhou et al., 2013a)
Fructus viticis	Chaste tree	Mang Jing Zi	Fruit	Anticancer, inflammation	(Hu et al., 2007b; Guan et al., 2010; Chen et al., 2011a; Zeng et al., 2012; Zhou et al., 2013a)
Vitex negundo	Five-leaved chaste tree	Huang jing zi	leaves	Rheumatoid arthritis, conjunctivitis, trachoma, gonorrhoea, toothache	(Diaz et al., 2003; Kunwar et al., 2010; Velpandian et al., 2013)
Crataegus pinnatifida	Chinese Haw	--	Leaves/Fruit	Declining cardiac performance, Deficiency in coronary blood supply	(Kao et al., 2005)
Pavetta crassipes	Chiwowo	--	Leaves/Aqueous	Asthma	(Mali and Dhake, 2011)
Nelsonia canescens	Blue Pussyleaf	--	Leaf	-----	(Mali and Dhake, 2011)
Butea frondosa Koen	Forest flame	--	Leaves/Aqueous	-----	(Mali and Dhake, 2011)
Dalbergia odorifera	Dalergia , dalbergia	Jiang xiang huangtan	Heart wood	-----	(Mali and Dhake, 2011)
Bryonia laciniosa	Native bryony	--	Leaves/Chloroform extract	Inflammation	(Aggarwal et al., 2011)
Citrus unshu	Tangerine	Wenzhou Migan	Peels	Cancer and inflammation	(Mali and Dhake, 2011; Nagoor et al., 2011)
Centipeda minima	Spreedind sneezeweed	--	Aerial parts	-----	(Mali and Dhake, 2011)
Clausena excavata	Clausena	Jia juang pi	Wood/Aqueous	-----	(Manosroi et al., 2005)
Croton betulaster	----	----	Leaves	Cerebral Cortical Progenitors, cancer, constipation, diabetes	(de Sampaio e Spohr et al., 2010; Freitas et al., 2011)
Dimorphandra mollis	----	----	Seeds	vascular disorders; hypertension	(Freitas et al., 2011)
Artemisia abrotanum L.	Lad's love, Old Man, Maiden's Ruin	----		Allergic rhinitis	(Remberg et al., 2004)

in cancerous cells (Rasul et al., 2011a; Rasul et al., 2011b; Shi et al., 2011; Rasul et al., 2012a; Rasul et al., 2012b; Rasul et al., 2012c; Rasul et al., 2012d; Rasul et al., 2013). Casticin induced early or late apoptosis in a dose dependent manner. Sub-G1 accumulation is usually considered as an apoptotic death profile, this evidence is in sync with mode of cell death and characterized by a set of physiological phenomena, including mitotic catastrophe

(Shen et al., 2009), cell membrane blabbing, chromatin condensation, and nuclear fragmentation (Hengartner, 2000; Haidara et al., 2006; Elmore, 2007b). The positive impact of this phenomenon signaling has been recognized in cell regulation (Hanahan and Weinberg, 2000; Evan and Vousden, 2001).

Casticin's inhibitory effects on cell proliferation (Song et al., 2010) and induction of apoptotic cell death of human cervical cancer HeLa cells (Zeng et al., 2012) are primarily mediated by mitochondrial dependent ROS generation and activation of caspase -3 and -9 (Chen et al., 2011a). Apoptotic cells characteristics such as translocation of phosphatidylserine (PS) from internal cell surface to external cell surface, in the early stage of apoptosis (Jiang et al., 2013). Further, after activation of a cascade of various caspases, caspase-3, and PARP are cleaved and activated, followed by DNA fragmentation, nuclear fragmentation, the appearance of apoptotic bodies and cellular shrinkage are considered essential features of apoptosis (Shen et al., 2009). In the late stage of apoptosis, major DNA with formation of typical DNA ladder of 180 – 220 bp can be seen (Collins et al., 1997).

Casticin inhibits the growth of PANC-1 cells by arresting the cell cycle at G2/M phase and inducing apoptosis through upregulation of Bax protein expression, down-regulation of Bcl-2 protein expression and cleavage of caspase-3 (Ding et al., 2012). Casticin triggers anti-proliferative effects and apoptosis in various cancer cells including human prostate (Diaz et al., 2003), colon (Tang et al., 2013), oral epidermoid carcinoma (Kobayakawa et al., 2004), breast cancer (Song et al., 2010), and leukemia cells (Diaz et al., 2003; Shen et al., 2009; Righeschi et al., 2012).

Targeting cancer cells by mitochondria-mediated apoptosis

Disruption of mitochondrial integrity is an important component of the apoptosis execution machinery. It is also one of the early events leading to apoptosis, which contain pro-apoptotic proteins such as cytochrome c. Extensive studies have revealed a rapid release of cytochrome c from the mitochondria to the cytoplasm triggered by casticin and activation of its signaling in activation of mitochondrial signaling in a ROS-dependent manner in HeLa cells (Zeng et al., 2012). It has no significant effect on Bcl-2 expression but caused decreases in Bcl-XL and XIAP (Elmore, 2007b; Chen et al., 2011a). Further, apoptosis can be initiated through two alternative signaling pathways: the death receptor-mediated extrinsic apoptotic pathway and the mitochondrial-mediated intrinsic apoptotic pathway (Kok et al., 2005; Reuter et al., 2008). It is increasingly becoming apparent that the mitochondria play critical roles in the regulation of various apoptotic processes leading to cell death (Birt et al., 2001), including drug-induced apoptosis (Cory and Adams, 2002).

It is considered that the mitochondrial, death pathway is controlled by members of the Bcl-2 family (Brunelle and Letai, 2009; Leibowitz and Yu, 2010), which play a central regulatory role to decide the fate of the cells via the interaction between pro- and anti-apoptotic members. Casticin triggers mitochondrial permeability transition (Bradham et al., 1998; Reed et al., 1998; Yang and Cortopassi, 1998a; Yang and Cortopassi, 1998b; Antonsson and Martinou, 2000), which is required for the complete release of cytochrome c. Casticin resulting in production of ROS in a caspase-dependent through reduction of the ratio Bcl-2/Bax and thus favor apoptotic pathways; that are mean Bax, a pro-apoptotic protein in the Bcl-2 family, are up-regulated and this induction of Bax, release cytochrome c in the cytosol, caspase-3 activation and PARP cleavage. In addition, the research carried out by (Chen et al., 2011a) reports that the downregulation of XIAP likely reflects an increase in protein degradation and concluded casticin-induced apoptosis of human cervical cancer cells via the mitochondrial death pathway.

Targeting cancer cells by ROS-mediated apoptosis

ROS, active, transitory and oxygenic compounds are known mediators of intracellular signaling of cascades, including H_2O_2, O_2, and hydroxyl radicals, are metabolites of biochemical processes in the body. In the genesis, ROS is the result of disordered mitochondria function and metabolite augmentation, and there may be ways to regulate ROS selectively in cancer cells (Kim et al., 2010). It is an integrated system to clear ROS in the body to maintain balance. Oxidation of cell membrane phospholipids, enzymes and DNA (Lin et al., 2007a; Appierto et al., 2009) by excessive generation of ROS can induce oxidative stress, alter the function of signal transduction pathways, platelet aggregation, immune control, and the regulation of cell growth, and in some cases can also cause necrosis or apoptosis (Chen and Chan, 2009; Wei et al., 2010). Moreover, casticin generates ROS in human cervical cancer cells and places special emphasis that NAC suppressed the apoptosis of HeLa cells by casticin which indicated that its apoptotic effect is dependent on ROS generation (Chen et al., 2011a).

Targeting cancer cells by caspase-mediated apoptosis

Caspases play important role in apoptosis via triggering of the death receptors and mitochondrial pathways to emit various pro-apoptotic signals to accomplish the programmed cell death (Nunez et al., 1998; Thornberry and Lazebnik, 1998). For the overall functional aspect of caspases, the activation of the caspase cascade requires both initiator caspases, such as caspase-8, and -9, and effector caspases, such as caspase-3. It is generally recognized that there are two major apoptotic pathways: one involves death signals transduced through death receptors, and the other relies on a signal from the mitochondria (Nunez et al., 1998; Thornberry and Lazebnik, 1998; Woo et al., 2003; Li et al., 2005).

Several studies reveal that both pathways are involved in an ordered activation of a set of caspases, which in turn cleave cellular substrates leading to the morphological and biochemical changes of apoptosis (Woo et al., 2003; Yang et al., 2010). The dissipation of $\Delta\Psi m$, rapid release of cytochrome c from the mitochondria to the cytosol, activated caspase-9, -8 and -3 and DNA fragmentation are triggered by casticin (Chen et al., 2011a). Furthermore, the presence of the inhibitors such as z-VAD-FMK for caspase-8 and z-LEHD-FMK for caspase-9 attenuated the

apoptosis induced by casticin in human cervical cancer PLC-PRF-5 cells (Yang et al., 2011).

The chemotherapeutic agents cause the dissipation of ΔΨm, along with cytochrome c release from the mitochondria and the subsequent activation of caspase-9 through binding to the protein Apaf-1 mediates apoptosis (Li et al., 1997; Thornberry and Lazebnik, 1998). Casticin is an effective apoptosis-inducing agent in human hepatocellular carcinoma (HCC) cells, which acts through depleting intracellular GSH content and up-regulating DR5, and subsequent activation of caspase-3, -8 and -9. It has been shown that that casticin can inhibit the growth of HCC cells independent of p53 status and thus can be suggested as a good candidate for additional evaluation as a cancer therapeutic agent for human HCC as well as other types of cancer (Yang et al., 2011).

Targeting cancer cells by regulating apoptosis related proteins

p53: The cancer suppressor p53, considered as a guardian of the genome, is an important factor affects the cell response to drug effects on growth inhibition and apoptosis induction (O'Connor et al., 1997; Pirollo et al., 2000). It has also been demonstrated that casticin induced apoptotic cell death in p53 mutant or null breast cancer cell lines (Haidara et al., 2006) and in p53 mutated human cervical cancer HeLa cells (Csupor-Loffler et al., 2009; Chen et al., 2011a). Many studies have been carried out to supports the notion that cells with wild-type p53 exhibit increased sensitivity to radiation or chemotherapeutic agents and revealed that cells with mutant p53 sequence tends to exhibit less growth inhibition in the screen than the wild-type p53 cell lines when treated with the majority of clinically used anticancer agents including DNA cross-linking agents, anti-metabolites, and topoisomerase I and II inhibitors (O'Connor et al., 1997). Whereas, cells lacking wild-type p53 expression still undergoes apoptosis but need a relatively high doses of radiation or chemotherapeutic drugs (Bae et al., 1996).

Casticin acts in a p53-independant manner with regards to its interaction with tubulin, cell cycle arrest in G2/M, p21 induction, Cdk1 activity inhibition, cyclin A down-regulation and finally induction of apoptotic death (Hofseth et al., 2004). As a multi-tasking and multi-directional agent in different cells, it is important for the suppression of tumor formation. The suppressing mechanism of casticin for malignant tumors occurs through c-Myc in p53 mutated Hs578T cells (Song et al., 2010). Furthermore, striking apoptosis was also confirmed in human glioma cells, accompanied by the up-regulation of caspase-3, p53 and pro-apoptotic protein Bax. These effects were absent when the caspase inhibitor z-VAD-

Table 2. Molecular Targets of Casticin in Different Cancer Types

Type of cancer	Cell lines	EC50/Concent	Targets	References
Cervical	HeLa, CasKi, SiHa	4μM or 2μM - 4μM	ROS↑, JNK↑, Bcl-2↓, Caspace-3- 9↑, Cyclin B1↓, Bax↑, Bcl-xL↓, XIAP↓, MMP↑	(Yang et al., 2010; Chen et al., 2011a; Xie et al., 2011; Zeng et al., 2012)
Pancreatic	PANC-1	40μM or 20μM-40μM	Bcl2↓, Bax↑, Caspace-3↑	(Ding et al., 2012)
Colon	Col2	8.6 +/- 0.3 ng/ml	TRAIL↑, Bcl-xL↓, Bcl-2↓, survivin↓, XIAP↓, cFLIP↓, DR5↑	(Tang et al., 2013)
Breast	MCF-7, Hs578T	0.25 and 0.53 μM/L	c-Myc↓, p21↑, Bcl-2↓	(Song et al., 2010)
Lung	A549, H460, H157	1.8 to 3.2 and 10.32 μM/L	DR5↑; NF-kB↓, MMP↑, cytochrome c↓, IκB-α↓, procaspase-9 and -3↑, XIAP↓, Bcl-XL↓, Bax↑, Bid↑	(Koh et al., 2011; Zhou et al., 2013a)
Gastric	BGC-823, SGC-7901 and MGC-803	1 or 5.6 μM	DR5↑, ROS↑, cFLIP↓, Bcl-2↓, XIAP↓, survivin↓	(Wang et al., 2010; Zhou et al., 2013b)
Hepatocellular carcinoma	HepG2, PLC/PRF/5	2.0 μM/L	CDK1↓, cdc25B↓, cyclin B↓, FOXO3a↓, FoxM1↓, CDK1↓, $p27^{KIP1}$↑, DR5↑	(Yang et al., 2011; He et al., 2013)
Glioma	U251, GL-15, U87, U373	50-100 μM	p53↑, Bax↑, Caspace-3↑	(Freitas et al., 2011; Feng et al., 2012; Liu et al., 2013)
Leukemia	CCRF-CEM; CEM/ADR5000, K562, Kasumi-1, HL-60	1.57 μM; 10μM; 5.95, 4.82 and 15.56μM	NF-κB↓, $p21^{waf1}$↑, $p27^{kip1}$↑, PI3K/Akt↓, caspace-3↑, PAPR↑	(Shen et al., 2009; Righeschi et al., 2012)
Prostate	KB, LNCaP, Lu1, PC3	0.5-0.7μM , 28.8μM	ROS↑, Bcl-2↓, Caspace-3↑, Bax↑	(Diaz et al., 2003; Meng et al., 2012)

fmk or p53 inhibitor PFTα were applied, suggesting that casticin could trigger cell apoptosis in a caspase-3 and p53-dependent manner (Liu et al., 2013). Accumulated data support that casticin can induces in p53-dependent and –independent manner in various cancer cells. Further studies are required to confirm these effects on p53 signaling pathways.

NF-κB: Detail study of literature validated that casticin may act in part by affecting NF-κB signaling pathway (Gillet et al., 2004; Nam, 2006). The expression of the inflammatory mediators is regulated by NF-κB (Ghosh et al., 1998). It has been described to inhibit NF-κB along with many other flavonoids known as NF-κB inhibitors (Gillet et al., 2004; Nam, 2006). NF-κB plays critical role in wide variety of physiological and pathological processes, such as regulating immune response, cell proliferation and apoptosis. They go on to say that a number of proteins in case (NF-κB1 and NF-κB2, each with two alternatively spliced forms, and REL-A, REL-B and c-REL) can form dimers, which are able to bind specific DNA motifs in the promoters of target genes (Brasier, 2006; Gilmore, 2006; Nam, 2006). These heterodimers can activate the transcription of about 200 target genes (Perkins, 2007). Inactive NF-κB1 or NF-κB2 proteins are complexes with IκBα (inhibitory κB) proteins in the cytosol and the phosphorylation of IκBα by IκBα kinase (IKK) leads to IκB degradation and translocation of NF-κB1 and NF-κB2 into the nucleus (Kobayakawa et al., 2004).

Further, the ROS-mediated NF-κB pathway is required for activation of endothelial cell adhesion molecules (Chen et al., 2003). Casticin significantly downregulated vascular inflammation, through inhibition of ROS–NF-κB pathway in vascular endothelial HUVEC cells (Lee et al., 2012). In clinical point view, NF-κB activation is involved in many chronic disease conditions, mainly in the development of atherosclerosis (Kanters et al., 2003). Nuclear NF-κB, p65 translocation and phosphorylation of IκB-α lead to the activation of specific target genes including VCAM-1, ICAM-1, and E-selectin (Liang et al., 2004). Furthermore, the expression of eotaxin, RANTES, VCAM-1, ICAN-1 and activation of eosinophilic inflammation involved in the pathogenesis of asthma is known to be mediated by the NF-kB signaling cascade (Wong et al., 2002; Kuldo et al., 2005; Zerfaoui et al., 2008; Li et al., 2009).

PI3K-Akt: Phosphatidylinositol 3-kinase/Akt signaling pathway is implicated to be one of the most important pathways for cell survival and inhibition of apoptosis (Carnero et al., 2008). It has been demonstrated that Akt can regulate a number of cellular processes, such as cell proliferation and cell growth (Klippel et al., 1996). Inhibition of phosphate-Akt (pAkt) will induce acute myeloid leukemia apoptosis (Papa et al., 2008). Inactivating Akt is a key mechanism for apoptosis induced by various anti-leukemia drugs (Lee et al., 2005; Loges et al., 2006). The PI3K/Akt signaling pathway can override G2/M cell cycle arrest induced by anti-cancer agents (Lee et al., 2005). Casticin inhibited PI3K/Akt signaling pathway in K562 cells and PI3K/Akt inhibitor enhanced casticin-induced cell death (Shen et al., 2009). Another complementary element ERK and PI3K/Akt signal pathway are two important signal pathway associated with cell survival (Xia et al., 1995; Kennedy et al., 1997).

Casticin and its synergistic activity with other chemotherapeutic drugs

Casticin is a multi-targeting molecule that enhances TRAIL-induced apoptosis and triggers G2/M growth arrest through the downregulation of cell survival proteins and the upregulation of DR5 receptors through actions on the ROS-ER stress-CHOP pathway (Zhou et al., 2013b). It is also shown that casticin potentiates TRAIL-induced apoptosis through downregulation of cell survival proteins and induction of DR5 mediated by ROS (Tang et al., 2013). In other hand, a direct effect of casticin on cyclin-A could also be involved in Cdk1 inhibition. The same author also reported that the anti-apoptotic protein Bcl-2 is down regulated, leading to apoptotic cell death (Haidara et al., 2006).

Conclusions and future perspectives

Casticin, naturally occurring compound, has been shown a good pharmacological potentially promising therapeutic effect including anti-inflammatory and anti-tumor effects. Casticin is located in fruits, vegetables, seeds, herbs, stems, roots, wood, and flowers of the many plants. The previous *in vitro* and *in vivo* studies demonstrated the potential applications of casticin to inhibit the growth of several human cancers by targeting cancer cells through a number of parameter including ROS, and capase-mediated apoptosis or by regulating apoptosis related proteins such as NF-κB, p53, and PI3K-Akt. Furthermore, casticin has synergistic activity with other chemotherapeutic drugs such as TRAIL, which enhance to induce apoptosis and triggers G2/M growth arrest through the downregulation of cell survival proteins and the upregulation of DR5 receptors through ROS-ER stress-CHOP pathway (Tang et al., 2013; Zhou et al., 2013b).

Having regard to the foregoing investigations, this review suggests that casticin may represent a novel therapeutic agent for the treatment of human cancers. This review elaborates the current understanding of the chemopreventive effects of casticin through its multiple molecular pathways and highlights its therapeutic value in the treatment and prevention of a wide range of cancers. To support our remarks of the anti-cancer potential of casticin, additional studies and preclinical trials are required to determine its specific intracellular sites of action and derivative targets in order to fully understand the mechanisms of its antitumor activity to validate this compound as medicinal agent in the prevention and treatment of various diseases including cancer.

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