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Review

Modulation of multiple cellular signalling pathways as targets for anti-inflammatory and anti-tumorigenesis action of Scopoletin

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Abstract

Objectives Scopoletin (6-methoxy-7-hydroxycoumarin) is a naturally occurring coumarin belonging to the category of secondary metabolites. Coumarins are commonly found in several herbs and play a prominent role in the defense mechanism of plants. Beneficial effects of scopoletin including antioxidant, anti-diabetic, hepatoprotective, neuroprotective and anti-microbial activity induced via numerous intracellular signalling mechanisms have been widely studied. However, anti-inflammation and anti-tumorigenesis properties of scopoletin are not well documented in the literature. Therefore, the primary focus of the present review was to highlight the plethora of research pertaining to the signalling mechanisms associated with the prevention of the progression of disease condition by scopoletin.

Key findings Multiple signalling pathways like nuclear erythroid factor-2 (NEF2)-related factor-2 (NRF-2), apoptosis/p53 signalling, nuclear factor-κB (NF-κB) signalling, autophagy signalling, hypoxia signalling, signal transducer and activator of transcription-3 (STAT3) signalling, Wnt-β signalling, Notch signalling are coupled with the anti-inflammation and anti-tumorigenesis potential of scopoletin.

Summary Understanding crucial targets in these molecular signalling pathways may support the role of scopoletin as a promising naturally derived bioactive compound for the treatment of several diseases.

Keywords: scopoletin; coumarin; inflammation; cancer; signalling; pharmacological property

Introduction

The principal metabolite compounds existing in almost all the plants are coumarins and they have specific therapeutic properties. A significant diversity of safe and efficient coumarin derivatives is being determined to be latent drug candidates.[1] The bioactive compounds or phytochemicals commonly seen belong to the classes of phenolics, terpenoids and coumarins. Coumarins are heterocyclic in structure that occurs because of the condensation of pyrone ring with benzene ring (benzopyrenes), produced at the time of injury, disease state and stress. The primary sites of synthesis are young and actively growing leaves, the shoot and the roots. Their accumulation varies in organisms and is mainly based on the class of coumarin preferred. There are numerous types of

coumarins identified, belonging to more than 150 plant species from 30 different families.[2]

6-Methoxy-7-hydroxycoumarin, called scopoletin, is a derivative of coumarin occurring naturally in many of the medicinal herbs, namely *Scopolia* spp., *Artemisia* spp., *Solanum* spp., *Crossostephium* spp. (spp. refers to specific pluralis) and edible foods like pepper, chicory, and oats. Various pharmacological applications of scopoletin observed are anti-microbial, antioxidative, anti-inflammatory and analgesic functions. It also exerts anti-thyroid, anti-hyperglycaemic and endocrinal activities. A considerable role of scopoletin to be highlighted is its cytotoxic activity exhibited specifically in cancers.[3–5]

Scopoletin was first isolated and discovered by Eykman in 1884. He studied the monomethyl ester of esculetin which has a melting point at 204°C to be designated as scopoletin. He used the rhizome of *Scopolia japonica,* a member of Solanaceae family.[6] Other efficient works in the family of Solanaceae include isolation of scopoletin by Kunz and co-workers in 1885 from *Atropa belladonna*. In 1909, Power and Moore worked on *Prunus serotina*, a Rosaceae family member. This was followed by Moore (1911) who isolated scopoletin from *Gelsemium sempervirens* of Loganiaceae family and from the Convolvulaceae family member *Ipomoea purga*. Goodson in 1922 established that the Asteraceae family member *Artemisia afra* to contain scopoletin as well. [6, 7]

In 1893, Kunz-Krause provided an assumption that scopoletin is 4-hydroxy 5-methyl coumarin. Scopoletin was earlier noted as β-methyl esculetin, chrysatropic acid and gelseminic acid.[6] Major works from various researchers at different periods stated that scopoletin is an ether of esculetin.[8] It was significant when an alcoholic extract of a plant resin was biochemically analysed to contain scopoletin along with glucosides, gelsemine, gelsemine and other sugars.[9] Edwards and Rogerson (1927) found a new glucoside form of scopoletin called 'fabriatin' from *Fabiana* sp. (sp., singular).[10] Scopoletin was first studied by Gentner (1928), who discovered the occurrence of blue fluorescent compounds in germinating seeds when observed under UV light. Best Rupert (1944) identified that the fluorescent compound as 7-hydroxy 6-methoxy coumarin for the first time.[11] Scopoletin was isolated as a blue fluorescent protein from the potato tubers infected with leafroll virus.[12] Similarly, the roots of *Avena sativa* also yielded the blue fluorescent scopoletin protein.[13]

Scopoletin is derived from the carbon skeleton C6-C3 and contains the flavonoid backbone core of 1,2-benzopyrone structure.[14] Scopoletin has shown to exhibit greater fluorescence in water. It is a photoacid presenting an excited-state proton transfer (ESPT) in competence with fluorescence, whose absorbance and emission spectrum yield ground-state and excited-state pK_a values ranging from 7.4 ± 0.1 and 1.4 ± 0.1 accordingly. The Stokes shift is measured to be less than 100 nm for aqueous scopoletin at pH ranging from highly acidic to neutral (pH 3 to 7).[15] Figure 1 depicts the timeline for the discovery of scopoletin.

Sources and Occurrence of Scopoletin

Scopoletin is a low-molecular-weight phytoalexin biosynthesised in various plants to overcome microbial attacks.[16] Scopoletin belongs to the first classification comprising of simple coumarins and is widely distributed among plants.[2] Almost all the plant families yield scopoletin in higher amounts. Some of which are Solanaceae, Rosaceae, Loganiaceae, Asteraceae, Aceraceae, Euphorbiaceae, Rubiaceae, Rutaceae, Fabaceae, Combretaceae, Meliaceae, Gentianaceae, Leguminoseae etc. They possess valuable medicinal properties.[17]

A few renowned plant species known for their pharmacological applications that are studied to contain scopoletin are listed: *Artemisia annua* L (Asteraceae),[18] *Angelica dahurica* (Apiaceae),[19] *Aleurites moluccana* (Euphorbiaceae),[20] *Sorbus commixta* Hedl. (Rosaceae),[21] *Argyreia speciosa* (Convolvulaceae),[22] *Manihot esculenta* (Euphorbiaceae),[23] *Sphaeralcea angustifolia* (Malvaceae),[24] *Spilanthes acmella* (Asteraceae),[14] *Tetrapleura tetraptera* (Fabaceae), *Physalis alkekengi* (Solanaceae), *Cedrelopsis rakotozafyi* (Rutaceae), *Terminalia trophophylla* (Combretaceae), *Lasianthus lucidus* (Rubiaceae),[17] *Erycibe schmidtii* (Convolvulaceae),[25] *Morinda citrifolia* (Rubiaceae),[26] *Canscora decussata* Schult. (Gentianaceae), *Clitorea ternatea* Linn. (Leguminoseae), *Convolvulus pluricaulis* Choisy. (Convolvulaceae),[27] *Chenopodiastrum murale* (Chenopodiaceae)[28] and *Melia azedarach* L. (Meliaceae).[29] All these plants display an array of excellent anti-tumour, anti-inflammatory, anti-microbial effects.

There are several environmental factors directing the quantity of scopoletin occurring in plants, which include the geographical region, altitude and season. It is reported that scopoletin

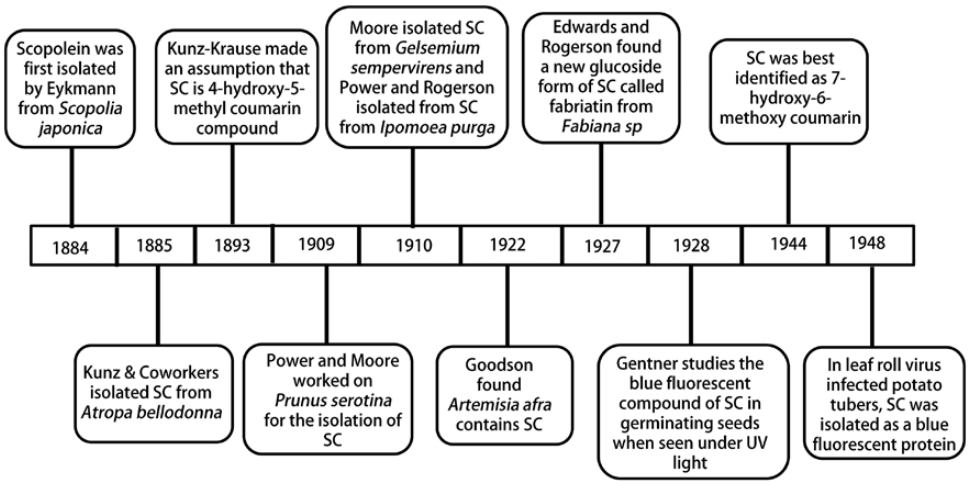

Figure1 Timeline for the discovery of Scopoletin.

accumulates more when plants grow in high altitudes.[2] Among different plant hormones like abscisic acid and gibberellin, the accumulation of scopoletin was reported to be high with the action of ethylene. Popular studies carried out in *Phleum pratense* proved that the action of scopoletin slightly varied with that of other coumarins with regard to plant growth. They showed exact patterns of inhibition of cell elongation while differing in the cell division, polarity and trichoblast differentiation, thereby, suggesting that the action of scopoletin and that of other coumarins alter differentially the growth mechanism.[30] Leaves of boron-deficient tobacco plants yielded scopoletin 20 times higher than that of normal plants. This provides a relationship between scopoletin and lignin synthesis.[31] Scopoletin is the natural compound that potentially regulates the activity of plant hormone indole acetic acid (IAA) oxidase. The crucial role of scopoletin in the *in vivo* modulation of IAA levels in plants is variedly studied.[32]

Biosynthesis or Isolation of Scopoletin

Biosynthesis of scopoletin occurs via phenylpropanoid metabolism and is also studied in the cassava plants where scopoletin is synthesised as the secondary metabolite. Three pathways of phenylpropanoid metabolism that leads to the production of scopoletin are (1) hydroxycinnamate, (2) hydroxy caffeate and (3) ferulate intermediates.[33] All the three pathways start with the deamination of amino acid l-phenylalanine, the end product of the shikimate synthesis pathway that produces (*E*)-cinnamate with the help of the enzyme called phenylalanine ammonia-lyase (PAL). From here, the pathways get diverges for the production of scopoletin.

Bioavailability, Safety and Toxicity

Generally, scopoletin is orally administered and the mode is undoubtedly considered as the most suitable and admissible route of drug administration route. It is especially favoured for patients who require long-term medication. Nevertheless, the complete bioavailability of scopoletin is only about 6.0%. This is due to the extremely moderate water solubility and instability in physiological media.[34] An experiment was performed using scopoletin extracted from *S. angustifolia* to study the pharmacokinetic parameters including the elimination mode of scopoletin. When administered orally at 400 mg/kg of a standardised active fraction (SaTES) of *S. angustifolia* in female ICR strain of albino mice (preserved in the bioterium at 25°C and 12 h light/dark cycle), detailed a bioavailability of scopoletin absorbed in plasma and distributed to blank organs. None of the derivatives after the biotransformation of scopoletin were detected while the elimination was through urine.[24]

Numerous studies have used only a simple scopoletin suspension to attain a pharmacological effect; it is administered through parenteral routes. Parenteral administration provides preferable bioavailability. It has certain disadvantages including low degrees of acceptance, safety concerns and high costs. Experiments prove that using micelles can profoundly increase the water solubility and oral bioavailability of poorly soluble drugs. The copolymer grafted in the combination of polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol (57/30/13) is an amphipathic copolymer known as Soluplus and has a property of self-assembly above the critical micelle concentration (CMC). Soluplus, having a molecular weight: 90–140 000 g/mole, is commonly employed in forming amorphous solid dispersions; producing micelles in aqueous media; and solubilising weakly water-soluble drugs. Since Soluplus-based micelles are used as excellent nano-drug carriers and delivery system, they were encouraged to study Scopoletin encapsulation for drug delivery.[34]

Scientists enhanced the formulation of Soluplus-based micelles for Scopoletin and denoted as Sco-Ms. They developed a thin-film hydration method and evaluated its oral bioavailability and distribution *in vivo*. It was revealed that scopoletin was only slightly soluble in water (~0.259 mg/mL in distilled water at 25°C), while Sco-Ms achieved a saturated scopoletin solubility of 8.512 mg/mL at 1:15 (w/w) of scopoletin/soluplus. Scopoletin formed intermolecular hydrogen bonding with the carriers that increased solubility of scopoletin. Sco-Ms increased scopoletin absorption (adhere and expand the contact area and contact time between the drug and GI tract), bioavailability and tissue distribution by 33 times.[34] In a study conducted by Jamuna *et al.*, leaf and root extracts of *Hypochaeris radicata* were subjected to acute toxicity study. The data showed that scopoletin showed a high safety profile and deaths were not observed in mice models up to doses of 100 mg/kg b.w. [35] Concurrent with the above findings, studies conducted by Pradhan *et al.* also showed that Scopoletin treatment with HeLa cells was non-toxic at even higher concentrations.[36]

Pharmacological/Therapeutic Properties of Scopoletin

Anti-inflammation effect of scopoletin

6-Methoxy-7-hydroxycoumarin (scopoletin) is found to have excellent anti-inflammatory and anti-allergic properties as mentioned in various reports. The expression levels of interleukin-1β (IL-1β), IL-6, iNOS, TNF-α and COX-2 were reduced in cells treated with scopoletin extracts.[21] The extracts from *Sphaeralcea angustifolia* were used traditionally for treating inflammation and arthritis. It is identified that the inflammatory activity in the plant was due to the occurrence of scopoletin.[24] Conditions like anxiety and stress may encounter inflammatory reactions at a considerable rate. In such cases, compounds like scopoletin having an anti-inflammatory action can be used to reduce the risk of anxiety. The lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cell lines were used to study the inhibitory activity of scopoletin in the accumulation of pro-inflammatory cytokines such as TNF-α. It is found that scopoletin negatively regulates the transcription of these pro-inflammatory cytokines.[19] In ICR male mice (20–25 g), croton-oil induced oedema in the ear was inhibited by scopoletin, which decreases the vascular leakage with the constant decrease in myeloperoxidase (MNO), polymorphonuclear (PMN) infiltration, PGE_2 and TNF-α. This showed the anti-inflammatory effects of scopoletin, which involve the prevention of eicosanoid biosynthesis, peroxidation and cell influx.[37] Scopoletin isolated from *Canarium patentinervium* was tested for the 5-LOX assay performed, which showed the inhibition of lipoxygenases at a higher rate.[38] Confertin and scopoletin are the two compounds isolated from *Hypochaeris radicata*. Cytokine assay was performed, which showed the prevention in the generation of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6.[35] In the carrageenan (Carr)-induced paw oedema, the administration of scopoletin lead to the suppression of malondialdehyde (MDA) and it significantly decreased the nitric oxide, TNF-α, PGE2 levels and also COX-2 expressions in the ICR mice models, which indicated the anti-inflammatory effects of scopoletin.[5] In the murine gout air pouch model (comprising of bursal space, that resembles the human synovium), monosodium urate (MNS) crystal-induced inflammation was assessed with regard to the scopoletin efficacy *in vitro*. The MNS

induction increased the neutrophil and phagocyte numbers that were constantly inhibited by the addition of scopoletin (100 and 200 mg/kg), modulating the levels of IL-1β, TNF-α, IL-6, PGE2 and NO.[39]

Antioxidant effects of scopoletin

The antioxidant effect of scopoletin is due to the presence of hydroxyl groups. These hydroxyl groups are better donors of hydrogen and help scopoletin to scavenge free radicals at a higher rate. The cytotoxic studies were carried out in Neuro-2A cells to prove the antioxidant effects of scopoletin. The H_2O_2-induced neuronal cytotoxicity was studied to be prevented by the scavenging effects of scopoletin. In the study conducted by Gay *et al.* (2020), it has been demonstrated that treatment with scopoletin of SH-SY5Y cells exposed to H_2O_2 showed decreased levels of apoptotic cell death and reactive oxygen species which support neuronal cell survival. Scopoletin, in a concentration-dependent manner, scavenges superoxide anion of the xanthine–xanthine oxidase reaction system, which was studied in the RAW 264.7 osteoclastic macrophages.[19] Oxidation of NADPH catalysed by horseradish peroxidase is substantially increased by scopoletin and superoxide, respectively.[40] In the oxidation of NADPH by peroxidase/H_2O_2 system, the guaiacol and scopoletin-peroxidase substrates act as catalysts. In SC-mediated NADPH oxidation, the stoichiometry between H_2O_2 and oxidised NADPH was in the ratio of 1:14 where superoxide dismutase (SOD) increased the oxidation when compared to guaiacol-mediated oxidation, where superoxide dismutase did not have any effect. This proves that peroxidase/H_2O_2 system requires a mediator molecule like scopoletin for the oxidation of NADPH.[41] Moreover, studies have demonstrated that pathophysiological conditions of inflammation may be exerted by antioxidant mechanisms like lipid peroxidation initiation and inactivation of antioxidant enzymes. In several studies conducted, several researchers have noted that scopoletin isolated from various sources like *Crossostephium chinensis*,[5] *Canarium patentinervium*,[38] *Hypochaeris radicata*[35] demonstrated the suppression of inflammatory activities possibly by promoting antioxidant mechanisms. Scopoletin is also shown to attenuate neurodegeneration by promoting antioxidant enzymes. Interesting findings reported in the literature showed that scopoletin possesses blood pressure-lowering capacity in oxidative stress rats with hypertension.[42]

Scopoletin was isolated from *Sinomonium acutum* extracts which combated superoxide anion in the system of xanthine–xanthine oxidase in a dose-dependent manner, but it did not suppress the levels of xanthine oxidase and may help in the suppression of the superoxide anion-induced damage in mice.[43] Scopoletin also showed a reduction in the oxido-lipidemic stress by increasing the antioxidant activity as well as mitigating the lipid profiles. It is further assumed that to sustain the normal morphology of the aorta, scopoletin could standardise the endothelial factor (NO) by suppressing the mRNA expression of *iNOS* gene.[44]

Anti-diabetic potential of scopoletin

Generally, most of the coumarins are proved to decrease the levels of blood glucose. Similarly, scopoletin is noted for its hypoglycaemic activity. Scopoletin negatively modulates insulin signalling by inhibiting JNK.[45] Scopoletin's effect on resistance to insulin was studied by treating high-glucose-induced and insulin-resistant HepG2 cells with scopoletin. Scientists then measured phosphatidylinositol 3-kinase (PI3 K)-associated protein kinase B (Akt/PKB) phosphorylation and found that there was significant reactivation of insulin-linked Akt/PKB phosphorylation. It is also noted that scopoletin increased the up-regulation of peroxisome proliferator-activated receptor γ2 (PPARγ2) and adipocyte-specific fatty acid-binding protein, thus ameliorating insulin resistance. Its lower adipogenic property helps in dealing with metabolic disorders, mainly type 2 diabetes mellitus.[46]

Another set of experiments dealt with the anti-diabetic effect of scopoletin using 3T3-L1 adipocytes. Most significant results were noted, including phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) and greater expression of glucose transporter type-4 on the cell plasma membrane by the activation of PI3K and subsequent PKB phosphorylation. This directly indicated the increase in the intracellular uptake of glucose. The addition of wortmannin, an inhibitor of PI3K signalling and AMPK inhibitor compound C, suppressed the cell's ability to uptake glucose, which was induced by scopoletin, proving predominantly the anti-diabetic effect of scopoletin.[47] Scopoletin was studied to down-regulate the digestive enzymes, namely α-glucosidase and α-amylase in mice models that externally induced diabetes with the application of streptozotocin. A markedly definite inhibitory effect on α-glucosidase and α-amylase was observed along with the decrease in postprandial blood glucose level. This confirms that scopoletin acts to bring down the postprandial hyperglycaemic condition via acting on the carbohydrate digestive enzyme.[48] The methanol extract from *Heracleum dissectum* consists of four different compounds of coumarins. It is shown that the scopoletin in methanolic extracts (125 and 250 mg/kg) had the potential for declining the blood glucose levels in plasma in the glucose-loaded mice.[49] *Morinda citrifolia* fruit (MCF) is mostly used in all parts of countries as it serves as a treatment for diabetes and the dosage of MCF and scopoletin were at 1 mg/ml and 0.2 μM, respectively. It has been studied in HepG2 cells that scopoletin as the biomarker showed the inhibitory effects on α-glucosidase by 57% and α-amylase by 43.5% and increased the levels of blood glucose uptake by 59.5% compared with metformin.[50] Anti-diabetic activity was measured in *Cydonia oblonga* Mill, *Allium porrum* L. ethanol extract was prepared from all these plants and tested for blood glucose level using glucose oxidase method where the study was carried out in normal and streptozotocin-induced rats in the dose range of 250 and 500 mg/kg. There was a declined rate in the blood glucose levels by 18.0% and 33.8%, respectively.[51]

Anti-cancer effect of scopoletin

Scopoletin causes an arrest of cell cycle, thereby promoting anti-tumour and anti-angiogenesis properties as studied in human prostate cancer and leukaemia cells.[18] Scopoletin governs calcium ion channels. It can suppress Ca^{2+}-ATPase by changing the concentration of intracellular and extracellular Ca^{2+} ions to maintain blood pressure. This also causes programmed cell death in tumour cells.[52] Alcohol is likely to bring about neuronal death. A study in the primary hippocampal neurons of rats stated that scopoletin triggers the inhibition of Bid proteins that correlates between the extrinsic and intrinsic pathways of apoptosis. The Bid proteins (BH3- interacting domain death agonist) belongs to the class of Bcl-2 family that governs the mitochondrial outer membrane permeabilisation during apoptosis. Eventually, the Bax proteins also get suppressed leading to the disruption of apoptotic pore formation and apoptosome assembly. Scopoletin also down-regulates caspase-9 activation. All these mechanisms may lead to inhibition of caspase-3 activation, PARP disruption and a significant inhibition of the mitochondrial apoptosis pathways.[53] The pro-apoptotic effects of scopoletin were tested in fibroblast-like synoviocytes (FLS) isolated from the rat

arthritis. Scopoletin in increasing concentrations declined the FLS cell viability, thereby inducing apoptosis. This is associated with the stimulation of caspase-3, mitochondrial membrane depolarisation and considerable Bcl-2 regulation.[54] Scopoletin is employed as a potential cure for angiogenesis-mediated diseases. The anti-arthritic effects of scopoletin were studied *in vivo,* the action was arbitrated by the anti-angiogenic changes with the up-regulation of IL-6, VEGF and FGF-2. Thus, scopoletin can be administered to arthritic implications for decreasing the neovascularisation, respectively.[55]

The selective down-regulation of serum activated ERK1/2 phosphorylation is associated with scopoletin action, without interfering with the p38 MAPK or JNK phosphorylation. These researches suggest the anti-angiogenic property of scopoletin demonstrated through the inhibition of endothelial cell migration and tube formation via ERK1/2 down-regulation accordingly.[56] Scopoletin also has excellent vasorelaxant property. A study conducted by Pan *et al*.[57] showed the anti-angiogenic potential of scopoletin in rats by means of aortic ring assay and *in vitro* angiogenesis assay proved that scopoletin elicit directly into the intervention of proangiogenic factor FGF-2 activity and indirectly prevents the expression of VEGF and resultant angiogenesis. These mechanisms are noted to be NF-κB dependent.

Hepatoprotection action of scopoletin

An inhibition in the hepatic lipid peroxidation makes scopoletin a good candidate for functioning in hepatoprotection.[28] The hepatoprotective attributes of scopoletin were detailed in a study conducted using mice with acute pancreatitis (AP) and associated lung injury (LI) by cerulein injection. Upon treatment with scopoletin, a reduction in the alleviation of AP and associated LI was observed. Histological reports proved a fall in the activity of myeloperoxidase and serum amylase, respectively. In addition, it was reported that the cerulein-induced NF-κB activation was also regulated by scopoletin in the pancreas and lungs. Moreover, scopoletin intensified the reduction of mast cell activation through decreased expression of monocyte chemoattractant protein 1, IL-33 and preprotachykinin A (encodes for neuropeptide substance P).[58] Scopoletin abides in improving the liver sensitivity to insulin and maintains the cellular glucose and lipid metabolism in C57BL/6J mice supplied with a high-fat diet (a model of the fatty liver).[45] Scopoletin can inhibit xanthine oxidase in the liver. It showed greater anti-hyperuricemic effects with notable increases in scopoletin concentrations in the liver.[34] The hepatoprotective activity of scopoletin was assessed by evaluating the release of glutamic pyruvic transaminase and sorbitol dehydrogenase from carbon tetrachloride-intoxicated rat hepatocytes. The presence of scopoletin greatly decreased the release of those enzymes by 53% and 58%, respectively, in a dose-dependent manner.[59]

The ethanolic extract of *Torilis radiata* Moench is shown to possess many secondary metabolites that include scopoletin which had been assessed and observed as the reduction in histological damage with a decrease of the hepatic malondialdehyde in association with an increase in the hepatic catalase, glutathione peroxidase and glutathione activity.[60] Scopoletin was also found in the chestnut (*Castanea crenata*) inner shell extract (CISE). In the chronic ethanol-induced oxidative stress in C57BL/6 mice, it was observed that CISE provides protection by suppressing the accumulation of lipids and peroxidation, and enhances the antioxidant system in the liver.[61] Scopoletin modulates AMPK–SREBP pathway, mediating lipogenesis in alcohol-induced hepatic lipid accumulation in mice fed with a high-fat diet. Scopoletin elevated the activities of antioxidant genes like SOD, CAT, GSH-Px and GST and hepatic AMPK. It also inhibited the DNA-binding proteins such as ACC, SREBP-1c, hepatic CYP2E1 activity and the activities of lipogenic enzymes like FAS, PAP and G6PD. The hepatic lipid peroxide level was lowered in alcohol-administered obese mice.[62]

Neuroprotection effect of scopoletin

Scopoletin exhibits protection against oxidative stress-induced neuronal damage. Conditions like alcohol-induced neurotoxicity were studied for the protective effects of scopoletin in primary hippocampus neurons. It was successfully reported that scopoletin remarkably reduced neuronal cell death by safeguarding the mitochondrial membrane from potential loss and by the suppression of Bid/Bax proteins and reduced caspase activity.[53] A study provided evidence that scopoletin can act as a potential multitarget-directed ligand that can be manifested against various enzymes like amyloid β protein (Aβ42), acetylcholinesterase (AChE) involved in Alzheimer's disease (AD) and oxidative stress. Moreover, SC can provide modifying implications in AD conditions.[16] In adult male Sprague-Dawley rats, Parkinson's disease was induced by rotenone that brings about an oxidative injury in the brain. Scopoletin translocates Nrf2 to nucleus via DJ-1 up-regulation that activates antioxidant genes, which relies on combating oxidative stress in the brain.[63] Regarding the dopamine levels in the brain, scopoletin can partially inhibit monoamine oxidase B eventually increasing the dopamine levels.[34]

The neuromuscular activity of scopoletin has been studied for its effects in animal models. It behaved as a depressor having hypotensive effects in rats. Scopoletin inhibited ACh-stimulated contractures of the toad rectus abdominis muscle, and diminished electrically evoked twitches of the *Biventer cervicis* muscle isolated from a chick.[64, 65] In a virtual screening study, Scopoletin and scopolin (isolated from the medicinal plant *Scopolia carniolica* Jaqc.) acted as potential AChE inhibitors.[66] When studied in *T. cinnabarinus*, Scopoletin provided excellent results as a neurotoxic agent as it affected the action of AChE and is thus considered as an insect repellent as well.[52] Furthermore, *in vivo* studies carried out in mice models revealed that scopoletin had a long-term potentiation effect on hippocampus, while its effect on neuronal plasticity was studied to be interfered with by nAChRs (non-competitive antagonist of the nicotinic acetylcholine receptors) like mecamylamine.[67] The neuroprotective effect of Scopoletin was studied against neurotoxicity, induced by oxygen and glucose-deprivation in a rat organotypic hippocampal slice culture with the compound extracted from *Angelica dahurica*. Scopoletin decreased the propidium iodide (PI) uptake that is directly proportional to the impairment of membrane integrity and inhibited the neuronal cell loss, thereby showing potential neuroprotection.[68]

Signalling Pathways Associated with Anti-inflammation and Anti-tumorigenesis Action of Scopoletin

Scopoletin and related factor-2 signalling

Nuclear erythroid Factor-2 (NEF2)-related factor-2 (Nrf-2) is the defense mechanism against oxidative injury and inflammation. Scopoletin along with Nrf-2 plays an accentuated role as agents of antioxidant and anti-inflammation.[3] Scopoletin is known to regulate oxidative stress by actively taking part in Nrf-2 signalling pathway (Figure 2). In Parkinson's disease (PD), Scopoletin can regulate the disease-associated protein DJ-1, translocation of Nrf2

into the nucleus and antioxidant gene transactivation to protect the brain from oxidative stress. It is also postulated that Scopoletin can be employed as an able drug to treat PD.[26] The anti-ageing property of Scopoletin was documented by the influence of Scopoletin over the manifestation of Nrf-2 transcription factor as well.[14] In mice with acute pancreatitis (AP) induced by cerulein and lung injury, SC decreased the level of pro-inflammatory cytokines in both pancreas and lungs. It also prevented the initiation of NF-κB along with Nrf-2.[58] SC as an anti-inflammatory agent was involved in the inhibition of TNF-α, IL-1β, myeloperoxidase (MPO) through the prevention of p88 MAP kinase phosphorylation.[69] Scopoletin repressed the activity of COX-2 which is responsible for the production of pro-inflammatory cytokines, prevented the PGE_2, TNF-α, IL-1β release in the LPS-stimulated RAW 264.7 cell line in a dose-dependent manner.[70] The *Morinda citrifolia* leaf extracts highly rich in Scopoletin was identified to account for its anti-angiogenic property and reducing the COX-2 levels. The leaf extract was fed to the BALB/c lung-tumour induced mice that consequently down-regulated the lung adenocarcinoma biomarker called epidermal growth factor receptor (EGFR) and prevented inflammation by reducing COX-2 levels, respectively.[71] Similarly, Scopoletin present in the extract of *Artemisia annua* showed an anti-cancer effect by suppressing the cancer cell lines irrespective of the EGFR expression, stating that this oncogene is not a resistance factor for the action of Scopoletin.[72] Nrf-2 expression increases the induction of hepatocellular carcinoma. Coumarin derivatives extracted from *Excoecaria formosana* has Scopoletin which suppresses the role of Nrf-2 expression that down-regulates the cancer cells induction.[73]

Scopoletin and apoptosis/p53 signalling

Apoptosis is a form of programmed cell death that is initiated by death receptors namely Fas/CD95, TNFR1, DR3, DR4 and DR5, which will in turn results in the caspase cascade pathway. p53 is the important tumour suppressor gene that is connected with apoptosis. It is involved in cell cycle arrest and maintains DNA integrity by declining the damage of the gene.[74] Scopoletin in human HeLa cervical cancer cells stimulated the apoptotic proteins like Bax, Bid, caspase-3, caspase-9 and decreased the expression of Bcl-2 that induced apoptosis. It suppresses the PI3K/AKT pathway and enhances the cell cycle arrest at the G2/M phase.[75] Kwon et al. (2005) analysed the apoptotic activity of Scopoletin using human hepatoma HepG2 cells.[76] Eventually, they observed that Scopoletin induced apoptotic cell death (confirmed via chromatin condensation) that included a series of reactions, which are a production of H_2O_2 followed by caspase-3 activation and the degradation of PARP, respectively. The leaf extract (100 or 200 mg/kg BW) from *Morinda citrifolia* containing Scopoletin was employed for its apoptotic and anti-inflammatory activity in the Jurkat human leukaemia cells and also in leukaemia induced BALB/c mice. It was found that the tumour suppressor genes namely CSF3, SOCS1, PTEN and TRP53 were upregulated and anti-apoptotic genes were down-regulated. Also, there was an induction of anti-inflammatory genes like IL-10 and IL-4 and declined level of pro-inflammatory genes like NF-κB.[77] Earlier, chemotherapy with cisplatin was used to study the anti-cancer activity that brought about adverse effects in human and the development of resistance by tumour cells. In this study, cisplatin along with Scopoletin induced the cell cycle arrest in the phase of G0/G1 and apoptosis in the cholangiocarcinoma cells which was

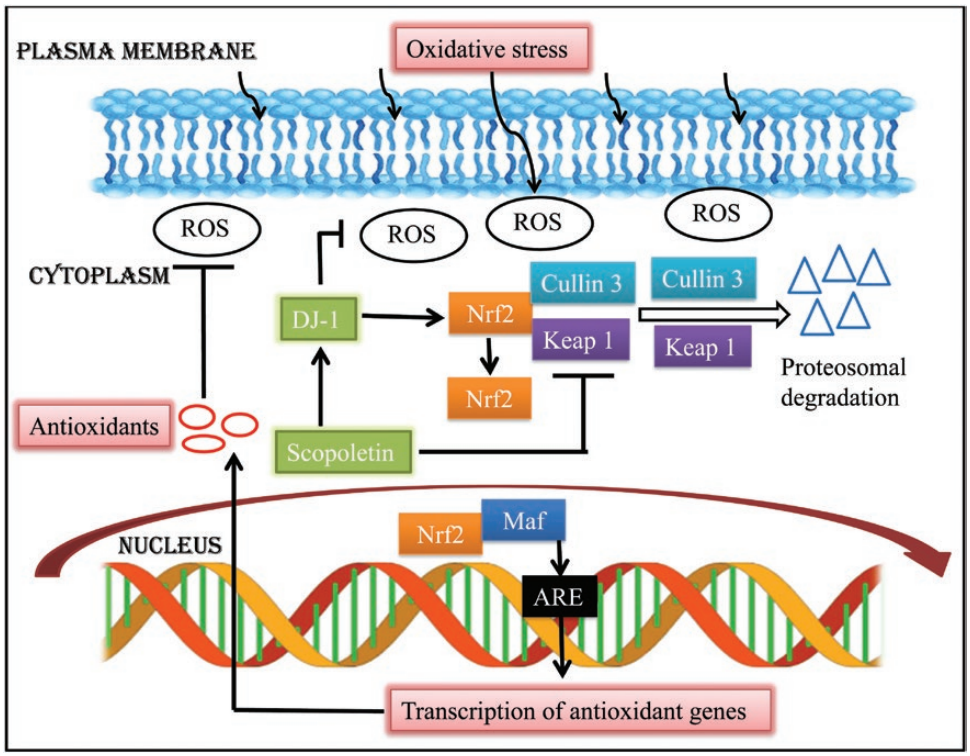

Figure 2 Scopoletin and Nrf-2 signalling pathway. Accumulation of reactive oxygen species (ROS) as a result of oxidative stress within the cell is controlled by the action of disease-associated protein (DJ-1) and antioxidants produced via the Nrf-2/ARE signalling. Certain disease states like Parkinson's manifests an increase in the ROS species generation and production of neurodegenerative bodies. Scopoletin administration can largely stabilise the DJ-1 protein and can also disrupt the Keap1-cullin3 proteosome complex, thereby setting the Nrf-2 DNA-binding protein-free.The Nrf-2 then enters the nucleus wherein it forms a complex with the Maf transcription factor to bind at the antioxidant response element (ARE) region, signalling the transcription of antioxidant genes, respectively.

studied using cell viability assays.[78] In LNCaP prostate cancer cells, Scopoletin decreased the Cyclin D expression and enhanced cell cycle arrest in the G2/M phase. Apoptosis is initiated by different concentrations of Scopoletin in the range of 40, 80 and 100 μM that shrinks the cells by creating blebbing in the membrane which is a key characteristic feature.[79] Scopoletin acting on the calcium-signal transducing pathway is related to various genes accounting for a large proportion. In *T. cinnabarinus*, the acaricidal mechanism of Scopoletin studied is due to the disruption of Ca^{2+} homeostasis inside the cell-mediated by G-protein coupled receptors, Bcl-2 protein receptors and guanylate kinase (GUK) receptors.[52] Scopoletin in the insect Sf9 cells induced the considerable rise of Ca^{2+} level in a concentrated-dependent manner, which significantly increases the acaricidal activity and brings about apoptosis in the tumour cells of the insect.[80] (Figure 3).

Scopoletin and nuclear factor κB signalling

Nuclear factor κB belongs to the family of transcription factors that regulates the wide variety of biological responses such as immune responses and inflammation. Also, it draws its main attention in oncogenesis where it is involved in the regulation of genes necessary for the development and progression of cancer.[81] The NF-κB and p38 MAPK pathways together influence the manifestation of pro-inflammatory cytokines. The GABA class of neurotransmitters (especially GABA-T) is reported to modulate the degree of pro-inflammatory cytokines produced via the NF-κB and p38 MAPK signalling. Scopoletin acts on GABA-T thereby inhibiting the activation of the canonical NF-κB pathway and the phosphorylation of MAPK, which in turn down-regulates the transcription of the pro-inflammatory mediators (Figure 4). However, Scopoletin alone can suppress p-JNK and p38 MAPK needed for the activation of NF-κB.[19] The skin fibroblasts witness the signalling of p38 MAPK and NF-κB pathways which enhances MMP-1 (matrix metalloproteinase-1) production that functions to regulate the ageing effects of the skin by degrading collagen. Scopoletin invariably acts on these signalling pathways to limit the expression of MMP-1 protein in fibroblasts thereby contributing to the anti-ageing effect.[29] Three triterpenoids and one coumarin derivative were isolated from the *Sorbus commixta*. The study conducted in EA.hy926 cells stated that Scopoletin showed the inhibitory effect on TNF-α in addition with endothelium-induced cell adhesion molecule namely ICAM-1, VCAM-1 and E-selectin, which as an inflammatory cytokine that targets the vascular cells. Scopoletin largely influenced NF-κB nuclear translocation by reducing the phosphorylation of Iκ-Bα.[21]

Scopoletin was found to inhibit the NF-κB and p38 MAPK pathway decreasing the effect of inflammation in mice models. This was proved by a study conducted by Pereira et al. (2016) reporting the inhibitory action of Scopoletin on NF-κB and p38 MAPK pathways accordingly.[82] They used carrageenan seaweeds to induce inflammation in mouse models of pleurisy. Scopoletin administration at a dose of 1 mg/kg was able to significantly lower the leukocyte migration and pleural fluid exudation. It reduced greatly the phosphorylation of p65 and p38. Also, on analysing the activities of myeloperoxidase, adenosine deaminase, nitric oxide, TNF-α and

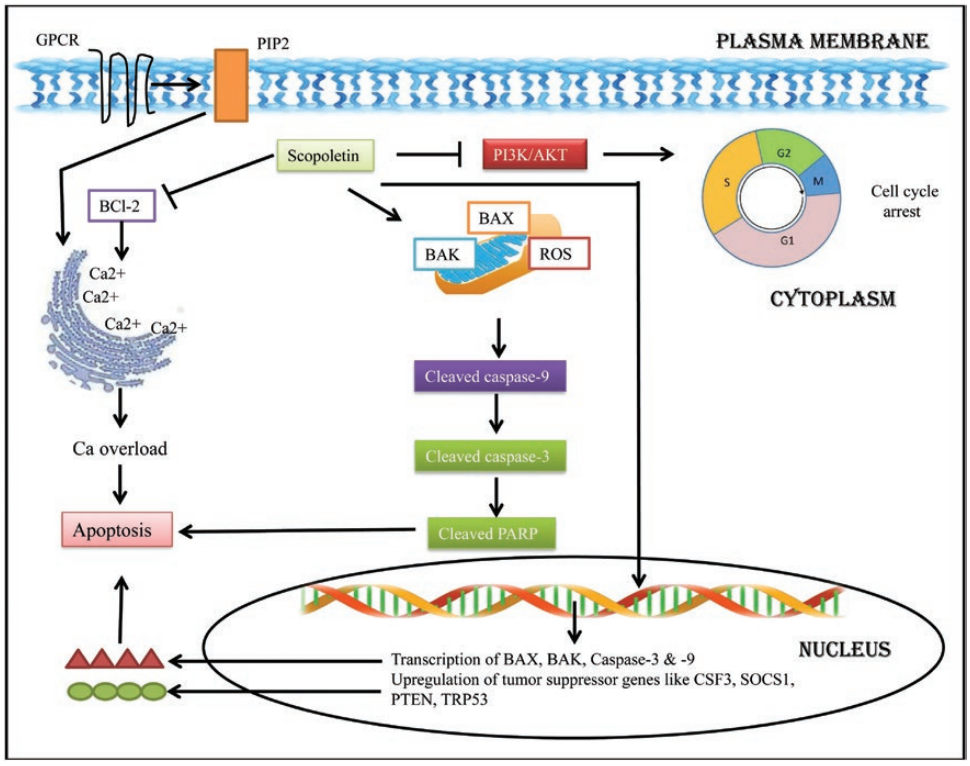

Figure 3 Scopoletin and Apoptosis/p53 signalling pathway. The caspase cascade pathway involves the action of apoptotic genes like BAX, BAK, caspase-3 and caspase-9 to result in apoptosis. Scopoletin favours programmed cell death in tumour cells by enhancing the transcription and activation of the apoptotic genes, aiding the promotion of anti-tumour genes including CSF3, SOCS1, PTEN etc., inhibiting the PI3K/AKT signalling to arrest cell cycle at various stages, suppressing BCl-2 and increasing the calcium metabolism in the cell. The scopoletin triggered apoptosis is mediated by the rise of ROS species like H_2O_2 and accumulation of the BAX/BAK proteins in the mitochondria that signals the activation of caspases to cleave the PARPs (poly(ADP-ribose) polymerases) leading to apoptosis.

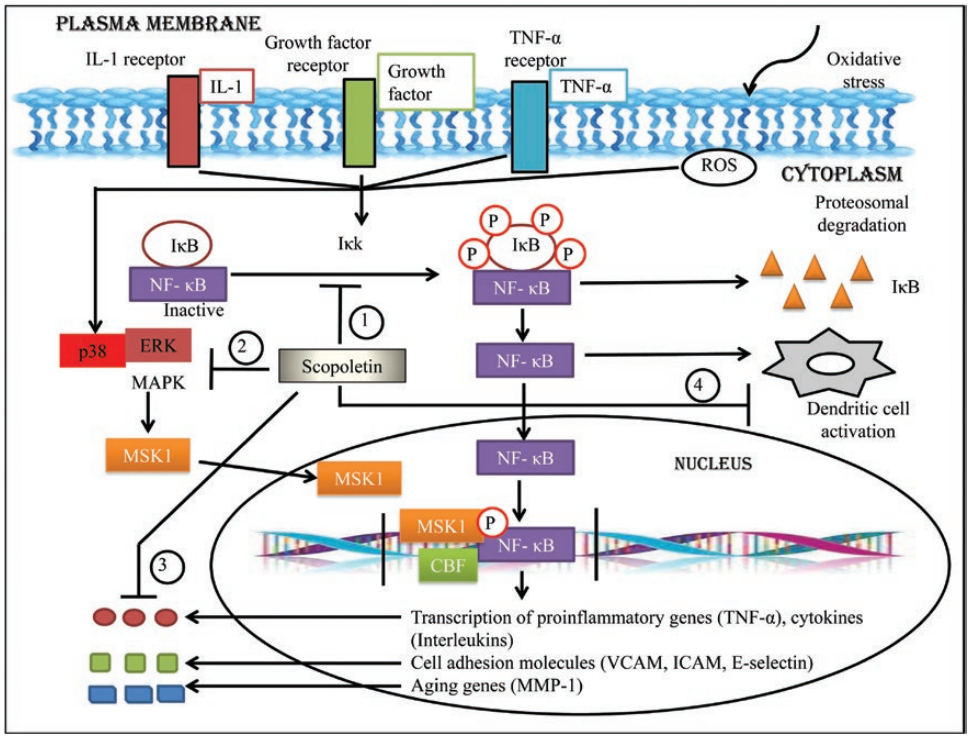

Figure 4 Scopoletin and NF-κB signalling pathway. Cell signalling molecules such as cytokines (interleukins), growth factors and oxidative stress may induce the NF-κB and p38MAPK signalling cascade to further induce the activation and production of more of inflammatory proteins. It is the IκB kinase (IKK) enzyme complex that phosphorylates the inhibitor of κB (IκB) to activate NF-κB which then triggers several inflammatory mechanisms, activates dendritic cell and transcribes regulatory genes. The mitogen stress-activated kinase (MSK1) triggered by the p38 MAPK pathway enters the nucleus to phosphorylate and bind with NF-κB, which then forms the DNA-binding complex along with the core binding factor (CBF). Scopoletin acts by inhibiting (1) phosphorylation of IκB such that Nf-κB remains inactive, (2) p38 MAPK signalling, (3) pro-inflammatory cytokines (anti-inflammatory effect), ageing proteins like matrix metallopeptidase-MMP-1 (anti-ageing effect) and cell adhesion molecules and (4) dendritic cell activation, accordingly.

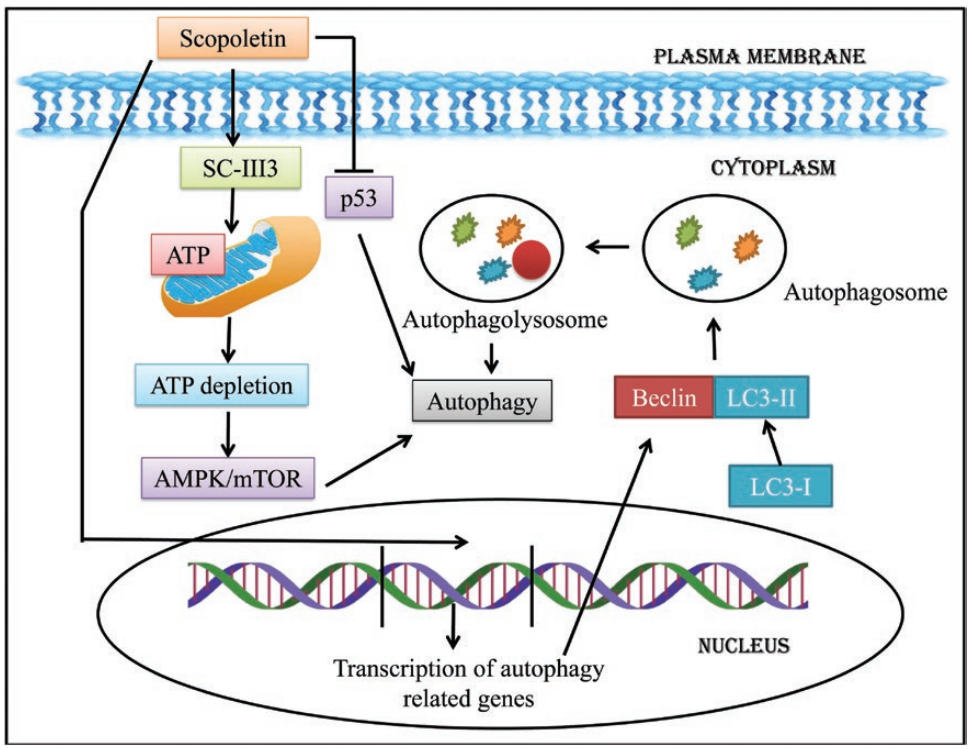

Figure 5 Scopoletin and Autophagy signalling pathway. The autophagy mechanism involves the association of Beclin1 and LC3 proteins to form the autophagolysosomal complex which can be mediated by the AMPK/mTOR pathway. Administration of scopoletin to the cells accumulates the autophagic proteins and also inhibits p53, thereby triggering autophagy. A derivative of scopoletin called SCIII3 supports autophagy by activating the AMPK/mTOR pathway via ATP depletion.

IL-1β levels, Scopoletin largely decreased their effects. Thus, these results reinforce that Scopoletin exhibits excellent anti-inflammatory activities that are attributed to its effectiveness to inhibit the phosphorylation of NF-κB and p38 MAPK, respectively. In dendritic cells (DC), Scopoletin also inhibited NF-κB activity that ameliorated experimental autoimmune encephalomyelitis (EAE) in mice through DC activation and decreased regulation of inflammation and demyelination of the central nervous system. It was shown that Scopoletin markedly suppressed the phosphorylation of NF-κB, which plays a significant role in the activation and maturation of DC. The reduction in IL-6 gene expression and the promoter activity of IL-23 were regulated by the NF-κB pathway which is vitally involved in producing pro-inflammatory cytokines.[83] In HL-60, a human pro-myeloleukaemic cell line, apoptosis was sequentially induced by Scopoletin. First, Scopoletin promoted the translocation of NF-κB from cytosol to nucleus for IκBα degradation. This, in turn, activates apoptosis by activating caspase-3 that leads to the proteolytic cleavage of 116 kDa PARP to 85 kDa protein.[84]

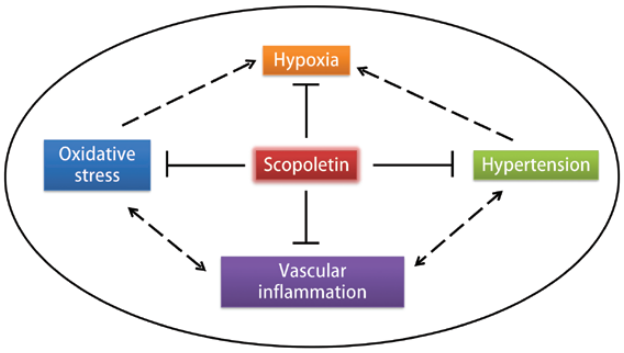

Figure 6 Scopoletin and hypoxia signalling pathway. Hypoxic conditions can be contributed by excessive oxidative stress, vascular inflammation and hypertension. Scopoletin possesses anti-hypoxic activity by inhibiting the conditions of oxidative stress (antioxidant effect), vascular inflammation (anti-inflammatory effect) and hypertension (anti-hypertensive effect) accordingly.

Scopoletin and autophagy signalling

Autophagy is an intracellular digestion process that transports the cytoplasmic constituents to the lysosome via various steps such as sequestration, autophagosome formation, autophagolysosomal formation, degradation and utilisation of degraded products with the help of autophagy-related genes.[85] A new SC derivative called SC-III3 was collectively known for the reduction of tumour development in HepG2 liver cancer cell lines. When HepG2 cells were treated with SC-III3, the autophagic proteins namely LC3-I, LC3-II and Beclin1 form the autophagosome. Also, the AMPK/TSC2-mTOR-p70s6k pathway was activated along with mitochondrial dysfunction and ATP depletion that shows the anti-cancer effects on HepG2 cells.[86] In the human lung fibroblast cell line, IMR 90 was tested for the Scopoletin effects on autophagy and anti-ageing. Scopoletin suppresses p53, reduces the level of ageing biomarker namely SA-β-Gal staining and histone acetyltransferases by increasing the levels of histone deacetylases. Thus, Scopoletin brings about autophagy in IMR 90 cell line.[87] In spinal cord injury (SCI)-induced rats, Scopoletin (100 mg/kg) exerts neuronal protection by the accumulation of autophagy-related genes such as Beclin1 and LC3B-II in positive neuronal cells. Also, the activation of adenosine monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin

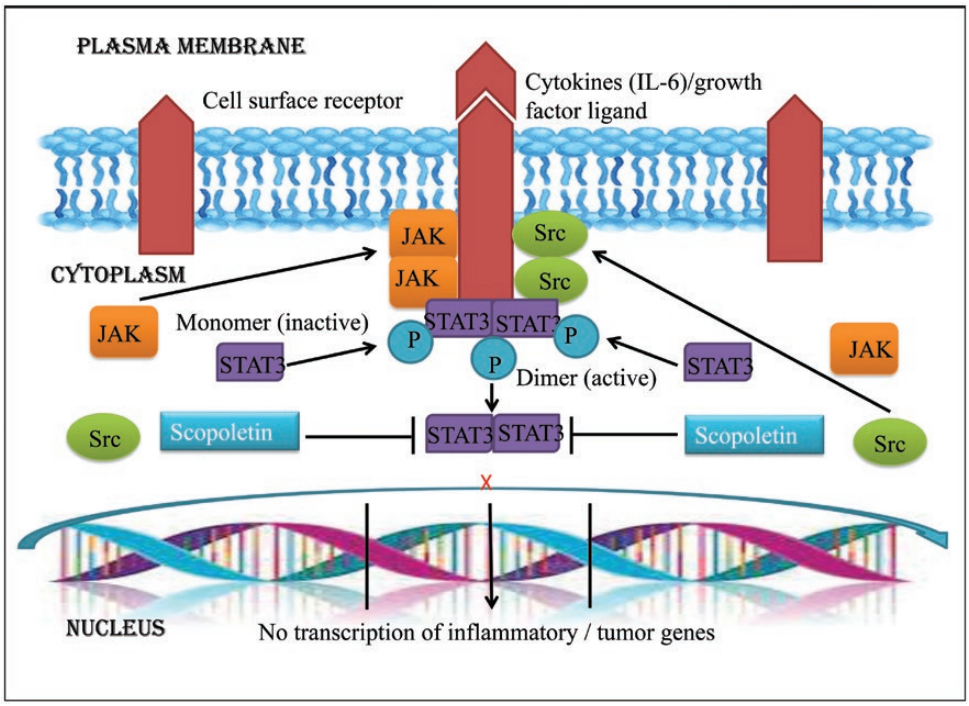

Figure 7 Scopoletin and STAT3 signalling pathway. The signalling ligands such as cytokines (IL-6) and certain growth factors find their specific cell surface receptors to bring out the STAT3 activation cascade. Firstly, the localisation of Janus kinases (JAKs) and Src tyrosine kinases (Src) at the cytoplasmic domain of the receptor proceeds, followed by the recruitment of inactive STAT3 monomers to form active STAT3 dimers, that is, by the phosphorylation of JAK and Src kinases, respectively. The activated dimers enter the nucleus to express their target inflammatory or tumour genes. Scopoletin showcases its anti-inflammatory and anti-tumour action by inhibiting the STAT3-activated dimers. Therefore, the target genes of the STAT3 transcription factor are not transcribed.

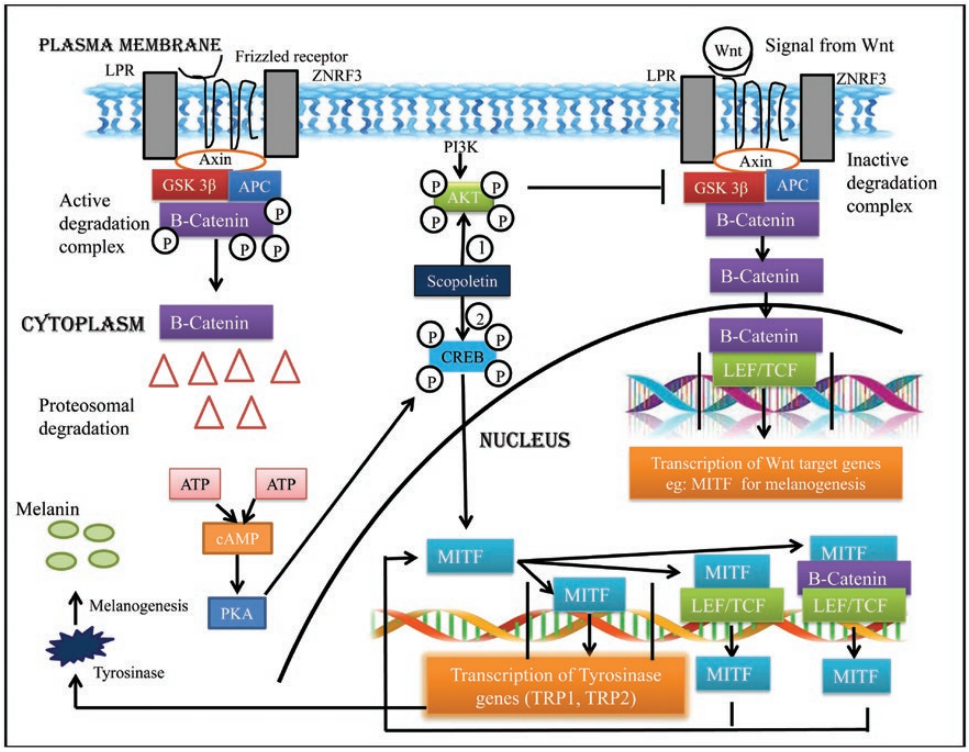

Figure 8 Scopoletin and Wnt-β signalling pathway. The classical Wnt-β catenin signal transduction takes place when the Wnt signal reaches its transmembrane receptor units comprising of lipoprotein-related protein receptors (LPR), frizzled receptors and E3 ubiquitin ligase zinc and ring finger 3 (ZNRF3) receptors. The signal then inactivates the β-catenin degradation complex, that is, axin, glycogen synthase kinase-3-β (GSK-3β) and adenomatous polyposis coli (APC) (regulator of Wnt pathway) proteins. Thus, the β-catenin escapes from proteasomal degradation enters the nucleus to complex with T-cell factor (TCF) or lymphoid enhancer factor (LEF) to transcribe the target genes. One of the target genes is the microphthalmia-associated transcription factor (MITF) needed for melanogenesis. MITF is a self-regulator of itself binding back to the LEF/TCF region separately or with the β-catenin/LEF complex. MITF stimulates the expression of tyrosinase genes (TRP-1, TRP-2) needed for melanin synthesis as well. The PI3K/Akt pathway may regulate the Wnt pathway, as activated protein kinase Akt (phosphorylated) can inhibit the action of GSK-3β invariably, thereby promoting the β-catenin protein to not get degraded. Scopoletin is said to enhance the phosphorylation of Akt (1), indirectly supporting the Wnt-β-catenin pathway to occur. Scopoletin does also phosphorylate the cAMP-responsive element-binding protein (cREB) (2). The cREB protein increases the action of MITF thereby, favouring the tyrosinase gene expression.

(mTOR) activated autophagy, thus it helps in the recovery of SCI-treated rats.[88] In U937-derived human macrophage cells, Scopoletin reduces the role of phagocytosis by inducing the formation of autophagosome thereby initiating autophagy. It acts as an immune booster and also as an adjuvant in some cases of autoimmune disorders.[89] (Figure 5).

Scopoletin and hypoxia signalling

Hypoxia is a condition in which adequate oxygen is absent in the tissues to maintain body functions. This condition can be overcome by several mechanisms like restoring oxygenation or helping the body to adopt hypoxia.[90] Chen *et al.* (2004) conducted a study to find the effects of Scopoletin on hypoxic conditions.[91] They observed the tolerance rate in forced swimming mice by testing for the tolerance to anoxia under normal pressure and myocardial specificity under hypoxia. Results were significant as Scopoletin treatment showed prolonged tolerance in both cases proving the anti-hypoxic function of Scopoletin correspondingly.[91] Hypoxia is said to result in highly prevalent hypertension. Oxidative stress and vascular inflammation are significant among the underlying causes reported. *Malva parviflora* extracts that contain Scopoletin showed anti-hypertensive activity in chronic and acute hypertensive models of mice. The plant treatments counteracted the inflammation and associated oxidative stress.[92] Another study added evidence for how Scopoletin elicits anti-hypertensive activity reducing systolic and diastolic blood pressure without affecting the heart rate, which is significant in hypoxia models.[93] A new synthetic co-drug called UPEI-400 is obtained from a covalent conjugate between lipoic acid and Scopoletin. It is studied to reduce ischaemic effects in mouse models. The effects of Scopoletin alone and UPEI-400 compound was tested by administering in acute stroke and reperfusion injury (I/R) in rats. UPEI-400 provides neuroprotection against reperfusion injury by scavenging free radicals and inhibiting inflammation pathways. These results suggest that the combination of Scopoletin and lipoic acid produced a 1000-fold increase in I/R-induced neuroprotection compared to Scopoletin alone [94] as shown in Figure 6.

Scopoletin and signal transducer and activator of transcription-3 signalling

Signal transducer and activator of transcription-3 (STAT3) belongs to the family of cytoplasmic proteins –STAT. They in response to cytokines and various growth factors regulate a variety of genes. STAT3 specifically activates a number of inflammatory responses and signalling pathways. Hyperphosphorylation of STAT3 occurs with Src tyrosine kinase or mutations in JAK proteins. However, the accurate mechanism of action of STAT3 is not clear. Further, the identification of natural compounds that inhibit STAT3 activation will play a major role in treating inflammatory diseases.[95]

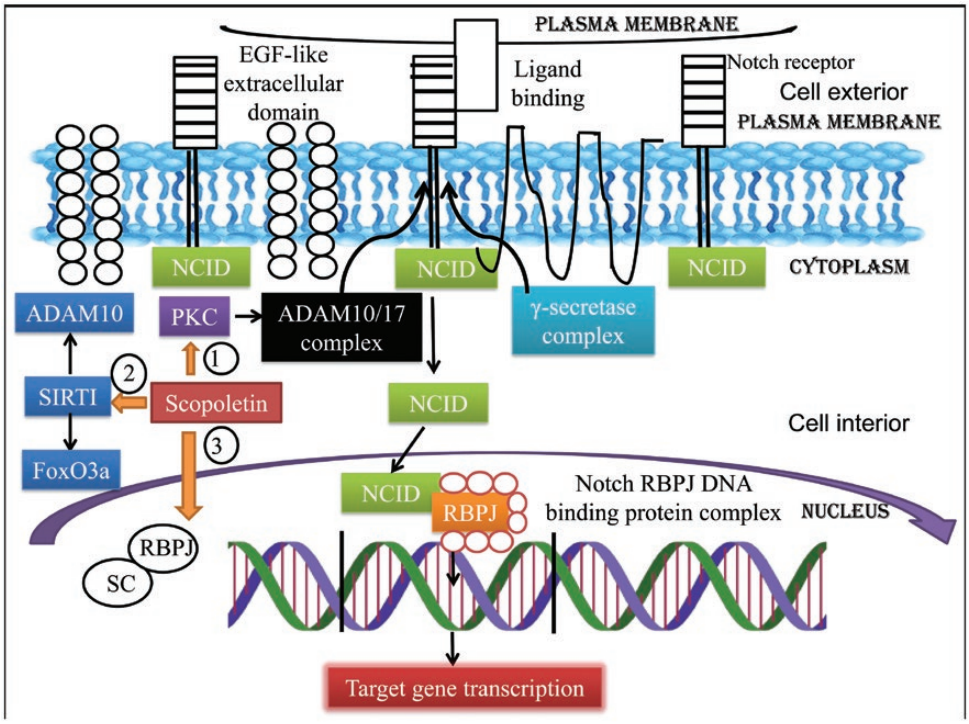

Figure 9 Scopoletin and Notch signalling pathway. Establishing a specific notch receptor-ligand binding causes the proteolytic cleavage of the notch intracellular domain (NCID) by the action of metalloproteases (ADAMs) and γ-secretase complexes. NCID enters the nucleus binds with RBP-J and other proteins forming the Notch-RBPJ DNA-binding protein complexes, to activate the target gene expression, e.g. Genes needed for erythrocyte proliferation. Scopoletin can regulate (1) Protein kinase C (PKC), which differentially modulates ADAM10/17 metalloproteases, (2) SIRT1 deacetylase/FoxO3a/ADAM10 mechanisms and (3) Recombination binding protein suppressor of hairless (RBP-J), thereby reducing its binding to NCID, limiting the acute inflammatory gene expression in arthritic conditions (anti-inflammation). Scopoletin can also regulate the TCR/CD28 mediated notch signalling in T-lymphocytes.

The regulation of STAT3 and signalling pathway was studied in *Xanthium sibiricum* extracts (MXS) which were reported to contain Scopoletin. The study was conducted in LPS-stimulated RAW 264.7 macrophages, to demonstrate the anti-inflammatory effect regulated by the combination of NF-κB and STAT3 signalling pathways. MXS at a concentration of 200 μg/ml caused the reduction in phosphorylation of the STAT3 and inhibitor of I kappa Bα (IκBα). This activity did not affect MAPK phosphorylation, respectively.[96] STAT3 signalling is vital in the development and progression of hepatocellular carcinoma (HCC). *Artemisia capillaris* extracts (ACE) are used for treating HCC. ACE contains Scopoletin and many other phytochemicals that inhibit the activity of STAT3. ACE significantly suppressed the growth, migration and colony formation of HCC cells, and inhibited the activation of STAT3 by IL-6. Collectively, the findings suggest that AC extract confers various antitumor effects against HCC through the regulation of the IL-6/STAT3 pathway.[97] The noni fruit which has a high quantity of Scopoletin was shown to inhibit its anti-cancer properties. It down-regulates pro-tumorigenic genes like BIRC5 and JAK2/STAT3/STAT5. It also increases the pro-apoptotic TRP53 genes, anti-inflammatory biomarkers like IL-4, IL-10 and glucocorticoid receptor (NR3C1)[98] (Figure 7).

Scopoletin and Wnt-beta signalling

The Wnt family of highly conserved signalling proteins comprises secreted glycoproteins that regulate various signal transduction mechanisms. They maintain the processes of development and disease manifestations. It is the β-catenin destruction complex (composed of glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC) and Axin) that phosphorylates the β-catenin protein to cause proteasomal degradation. This occurs with the lack of an active Wnt pathway when antagonised by the non-canonical Wnt pathway. While the enhancement of the canonical Wnt signalling pathway inhibited the β-catenin destruction complex to associate, through which the accumulation and subsequent translocation of β-catenin into the nucleus take place. Later the nuclear β-catenin interacts with the T-cell factor (Tcf)/lymphoid enhancer factor (LEF) family members to activate the expression of Wnt target genes.[99] Melanocyte- specific microphthalmia-associated transcription factor (MITF) isoform (MITF-M) expression (regulated by a melanocyte-specific promoter (M-promoter)) is studied to be transcribed by the LEF-1 signals from the Wnt pathway. MITF-M, self-regulator of its own manifestation during melanocyte development plays dually in the Wnt signalling pathway: a downstream target and a nuclear mediator respectively.[100]

Melanogenic enzymes called tyrosinase, tyrosinase-related protein 1 (TRP-1) and TRP-2, important for melanogenesis are regulated by MITFs, which in turn can be modulated by the cAMP-responsive element-binding protein (CREB) (by the activation of cAMP and protein kinase A (PKA)). cAMP/PKA signalling activates the canonical Wnt pathway through the inactivation of GSK-3β. The activation of the p38 mitogen-activated protein kinase (MAPK) pathway is shown to activate MITF and tyrosinase expression. Scopoletin favours melanin synthesis by activating the CREB phosphorylation and tyrosinase expression, which was proved by using PKA inhibitors that inhibited the Scopoletin-induced melanin synthesis interfering with the cAMP/PKA pathway.[101] Noni leaf extract that is a rich source

of coumarins namely, Scopoletin was tested for the underlying mechanism that induces osteogenic differentiation. Wnt proteins and β-catenin are considerable markers in osteogenesis, whose expression was found high with Noni extract. It phosphorylated Akt and GSK3*β*, enhancing the nuclear translocation and transcriptional activity of β-catenin. Thus, osteogenic differentiation has resulted via PI3K/Akt-dependent activation of Wnt/β-catenin signalling [102] as shown in Figure 8.

Scopoletin and Notch signalling

Notch signalling is a highly conserved signalling framework that supervises signal transduction of various biological processes. The Notch receptor delta and its ligands are transmembrane proteins. They have a large extracellular domain made up of epidermal growth factor (EGF)-like repeats. Signal transduction takes place via the proteolytic actions of Notch delta (catalysed by ADAM-family metalloproteases and γ-secretase enzyme complexes that contains presenilin, nicastrin, PEN2 and APH1), when the ligand binds to it.[103] Protein kinase C (PKC) is needed for Notch activation by differentially inducting ADAM10 and ADAM17. Notch activation is also reported to occur by T-cell receptor (TCR) complex/CD28 signalling pathways.[104] Researches have dealt with the molecular mechanism of Scopoletin in inducing T-cell proliferation and its particular interaction with PKC is needed. As the PKC activity outbreaks the haematopoietic cell proliferation and differentiation, it is hypothesised that Scopoletin may act on PKC to elaborate erythrocyte cell proliferation, wherein the Notch signalling could bridge the connection between the endothelial and haematopoietic lineages. Thus, it is proposed that Scopoletin treatment may raise the number of erythrocytes through levelled Notch activity.[105] The SIRT1 deacetylase belonging to the human sirtuin protein family acts by cleaving the acetyl groups from both histone and non-histone proteins, which is known to aid ADAM10 expression. Scopoletin was reported to regulate Notch and different other signalling pathways by up-regulating the SIRT1-FoxO3a-ADAM10 signalling pathway[14] as shown in Figure 9.

RBP-J (recombination binding protein suppressor of hairless) participates in Notch signalling and toll-like receptor signalling pathways, which are necessary for the development and acute inflammatory responses respectively. Thus, the RBP-J protein was speculated as a target for treating rheumatoid arthritis (RA). It is proven that the absence of RBP-J resulted in a decrease of Notch target gene expression and IL-6 production. Coumarins present in *Kirganelia reticulata,* a herb used to treat arthritis, were tested as a ligand for molecular docking analysis with regard to RBP-J protein. The Binding energy and number of hydrogen bonds formed were studied to interpret the results. Among the compounds obtained from the extract, Ellagic acid proved as an efficient drug for arthritis showing minimum binding energy of −7.65 g/mol forming seven hydrogen bonds. While Scopoletin ended with an average binding energy of −6.12 g/mol forming four hydrogen bonds primarily which was considered a favourable result.[106]

Conclusion

Numerous scientific literature has led us to tremendous knowledge pertaining to the beneficial activity of the naturally derived Scopoletin. These findings provide us with crucial aspects of the targets that Scopoletin acts to exert compelling insight like antioxidant and anti-inflammation properties. Studies also demonstrate how a multitude of factors interlinks to derive significant bioactivity. Considering the knowledge derived from the literature Scopoletin still has much potential in unravelling its use for different other disease conditions which could demonstrate its potential using *in vitro*/*in vivo* models. Focusing on signalling pathways could lead us to acquire customised targets for several diseases.

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Author contributions

Kunnathur Murugesan Sakthivel, Conceptualisation; Data curation; Manuscript writing, review and editing; Selvaraj Vishnupriya: Data curation; Manuscript writing; Loganathan Chandramani Priya Dharshini: Data curation; Manuscript writing, Rajan Radha Rasmi: Data curation; Manuscript writing and Balasubramanian Ramesh: Conceptualisation and Manuscript review.

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Conflict of Interest

The authors have no competing interest to declare.

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